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Nitrogen fixation does not axiomatically lead to phosphorus limitation in aquatic ecosystems

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A long-standing debate in ecology deals with the role of nitrogen and phosphorus in management and restoration of aquatic ecosystems. It has been argued that nutrient reduction strategies to combat blooms of phytoplankton or floating plants should solely focus on phosphorus (P). The underlying argument is that reducing nitrogen (N) inputs is ineffective because N_2-fixing species will compensate for N deficits, thus perpetuating P limitation of primary production. A mechanistic understanding of this principle is, however, incomplete. Here, we use resource competition theory, a complex dynamic ecosystem model and a 32-year field data set on eutrophic, floating-plant dominated ecosystems to show that the growth of non-N_2-fixing species can become N limited under high P and low N inputs, even in the presence of N_2 fixing species. N_2-fixers typically require higher P concentrations than non-N_2-fixers to persist. Hence, the N_2 fixers cannot deplete the P concentration enough for the non-N_2-fixing community to become P limited because they would be outcompeted. These findings provide a testable mechanistic basis for the need to consider the reduction of both N and P inputs to most effectively restore nutrient over-enriched aquatic ecosystems.

Keywords: eutrophication, nitrogen fixation, R*

Introduction

Eutrophication due to anthropogenic nutrient over-enrichment threatens the biodiversity, function and sustainability of freshwater ecosystems worldwide (Smith 2003), by promoting surface dwelling toxic cyanobacterial blooms (Paerl 1988, Huisman et al. 2005) and through the invasion by dense mats of free-floating plants (Scheffer et al. 2003). Both lead to aphotic, anoxic sub-surface conditions that severely constrain aquatic life (Heisler et al. 2008). To combat these eutrophication-related declines in ecosystem function, the question arises as to whether to focus on reducing inputs of nitrogen (N) or phosphorus (P) or both (Conley et al. 2009, Paerl et al. 2016).

Based on evidence from long-term whole-ecosystem experiments, it has been proposed that nutrient reduction strategies should focus solely on P, because N reduction

favours N_2-fixing cyanobacteria that draw on the vast atmospheric N_2 reserve, eventually providing the eutrophic ecosystem with enough N to compensate for N deficits, thus perpetuating P-limitation (Schindler 1977, Hecky and Kilham 1988, Schindler et al. 2008, Schindler and Hecky 2009). The general applicability of this principle has been questioned however, as it has been argued that N_2-fixers cannot meet the N requirements of eutrophic ecosystems due to ecological and geochemical constraints on N_2-fixation (Howarth et al. 1988, Paerl 1990, 2017, Vitousek and Howarth 1991), and because the N input by N_2-fixers is offset by denitrification in eutrophic waters (Lewis and Wurtsbaugh 2008, Paerl et al. 2010, 2016, Scott and McCarthy 2010, Lewis et al. 2011), thus perpetuating N-limitation (Paerl et al. 2016). A far-reaching implication is that ecosystem managers should also consider the reduction of N inputs, rather than focusing solely on P (Conley et al. 2009). However, a mechanistic understanding of how N_2-fixation affects nutrient limitation and eutrophication in aquatic ecosystems remains incomplete.

Here, we use classical resource competition theory (Tilman 1982, Huisman and Weissing 1995) to explore whether N_2-fixers are capable of perpetuating P limitation when lowering external N supply. To address this, we built a resource competition model and analyzed for different combinations of external N and P supply how N_2-fixation influences the structure, productivity and nutrient limitation characteristics of a eutrophic community dominated by floating macrophytes. Then, we implemented the resource competition model in an established complex freshwater ecosystem model to test the robustness of our results in a more realistic setting including various biogeochemical processes and biotic interactions. Finally, we compared the model predictions with a 32-year field data set on eutrophic floating-plant dominated ecosystems to validate the results, and discussed the role of N_2-fixation and its implication in developing nutrient management strategies for aquatic ecosystems undergoing cultural eutrophication.

