



Response of a submerged macrophyte (*Vallisneria natans*) to water depth gradients and sediment nutrient concentrations



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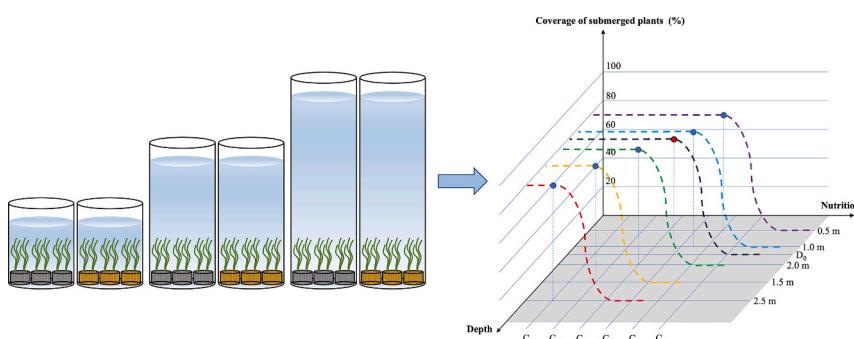
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HIGHLIGHTS

- *V. natans* responses to water depth and sediment nutrient content were studied.
- The influence of water depth on submerged plants is greater than that of sediment.
- Sediment nutrient concentration has more significant impact on root morphology.
- Increasing water depth promotes phosphorus release, further affecting plants.

GRAPHICAL ABSTRACT



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ABSTRACT

Submerged plants constitute a vital component of shallow lake ecosystems, where water depth and sediment nitrogen-phosphorus content are two key factors influencing their growth. This study focuses on *Vallisneria natans* and investigates the morphological and physiological changes of *V. natans* under the interaction of three water depth gradients and two different sediment nutrient levels. It explores the mechanisms through which varying sediment nutrient conditions under different water depths affect the growth of *V. natans*. The results indicate that both independent and interactive effects of water depth and sediment nutrient status significantly impact the morphology, antioxidant enzyme activity, and photosynthetic pigment content of *V. natans*, with water depth having a greater influence. To adapt to increased water depth-induced light stress, *V. natans* responds morphologically by increasing leaf length, leaf width, and decreasing maximum root length. Physiologically, it enhances its antioxidant regulation capacity and photosynthetic efficiency by increasing antioxidant enzyme activity, root vitality, and photosynthetic pigment content to counter weak light stress. However, these adaptations are insufficient to cope with excessively deep waters (200 cm). Sediment nutrient levels primarily control the growth of *V. natans* by affecting its root system. When sediment nitrogen and phosphorus content is

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lower, *V. natans* exhibits greater total root volume and surface area to enhance nutrient absorption efficiency. Water depth not only directly influences the growth of submerged plants but may also impact the migration and transformation of phosphorus in sediments, further exacerbating its effects on the growth of these plants, thus accelerating the regime shift of shallow lakes. Therefore, this study reveals *V. natans'* response strategies to varying water depths and sediment nutrient levels, determining suitable water levels and sediment nutrient conditions for its growth. These research findings provide a scientific basis for water level management and ecological restoration of submerged aquatic plants in shallow lakes.

1. Introduction

Submerged plants, as important primary producers in shallow lakes, play a vital role in maintaining the structure and function of lake ecosystems (Kolada, 2010; Scheffer et al., 1993). On the one hand, submerged plants release oxygen into the water through photosynthesis, increasing the dissolved oxygen content in the water (Cao et al., 2019; Caraco et al., 2006). Respiration, on the other hand, consumes oxygen and creates a low-oxygen environment in localized areas of the plant leaves, promoting microbial redox reactions and nutrient transformations among different forms. Submerged plants also produce allelopathic substances that inhibit the growth of algae, demonstrating strong algal inhibition abilities (Pakdel et al., 2013; Zhou et al., 2017b). Additionally, submerged plants absorb nutrients such as nitrogen and phosphorus from the water and sediment during their growth, leading to a significant decrease in nutrient levels in the water (Ersoy et al., 2020; Lu et al., 2018; Zhou et al., 2017a). In particular, for eutrophic lakes, when submerged plants are present, the ecosystem remains in a clear-water state (Sandjensen and Borum, 1991). Therefore, submerged plants are widely used for the ecological restoration of eutrophic lakes and the construction of benign ecosystems (Blindow et al., 2014; Sayer et al., 2010).

Water depth can change a series of environmental factors in the water, especially underwater light intensity, which has a significant impact on the growth of submerged aquatic plants (Chen et al., 2016; Chen et al., 2020). In shallow water lakes, plants can adapt to increasing water depth by enhancing their aboveground biomass through factors such as increasing plant height, internode length, and leaf area (Mommert et al., 2005; Yang et al., 2004); in the weak light environment of deep-water areas, the photosynthetic efficiency of submerged plants is usually greatly reduced (Zhang et al., 2014). Different submerged plants have specificity in their response and adaptability to water depth, and there have been many studies on the functional traits of submerged plants under different water depth conditions. For example, the canopy-type species *Potamogeton maackianus* responds to low light conditions by elongating its stems, while the rosette-type species *Vallisneria natans* produces more chlorophyll *a* in deeper water to tolerate shading (He et al., 2019). *Potamogeton crispus* promotes the growth of aboveground vegetation and root tissue, as well as reproductive ability, at water depths between 60 and 120 cm. When the water depth reaches 200 cm or more, the lack of formation of turions and the inhibition of ramet production limits the distribution of *P. crispus* in the following year (Zhou et al., 2017a).

Sediments can also significantly affect the growth performance of submerged plants, as they provide the foundation for the rooting, growth, and reproduction of these plants (Liu et al., 2017), as well as the main source of nutrients and trace elements (Xiao et al., 2007). The nutritional level of sediments can influence the biomass distribution of submerged plants, with the root/leaf mass ratio of *V. natans* being higher in low-nutrient sediments than in sediments that are more nutrient-rich (Bai et al., 2015). *Myriophyllum spicatum* can balance the tradeoff between internal aeration and nutrient acquisition by adjusting its root structure and biomass allocation patterns among different root types (Xie et al., 2007). In nutrient-rich patches, the length and diameter of the stolons as well as the length and width of leaves of *Vallisneria spiralis* L. dramatically increased (Wang and Yu, 2007). In addition to directly

affecting the growth and development of submerged plants, substratum types can also interact with water depth, resulting in more complex effects on submerged plants (Xiao et al., 2007).

