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Responses of Different Submerged Macrophytes to the Application of Lanthanum-Modified Bentonite (LMB): A Mesocosm Study

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Abstract: Lanthanum-modified bentonite (LMB) has remarkable efficacy on eutrophication control, but the reduced bioavailable phosphorus and formed anaerobic horizon from LMB may be harmful to submerged macrophytes. We conducted this study to explore the influence of LMB on *Hydrilla verticillata* and *Vallisneria natans* in mixed-species plantings. The concentrations of TP, TDP, SRP, and TDN in the LMB treatments were lower than the Control, but the Chl *a* concentration in the HLMB treatment (850 g m^{-2}) was higher than the Control by 63%. There were no differences of *V. natans* growth among the treatments. For *H. verticillata*, its biomass, RGR, height, branch number, root number, and length in the LLMB treatment (425 g m^{-2}) were lower than the Control by 48%, 22%, 13%, 34%, 33%, and 8%, respectively. In addition, the biomass of *H. verticillata* was 62%, the RGR was 32%, the height was 19%, the branch number was 52%, the root length was 40%, and the root number was 54% lower in the HLMB treatment than those in the Control. In summary, LMB had negative effects on submerged macrophytes with underdeveloped roots. Submerged macrophytes with more developed roots are preferred when using combined biological–chemical methods for water restoration.

Keywords: subtropical lakes; restoration; Phoslock®; *Hydrilla verticillata*; *Vallisneria natans*; interspecific competition

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1. Introduction

Eutrophication in freshwater lakes, caused by the excessive input of nutrients (nitrogen and phosphorus), has become a worldwide problem in recent decades [1,2]. It is generally accepted that phosphorus input control is the key to effectively mitigate eutrophication [3,4]. However, abundant research has shown that a lake recovery is often delayed after the successful reduction of the external P input [5–7] due to the internal release of P, promoting algae reproduction [8,9]. Therefore, increasing attention has been paid to internal loading management and many techniques have been developed [10–13]. For instance, lanthanum-modified bentonite (LMB), a type of phosphorus capping agent, has been widely used for internal loading management in the past decade. LMB has a wide range of applications [14–17] and, significantly, inhibits the release of bioavailable P from sediment by transforming mobile P into immobile P through forming an insoluble P form ($LaPO_4$), thereby effectively controlling internal loading and inhibiting algae growth [18–22].

Submerged macrophytes are important producers in freshwater ecosystems and play an essential role in structuring shallow lake communities [23,24]. The re-establishment of submerged macrophytes is beneficial to the restoration and maintenance of a clear lake water state under the condition of submerged macrophyte degradation and gradual eutrophication [25–28]. In order to achieve better efficacy, submerged macrophyte transplantation is often applied together with other methods for eutrophication control, e.g., dredging, fish removal, and geoengineering materials [22,29,30]. However, studies have found that LMB has marginal effects on submerged macrophytes [31]. Submerged macrophytes mainly absorb nutrients from sediment through their roots to maintain their growth [23]; a reduction in bioavailable P and the possible formation of an anaerobic layer on the sediment surface by the application of LMB has, therefore, been suggested to negatively affect the growth and function of the roots, thereby inhibiting the growth and reproduction of submerged macrophytes [22,31,32]. This is not of benefit to the rapid establishment of submerged macrophytes in the early stage of restoration [22,33] and also indicates that submerged macrophytes with different morphological and structural roots may have different responses to the application of LMB [32,34].

From their physiological structure, submerged macrophytes can be divided into C_3 species, such as *Vallisneria natans*, and C_4 species, such as *Hydrilla verticillata* [35]. Both *H. verticillata* and *V. natans* are common species in freshwater ecosystems in China [36,37] and are also frequently used in water restoration [13,24,28]. They have similar habitat requirements and are often simultaneously observed in freshwater habitats [38]. However, there are significant morphological differences between them. *H. verticillata* usually forms a canopy over the water whereas *V. natans* produces a basal rosette of leaves, relying more on the light near the sediment to grow [39]. In terms of the root system, that of *H. verticillata* is relatively undeveloped compared with *V. natans* [39–41]. However, due to its typically high growth rate, *H. verticillata* is more competitive than *V. natans* when growing in the same area [39,41]. Thus, considering the underdeveloped root system of *H. verticillata*, the negative effects of LMB on *H. verticillata* may be higher than on *V. natans*, potentially affecting the competition between them: this observation remains to be verified.

In the present study, three doses of LMB (absent, low, and high) were administered to study the influence of the application of LMB on *V. natans* and *H. verticillata* in mixed-species plantings and also to choose a more suitable submerged macrophyte species to be used with LMB in water restoration. We hypothesized that different types of submerged macrophytes have different responses to the application of LMB and that *V. natans* is more adaptable due to its high asexuality and developed root system.

