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# Submerged Macrophytes Can Maintain Stable Dominance Over Free-Floating Competitors Through High pH

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## ABSTRACT

1. Submerged and free-floating macrophytes are the two main vegetation types that can alternatively dominate in small shallow ponds and ditches. Submerged macrophytes provide more aquatic ecosystem services and cause fewer problems with anoxia and greenhouse gas emissions than free-floating plants, but are inferior competitors for light. High pH values have been proposed as a contributor to submerged plant dominance, but threshold values for pH-induced growth reduction of floating plants by submerged macrophytes are not known.
2. In this study, we conducted laboratory experiments to test whether submerged plants (*Ceratophyllum demersum*) can effectively limit the growth of free-floating competitors (*Lemna gibba*) by exceeding a critical threshold pH value. We used field data to test if and when such pH values can be achieved within dense submerged macrophyte stands.
3. The pH values that caused 50% reduction in fresh weight- and chlorophyll-based growth of *L. gibba* were 9.6 and 9.8, respectively, and the growth was negligible above pH 10.0. The photochemical efficiency of photosystem II and chlorophyll content of the floating plants declined rapidly above pH 9 and 9.5, respectively. Similarly, phosphorus concentration decreased and dry matter content increased sharply in *L. gibba* fronds above pH 9.5, respectively. We thus expect a critical pH threshold for *L. gibba* at around 9.5.
4. Our mesocosm and field data show that the critical pH threshold for *L. gibba* growth is regularly exceeded within dense stands of bicarbonate-using submerged macrophytes in lentic water-bodies. Such conditions can be prevalent in the upper water layer during most of the day (12 h) in summer.
5. The literature showed that many duckweed species (and other common free-floating plants) in temperate and tropical water bodies show growth inhibition above pH 8. Therefore, high pH created by submerged macrophytes that use $HCO_3^-$ in photosynthesis can be a major mechanism by which dominance over free-floating competitors is maintained in densely vegetated lentic eutrophic water bodies. We propose that alternative stable states occur between free-floating and submerged macrophytes along a pH gradient. Active management of pH may, thus, help in maintaining the desired state.

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## 1 | Introduction

Different types of stable dominance can exist among primary producers in freshwater ecosystems (Scheffer and van Nes 2007; Szabó, Koleszár, Braun, et al. 2022; Szabó, Koleszár, Zavanyi, et al. 2022). Shifts between these stable equilibria significantly alter important functions and services of these ecosystems (Janssen et al. 2021). In shallow freshwater ecosystems, primary producers compete for light and nutrients as major resources along opposing vertical supply axes (Szabó et al. 2010; van Gerven et al. 2015). Pelagic phytoplankton and free-floating macrophytes shade submerged macrophytes, which, in turn, are better at intercepting nutrient fluxes from sediment to water (Jäger and Diehl 2014). Increasing the external supply of nutrients to the water leads to a loss of submerged macrophytes when a critical threshold of light availability is exceeded. Consequently, high nutrient loading often results in dominance of either phytoplankton in larger water bodies (Janssen et al. 2014; Scheffer and van Nes 2007), or free-floating plants in small lentic waters (Scheffer et al. 2003; Szabó et al. 2022a). In the latter case, free-floating plants can shade out both phytoplankton (de Tezanos Pinto, Allende, and O'Farrell 2006; de Tezanos Pinto and O'Farrell 2014) and submerged macrophytes (Lu et al. 2013; Szabó et al. 2024). In contrast, at low and intermediate nutrient concentrations, submerged plants can outcompete both free-floating macrophytes and phytoplankton (Szabó et al. 2010, 2022a; van Gerven et al. 2015).

pH has also been found to play an important role in the successful competition of submerged plants with their free-floating opponents (Szabó et al. 2022b). Inland waters are typically well-buffered and, in most systems, their pH normally stays within the range of 6–9. However, abiotic and biotic processes acting both within and outside of these surface water bodies can alter the pH on hourly to much longer timescales (Finlay and Bogard 2022). Submerged plants increase water alkalinity through their photosynthesis and bicarbonate uptake (Pedersen, Colmer, and Sand-Jensen 2013). This may give them a competitive advantage if their free-floating competitors have (1) a lower optimum pH range, (2) lower uptake efficiency for anions such as nitrate and phosphate, because their uptake is blocked above pH 9.5 (Loeppert, Kronberger, and Kandeler 1977; Ullrich-Eberius et al. 1981) and/or (3) a lower uptake efficiency to manganese, iron or phosphate which precipitate at alkaline pH (Otsuki and Wetzel 1972; Stumm and Morgan 1996; Szabó et al. 2022b). Indeed, McLay (1974) hypothesized that the lowered growth rate of the floating minute duckweed *Lemna perpusilla* in zones of water with the submerged *Stuckenia pectinata* was attributable to higher pH in the water; probably as result of photosynthesis by the submerged plants. For hornwort (*Ceratophyllum demersum*), as one of the most abundant submerged species in small ponds and ditches in Europe (Birk and Willby 2010; Hilt et al. 2018; Szabó et al. 2024), high pH together with nitrogen limitation were found to be the most important growth-limiting factors maintaining its dominance over the free-floating duckweed (Szabó et al. 2022b). In laboratory experiments, hornwort-induced rise in alkalinity to pH 10 reduced the growth and tissue nitrogen and phosphorus content in duckweed (Szabó et al. 2022b). However, pH values can show strong spatial and temporal variation within submerged macrophyte stands (Frodge, Thomas, and Pauley 1990; Spencer, Teeri, and Wetzel 1994; O'Sullivan and Reynolds 2004). Yet, the threshold alkalinity for pH-induced growth reduction of floating plants by submerged macrophytes is still not known.

In this study, we hypothesized that (1) exceeding a threshold pH can limit the growth of free-floating duckweed and that (2) this threshold pH level can be exceeded within dense submerged macrophyte stands during daylight and in the upper water layer. We tested these hypotheses by combining laboratory experiments on the growth of duckweed (*L. gibba*) cultivated over a wide pH range with an outdoor mesocosm study on the spatial and temporal pH variation within *C. demersum* stands and with field studies on the pH within dense submerged macrophyte stands in Hungarian and Croatian water bodies with water temperatures between 25°C and 37°C.

