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Effects of Planting Density on Water Restoration Performance of *Vallisneria spinulosa* Yan Growth System Constructed by Enclosure

Weiguang Sun [1], Jia Xing [2,*], Xinyu Li [3] and Suqing Wu [3,*]

1 School of Intelligent Building Engineering, Nanchang Institute of Technology, Nanchang 330044, China; swg0120@163.com
2 Heilongjiang Academy of Environmental Sciences, Harbin 150090, China
3 College of Life and Environmental Science, Wenzhou University, Wenzhou 325000, China; 17320025003@163.com
* Correspondence: xingjia0827@163.com (J.X.); wusuqing@wzu.edu.cn (S.W.)

Abstract: Submerged macrophytes play a crucial role in the ecological restoration of aquatic environments, and enclosed plot planting technology is one of the economical and effective methods to establish submerged macrophyte communities in high-turbidity water bodies. This study focused on *Vallisneria spinulosa* Yan (*V. spinulosa*), examining the impact mechanism of planting density on the water restoration effectiveness of *V. spinulosa* growth systems constructed within enclosed plots, based on its growth and physiological characteristics as well as the water purification effects of its growth system. The research results indicate that low to medium planting densities (50–100 plants/m^2) favor leaf elongation and expansion, as well as the growth of root diameter, surface area, and volume, while high densities (150–200 plants/m^2) inhibit leaf and root growth. The content of photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids) in *V. spinulosa* increased with planting density. At high densities, significant increases in superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) levels in *V. spinulosa* suggest enhanced antioxidant activity. High protein content at low densities indicates stronger metabolic activity. Medium planting density (100 plants/m^2) had significant effects on increasing dissolved oxygen (DO), regulating pH, and reducing electrical conductivity (EC), and exhibited the optimum removal loadings for total phosphorus (TP), phosphate (PO_4^{3-}-P), total nitrogen (TN), and nitrate (NO_3^-), achieving the average value of 0.44, 0.42, 6.94, 0.83 mg m^{-2} d^{-1}. The findings of this study can provide a theoretical basis and technical support for practical ecological restoration projects involving submerged macrophytes in aquatic environments.

Keywords: enclosed; *Vallisneria spinulosa* Yan; water restoration; planting density

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1. Introduction

Submerged macrophytes play a crucial role in the ecological restoration of water bodies. However, some open water areas maintain high pollutant loading and frequent hydraulic disturbance, which increases difficulties in the construction of a submerged macrophyte community. In addition, hydraulic disturbance caused by fish and wind waves in these areas may lead to high concentrations of suspended solids (SSs), and consequently high water turbidity in water bodies [1,2]. SSs in water bodies reduce underwater light intensity, which inhibits photosynthesis of submerged macrophytes, thus exerting a significant inhibitory effect on their growth [3]. Furthermore, water hardness (Ca^{2+} and Mg^{2+}) will affect the precipitation of SSs, further influencing underwater light intensity, which also significantly affects the growth of submerged macrophytes. Therefore, overcoming these unfavorable factors and effectively constructing a submerged macrophyte community is significant for enhancing the water restoration effect of submerged macrophytes [4].

To solve these issues, ecological enclosure planting technology was applied in constructing a community of some submerged macrophytes (i.e., *Vallisneria natans*, *Elodea nuttallii*, *Potamogeton crispus*) in real ecological projects for water restoration [5]. It can reduce pollutant loading from external sources and hydraulic disturbance caused by fish and wind waves, thus enhancing effective construction of submerged macrophyte communities and water quality of partial water areas, and finally, improving the water quality of the entire water area [6]. Globally, approximately 35% of lake restoration projects have adopted ecological enclosure planting technology [7]. Its core advantage lies in achieving cascading restoration through hydraulic exchange regulation, namely "pollution isolation—habitat optimization—biological response" [8]. In China, monitoring data from 2022 showed that lakes using ecological enclosure planting technology had a 40–60% increase in total phosphorus removal load compared to traditional methods, with an average annual increase in transparency of 0.8–1.2 m [9]. In Denmark's Væng Lake, the coverage of submerged macrophytes within the enclosed area recovered from 5 to 65%, and the benthic animal diversity index rose from 1.2 to 2.8 [10].