2. Materials and Methods

2.1. Experimental Design

A single factorial experiment was conducted near Taihu Lake (31°30′ N, 120°30′ E) from 1 June to 5 July 2019 with three doses of LMB: (1) the absence of LMB (Control); (2) low-dose LMB (LLMB, 425 g m^{-2}); and (3) high-dose LMB (HLMB, 850 g m^{-2}). Each treatment consisted of four replicates. All mesocosm systems were planted with *H. verticillata* and *V. natans*. Young submerged macrophytes were collected from Taihu Lake and cultivated for three weeks before the experiment. LMB was purchased from Phoslock Water Treatment Technology Co., Ltd. (Shanghai, China). The low (425 g m^{-2}) and high doses (850 g m^{-2}) were based on a mobile P pool in a 5 cm thick layer of surface sediment (mobile P: 0.26 ± 0.02 mg g^{-1} DW) with ratios of 50:1 and 100:1 (LMB: mobile P), respectively.

Twelve barrels (bottom diameter: 0.77 m; upper diameter: 0.97 m; height: 0.95 m; and volume: 0.5 m^{-3}) were used in the present study. Prior to the study, sediment (10 cm) and lake water (400 L) were filled into each barrel, which had been gathered from Taihu Lake and filtered by 0.50 cm and 380 μm aperture sieves, respectively, and then separately, but evenly, mixed. Fifteen *V. natans* (height: 20.87 ± 1.09 cm; number of leaves: 9.8 ± 1.64; total wet weight: 49.75 ± 2.03 g) and fifteen *H. verticillata* (height: 20.16 ± 1.65 cm; total wet weight: 16.47 ± 0.89 g) were then uniformly transplanted into each barrel. Subsequently, the exact amount of LMB was evenly added to the LLMB (200 g) and HLMB (400 g) treatments in the form of slurry after seven days. During the experiment, the experimental water was not changed and the temperature of it was maintained between 21.6 and 25.2 °C with a 14 h/10 h light/dark photoperiod. In addition, there was no strong disturbance caused by other factors.

2.2. Sampling

The experiment lasted for five weeks and the water physicochemical parameters were measured once a week. Conductivity and pH were monitored by a YSI 9500 photometer (YSI Inco, Yellow Springs, OH, USA), and turbidity was measured by a 1900c spectrophotometer (Hach Company, Loveland, CO, USA). The water chlorophyll a (Chl *a*) was measured by a BBE FluoroProbe (BBE Moldaenke GmbH, Schwentinental, Germany). A mixed water sample was then gathered from each barrel to analyze the nutrients. Total phosphorus (TP), total dissolved phosphorus (TDP), soluble reactive phosphorus (SRP), total nitrogen (TN), and total dissolved nitrogen (TDN) were measured according to [42].

Zooplankton were gathered by filtering (64 μm aperture sieve) and concentrating 10 L of mixed water into a 50 mL vial at the end of the experiment, which was then preserved with 4% formaldehyde. The identification and count of the zooplankton species were as referred to in [43–46]. Copepods and cladocerans were counted at a ×40 magnification; rotifers were counted at a ×100 magnification.

All submerged macrophytes were then carefully collected from the barrels and thoroughly washed. The *H. verticillata* and *V. natans* were dried with absorbent paper to calculate the ramet number of *V. natans* as well as the total biomass of *H. verticillata* and *V. natans* per barrel. The relative growth rate (RGR, mg g^{-1} d^{-1}) of *H. verticillata* and *V. natans* per barrel was calculated; the calculation formula was as follows:

$$RGR = \ln (W_f/W_i)/\text{days} \quad (1)$$

W_i (g) and W_f (g) were the initial and final total biomass of *V. natans* and *H. verticillata* per barrel, respectively. In addition, in order to show the competition between *H. verticillata* and *V. natans*, a relative competitiveness indicator (RCI) was adopted. The calculation formula was as follows:

$$RCI = R/RCH \quad (2)$$

R represented the RGR of *V. natans* and *H. verticillata* per barrel and RCH represented the RGR of *H. verticillata* in the Control treatment. This denoted that the RCI of *H. verticillata* in the Control treatment was 1. Finally, we measured the height, leaf number (branch number), root length, stolon length, malondialdehyde (MDA), and leaf chlorophyll *a* and *b* of the submerged macrophytes by randomly selecting five *H. verticillata* and five first ramets of the initial *V. natans* from each barrel [47,48].

