Research

Oikos 119: 567–575, 2010
doi: 10.1111/j.1600-0706.2009.17924.x,

Subject Editor: Enrique Chaneton. Accepted 1 September 2009

Interactive effects of N:P ratios and light on nitrogen-fixer abundance

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Nitrogen-fixers can contribute significant amounts of nitrogen (N) and impact ecosystem functioning in diverse aquatic and terrestrial ecosystems. What determines N-fixer abundance still remains poorly understood. Here we experimentally investigate major environmental controls on the abundance of N-fixers: nitrogen to phosphorus (N:P) ratio and light. We grew a N-fixer, cyanobacterium *Anabaena flos-aquae*, in a multispecies community of freshwater phytoplankton in replicated factorial design treatments with two N:P ratios and two light levels. We show that low N:P ratios promote the dominance of the N-fixer in the community, but only under high light. Under low light, N:P ratio did not have a significant effect on the abundance of the N-fixer. N fixation occurred at low N:P only and increased with increasing light. In contrast, the density of non N-fixing cyanobacteria did not depend on N:P ratios. Green algae dominated under high N:P and high light only, exhibiting the opposite pattern of dominance to N-fixers. These results are consistent with patterns observed in nature and help explain the N-fixer distribution along the environmental gradients of nutrients and light.

Nitrogen, phosphorus and light are major resources for most primary producers. Both the absolute levels and ratios of these resources impact total biomass dynamics and community structure (Tilman 1982). Nitrogen to phosphorus (N:P) ratio is often viewed as a key factor determining the outcomes of competition for nitrogen and phosphorus and, consequently, species composition (Tilman 1982, Miller et al. 2005). Low N:P supply ratios and limitation by N should favor free-living or symbiotic nitrogen fixers, thus shifting communities towards dominance by legumes in terrestrial and cyanobacteria in aquatic ecosystems (Schindler 1977, Smith 1992, Vitousek et al. 2002a). An increase in N-fixer abundance greatly impacts community structure of primary producers, ecosystem stoichiometry and biogeochemical cycling (Sterner and Elser 2002). Consequently, understanding the mechanisms controlling the abundance of N-fixers should improve our understanding of the functioning of many terrestrial and aquatic ecosystems, especially under N:P ratios changing rapidly due to human activities (Vitousek et al. 2002b).

The role of N:P ratios in controlling community composition of primary producers is especially important in aquatic ecosystems, as many N-fixers (cyanobacteria) are capable of producing toxins that negatively affect water quality and higher trophic levels (Huisman et al. 2005). Moreover, nitrogen fixation can be a main source of nitrogen in the oligotrophic ocean (Capone et al. 1997). An empirical analysis by Val Smith showed that in natural water bodies cyanobacteria tend to dominate more frequently when N:P ratios do not exceed 29 by weight (Smith 1983). Other studies report different critical N:P ratios (Howarth et al. 1988, Noges et al. 2008), but the qualitative pattern remains: low N:P ratios tend to favor cyanobacteria. A possible mechanistic explanation is that many cyanobacteria are N-fixers and when N is limiting (low N:P), they have a competitive advantage. However, several studies report a lack of or only a weak influence of N:P ratios on cyanobacterial abundance (Reynolds 1999, Downing et al. 2001). Smith and Schindler (2009) assert that ratios of total and inorganic N:P are imperfect predictors of cyanobacteria dominance because they represent the overall result of external nutrient inputs, nitrogen fixation and within-system recycling.

How universal is the role of N:P ratios in controlling cyanobacterial and N-fixer abundance? In addition to observational studies, experimental tests of the effect of N:P ratios on cyanobacteria and N-fixers are needed. Surprisingly, there are very few experimental studies investigating the effect of N:P supply ratio on phytoplankton community structure and cyanobacterial abundance. Hu and Zhang (1993) looked at pairwise competition between a N-fixer and a diatom under different combinations of N, P and Si and found that N-fixers dominated under low N:P ratios. Levine and Schindler (1999) manipulated total N:P ratio in mesocosms and found that cyanobacterial N-fixers

were stimulated by low N:P, while non-heterocystous (and presumably non N-fixing) cyanobacteria were more abundant at high N:P. Due to the nature of mesocosm experiments, there was a high variability in light and temperature conditions, as well as a substantial deviation from the target N:P ratios in treatments (Levine and Schindler 1999). This makes interpreting the results less straightforward. Recently, Vrede et al. (2009) applied different N:P ratio loadings to a natural phytoplankton assemblage on a weekly basis, and observed that the N fixer abundance increased more in the lower N:P ratios.

An experimental investigation of the role of N:P ratio in promoting N-fixers and other cyanobacteria in a more controlled multispecies environment would provide a more direct test and a more solid foundation for managing cyanobacterial blooms in nature. Currently there is an even greater need to better understand the connection between low N:P ratios and cyanobacterial blooms, as N:P ratios are declining in many lakes worldwide (Weyhenmeyer et al. 2007, Noges et al. 2008).