Methods

Resource competition model

We developed a mechanistic model that describes a community of floating plant species that compete for N, P and light (Table 1, Supplementary material Appendix 1 Fig. A1). One species is able to fix N_2 from the atmosphere while the other is completely reliant on combined N in the water column. The

Table 1. Resource competition of two competing floating plant species in a water body.

Description	Mathematical formulation	Eq.
Differential equations		
Biomass dynamics non-N_2-fixer	$\dfrac{dL}{dt} = (p_L - m_L)L$	1
Biomass dynamics N_2-fixer	$\dfrac{dA}{dt} = (p_A - m_A)A$	2
N dynamics in the water column	$\dfrac{dN}{dt} = \dfrac{n_{in}}{z_B} - DN - \dfrac{1}{z_B}c_{N,L}p_L L + \dfrac{a}{z_B}\left(c_{N,L}m_L L + c_{N,A}m_A A\right)$	3
P dynamics in the water column	$\dfrac{dP}{dt} = \dfrac{p_{in}}{z_B} - DP - \dfrac{1}{z_B}\left(c_{P,L}p_L L + c_{P,A}p_A A\right) + \dfrac{a}{z_B}\left(c_{P,L}m_L L + c_{P,A}m_A A\right)$	4
Auxiliary equations		
Growth rate of the non-N_2-fixer	$p_L = \min[f_{N,L}, f_{P,L}]f_{I,L}p_{\max,L}$	5
Growth rate of the N_2-fixer	$p_A = f_{P,A}f_{I,A}p_{\max,A}$	6
N limitation function	$f_{N,L} = \dfrac{N}{N + M_{N,L}}$	7
P limitation function	$f_{P,i} = \dfrac{P}{P + M_{P,i}}$	8
Light limitation function	$f_{I,i} = \dfrac{1}{k_L L + k_A A}\displaystyle\int_{I_{out}}^{I_{in}}\dfrac{1}{H_i + I}\,dI = \dfrac{1}{k_L L + k_A A}\ln\left(\dfrac{H_i + I_{in}}{H_i + I_{out}}\right)$	9
Light intensity below the plants	$I_{out} = I_{in}e^{-(k_L L + k_A A)}$	10

model builds on existing theoretical frameworks for resource competition (Huisman and Weissing 1995, Van Gerven et al. 2015a) and simulates the dynamics of plant biomass (g DW m^{-2}) and the N and P concentrations (g N m^{-3} and g P m^{-3}) in the water column (Eq. 1–4). The water column N and P concentrations are affected by supply, outflow, uptake and remineralization. The biomass dynamics depend on loss rate m and growth rate p. The growth rate is co-limited by light and nutrients (Eq. 5 and 6). Nutrient limitation follows the Michaelis–Menten function (Eq. 7 and 8). The non-N$_2$-fixer is limited by either N or P according to Liebig's law of the minimum, whereas the N$_2$ fixer is only limited by P as its N demand is assumed to be met by N$_2$ fixation. Light limitation is also characterized by the Michaelis–Menten function (Eq. 9) and follows from integrating over the plant depth, as the light intensity I decreases with depth due to light attenuation by the plants according to Lambert Beer's law (Eq. 10). Via the remineralization process the N$_2$ fixer is capable of enriching the system with N, as part of the nutrients in dead plant tissue ends up in the water while the remaining part is lost to the sediment by burial.