2.3. Statistical Analysis

The time series data of the water physicochemical parameters (conductivity, DO, pH, turbidity, TN, TP, TDN, TDP, SRP, and Chl *a*) were analyzed by repeated measures analysis of variance (rANOVA) and a simple effect analysis. The biological indicators (e.g., the biomass of the zooplankton and the physiological and morphological indicators of *H. verticillata* and *V. natans*) were tested by a one-way analysis of variance (one-way ANOVA). A result of $p < 0.05$ was significant in the ANOVA. All statistical analyses were conducted using SPSS 24.0 software (IBM Corp., Armonk, NY, USA); the drawing was conducted using Origin 9.1 software (OriginLab Corp., Northampton, MA, USA).

3. Results

3.1. Physicochemical Parameters in the Overlying Water

LMB had no significant influence on pH and DO (Figure 1a,b; Table 1), but significantly improved water conductivity, which increased with the increase in the LMB dose (Figure 1c; Table 1). Turbidity in the LMB treatments was lower than that in the Control treatment at the early stage of the experiment, but there were no significant differences between the HLMB treatment and the Control treatment at the end of the experiment (Figure 1d; Table 1).

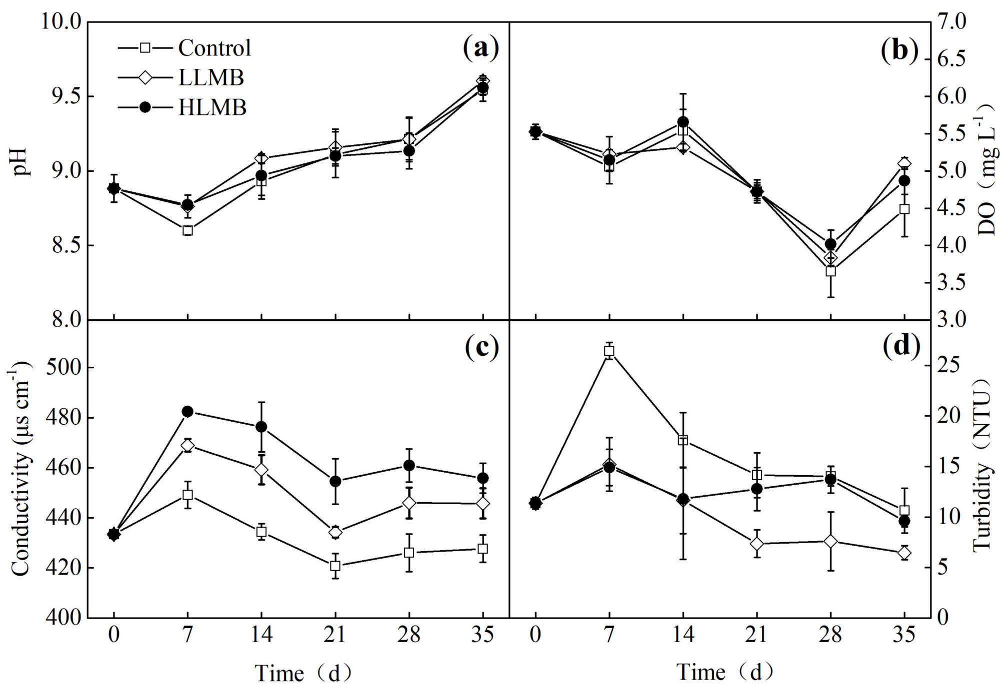

Figure 1. Time series of pH (**a**), dissolved oxygen (DO) (**b**), conductivity (**c**), and turbidity (**d**) in different treatments. Factor was LMB (0, 425 g m^{-2}, and 850 g m^{-2}). The values represented a standard deviation (n = 4).

Table 1. rANOVA results of the physicochemical parameters in the overlying water during the experiment.

	df	pH		DO		Conductivity		Turbidity		Chl *a*	
		F	Pr > *F*	*F*	Pr > *F*	*F*	Pr > *F*	*F*	Pr > *F*	*F*	Pr > *F*
L	2	3.32	n.s.	2.70	n.s.	39.10	**	53.37	**	21.65	**
T	5	53.86	**	150.11	**	94.65	**	18.39	**	133.65	**
L × T	10	0.89	n.s.	3.34	n.s.	9.03	*	6.03	*	1.66	n.s.
		TN	TP	TDN	TDP	SRP	TN	TP	TDN	TDP	SRP
L	2	4.23	n.s.	13.52	*	21.79	*	17.33	*	99.30	**
T	5	59.14	**	23.18	**	10.86	*	57.36	**	18.90	*
L × T	10	1.63	n.s.	2.24	n.s.	5.81	*	3.62	n.s.	13.42	*

** $p < 0.01$; * $p < 0.05$; n.s.: not significant. Factor was LMB (0, 425 g m^{-2}, and 850 g m^{-2}). L: LMB; T: time; DO: dissolved oxygen; TN: total nitrogen; TP: total phosphorus; SRP: soluble reactive phosphorus.

