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PRIMARY RESEARCH PAPER

Synergistic effects of nutrients and light favor Nostocales over non-heterocystous cyanobacteria

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Abstract Blooms of Nostocales (Cyanobacteria) are thought to be invading subtropical and temperate water bodies. According to nutrient stoichiometry and physiological differences between cyanobacterial groups, the replacement of non-heterocystous species by Nostocales is favored when dissolved inorganic nitrogen decreases. However, some studies have shown different trends. We used laboratory bioassays to evaluate the concomitant effects of light and nutrient enrichment on phytoplankton assemblages dominated by non-heterocystous filamentous cyanobacteria. Three nutrient conditions (no addition, addition of phosphate, and addition of nitrate and phosphate) and two light intensities (40 and 80 µmol photon m^{-2} s^{-1}) were assayed. Nostocales replaced or co-dominated with non-heterocystous species in all treatments by the end of the study. The shift in community composition towards Nostocales dominance led to an increase in species richness, which suggests that species with different eco-physiological traits may have differential impacts on diversity. The highest saxitoxin concentrations were measured in no addition treatments, which could link production to nutritional stress. Nostocales featured high phenotypic plasticity in terms of changes in average trichome

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volume and growth rates. These results help to have a better understanding of the conditions under which Nostocales predominance can occur.

Keywords Cyanobacterial blooms · Eutrophication · Saxitoxin · Invasive species · Nutrients · Species diversity

Introduction

Nutrient concentration and light intensity play a key role in shaping phytoplankton communities, since they constitute major resources for phytoplankton growth (Tilman, 1982). Freshwater cyanobacterial blooms are clear results of the interaction between the effects of climate change and anthropogenic eutrophication (O'Neil et al., 2012; Paerl & Paul, 2012). In this framework, a focal point of limnological research has been to comprehend and predict the causes of cyanobacterial dominance in eutrophic ecosystems, characterized by high nutrients and low light availability (Paerl & Otten, 2016). High biomass of cyanobacteria is often observed in such environments, which is ascribed to an adaptive strategy of many species to efficiently utilize low light (Scheffer et al., 1997; Schwaderer et al., 2011).

Cyanobacteria represent a highly heterogeneous and diversified group that comprises phylogenetically diverse lineages with unique eco-physiological traits (Carey et al., 2012; Komárek et al., 2014). Filamentous cyanobacteria can be grouped into heterocystous (N_2-fixers, Order Nostocales) and non-heterocystous species. Even though both types form dense and dispersive blooms in eutrophic freshwaters, the eco-physiological differences between them drive their dominance and distribution (Paerl et al., 2001, 2014). The dominance of non-heterocystous filamentous cyanobacteria, like *Planktothrix agardhii* (Gomont) Anagnostidis & Komárek, is commonly attributed to their ability to maintain net growth under self-shading conditions (Scheffer et al., 1997; Bonilla et al., 2012). In temperate freshwaters, *P. agardhii* commonly outcompetes Nostocales under extreme low underwater irradiance and high nutrient concentrations (Wiedner et al., 2002; Havens et al., 2003). Conversely, Nostocales represent a large monophyletic cluster of species characterized by high ecological plasticity and the capacity to differentiate specialized cells (heterocysts and akinetes). The above-mentioned characteristics give this group a unique advantage to anticipate population collapse (Kaplan-Levy et al., 2010; Sukenik et al., 2012; Mantzouki et al., 2015). Under low N:P ratios and optimal energy conditions, the ability to fix N_2 within the heterocysts would give diazotrophic genera a competitive advantage over other phytoplankton groups (Schindler, 2012). Nostocales, such as *Cylindrospermopsis raciborskii* (Woloszyńska) Seenayya et Subba Raju and species of the genus *Aphanizomenon*, are invasive organisms commonly found in subtropical and temperate water bodies (Sukenik et al., 2012), which raise concern due to their capacity to produce potent cyanotoxins like saxitoxins (STX) (Burford et al., 2016; Cirés & Ballot, 2016).

A decrease in phytoplankton diversity is usually associated with the development of cyanobacterial blooms (Paerl & Otten, 2013). However, different consequences in species richness have been reported under the dominance of non-heterocystous and heterocystous filamentous cyanobacteria (Bonilla et al., 2012; Olli et al., 2015), suggesting that community diversity is also dependent on the present dominant species.

The exact nutrient conditions that potentially drive shifts in cyanobacteria species during blooms remain poorly understood (Harke et al., 2016). Using mesocosm and laboratory bioassays, many studies have examined the effect of increasing nutrient concentrations on phytoplankton community to enhance our understanding of the factors that trigger cyanobacterial dominance and species replacement in eutrophic ecosystems (Donald et al., 2013; Chislock et al., 2014; Paerl et al., 2014; Davis et al., 2015). Nonetheless, only a few have explored the interaction between light intensity and nutrients under controlled laboratory conditions using natural phytoplankton communities (Dickman et al., 2006; de Tezanos Pinto & Litchman, 2010). The effect of the interaction between nutrients and light provides information that could be useful to predict cyanobacterial blooms and species composition. The incorporation of cyanobacterial physiology into bloom predictions is essential to comprehend how cyanobacteria will respond to environmental changes (Carey et al., 2012). In addition, controlled experiments are useful to document plastic responses to specific environmental variables (Miner et al., 2005).

In this work, laboratory bioassays were used to investigate the combined effects of nutrient addition and two light intensities on the structure, morphology, and growth of a phytoplankton community dominated by filamentous cyanobacteria.

Materials and methods

Sample collection

Sampling was performed on October 2012 in Lake Javier, Canelones, Uruguay (34°51′S, 56°20′W), a eutrophic artificial lake (area: 0.10 km^2, Z_{max}: 11 m) dominated by filamentous and colonial cyanobacteria (Fabre et al., 2010). At the sampling date (late spring, Southern hemisphere), lake stratification was beginning. Thus, the lake water column was partially mixed, with dissolved oxygen concentration decreasing from 3 m deep to hypoxic and anoxic conditions at the deepest layers. In addition, the vertical light attenuation coefficient was approximately 2 m^{-1}, the euphotic zone about 2.5 m, and the Z_{eu}/Z_{mix} ratio < 1 (Somma, pers. comm.).

Temperature, conductivity, pH, and dissolved oxygen were measured in situ using a Horiba D-24 and 25 sensors (Horiba Ltd., Kyoto, Japan). A surface water sample (20 l) was taken to perform laboratory incubations (see next section) and additional samples were collected for nutrient analysis, saxitoxins, and chlorophyll *a* determination.

Laboratory incubations and experimental design

Eleven-day experiments were carried out to determine the effect of light intensity and nutrient enrichment on the natural phytoplankton assemblages. The surface water sample was subdivided into 800 ml aliquots and placed in sterile 1-l Schott bottles. Zooplankton was not removed since total zooplankton biomass was low and dominated by copepods and rotifers (Carballo, pers. comm.), which generally do not prefer grazing on large cyanobacteria.

Aliquots in triplet (treatment) were incubated for 11 days in a fully factorial design under three nutrient conditions and two light intensities. The applied treatments were no addition (NA), addition of phosphate (+P), and addition of nitrate and phosphate (+N+P), under two light conditions, 40 and 80 µmol photon m^{-2} s^{-1}, hereafter low light (LL) and high light (HL) (i.e., 6 treatments and 18 bottles per experiment). Experiments were carried out under white fluorescent tubes and intensities were measured with a LI-250 light sensor. To avoid nutrients depletion, N and P were supplied in pulses (Moisander et al., 2008; Aubriot & Bonilla, 2012) applied every 24 h during the first four days of the experiment. Four pulses of 11.5 µmol K_2HPO_4–P l^{-1} were added to the (+P) treatment (total P added = 46 µmol K_2HPO_4–P l^{-1}), whereas 11.5 µmol K_2HPO_4–P l^{-1} plus 364 µmol $NaNO_3$–N l^{-1} were added per pulse to the (+P+N) treatment (total P added = 46 µmol K_2HPO_4–P l^{-1}; total N added = 1.456 mmol $NaNO_3$–N l^{-1}). The purpose of total amount of nutrients added was to avoid nutrient limitation during the incubation period, and it was based on previous knowledge of the hypolimnetic nutrient conditions of Lake Javier and test runs (Fabre et al., 2010).