Model analysis

To examine the interplay between external N supply and N$_2$-fixation on nutrient limitation and productivity in the aquatic ecosystem, we analyzed the basic configuration of the model, after which we added complexity in a stepwise manner. We first considered the hypothetical situation of having two non-N$_2$-fixing species with identical resource requirements, using parameter values of duckweed (*Lemna* spp., Table 2). De facto we were thus modelling growth of a single species resulting from intraspecific competition. This exercise provided an important contrast for the next step, where we granted one of the two species the capability to fix N$_2$. This second step exposes the single effect of N$_2$ fixation on the ecosystem. As a third step, we also changed the other parameter values of the N$_2$-fixer, implementing values of the water fern (*Azolla* spp.), a floating plant species living in symbiosis with the N$_2$-fixing cyanobacterium *Anabaena azollae* (Wagner 1997, Table 2). This third step allowed us to evaluate the effect of N$_2$-fixation in relation to the different resource requirements of *Lemna* and *Azolla*. For each of the steps, we determined the competition outcome for a range of N (n_{in}) and P (p_{in}) inputs and assessed which nutrient was limiting the growth rate of the non-N$_2$-fixing species. We also took a cross section in the N and P plane, focusing on the effect of decreasing N input while keeping the P input constant at a fairly high level representing eutrophic conditions. We calculated the biomass of both species to analyze how external N supply controls the productivity in the system and reported which resource was limiting the growth of the non-N$_2$-fixing species. To compare the effects of reducing the supply of P instead of N, we present a similar analysis in Supplementary material Appendix 1 Fig. A7, focusing on the effect of decreasing P input while keeping the N input constant. Additionally, to gain more insight into the competitive ability of the species, we calculated and analyzed the zero net growth isoclines of the macrophytes for the different resources, which is presented in Supplementary material Appendix 1 Fig. A9.

Complex ecosystem model

To test whether the results of our resource competition model remain the same in a more realistic setting, we implemented the equations of the resource competition model in a full-scale and well-tested dynamic ecosystem model PCDitch (Janse 1998, Janse and Van Puijenbroek 1998), and repeated the analyses. PCDitch has previously been used to

Table 2. Parameter values of the resource competition model.

Description	Symbol	Unit	*Lemna*	*Azolla*
Species parameters				
Maximum growth rate	$p_{max,i}$	day^{-1}	0.4[a]	0.25[b]
Loss rate	m_i	day^{-1}	0.05[a]	0.03[c]
Light attenuation coeff.	k_i	m^2 g DW^{-1}	0.07[a*]	0.07[d]
Half-sat. constant for light	H_i	J m^{-2} s^{-1}	25[a*]	35[e]
Half-sat. constant for N	$M_{N,L}$	g N m^{-3}	2.5[a]	–
Half-sat. constant for P	$M_{P,i}$	g P m^{-3}	0.25[a]	0.45[f]
N to dry weight ratio	$c_{N,i}$	g N g DW^{-1}	0.07[a]	0.03[g]
P to dry weight ratio	$c_{P,i}$	g P g DW^{-1}	0.015[a]	0.01[g]
Environmental parameters				
N loading	n_{in}	g N m^{-2} day^{-1}	0–0.5	
P loading	p_{in}	g P m^{-2} day^{-1}	0–0.05	
Incident light intensity	I_{in}	J m^{-2} s^{-1}	200	
Dilution rate of water column	D	day^{-1}	0.1	
Remineralization fraction of died-off plants	α	–	0.5	
Water column depth	z_B	m	0.5	

[a]PCDitch; Janse (1998), [a*]the values of PCDitch were raised to better match the values found for *Azolla*, [b]Van der Heide et al. (2006), [c]Chosen such that maximum biomass is realistic, [d]Forchhammer (1999), [e]Moretti and Siniscalco Gigliano (1988), [f]Bieleski and Lauchli (1992), [g]Costa et al. (1999).