LMB had no significant influence on TN, but this decreased significantly with time (Figure 2a; Table 1). The TP, TDP, TDN, and SRP in the LLMB and HLMB treatments were significantly lower than the Control treatment, but there were no significant differences in those between the LLMB and HLMB treatments (Figure 2b–e; Table 1). At the end of the experiment, the concentrations of TP, TDP, TDN, and SRP in the LLMB treatment were 42%, 18%, 39%, and 78% lower than the Control treatment, respectively. In the HLMB treatment, TP was 45%, TDP was 19%, TDN was 46%, and SRP was 84% lower than the Control treatment. We also found that the concentrations of Chl *a* in the overlying water decreased with time and were higher in the HLMB treatment than the Control and LLMB treatments at the end of the experiment (Figure 2f; Table 1).

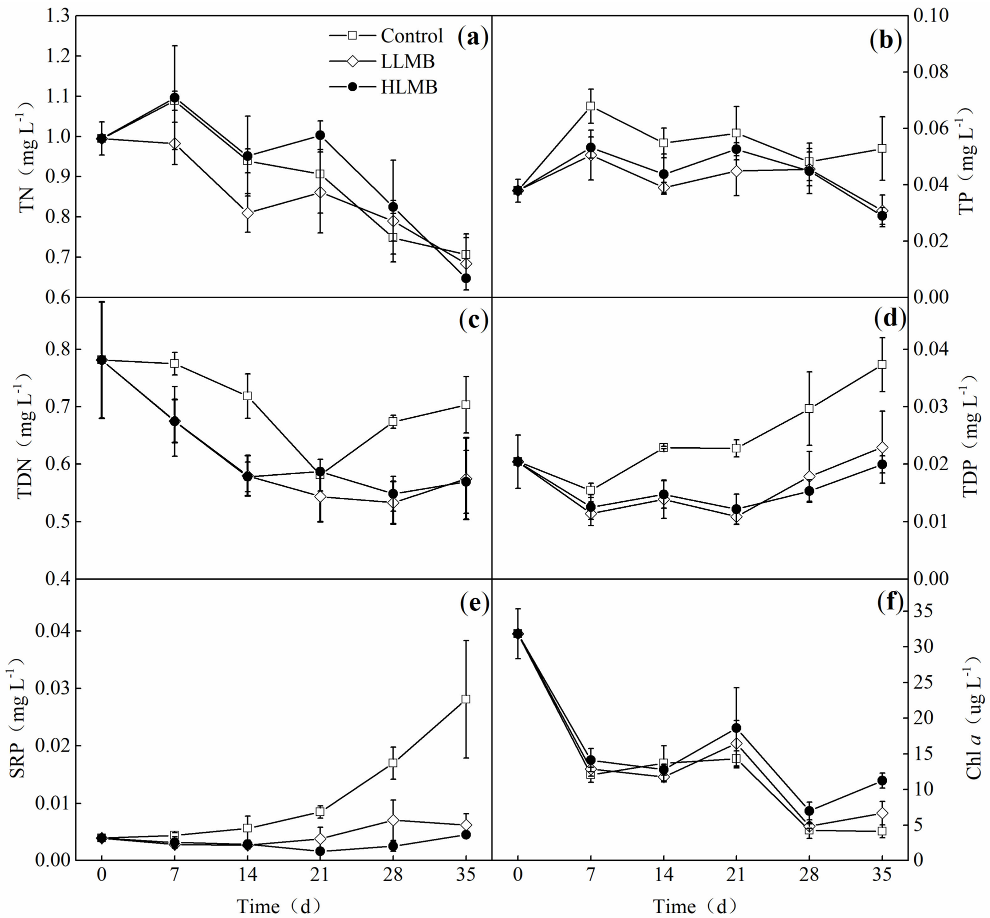

Figure 2. Time series of total nitrogen (TN) (**a**), total phosphorus (TP) (**b**), total dissolved nitrogen (TDN) (**c**), total dissolved phosphorus (TDP) (**d**), soluble reactive phosphorus (SRP) (**e**), and chlorophyll *a* (Chl *a*) (**f**) in different treatments. Factor was LMB (0, 425 g m^{-2}, and 850 g m^{-2}). The values represented a standard deviation (n = 4).

3.2. Zooplankton

The application of LMB significantly reduced the zooplankton biomass (Figure 3). In terms of the zooplankton community, the copepods dominated the zooplankton community followed by nauplii and cladocerans. Rotifers constituted the smallest proportion of the zooplankton. Compared with the Control treatment, the biomass of copepods in the LLMB treatment was significantly reduced by 67%; the biomass of cladocerans and copepods in the HLMB treatment were also significantly reduced by 46% and 54%, respectively.