Experiments were conducted at room temperature (22 ± 2°C) and bottles hand-shaken twice a day. Phytoplankton growth was monitored daily by optical density (absorbance at 750 nm) using an Evolution 60S UV–Visible Spectrophotometer (Thermo Scientific). Chlorophyll *a* and phycocyanin fluorescence were measured on a daily basis as surrogates for biomass, using an Aquafluor Handheld Fluorometer (Turner Designs). To calculate the mean light intensity perceived by organisms (I_{est}), the following equation was applied (Kenesi et al., 2008):

$$I_{est} = \sqrt{I_o * I_c},$$

where (I_0) and (I_c) are the irradiance on the surface and in the center of the vessels, respectively. I_c was calculated from absorbance at 440 nm, which was measured daily in each aliquot (Kirk, 2011).

Aliquots of 10 ml were taken from each replicate for phytoplankton identification and count at $T = 0$ (before nutrient addition), $T = 5$, $T = 8$, and $T = 11$ days and preserved in Lugol's iodine solution. Samples were observed under an inverted microscope (AXIOVERT 40 C, Carl Zeiss, Jena, Germany) using sedimentation chambers. Cell counting was performed following the Utermöhl (1958) methodology, and phytoplankton was identified to species level when possible. If present, heterocysts of *C. raciborskii* were also quantified. At least 100 individuals of the most frequent species or 400 individuals in total were counted in random fields. Species-accumulation

curves were constructed to determine the optimal sample size (when the curve became asymptotic) which was used to estimate the species richness of samples.

Individual volume was obtained by measuring at least 30 individuals of each taxa and following volumetric formulae (Hillebrand et al., 1999). Biovolume was calculated by multiplying abundance per individual volume and expressed as $mm^3\ l^{-1}$. The Shannon index (H′) was applied to biovolume at species level for each replicate, and then converted using the function D that transforms H′ into true diversity, according to Jost (2007), for $T = 0$ and for every treatment at days 5, 8, and 11.

Nutrient and chlorophyll *a* content was determined at the beginning ($T = 0$, before nutrient addition) and at the end of the experiments ($T = 11$). Total nitrogen (TN) and total phosphorus (TP) were determined following Valderrama (1981). Phosphate (Pi), nitrate, and ammonium were analyzed as previously described by Murphy & Riley (1962), Müller & Widemann (1955), Koroleff (1970), respectively. For chlorophyll *a* determination, samples were filtered through Whatman GF/C glass fiber filters and frozen. Pigments were extracted with 90% hot ethanol and measured spectrophotometrically (ISO-10260; ISO, 1992).

Saxitoxin quantification

Regarding saxitoxin analysis, 1 ml aliquots were collected in microfuge tubes. The aliquots were preserved (9:1 volume sample:volume fixator) to prevent adsorptive loss of saxitoxin, and kept at −20°C prior to analysis. The cells were disrupted by three cycles of freezing and thawing, and thus the results display the total toxin content (intracellular plus extracellular) (Nicholson & Burch, 2001). Saxitoxin concentration was determined by ELISA (Abraxis, LLC, Warminster, PA, USA) with a detection limit of 0.015 ng ml^{-1}. Cross-reactivity of the assay for the different toxin congeners were 100% for STX (per definition); Decarbamoyl STX, 29%; GTX-5B 23%; Sulfo GTX 1 & 2, 2.0%; Decarbamoyl GTX 2 & 3, 1.4%; Decarbamoyl Neo STX 0.6%; and GTX 1 & 4, < 0.2%. Each sample was analyzed in two dilutions in the working range of the assay, each dilution in duplicate. The fixator and dilution buffer were provided by the ELISA kit manufacturer. Results were expressed as ng STX equivalents per ml and then standardized to biomass parameters (total biovolume of N_2-fixers).

Data analysis

All experiments were conducted with three independent replicates, and the results are presented as the mean value (±SD). Bulk analysis of the community was performed using OD and in vivo pigments. Changes in phytoplankton assemblages were analyzed using the biovolume of the main groups and species. In order to quantitatively compare growth under tested conditions, specific growth rates (μ, day^{-1}) for non-heterocystous and heterocystous species were calculated in the exponential phase following the equation below:

$$\mu = \frac{\ln\left(\frac{BV_{t_f}}{BV_{t_i}}\right)}{t_f - t_i},$$

where t_f and t_i represent the final and initial time of the exponential phase, respectively. The average growth rate was estimated for each treatment. Negative slopes in the growth curves were considered as decay phases, for which the decay constant (k, day^{-1}) was calculated based on the equation:

$$k = \frac{\ln\left(\frac{BV_{t_f}}{BV_{t_i}}\right)}{t_f - t_i},$$

where t_f and t_i represent the final and initial time of the decay phase, respectively (Blanch & Clark, 1996).

Effects of nutrient and light treatments on phytoplankton and cyanobacterial biovolume across all dates were tested using repeated measures analysis of variance (RM-ANOVA). Log_{10}-transformations were applied when data did not meet the homoscedasticity assumption. The Bonferroni's multiple comparison test was applied to compare cyanobacterial biovolume measured over time. Two-way ANOVA was performed to determine the significant effects of the treatments on saxitoxin and chlorophyll *a* concentrations at the end of the experiments. The same approach was repeated to specify the effects on the relative abundances of trichomes of *Cylindrospermopsis raciborskii* with heterocysts (arcsine-transformed data). When significant ($P < 0.05$), the Holm-Sidak's post hoc test was used for multiple comparisons. Analyses were performed using the statistical software STATISTICA 7 (Statsoft Inc, 2004).

Results

Physical and chemical conditions of the lake and experiments

Surface water temperature in Lake Javier was 16.6°C on sampling date, while pH, conductivity, and dissolved oxygen were 9.2, 492 μS cm^{-1}, and 9.28 mg l^{-1}, respectively. TN and TP concentrations were 1.838 and 0.077 mg l^{-1}, respectively. Regarding dissolved nutrients, nitrate, and ammonium concentrations were 0.09 and 0.035 mg l^{-1}. Phosphate concentration was closed to the detection limit (0.01 mg l^{-1}). Nutrient atomic ratios were high (TN:TP = 58 and DIN:Pi = 25) (Table 1).

At day 8, pH values in the vessels reached 8.5 in the (+N+P) treatment, under low and high light, while pH values did not exceed 7.5 in the rest of the treatments [(NA) and (+P)]. By the end of the experiment, TP concentrations were the highest in the (+P) and the (+N+P) treatments as a results of Pi addition, under both light conditions (Table 1). As expected, the growth condition of phosphate sufficiency was fulfilled in all P addition treatments until the end of the incubation period. When comparing (+N+P) treatments, phosphate concentrations were significantly lower under HL than under LL, presumably owing to higher phosphate uptake rates under HL. Despite the higher uptake activity, Pi was still in high concentration levels at the end of incubations. High concentrations of Pi were confirmed in both (+P), while values were near the detection limit in both (NA). Ammonium concentration under (+N+P)/HL was significantly higher than in the other treatments, and below the detection limit (0.010 mg l^{-1}) in the NA/HL and both (+P) treatments. Nitrate concentrations were the highest in both (+N+P) treatments, which indicates that these treatments fulfilled sufficiency of dissolved inorganic nitrogen along the incubation period (at concentration levels of milligram l^{-1}). Nitrate was close or below the detection limit (0.060 mg l^{-1}) in the other treatments. Nitrate concentration was 44 and 41% less than initial nutrient addition in (+N+P)/LL and (+N+P)/HL, respectively. Total N increased in both (NA) and (+P)/HL treatments as compared to initial conditions. TN:TP ratios were the highest in both (NA) and (+N+P) treatments, while the ratios in both (+P) treatments yielded the lowest values (Table 1).