predict a nutrient-driven regime shift from submerged plants to floating-plant dominance (Van Gerven et al. 2015b). PCDitch describes multiple biotic groups that compete for nutrients and light and have a different competition strategy due to their distinct growth forms, including phytoplankton and several submersed and emergent macrophytes (Supplementary material Appendix 1 Fig. A2). Furthermore, PCDitch comprises a sediment layer, includes all key nutrient cycling processes, including sediment-P release and denitrification, and accounts for seasonal variation in water temperature, light intensity and daylength (Janse 2005 for detailed process descriptions). We used the parameter settings of the calibrated model (Janse 1998), resembling an 'average' macrophyte-dominated freshwater ecosystem in the temperate zone. Because PCDitch accounts for seasonality, we slightly extended the equations of the resource competition model as presented in Table 1 to account for the phenology of *Lemna* and *Azolla*, by making the growth dependent on temperature and by incorporating an overwintering period and a growing season. We adopted the associated phenological parameters from the original *Lemna* group in PCDitch, and kept these parameters the same for *Lemna* and *Azolla*. We used summer averaged results to evaluate the outcome of the competition experiments and again reported which resource was limiting the growth of the non-N_2-fixing floating plant species together with the biomass of both species. Additionally, we performed a sensitivity analysis to better understand the effect of denitrification on the results, which is presented in Supplementary material Appendix 1 Fig. A8.

Empirical validation

The results of the models used in this study were compared with an extensive 32-year data set (from 1980 to 2012) of occurrences of vegetation in eutrophic aquatic ecosystems in the Netherlands. These data are part of Limnodata Neerlandica, a large database collected by Dutch water authorities in drainage ditches, canals, streams, ponds and lakes (STOWA 2015). This database contains both macrophyte abundances and water column nutrient concentrations. We extracted data on the occurrence of *Azolla* spp. and *Lemna* spp., from locations where nutrient concentrations were also measured. A Welch's t-test was used to check for differences in occurrence of *Azolla* spp. and *Lemna* spp. in relation to total N and total P in the water column.

Results

For a community of non-N_2-fixing floating macrophytes, the resource competition model shows that a decrease of the external N supply (n_{in}) leads to a shift from P to N limitation in the growth rate (Fig. 1a, Supplementary material Appendix 1 Fig. A3). Once the growth is limited by N, a further reduction of the external N supply leads to a decrease of productivity (Fig. 1a, right panels).

When considering a hypothetical community of N_2-fixers and non-N_2-fixers that have the same resource requirements, the resource competition model predicts that N_2-fixers have the potential to keep the system P-limited when the system's
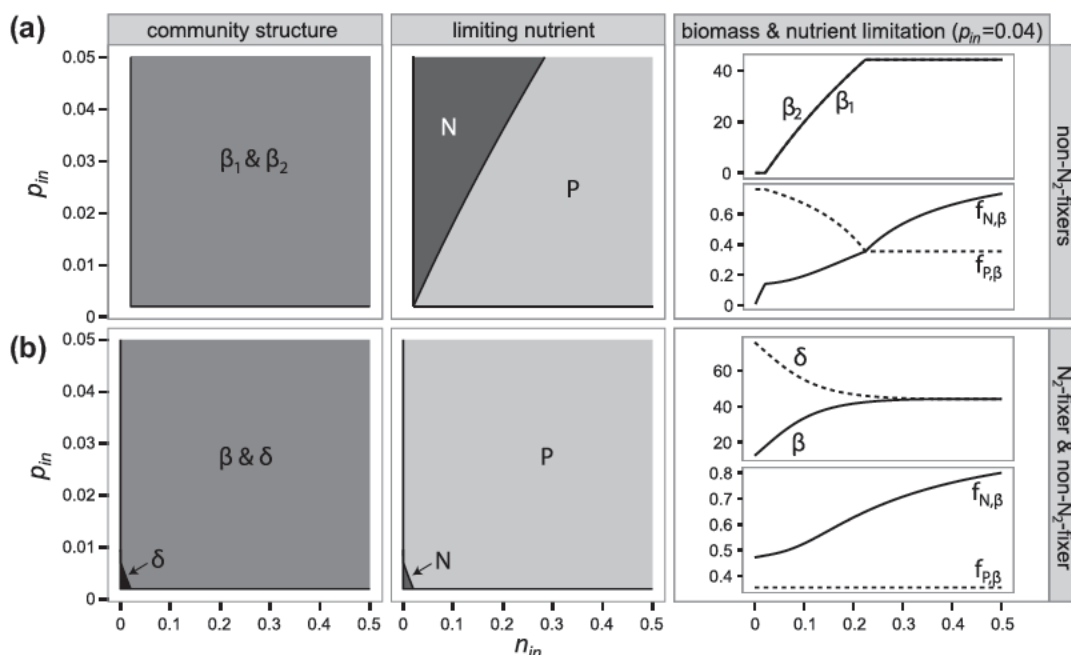

