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Strong growth limitation of a floating plant (*Lemna gibba*) by the submerged macrophyte (*Elodea nuttallii*) under laboratory conditions

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SUMMARY

1. The asymmetric competition for light and nutrients between floating and submerged aquatic plants is thought to be key in explaining why dominance by either of these groups can be stable and difficult to change.
2. Although the shading effect of floating plants on submerged plants has been well documented, the impact of submerged plants on floating plants has been poorly explored hitherto.
3. Here, we used laboratory experiments to examine how submerged plant (*Elodea nuttallii*) alter nutrient conditions in the water column and how this affects the growth of floating plants (*Lemna gibba*).
4. We demonstrate that, at higher nutrient concentrations, *Lemna* is increasingly likely to outcompete *Elodea*.
5. Under low nutrient concentrations (0.1–2 mg N L^{-1}) *Elodea* can strongly reduce the growth of *Lemna*. Growth of floating plants virtually stopped in some of the experiments with *Elodea*.
6. Extremely reduced tissue N, Mn, chlorophyll and elongated roots indicated that the growth inhibition of *Lemna* by *Elodea* was predominantly caused by the latter's impact on the nutrient conditions for floating plants.
7. These results strengthen the hypothesis that submerged plants can prevent colonization of a lake by floating plants.

Keywords: competition, duckweed, nutrient limitation, submerged plants

Introduction

A trade-off in aquatic macrophytes between maximizing the capacity to take up nutrients versus optimizing light may largely determine the relative competitive advantage of floating and submerged plants along a gradient of nutrient richness. With respect to light, floating plants are simply on top. Dense mats cast a shade that makes it impossible for submerged plants to survive. On the other hand, submerged rooted plants are likely to affect the growth of free-floating plants through a reduction of available nutrients in the water column. Submerged macrophytes may take up their nutrients from the

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sediment through their roots (Hutchinson, 1975; Chambers *et al.*, 1989). However, they also take up nutrients effectively through their shoots (Sculthorpe, 1967; Carignan & Kallf, 1980; Robach, Thiébaut & Trémolières, 1995).

Competition is likely to be especially strong for nitrogen. Inorganic nitrogen concentrations in the water column of submerged vegetation stands are often below detection levels (Goulder, 1969; Van Donk *et al.*, 1993). Low nutrient concentrations in vegetation stands result not only from uptake by the plants themselves but also by attached periphyton (Eriksson & Weisner, 1997; Scheffer, 1998). The availability of nutrients may be further reduced by the precipitation of phosphates and trace minerals caused by the high pH associated with photosynthesis of algae and submerged plants (Otsuki & Wetzel, 1972; Wetzel, 1983; Spencer, Terri & Wetzel, 1994).

Such asymmetric competition for light and nutrients may well explain why floating plants tend to be dominant under more eutrophic conditions and submerged plants under lower nutrient levels (Portielje & Roijackers, 1995; Scheffer *et al.*, 2003; Morris *et al.*, 2004). A few studies have addressed the interactions between these two groups of macrophythes (Janes, Eaton & Hardwick, 1996; Forchhammer, 1999; Scheffer *et al.*, 2003; Morris *et al.*, 2004). However, these studies mainly focussed on the impact of floating plants on submerged plants, and little empirical work has been done on the effect of submerged vegetation on floating plants. According to Forchhammer (1999), the impact of submerged macrophytes on the growth of floating plants may be insignificant. However, McLay (1974) already noted that very low abundances of floating plants above dense stands of submerged vegetation, suggested a negative effect. In an earlier study, we have suggested that the effect of submerged plants on floating plant growth is crucial in shaping the plant community structure in many fresh waters, as its interaction with competition for light may lead to alternative stable states of dominance by either group (Scheffer *et al.*, 2003).

Here, we studied the effect of submerged plants on floating plants experimentally. We tested the hypothesis that the submerged plant [*Elodea nuttallii* (Planch.) St. John] can reduce nutrient concentrations in the water column sufficiently to suppress the growth of a common floating plant (*Lemna gibba* Linné.

Methods

Apical shoots of *E. nuttallii* and fronds of *L. gibba* were collected from ditches near Wageningen, The Netherlands. The plants were pre-incubated under experimental conditions for 7 days. For pre-incubation, *Elodea* (15 cm long) shoots and *Lemna* fronds were cultivated in aquaria containing a growth medium modified from Barko & Smart (1985) by adding NH_4NO_3 to a final concentration of 0.5 mg N L^{-1} Phosphorus was added as K_2HPO_4 to a final concentration of 0.083 mg P L^{-1} and a supply of micronutrients was ensured by adding 0.1 mL L^{-1} TROPICA micronutrient solution. The concentrations after dilution were: Fe 0.07, Mn 0.04, Zn 0.002, Cu 0.006 and Mo 0.002 mg L^{-1} respectively.

