

Oikos

**OIK-05246**

van Gerven, L. P. A., Kuiper, J. J., Mooij, W. M., Janse, J. H., Paerl, H. W. and de Klein, J. J. M. 2018. Nitrogen fixation does not axiomatically lead to phosphorus limitation in aquatic ecosystems. – Oikos doi: 10.1111/oik.05246

## Appendix 1

Fig. A1–A6

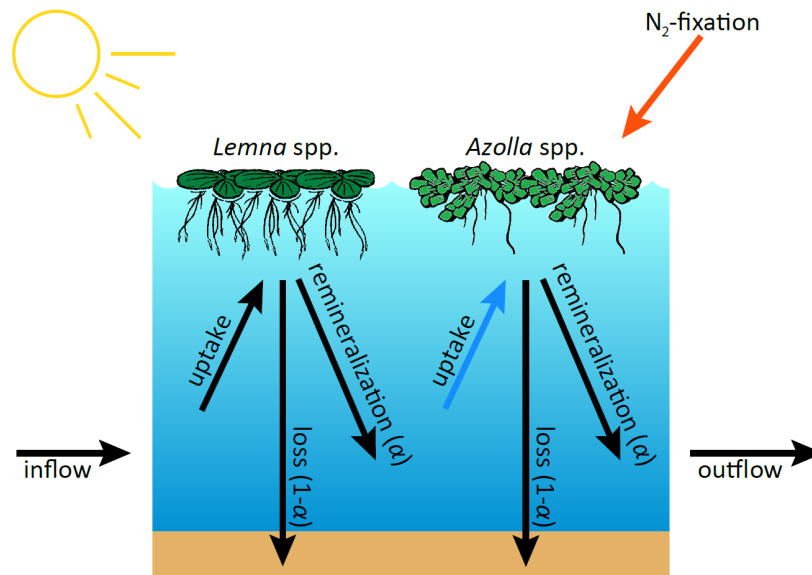


Figure A1. Schematic overview of the resource competition model. The arrows denote the nutrient fluxes; black = N and P, blue = P, red = N. The non-N<sub>2</sub>-fixer (*Lemna*) takes up N and P from the water, whereas the N<sub>2</sub>-fixer (*Azolla*) takes up only P from the water and fulfills its N demand by N<sub>2</sub>-fixation. When the plants die-off, their nutrient content partly ends up in the water through remineralization ( $\alpha$ ), and the remaining part ( $1-\alpha$ ) is lost to the sediment by burial.