## 2 | Material and Methods

### 2.1 | Culturing and Experimental Conditions

#### 2.1.1 | Plant Collection and Preincubation

Free-floating (*Lemna gibba* L., subsequently termed as *Lemna*) and rootless submerged (*Ceratophyllum demersum* L., subsequently termed as *Ceratophyllum*) plants were collected from the Igrice canal near Nyíregyháza (NE Hungary; N 47.99646, E 21.73389). *Lemna* and *Ceratophyllum* plants were preincubated for 4 weeks under the experimental conditions on a general-purpose BS medium (Smart and Barko 1985). This BS medium was supplemented by adding $KNO_3$ to a final concentration of $4\,mg\,N\,L^{-1}$. Phosphorus was added as $K_2HPO_4$ to a final concentration of $0.8\,mg\;P\;L^{-1}$ and a supply of micronutrients was ensured by adding $0.1\,mL\,L^{-1}$ TROPICA supplier micronutrient solution (final concentrations: $0.08\,mg\;Fe\;L^{-1}$, $0.03\,mg\;Mn\;L^{-1}$, $0.002\,mg\;Zn\;L^{-1}$, $0.006\,mg\;Cu\;L^{-1}$and $0.002\,mg\;Mo\;L^{-1}$). The initial pH of the medium was adjusted to 7.0 by adding 0.2 M KOH or 0.2 M HCl. The plant cultures were kept at a water temperature of 25°C, a photon flux density of $280\,\mu mol\,m^{-2}\,s^{-1}$ and a 16 h light/8 h dark cycle both under preincubation and laboratory experiment.

#### 2.1.2 | Laboratory Experiment

A laboratory experiment was performed to determine the potential threshold pH value above which the growth rate of *Lemna* was significantly reduced. For preparing the culture media, three 20 L grey plastic containers (surface area $0.125\,m^2$) filled with culture medium were used. Portions of 200 ± 2 g (fresh weight) biomass of *Ceratophyllum* plants were placed into each container and co-cultured with 2 g biomass (fresh weight) of *Lemna*. The medium was analysed for pH daily after 8 h of irradiation using a pH meter (Orion Star 2000, Thermo Scientific). After 4 days, when the maximum daily pH exceeded 10.3, the culture medium was gently decanted from the containers (in total 54 L), and stored at 5°C in dark. After 24 h of sedimentation, this medium was decanted again, filtered (10 µm pore size) and analysed for $PO_4^{3-}$, $NO_3^-$, Fe and Mn concentrations (Szabó et al. 2022b). This filtrate was used for the subsequent pH treatment experiment.

Nutrient concentrations (N, P, Fe, Mn) of the *Ceratophyllum*-treated medium were adjusted to the non-treated "BS" medium. Nutrients were supplemented as $KNO_3$, $K_2HPO_4$, $FeSO_4$ and $MnCl_2$, respectively. For measuring the effects of pH, 2 L of this nutrient-supplemented medium was portioned into 2 L aquaria containing *Lemna* enclosures. Aquaria were covered by a black plastic foil on the sides to avoid algal competition (Szabó, Roijackers, and Scheffer 2003). To measure the potential effect of pH on the growth of *Lemna*, the pH was adjusted daily to 7, 8, 8.5, 9, 9.5, 10, 10.5 and 11 by 0.2 M KOH or 0.2 M HCl. *Ceratophyllum*-treated *Lemna* fronds (200 mg fresh weight) were placed in the *Lemna* enclosures (5 cm in diameter) in each aquarium (3 replicates per treatment). The surface of the aquaria outside the enclosures was covered by grey plastic sheets to avoid algal growth. In order to avoid overcrowding, as soon as *Lemna* fronds reached a 100% cover, we replaced the enclosure with a bigger one in diameter (Szabó, Roijackers, and Scheffer 2003). *Lemna* cultures were incubated for 8 days and biomass (fresh weight after blotting) of the fronds was measured on the 4th, 6th and 8th days, respectively. Relative growth rates (RGR) were calculated for the period between the 0th and 8th days (Szabó et al. 2022a). After 8 days, the *Lemna* plants were harvested and weighed (FW). Fresh weight (50–100 mg) of the samples was used for determining the in vivo photosynthetic efficiency, and subsequently to measure the chlorophyll and carotenoid concentrations. The remaining biomass was used for determining the dry weight (80°C for 48 h) and C, N, P, Fe and Mn contents, respectively (see below).

The photochemical efficiency of photosystem II ($\Phi_{PSII}$) was measured by means of chlorophyll fluorescence induction method with a MINI-PAM fluorometer (Heinz Walz GmbH, Germany). The *Lemna* fronds were placed in a plastic holder facing perpendicularly towards the fiberoptics of the fluorometer, and irradiated with 120 $\mu mol\, m^{-2} s^{-1}$ actinic light. The actual photochemical efficiency of photosystem II was calculated as $\Delta F/F_m' = (F_m' - F_s)/F_m'$ with $F_m'$ the maximal fluorescence and $F_s$ is the steady-state fluorescence of the illuminated plants (Genty, Briantais, and Baker 1989; Szabó et al. 2020). The average of three chlorophyll fluorescence measurements represented the photochemical efficiency of the plants in each aquarium.

### 2.1.3 | Mesocosm Study

Vertical pH profiles and the diurnal variation of pH and temperature were measured in mesocosms densely covered with *Ceratophyllum*. A 7 cm thick layer of clayey lake sediment was spread on the bottom of four plastic containers (0.7 m in diameter, 0.38 $m^2$ total area) filled with 200 L of well water. After 2 days of settling, 350 g biomass (921 $g\, m^{-2}$) of *Ceratophyllum* shoots (20–30 cm length) were added to each of the four mesocosms. After 14 days of pre-incubation under outdoor conditions (24th June, 2023), the water was analysed for nitrate and phosphate, and then supplemented by adding $KNO_3$ to a final concentration of 2 $mg\, N\, L^{-1}$. Phosphorus was added as $K_2HPO_4$ to a final concentration of 0.4 $mg\ P\ L^{-1}$. After 2 days of incubation, the pH of the water in the mesocosms was monitored for 1 day by taking hourly samples from 2 cm depth between 4 AM and 10 PM. The 15 mL samples were taken in quadruplicate from each container using a 20 mL syringe and then pooled, giving a total of 60 mL per sampling and container. The next day at 2 PM, we also took 60 mL samples from the water at increasing depths (1, 5, 10, 20, 30, 40 and 50 cm from the surface) to measure the pH and temperature. The *Ceratophyllum* plants were then cultivated with weekly nutrient addition, and the sampling to monitor daily pH and temperature trends was repeated on 12th of September 2023.