Figure 1. Predicted structure and nutrient limitation characteristics of a community of (a) identical non-N_2-fixers β_1 and β_2, and (b) non-N_2-fixer β and N_2-fixer δ, for different supply of N (n_{in}) and P (p_{in}). Left panel, competition outcome where '&' denotes coexistence. Middle panel, nutrient that limits the growth of the non-N_2-fixer (Eq. 5). Right panel, underlying biomasses in g DW m^{-2} and nutrient limitation factors of the non-N_2-fixer (Eq. 7 and 8) along a gradient of N supply (p_{in} = 0.04). All species were parametrized as *Lemna* sp. (Table 2) except for M_N which does not apply to N_2-fixer δ.

external N supply is lowered (Fig. 1b). We find that P limitation is much more pronounced in the presence of a N_2 fixer (Fig. 1b, middle panel) compared to a community where N_2-fixation does not occur (Fig. 1a, middle panel). The presence of the N_2-fixer makes it possible for the non-N_2-fixing species to maintain relatively high levels of biomass at low external N supply, compared to the situation without N_2-fixers (Fig. 1, right panels). However, the biomass of the non-N_2-fixing species decreases when the external N supply is lowered. The N_2-fixing species, which does not compete for N in the water column, benefits from a reduction of the external N supply and becomes dominant, offsetting the reduction of the non-N_2-fixing species. As a result, the external N supply has no control on the total productivity (Fig. 1, right panels). This is even the case when N_2-fixers do not 'fertilize' the water with fixed atmospheric N through remineralization of died-off biomass (Supplementary material Appendix 1 Fig. A4).

The ability of N_2-fixers to perpetuate P-limitation disappears when we consider the species of interest, the N_2-fixing *Azolla* and non-N_2-fixing *Lemna*, by implementing the different growth parameters and taking into account the respective differences in resource requirements. Our modelling results indicate that *Azolla* is unable to coexist with *Lemna* as long as the growth rate of *Lemna* is limited by P (Fig. 2a). Only when the growth limitation of *Lemna* switches from P to N, following a reduction of external N input, is *Azolla* able to coexist. Thus, the N_2-fixing capacity of *Azolla* does not prevent *Lemna* from becoming N limited. These findings remain even if we assume remineralization and associated

'N-fertilization' to be very high (Supplementary material Appendix 1 Fig. A5). Consequently, a further reduction of external N input reduces the biomass of *Lemna* (Fig. 2a). The *Azolla*, however, relieved from competitive exclusion, can benefit from the poor growth conditions for *Lemna* and builds up considerable biomass under low N and high P input conditions (Fig. 2a, right panel). As a result, there is no consistent control of external N input on the total productivity in the system. These results are confirmed when we analyze the competition outcome along a gradient of external P supply while keeping the N supply constant (Supplementary material Appendix 1 Fig. A7, right panel). When the external input of N is high, the growth of *Lemna* is limited by P and *Azolla* is unable to coexist. When the N supply is relatively low, *Azolla* dominates as long as the growth of *Lemna* is limited by N. For both high and low N supply, the reduction of external P supply has a clear negative effect on the total biomass in the system.