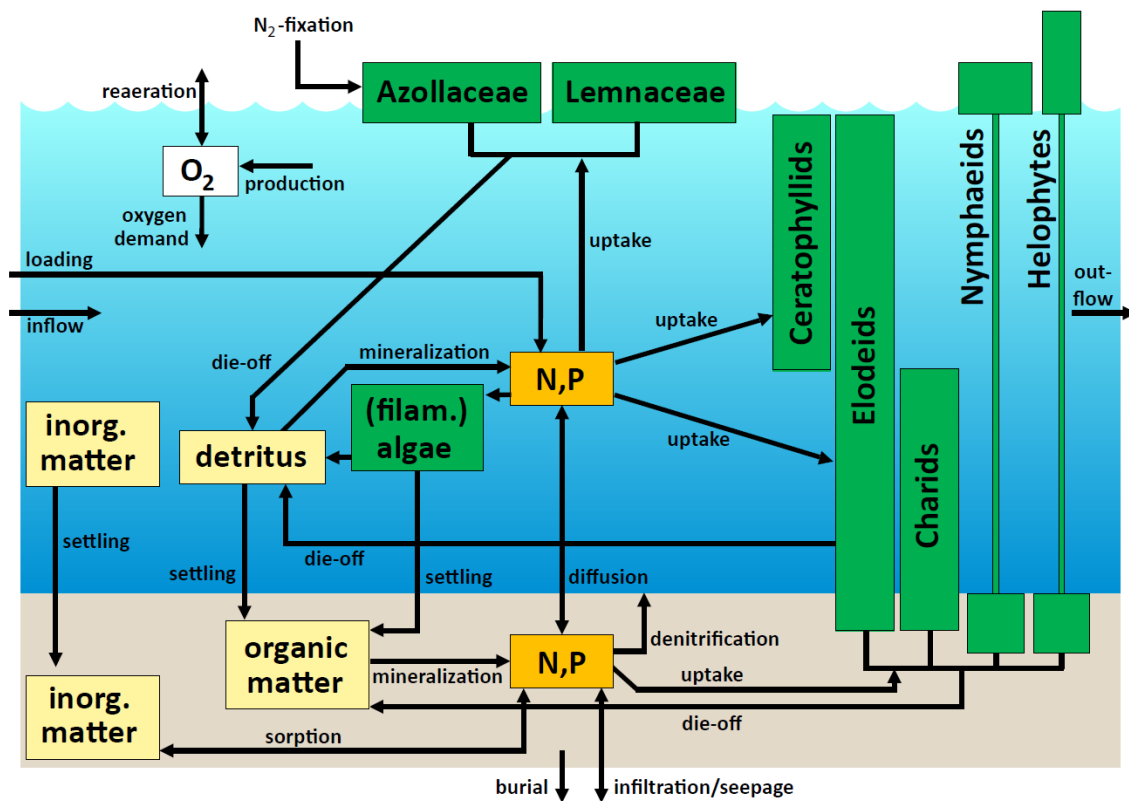


Figure A2. Schematic overview of the complex ecosystem model. A full description of PCDitch is presented in Janse, J. H. 2005. Model studies on the eutrophication of shallow lakes and ditches. – PhD thesis, Wageningen University.