Co-cultures on semi-static media

Apical shoots (8 cm length) of *Elodea* plants were placed in 2 L plastic aquaria (11.5 × 11.5 × 18 cm) in an upright position on a plastic mesh. Initial biomass was as follows: 0 g (control), 10 g wet weight (WW). The sides of the aquaria were covered by black foil to avoid light penetration from the sides. The plants were co-cultured with 1 g WW of *Lemna*. The plants were grown at five different nitrogen concentrations by adding NH_4NO_3 to 0.1, 0.5, 2, 5 or10 mg L^{-1} nitrogen. Total P concentrations were increased concomitantly with total N, keeping the N : P weight ratio at 6 (0.017, 0.083, 0.333, 0.833, 1.667 mg P L^{-1}). The concentration of microelements was the same as in the pre-incubation. Three aquaria were used per treatment. The cultures were incubated for 10 days under the following conditions: 180 μmol m^{-2} s^{-1} photon flux density, 16-h light/8-h dark, 25 °C. The medium was renewed on the 3rd, 6th and 9th days. On the 10th day of incubation, the wet and dry mass of duckweed plants was measured. A portion of 0.1 g WW *Lemna* was used for chlorophyll determination. Chlorophyll was extracted in 95% ethanol and the content determined by spectrophotometry, according to Lichtentaler (1987). The main portion of *Lemna* plants was used for dry mass determination. Dry mass was used to calculate the relative growth rate of *Lemna* during 10 days of incubation (RGR): $RGR = (\ln DW_t - \ln DW_0)/t$ in which DW_t and DW_0 are the dry masses at time t and time 0 respectively.

Further, a *Lemna–Elodea* co-culture experiment was carried out in 8 L of black plastic cylinders (20 cm in

diameter) containing 8 L culture media. Apical shoots (8 cm length) of *Elodea* plants were placed in upright position on a plastic mesh. Initial WW biomass was 0 g (control) and 10 g for *Elodea*, co-cultured with 1 g *Lemna*. The plants were grown at five different nitrogen concentrations (0.1, 0.5, 2, 5 and 15 mg N L^{-1}). Total P concentrations were increased concomitantly with total N, keeping the N : P weight ratio of 6. Half of the medium was replaced every 3 days. Treatments were replicated twice. The cultures were grown for 23 days and the wet mass of duckweed and *Elodea* was measured on the 10th and 23rd days. RGR of *Lemna* was calculated.

Co-cultures on static media

For the co-cultivation of duckweed and *Elodea*, black plastic aquaria (11.5 × 11.5 × 18 cm) containing 2 L of culture mediaum were used. A PVC-tube (9 cm length) with a diameter of 2 cm was placed vertically in each of the aquaria. It served as a duckweed enclosure (Szabó, Roijackers & Scheffer, 2003). Portions of pre-incubated *Lemna* fronds (0.1 g WW) were put inside the enclosures. This method allowed us to culture duckweeds in a static medium under optimal conditions, avoiding overcrowding as well as algal inhibition (Szabó *et al*., 2003, 2005). *Elodea* plants were placed in the aquaria outside the *Lemna* enclosures as follows: 0 g (control), 2 and 10 g WW. The plants were grown at five different nitrogen concentrations (0.1, 0.5, 2, 5 and 15 mg N L^{-1}). The mass ratio of nitrogen to phosphorus was 6 (0.017, 0.083, 0.333, 0.833, 2.5 mg P L^{-1}). The concentration of microelements was the same as in the pre-incubation. Three aquaria were used per treatment.

The cultures were incubated for 10 days. The WW of *Lemna* fronds was measured on days 4, 6, 8 and 10. On the 10th day *Lemna* plants were harvested. A portion (0.10 g FW) of *Lemna* was used for chlorophyll determination. The main fraction of the *Lemna* plants was used for dry weight determination. Dry masses were used to calculate the relative growth rate of *Lemna* over 10 days of incubation. The RGR based on wet masses was followed in time and was calculated for the 4th, 6th, 8th and 10th days of incubation: $RGR_6 = (\ln WW_6 - \ln WW_4)/2$.