Table 1 Mean (± standard deviation of three replicates) of chlorophyll *a* (Chlo *a*) and nutrient concentration at day 0 and in all treatments at day 11

Treatment	Day	Chlo *a* (μg l^{-1})	TN (mg l^{-1})	TP (mg l^{-1})	Ammonium (mg l^{-1})	Nitrate (mg l^{-1})	Phosphate (mg l^{-1})	TN:TP (mass ratio)	Dissolved N:P ratio
–	0	67.0 (19.7)	1.838 (2.200)	0.077 (0.00)	0.035 (0.01)	0.09 (0.02)	0.010 (0.00)	58	25
LL-No addition	11	57.0 (4.4)^{a}	2.758 (0.15)^{a}	0.075 (0.00)^{a}	0.018 (0.02)^{a}	~0.060 (0.04)^{a}	~0.010 (0.00)^{a}	86	15
LL + P	11	87.0 (17.7)^{ab}	1.841 (0.05)^{b}	1.139 (0.04)^{b}	<0.010 (0.00)^{a}	<0.060 (0.00)^{b}	0.873 (0.04)^{b}	5	0.10
LL +N+P	11	312.0 (32.2)^{c}	25.284 (6.78)^{d}	1.105 (0.05)^{b}	0.013 (0.00)^{a}	9.03 (0.18)^{c}	0.466 (0.07)^{c}	55	43
HL-No addition	11	51.1 (8.4)^{a}	2.511 (0.11)^{a}	0.072(0.01)^{a}	<0.010 (0.00)^{a}	<0.060 (0.01)^{a}	~0.010 (0.00)^{a}	82	9
HL + P	11	160.2 (3.1)^{d}	2.561 (0.21)^{a}	1.125 (0.03)^{b}	<0.010 (0.00)^{a}	<0.060 (0.00)^{b}	0.793 (0.02)^{b}	5	0.11
HL + N+P	11	177.7 (148.2)^{d}	19.740 (4.55)^{d}	1.062 (0.10)^{b}	0.220 (0.12)^{d}	8.38 (0.58)^{c}	0.163 (0.05)^{d}	44	117

^{a,b,c,d} Designates significant differences between treatments at $T = 11$ ($P < 0.05$)

Along the experiment, light intensity inside the experimental units was similar in the (NA) and (+P) treatments under LL. In the (+N+P) treatment, 28 µmol photon m^{-2} s^{-1} was the lowest I_{est} registered. Under HL, I_{est} varied in both (+P) and (+N+P) treatments although values were always higher than 55 µmol photon m^{-2} s^{-1} (Fig. 1a–b).

Phytoplankton assemblage and growth rates

The initial assemblage was primarily composed by filamentous cyanobacteria and cryptophytes (~12% of total biovolume). Chlorophyceae, Bacillariophyceae, Euglenophyceae, and Dinophyceae were present in low proportions (~1.3% altogether). Non-heterocystous *P. agardhii* (Order Oscillatoriales) was the dominant species (75% of the total). Other non-heterocystous cyanobacteria were *Pseudanabaena raphidioides* (Geitler) Anagnostidis & Komárek, *Pseudanabaena* aff. *limnetica* (Lemmermann) Komárek, and *Planktolyngbya limnetica* (Lemmermann) Komárková-Legnerová & Cronberg (Order Synechococcales, hereafter PPP when considered together). Nostocales (mainly *C. raciborskii* and *Aphanizomenon* aff. *gracile* Lemmermann) were present in low percentages (~6% of the total altogether) and coccoids forms, such as *Microcystis*, were nearly absent (<0.1% of total biovolume).

Interaction between time and treatments was highly significant on phytoplankton, total cyanobacteria, and Nostocales biovolume (RM-ANOVA, $P < 0.01$). Throughout the experiment, the cyanobacteria dominated in all treatments, with a relative contribution always above 80% (Fig. 2).

Cyanobacterial biovolume significantly increased under both (+N+P) treatments, thereby indicating that P was not the only limiting nutrient in Lake Javier (RM-ANOVA, Bonferroni's post hoc test $P < 0.05$) (Fig. 2a–b). The proliferation of non-heterocystous cyanobacteria was promoted mainly under (+N+P) treatments. The highest growth rate was achieved under HL (Fig. 2e–f; Table 2). Growth of heterocystous species was significantly promoted under (+P)/HL and (+N+P) in both light treatment (RM-ANOVA, Bonferroni's post hoc test $P < 0.05$), although the highest biovolume and growth rates were reached under HL (Fig. 2c–d; Table 2). In terms of replacement and dominance, all treatments remained dominated by non-heterocystous species by day 5 (Fig. 3, S1), except for (+P)/HL, in which phosphorus addition led to a significant replacement of non-heterocystous cyanobacteria with Nostocales (Fig. 3d). By day 8, only the (+N+P)/LL remained dominated by non-heterocystous cyanobacteria, while both groups co-dominated in the rest of treatments. PPP and *P. agardhii* contributed equally to total biovolume of non-heterocystous cyanobacteria in the (+N+P)/HL treatment (Fig. 3f). At the end of the experiment ($T = 11$). Nostocales dominated in both (NA) (Fig. 3a, b), with *C. raciborskii* accounting for

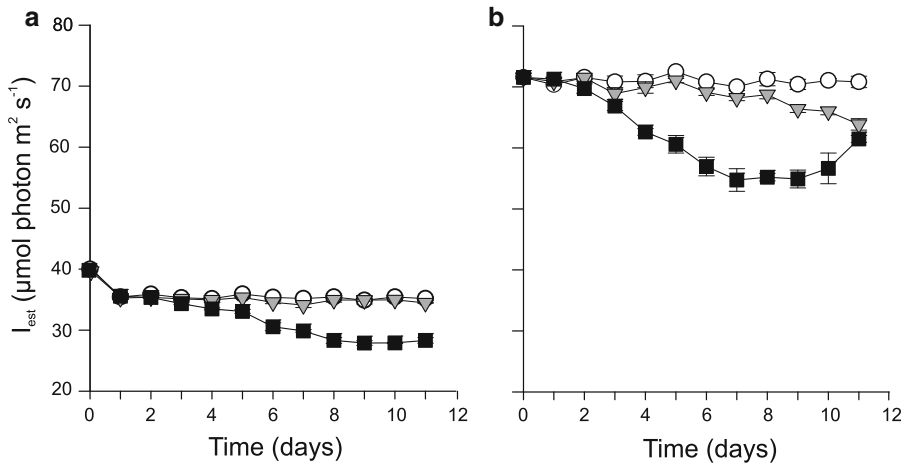


Fig. 1 Light perceived by organisms after nutrient addition treatments under low (**a**) and high light (**b**), over eleven-day experiments. *Symbols* represent mean and standard deviation ($n = 3$) for each nutrient treatment: addition of P (*filled triangle*), addition of P and N (*filled square*), no addition (*open circle*). Low light treatments, 40 µmol photon m^{-2} s^{-1}; high light treatments, 80 µmol photon m^{-2} s^{-1}