The construction parameters are crucial factors in establishing submerged macrophyte communities using ecological enclosure planting technology, as they influence the water purification effectiveness of submerged macrophytes, water transparency, water turbidity, and aquatic landscape effects [11]. These parameters play significant roles in the structure and function of ecosystems, competition among species, and plant responses to environmental changes. Among them, the planting density of submerged macrophytes is an important parameter that affects the structure of the entire community by influencing the growth space and resource allocation of submerged macrophytes [12]. Studies have shown that if the initial planting density of submerged macrophytes is too low, the risk of individual plant death increases, and the plant community lacks stability. Conversely, if the initial density is too high, plants may be suppressed or even killed due to competition for space and resources [13]. Additionally, this can increase construction costs and result in resource wastage. Liu et al., through a 150-day study, established populations at two density levels and found that populations with higher plant densities may facilitate the restoration of submerged macrophytes in degraded wetlands [14]. Furthermore, the density of submerged macrophytes is related to the number of plankton species. As the density of submerged macrophytes increases, the biomass of phytoplankton decreases, and the total number of plankton species tends to increase. When submerged macrophytes are uniformly distributed at a density of 60%, the number of plankton species is highest, allowing for balanced development among various plankton species [15].

Previous studies have indicated that the planting density of submerged macrophytes is one of the key parameters in establishing submerged macrophyte communities using ecological enclosure planting technology. However, the mechanism through which it affects the water restoration effectiveness of the enclosed submerged macrophyte growth system remains unclear. Based on this, this study focused on *V. spinulosa*, a common submerged macrophyte in surface water bodies, and investigated the impact of its planting density on the water restoration effectiveness of the enclosed *V. spinulosa* growth system, in terms of the growth and physiological characteristics of *V. spinulosa* and the water purification effectiveness of its growth system. The aim of this study is to provide a theoretical basis and technical support for practical submerged macrophyte-based aquatic ecological restoration projects.

2. Materials and Methods

2.1. Experimental Materials

V. spinulosa was collected from Honghu Lake (Hubei, China). Plants of similar length, biomass, and growth status, which had not yet produced offspring tillers, were selected. Firstly, the *V. spinulosa* plants were rinsed twice with tap water to remove any epiphytes and snails adhering to their surfaces. Subsequently, they were domesticated and cultivated in flowerpots for 15 days, using actual lake sediment as the planting substrate and actual lake water for irrigation, with specific water quality details provided in Table 1.

Table 1. Influent water quality.

Index	TN (mg/L)	NH_4^+-N (mg/L)	NO_3^--N (mg/L)	TP (mg/L)	pH	Turbidity (NTU)
Numeric value	0.9–1.3	0.5–0.7	0.4–0.6	0.07–0.14	6.8–7.8	100–110

2.2. Experimental Design

In this experiment, five relatively independent enclosed plots (1.0 m × 1.0 m) were constructed in an actual lake using impermeable enclosures, as shown in Figure 1. The bottom of each enclosure was embedded in the sediment with galvanized iron chains, and quartz sand bags were laid inside to prevent water exchange between the enclosed area and the surrounding water body. *V. spinulosa* was planted in PP material flowerpots (diameter of 19.8 cm, height of 18.5 cm), with a 12 cm thick layer of lake sediment as the planting substrate. The planting densities of *V. spinulosa* were set at 50, 100, 150, and 200 plants/m^2, with a natural settlement group without *V. spinulosa* serving as the control (CK). A vacuum pump was used to mix and homogenize a sediment solution (lake bottom mud sieved to 100 μm), which was then added to the water inside the enclosures to adjust the turbidity to 100 NTU. The water and plant samples were selected at three places in each enclosure as three replicates, and the water treatment by each enclosure was conducted for six influent cycles. The hydraulic retention time of each influent cycle in each enclosure was 7 d.