We recorded pH on the 2nd, 4th, 6th, 8th and 10th days. Samples of water were taken on the 1st, 2nd, 4th 6th and 10th days, filtered and analysed for PO_4^{3-}, NO_3^-, NH_4^+ (Technicon Auto Analyser, Skalar, Breda, the Netherlands) and Fe and Mn (by inductively coupled plasma atomic emission spectroscopy (ICP AES) as described by Szabó, Braun & Borics, 1999). At the end of the experiment the chemical composition of the fronds was analysed. Nitrogen was analysed using a VARIO EL elemental analyser (Elementar Analysensysteme GmbH, Hanau, Germany), Mn was analysed by ICP AES.

Statistics

The significance of the factors (*Elodea* densities, nitrogen and phosphorus concentration) on the RGR and chlorophyll concentration of *Lemna* was evaluated by ANOVA using SPSS 11.0 (SPSS 1999) software. RGR and the chlorophyll concentration of *Lemna* cultures grown in static media with *Elodea* plants were compared with their control cultures using the Dunnett *t*-test. In semi-static media, *t*-tests and Dunnett's T3 pairwise comparison tests were used for comparing the means. The normal distribution of the variables was checked by the Kolmogorov–Smirnov test. Levene's test was used for checking the equality of error variances of dependent variables across groups.

Results

Elodea density and nutrient (N, P) concentrations of the medium and their interactions had significant ($P < 0.001$; ANOVA) effects on the growth and on the chlorophyll concentration of *Lemna* plants grown in semi-static as well as in static media (Table 1). The growth of *Lemna* (expressed as RGR) increased significantly ($P < 0.001$; Dunnet's test) with increasing nutrient concentration. The presence of *Elodea*, reduced the growth of duckweed significantly ($P < 0.01$–0.001; *t*-test, Dunnet's test) (Figs 1, 3 & 4).

Tissue chlorophyll concentrations of *Lemna* significantly increased with increased N concentration of the medium ($P < 0.001$; Dunnet's test). Chlorophyll concentration of the fronds appeared to be a particularly sensitive indicator of inhibitory effects of submerged plants. It was significantly ($P < 0.01$–0.001; *t*-test, Dunnet's test) reduced in *Lemna* co-cultured with *Elodea* (Figs 1b & 4c).

Co-cultures on semi-static media

Depending on the initial nutrient (N, P) concentration, over 10 days incubation *Elodea* plants reduced the

Source of variation	Co-cultures on semi-static medium			Co-cultures on static medium		
	d.f.	*F*	*P*-value	d.f.	*F*	*P*-value
Relative growth rate						
Nutrient level	4	81.1	<0.001	4	217.6	<0.001
Elodea	1	561.9	<0.001	2	647.9	<0.001
Interaction	4	34.1	<0.001	8	18.3	<0.001
Error	20			30		
Chlorophyll cocncentration						
Nutrient level	4	54.7	<0.001	4	89.4	<0.001
Elodea	1	697.2	<0.001	2	371.3	<0.001
Interaction	4	17.7	<0.001	8	12.1	<0.001
Error	20			30		

Lemna and *Elodea* were co-cultured on semi-static and on static media in 2 L aquaria under different initial nutrient (N, P) concentrations in the water combined with different *Elodea* densities.

Table 1 ANOVA of the relative growth rates and of the chlorophyll concentration of *Lemna* cultures against nitrogen concentration and *Elodea* densities.