3.3. Macrophytes

LMB significantly inhibited *H. verticillata* growth, but had no significant influence on *V. natans* (Figure 4). At the end of the experiment, the biomass and RGR of *H. verticillata* in the HLMB treatment were 104 g and 53 mg g^{-1} d^{-1}, respectively, which were 62% and 32% lower than the Control treatment (Figure 4a,b). In the LLMB treatment, the biomass and RGR of *H. verticillata* were 143 g and 61 mg g^{-1} d^{-1}, respectively; lower than the Control treatment by 48% and 22%, respectively. For *V. natans*, the biomass and RGR in each treatment were close to 500 g and 70 mg g^{-1} d^{-1} at the end of the experiment, respectively (Figure 4a,b). In addition, the biomass proportion and relative competitive indicator of *H. verticillata* gradually decreased with the increase in LMB dose (Figure 4c,d).

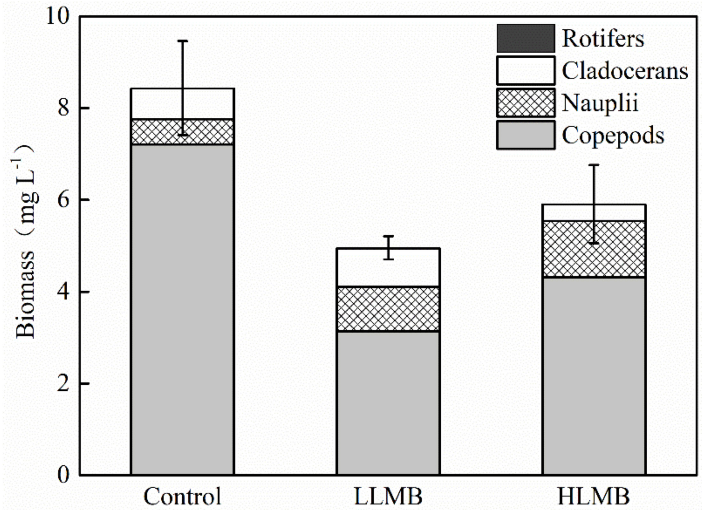

Figure 3. Biomass of zooplankton in different treatments. Factor was LMB (0, 425 g m^{-2}, and 850 g m^{-2}). The values represented a standard deviation (n = 4).

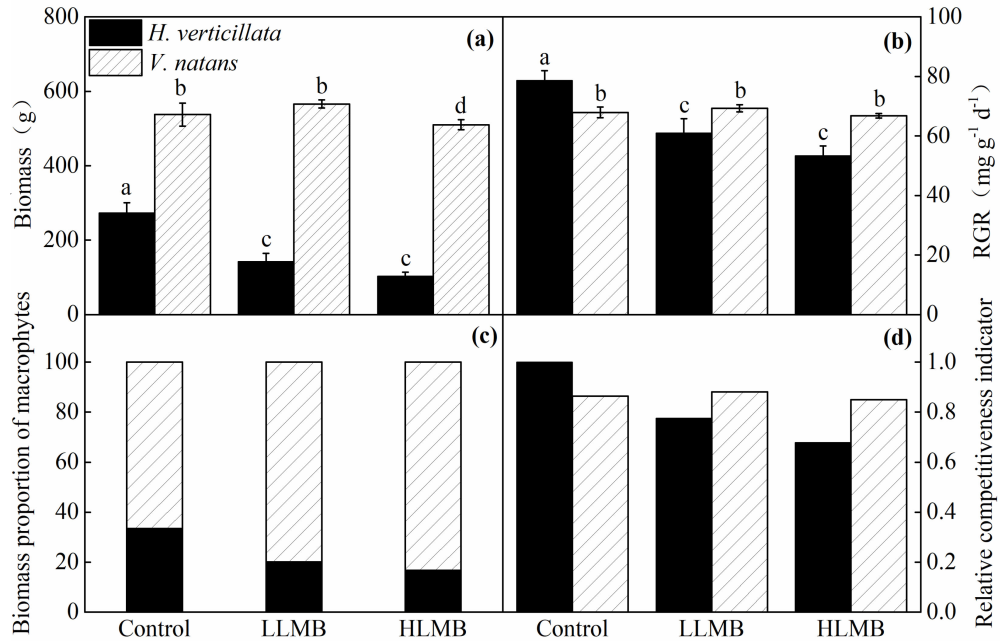

Figure 4. Biomass (**a**), relative growth rate (**b**), biomass proportion (**c**), and relative competitiveness indicator (**d**) of *H. verticillata* and *V. natans*. Factor was LMB (0, 425 g m^{-2}, and 850 g m^{-2}). The values represented a standard deviation (n = 4). Means with different letters (a, b, c, and d) were significantly different ($p < 0.05$).