When we included the resource competition model in the full-scale ecosystem model PCDitch we found similar results: namely, that when lowering the N supply, the N_2-fixing *Azolla* cannot keep the growth of non-N_2-fixing *Lemna* P-limited (Fig. 2b, Supplementary material Appendix 1 Fig. A6). Both models predict that *Azolla* can only exist if the growth of *Lemna* is limited by N and there is sufficiently P available for *Azolla* to grow. To validate these qualitative results, we analysed the field data, which showed that N_2-fixing *Azolla* spp. indeed occurred at significantly higher P concentrations than *Lemna* spp. (Fig. 3a: $p < 0.001$). Note that in the
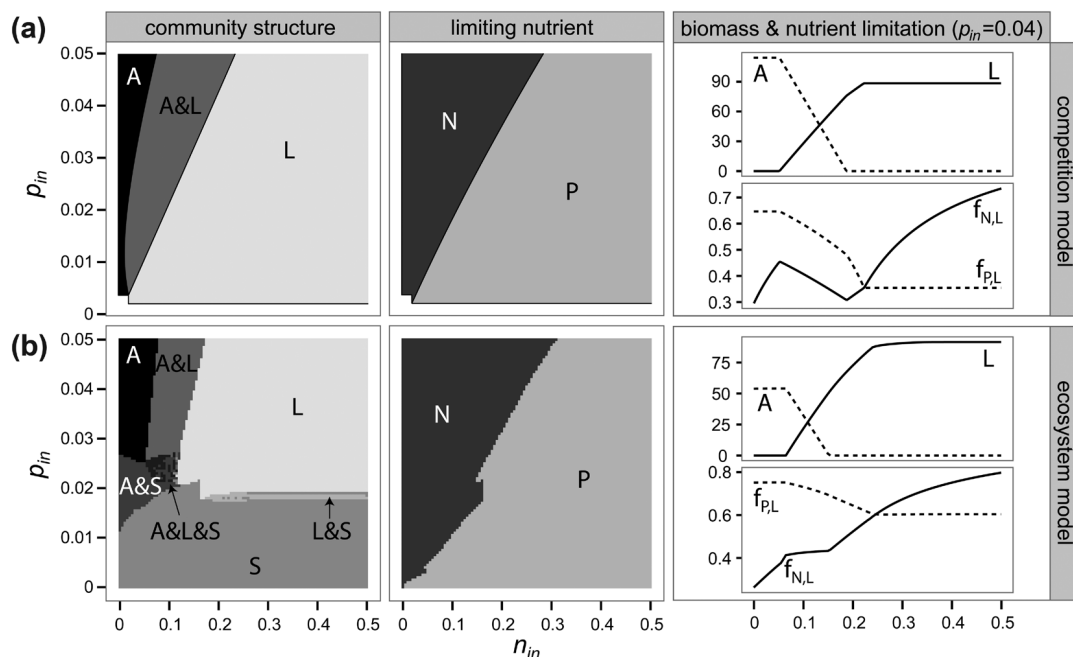