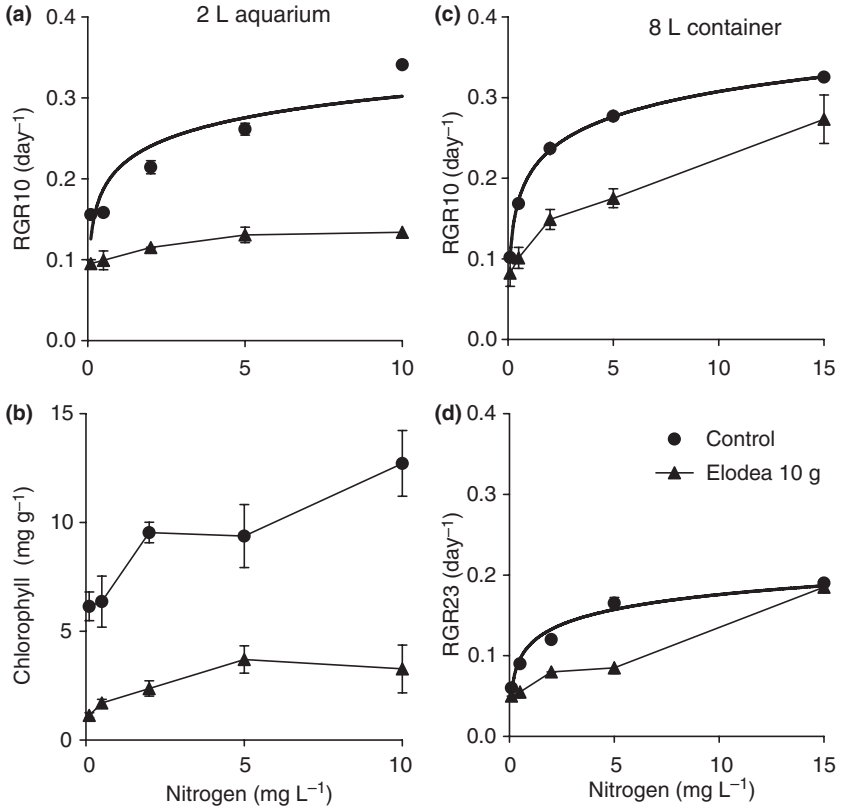

Fig. 1 The impact of *Elodea* on the relative growth rate (RGR) (a, c &d) and on the tissue chlorophyll concentration (b) of *Lemna* grown on semi-static medium (2 L aquaria, 8 L containers). The data for RGR10 (a) were based on the dry mass of the fronds at time 0 and on the10th day. The data for RGR10 (c) and RGR23 (c) were based on the wet mass of *Lemna* measured on the 10th and 23rd days of incubation. *Lemna* was co-cultured with *Elodea* grown in media containing different nutrient (N, P) concentrations. Error bars indicate the standard deviations of data (panels a, b: $n = 3$; panels c, d: $n = 2$). Error bars are sometimes too small to be visible.

RGR of *Lemna* by 38–60% and 17–40% in 2 and 8 L containers, respectively (Fig. 1a,c). However, in 8 L containers with increasing nutrient concentration (5–15 mg N L^{-1}), the growth inhibition of *Elodea* weakened (Fig. 1c). After 18 days incubation at the highest nutrient concentration, *Lemna* had covered the surface totally even in the presence of *Elodea*. At the highest nutrient concentration, after 23 days *Elodea* had no significant ($P > 0.05$; *t*-test) impact on the growth of *Lemna* (Fig. 1d).

Nutrient concentration of the media had significant impact on the biomass of *Elodea* ($P < 0.01$, ANOVA).

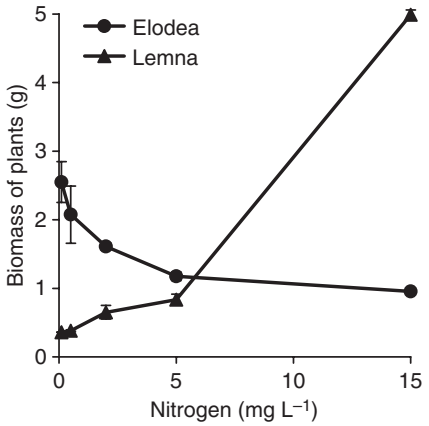

Fig. 2 Plant biomass in *Lemna–Elodea* co-cultures grown on semi-static media with different nutrient concentrations. The points represent the mean of dry weight of the plants; error bars indicate the standard deviations of the data (n = 3).

The biomass of *Elodea* in *Lemna–Elodea* co-cultures showed a gradual decrease with increasing nutrient concentration. Although the initial biomass ratio was low (*Lemna:Elodea* 1 : 10), under the highest nutrient concentration *Lemna* outcompeted *Elodea*. Its biomass was five times higher at the end of the experiment (Fig. 2).