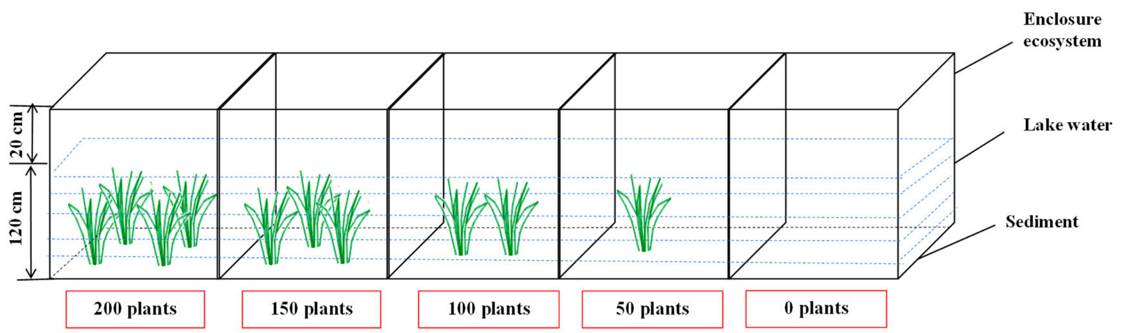

Figure 1. Schematic diagram of the construction of the enclosed block.

2.3. Monitoring and Analysis Methods

2.3.1. Water Quality

The pH, dissolved oxygen (DO), water temperature (WT), and oxidation–reduction potential (ORP) of the water surrounding *V. spinulosa* were measured using a YSI water quality analyzer (YSI ProQuatro, Marion, MA, USA). The concentrations of total nitrogen (TN), ammonia nitrogen (NH^{4+}-N), nitrate (NO_3^--N), nitrite (NO_2^--N), total phosphorus

(TP), phosphate (PO_4^{3-}-P), and chemical oxygen demand (COD) in the water samples were determined using the alkaline potassium persulfate digestion-UV spectrophotometric method, Nessler's reagent spectrophotometric method, phenol-disulfonic acid spectrophotometric method, N-(1-naphthyl)-ethylenediamine spectrophotometric method, potassium persulfate digestion-ammonium molybdate UV spectrophotometric method, ammonium molybdate UV spectrophotometric method, and Hach COD test method, respectively. The turbidity of the water was measured using a Hach turbidity meter (2100Q, HACH, Loveland, CO, USA).

2.3.2. Growth Characteristics of *V. spinulosa*

The leaf length of *V. spinulosa* was measured using a 20 cm ruler. The leaf of *V. spinulosa* was collected from three basins in each enclosure, and three leaves were collected from each basin, which were used to measure the leaf length of *V. spinulosa*. Their average value in each basin was calculated. The number of leaves on *V. spinulosa* was counted manually, with any leaf longer than 5 cm counted as one, and the average number of leaves was taken as the average for that group. Leaf width was analyzed using a Wanshen root analyzer.

The root length, root diameter, root surface area, and root volume of *V. spinulosa* were analyzed using a Wanshen root analyzer system (LA-S, Hangzhou, China), including a dual light source color scanner, a color imaging high-speed camera, and an ultra-thin backlight board. The specific operation process involved selecting an appropriate amount of *V. spinulosa* plants, washing their roots and leaves with distilled water, laying them flat on a transparent imaging root tray with an appropriate amount of distilled water added, scanning the roots and leaves using a light source scanner to obtain corresponding scan images, and then processing the images using software. The data processing was conducted using LA-S series plant image analysis software to obtain the corresponding data.

2.3.3. The Physiological Characteristics of *V. spinulosa*

Fresh leaves of *V. spinulosa* were collected, their surfaces wiped clean, and 0.2 g of the leaves were weighed and their mass recorded. The leaves were then cut into small pieces and placed in a 10 mL centrifuge tube, followed by the addition of 10 mL of 95% ethanol. The tube was left to soak in the dark for 24 h, with shaking occurring four times during this period. After soaking, the wavelength values at 665, 649, and 470 nm were immediately measured using a UV spectrophotometer, avoiding the leaf veins during the measurement process. After the measurements, the concentrations of chlorophyll and carotenoids, as well as the content of each pigment, were calculated.