LMB significantly reduced the height, branch number, root length, and number of *H. verticillata*, but it had no significant influence on the morphological indicators of *V. natans* (Figure 5). At the end of the experiment, the height, branch number, root length, and number of *H. verticillata* in the HLMB treatment were 19%, 52%, 40%, and 54% lower than those in the Control treatment, respectively. In the LLMB treatment, the height was 13%, the branch number was 34%, the root length was 33%, and the number of *H. verticillata* was 8% lower than those in the Control treatment (Figure 5a–d). There were no significant differences of height, leaf number (branch number), root length, ramet number, and stolon length of *V. natans* among the treatments (Figure 5a–f).

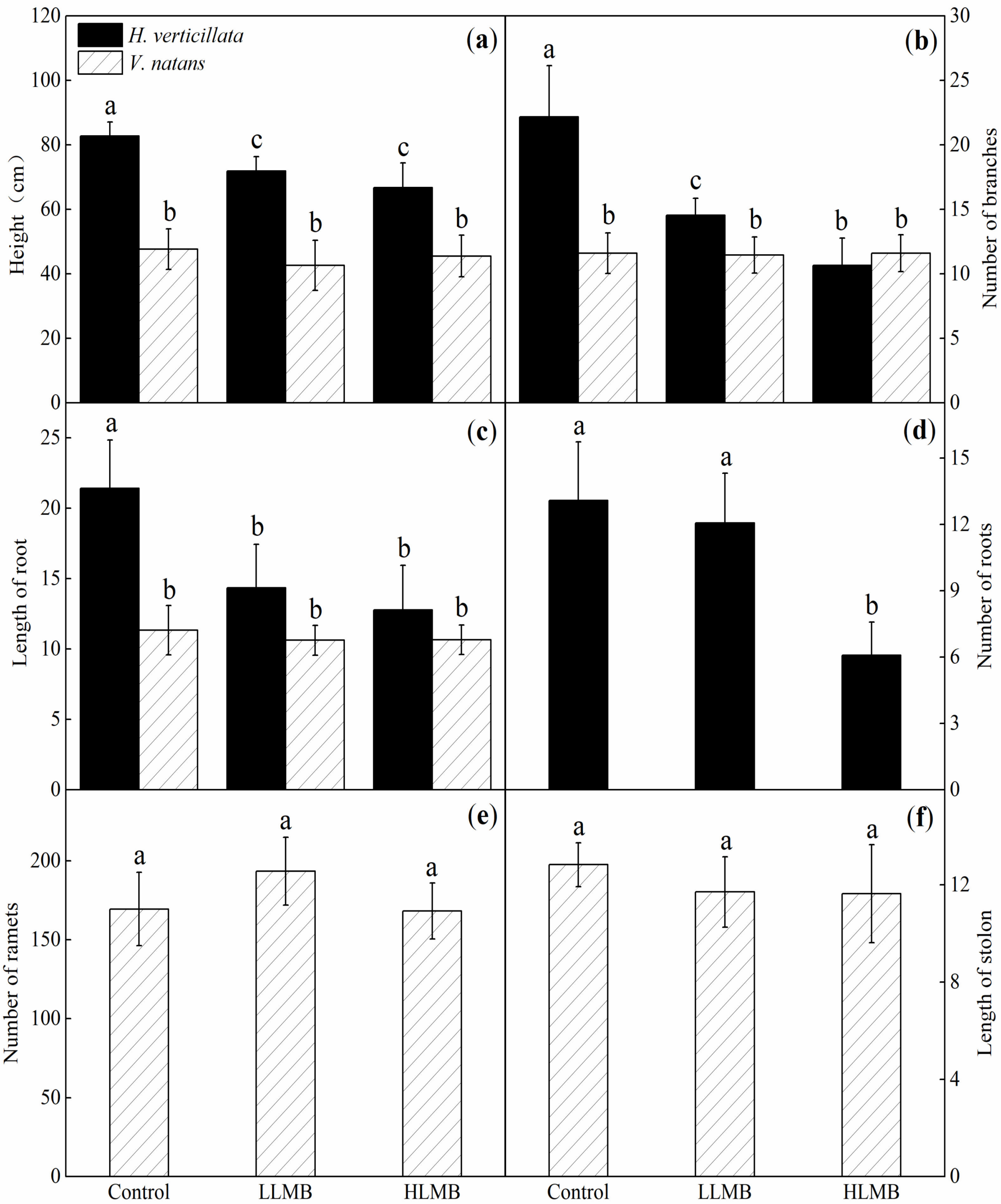

Figure 5. Height (**a**), branch number (**b**), root length (**c**), root number (**d**), ramet number (**e**), and stolon length (**f**) of *H. verticillata* and *V. natans*. Factor was LMB (0, 425 g m^{-2}, and 850 g m^{-2}). The values represented a standard deviation. Means with different letters (a, b, and c) were significantly different ($p < 0.05$).