Co-cultures on static media

Competition for nutrients. In the control culture at higher nitrogen concentrations (2–15 mg N L^{-1}), the growth of *Lemna* was relatively high (0.210–0.362 day^{-1}). The biomass of *Lemna* increased exponentially during the incubation (Fig. 3a,b). At 5 mg N L^{-1}, its growth rate increased up to 6 days,

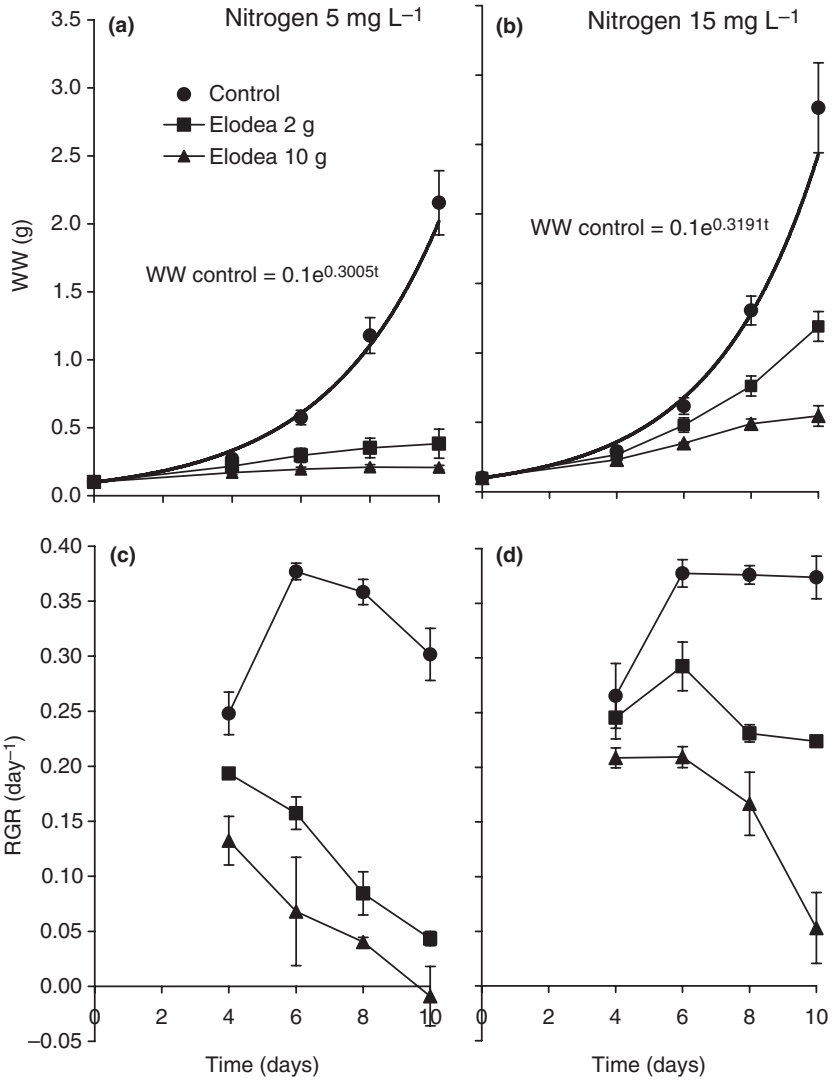

Fig. 3 The impact of *Elodea* on the biomass (a, b) and on the relative growth rate (RGR) (c, d) of *Lemna* grown on 5 and 15 mg N L^{-1} static media. The points represent the mean of wet weight and RGR of duckweed cultures. Data for RGR were based on the wet mass of *Lemna*; error bars indicate the standard deviations (n = 3).