The morphology and physiological indicators of submerged plants can reflect their response to different environments. Morphological indicators such as plant height and leaf length can directly reflect the energy resource investment strategy of plants under different environments (Vojtiskova et al., 2004), while physiological indicators such as chlorophyll concentration and antioxidant enzyme activity can more accurately reflect the physiological response of plants to the environment. Chlorophyll concentration is closely related to photosynthesis, which can reflect the photosynthetic efficiency of plants and their adaptation to changes in light intensity (Frostchristensen and Sandjensen, 1992; Hussner et al., 2011). Antioxidant enzyme activity can indirectly reflect the level of reactive oxygen species in plant bodies, indicating the condition of plant oxidative stress (Jiang et al., 2019; Shabnam and Pardha-Saradhi, 2016). Therefore, investigating the variations in morphology and physiological responses of submerged aquatic plants under different water depths and substrate conditions holds significant importance in enhancing our understanding of the mechanisms governing their responses to environmental changes. Moreover, such studies can provide valuable insights to tailor appropriate strategies for guiding the restoration of submerged vegetation based on specific local conditions.

Vallisneria natans is a common submerged plant species in shallow lakes of the middle and lower reaches of the Yangtze River in China. Due to its strong pollutant absorption capacity and tolerance to low light conditions, it is often used in wetland and shallow lake vegetation restoration. In response to adverse conditions, *V. natans* undergoes changes in its morphology and physiological state. Currently, there have been related reports on the influence of water depth variation and substrate types on the growth of submerged plants. However, there is relatively limited research on the indirect negative impacts of increased water depth on the release of phosphorus from polluted sediment on the growth of submerged plants. Therefore, this study selected *V. natans* as the target species and cultivated it under different water depths and sediment nutrient conditions. The effects of water depths and sediment nutrient conditions on the morphology and physiological parameters of *V. natans* were observed during the experiment process, in order to explore the synergistic effects and feedback mechanisms of increased water depth and sediment nutrient status on submerged plants, providing scientific evidence for lake water level management and submerged plants restoration in complex environments.

2. Materials and methods

2.1. Experimental design

The experimental setup employed high-density polyethylene white plastic buckets with a diameter of 70 cm and heights of 80 cm, 150 cm, and 220 cm, respectively. Considering the substantial water requirement for the experiments, approximately 3.50 m³, it was impractical to conduct in-situ water collection on a large scale. Therefore, the overlying water used in the experimental setup was sourced locally from Xuanwu Lake in Nanjing. It was blended with tap water that had been aerated for 48 h in a 1:9 ratio to ensure that the initial chlorophyll *a*

content within the experimental units fell within the range of 25 to 30 µg/L. This approach ensured uniform initial conditions among the experimental groups for the overlying water while also providing a more accurate simulation of shallow lake habitat conditions.

Sediments were collected from two locations in Dongshan Bay of Taihu Lake, where abundant submerged plants grow, each with varying nutrient levels and labeled as S1 and S2. To remove large plant debris and coarse sand and gravel impurities, the sediments were sieved through a 1 cm × 1 cm mesh and homogenized before use. The prepared sediments were then transferred to planting pots with a diameter of 24 cm and height of 10 cm, with a height of 8 cm of sediment in each pot. The nitrogen (TN-s), phosphorus (TP-s), and organic carbon (W_{c,o}) contents of the sediments at the beginning (day 0) and end (day 70) of the experiment was shown in Table S1.

V. natans for test was collected from Dongshan Bay of Lake Taihu. To ensure uniformity in the study, morphologically consistent plants were handpicked for the experimental setup.

The experiment was conducted at the Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences (118°41'21"N, 32°3'28"E) from July to September 2021 (Fig. 1), with a duration of 70 days. Polyethylene white plastic barrels with diameters of 70 cm and heights of 80 cm, 150 cm, and 220 cm were selected as the experimental devices. Three water depth gradients of 60 cm, 130 cm, and 200 cm were established, labeled L, M, and H, respectively. A total of six polyethylene containers were employed, each wrapped externally with black shade nets to prevent thermal effects and replicate the underwater light conditions of Lake Taihu. Under each water depth condition, there were two treatment groups, labeled s1v and s2v. The sediment nutrient level in the s1v group was higher than that in the s2v group (Table S1). Each treatment group contained 7 planting pots, with 9 *V. natans* plants in each pot. Nylon ropes were attached to the planting pots to facilitate subsequent sampling and analysis. Additionally, a transparent plastic canopy was erected above the experimental setup to prevent rainwater ingress while allowing sunlight penetration.

Upon commencing the experiment, water temperature in the overlying water was measured every 10 days. Total nitrogen (TN-w), total phosphorus (TP-w), and chlorophyll a (Chl a-w) content in the overlying water were determined using standard methods (Rice and Association, 2012). We measured the underwater light intensity at the water surface and the positioning of the planting bowls at 11:00 every 10 days, using a micro Li-Cor quantum sensor (Walz GmbH Effeltrich, Germany). The specific points of light intensity measurement are denoted by triangular markers in Fig. 1. Subsequently, one planting bowl was chosen from

each treatment group, and the grown *V. natans* specimens were collected. We randomly selected three or more different plants from each group for further analysis of morphological and physiological indicators.

2.2. Determination of plant indicators

The height of the plants and the maximum root length of *V. natans* were measured using a measuring tape, while the maximum leaf width was measured using a vernier caliper. Total root length (Length), total projected area (ProjArea), total root surface area (SurfArea), mean root diameter (AvgDiam), total root volume (RootVolume), number of root tips (Tips), number of forks (Forks), and number of crossings (Crossings) were analyzed using root scanning instrument and software (WinRhizo Pro 2007, Régent Instruments, Québec, Canada).

Fresh leaves of the sampled *V. natans* were collected and cut into small pieces, with 0.2 g of each sample transferred to a mortar with a small amount of quartz sand, calcium carbonate powder and 95 % ethanol. The leaves were then ground until the tissue turned white, and the extraction liquid was filtered into a brown volumetric flask until no green color remained in the filter paper or residue. Finally, the extract was adjusted to 25 mL with ethanol. The chlorophyll extract was poured into a colorimetric dish with a path length of 1 cm. The absorbance was measured at wavelengths of 665 nm, 649 nm and 470 nm using 95 % ethanol as a blank to calculate chlorophyll a (Chl a), chlorophyll b (Chl b), carotenoids (Car) and total pigment (Chl) content in leaves (Lichtenthaler and Wellburn, 1983).

To determine malondialdehyde (MDA) content, approximately 0.8 g of fresh *V. natans* leaves were taken, added to 5 mL of 5 % trichloroacetic acid (TCA), and homogenized at 3000 r/min for 10 min; then, 2 mL of the supernatant was added to 2 mL of 0.67 % tri-n-butylamine (TBA). The mixture was boiled for 15 min in a water bath at 100 °C, cooled, and centrifuged again for 10 min. The absorbance values of the supernatant were measured at 450 nm, 532 nm, and 600 nm, with 2 mL of distilled water and 2 mL of 0.67 % TBA used as a control. According to the reference (Du and Bramlage, 1992), MDA equivalents were calculated.

To determine the activities of peroxidase (POD) and catalase (CAT), the high potassium permanganate titration method and the pyrogallol method were used, respectively (AMAKO et al., 1994). Root vitality was determined by measuring the amount of 2,3,5-triphenyltetrazolium chloride (TTC) present in the roots (Chen et al., 2000).