### 2.1.4 | Field Survey

We sampled water (60 mL) from 5 cm below the surface for pH measurement in dense submerged macrophyte stands during the summers (July–August) of 2022 and 2023, between 10 AM and 4 PM (Table S1). Samples were measured for pH within 5 min after sampling and were collected from predominantly stagnant waters of drainage ditches and channels, oxbow lakes and ponds from 130 sites in the Northern and Southern Great Plain (Hungary), the Kis-Balaton wetland area (SW Hungary) and the Baranja region (NE Croatia), respectively. The submerged vegetation was dominated by either *C. demersum* ($N=74$), *Utricularia vulgaris* ($N=5$), *Elodea nuttallii* ($N=16$), *Potamogeton crispus* ($N=7$), *Myriophyllum verticillatum* ($N=3$) or *Myriophyllum spicatum* ($N=8$). We also collected pH data (from June to August) from lowland sites of Hungary and Croatia, where free-floating plants densely (> 82%) covered the water surface. These sites were dominated by either *Salvinia natans* ($N=17$), *Lemna minor* ($N=73$), *L. gibba* ($N=11$) or *Spirodela polyrhiza* ($N=17$), respectively. We also took diurnal samples (six times between 6 AM and 9 PM), and measured the daily pH-course in two dense submerged macrophyte stands at the shore of Lónyay channel (Northern Great Plain, NE Hungary, N 48.064021; E 21.75457) dominated by either *C. demersum* or *E. nuttallii* on 18th of August 2019.

## 2.2 | Analytical Methods

Chlorophyll (chl) of *Lemna* fronds was extracted in 6 mL 95% ethanol for 24 h at 4°C in dark. Concentrations of chl a, chl b and carotenoids were measured by spectrophotometry (T80+ Spectrometer, PG Instruments Limited, UK) and calculated according to Lichtenthaler (1987). Total carbon and nitrogen contents in the *Lemna* plants were determined by dry combustion using a Vario Max Cube elemental analyser (Elementar GmbH, Germany). Phosphorus, iron and manganese content of *Lemna* was measured by Agilent 4200 Microwave plasma atomic emission spectroscopy (MP-AES) after acidic digestion in a mixture of 5 mL of 67% (m/m) nitric acid and 5 mL 30% (m/m) hydrogen peroxide at 90°C for 1 h. For these elemental analyses, *Lemna* plant samples from the three replicates were pooled to obtain sufficient biomass.

## 2.3 | Data Analysis

The impact of pH on *Lemna* traits (biomass, RGR, chl concentration, dry matter content) was tested by one-way analysis of variance (ANOVA). Normal distribution of the variables was tested using Kolmogorov–Smirnov test. Homogeneity of variances of the dependent variables was tested using Levene's

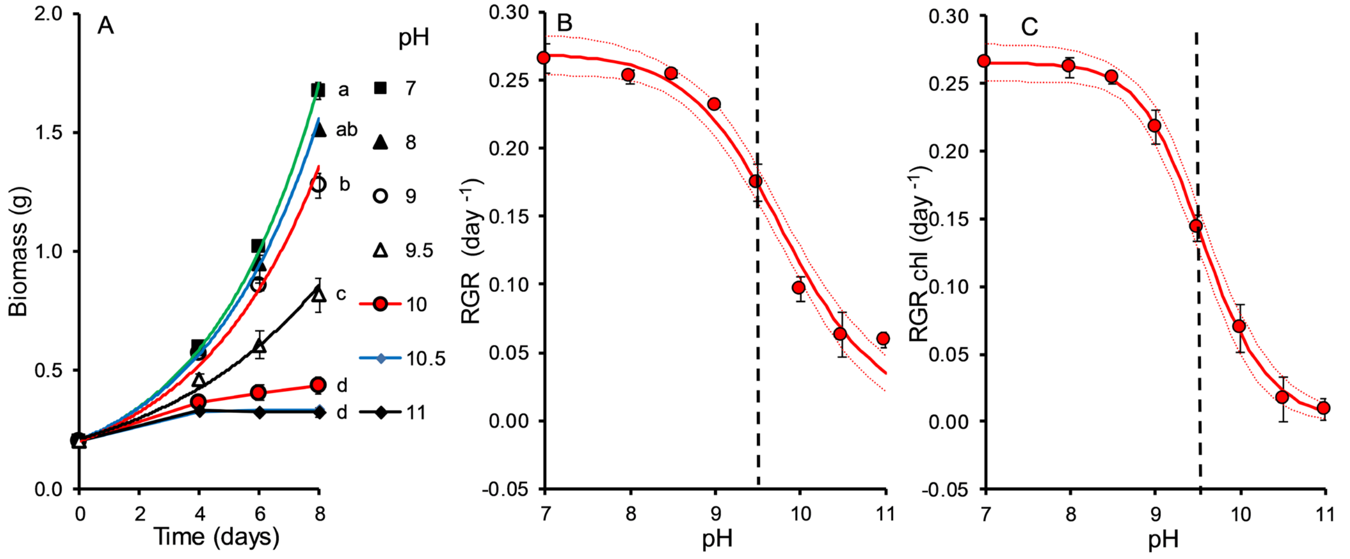

**FIGURE 1** | (A) Changes in the biomass of *Lemna gibba* cultivated at different pH (means ± SE, $n = 3$). Lines of pH 10.5 and pH 11 are overlapping. Significant differences among pH treatments on the 8th day are indicated with different lowercase letters (Tukey's test, $p < 0.05$). Impact of pH on relative growth rates of *Lemna* cultures based on (B) biomass (fresh weight) and (C) on chlorophyll content (means ± SE of $n = 3$ samples, dotted lines are 95% confidence intervals).