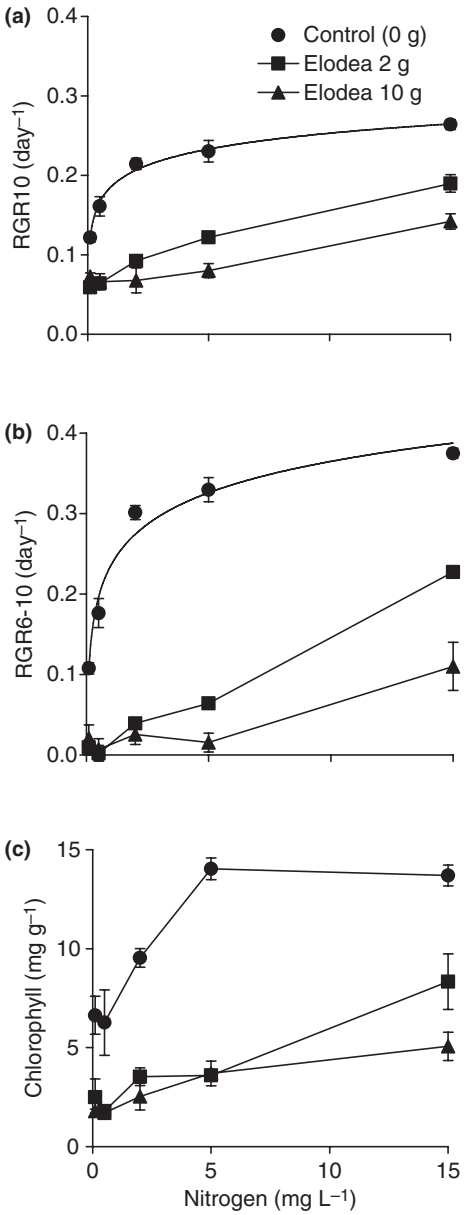

Fig. 4 The relative growth rate (a, b) and tissue chlorophyll concentration (c) of *Lemna* co-cultured with *Elodea* as a function of the initial nitrogen concentration and *Elodea* biomass. The data for RGR10 (a) were based on the dry mass of the fronds at time 0 and on the10th day, RGR6-10 (b) was based on the wet mass of *Lemna* measured on the 6th and 10th days of incubation. *Lemna* was co-cultured with *Elodea* grown on static media containing different nutrient (N, P) concentrations. Error bars indicate standard deviations (n = 3).

then decrease slightly after 8 days incubation (Fig. 3c). In 15 mg L^{-1} nitrogen, the growth rate showed the same increase as in 5 mg L^{-1}, then it remained constant during the incubation (Fig. 3d). In control cultures, the growth was saturated above 5 mg N L^{-1} and intraspecific competition among the fronds could not be detected, since the RGR and N concentration of the fronds were more or less constant (Figs 3d & 6a).

Depending on the initial nutrient (N, P) concentration, *Elodea* plants (10 g WW) reduced the RGR (days 0–10) by 40–68% (Fig. 4a). During the last 4 days of incubation, the growth inhibition of *Elodea* on *Lemna* was even stronger than it was calculated for the overall experiment. Here, *Elodea* reduced the growth ($RGR_{6–10}$) by 71–96% (Fig. 4b). In general, the most intensive growth reduction of *Lemna* was measured in the range of 0.5–2 mg N L^{-1} where the RGR (days 6–10) was reduced to below 0.05 day^{-1}. The RGR of *Lemna* co-cultured with *Elodea* showed a gradual decrease during the incubation and was significantly lower ($P < 0.05$; t-test). Under 5 mg L^{-1} initial nitrogen, on the final day of incubation, RGR values were reduced below zero in the presence of *Elodea* (Fig. 3c). With increasing nutrient (N, P) concentration (5–15 mg N L^{-1}), the growth inhibition of *Elodea* weakened (Fig. 4). In the range 5–15 mg N L^{-1}, the inhibitory effect of *Elodea* on RGR of *Lemna* was significantly ($P < 0.05$, t-test) stronger under high than low plant density.

Changes in elemental composition. In control aquaria initially containing 5 mg N L^{-1}, the nitrogen concentration of the medium was reduced to 0.97 mg L^{-1} by *Lemna*. In the presence of submerged plants, nitrogen dropped below 0.27 mg L^{-1} (Fig. 5a). In control cultures initially containing 5 mg N L^{-1}, the PO_4^{3-}–P concentration of the water was reduced from 0.83 to 0.17 mg L^{-1} after 10 days. In *Lemna–Elodea* co-cultures, the PO_4^{3-}–P concentration of the medium was reduced to 0.01 mg L^{-1} (Fig. 5b). The iron concentration was reduced from 0.081 to 0.025 mg L^{-1} in control aquaria, whereas in *Lemna–Elodea* co-cultures, it dropped below 0.01 even after 2 days (Fig. 5c). The concentration of manganese in the water showed a similar sharp drop (Fig. 5d). The depletion of nutrients (N, P) was faster when the biomass of *Elodea* was higher.

In aquaria containing *Elodea*, pH increased above 10.0 after 4 days of incubation, whereas in control aquaria it dropped slightly from 7.8 to 7.6. The increase in pH was more marked at high densities of *Elodea*.