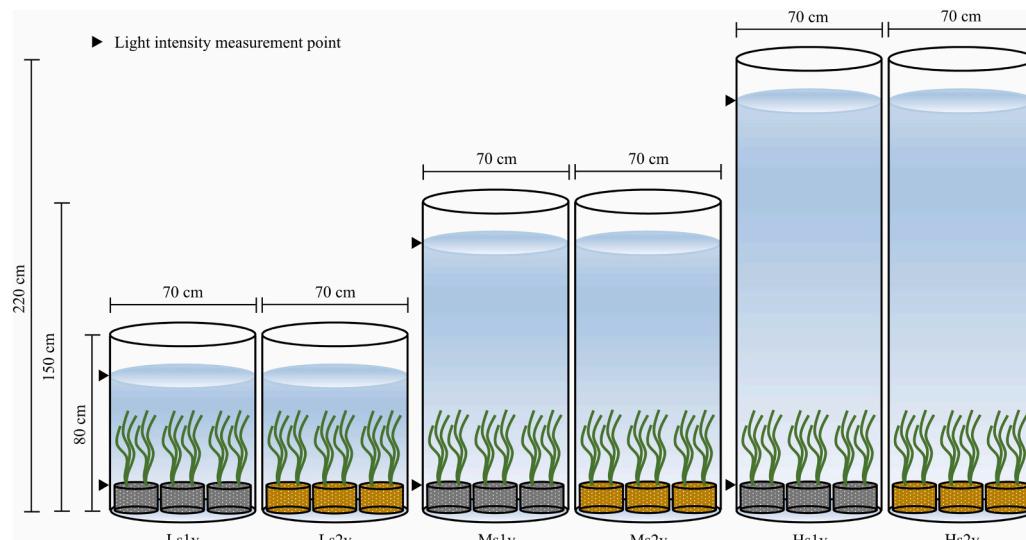


Fig. 1. Sketch of reactor size and six experimental groups.

2.3. Data processing and statistical analysis

Data graphs were plotted using GraphPad software (Prism 9 for macOS), and one-way ANOVA and two-way ANOVA were performed using SPSS 26.0 software (IBM, New York, NY, USA). One-way ANOVA was used to represent the significance level, with significance levels set at $p < 0.01$ (**), $p < 0.05$ (*), and $p > 0.05$. Two-way ANOVA was employed to investigate the interactive and independent effects of water depth and sediment conditions on the morphological and physiological indicators of *V. natans*. The data residuals were tested for normality using the Shapiro-Wilk test, and homogeneity of variance was confirmed by applying the Levene's test. In the presence of an interaction effect, separate analyses were conducted to examine the individual effects of water depth and sediment conditions, followed by Bonferroni adjustments for multiple comparisons among groups. In the absence of an interaction effect, the main effects of water depth and sediment conditions were analyzed individually.

3. Results

3.1. Indicators of water environment and underwater light intensity

At the commencement of the experiments, there were no significant differences in water temperature (WT), TN-w, TP-w, and Chl *a*-w concentrations among the experimental groups (Table 1, $p > 0.05$). Throughout the course of the experiment, water temperatures in the different treatment groups remained approximately within the range of 27–34 °C, while TN-w, TP-w, and Chl *a*-w in the overlying water of the various treatment groups showed significant reductions (Table 1). The mean TP-w content in the overlying water at a depth of 200 cm was

Table 1

Water temperature (WT), total nitrogen (TN-w), total phosphorus (TP-w) content and chlorophyll *a* concentration of overlying water (Chl *a*-w) in different treatment groups in the experiment.

Indicator	Group	Day 0	Day 70	All
WT (°C)	Ls1v	28.52 ± 0.31a	28.40 ± 0.48a	29.51 ± 1.70a
	Ls2v	28.69 ± 0.36a	28.54 ± 0.09a	29.99 ± 1.92a
	Ms1v	28.74 ± 0.32a	28.35 ± 0.20a	29.94 ± 1.84a
	Ms2v	28.85 ± 0.20a	28.45 ± 0.14a	30.24 ± 1.92a
	Hs1v	28.46 ± 0.34a	28.42 ± 0.25a	29.78 ± 1.27a
	Hs2v	28.63 ± 0.28a	28.50 ± 0.29a	29.98 ± 1.19a
TN-w (mg/L)	Ls1v	1.745 ± 0.006a	0.511 ± 0.017c	0.963 ± 0.473a
	Ls2v	1.659 ± 0.024a	0.327 ± 0.016f	0.745 ± 0.477a
	Ms1v	1.731 ± 0.012a	0.573 ± 0.015b	0.999 ± 0.498a
	Ms2v	1.714 ± 0.007a	0.477 ± 0.017d	0.907 ± 0.537a
	Hs1v	1.678 ± 0.067a	0.623 ± 0.009a	0.751 ± 0.406a
	Hs2v	1.731 ± 0.056a	0.437 ± 0.004e	0.710 ± 0.439a
TP-w (mg/L)	Ls1v	0.094 ± 0.001a	0.034 ± 0.001	0.048 ± 0.017c
			cd	
	Ls2v	0.094 ± 0.002a	0.034 ± 0.001d	0.059 ± 0.020bc
	Ms1v	0.095 ± 0.004a	0.034 ± 0.001	0.063 ± 0.017b
			cd	
	Ms2v	0.093 ± 0.001a	0.035 ± 0.000c	0.056 ± 0.018bc
Chl <i>a</i> -w (μg/L)	Hs1v	0.092 ± 0.003a	0.158 ± 0.001a	0.079 ± 0.029a
	Hs2v	0.093 ± 0.003a	0.135 ± 0.001b	0.062 ± 0.029b
	Ls1v	28.883 ± 1.498a	2.730 ± 0.746e	13.679 ± 11.95a
	Ls2v	30.371 ± 0.803a	3.264 ± 0.732e	10.854 ± 10.912a
	Ms1v	30.411 ± 1.396a	7.826 ± 0.376c	15.594 ± 10.741a
	Ms2v	29.125 ± 1.162a	5.005 ± 0.717d	15.169 ± 11.835a
	Hs1v	29.960 ± 0.564a	18.751 ± 0.606a	14.694 ± 7.284a
	Hs2v	29.951 ± 1.374a	11.977 ± 0.340b	13.300 ± 8.950a

Note. Different letters indicate that there are significant differences among the treatments at significant level of $p = 0.05$, and same letters indicate no significant differences among the treatments.

0.031 mg/L higher in the sediment high-nitrogen phosphorus group compared to the depth of 60 cm (Table 1, $p < 0.05$). By the conclusion of the experiment (day 70), the TN content in the overlying water at various depths was significantly higher in the sediment high-nitrogen phosphorus group compared to the low-nitrogen phosphorus group ($p < 0.05$), with the greatest difference observed at a depth of 200 cm, reaching 0.186 mg/L. Additionally, at a water depth of 200 cm, both TP-w and Chl *a*-w contents in the overlying water were significantly higher compared to other water depths (Table 1, $p < 0.05$), with the high-nitrogen phosphorus group at a depth of 200 cm having a TP-w content 0.023 mg/L higher than the low-nitrogen phosphorus group ($p < 0.05$), resulting in a 6.744 mg/L increase in Chl *a*-w content ($p < 0.05$).