**TABLE 1** | The calculated effective pH with 95% confidence intervals in brackets (95% CI) that resulted in 10%, 20% and 50% inhibition of the fresh weight ($RGR_{FW}$) and chlorophyll-based relative growth rates ($RGR_{Chl}$) of *Lemna gibba* at the end of the 8 days-long treatments.

| Variable | 10% inhibition | 20% inhibition | 50% inhibition | RSE | Pseudo-$R^2$ |
|---|---|---|---|---|---|
| $RGR_{FW}$ | 8.63 (8.34–8.93) | 9.06 (8.83–9.29) | 9.83 (9.70–9.97) | 0.017 | 0.98 |
| $RGR_{Chl}$ | 8.77 (8.56–8.98) | 9.06 (8.89–9.22) | 9.57 (9.46–9.67) | 0.017 | 0.99 |

test. In the laboratory experiment, *Lemna* biomass at Day 8 ($FW_8$), chl concentration, chl/carotenoid ratio and photosynthetic efficiency were compared between treatments using Tukey post hoc tests. The effect of pH on the elemental composition of *Lemna* fronds was evaluated using linear regression analysis. A Kruskal–Wallis test was used to compare the pH values between June and September in the mesocosms covered with *Ceratophyllum*, and in the field survey to compare the pH values between submerged and free-floating vegetation stands. Linear regression analysis was used to test the correlation between water temperature and pH in submerged vegetation stands. All the above statistical analyses were carried out using SPSS 16.0.

The pH-dependent responses of *Lemna* in terms of FW- and chl content-based RGR, and chlorophyll concentrations in the biomass were described by three-parameter log-logistic functions using results from the laboratory experiment. The functions were fitted using the "drc"-package (version 3.0–1, Ritz et al. 2015) in RStudio (version 2022.12.0.353, RStudio Team 2020). After visual inspection of the model fittings, pseudo-$R^2$ and effective pH resulting in 10%, 20% and 50% inhibition in the respective parameter were calculated based on the fitted regression models using the "cor" and "ED" functions of the "drc"-package.

To analyse the dry matter content and tissue concentrations of N, P, and Mn in *Lemna* as a function of pH, we fitted linear regression models using the LAB Fit (v. 7.2.51) curve-fitting software (Silva and Silva 2018). To describe the diurnal variation of pH in the mesocosm study, a spline function was also fitted by means of by Past 4.04 software (Hammer and Harper 2006).

## 3 | Results

### 3.1 | Laboratory Experiment

*Lemna gibba* plants grown at pH 7.0–9.5 showed exponential growth during the incubation period (Figure 1A). There was no significant difference in the growth rates of cultures grown at pH 7–8.5 (Figure 1, Table 1.). With increasing pH, plant growth rate based on fresh weight (Figure 1B) and on tissue chlorophyll concentration (Figure 1C) showed a strong decrease above pH 9. The calculated 50% growth inhibition for RGR based on fresh weight and chlorophyll was at pH 9.8 and pH 9.6, respectively (Figure 1B,C, Table 1). Above pH 10.0, the growth of *Lemna* almost stopped and at pH 11 the RGR became negative (Figure 1, Figure S1).

With increasing pH, the concentration and ratio of photosynthetic pigments (chlorophyll and carotenoids) of the *Lemna* plants changed significantly (Figure 2A,B). The concentration of total chlorophyll decreased linearly above pH 8.5 (Figure 2A) and the ratio of chl to carotenoids showed a sudden decrease

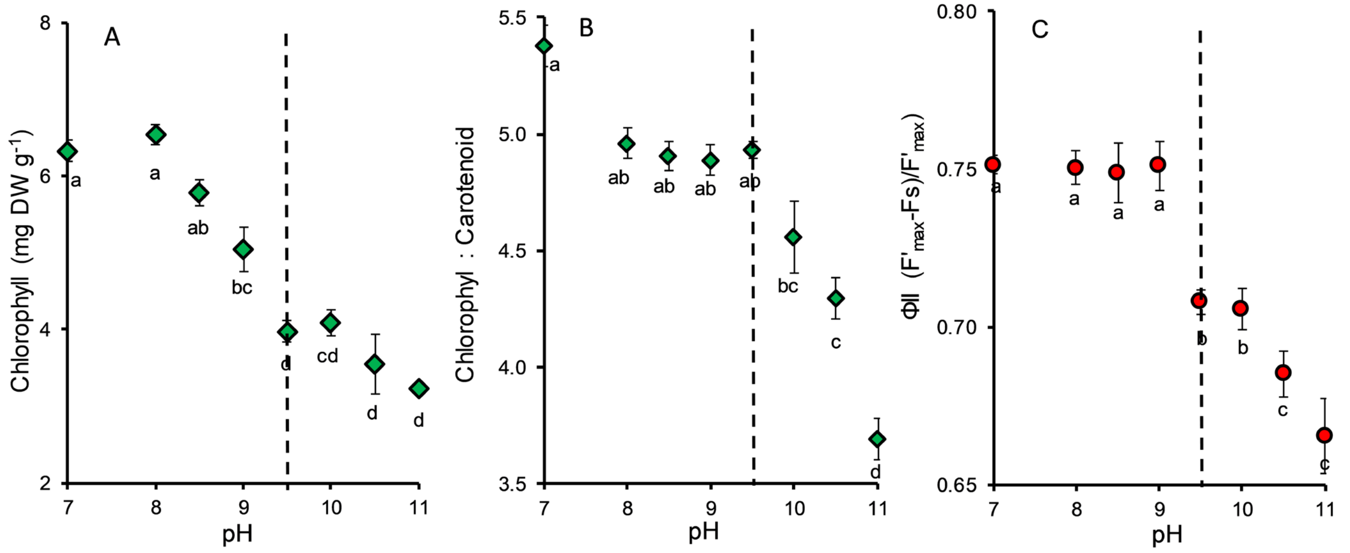

**FIGURE 2** | Effect of pH on (A) chlorophyll (chl) concentrations, (B) chlorophyll: carotenoid ratio and (C) photosynthetic efficiency ($\Phi_{PSII}$) of *Lemna gibba* cultures (Means ± SE, $n = 3$). Significant differences (Tukey's test, $p < 0.05$) of the variables among pH treatments are indicated with different lowercase letters. Dashed lines indicate thresholds for 50% reductions in growth rate of *L. gibba* (see Table 1).

above 9.5 (Figure 2B). The photochemical efficiency ($\Phi_{PSII}$) of plants also showed a steady decrease above pH 9 (Figure 2C).