Nitrogen fixation is an energetically costly process that depends on light; the rates of N fixation increase with increasing light levels (Mugidde et al. 2003, Agawin et al. 2007). Consequently, light levels may mediate the effects of N:P ratios on N-fixers and affect their success. In the analysis of data from 22 lakes, Smith (1986) found that light levels mediated the effects of N:P ratios: at fixed light levels, decreasing TN:TP (total N:total P) ratio increased abundance of cyanobacteria and at a fixed TN:TP, decreasing light increased relative cyanobacterial abundance, due to low light requirements of cyanobacteria. Despite the observational evidence for the interaction of N:P ratios and light in determining the biomass of cyanobacteria (Smith 1986), to our knowledge, there are no published experimental tests of this interaction. It is unknown whether manipulating N:P ratios and light levels affects all cyanobacteria or N-fixers only. Under high light and low N:P ratios, N-fixers may gain a competitive advantage due to their ability to fix atmospheric N and dominate the community. Under low light and low N:P ratios, there may not be enough energy to maintain N-fixation, but cyanobacteria, not necessarily N-fixers, may still dominate the community because of their low light requirements (Richardson et al. 1983, Smith 1986, A. Schwaderer pers. comm). Consequently, under low light we may observe cyanobacterial dominance under high N:P ratios as well, and there should be less difference in cyanobacterial abundance between contrasting N:P ratios because of the overriding effect of light limitation.

Zooplankton may also mediate the effects of light and N:P ratios (Smith 1986), making it harder to interpret field observations. An experimental simultaneous manipulation of light levels and N:P ratios in the absence of zooplankton or other confounding factors would allow us to address the exact mechanisms by which light and N:P ratios structure phytoplankton communities. Here we experimentally examine whether there is an interaction between N:P supply ratios and light levels in determining N-fixer abundance and phytoplankton community composition, using an experimental multispecies assemblage that includes representatives from major taxonomic groups of freshwater phytoplankton.

Material and methods

Species selection

We chose eleven species from major freshwater taxonomic groups ranging in size and light and nutrient competitive abilities, including a cyanobacterial N-fixer (*Anabaena flos-aquae*), two non N-fixing cyanobacteria (*Microcystis aeruginosa* and *Planktothrix rubescens*, four green algae (*Chlamydomonas reinhardtii*, *Ankistrodesmus falcatus*, *Oocystis* sp. and *Scenedesmus quadricauda*, two diatoms (*Asterionella formosa* and *Fragilaria crotonensis*) and two cryptomonads (*Cryptomonas erosa* and *Rhodomonas minuta*). These species cover a wide phylogenetic range and a wide diversity of ecophysiological characteristics, and are common in lakes worldwide (Reynolds 1984). They were obtained either from culture collections or isolated from local lakes. To distinguish the effects of N:P ratios on all cyanobacteria versus N-fixers only, we included both a N-fixer and non-fixers to represent cyanobacteria in the community. In each vessel, the assemblage was built by inoculating similar low biovolumes for each species (approximately 120 000 $\mu m^3 ml^{-1}$). Initial total and individual species biovolumes were similar across treatments ($p > 0.05$)

Experimental design

We used two light levels (15 and 100 µmol quanta $m^{-2} s^{-1}$) and two N:P ratios (4 and 32) in a factorial design. The chosen light levels and N:P ratios, as well as absolute nutrient concentrations, are common in epilimnia (upper part of the water column) of many lakes (Wetzel 1983, Kirk 1994). Each treatment was run in duplicate in 125 ml flasks with 100 ml of medium in semi-continuous regime (daily dilutions of 0.2 day^{-1}).

Light was measured using a quantum sensor immersed in distilled water at the onset and in the algal suspensions directly in the end of the experiment, after mixing the cultures. The light sensor was positioned in the middle of the experimental vessel and secured with tape; measurements were taken when the door of the environmental chamber was closed. For obtaining the two N:P ratios, we modified regular WC medium (Guillard 1975) by changing the N levels (8 and 64 µmol l^{-1} N) and holding P levels constant (2 µmol l^{-1} P), a common way to manipulate N:P ratios (Levine and Schindler 1999, Vrede et al. 2009). To prevent carbon or iron limitation (due to high iron demands for N-fixation), we aseptically added extra HCO_3^- (×2 the standard concentration) and extra trace metals (×1.5) after autoclaving the modified WC medium. The experimental flasks were randomly arranged within one environmental chamber at 20°C and 16:8 light:dark photoperiod. They were swirled and randomly re-arranged once a day. The experiment was run for 50 days to allow sufficient time for competitive outcomes to emerge (Litchman 2003).

We quantified the response of the assemblage to the combined effects of light and N:P ratios in terms of species composition, productivity (biomass = biovolume) and nutrient availability (both dissolved inorganic and total fractions). Samples for dissolved inorganic nutrient analyses were taken

once a week and for algae enumeration twice a week. Total N and total P were measured at the end of the experiment. Nitrate was measured following Crumpton et al. (1992) and TN following Bachmann and Canfield (1996). Phosphate and TP were measured using the standard molybdate method on a nutrient analyzer.

We calculated the rates of N fixation in each treatment by solving the total nitrogen dynamics equation at equilibrium:

$$\frac{dN}{dt} = a(N_{in} - N) + N_{fix}$$

where N is total nitrogen measured in the system (μmol l^{-1}), a is the dilution rate (day^{-1}), N_{in} is total N supplied in the medium and N_{fix} is the rate of N fixation (μmol $l^{-1}day^{-1}$) determined as follows:

$$N_{fix} = a\left(N - N_{in}\right)$$

Phytoplankton samples were fixed with Lugol's solution and counted microscopically in a Palmer–Maloney counting chamber using a microscope (400× magnification). The counting unit was the individual (cell, colony, coenobium or filament). At least 80 fields were counted for the dominant species and the whole chamber was scanned for rare species. Likewise, the density of heterocysts (N-fixing cells) was quantified for the N fixer. As unit volumes differed several orders of magnitude, we used biovolumes to describe species dynamics. Biovolume calculations followed Hillebrand et al. (1999) and Jun and Dongyan (2003). Assemblages were not grown axenically; regular inspection in the light microscope revealed that heterotrophic bacteria remained well under 1% of total biomass. This method is less precise than e.g. fluorescent staining techniques but gives an estimate of bacterial contribution to biomass.