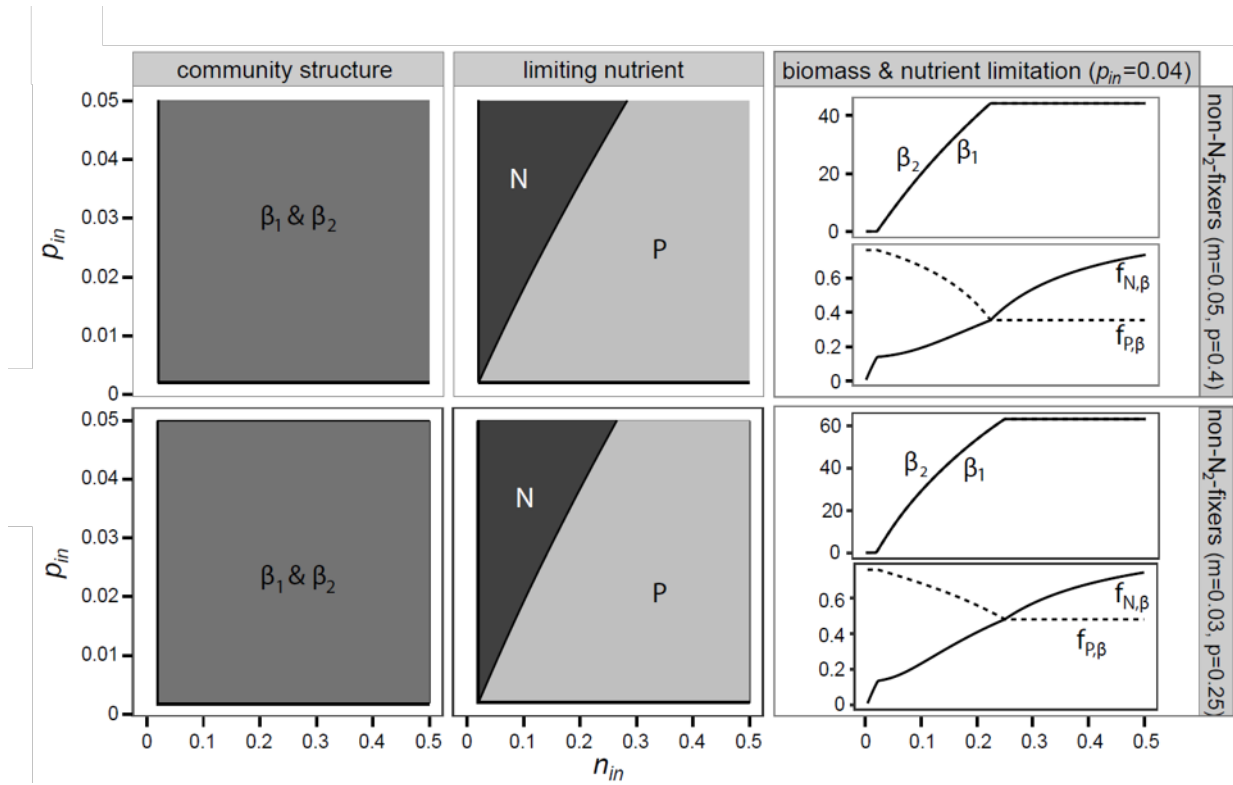


Figure A3. Sensitivity analyses of the structure and nutrient limitation of a community of identical non-N<sub>2</sub>-fixers  $\beta_1$  and  $\beta_2$  for different supply of N ( $n_{in}$ ) and P ( $P_{in}$ ). The upper panels show the results of the default analysis, describing *Lemna* (Table 2). The lower panels show the results when the values of  $p_{\max,i}$  (maximum growth rate) and  $m_i$  (loss rate) are used of *Azolla* instead of *Lemna* (Table 2). Left panel, competition outcome where ‘&’ denotes coexistence. Middle panel, nutrient that limits the growth of the non-N<sub>2</sub>-fixer (Table 1, Eq. 5). Right panel, underlying biomasses in gDW m<sup>-2</sup> and nutrient limitation factors of the non-N<sub>2</sub>-fixer (Table 1, Eq. 7–8) along a gradient of N supply ( $p_{in} = 0.04$ ).

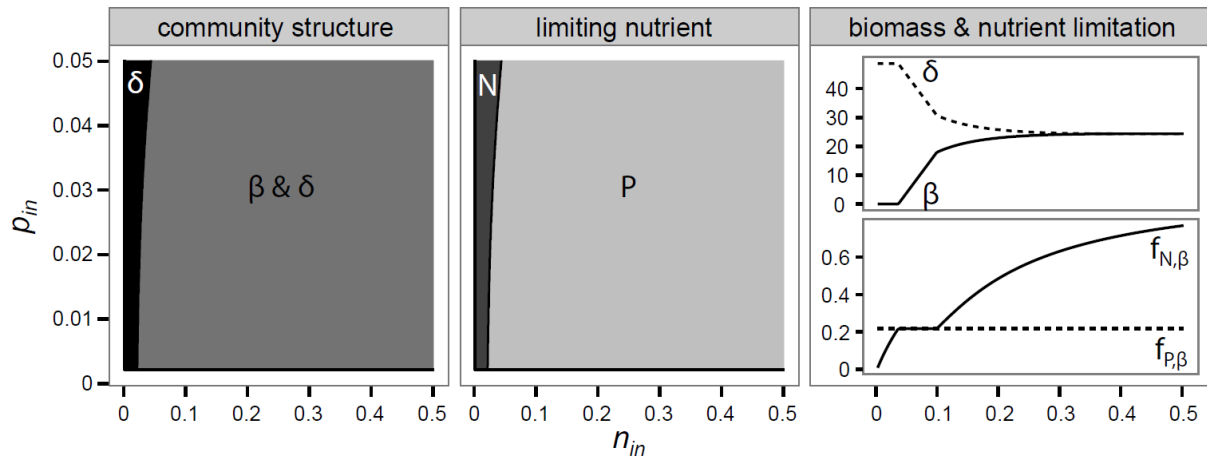


Figure A4. Predicted structure and nutrient limitation of a community of non-N<sub>2</sub>-fixer  $\beta$  and N<sub>2</sub>-fixer  $\delta$ , for different supply of N ( $n_{in}$ ) and P ( $P_{in}$ ), at zero remineralization ( $\alpha = 0$ ). Left panel, competition outcome where ‘&’ denotes coexistence. Middle panel, nutrient that limits the growth of the non-N<sub>2</sub>-fixer (Eq. 5). Right panel, underlying biomasses in gDW m<sup>-2</sup> and nutrient limitation factors of the non-N<sub>2</sub>-fixer (Eqs. 7-8) along a gradient of N supply ( $p_{in} = 0.04$ ). Both species were parametrized as *Lemna* sp. (Table 2).