Figure 2. Structure and nutrient limitation characteristics of a floating-plant community of *Lemna* (L) and *Azolla* (A) as predicted by (a) the resource competition model and (b) the complex ecosystem model. Left panel, competition outcome where 'S' denotes submerged plants. Middle panel, nutrient that limits the growth of the non-N_2-fixing *Lemna*. Right panel, underlying biomasses in g DW m^{-2} and nutrient limitation factors of *Lemna* along a gradient of N supply ($p_{in} = 0.04$). Table 2 for parameter values. Note that for the ecosystem model it concerns summer-averaged results.
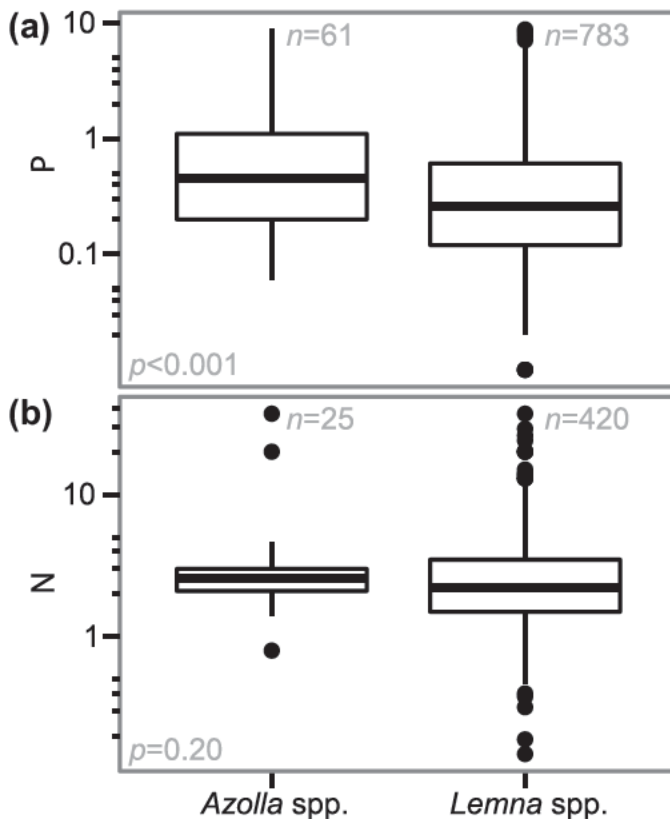
Figure 3. Box-whisker plots of (a) the total P concentrations and (b) the total N concentrations at which *Azolla* spp. and *Lemna* spp. were observed in Dutch water bodies (1980–2012). The shown p-values result from a Welch's *t*-test for which the data were log-transformed.

complex ecosystem model the reduction of external N supply does lead to a decrease of the total biomass of floating macrophytes. The potential growth of *Azolla* can only partly compensate for the reduced growth of *Lemna* (Fig. 2b, right panel). Switching-off the denitrification process in PCDitch shows that denitrification is not the main driver of the observed reduction of total biomass (Supplementary material Appendix 1 Fig. A8).

Discussion

This study has helped clarify the potential roles N and P input reductions play in freshwater ecosystem restoration strategies (Lewis and Wurtsbaugh 2008, Schindler and Hecky 2008, 2009, Lewis et al. 2011, Paerl et al. 2016, Schindler et al. 2016, 2017, Cotner 2017). In this regard, a key question we have addressed is whether N$_2$-fixers are capable of perpetuating P limitation by compensating for ecosystem-scale N deficiencies, as this would make controlling N ineffective. Here, we used mechanistic modelling and classical resource competition theory to demonstrate that, for a eutrophic water

body dominated by floating macrophytes, N$_2$-fixation does not completely fulfill this ecosystem's N demands. The capacity of the N$_2$-fixer to provide the system with combined N appears to be restricted by the resource requirements of the non-N$_2$-fixing community. As soon as the addition of fixed N would drive the non-N$_2$-fixing species into P limitation, the N$_2$-fixers would be outcompeted by the non-N$_2$-fixers. This becomes clearer when examining the lowest level at which the species can deplete resources, referred to as R* in competition theory (Tilman 1982, Huisman and Weissing 1995). When we calculate the lowest levels to which the different species can deplete P (P^*) and the light availability (I_{out}^*) in the water column, we find that *Azolla* has a higher value for P^* and I_{out}^*, making *Azolla* an inferior competitor for P and light (Supplementary material Appendix 1 Fig. A9). Therefore, *Azolla* cannot drive the entire system into P-limitation as it can never deplete the P concentration to a level low enough for *Lemna* to become P-limited. A fortiori, *Lemna* competitively excludes *Azolla* when P is limiting. The limited competitive power of *Azolla*, being only a superior competitor for N due to its ability to fix N$_2$, also explains why *Azolla* is abundant only when the supply of N is sufficiently low compared to the P supply (Fig. 2a, left panel).