Statistical analyses

The effects of light level and N:P ratio and their interaction on total biovolume, the absolute and relative biomasses of different taxonomic groups and of the N-fixer (*Anabaena flos-aquae*) specifically, as well as rates of N fixation were analyzed using two-way ANOVA (JMP, SAS Inst.) in the end of the experiment. Tukey-Kramer HSD comparisons were used to test for significant differences ($p < 0.05$) between treatments. The data on proportions were arcsine square root transformed (Sokal and Rohlf 1981). The effects of light level and N:P ratio on dissolved nutrient variables were analyzed using one-way ANOVA at each sampling date.

The similarity between replicates was assessed using Stander's similarity index (Stander 1970) ($0 \leq SIMI \leq 1$) frequently used to compare algal assemblages (Johnson et al. 1982). The index was calculated for every sampling date and the median and the range were determined.

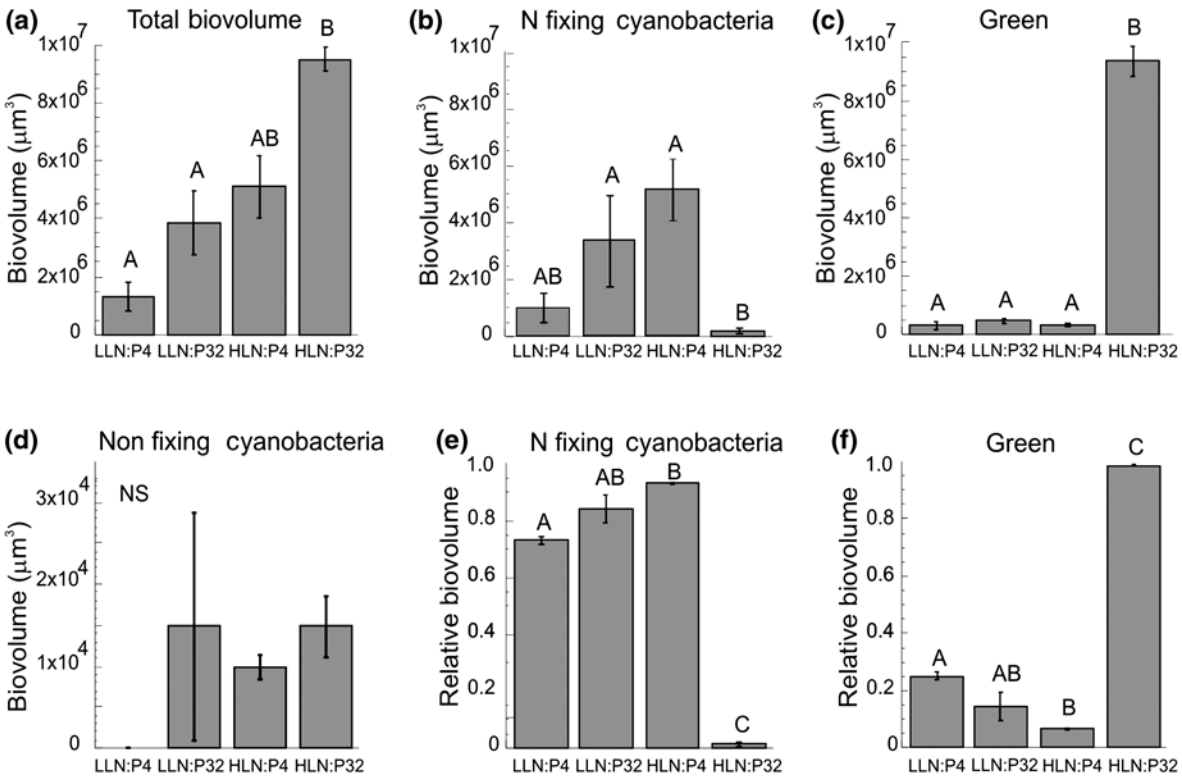

Figure 1. The effects of N:P ratios and light levels at the end of the experiment on (a) total biomass (biovolume), (b) absolute biomass of the N-fixing cyanobacteria (*Anabaena flos-aquae*), (c) biomass of green algae, (d) biomass of non-fixing cyanobacteria, (e) relative biomass of the N-fixing cyanobacteria and (g) relative biomass of green algae. Different letters denote significant differences ($p<0.05$) based on the Tukey-Kramer HSD comparisons (significantly different treatments do not have letters in common). LL= low light, HL=high light, N:P 4=low N:P, N:P 32=high N:P. Error bars are standard error of the duplicate cultures.