The ratio of underwater light intensity to surface light intensity in different treatment groups is shown in Fig. 2. At water depths of 60 cm, 130 cm, and 200 cm, the light intensity at the planting bowls was approximately 40.21 %, 17.84 %, and 3.52 % of the surface light intensity, respectively. Underwater light intensity exhibited a significant attenuation with increasing water depth ($p < 0.05$). However, the variation in sediment nutrient levels across different water depths did not have a significant effect on the ratio of underwater light intensity to surface light intensity (Fig. 2, $p > 0.05$).

3.2. Changes in morphological indices of *V. natans*

The water depth had a significant independent effect on the number of *V. natans* ramets during the experimental period (Table S2). The number of *V. natans* ramets at 60 cm and 130 cm water depths was significantly higher than at 200 cm ($p < 0.001$). *V. natans* at the 60 cm and 130 cm depths exhibited ramets as early as the 10th day of the experiment, while those at the 200 cm depth did not show ramets until the 40th day, indicating a delayed growth and reproduction pattern for *V. natans* in the higher water depth group. Nevertheless, *V. natans* in all experimental groups were able to survive throughout the experiment.

There were significant differences in the leaf length of *V. natans* among different treatment groups as growth days increased ($p < 0.01$). Starting from the 20th day of the experiment, water depth began to significantly influence leaf length (Table S2, $p < 0.01$), continuing until the end of the experiment. Under the same nitrogen and phosphorus sediment conditions, the leaf length of *V. natans* at a water depth of 60 cm and 130 cm was significantly greater than at a depth of 200 cm ($p < 0.01$) due to the availability of ample light, with the highest mean leaf length observed at a depth of 130 cm. At water depths of 60 cm and 130 cm, where nutrients were more abundant, the leaf length of *V. natans* in the high nitrogen-phosphorus sediment group was greater than that in

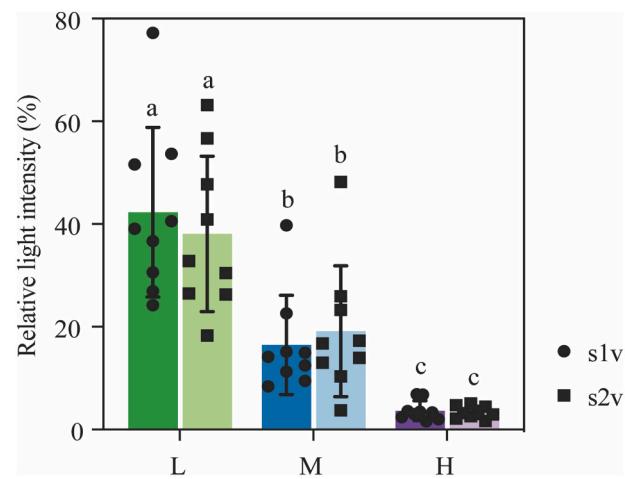


Fig. 2. Ratio of underwater light intensity to surface light intensity for different groups during the simulation experiment. Columns denoted by a different lower case letter differ significantly by ANOVA at $p < 0.05$.

the low nitrogen-phosphorus group (Fig. 3b, $p > 0.05$).

During the experiment, significant differences in the leaf width of *V. natans* were observed among different treatment groups ($p < 0.01$), with sediment and water depth showing an interactive effect on *V. natans* leaf width (Table S2). Moreover, water depth had a sustained significant impact on *V. natans* leaf width ($p < 0.05$), especially notable after the 50th day ($p < 0.01$). The leaf width at a depth of 200 cm was significantly lower compared to other water depth groups (Fig. 3d, $p < 0.05$). Individual analysis of sediment and water depth effects revealed that at a depth of 130 cm, the high nitrogen-phosphorus sediment group had significantly wider *V. natans* leaves than the low nitrogen-phosphorus group ($p < 0.01$), consistent with the pattern observed for leaf length.

Water depth exhibited a sustained significant impact on the maximum root length of *V. natans* throughout the experimental period (Table S2, $p < 0.05$). The maximum root length of *V. natans* at water depths of 60 cm and 130 cm gradually increased over time and remained relatively stable from the 40th day onwards. In contrast, the changes in maximum root length at a depth of 200 cm were less pronounced, suggesting that *V. natans* in all groups exhibited normal growth during the experiment. During the experiment, *V. natans* at a 200 cm depth experienced intense light stress, resulting in a mean maximum root length that was 10.692 cm and 8.647 cm shorter compared to depths of 60 cm and 130 cm, respectively ($p < 0.01$). At a depth of 130 cm, *V. natans* in the low nitrogen-phosphorus sediment group extended their roots to absorb more nutrients from relatively nutrient-poor sediment to support their growth, resulting in a mean maximum root length 1.348 cm longer than that of the high nitrogen-phosphorus group.

The interaction between water depth and sediment had no statistically significant effect on the root morphology of *V. natans* ($p > 0.05$). The total root length of *V. natans* significantly decreased as water depth increased and underwater light decreased ($p < 0.01$). The mean Length

of *V. natans* at water depths of 60 cm and 130 cm was 395.494 cm ($p < 0.01$) and 294.098 cm ($p < 0.01$) greater, respectively, than at a depth of 200 cm. Sediment had a significant effect on the ProjArea, SurfArea, and RootVolume of root system (Table S3, $p < 0.05$). Due to the relatively abundant nutrients available in the sediment, under conditions of high nitrogen-phosphorus sediment content, the mean ProjArea was smaller by 5.35 cm² ($p < 0.05$), the mean SurfArea was smaller by 16.807 cm² ($p < 0.05$), and the mean RootVolume was smaller by 0.164 cm³ ($p < 0.05$) compared to conditions with low nitrogen-phosphorus sediment content.

3.3. Changes in physiological indices in the different treatment groups

Overall, the effect of sediment nutrients on the MDA content of *V. natans* leaves was not statistically significant, while water depth had a significant impact on the MDA content of *V. natans* leaves (Table S4). The average MDA level at a depth of 200 cm was 0.386 nmol/g.FW higher than that at a depth of 60 cm ($p < 0.05$). On the 30th day of the experiment, the accumulated MDA in *V. natans* at different water depths began to increase, with the most rapid increase observed at a water depth of 200 cm. This suggests that under these specific water depth conditions, the growth of *V. natans* was subjected to stress (Fig. 4a).