LMB significantly affected the chlorophyll of *H. verticillata* as well as the MDA of *V. natans* and *H. verticillata* (Figure 6). At the end of the experiment, the Chl *a* and *b* and MDA of *H. verticillata* in the HLMB treatment were 15%, 20%, and 45% higher than the Control treatment, respectively. In the LLMB treatment, Chl *a* was 4%, Chl *b* was 13%, and MDA was 30% higher than the Control treatment. In addition, compared with the Control treatment, the MDA of *V. natans* in the HLMB and LLMB treatments significantly decreased by 63% and 53%, respectively (Figure 6c).

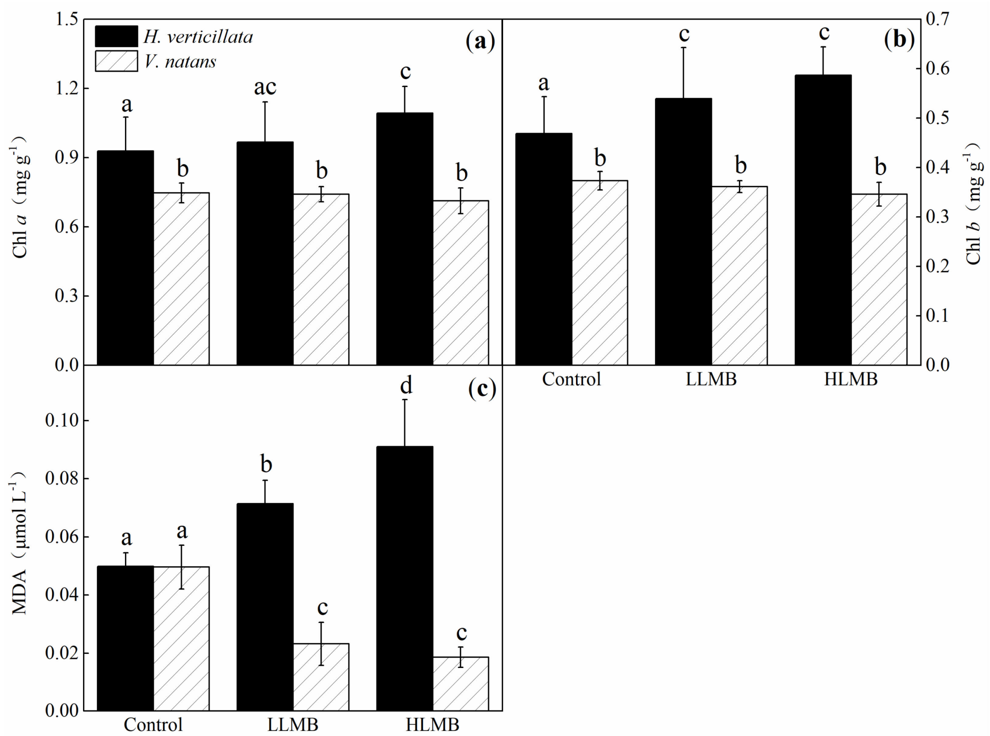

Figure 6. Chlorophyll *a* (**a**), Chlorophyll *b* (**b**), and MDA (**c**) of *H. verticillata* and *V. natans*. Factor was LMB (0, 425 g m^{-2}, and 850 g m^{-2}). The error bars represented a standard deviation. Means with different letters (a, b, c, and d) were significantly different ($p < 0.05$).

4. Discussion

In this study, we explored the influence of the application of LMB on *H. verticillata* and *V. natans* in mixed-species plantings, and also chose a more suitable submerged macrophyte species to be used with LMB in water restoration. As hypothesized, different types of submerged macrophytes had different responses to the application of LMB. Submerged macrophyte *V. natans* with well-developed roots was more adaptable to LMB.