Results

Both N:P supply ratios and light levels had a significant effect on community composition, absolute and relative biomasses of major groups of phytoplankton, including the N-fixer dominance (Fig. 1). Because the dominant species reached their equilibrium densities and major competitive trends were evident by the end of the experiment (Fig. 2), we report mostly the results across treatments from the final sampling time (day 50). The two replicates of each treatment showed high similarity throughout the experiment, as evidenced by high values of Stander's similarity index in all treatments (median SIMI ≥ 0.93). Total biomass (estimated as biovolume) increased significantly with increasing N:P ratios and light levels and was the highest at high light and high N:P ratio (Fig. 1a), however the interaction effect of light and N:P ratio on biomass was not significant (Table 1). Green algae and the N-fixer contributed the most to the total biomass in all treatments, whereas diatoms, cryptomonads and non-N fixing cyanobacteria did not exceed 4% of the total biovolume in any treatment.

The absolute and relative biomass of the N-fixer was high in all treatments except for the high light high N:P ratio (Fig. 1b, 1e, 2). For green algae the pattern was reverse: their absolute biomass and relative biomass was the highest in this treatment (Fig. 1c, 1f) and quickly (days) overwhelmed the N-fixer (Fig. 2d). The low N:P ratio had a significant positive effect on the absolute and relative biomass of the N-fixer, but only at high light (Fig. 1b, 1e); the assemblage shifted to N-fixer dominance only after several weeks of low N:P supply (Fig. 2c). In contrast to the N-fixer, the non-fixing cyanobacteria (*Planktothrix* and *Microcystis*) showed a different response to the light and N:P combinations: they were excluded in the low light and low N:P ratio treatment and persisted at low biomasses in all other treatments (Fig. 1d), however, neither N:P ratio, nor light had significant effects on their absolute or relative biomass. Thus, non N-fixing cyanobacteria were not stimulated by low N:P ratio in our experiment. Throughout the experiment, the diatom *Fragilaria* performed better in low light (Fig. 2a–b) than in the high light treatments (Fig. 2c–d) while both cryptomonads (*Cryptomonas* and *Rhodomonas*) and the diatom *Asterionella* performed poorly in all situations (Fig. 2). On day 50, for both diatoms and cryptophytes, the effect of light and N:P ratio and their interaction on biomass was not significant ($p > 0.05$) (Table 1); their relative contribution to the final assemblages was very low (< 0.025%).

At high light, low N:P ratio lead to the dominance by the cyanobacterial N-fixer (Fig. 1b, 2c), while the high N:P ratio lead to the dominance by a mixture of green algae, mostly *Chlamydomonas, Oocystis* and *Scenedesmus* (Fig. 1c, 1f, 2d). Under low light, both N:P treatments had a high relative biomass of the N-fixer (Fig. 1e); their absolute biomass was lower in the low N:P ratio but not significantly different from the high N:P ratio (Fig. 1b). The green alga *Ankistrodesmus* performed better in low light scenarios throughout the experiment (Fig. 2a–b); all other green algae were very scarce.

Light declined during the experiment. In the low light flasks, light at the end of the experiment dropped from 15 µmol quanta m^{-2} s^{-1} to (mean ± SE) 9.5 ± 0.7 and 8.6 ± 0.1 µmol quanta m^{-2} s^{-1} in the low and high N:P treatments, respectively. In the high light treatments light decreased from 100 µmol quanta m^{-2} s^{-1} to 78.5 ± 1.3 and 74 ± 1.0 µmol quanta m^{-2} s^{-1} in the low and high N:P treatments, respectively.