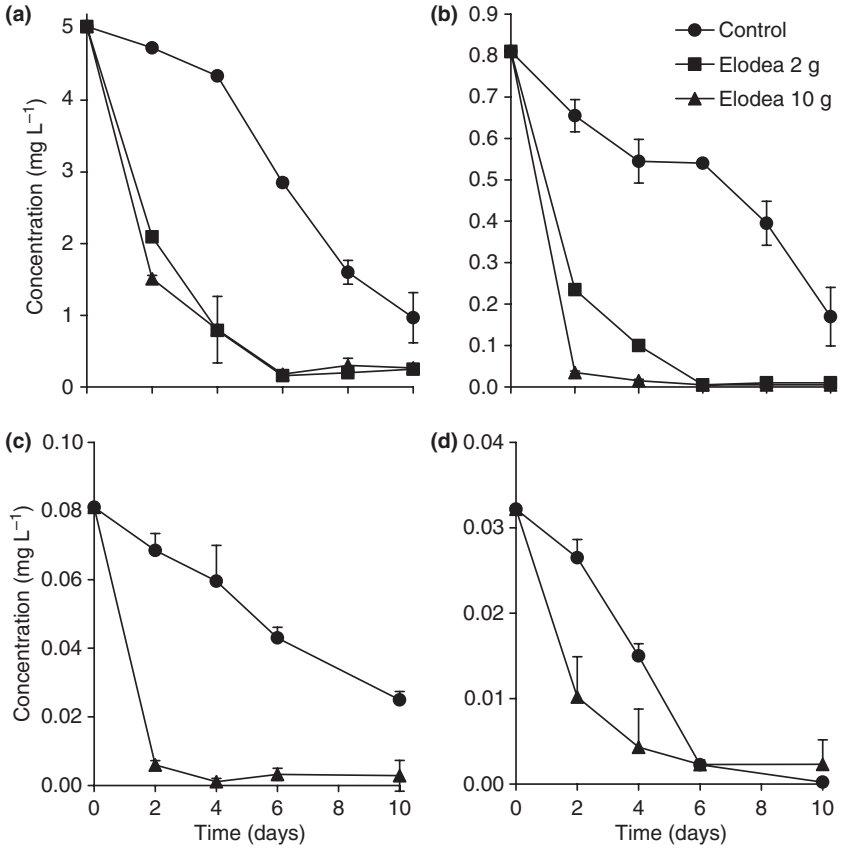

Fig. 5 Nutrient concentration [(a) nitrogen (NO_3^-–N, NH_4^+–N), (b) phosphorus (PO_4^{3-}–P), (c) total iron, (d) total manganese] of the water in *Lemna–Elodea* co-cultures grown on medium containing 5 mg N L^{-1}. Error bars indicate standard deviations (n = 3). Iron and manganese concentration with 2 g of *Elodea* are not measured.

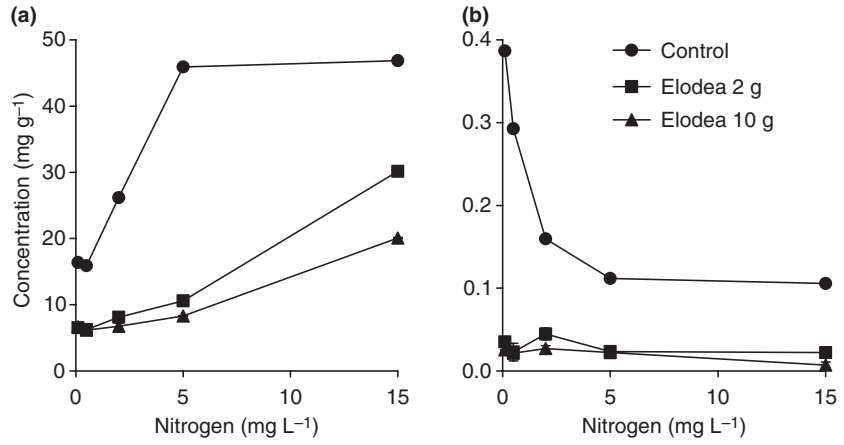

Fig. 6 Nitrogen (a) and manganese (b) concentration of *Lemna* fronds co-cultured with *Elodea* grown on media with different nitrogen concentrations. Points indicate the mean of the three replicates. Standard deviations are not shown.

In the control cultures, the N concentration of the fronds increased with increasing N concentration of the medium up to 5 mg L^{-1}, above which it remained constant. In the treatment with *Elodea* (10 g WW) at 2 and 5 mg N L^{-1}, the nitrogen concentration of *Lemna* fronds was reduced by 74–82% (Fig. 6a). In control cultures, the manganese concentration of the frond was much higher at low nitrogen concentrations and showed a gradual decrease with increasing nitrogen concentration. However, in the treatment with *Elodea* (10 g WW) at 5 mg N L^{-1}, the manganese concentration of *Lemna* fronds was reduced by 80% (Fig. 6b).