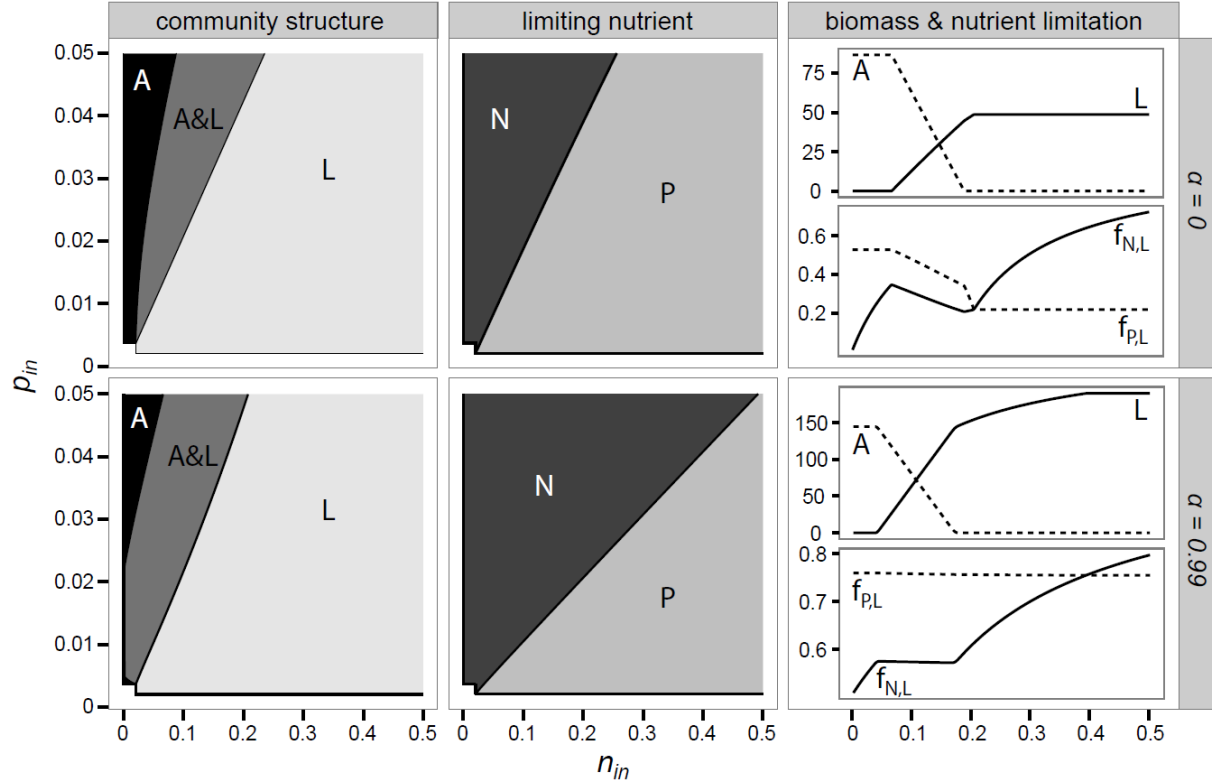


Figure A5. Structure and nutrient limitation of a community of *Lemna* (*L*) and *Azolla* (*A*), as predicted by the resource competition model at zero mineralization (upper panels:  $\alpha = 0$ ) and high mineralization (lower panels:  $\alpha = 0.99$ ). Left panel, competition outcome. Middle panel, nutrient that limits the growth of *Lemna*. Right panel, underlying biomasses in gDW m<sup>-2</sup> and nutrient limitation factors of *Lemna* along a gradient of N supply ( $p_{in} = 0.04$ ). See Table 2 for parameter values. Note that the nutrient limitation in the  $n_{in}$ - $p_{in}$  plane at zero remineralization (upper middle panel) is directed by the nutrient uptake and therefore follows (in absence of the N<sub>2</sub>-fixer) the ratio of the non-N<sub>2</sub>-fixer's nutrient content ( $c_{N,L} : c_{P,L} \approx 