Nitrogen (Figure 3A), phosphorus (Figure 3B) and manganese (Figure 3C) concentrations in *Lemna* fronds decreased linearly (ANOVA $F_{1,6} = 41.48$, $p = 0.001$; $F_{1,6} = 25.45$, $p = 0.002$ and $F_{1,6} = 35.66$, $p = 0.001$, respectively), while iron concentrations showed no significant trend ($F_{1,6} = 2.97$, $p = 0.135$; ANOVA) with increasing pH (data not shown). On the other hand, high pH (> 9.5) increased the dry matter content (DMC) of *Lemna* by 25%–31% (Figure 3D).

### 3.2 | Mesocosms Study

In June of 2023, pH in the upper layer of the mesocosm water with a dense *Ceratophyllum* stand rose steeply from early morning, then remained fairly constant between 10 AM and 5 PM, after which it fell steeply (Figure 4A). Accordingly, the pH near the water surface was above the threshold for *Lemna* growth inhibition (pH 9.5, see Table 1) for 11 h (between 8:30 AM and 7:30 PM), and exceeded pH 10 for 7 h (between 10:00 AM and 5:00 PM). In September, the pH within the *Ceratophyllum*-covered mesocosm water was significantly lower than in June ($\chi^2 = 40.02$, $p < 0.001$, Kruskal–Wallis test). However, the threshold for significant growth reduction in *Lemna* was still exceeded for about 5 h (between 11:00 AM and 4:00 PM). In June, the highest pH (10.35 ± 0.02 SE) was measured in the top 5 cm of the mesocosm water column, and the threshold value was exceeded in the top 14 cm (Figure 4B).

### 3.3 | Field Survey

Among the 130 sampling sites of dense submerged vegetation stands, the highest pH values were measured in the Northern Great Plain, followed by the Kis-Balaton wetland area and Baranja (Croatia) together with the Southern Great Plain region (Figure S2). The lowest pH was found in submerged vegetation stands located in flowing channels (Figure S2). The highest pH was found in submerged vegetation stands dominated by *Potamogeton crispus*, followed by *Elodea nuttallii* and *Ceratophylum demersum* (Figure 5). The pH was significantly higher ($\chi^2 = 148.2$, $p < 0.001$, Kruskal–Wallis test) under dense submerged vegetation stands compared to dense free-floating vegetation (Figure 5).

Within the Lónyay channel (Northern Great Plain, Hungary), surface water pH varied from 7:30 to 11:00 h in submerged macrophyte stands dominated by *Ceratophyllum*, while maximum pH values were lower within *Elodea* stands (Figure 6A). The threshold pH level for significant growth reduction of *Lemna* (see Table 1) was exceeded for 11 and 9 h in *Ceratophyllum* and *Elodea* stands, respectively (Figure 6A). For samples collected in the Kis-Balaton area, the water pH showed a strong positive correlation (ANOVA, $F_{1,26} = 31.819$, $p < 0.001$) with water temperature (Figure 6B).

## 4 | Discussion

Our laboratory results clearly showed that water pH above 9.5 strongly limits the growth of *Lemna gibba* as a common free-floating plant species in stagnant, highly eutrophic temperate water bodies, supporting our first hypothesis. Our mesocosm and field data also supported Hypothesis 2, showing that this pH threshold had often been exceeded within dense submerged macrophyte stands of stagnant water bodies during most of the summer daylight hours and in the upper water layer. We thus conclude that high pH may be an important mechanism for submerged macrophytes to maintain dominance over competing free-floating plants in stagnant eutrophic temperate water bodies.