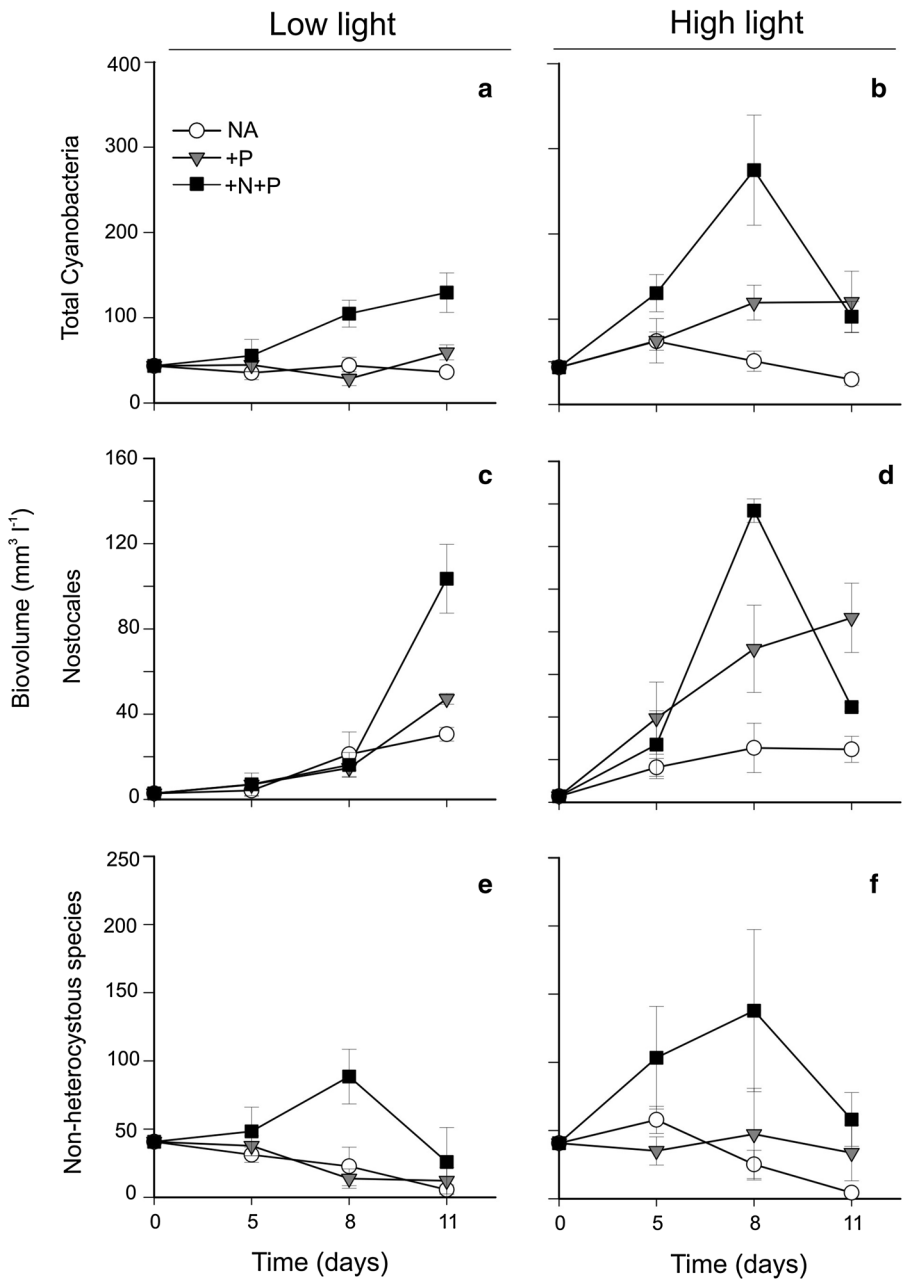


Fig. 2 Biomass responses of total cyanobacteria (**a**–**b**), Nostocales (**c**–d), and non-heterocystous cyanobacterial species (**e**–**f**) to light and nutrient treatments. Biovolume is presented as $mm^3\ l^{-1}$. *Symbols* represent mean and standard deviation ($n = 3$) for each nutrient treatment: addition of P (*filled triangle*), addition of P and N (*filled square*), no addition (*open circle*). Low light treatments (40 µmol photon $m^{-2}\ s^{-1}$) are on the left and high light treatments (80 µmol photon $m^{-2}\ s^{-1}$) on the right

∼60%, while *P. agardhii* contribution dropped to ∼5% (Fig. 3a–b). Under both (+P) treatments, Nostocales also dominated with *C. raciborskii* accounting for ∼50% and *A.* aff. *gracile* for ∼20% (Fig. 3c–d). The percentage of *C. raciborskii* trichomes with heterocysts was statistically higher in (+P)/HL treatment (32.1 ± 5.7%) with respect to the other treatments (two-way ANOVA, Holm-Sidak's post hoc test

Table 2 Growth rates (μ) and decay constant (k) of Nostocales and non-heterocystous cyanobacteria calculated from biovolume data

Treatment	μ (day^{-1})		K (day^{-1})	
	Nostocales	Non-heterocystous	Nostocales	Non-heterocystous
LL-No addition	0.207 ± 0.03	–	–	−0.142 ± 0.04
LL + P	0.233 ± 0.00	–	–	−0.119 ± 0.04
LL +N+P	0.273 ± 0.06	0.076 ± 0.02	–	−0.484 ± 0.32
HL-No addition	0.237 ± 0.02	0.069 ± 0.03	–	–
HL + P	0.364 ± 0.02	–	–	−0.023 ± 0.06
HL + N+P	0.470 ± 0.04	0.155 ± 0.02	−0.373 ± 0.02	−0.280 ± 0.10

$P < 0.05$) (Fig. S2). A shift in dominance was noticed in the (+N+P)/LL treatment, as Nostocales became dominant, with *C. raciborskii* accounting for 41% and *Aphanizomenon* for 30% (Fig. 3e). However, non-heterocystous cyanobacteria and Nostocales (only *C. raciborskii*) co-dominated the phytoplankton under (+N+P)/HL treatment (Fig. 3f).

The specific diversity of the community increased almost twice along the experiment if compared to the initial value ($D = 2.64$). Remarkably, D index was found to be the lowest when non-heterocystous species (mainly *P. agardhii*) contributed more than 60% to total biovolume. On the contrary, the increase in the contribution of Nostocales resulted in higher D index values (Fig. 4). Morphological traits of cyanobacterial species responded differently to the tested conditions. *Planktothrix agardhii* and *P. raphidioides* varied slightly their specific volumes, while *Cylindrospermopsis raciborskii* trichome size was the most influenced by treatments. Its size increased two and a half times under HL, regardless of the nutrient added. Under LL, the highest volume was attained in the (+N+P) treatment (Fig. 5).

Saxitoxin production

Saxitoxin concentration increased significantly from 0.51 ± 0.02 ng STX eq. ml^{-1} at $T = 0$ to maximum values in the (+P)/HL treatment at the end of the experiments (0.95 ± 0.06 ng STX eq. ml^{-1}). Significantly low values were detected in the (+N+P)/HL (0.356 ± 0.13 ng STX eq. ml^{-1}) (two-way ANOVA, Holm-Sidak's post hoc test $P < 0.05$). When standardized to total Nostocales biovolume, mean saxitoxin contents in both (NA) were significantly higher than in nutrient addition treatments (two-way ANOVA, Holm-Sidak's post hoc test $P < 0.05$) (Fig. 6).

Discussion

The results presented in this study indicate that the interaction between nutrients and light intensity promotes changes in the dominance of non-heterocystous cyanobacteria and Nostocales. These two groups displayed significant differences in their eco-physiological traits with consequences on their tolerance to nutritional stress and divergent effects on overall community diversity.

In our study, P addition treatments resulted in Nostocales dominance, which was enhanced by available energy (light intensity). Despite the fact that P addition failed to promote significant proliferation under LL, Nostocales displayed significant increases in biomass and benefited from the high growth rates under HL. Similar shifts in phytoplankton composition towards the dominance of Nostocales have been reported under low N:P ratios (Levine & Schindler, 1999; Schindler et al., 2008), while rapid growth of Nostocales, high N_2 fixation rates, and heterocyst differentiation were obtained in experiments with non-dissolved inorganic nitrogen (DIN) addition or P addition only (Moisander et al., 2008; Muhid et al., 2013; Burford et al., 2014). In our experiments, low DIN concentrations at the end of the experiment (close to detection limits) indicate dissolved nitrogen consumption under both light conditions, allowing only Nostocales to grow. The high percentage of *C. raciborskii* trichomes with heterocysts suggests that Nostocales proliferation could have been partly supported by N_2 fixation. However, heterocysts differentiation may not necessarily entail N_2 fixation since many factors control and regulate this process (Howarth et al., 1988). Previous experimental approaches have demonstrated that treatments subjected to limiting amounts of N were able to