5$ ). On the other hand, at high mineralization rates, almost all nutrients are recycled, leading to almost no net uptake, making that nutrient limitation is reflected by the ratio of the N:P half-saturation concentrations ( $M_{N,L} : M_{P,L} = 10$ ).

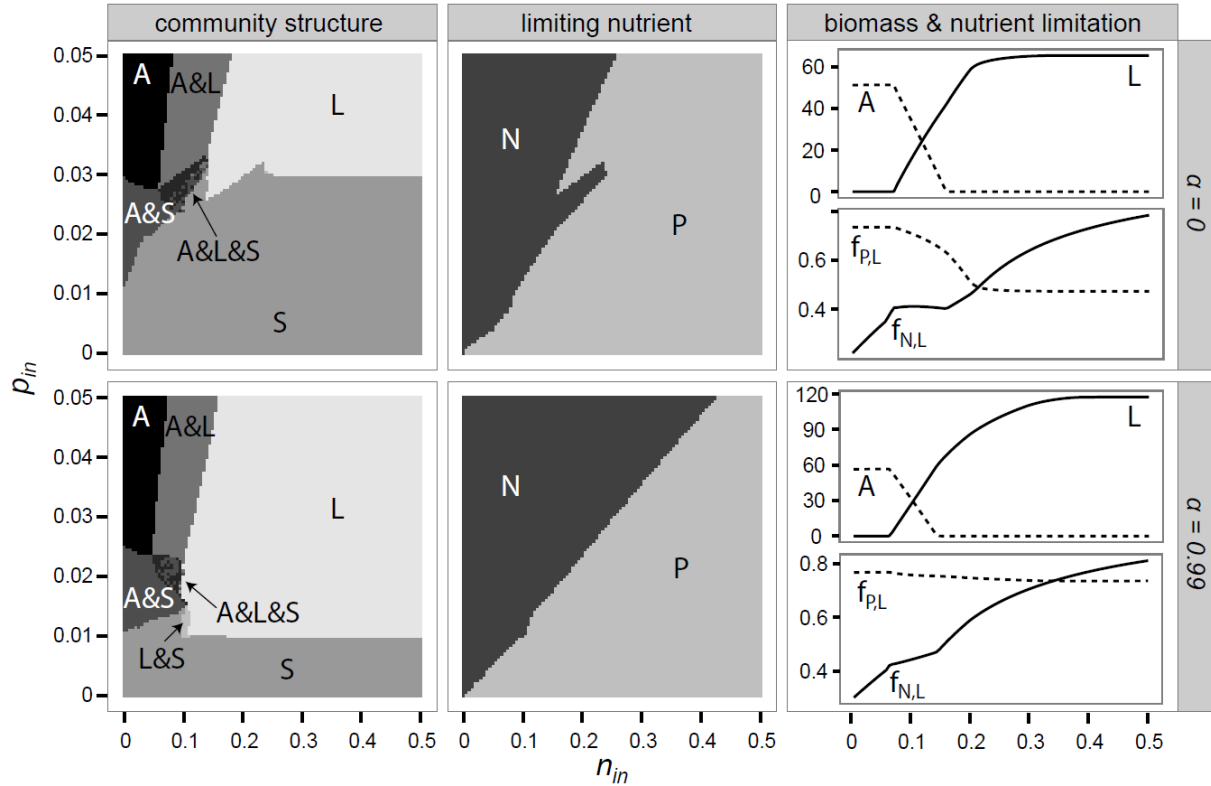


Figure A6. Structure and nutrient limitation of a community of *Lemna* (*L*) and *Azolla* (*A*), as predicted by the complex ecosystem model at zero mineralization (upper panels:  $\alpha = 0$ ) and high mineralization (lower panels:  $\alpha = 0.99$ ). Left panel, competition outcome where ‘S’ denotes submerged plants. Middle panel, nutrient that limits the growth of *Lemna*. Right panel, underlying biomasses in gDW m<sup>-2</sup> and nutrient limitation factors along a gradient of N supply ( $p_{in} = 0.04$ ). See Table 2 for parameter values.