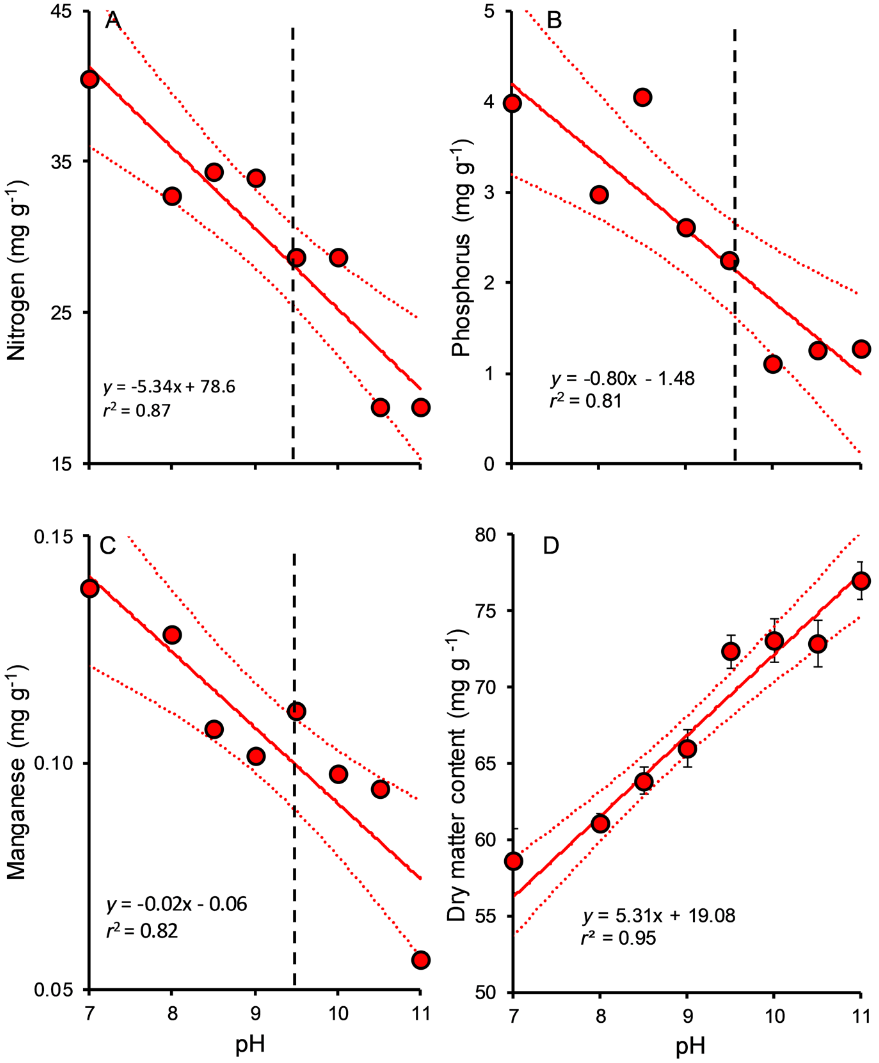

**FIGURE 3** | Effect of pH on the tissue concentrations of nitrogen (A), phosphorus (B), manganese (C) and dry matter content (D) in *Lemna gibba* fronds. Means ± SE of $n = 3$ samples, dotted lines are 95% confidence intervals. Dashed lines indicate thresholds for significant reductions in growth rate of *L. gibba* (see Table 1).

Positive feedback loops between high pH and submerged plants or low pH and free-floating plants, respectively, can result in alternative stable equilibria along a pH gradient (Figure 7). Although this link has not been directly proven in this study, we can hypothesize that it as a reasonable possibility based on our results.

## 4.1 | Threshold pH for Growth of Duckweeds

The optimum pH range for the growth of many duckweed species is 5–9 (Landolt and Kandeler 1987; Table 2), which is well below the threshold measured in our experiments with *L. gibba*. Phosphorus concentrations decreased while dry matter content increased sharply in the *L. gibba* fronds above a pH of 9.5, supporting a critical pH threshold for *L. gibba* around 9.5.

Growth rates of *Lemna* showed threshold-like rather than gradual response to increasing pH. Tissue concentrations of phosphorus were significantly lower above this threshold, suggesting that phosphate uptake by *Lemna* may be limited above pH 9.5. In fact, Ullrich-Eberius et al. (1981) had also demonstrated an inhibited uptake of phosphate by *L. gibba* above pH 9.5. In contrast, nitrogen concentrations in *Lemna* tissues decreased more gradually with increasing pH, but were not significantly lower even above the pH threshold levels for growth inhibition. Blocked nitrate uptake above pH 9.5, as suggested by Loeppert, Kronberger, and Kandeler (1977), may therefore contribute to the reduced growth of *Lemna*, but phosphorus appears to be of greater importance. In addition, precipitation of manganese and phosphate at high pH combined with high oxygen concentrations (Otsuki and Wetzel 1972; Stumm and Morgan 1996; Szabó et al. 2022a) may contribute to reduced *Lemna* growth rates at higher pH, whereas iron precipitation had no significant effect in the pH range tested. Due to the combined effects of pH on both phosphate and nitrate uptake and phosphate and manganese precipitation, it is not possible to fully disentangle the effect of high pH from nutrient availability.

Although our laboratory experiment was based on the interaction between two specific species, *Ceratophyllum* as a

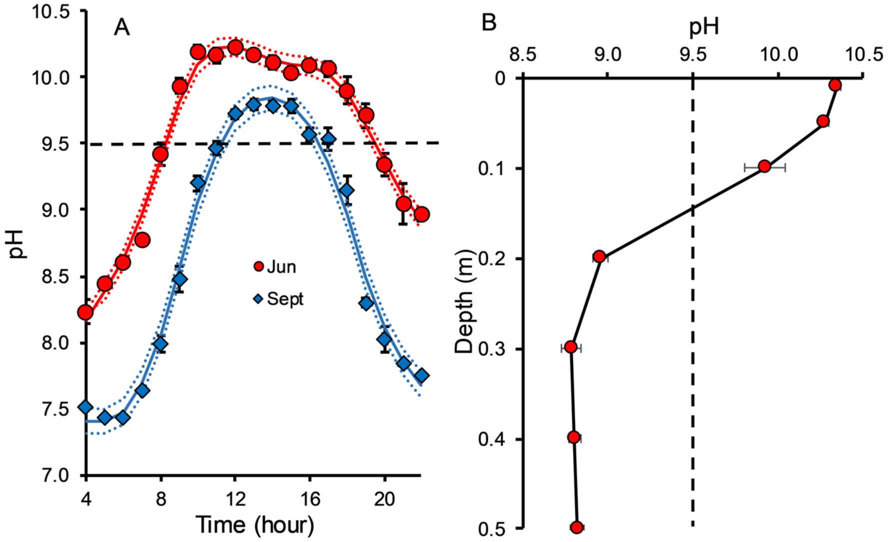

**FIGURE 4** | (A) Daily course of pH at 5 cm below the water surface in a mesocosm covered with *Ceratophyllum demersum*, the dotted lines are 95% confidence intervals. Spline function was fitted to the data lines calculated by Past 4.04 software (Hammer and Harper 2006). (B) Change of pH along increasing water depth below dense *C. demersum* cover in June 2023. Means ± SE, $n = 4$. Dashed lines indicate the pH thresholds for significant reduction in growth rate of *Lemna gibba* (see Table 1).