Between the 10th and 40th day of the experiment, the POD activity in *V. natans* leaves generally increased with increasing water depth (Fig. 4c). There was a significant interaction between water depth and sediment for POD activity in *V. natans* leaves (Table S4, $p < 0.05$). Under fixed sediment conditions, the POD activity of *V. natans* leaves at a water depth of 200 cm was significantly higher than at other water depths ($p < 0.05$). This effect was particularly pronounced under low-nitrogen and low-phosphorus sediment conditions, attributed to the significant lack of light exposure and nutrient availability ($p < 0.01$).

During the experiment, the CAT activity of *V. natans* leaves in all

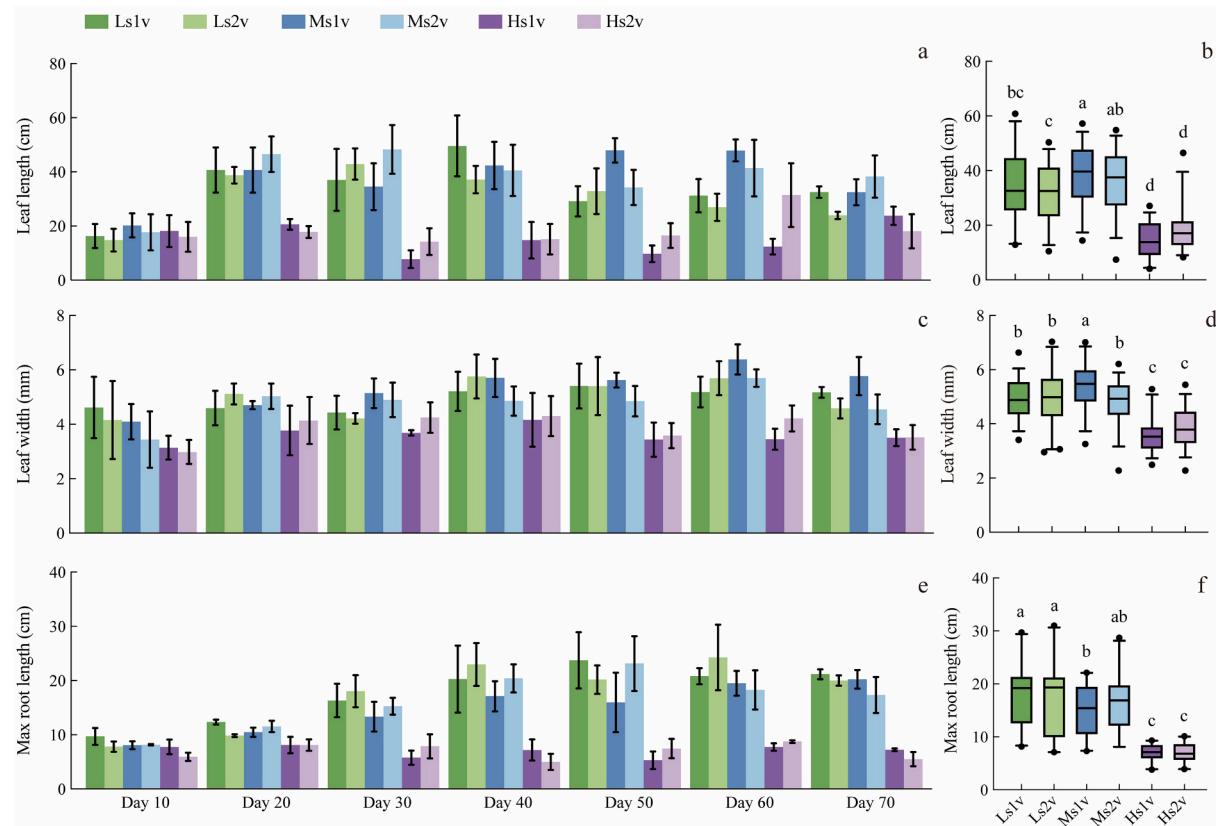


Fig. 3. Changes in leaf length, leaf width, leaf thickness and maximum root length in the different treatment groups during the experiment. Boxes denoted by a different lower case letter differ significantly by ANOVA at $p < 0.05$.