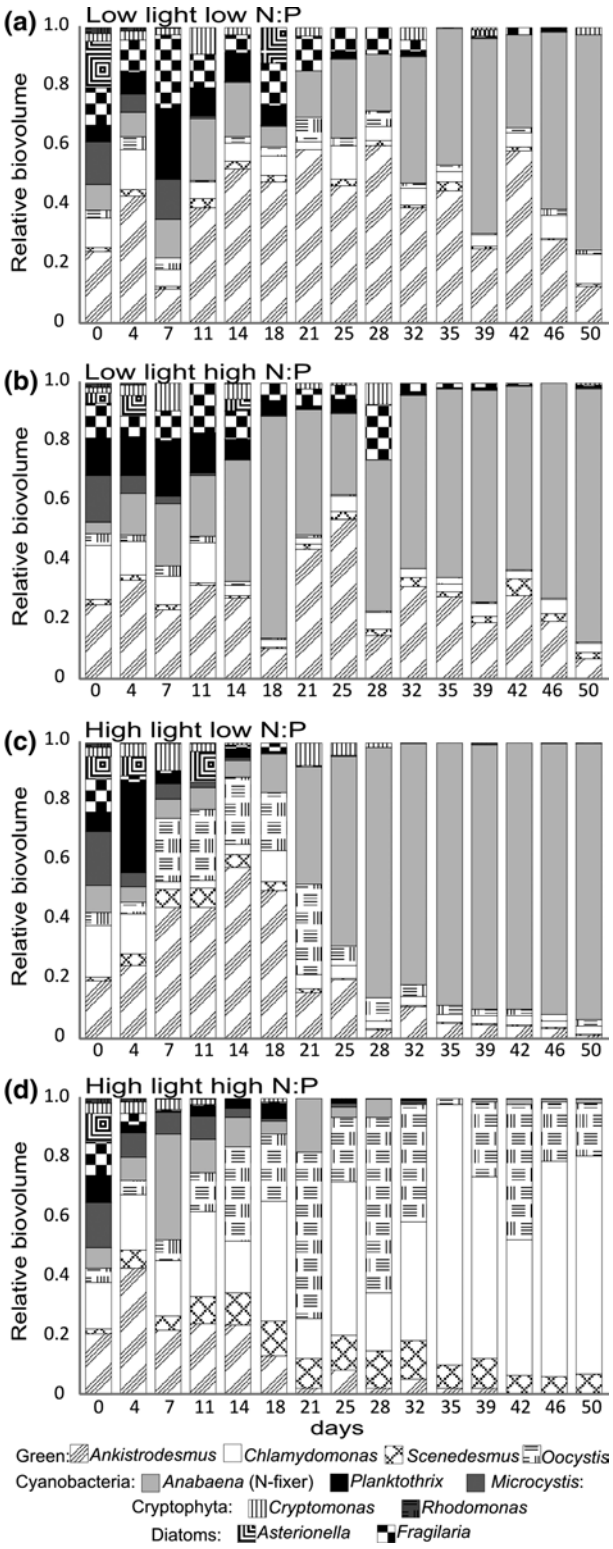

Figure 2. Relative biomass (biovolume) of each species in the course of experiment. a) low light low N:P, b) low light high N:P, c) high light low N:P and d) high light high N:P.

Table 1. Results of two-way ANOVAs testing the effects of light levels and N:P ratios and their interaction on community variables measured in the end of experiment (day 50). Species biomasses of different groups were log-transformed when needed to achieve normality. The relative biomasses were arcsine square root-transformed. p-values for the model, as well as for individual effects and their interaction are given. The direction of the effects and their interaction is shown with a sign in parentheses. Significant p-values (<0.05) are in bold.

	Model p-value	Model R^2	N:P ratio	Light level	N:P×light
Total biomass (biovolume)	**0.01**	0.93	**0.01 (+)**	**0.005 (+)**	0.33 (+)
N fixation rate	**0.0032**	0.96	**0.0012 (–)**	**0.0023 (+)**	**0.024 (–)**
All cyanobacteria biomass	**0.01**	0.92	**0.04 (–)**	0.13 (–)	**0.004 (–)**
N-fixer (*A. flos-aquae*) biomass	**0.01**	0.92	**0.047 (–)**	0.13 (–)	**0.005 (–)**
Non-fixing cyanobacteria biomass	0.50	0.42	0.24 (+)	0.53 (+)	0.53 (–)
Green algae biomass	**0.001**	0.97	**0.001 (+)**	**0.003 (+)**	**0.004 (+)**
Diatom biomass	0.61	0.33	0.93 (+)	0.93 (–)	0.23 (–)
Cryptophyte biomass	0.8	0.20	0.60 (+)	0.60 (–)	0.57 (+)
All cyanobacteria proportion	**0.0001**	0.99	**0.0002 (–)**	**0.0007 (–)**	**<0.0001 (–)**
N-fixer proportion	**<0.0001**	0.99	**0.0002 (–)**	**0.0006 (–)**	**<0.0001 (–)**
Non-fixing cyanobacteria proportion	0.36	0.96	0.21 (+)	0.28 (+)	0.13 (–)
Green algae proportion	**0.0002**	0.99	**0.0005 (+)**	**0.0013 (+)**	**0.0002 (+)**
Diatom proportion	0.56	0.37	0.59 (+)	0.59 (–)	0.27 (–)
Cryptophyte proportion	0.70	0.27	0.94 (–)	0.35 (–)	0.58 (+)

At high light, light levels in the end of experiment were significantly lower ($p = 0.02$) at the high N:P ratio, possibly due to higher biomass in this treatment.

Nutrient levels dropped within the first and second week of the experiment and remained low throughout the experiment (Fig. 3). In the low light treatments, for most of the time, nitrate levels were significantly higher in high N:P ratio treatment (Fig. 3a) and phosphate levels were significantly higher in low N:P ratio (Fig. 3b), indicating weaker relative limitation by nitrogen and phosphorus in those treatments, respectively. In all treatments phosphate concentrations increased after day 35 (≥ 0.3 µmol l^{-1}, Fig. 3b) to values above phytoplankton growth limitation (≤ 0.1 µmol l^{-1}, Reynolds 1984). This increase in phosphate concentration coincided with shifts in community composition, either towards the N fixer dominance (Fig. 2a–c) or its exclusion (Fig. 2d) and could possibly be related to changes in community uptake patterns.

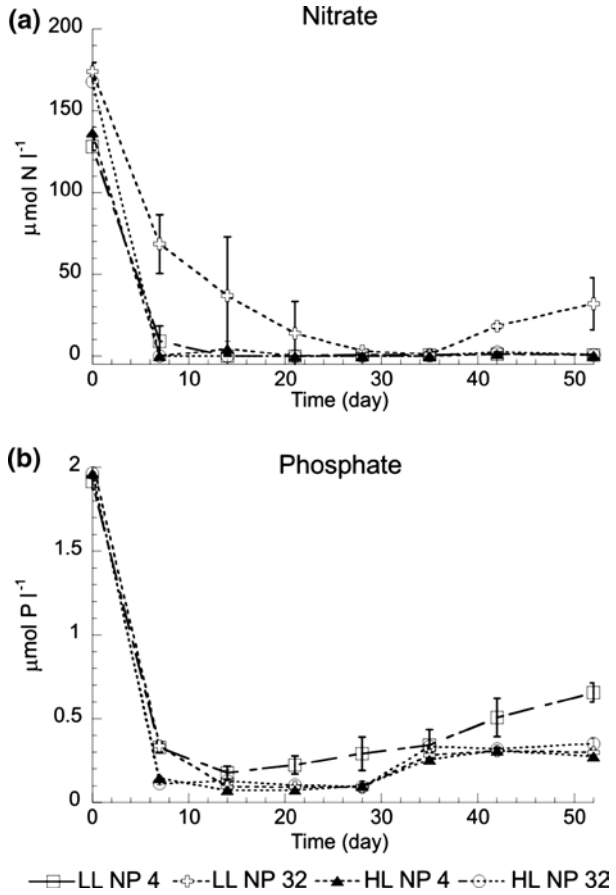

Figure 3. The time series of a) nitrate and b) phosphate in the experiment. LL= low light, HL=high light, N:P 4=low N:P, N:P 32=high N:P. Error bars are standard error of the duplicate cultures.