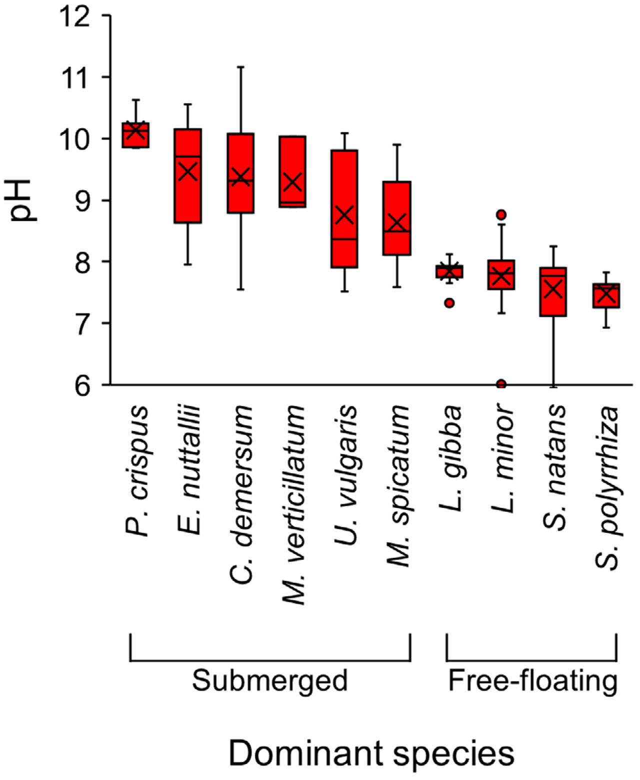

**FIGURE 5** | Upper water layer (5 cm) pH measured within dense submerged and free-floating macrophyte stands at four different field sampling sites (Northern Great Plain, Kis-Balaton wetland area, Southern Great Plain, Baranja) during the summers (July–August) of 2022 and 2023, between 10 AM and 4 PM.

submerged and *Lemna* as a free-floating macrophyte, previous laboratory and field studies (Frodge, Thomas, and Pauley 1990; Frost-Christensen and Sand-Jensen 1995; James, Eaton, and Hardwick 1999; Szabó et al. 2010) and the field survey of the present study showed that not only *Ceratophyllum*, but also dense stands of several other submerged macrophytes (*Elodea, Utricularia, Potamogeton, Myriophyllum, Lagarosiphon*) were able to increase the pH in the water, potentially inhibiting *Lemna* growth. Therefore, we believe that our results can be generalised to other submerged macrophytes. On the other hand, literature data show that other common free-floating plants (including *Lemna, Spirodela, Azolla, Eichhornia, Pistia* and *Salvinia* sp.) in temperate and tropical waters also show growth inhibition above pH 8. We, therefore, expect critical pH thresholds to be a potentially general pattern in shaping competition between different groups of aquatic plants.

Although several studies have previously shown that elevated pH can facilitate the release of phosphorus from the sediment via ligand change reaction, thus providing nutrients to primary producers (e.g. Holmroos et al. 2009; Koski-Vähälä, Hartikainen, and Tallberg 2001), the plant-related daily increase in pH was essentially limited to the upper 0.2 m water layer of macrophyte stands. Consequently, it could not play a significant role in reducing competition for nutrients between submerged and free-floating macrophytes.

### 4.2 | Dense Stands of Submerged Macrophytes Can Increase pH Above Threshold Levels

Our results from both mesocosm and field measurements in dense submerged plant stands confirmed that elevated pH above the growth inhibition threshold is a relevant factor in outcompeting the free-floating duckweed. Higher pH values than 9.5 had often been found in the upper layers of dense submerged vegetation stands (O'Sullivan and Reynolds 2004; Lindholm, Rönnholm, and Häggqvist 2008; Stiers, Njambuya, and Triest 2011; Pedersen, Colmer, and Sand-Jensen 2013). However, in our study, such high pH values were restricted to the upper 10 cm of the water column. Consequently, stable high pH above

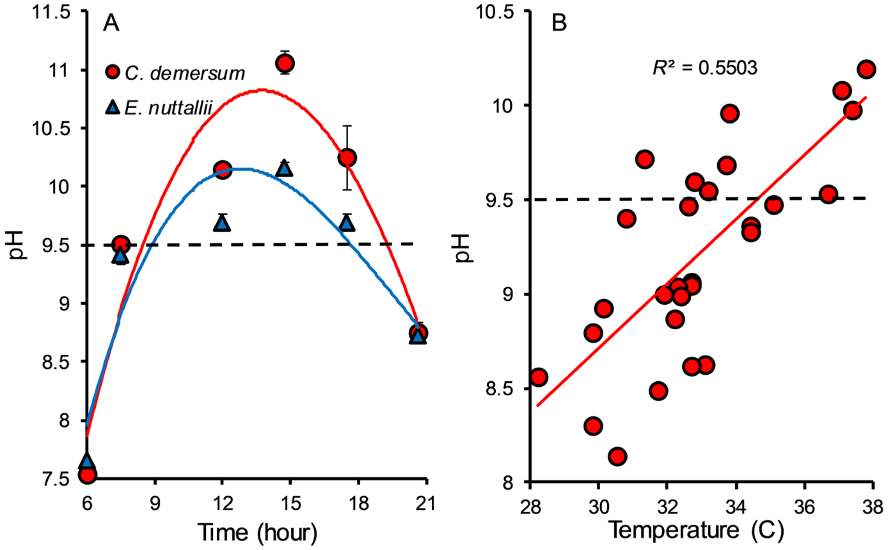

**FIGURE 6** | (A) Daily variation of pH 5 cm below the water surface in submerged macrophyte stands covered by *Ceratophyllum demersum* and *Elodea nuttallii* in NE Hungary, 19 August 2019. Means ± SE, $n = 3$. (B) Correlation between water temperature and pH at sampling sites of the Kis-Balaton wetland area ($n = 28$). Dashed lines indicate threshold levels for significant reduction of growth rates of *Lemna gibba* (see Table 1).

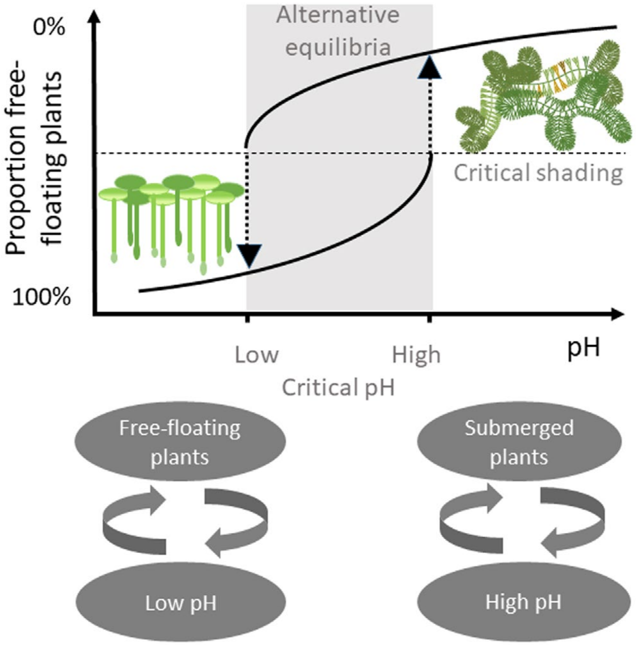

**FIGURE 7** | Proposed alternative equilibria between free-floating and submerged macrophytes along a pH gradient due to positive feedbacks between high and low pH and the respective vegetation.