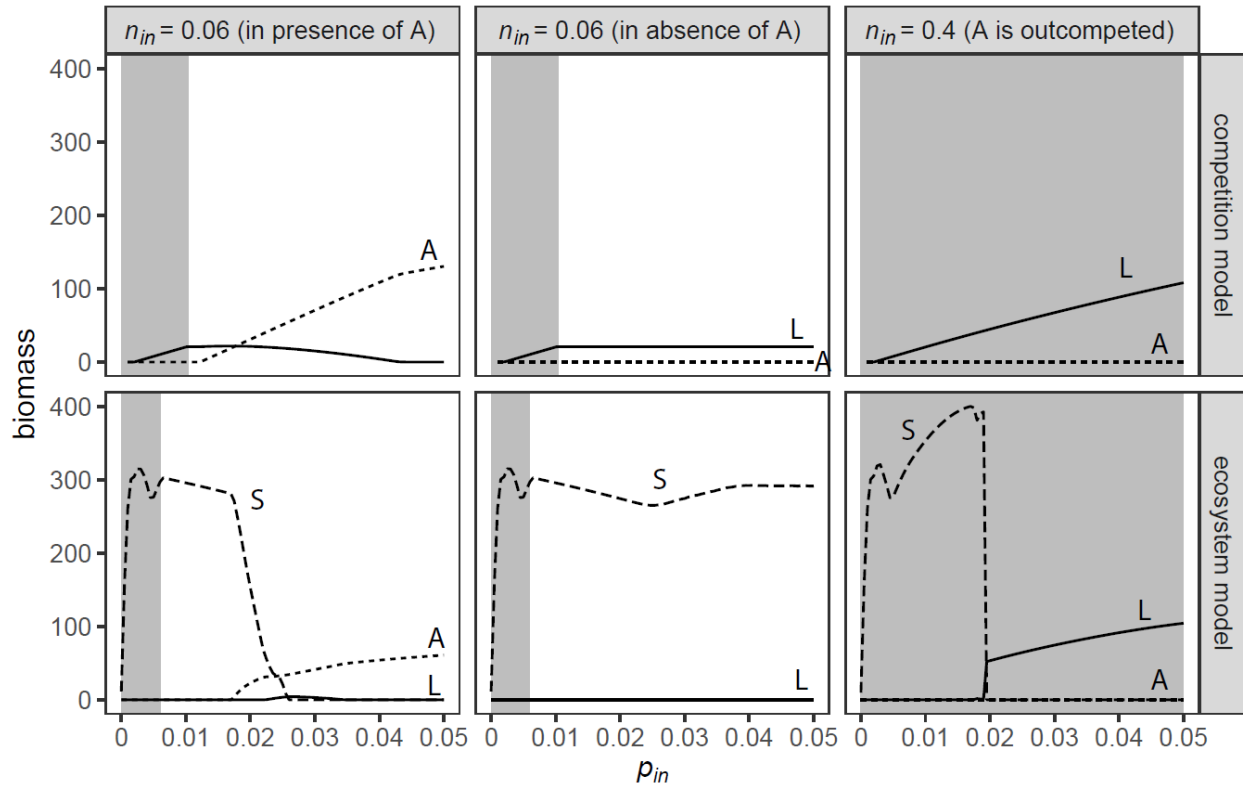


Figure A7. Biomass in gDW m<sup>-2</sup> of a floating-plant community of *Lemna* (L) and *Azolla* (A) as predicted by the resource competition model (upper panel) and the complex ecosystem model (lower panel) along a gradient of P supply, at low N supply (left, middle) and high N supply (right), and where the N<sub>2</sub>-fixer A is present (left) or absent (middle, right). ‘S’ denotes submerged plants. The grey-shaded area denotes P-limitation, whereas the non-shaded zone is N-limited. The model settings are similar to the ones used in Fig. 2.