Heterocyst densities were higher in the treatments with low N:P supply (Fig. 4a). Regardless of the TN supplied, whenever nitrate concentration was low, the N fixer developed heterocysts (N-fixing cells) (Fig. 4b). Low nitrate per se did not ensure N-fixer dominance: in the high light and high N:P ratio treatment, nitrate was low (Fig. 3a) and the N fixer developed the N-fixing cells (Fig. 4b), but the assemblage changed to the green algae dominance (Fig. 1c, 1f, 2d). In the low light and high N:P treatment, where nitrate concentrations were high throughout most of the experiment, heterocysts were only observed during the period when nitrate was low (Fig. 4 a–b), thus strongly suggesting that dissolved inorganic nitrogen and not the total nitrogen concentration is the environmental cue that triggers heterocyst development.

According to the mass balance calculations (TN measured-TN supplied) at the end of the experiment (Fig. 5), N fixation was significantly greater than zero only in the low N:P ratio treatments, and the rate of fixation increased with increasing light (Fig. 5a). The rates were directly proportional to the density of heterocysts (N fixing cells) (Fig. 5b–c). N fixation increased TN concentrations in the low N:P treatments and thus raised the N:P ratio from 4 (inflow N:P ratio) to 9 and 21 on average in low and high light treatments, respectively. TP in all treatments matched the supplied P (ca 2 µmol l^{-1}).

Discussion

Our experimental results confirm previous observational findings that cyanobacteria tend to increase in abundance

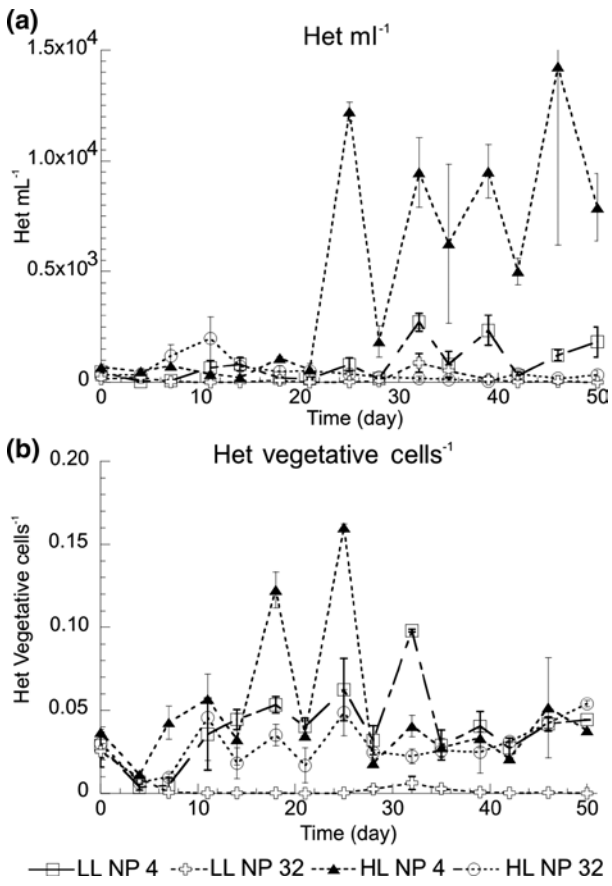

Figure 4. The time series of a) density of heterocysts (Het mL^{-1}) and b) the ratio of heterocysts to vegetative cells in the experiment. LL= low light, HL=high light, N:P 4=low N:P, N:P 32=high N:P. Error bars are standard errors of the duplicate cultures.

under low N:P ratios (Smith 1983, Schindler et al. 2008), but also reveal that this effect of N:P ratio holds under high light only. The dominance by cyanobacteria at low N:P ratio at high light was due to the abundance of the N-fixer only and not of the non-fixing cyanobacteria, thus supporting the mechanistic explanation that the ability to fix atmospheric N affords a competitive advantage at low N:P ratio. Levine and Schindler (1999) and Vrede et al. (2009) observed a similar stimulation of N-fixers by low N:P ratio in mesocosm experiments and Schindler et al. (2008) in a whole lake experiment. Non-fixing cyanobacteria may also differ in their responses to N:P ratio and some are superior competitors at low N:P ratio (Fujimoto et al. 1997), but this was not observed in our study.

Under low light, N:P ratio did not significantly influence the N-fixer biomass, probably due to the overriding effect of light limitation, even though N-fixation depended on N:P. The high abundance of cyanobacteria (N-fixer *A. flos-aquae*) can be due to the low light requirements of this species (Reynolds 1984, Litchman 2000, 2003) resulting in it being a superior competititor for light (De Nobel et al. 1998), coupled with buoyancy regulation. In dim light situations, these traits appear more ecologically relevant than the ability to fix N.

The rates of N-fixation did, however, depend on N:P ratio even at low light; they were significantly greater than zero only at low N:P ratio and higher at increased light.