4.1. Effects of LMB on Hydrilla verticillata and Vallisneria natans

In the present study, LMB significantly inhibited *H. verticillata* growth, but had no significant influence on *V. natans*. The significantly inhibited growth of *H. verticillata* by LMB was associated with the inhibited root growth and restricted availability of bioavailable phosphorus [22,31,32]. Compared with *V. natans*, the root system of *H. verticillata* is relatively underdeveloped [40]. LMB capping could form an anaerobic layer on the surface sediment to reduce oxygen diffusion to the sediment, which has been suggested to be harmful to the root growth [32]. Thus, reductions in root length and the number of *H. verticillata* were recorded after the application of LMB. The efficacy of LMB on the immobilization of P reduced the content of bioavailable P in the surface sediment and water [18,20,22,31], thereby potentially leading to a restriction of the nutritional supply of *H. verticillata*. *V. natans* has a relatively developed root system and can also continuously generate new individuals through the elongation of the stolon to form large clonal populations [22,49], thereby expanding the absorption range of nutrients in the sediment and avoiding the influence of LMB. This result was contrary to other studies where the application of LMB inhibited *V. natans* growth [22]. The differences may be related to the different doses of LMB and the experimental environment. In terms of physiology, the application of LMB significantly increased the content of MDA, indicating that *H. verticillata* was under physiological stress [50]. At the end of the experiment, we also clearly observed that *H. verticillata* in the LMB treatments had more leaves than the Control treatment.

The different responses of *V. natans* and *H. verticillata* to the application of LMB also resulted in a change in their competitive ability. Under normal circumstances, the typically high growth rates and the canopy formation of *H. verticillata* could reduce the penetration of sunlight to the zones beneath, thus inhibiting the growth of *V. natans* and leading to a competitive advantage of *H. verticillata* [39,41]. The growth of *V. natans* was not inhibited or promoted after the application of LMB in the present study, but the biomass proportion of *V. natans* increased as the growth of *H. verticillata* was significantly inhibited, which increased the competitive ability of *V. natans* versus *H. verticillata* to an extent. Studies have shown that, under nutritionally restricted environments, the growth and competitive abilities of *H. verticillata* can be inhibited in comparison with *Vallisneria americana* [51]. In addition, *H. verticillata* can reproduce asexually by a branch in nature [40]; it can, therefore, be speculated that LMB may inhibit the growth and development of branch roots, thus inhibiting the formation of new individuals, which is not conducive to the population expansion of *H. verticillata* [22,33].

4.2. Effects of LMB on the Water Purification Ability of Submerged Macrophytes

The negative effects of the application of LMB reduced the inhibitory effect of submerged macrophytes on algae growth despite the P concentration being decreased by the application of LMB. This was related to the inhibition of LMB on *H. verticillata* growth. The decreased biomass of *H. verticillata* reduced the total biomass of the plants in the LMB treatments, which weakened the competitive inhibition of the submerged macrophytes with algae [52,53]. The negative effects of LMB on the water purification ability of submerged macrophytes may be even more severe when *H. verticillata* are grown alone. In addition, the negative effects of LMB on other organisms also contributed to a deterioration in the water quality; a reduction in the zooplankton biomass from the application of LMB [54–56] reduced the grazing pressure of zooplankton on phytoplankton [57–59]. Speculatively, this phenomenon may also have been related to the low level of Chl *a* concentration in the present study (<30 μg L^{-1}); it seems unlikely to occur in water bodies with a high Chl *a* concentration where the inhibition of organisms on algae is naturally weak [60].

4.3. Implications for the Restoration of Subtropical Lakes

In water restoration, the combination of various restoration measures such as biological–chemical methods [22,61–63] has become a normal practice. The prerequisite for giving full play to this combined effect is that there is no significant mutual interference or inhibition between the two. It indicates that submerged macrophytes, which are less negatively affected by LMB (for example, the root systems are more developed), should be selected to combine with LMB to achieve greater efficacy in water restoration. This study showed that *H. verticillata* was strongly inhibited by LMB. Compared with *H. verticillata*, the root system of *V. natans* is more developed and is more suitable for use with LMB. In addition, new plants of *V. natans* can be quickly formed through tillering; thus, the coverage can rapidly increase, significantly improving nutrient absorption and sediment stabilization [39,53]. Thus, such combinations are also suitable for water bodies with frequent hydrodynamic disturbances based on the stabilization of sediment by LMB and submerged macrophytes [15,23,64]. Only two typical submerged macrophytes, *V. natans* and *H. verticillata*, were analyzed in the present study. Considering the diversity and difference of submerged macrophytes, larger scale research with diverse submerged macrophytes is needed to test in other seasons or for a longer time.

5. Conclusions

LMB had no significant influence on *V. natans* growth. However, due to the inhibition of the *H. verticillata* root by the application of LMB, the biomass, RGR, height, and branch number of *H. verticillata* significantly decreased with an increase in LMB doses. Thus, the competitive ability of *H. verticillata* versus *V. natans* was reduced. In addition, the water quality showed unexpected changes such as an increased Chl *a* concentration in the overlying water due to the negative effects of LMB on *H. verticillata*. Therefore, submerged macrophytes with more developed roots such as *V. natans* should be preferred when using combined biological–chemical methods for water restoration.

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Data Availability Statement: Data are presented in the text.

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Conflicts of Interest: The authors declare no conflict of interest.

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