Discussion

Our experiments show that submerged plants can indeed affect the chemical composition of the water strongly. For instance, N, P, Fe and Mn were

exhausted to below detection limits in the medium. This depletion may well explain the suppression of duckweed growth, because other studies have found that substantial concentrations of these elements (N 0.22, P 0.03, Fe 0.028, Mn 0.0005 mg L^{-1}) are needed to sustain growth of duckweeds (Hopkins, 1931; Steinberg, 1946; Eyster, 1966; Landolt, 1986). In addition, *Elodea* raised the pH beyond the range generally reported for optimal growth of *Lemnaceae* (McLay, 1976; Landolt & Wildi, 1977; Landolt & Kandeler, 1987).

Our results show that, while *Elodea* inhibited *Lemna* growth in the low nutrient range, *Lemna* was increasingly less affected by *Elodea* at higher nutrient concentrationss (Figs 1, 2 & 4). In fact, *Lemna* outcompeted *Elodea* at the highest concentrations. This is well in line with the view that asymmetric competition for light and nutrients allows submerged plants to dominate at low nutrient concentrations, but not at higher concentrations.

The idea that *Elodea* suppresses the growth of *Lemna* via the depletion of nutrients is further supported by the finding that, with increasing density of *Elodea*, suppression of growth, and also the decline in the N concentration of *Lemna* leaf tissue, was more pronounced. Also the decrease in the nutrient concentration of the water corresponded well with the very low N and Mn and chlorophyll concentration of the fronds and with the reduction in *Lemna* growth.

It is plausible that the reduced N concentration of the fronds (45–8 mg g^{-1}), associated with the presence of *Elodea*, was a factor causing the reduction of growth, because the range of RGR reported from duckweeds growing optimally (25–45) are substantially higher (Landolt & Kandeler, 1987; Vermaat & Hanif, 1998).

Although direct nutrient depletion seems to have been an important process in our experiments, it is probably not the only factor explaining the growth reduction of *Lemna*. The fact that the growth and N concentration of the fronds was much lower in the culture with *Elodea* in 5 mg N L^{-1} than in the control culture at the lowest nutrient concentration, suggests that, in addition to the exhaustion of nutrients (N, P), pH-related inhibition may have played a role. A high pH may reduce the bioavailability of nutrients (nitrate, phosphate) and cause phosphate and trace elements to precipitate from the water (Otsuki & Wetzel, 1972; Loeppert, Kronberger & Kandeler, 1977; Novacky & Ullrich-Eberius, 1982; Wetzel, 1983). Indeed, in competition experiments between duckweeds and planktonic algae, we found that algal activity, by raising pH, resulted in the depletion of trace elements (iron) and reduced the growth of duckweed (Szabó *et al*., 1999, 2003, 2005; Roijackers, Szabó & Scheffer, 2004). Such pH-related mechanisms may well play a role in the field, as high pH has frequently been found above dense stands of submerged plants (*Elodea*, *Ceratophyllum*, *Potamogeton*) (Goulder, 1969; McLay, 1974; Forchhammer, 1999). Obviously, these synergistic negative effects through pH will tend to enhance, rather than weaken, the effect of submerged plants on floating plants, implying an even stronger potential for hysteresis (Scheffer *et al*., 2003).

In conclusion, our study demonstrates that, at low-to-moderate nutrients, submerged macrophythes can strongly reduce the growth of floating plants. Growth of *Lemna* virtually stopped in some of the experiments, and the extremely reduced tissue N, Mn chlorophyll, yellowish colour and elongated roots indicated that the growth inhibition induced by submerged plants was predominantly realized through their impact on the nutrient conditions for floating plants.

Obviously, our laboratory experiments give a rather extreme image of the interaction between floating and submerged plants, as various buffering mechanisms were excluded. For instance, competitive impacts of submerged vegetation on floating plants in the field can be ameliorated by nutrient release from sediments and by decomposition (Szabó *et al*., 2000). On the other hand, in more open waterbodies, winds blow the mats of free-floating vegetation to the shore, and therefore their shading effect plays a lesser role in competition for light.

Nonetheless, our results are well in line with the idea that submerged plants can sustain their dominance over floating plants until nutrient loading becomes too high. With increasing nutrients, growth of floating plants becomes less limited and they may gradually increase (Figs 1d & 2). This can then lead to a positive feedback, as their shading effect reduces nutrient uptake by submerged plants. The system may then 'flip' to a stable state of dense floating plant cover under which submerged plants vanish (Scheffer *et al*., 2003). Anoxic conditions in such situations may allow little life at all under the floating plant beds (Morris *et al*., 2003, 2004).

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