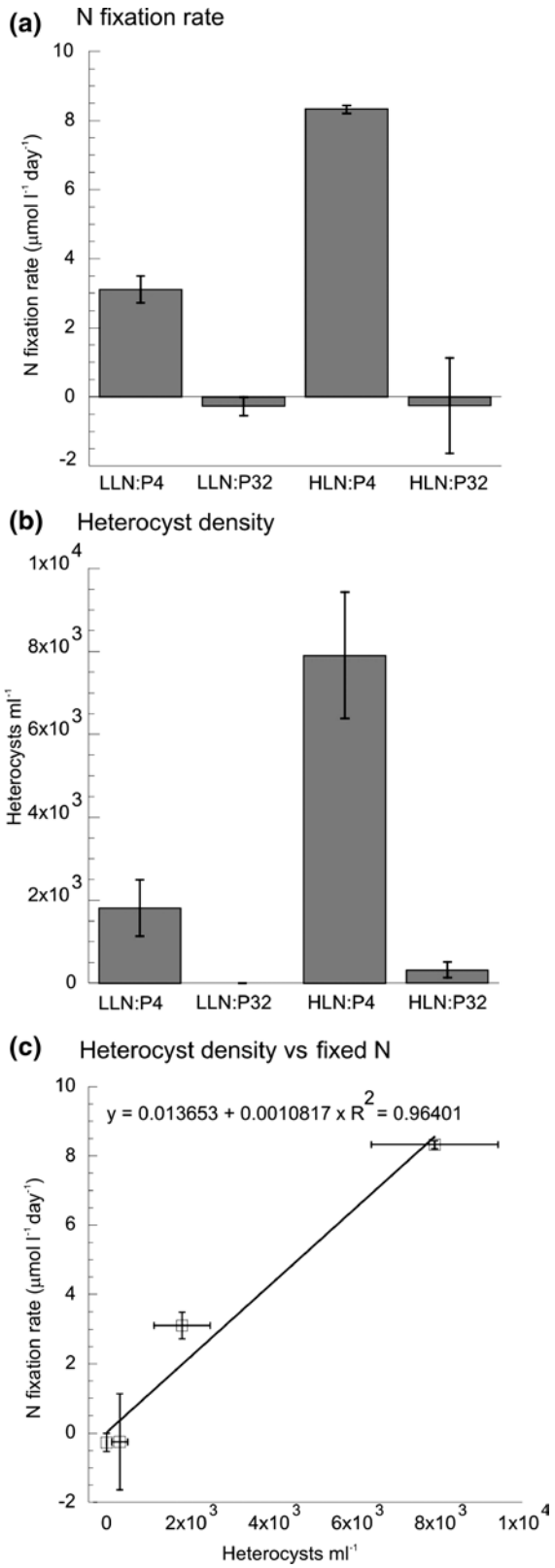

Figure 5. The effect of N:P ratio and light on nitrogen fixation at the end of experiment. a) Rates of N fixation, calculated based on mass balance of TN (measured–supplied) and dilution rate in the end of experiment (see Methods), b) density of heterocysts (N-fixing cells) and c) the relationship between the density of heterocysts and rate of N fixation in the end of experiment. LL= low light, HL=high light, N:P 4=low N:P, N:P 32=high N:P. Error bars are standard error of the duplicate cultures.

The results underscore the importance of light levels in predicting the effect of N:P ratios on cyanobacterial abundance and N-fixation (Smith 1986, Ferber et al. 2004). The observed dependence of the N:P ratio effects on light may contribute to the scatter in the relationship between the relative cyanobacterial abundance and N:P ratio found in some observational studies (Downing et al. 2001). Consequently, future analyses of the effects of N:P ratio on N-fixer abundance should explicitly include the effect of light. Another interesting question to address in the future is whether the light effect on N-fixation is gradual or if there is a threshold light level below which N-fixation is not possible. Other experiments show that the N:P effect on N-fixers is gradual, probably related to internal N quotas and fixation needs (de Tezanos Pinto and Litchman unpubl.)

The ratios of total N to total P are commonly used to infer relative availabilities of the two nutrients and often are good predictors of the N-fixer dominance patterns. Nevertheless, N-fixers are frequently underrepresented in highly productive but consistently N-limited lakes (Lewis et al. 2008). Our results show that low dissolved inorganic N concentration triggers heterocyst (N-fixing cells) development, yet does not necessarily lead to the dominance of N-fixers. Thus, the TN:TP ratio may better predict the N-fixer abundance than dissolved inorganic N in high light. In addition to the TN:TP ratio, we hypothesize that the absolute value of TN plays an important role in determining the ecological success of N fixers. Monoculture experiments with this N fixer under a wide range of N:P ratios at high light indicate that fixation only occurs up to ca 64 µmol l^{-1} TN (32 N:P); fixation gradually decreases with increasing N:P ratio (from 0 to 32 N:P) (de Tezanos Pinto and Litchman unpubl.). Ambient TN concentrations above those that trigger N fixation would result in a loss of competitiveness of N-fixers relative to other phytoplankton, in part due to lower growth rates of N-fixers compared to green algae under high N levels.