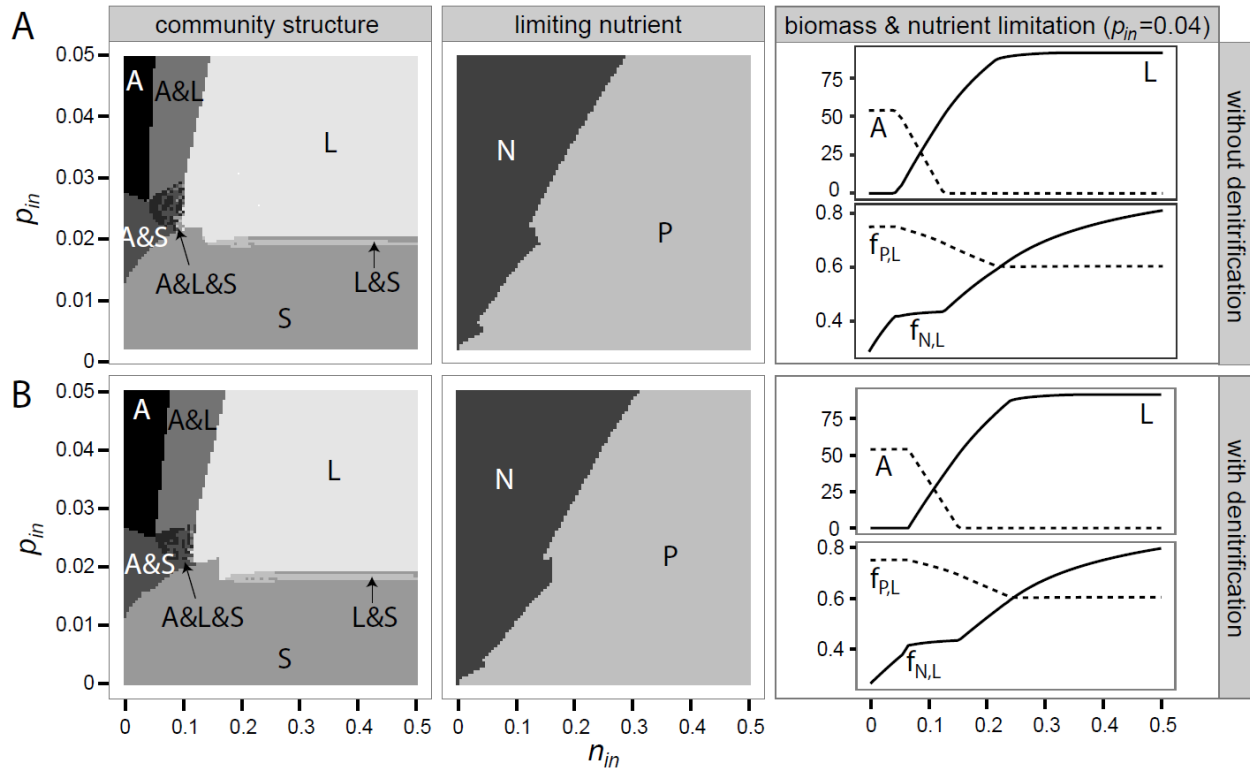


Figure A8. Structure and nutrient limitation of a floating-plant community of *Lemna* (*L*) and *Azolla* (*A*) as predicted by the complex ecosystem model for a situation without (upper panel) and with (lower panel) denitrification. Left panel, competition outcome where ‘S’ denotes submerged plants. Middle panel, nutrient that limits the growth of the non-N<sub>2</sub>-fixing *Lemna*. Right panel, underlying biomasses in gDW m<sup>-2</sup> and nutrient limitation factors of *Lemna* along a gradient of N supply ( $p_{in} = 0.04$ ). We calculated that the denitrification flux amounted to 5% till 40% of the N input, with an average of about 30%. See Table 2 for parameter values. Note that the lower panel is equal to Fig. 2b.