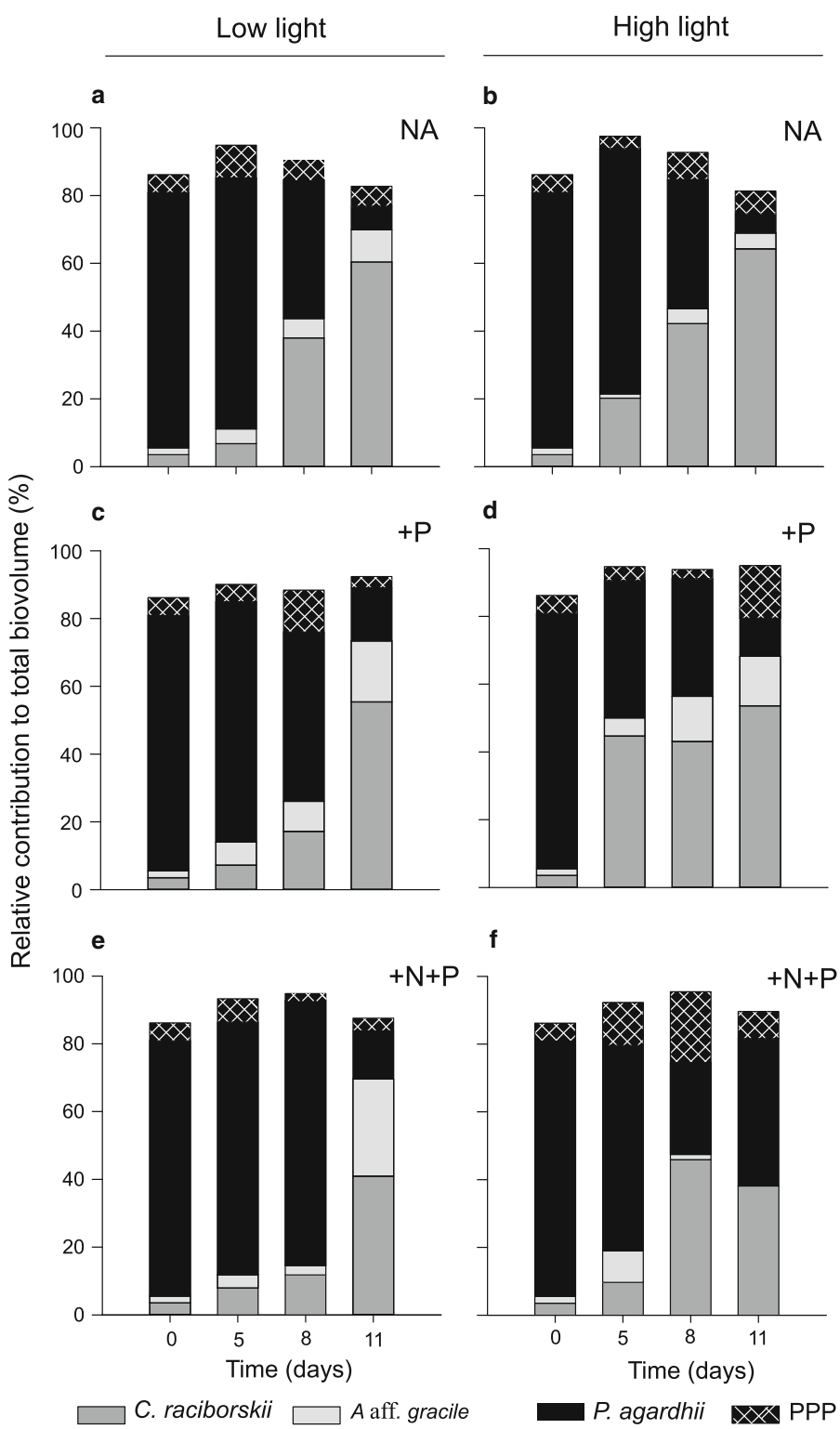


Fig. 3 Relative contribution to total biovolume (%) of cyanobacterial species at $T = 0$ and on $T = 5$, 8, and 11, under tested conditions**. a–b** No nutrient addition (NA); **c–d**, addition of P(+P); **e–f** addition of N and P (+N+P). Low light treatments (40 µmol photon m^{-2} s^{-1}) are on the left and high light treatments (80 µmol photon m^{-2} s^{-1}) on the right. *C. raciborskii, Cylindrospermopsis raciborskii*; *A.* aff. *gracile, Aphanizomenon* aff. *gracile; P. agardhii, Planktothrix agardhii;* PPP, *Pseudanabaena raphidioides*, *Pseudanabaena* aff. *limnetica* and *Planktolyngbya limnetica*

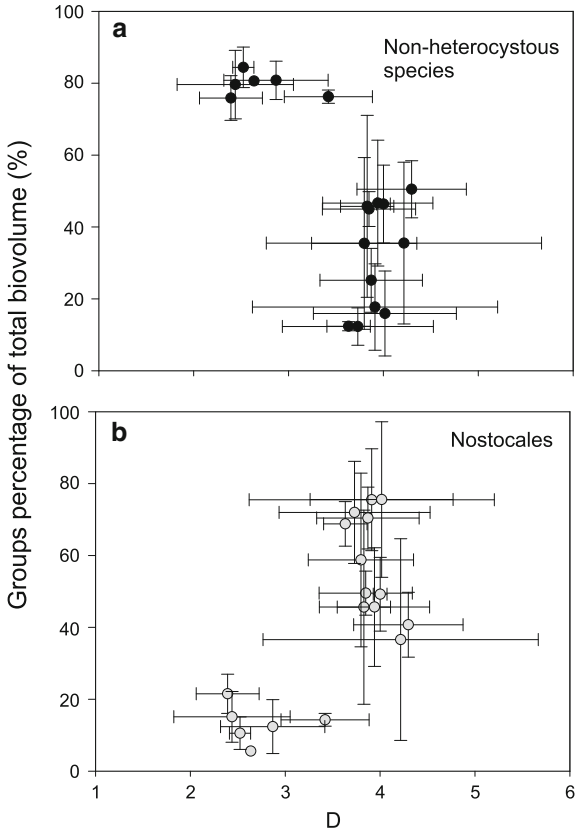


Fig. 4 Contribution of non-heterocystous cyanobacteria (**a**) and Nostocales (**b**) to total biovolume in relation to community diversity (*D*)

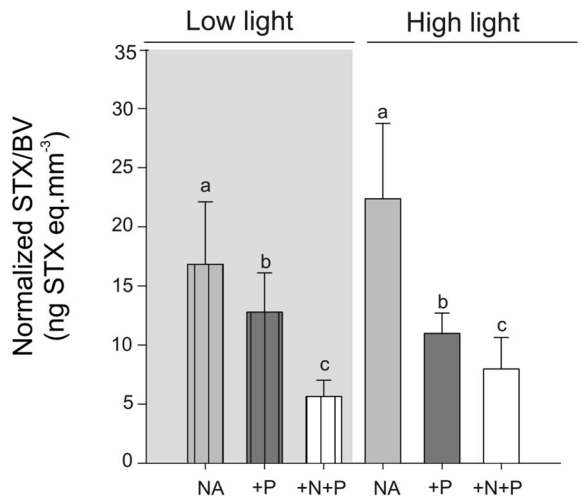
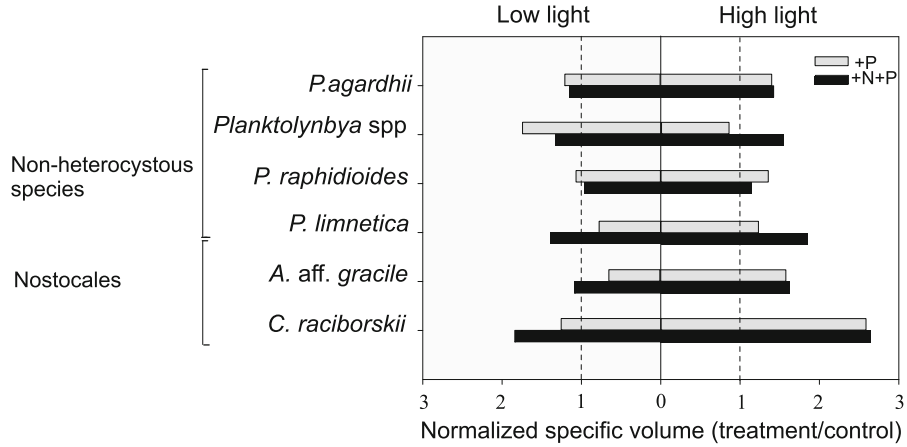


Fig. 6 Mean saxitoxin content (STX) normalized to N_2-fixers biovolume at $T = 11$, under tested treatments. Values significantly different are denoted with *a*, *b*, and *c* (two-way ANOVA, Holm-Sidak's post hoc test $P < 0.05$)

compensate for nutrient deficiencies by fixing atmospheric N (Vrede et al., 2009). The low biovolume and lower growth rates of Nostocales achieved under LL compared to HL could be partially attributed to energy limitation for dissolved nitrogen consumption and also fixation. Nitrogen fixation is energetically costly, and the rate of fixation increases as light intensity does (Agawin et al., 2007).

Non-heterocystous species were unable to grow in the (+P) treatments, which clearly indicates that the original phytoplankton from Lake Javier was co-limited by N. Even though *P. agardhii* is known to have high P requirements (Bonilla et al., 2012), the species was unable to proliferate under (+P) treatments, indicating that the N available was not enough to maintain its growth. On the contrary, the proliferation of non-heterocystous cyanobacteria was significantly promoted under both (+N+P), which is

Fig. 5 Mean specific volume (μm^3) of non-heterocystous cyanobacteria and Nostocales obtained under tested conditions at $T = 11$, normalized against respective no addition treatment



consistent with previous reports that postulate that filamentous non-heterocystous species are preferentially found in water bodies with high NP enrichment (Dolman et al., 2012).

The concomitant addition of N and P resulted in a sudden increase in cyanobacterial biomass during the first incubation days, which caused an increase in pH. This rise in pH due to photosynthesis could have led to iron (or another metal trace such as manganese) precipitation, therefore limiting further population growth in the (+N+P) treatments. Moreover, oxidized iron could have reacted with and precipitated phosphorus (Wetzel, 2001; Olsen et al., 2006).