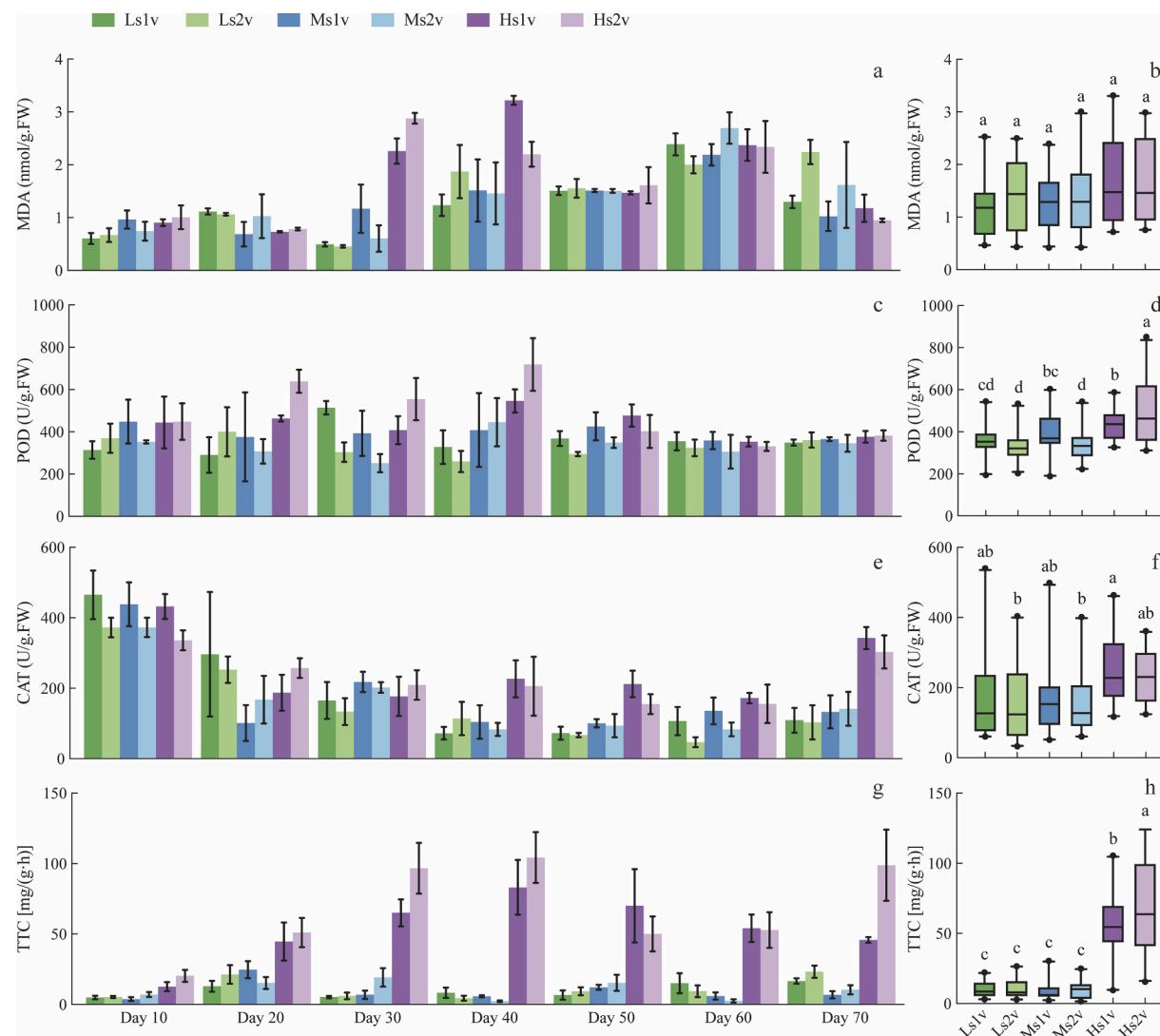


Fig. 4. Changes in malondialdehyde (MDA), peroxidase (POD), and catalase (CAT) contents and root vigor (TTC) of *V. natans* leaves in the different treatment groups. Boxes denoted by a different lower case letter differ significantly by ANOVA at $p < 0.05$.

treatment groups showed a significant decrease from day 10 to day 20, followed by an increase from day 70 in higher water depth (Fig. 4e). Water depth had a significant independent effect on CAT activity (Table S4, $p < 0.05$). The CAT at a depth of 200 cm was significantly higher than that at a depth of 60 cm ($p < 0.05$) and higher than that at a depth of 130 cm by 71.218 U/g.FW ($p < 0.05$).

The TTC content at a depth of 200 cm was markedly higher than at other depths, and under low nitrogen-phosphorus sediment conditions, the TTC content was significantly higher than under high nitrogen-phosphorus sediment conditions (Fig. 4h, $p < 0.05$), indicating that *V. natans* requires higher root vitality to survive when subjected to high water depth and low-nutrient stress conditions. Sediment nutrient content has a more pronounced independent effect on the TTC content of *V. natans* than water depth (Table S4, $p < 0.01$). Under low nitrogen-phosphorus sediment conditions, the mean root activity in *V. natans* exceeds that under high nitrogen-phosphorus conditions by 6.888 mg/(g·h) ($p < 0.05$). Additionally, at a water depth of 200 cm, the mean root activity is significantly higher, surpassing that at 60 cm and 130 cm by 49.13 mg/(g·h) and 50.767 mg/(g·h), respectively ($p < 0.01$).

During the experimental process, there was a general similarity in the overall trends of Chl *a*, Chl *b*, Car, and Chl content in *V. natans* across different treatment groups (Fig. S1). The Ms1v group, benefiting from suitable light conditions due to the appropriate water depth and ample

nutrients provided by the sediment, exhibited the highest photosynthetic pigment content, as photosynthesis was enhanced. Conversely, the Hs2v group, due to underwater light attenuation and inadequate nutrient availability, experiences reduced light energy absorption efficiency, resulting in the lowest photosynthetic pigment content. The independent and interactive effects of water depth and sediment had no statistically significant impact on Chl *a*, Chl *b*, Car, and Chl content in *V. natans* leaves (Table S4, $p > 0.05$). At water depths of 130 cm and 200 cm, the sediment high in nitrogen and phosphorus content led to higher average values of all photosynthetic pigments and total pigments, as it provided sufficient nutrients for growth and photosynthesis compared to the low nitrogen-phosphorus sediment group.

4. Discussion

4.1. Effect of water depth and sediment on the growth pattern of *V. natans*

The morphology of submerged plants often changes with environmental variations (Fu et al., 2014) and therefore, the morphological characteristics of plants reflect their adaptive strategies to the environment. *V. natans*, as a basal rosette-type submerged plant, has a strong tolerance to low light (Yu et al., 2016). Karl (Havens, 2003) believed that water depth is closely related to the decrease in light intensity, and

under certain water depth conditions, when the light intensity cannot reach the compensation point for plant growth, submerged plants respond to changing environments by altering their leaf characteristics, while submerged plants in shallow water areas often experience light inhibition due to direct strong light far exceeding the compensation point (Blanch et al., 1998). In this experiment, *V. natans* exhibited a series of positive responses to water depth, including leaf length, leaf width, clonal number, maximum root length, and total root length. As the water depth increases from 60 cm to 130 cm, *V. natans* increases its leaf length and width in order to obtain more light, thereby improving its photosynthetic production capacity. At a water depth of 200 cm, *V. natans* had shorter leaf length, narrower leaf width, fewer clonal numbers, smaller maximum root length, and smaller total root length, significantly different from other water depths ($p < 0.05$). This difference is due to the exponential attenuation of light upon entering the aquatic environment. With increasing water depth, photosynthesis and usable light for growth are greatly reduced, posing a considerable threat to the normal growth of *V. natans*. Photosynthesis and water vapor exchange in *V. natans* are constrained, and its capacity for morphological plasticity regulation is weakened, resulting in reduced plant height, leaf width, maximum root length, and other parameters. (Wang et al., 2021; Zhou et al., 2017b).

Submerged plants can absorb most of their mineral nutrients from the sediment through their root system. Studies have shown that fertile sediment stimulates plant growth (Zhu et al., 2012), indicating that sufficient nitrogen and phosphorus in the sediment can provide adequate nutrients for the growth and development of *V. natans*. In this study, the leaf width of *V. natans* was influenced by the interaction of sediment and water depth. At a water depth of 130 cm, the leaf width was greater under high-nitrogen and phosphorus conditions in the sediment, indicating positive feedback between leaf width and nutrient content in the sediment at this water depth. This study also found that sediment with different nutrient conditions significantly affected the root growth shape of *V. natans*, as the ProjArea, SurfArea, and RootVolume of *V. natans* in sediment with low nitrogen and phosphorus were significantly larger than those in sediment with high nitrogen and phosphorus. This is attributed to *V. natans* in the low nitrogen and phosphorus group increasing the exposed absorption surface area in the sediment to acquire nutrients (Barko et al., 1991). It is also possible that in the high nitrogen and phosphorus group of this study, the sediment nutrient condition was already sufficient to meet the growth demands of *V. natans* roots. When there is an adequate supply of nutrients, plants may not need to develop large root systems to absorb nutrients (Madsen and Cedergreen, 2002). Studies have also shown that an increase in phosphorus content in the sediment may inhibit the growth of submerged plants (Wang et al., 2023), leading to a reduction in average root length and primary root diameter (Wang et al., 2009).