Our experiments confirm that N-fixation strongly depends on N:P ratio and light availability and that the abundance of heterocysts can be used as an indicator of the N-fixation rates in heterocystous N-fixers as suggested by Ferber et al. (2004) and others. The limiting effect of light on the N-fixer abundance was observed in natural water bodies as well: in Lake Okeechobee, FL, N-fixers were restricted by low light availability, despite low N:P ratios (Havens et al. 2003). The fact that N fixation decreases with decreasing light (Mugidde et al. 2003, this study) may also explain the negative effect of deep mixing on N fixer abundances. Mixing (turbulence) can also inhibit N-fixer growth directly (Howarth et al. 1988). In addition to light, N-fixation may be constrained by the availability of macronutrients (phosphorus) and micronutrients (iron and molybdenum), temperature, the physical structure of the water column and predation (Howarth et al. 1988, Vitousek et al. 2002a, Staal et al. 2003, Fu et al. 2008, Smith and Schindler 2009). Experiments that address interactions of these factors in determining N-fixer abundance would improve our understanding of environmental controls on N-fixation.

In nature, the majority of N-fixer blooms occurs under N-fixing (low dissolved inorganic N or low TN:TP) conditions, yet occasionally blooms without heterocysts occur (Jacobsen and Simonsen 1993). We found both patterns in our experiment. Blooms with developed heterocysts most probably will occur at high light, low total and dissolved inorganic N, high total and dissolved inorganic P, and high temperatures. In contrast, blooms without heterocysts most likely will occur under low light, high TN:TP ratios and high dissolved inorganic N. Filamentous N-fixers (e.g. *Anabaena flos-aquae*, Nostocales) behaving as non fixers may play a similar ecological role to non fixing filamentous cyanobacteria (Oscillatoriales) that frequently develop persistent blooms under these conditions (Scheffer et al. 1997, de Tezanos Pinto et al. 2007).

Green algae were strongly stimulated by the combination of high N:P ratio and high light, achieving a very high abundance. This result agrees well with their ecophysiological preferences reported previously, as they tend to have high N and high light requirements (Rhee and Gotham 1980, Richardson et al. 1983, Litchman and Klausmeier 2008), and match well with observational evidence in nature (Schindler 1977).

The lowest species diversity occurred when N fixer dominated at high light (data not shown). Although this result agrees with the observations from nature, where cyanobacterial blooms result in low species diversity (Huisman et al. 2005, Reynolds 1984), our experimental design does not allow making strong inferences about diversity because we had only one species of N fixer. Utilizing comparable number of species from different functional groups should provide a better assessment of diversity responses to N:P ratios and light.

Different aquatic environments vary in how much light is available to primary producers, including N-fixers. Light limitation may be stronger in deep polymictic lakes or lakes with relatively shallow euphotic depths compared to mixing depths, possibly decreasing the potential for N fixation and hence the input of new N. Both low light and high dissolved nitrogen availability should decrease the success of heterocystous nitrogen fixers.

Global environmental change may alter both the absolute amounts and N:P ratios of nutrient loading, due to changes in atmospheric N deposition, fertilizer application, runoff treatment and other anthropogenically mediated processes (Tilman et al. 2001, Melillo et al. 2003, Weyhenmeyer et al. 2007). Light availability in aquatic ecosystems may also change due to climatic influences on cloudiness, precipitation and mixing regimes (Williamson et al. 1999, MacKay et al. in press, Adrian et al. 2009). Stronger stratification and shallower mixing depths resulting from warmer temperatures may increase light availability and stimulate N fixation. On the other hand, dissolved organic carbon (DOC), including colored dissolved organic matter (CDOM) input into lakes and other water bodies may increase, as a result of changes in climate and water chemistry (Monteith et al. 2007). This may strongly limit light availability to primary producers (Karlsson et al. 2009) and thus restrict the potential for N fixation. Therefore, anthropogenic influences on nutrient and light availability will likely alter N-fixer abundance in many aquatic ecosystems. This, in turn, can change phytoplankton community composition, water quality and nutrient cycling.

Little is known about the interaction of N:P ratios and light in regulating the abundance of N-fixers in terrestrial systems. Terrestrial N-fixation rates also depend on light

(Hardy and Havelka 1976, Sprent 1976) and were shown to increase at high light in legumes in a pine forest (Hiers and Mitchell 2007). It would be of interest to investigate experimentally how N:P ratios interact with light levels in determining the success of N-fixers in terrestrial systems and to compare the strength of the light level influence on N-fixers in terrestrial versus aquatic systems. It is possible that light control on N-fixation is weaker in terrestrial systems, as it is less direct due to the 'division of labor': light capture and N-fixation are carried out by different organisms (plant host and rhizobia). Alternatively, such 'division of labor' can render a less efficient coupling of photosynthesis and N acquisition by N fixation; thus increasing the amount of light needed for N fixation and resulting in an even stronger light control on N-fixation in terrestrial systems. In addition to light, water availability may constrain N-fixation on land (Aranjuelo et al. 2009).

N fixers play an important role in the global N cycle and ecosystem functioning in both aquatic and terrestrial ecosystems. Therefore, understanding the interactive effects of various environmental controls on N-fixer abundance will allow us to better predict how ecosystems function and will respond to global environmental change.

Acknowledgements – We thank P. Woodruff for running nutrient analyses. J. Lau and C. Klausmeier provided valuable comments and suggestions. This work was in part supported by the NSF grants DEB 06-10531, 06-10532 and 08-45932 and the J.S. McDonnell Foundation. PTP was also supported by the Kellogg Biological Station summer fellowships and PIP 5355 (CONICET) grant. This is W.K. Kellogg Biological Station contribution no. 1537.

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