In our study, the initial growth and high biomass of *P. agardhii* under both (+N+P), seemed to have prevented the early proliferation of Nostocales by creating appropriate light-limiting conditions, as reported by other authors for eutrophic freshwater systems (Scheffer et al., 1997; Wiedner et al., 2002). Under LL, the fast-growing response of *P. agardhii* could have depleted the available light to a critical value, therefore constraining its population and eventually leading to its own collapse. This collapse may have improved the light environment favoring Nostocales replacement by the end of the experiments. Under HL, however, non-heterocystous species and Nostocales (mainly *C. raciborskii*) co-dominated by the end of the experiments. These results indicate that *C. raciborskii* is able to grow despite the initial fast-growth of *P. agardhii* under high nutrient conditions. The high level of flexibility of *C. raciborskii* with respect to light and nutrients (Bonilla et al., 2012; 2016; Antunes et al., 2015; Pierangelini et al., 2015; Burford et al., 2016) can partly explain the shifts in community composition towards the dominance of Nostocales observed in both (+N+P) treatments.

Both control treatments tested in this work led to the replacement of non-heterocystous taxa by *C. raciborskii* and *Aphanizomenon* aff. *gracile,* albeit without significant biomass increase. *Cylindrospermopsis raciborskii* is known to have the ability to deal with low and fluctuating N and P (Présing et al., 1996; Istvánovics et al., 2000; Wu et al., 2009; Amaral et al., 2014). On the other hand, growth and N_2 fixation efficiency at low to moderate irradiance has been reported for *Aphanizomenon* sp. (Bradburn et al., 2012; Cirés & Ballot, 2016), together with its ability to grow on the basis of intracellular P stores (Walve & Larsson, 2007). These eco-physiological traits could have allowed Nostocales species to sustain net growth under the nutrient-limited conditions seen in both (NA) treatments. Along these lines, *C. raciborskii* tends to co-dominate with colonial cyanobacteria under low light and low P conditions in subtropical lakes Javier and Leandro (Fabre et al., 2010). In subtropical Peri Lake (Brazil), *C. raciborskii* dominance also occurs under low light and low P availability (Hennemann & Petrucio, 2016).

Considering that phosphate concentration in Lake Javier was close or below the detection limit and that nutrient ratios tended towards P limitation (Table 1), the addition of N alone could have resulted in similar trends to those observed in NA treatments. Alternatively, non-heterocystous species dominance could also have occurred in a (+N) treatment, but probably without biomass increase.

A general rule in ecology is the inverse effect between species diversity and total biomass accumulation in a community throughout its natural evolution. Field studies have demonstrated a decrease in species richness after cyanobacterial blooms (de Figueiredo et al., 2006; Crossetti et al., 2008; Toporowska & Pawlik-Skowrońska, 2014). Light-limiting conditions generated by non-heterocystous filamentous cyanobacteria can outcompete other phytoplankton species resulting in lower community diversity (Scheffer et al., 1997; Mischke, 2003). Notably, in our study, shifts in community composition towards the dominance of Nostocales led to a species diversity (*D*) increase, even at high total biovolume. These results support recent findings that cyanobacterial blooms do not have a unique effect at a community level (Olli et al., 2015), highlighting that cyanobacterial species with different eco-physiological traits may have differential impacts on specific diversity. Results obtained in this experimental study help explain the patterns observed by Fabre et al. (2010), who found that cyanobacterial predominance in all the studied Uruguayan lakes was not associated with a decrease in diversity, particularly in those dominated by *C. raciborskii* (Fabre et al., 2010). In line with this, the higher capacity of *C. raciborskii* for co-existence with other species has been proposed (Kokociński et al., 2010) and a field data analysis reported that higher community diversity was supported under *C. raciborskii* in relation to *P. agardhii* dominance (Bonilla et al., 2012). Moreover, perennial blooms of *P. agardhii* tend to negatively affect phytoplankton

species richness and diversity, while short-term blooms of Nostocales do not result in diversity loss (Toporowska & Pawlik-Skowrońska, 2014), thereby contradicting general assumptions.

Many organisms respond to environmental changes with physiological and morphological reversible changes, known as phenotypic plasticity. High plasticity enables growth over a wide range of environmental conditions (Agrawal, 2001). The phenotypic plasticity of *C. raciborskii* and *A.* aff. *gracile* (Shafik et al., 2003; Soares et al., 2013; Amaral et al., 2014; Cirés & Ballot, 2016) could explain the changes in growth and specific volume observed in this study.

In spite of the fact that the ecological role of cyanotoxins remains unknown (Neilan et al., 2013), environmental factors such as nutrients, light, and temperature have been proposed to cause a potential impact on toxins production (Holland & Kinnear, 2013). Nitrogen depletion, light intensity, and UV-B exposure appear to be key factors that influence STX production (Carneiro et al., 2009; Casero et al., 2014; Beamud et al., 2015), while other studies revealed no nutrient effect on its production (Stucken et al., 2014; Vico et al., 2016). It has been proposed that toxin production could confer ecological advantages to toxic strains (Holland & Kinnear, 2013). In this study, STX synthesis was clearly affected by nutrient conditions. STX concentrations showed an inverse trend with nutrient availability, suggesting that saxitoxin production may be connected to physiological stress conditions, as accounted for in previous studies (Pomati et al., 2003; Soto-Liebe et al., 2012; Beamud et al., 2015). The addition of N and P did not increment STX production, contrary to findings of Chislock et al. (2014), where high N:P treatments increased STX production nearly one time compared to low N:P treatments. Albeit not statistically significant, STX concentrations in this study were lower in (NA)/LL as compared to (NA)/HL. This could suggest that light intensity energetically limited toxin production, which is in line with previous reports indicating that STX production is regulated by light (Carneiro et al., 2009). Further research should be conducted to determine which environmental factors regulate STX production and also the possible effects of the cellular nutrient status on its production.

Given the fact that our approach is constrained by laboratory bioassays (Daehler & Strong, 1996), we are aware that extrapolations to natural ecosystems should be done cautiously. However, the use of this kind of experimental approach allows addressing questions under controlled conditions. In short, this study indicates that adaptive eco-physiological traits could favor Nostocales dominance, promoting species diversity. Our results also suggest that conditions of nutritional stress could enhance the toxicity of STX producers.

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References

Agawin, N. S. R., S. Rabouille, M. J. W. Veldhuis, L. Servatius, S. Hol, H. M. J. van Overzee & J. Huisman, 2007. Competition and facilitation between unicellular nitrogen-fixing cyanobacteria and non—nitrogen-fixing phytoplankton species. Limnology and Oceanography 52: 2233–2248.

Agrawal, A. A., 2001. Phenotypic plasticity in the interactions and evolution of species. Science 294: 321–326.

Amaral, V., S. Bonilla & L. Aubriot, 2014. Growth optimization of the invasive cyanobacterium *Cylindrospermopsis raciborskii* in response to phosphate fluctuations. European Journal of Phycology 49: 134–141.

Antunes, J. T., P. N. Leão & V. M. Vasconcelos, 2015. *Cylindrospermopsis raciborskii*: review of the distribution, phylogeography, and ecophysiology of a global invasive species. Frontiers in Microbiology 6: 473.

Aubriot, L. & S. Bonilla, 2012. Rapid regulation of phosphate uptake in freshwater cyanobacterial blooms. Aquatic Microbiological Ecology 67: 251–263.

Beamud, G., P. Vico, S. Haakonsson, G. Martínez de la Escalera, C. Piccini, B. Brena, M. Pirez & S. Bonilla, 2015. Influence of UV-B radiation on the fitness and toxin expression of the cyanobacterium *Cylindrospermopsis raciborskii*. Hydrobiologia 763: 161–172.

Blanch, H. W. & D. S. Clark, 1996. Biochemical Engineering. Marcel Dekker Inc, New York.

Bonilla, S., L. Aubriot, M. C. S. Soares, M. González-Piana, A. Fabre, V. L. M. Huszar, M. Lürling, D. Antoniades, J. Padisák & C. Kruk, 2012. What drives the distribution of the bloom-forming cyanobacteria *Planktothrix agardhii* and *Cylindrospermopsis raciborskii*? FEMS Microbiology Ecology 79: 594–607.

Bonilla, S., M. González-Piana, M. C. S. Soares, V. L. M. Huszar, V. Becker, A. Somma, M. M. Marinho, M. Kokociński, M. Dokulil, D. Antoniades & L. Aubriot, 2016. The success of the cyanobacterium *Cylindrospermopsis raciborskii* in freshwaters is enhanced by the combined effects of light intensity and temperature. Journal of Limnology 75: 606–617.

Bradburn, M. J., W. M. Lewis Jr. & J. H. McCutchan Jr., 2012. Comparative adaptations of *Aphanizomenon* and

Anabaena for nitrogen fixation under weak irradiance. Freshwater Biology 57: 1042–1049.