Based on the above analysis, it can be concluded that the critical factor influencing the morphology of *V. natans* is water depth. The attenuation of underwater light intensity due to increasing water depth affects the growth of *V. natans*. Nutrient conditions of sediment also significantly impact the growth morphology of *V. natans*. Under more suitable water depth conditions (130 cm), high nitrogen and phosphorus in the sediment promote the elongation and widening of *V. natans* leaves. The ProjArea, SurfArea, and RootVolume of roots under higher nitrogen and phosphorus sediment conditions are all smaller than those under low nitrogen and phosphorus sediment conditions. In addition, during the experimental process, the increase in water depth leads to an increase in total phosphorus content in the water under high nitrogen and phosphorus sediment conditions, subsequently affecting the growth of *V. natans*. Therefore, it is evident that an increase in water depth will affect the growth of submerged plants and will increase the degree of negative impact of polluted sediment on submerged plants, ultimately exacerbating the stress on their growth. Therefore, maintaining appropriate water depth conditions is particularly crucial for the growth and development of submerged plants. For *V. natans* in this study, water

depth condition of <130 cm were more suitable.

4.2. Effects of water depth and sediment nutrients on the physiological characteristics of *V. natans*

Antioxidant enzyme activity is a highly sensitive indicator that can intuitively reflect the condition of stress on plants. Submerged plants exhibit physiological and ecological adaptability at different water depths through the synergistic action of antioxidant enzymes (Islam and Macdonald, 2004). MDA is the final decomposition product of cytoplasmic membrane peroxidation. The higher the MDA content, the more severe the damage to the cytoplasmic membrane, and the more obvious the growth inhibition (Asaeda and Rashid, 2017). This is consistent with the monitoring data for MDA accumulation, leaf length, leaf width, and maximum root length in *V. natans* leaf tissues at a water depth of 200 cm in this study. During the 30th to 40th day period, the morphological indicators at a depth of 200 cm were lower than at other water depths, with significantly higher MDA content in the leaves. During this period, it was evident that *V. natans* in deep-water habitats experienced significant growth inhibition due to prolonged periods of insufficient light, leading to a diminished physiological and ecological adaptability.

POD is an enzyme that participates in the growth, development, and aging of plants, playing an important role in eliminating free radicals and reducing lipid peroxidation and membrane damage (Shah and Nahakpam, 2012; Zhu et al., 2020). *V. natans* at a water depth of 60 cm was exposed to sufficient underwater light intensity in this study, so it did not need to exhibit higher POD activity to eliminate free radicals and reduce lipid peroxidation and membrane damage. As a result, its POD activity was generally lower than that of plants grown at other water depths. The POD activity was influenced by the interaction between water depth and sediment in this study. At a water depth of 130 cm, the high-nitrogen and phosphorus sediment group had higher POD activity in the leaves of *V. natans*, which is consistent with previous studies that found that high nutrient levels in the substrate can lead to an increase in the concentration of antioxidant enzymes in plants (Kang et al., 2015). During days 20 to 40 of the experimental study, *V. natans* exhibited higher POD activity in the low nitrogen-phosphorus sediment group at a water depth of 200 cm. This suggests that under high water depth conditions, *V. natans* experiences more severe oxidative stress due to the low sediment nutrient conditions. Moreover, it indicates that during this period, *V. natans* at high water depths with low sediment nutrient levels is unable to acquire sufficient nutrients from the sediment to counteract the effects of low light stress.

V. natans possess a sophisticated stress regulation system to cope with adverse environments, maintaining dynamic balance of metabolites in the body. CAT is an important component of this system, catalyzes the conversion of intermediate metabolite H₂O₂ into H₂O and O₂, facilitating its expulsion from plant metabolic tissues. This process mitigates the damage caused by free radicals to organic molecules in plant cells, ensuring normal cellular activities. (Li et al., 2022; Shah and Nahakpam, 2012). In this study, it was observed that the CAT activity in *V. natans* was significantly higher at a depth of 200 cm under low-nitrogen and phosphorus sediment conditions than at other depths ($p < 0.05$). This result suggests that the growth of *V. natans* under low-nutrient conditions is significantly affected by low-light stress, which is consistent with the observed changes in POD activity. As environmental stress intensified during the experimental process, an excess of H₂O₂ oxidized the cytoplasmic membrane, resulting in the production of MDA, which led to impaired cellular function and inhibited the growth and metabolism of *V. natans*. This was evidenced by significant differences in leaf length and root length of *V. natans* at a depth of 200 cm on day 30 compared to other water depths ($p < 0.05$). CAT activity peaked on day 10 in this study, while MDA peaked after day 30, indicating that the accumulation of H₂O₂ exceeded the catalytic decomposition capacity of CAT. Some studies have shown that the response of MDA content in *V. natans* leaves to adverse environments lags behind that of

CAT (Chen et al., 2013), consistent with the results of this study. Therefore, in the actual recovery process of *V. natans* communities, monitoring CAT activity to reflect the growth status of plants has a better early response mechanism than monitoring MDA content.

The root system of plants is an active organ for absorption and synthesis, and the growth of roots directly affects the nutrient status of the aboveground parts of plants. In this study, both water depth and sediment nutrient status had significant independent effects on the root vitality of *V. natans*. The root vitality in the sediment with low nitrogen and phosphorus was considerably higher than that in the sediment group with high nitrogen and phosphorus ($p < 0.05$). This difference is because *V. natans* exhibited higher root vitality under lower nutrient levels to counteract adversity. Similarly, to counteract low-light stress, the root vitality at a depth of 200 cm in *V. natans* was significantly higher than at other depths ($p < 0.01$), and within the low-nitrogen-phosphorus sediment group, *V. natans* root vitality was significantly higher than in the high-nitrogen-phosphorus group ($p < 0.05$). This is likely attributed to *V. natans* displaying heightened root vitality under conditions of high depth and low nutrient sediment, enabling it to simultaneously cope with low-light and nutrient stress.

Chlorophyll is the main pigment used by plants for photosynthesis, and its concentration reflects the plant's ability to carry out photosynthesis to some extent (Ashraf and Harris, 2013). The main function of Chl *a* is to convert light energy into electrical energy for electron transfer, which is ultimately converted into chemical energy. Chl *b* is the main component of the plant's photosynthetic pigment system (Anderson and Aro, 1994), while Car is both photosynthetic pigment and endogenous antioxidant, and its presence protects and stabilizes the structure of the light-harvesting complex (LHC) in the photosynthetic apparatus (Xiao et al., 2011). During the experimental process, *V. natans* at a water depth of 130 cm exhibited an enhanced photosynthetic capacity and improved LHC structure compared to those at a depth of 60 cm by increasing the content of Chl *a*, Chl *b*, and Car in their leaves. This enhancement resulted in a higher light-capturing ability, promoting better growth and reproduction. Similarly, *V. natans* in the 200 cm water depth group responded to low light stress by increasing their photosynthetic pigment content and improving light energy absorption efficiency, although their pigment content remained lower than that of the 130 cm water depth group due to subdued light conditions. In this study, it was observed that the high-nitrogen and high-phosphorus groups had higher pigment content at water depths of 130 cm and 200 cm compared to the low nitrogen-phosphorus groups. This can be attributed to the direct influence of nitrogen, magnesium, and iron deficiency on chlorophyll synthesis (Giompi et al., 1996). Under the same light conditions, the low nitrogen-phosphorus groups had lower photosynthetic pigments due to the lack of nutrients provided by the sediment, resulting in lower photosynthetic rates.