Although illustrated for floating plants, it seems plausible that N$_2$-fixers in general have higher resource requirements than their non-N$_2$-fixing competitors, given the energetic costs of the N$_2$-fixation process and the associated need for heterocyst production (Paerl 1990, Paerl et al. 2016). This would imply that also in phytoplankton-dominated systems, such as eutrophic lakes, N$_2$-fixers cannot relieve the system from N limitation at low N inputs because of their higher resource requirements, and their inferior competitive capabilities for P and light. In this regard, competition experiments among cyanobacterial bloom formers show that N$_2$-fixing species are inferior competitors for light as they are competitively excluded by non-N$_2$-fixers when light is limiting (Zevenboom et al. 1981, Huisman et al. 1999). Additionally, under N-limited conditions, when N$_2$-fixers exclusively rely on N$_2$-fixation for their N demands, they may become even poorer competitors for light, as demonstrated by markedly increasing I_{out}^* values of a N$_2$-fixing species with decreasing N supply (Agawin et al. 2007). Furthermore, the observed P^* values of N$_2$-fixing cyanobacteria, though showing quite some plasticity, are on average higher than those of non-N$_2$-fixing species (De Nobel et al. 1997, Passarge et al. 2006), indicating that N$_2$-fixing cyanobacteria are also inferior competitors for P. This would help explain why at least half of the freshwater ecosystems on the world are either N-limited or N and P co-limited (Elser et al. 2007, Paerl et al. 2016).

Does this imply that reducing N inputs to restore aquatic ecosystems will be universally successful? Our mechanistic models dictate that caution must be practiced. The ultimate goal of controlling N input is not to create N-limitation per se, but to reduce the overall detrimental effects caused by nuisance species. Although our models indicate that N-fixers

appear unable to perpetuate P-limitation, they also show that N-fixers benefit from decreasing N inputs and may be able to increase their biomass considerably. Dense mats of *Azolla* can be just as harmful for the ecosystem and the provisioning of ecosystem services as the targeted non-N$_2$-fixing *Lemna* species (Janes et al. 1996, Hussner 2012). Moreover, the N$_2$-fixers provide extra N to the system and thereby can potentially provide combined N for harmful eutrophic species (Schindler et al. 2008).

On the other hand, our models probably overestimate the growth potential of N$_2$-fixers by ignoring other (in addition to P) ecological, geochemical and energetic constraints on N$_2$-fixation (Paerl 1990, 2017, Berman-Frank et al. 2007). For example, our models assume that the N$_2$-fixer's N demand is immediately fulfilled by N$_2$-fixation and thereby ignore most of the energetic costs associated with N$_2$-fixation (Paerl 1990, 2017). Such constraints could explain why N$_2$-fixation and the associated heterocyst-production was minimal at low N supply (Ferber et al. 2004) and that N$_2$-fixers do not always become dominant over non-N$_2$-fixers when reducing N inputs (Paerl et al. 2014, 2016). This conclusion is also supported by long-term field data on floating macrophytes, which showed that N$_2$-fixers do not necessarily occur at lower N availability when compared to non-N$_2$-fixers (Fig. 3b). Moreover, in our comprehensive ecosystem model we did observe a moderate decrease in macrophyte biomass in response to the reduction of N input. Based on these observations, we propose that controlling N can be effective, given enough constraints on N$_2$-fixation. Therefore, we advocate a balanced approach to ecosystem restoration by not solely focusing on reducing P inputs but also lowering N inputs (Conley et al. 2009, Paerl et al. 2016, Cotner 2017).

Given that excessive nutrient loading is one of the major drivers of global environmental change (Steffen et al. 2015), and that the societal costs associated with mitigating eutrophication are significant (Smith 2003, Dodds et al. 2009), there is a clear mandate for the scientific community to come up with an unambiguous standpoint on how to restore aquatic ecosystems. Here, we stress the importance of theoretical models, to ensure that the arguments used in the debate have a sound and testable mechanistic underpinning.

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