Burford, M. A., T. W. Davis, P. T. Orr, R. Sinha, A. Willis & B. A. Neilan, 2014. Nutrient-related changes in the toxicity of field blooms of the cyanobacterium *Cylindrospermopsis raciborskii*. FEMS Microbiology Ecology 89: 135–148.

Burford, M. A., J. Beardall, A. Willis, P. T. Orr, V. F. Magalhaes, L. M. Rangel, S. M. F. O. E. Azevedo & B. A. Neilan, 2016. Understanding the winning strategies used by the bloom-forming cyanobacterium *Cylindrospermopsis raciborskii*. Harmful Algae 54: 44–53.

Carey, C. C., B. W. Ibelings, E. P. Hoffmann, D. P. Hamilton & J. D. Brookes, 2012. Eco-physiological adaptations that favour freshwater cyanobacteria in a changing climate. Water Research 46: 1394–1407.

Carneiro, R. L., M. E. V. dos Santos, A. B. F. Pacheco & S. M. F. O. Azevedo, 2009. Effects of light intensity and light quality on growth and circadian rhythm of saxitoxins production in *Cylindrospermopsis raciborskii* (Cyanobacteria). Journal of Plankton Research 31: 481–488.

Casero, M. C., A. Ballot, R. Agha, A. Quesada & S. Cirés, 2014. Characterization of saxitoxin production and release and phylogeny of sxt genes in paralytic shellfish poisoning toxin-producing *Aphanizomenon gracile*. Harmful Algae 37: 28–37.

Chislock, M. F., K. L. Sharp & A. E. Wilson, 2014. *Cylindrospermopsis raciborskii* dominates under very low and high nitrogen-to-phosphorus ratios. Water Research 49: 207–214.

Cirés, S. & A. Ballot, 2016. A review of the phylogeny, ecology and toxin production of bloom-forming *Aphanizomenon* spp. and related species within the Nostocales (cyanobacteria). Harmful Algae 54: 21–43.

Crossetti, L. O., D. C. Bicudo, C. E. M. Bicudo & L. M. Bini, 2008. Phytoplankton biodiversity changes in a shallow tropical reservoir during the hypertrophication process. Brazilian Journal of Biology 68: 1061–1067.

Daehler, C. C. & D. R. Strong, 1996. Can you bottle nature? The roles of microcosms in ecological research. Ecology 77: 663–664.

Davis, T. W., G. S. Bullerjahn, T. Tuttle, R. M. McKay & S. B. Watson, 2015. Effects of increasing nitrogen and phosphorus concentrations on phytoplankton community growth and toxicity during *Planktothrix* blooms in Sandusky Bay, Lake Erie. Environmental Science & Technology 49: 7197–7207.

De Figueiredo, D. R., A. S. S. P. Reboleira, S. C. Antunes, N. Abrantes, U. Azeiteiro, F. Gonçalves & M. J. Pereira, 2006. The effect of environmental parameters and cyanobacterial blooms on phytoplankton dynamics of a Portuguese temperate Lake. Hydrobiologia 568: 145–157.

de Tezanos Pinto, P. & E. Litchman, 2010. Interactive effects of N:P ratios and light on nitrogen-fixer abundance. Oikos 119: 567–575.

Dickman, E. M., M. J. Vanni & M. J. Horgan, 2006. Interactive effects of light and nutrients on phytoplankton stoichiometry. Oecologia 149: 676–689.

Dolman, A. M., J. Rücker, F. R. Rick, J. Fastner, T. Rohrlack, U. Mischke & C. Wiedner, 2012. Cyanobacteria and cyanotoxins: the influence of nitrogen versus phosphorus. PLoS ONE 7: e38757.

Donald, D. B., M. J. Bogard, K. Finlay, L. Bunting & P. R. Leavitt, 2013. Phytoplankton-specific response to enrichment of phosphorus-rich surface waters with ammonium, nitrate, and urea. PLoS ONE 8: e53277.

Fabre, A., C. Carballo, E. Hernández, P. Piriz, L. Bergamino, L. Mello, S. González, G. Pérez, J. G. León, L. Aubriot, S. Bonilla & C. Kruk, 2010. El nitrógeno y la relación zona eufótica/zona de mezcla explican la presencia de cianobacterias en pequeños lagos subtropicales, artificiales de Uruguay. Pan-American Journal of Aquatic Sciences 5: 112–125.

Hennemann, M. C. & M. M. Petrucio, 2016. High chlorophyll *a* concentration in a low nutrient context: discussions in a subtropical lake dominated by Cyanobacteria. Journal of Limnology 75: 520–530.

Harke, M. J., T. W. Davis, S. B. Watson & C. J. Gobler, 2016. Nutrient-controlled niche differentiation of Western Lake Erie cyanobacterial populations revealed via metatranscriptomic surveys. Environmental Science & Technology 50: 604–615.

Havens, K. E., R. T. James, T. L. East & V. H. Smith, 2003. N:P ratios, light limitation, and cyanobacterial dominance in a subtropical lake impacted by non-point source nutrient pollution. Environmental Pollution 122: 379–390.

Hillebrand, H., C. D. Dürselen, D. Kirschtel, U. Pollingher & T. Zohary, 1999. Biovolume calculation for pelagic and benthic microalgae. Journal of Phycology 35: 403–424.

Holland, A. & S. Kinnear, 2013. Interpreting the possible ecological role(s) of cyanotoxins: compounds for competitive advantage and/or physiological aide? Marine Drugs 11: 2239–2258.

Howarth, R. W., R. Marino & J. J. Cole, 1988. Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 2. Biogeochemical control. Limnology and Oceanography 33: 688–701.

ISO, 1992. Water Quality Measurement of Biochemical Parameters Spectrophotometric Determination of Chlorophyll-a Concentration. International Organization for Standardization, Geneva: 1–6.

Istvánovics, V., H. M. Shafik, M. Présing & S. Juhos, 2000. Growth and phosphate uptake kinetics of the cyanobacterium, *Cylindrospermopsis raciborskii* (Cyanophyceae) in through flow cultures. Freshwater Biology 43: 257–275.

Jost, L., 2007. Partitioning diversity into independent alpha and beta components. Ecology 88: 2427–2439.

Kaplan-Levy, R. N., O. Hadas, M. L. Summers, J. Rücker & A. Sukenik, 2010. Akinetes: dormant cells of Cyanobacteria. In Lubzens, E., J. Cerda & M. Clark (eds), Dormancy and resistance in harsh environments. Springer, Berlin: 5–27.

Kenesi, G., H. M. Shafik, A. W. Kovács, S. Herodek & M. Présing, 2008. Effect of nitrogen forms on growth, cell composition and N_2 fixation of *Cylindrospermopsis raciborskii* in phosphorus-limited chemostat cultures. Hydrobiologia 623: 191–202.

Kirk, J., 2011. Light and photosynthesis in aquatic ecosystems. Cambridge University Press, New York.

Kokociński, M., K. Stefaniak, J. Mankiewicz-Boczek, K. Izydorczyk & J. Soininen, 2010. The ecology of the invasive cyanobacterium *Cylindrospermopsis raciborskii* (Nostocales, Cyanophyta) in two hypereutrophic lakes dominated by *Planktothrix agardhii* (Oscillatoriales, Cyanophyta). European Journal of Phycology 45: 365–374.

Komárek, J., J. Kaštovský, J. Mareš & J. R. Johansen, 2014. Taxonomic classification of cyanoprokaryotes (cyanobacterial genera) 2014, using a polyphasic approach. Preslia 86: 295–335.

Koroleff, F., 1970. Revised version of "Direct determination of ammonia in natural waters as indophenol blue". The International Council for the Exploration of the Sea C. M. 1969/C:9 ICES information on techniques and methods for sea water analysis. Interlaboratory Report No. 3, 19–22

Levine, S. N. & D. W. Schindler, 1999. Influence of nitrogen to phosphorus supply ratios and physicochemical conditions on cyanobacteria and phytoplankton species composition in the Experimental Lakes Area, Canada. Canadian Journal of Fisheries and Aquatic Sciences 56: 451–466.

Mantzouki, E., P. M. Visser, M. Bormans & B. W. Ibelings, 2015. Understanding the key ecological traits of cyanobacteria as a basis for their management and control in changing lakes. Aquatic Ecology 50: 333–350.

Miner, B. G., S. E. Sultan, S. G. Morgan, D. K. Padilla & R. A. Relyea, 2005. Ecological consequences of phenotypic plasticity. Trends in Ecology & Evolution 20: 685–692.

Mischke, U., 2003. Cyanobacteria associations in shallow polytrophic lakes: influence of environmental factors. Acta Oecologica 24: S11–S23.