4.3. Conceptual model of disaster points in shallow lake ecosystems under the synergistic influence of water depth and sediment nitrogen and phosphorus

During the process of transitioning from a macrophyte-dominated to an algal-dominated state in shallow lake ecosystems, water depth not only may serve as a crucial factor influencing the composition and growth of submerged vegetation communities (Scheffer et al., 1993), but also could impact the migration and transformation of nitrogen and phosphorus within sediments, consequently altering the characteristic factors and thresholds driving the state transition process. The primary features of a shift from macrophyte dominance to algal dominance in lakes under nitrogen and phosphorus nutrient stress are the progressive degradation of aquatic vegetation, with submerged vegetation degradation being particularly conspicuous. When total phosphorus and total nitrogen concentrations exceed 0.1 mg/L and 2 mg/L, respectively, aquatic vegetation coverage nearly approaches zero (Gonzalez Sagrario et al., 2005).

In this study, an increase in water depth resulted in elevated total phosphorus levels in the overlying water of sediments rich in nitrogen and phosphorus, further leading to an increase in Chl *a-w* content (Table 1). Increased phytoplankton biomass resulted in nutrient and spatial competition, reduced light availability due to phytoplankton shading, and physiological stress on submerged vegetation due to excess nutrient levels in the water, all negatively affecting submerged vegetation growth and leading to degradation (Iversen et al., 2019; Xu et al., 2020; Rodrigues et al., 2020). Under high water depth conditions in this study, especially in sediments with high nitrogen and phosphorus contents, the growth of submerged vegetation was found to be poor, characterized by shorter leaves, narrower leaf width, fewer shoots, higher antioxidant enzyme activity, and greater root vitality. At this point, not only did the increase in water depth inhibit submerged vegetation growth through light limitation, but the rising phosphorus concentration and chlorophyll content (indicative of algal biomass) in the overlying water due to increased water depth also imposed stress on submerged vegetation growth, exacerbating the stress on submerged vegetation. Hence, we propose a conceptual model of an ecological tipping point in shallow lake ecosystems influenced by water depth and the synergistic effects of sediment nitrogen and phosphorus (Fig. 5). This model suggests that changes in water depth, along with their combined impact on nitrogen and phosphorus nutrient levels in shallow lakes, affect submerged vegetation growth. Given the typical multistable characteristics of shallow lakes (Scheffer and van Nes, 2007), even slight changes in water levels when nutrient concentrations approach the threshold can profoundly impact the lake ecosystem (Leira and Cantoni, 2008; Smith and Brock, 2007; Selkoe et al., 2015). In eutrophic shallow lakes with significant contaminated sediment pollution, phosphorus release from the sediments may increase significantly with water depth, further accelerating the degradation of submerged plants. Therefore, in response to water depth, the nitrogen and phosphorus threshold for regime shift in shallow lakes can be altered. Subsequent experiments could further investigate how the nitrogen and phosphorus nutrient thresholds for state transitions change across different water depth gradients, contributing to a better understanding of state transitions under coupled conditions of water depth gradients and nutrient concentrations.

5. Conclusion

The development features and physiological parameters of *V. natans* were significantly influenced by the independent and combined impacts of water depth and sediment nutrient concentration. As the water depth increases, *V. natans* adapts to growth under weak light conditions by adjusting their material and energy allocation, such as increasing plant height and leaf width, and reducing root length. However, larger water depths (200 cm) will inhibit their growth. The nutrient concentration of sediment also has an impact on the morphology of *V. natans*, with a more significant impact on root morphology. Under conditions of higher water depth and low nutrient levels, the roots of *V. natans* are shorter but denser to cope with the dual stress of light and nutrition, and exhibit high antioxidant enzyme activity, root activity, and photosynthetic efficiency. *V. natans* grow better under moderate water depth and higher sediment nutrient conditions due to sufficient light and nutrient resources. Overall, the influence of water depth is greater than that of sediment. Increasing water depth can further affect the growth of submerged plants by promoting the release of phosphorus from sediment. Therefore, in lakes with more severe sediment pollution, the increase in water level exacerbates the stress of water depth on submerged plants, accelerating the shift from a clear water state dominated by submerged plants to a turbid water state dominated by phytoplankton in lakes. This study identifies the appropriate water level and substrate for the growth of submerged plants, which is of great significance for the restoration of submerged vegetation and benign ecological system management.

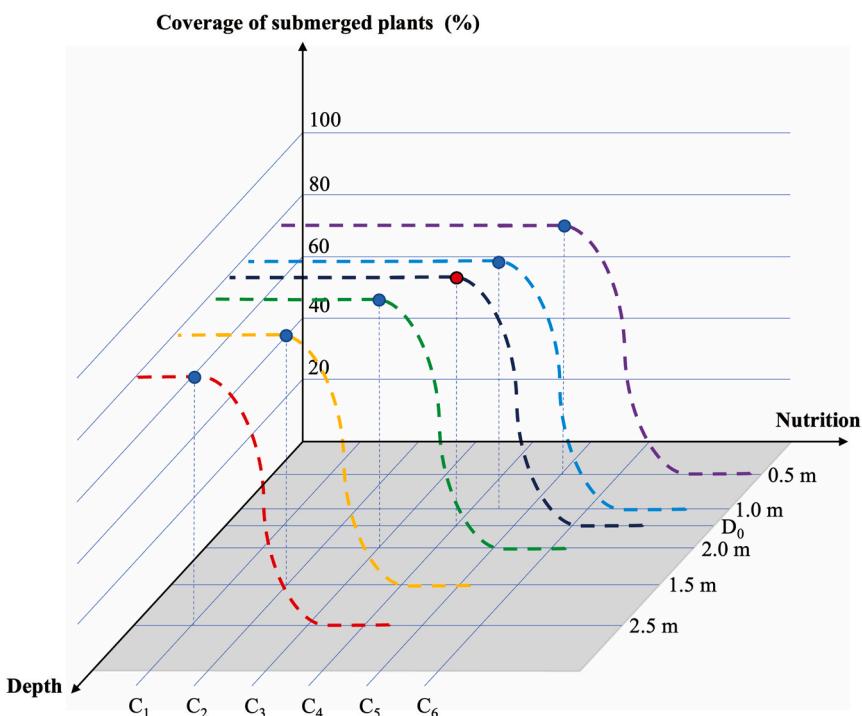


Fig. 5. Conceptual model of an ecological tipping point in shallow lake ecosystems influenced by water depth and the synergistic effects of sediment nitrogen and phosphorus.

CRediT authorship contribution statement

Siwen Chen: Writing – original draft. **Lei Jiang:** Writing – review & editing. **Shuzhan Ma:** Writing – review & editing. **Yue Wu:** Writing – review & editing. **Qi Ye:** Writing – review & editing. **Yiheng Chang:** Writing – review & editing. **Ye Ye:** Supervision. **Kaining Chen:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.169154>.

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