dense submerged vegetation is likely to be found only in small, shallow, lentic waters sheltered from wind. Dense submerged stands are known to reduce water movement (Scheffer 1998), thus further stabilising high daytime pH. In a study of 29 Patagonian ponds, water bodies with very alkaline water ($pH > 9$) were indeed dominated by submerged macrophytes (Manzo et al. 2020). It should also be noted that the measurements in the *Ceratophyllum*-covered mesocosms and the sampling for the field studies were mainly carried out under sunny conditions, representing the typical midsummer weather conditions in the lowland parts of the Carpathian Basin. On a cloudy summer day, however, water pH can be as much as 0.5–1 units lower. Therefore, it appears that not only light intensity but also the sum of sunshine hours and day length affect alkalinity in dense submerged vegetation.

Epiphytic algae can also contribute to high pH values (Szabó et al. 2005; Koleszár et al. 2022). Consequently, herbivores grazing on epiphyton may reduce the pH-increasing effects (Pinowska 2002; Koleszár et al. 2022). In turn, their predators such as molluscivorous tench (*Tinca tinca* L.) indirectly increase epiphytic algal biomass resulting in higher pH levels (Brönmark 1994). Thus, food web interactions can significantly alter the effects of submerged macrophytes on pH and thus strengthen or weaken the dominance of submerged vegetation over free-floating plants in temperate eutrophic freshwater bodies. High pH values can also alter the species composition of both phytoplankton and zooplankton (Beklioglu and Moss 1995). However, it may have little effect on the competitive outcome between submerged and free-floating plants.

## 5 | Conclusion

Our data support the existence of a critical pH threshold above which submerged plants may maintain a stable dominance over free floating competitors, while at medium pH, both equilibria seem possible, resulting in a hysteresis (Figure 7). This mechanism would add to previous theories on the role of nutrient loads for alternative equilibria (Scheffer et al. 2003; Scheffer and van Nes 2007). According to our findings, shifts between submerged and free-floating macrophytes as alternative equilibria in stagnant waters may also be triggered by gradual changes in the pH of the water (Figure 7). The latter can be affected not only by the photosynthetic activity of submerged plants, their epiphyton or phytoplankton, but also by changes in the quality and quantity of the run-off from the catchment or groundwater inflow. Actively managing the pH, thus, may help maintaining the desired equilibrium.

**TABLE 2** | Literature review on the effects of pH on the growth of different free-floating macrophyte species.

| Free-floating species | pH range with reported growth | pH range for optimum growth | Low pH with reported growth inhibition | High pH with reported growth inhibition | Reference |
|---|---|---|---|---|---|
| *Lemna gibba* | | | 7.0–8.5 | < 9.6 | This study |
| | | 5.5–8 | < 4.5 | > 8 | Guy, Granoth, and Gale (1990) |
| *Lemna minima* | | 5–8 | | | Boss, Dijkman, and Russell (1963) |
| *Lemna minor* | 3–10.5 | 5–8.2 | < 2 | | McLay (1976) |
| | 5–8 | | < 5 | > 8 | Hicks (1932) |
| | | 5–8 | | | Boss, Dijkman, and Russell (1963) |
| | | | | > 8 | Keddy (1976) |
| | | | < 4.5 | > 8 | Hicks (1932) |
| *Lemna perpusilla* | | 7.69 | 8.23 | | McLay (1974) |
| | | 5–8 | | | Boss, Dijkman, and Russell (1963) |
| *Spirodela oligorrhiza* | 3–10.5 | 5–8.2 | < 3 | | McLay (1976) |
| | | 5–8 | | | Boss, Dijkman, and Russell (1963) |
| *Spirodela polyrhiza* | 5–8 | | < 5 | > 8 | Hicks (1932) |
| | | 5–8 | | | Boss, Dijkman, and Russell (1963) |
| *Azolla filiculoides* | | 5–7 | < 4 | > 8 | Cary and Weerts (1992) |
| *Eichhornia crassipes* | 4.0–8.0 | 5.8–6.0 | < 4 | > 8 | El-Gendy, Biswas, and Bewtra (2004) |
| | | 6.9–7.0 | < 3 | > 8.2 | Chadwick and Obeid (1966) |
| | | | | 8–11 | Hadad et al. (2018) |
| *Pistia stratiotes* | 3.0 | 4.0 | | | Chadwick and Obeid (1966) |
| | | | | 8–11 | Hadad et al. (2018) |
| *Salvinia molesta* | 5.0–8.0<br>5.6–9.5 | | | | Madsen and Wersal (2008) |
| | | < 7.5 | | 8.5–10 | Owens et al. (2005) |
| *Salvinia herzogii* | | | | 8–11 | Hadad et al. (2018) |

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Conceptualisation: Sándor Szabó, Sabine Hilt and Viktor Oláh. Developing methods: Sándor Szabó and Mihály Braun. Conducting the research: Sándor Szabó, Noémi Fedor, Gergő Koleszár, Mihály Braun, Aleksandra Kocic and János Korponai. Data analyses: Sándor Szabó, Mihály Braun, Sabine Hilt and Viktor Oláh. Preparation of figures and tables: Sándor Szabó, Mihály Braun and Viktor Oláh. Data interpretation: Sándor Szabó, Sabine Hilt and Viktor Oláh. Writing: Sándor Szabó, Noémi Fedor, Gergő Koleszár, Mihály Braun, János Korponai, Aleksandra Kocic, Sabine Hilt and Viktor Oláh.

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### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## Supporting Information

Additional supporting information can be found online in the Supporting Information section.