Moisander, P. H., H. W. Paerl & J. P. Zehr, 2008. Effects of inorganic nitrogen on taxa-specific cyanobacterial growth and *nifH* expression in a subtropical estuary. Limnology and Oceanography 53: 2519–2532.

Muhid, P., T. W. Davis, S. E. Bunn & M. A. Burford, 2013. Effects of inorganic nutrients in recycled water on freshwater phytoplankton biomass and composition. Water Research 47: 384–394.

Müller, R. & F. Widemann, 1955. Die bestimmung des nitrats in wasser. Jahrb. Wasserchem. Wasserreinigungstechnik. Verlag Chimie 12: 247–271.

Murphy, J. & J. P. Riley, 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta 27: 31–36.

Neilan, B. A., L. A. Pearson, J. Muenchhoff, M. C. Moffitt & E. Dittmann, 2013. Environmental conditions that influence toxin biosynthesis in cyanobacteria. Environmental Microbiology 15: 1239–1253.

Nicholson, B. C., M. D. Burch, 2001. Evaluation of analytical methods for detection and quantification of cyanotoxins in relation to Australian drinking water guidelines, a report prepared for the National Health and Medical Research Council of Australia, the Water Services Association of Australia, and the Cooperative Research Centre for Water Quality and Treatment.

Olli, K., R. Klais & T. Tamminen, 2015. Rehabilitating the cyanobacteria – niche partitioning, resource use efficiency, and phytoplankton community structure during diazotrophic cyanobacterial blooms. Journal of Ecology 103: 1153–1164.

O'Neil, J. M., T. W. Davis, M. A. Burford & C. J. Gobler, 2012. The rise of harmful cyanobacteria blooms: the potential roles of eutrophication and climate change. Harmful Algae 14: 313–334.

Olsen, L. M., M. Öztürk, E. Sakshaug & J. Geir, 2006. Photosynthesis-induced phosphate precipitation in seawater: ecological implications for phytoplankton. Marine Ecology Progress Series 319: 103–110.

Paerl, H. W. & T. G. Otten, 2013. Harmful cyanobacterial blooms: causes, consequences, and controls. Microbial Ecology 65: 995–1010.

Paerl, H. W. & T. G. Otten, 2016. Duelling "CyanoHABs": unravelling the environmental drivers controlling dominance and succession among diazotrophic and non-N_2-fixing harmful cyanobacteria. Environmental Microbiology 18: 316–324.

Paerl, H. W. & V. J. Paul, 2012. Climate change: links to global expansion of harmful cyanobacteria. Water Research 46: 1349–1363.

Paerl, H. W., R. S. Fulton 3rd, P. H. Moisander & J. Dyble, 2001. Harmful freshwater algal blooms, with an emphasis on cyanobacteria. Scientific World Journal 1: 76–113.

Paerl, H. W., H. Xu, N. S. Hall, G. Zhu, B. Qin, Y. Wu, K. L. Rossignol, L. Dong, M. J. McCarthy & A. R. Joyner, 2014. Controlling cyanobacterial blooms in hypertrophic Lake Taihu, China: will nitrogen reductions cause replacement of non-$N(_2)$ fixing by $N(_2)$ fixing taxa? PLoS ONE 9: e113123.

Pierangelini, M., S. Stojkovic, P. T. Orr & J. Beardall, 2015. Photo-acclimation to low light – changes from growth to antenna size in the cyanobacterium *Cylindrospermopsis raciborskii*. Harmful Algae 46: 11–17.

Pomati, F., B. A. Neilan, G. Manarolla, T. Suzuki & C. Rossetti, 2003. Enhancement of intracellular saxitoxin accumulation by lidocaine hydrochloride in the cyanobacterium *Cylindrospermopsis raciborskii* T3 (Nostocales). Journal of Phycology 39: 535–542.

Présing, M., S. Herodek, L. Vörös & I. Kóbor, 1996. Nitrogen fixation, ammonium and nitrate uptake during a bloom of *Cylindrospermopsis raciborskii* in Lake Balaton. Archiv für Hydrobiologie 136: 553–562.

Scheffer, M., S. Rinaldi, A. Gragnani, L. R. Mur & E. H. van Nes, 1997. On the dominance of filamentous cyanobacteria in shallow, turbid lakes. Ecology 78(1): 272–282.

Schindler, D. W., 2012. The dilemma of controlling cultural eutrophication of lakes. Proceeding of the Royal Society B Biological Sciences 279(1746): 4322–4333.

Schindler, D. W., R. E. Hecky, D. L. Findlay, M. P. Stainton, B. R. Parker, M. J. Paterson, K. G. Beaty, M. Lyng & S. E. M. Kasian, 2008. Eutrophication of lakes cannot be controlled by reducing nitrogen input: results of a 37-year whole-ecosystem experiment. Proceedings of the National Academy of Sciences 105: 11254–11258.

Schwaderer, A. S., K. Yoshiyama, P. de Tezanos Pinto, N. G. Swenson, C. A. Klausmeier & E. Litchman, 2011. Eco-evolutionary differences in light utilization traits and distributions of freshwater phytoplankton. Limnology and Oceanography 56: 589–598.

Shafik, H. M., L. Vörös, P. Spröber, M. Présing & A. W. Kovács, 2003. Some special morphological features of *Cylindrospermopsis raciborskii* in batch and continuous cultures. Hydrobiologia 506: 163–167.

Soares, M. C. S., M. Lürling & V. L. M. Huszar, 2013. Growth and temperature-related phenotypic plasticity in the cyanobacterium *Cylindrospermopsis raciborskii*. Phycological Research 61: 61–67.

Soto-Liebe, K., M. A. Méndez, L. Fuenzalida, B. Krock, A. Cembella & M. Vásquez, 2012. PSP toxin release from the cyanobacterium *Raphidiopsis brookii* D9 (Nostocales) can be induced by sodium and potassium ions. Toxicon 60: 1324–1334.

Statsoft Inc, 2004. STATISTICA (data analysis software). Tulsa, Oklahoma

Stucken, K., U. John, A. Cembella, K. Soto-Liebe & M. Vasquez, 2014. Impact of nitrogen sources on gene expression and toxin production in the diazotroph *Cylindrospermopsis raciborskii* CS-505 and non-diazotroph *Raphidiopsis brookii* D9. Toxins 6: 1896–1915.

Sukenik, A., O. Hadas, A. Kaplan & A. Quesada, 2012. Invasion of Nostocales (cyanobacteria) to subtropical and temperate freshwater lakes – physiological, regional, and global driving forces. Frontiers in Microbiology 3: 86.

Tilman, D., 1982. Resource competition and community structure. Monographs in Population Biology 17: 1–296.

Toporowska, M. & B. Pawlik-Skowrońska, 2014. Four-year study on phytoplankton biodiversity in a small hypertrophic lake affected by water blooms of toxigenic cyanobacteria. Polish Journal of Environmental Studies 23: 491–499.

Utermöhl, M., 1958. Zur Vervollkommung der quantitativen Phytoplankton Methodik. Mitteilungen Internationale Vereinigung Limnologie 9: 1–38.

Valderrama, J. C., 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. Marine Chemistry 10: 109–122.

Vico, P., L. Aubriot, F. Martigani, N. Rigamonti, S. Bonilla & C. Piccini, 2016. Influence of nitrogen availability on the expression of genes involved in the biosynthesis of saxitoxin and analogs in *Cylindrospermopsis raciborskii*. Harmful Algae 56: 37–43.

Vrede, T., A. Ballantyne, C. Mille-Lindblom, G. Algesten, C. Gudasz, S. Lindahl & A. K. Brunberg, 2009. Effects of N:P loading ratios on phytoplankton community composition, primary production and N fixation in a eutrophic lake. Freshwater Biology 54: 331–344.

Walve, J. & U. Larsson, 2007. Blooms of Baltic Sea *Aphanizomenon* sp. (Cyanobacteria) collapse after internal phosphorus depletion. Aquatic Microbial Ecology 49: 57–69.

Wiedner, C., B. Nixdorf, R. Heinze, B. Wirsing, U. Neumann & J. Weckesser, 2002. Regulation of cyanobacteria and microcystin dynamics in polymictic shallow lakes. Archiv für Hydrobiologie 155: 383–400.

Wu, Z., J. Shi & R. Li, 2009. Comparative studies on photosynthesis and phosphate metabolism of *Cylindrospermopsis raciborskii* with *Microcystis aeruginosa* and *Aphanizomenon flos-aquae*. Harmful Algae 8: 910–915.

Wetzel, R. G., 2001. Limnology. Academic Press, San Diego.