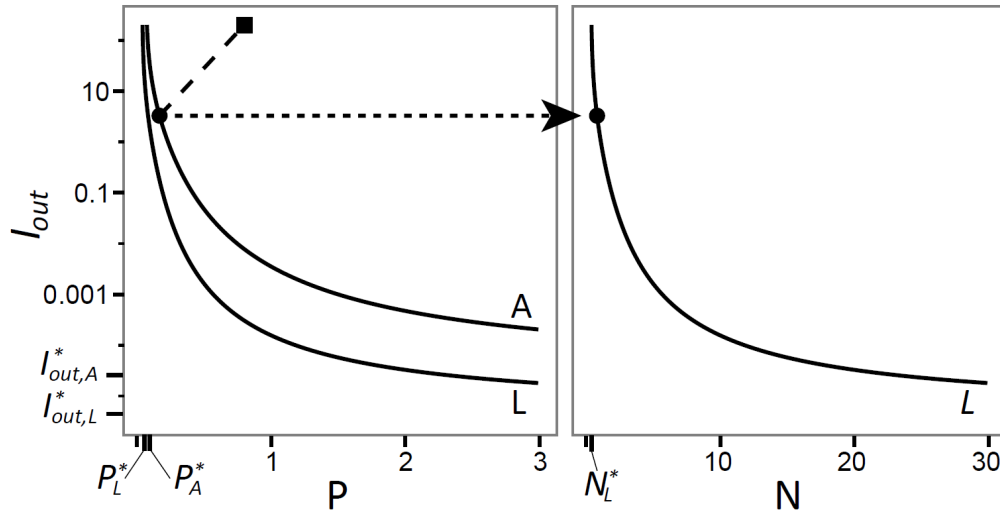


Figure A9. Graphical isocline approach showing why *Lemna* (*L*) cannot be P-limited in presence of *Azolla* (*A*). The solid lines depict the zero net growth isoclines (ZNGI's) of both species. The ZNGI represents combinations of light intensity at the bottom of the plant ( $I_{out}$ ) and concentrations of P (left panel) and N (right panel) in the water at which the net growth of a species is zero, so where its biomass is in equilibrium. The shape of the ZNGI follows from higher nutrient concentrations leading to higher equilibrium biomasses and therefore to more light attenuation and thus a lower  $I_{out}$ . The ZNGI captures the competitive ability of a species. The species with the 'lowest' ZNGI is the best competitor because it can deplete the resources to a level too low for the other species to persist. The lowest levels at which *Azolla* and *Lemna* can deplete a resource are denoted by  $N^*$  and  $P^*$  for nutrients, and  $I_{out}^*$  for light. Note that the  $N^*$  does not apply to *Azolla* because *Azolla* does not rely on N in the water for its growth, as it can fix  $N_2$  from the atmosphere and therefore is a superior competitor for N. *Lemna*, on the other hand, is the best competitor for P and light, due to its lower values of  $P^*$  and  $I_{out}^*$ . As a result, *Lemna* can never be P-limited in presence of *Azolla*, as *Lemna* would then lower the availability of light and P to a level too low for *Azolla* to persist (left panel). Therefore, if *Azolla* is present, then *Lemna* is N-limited. For *Azolla* to be present, the P:N supply ratio needs to be high enough such that *Lemna* keeps the P concentration and light availability high enough for *Azolla* to persist. This is the case for the chosen resource supply in this figure, denoted by the resource supply point (black square;  $p_{in}=0.04$ ,  $I_{in}=200$ , and  $n_{in}=0.15$ ), indicating the maximum resource availability. The consumption vector (dashed line) shows how the resource availability is depleted till *Azolla*'s biomass reached equilibrium (black dot in left panel). Given the realized light availability (dashed horizontal line), *Lemna* depletes the N concentration to a level corresponding with its equilibrium (black dot in right panel).