Fresh leaves of *V. spinulosa* were collected, and 0.1 g of leaves were precisely weighed and their mass recorded. The leaves were appropriately cut into small pieces and placed in a 1.8 mL freezer tube. A 0.9 mL of PBS buffer was added in a 1:9 ratio to the leaves, along with several 2–3 mm zirconia beads. The mixture was then ground in a high-throughput tissue homogenizer to prepare a 10% tissue homogenate. After grinding, the freezer tube was placed in a high-speed refrigerated centrifuge and centrifuged at 4000 revolutions per minute (rpm) for 10 min at 4 °C. The supernatant was collected for further testing. The activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and malondialdehyde (MDA) were measured using kits from Jiancheng Bioengineering Institute in Nanjing, following the instructions provided. The soluble sugar content, soluble protein content, and root activity of the *V. spinulosa* leaves were determined using the anthrone method, Coomassie Brilliant Blue G250 staining method (the kit with CAS number of 6104-58-1, pH of 6.4, potency of >5000 mg/kg LD50, solubility of 40 g/L), and triphenyltetrazolium chloride (TTC) method, respectively, with reference to "Experimental Techniques in Plant Physiology" [16].

The anthrone method was used to determine the soluble total sugar secreted by the leaves. Firstly, 1 mL of culture solution containing *V. spinulosa* was collected from the enclosure and added to a 10 mL centrifuge tube. Then, 5 mL of pre-prepared anthrone reagent was added, and the mixture was rapidly shaken to combine. The tube was then subjected to a boiling water bath for 10 min, followed by ice-water cooling. The absorbance was measured at a wavelength of 620 nm, and the content of soluble total sugar was calculated using a standard curve. Additionally, a kit was used to measure the soluble protein secreted by the leaves.

2.4. Data Analysis

The data were analyzed using SPSS 27.0. Analysis of variance (ANOVA) was employed to assess the significance among the groups with different planting densities. The significance threshold was set at $p < 0.05$, with a higher level of significance at $p < 0.01$. The Kruskal–Wallis nonparametric test was used to evaluate the correction between planting density and water restoration.

3. Results and Discussion

3.1. The Growth Characteristics of V. spinulosa

3.1.1. Leaf

As shown in Figure 2a,b, at the initial stage of the experiment, the leaf length of *V. spinulosa* decreased overall with increasing planting density, a trend consistent with the dynamic changes in average leaf length, indicating that high density had a certain inhibitory effect on leaf elongation during this early phase. As the experiment progressed, the leaf length growth rates of the D50 and D100 groups were significantly higher than those of the D150 and D200 groups, exhibiting similar upward trends, suggesting that lower densities were more conducive to sustained leaf growth. By the end of the experiment, compared to their initial states, the leaf lengths of each treatment group increased by 67.33 $\pm$ 0.23%, 78.17 $\pm$ 0.21%, 54.42 $\pm$ 0.22%, and 43.17 $\pm$ 0.19%, respectively, further confirming that planting density had a significant impact on the dynamic changes in *V. spinulosa* leaf growth. Additionally, as shown in Figure 2c, there were significant differences in leaf width among the treatment groups at the end of the experiment, specifically D100 (0.51 $\pm$ 0.07 cm) > D150 (0.50 $\pm$ 0.04 cm) > D50 (0.41 $\pm$ 0.02 cm) > D200 (0.33 $\pm$ 0.06 cm), with the difference between the D100 and D200 groups reaching statistical significance. This indicated that an appropriate density could promote the lateral expansion of *V. spinulosa* leaves, while a high-density environment might inhibit the normal development of leaf width. As shown in Figure 2d, the number of *V. spinulosa* leaves decreased with increasing planting density, with the D50 group having the highest average number of leaves at 18.26. This suggested that plants in low-density environments enhanced their light-capturing ability by increasing the number of leaves. Combined with the leaf length trends, it was evident that *V. spinulosa* exhibited a certain degree of morphological plasticity under different density conditions, optimizing resource acquisition strategies by adjusting leaf number and size traits. In low-density environments, *V. spinulosa* tended to increase light-capturing efficiency by increasing leaf number and promoting leaf elongation. However, under high-density conditions, limited by competition for resources such as light, nutrients, and space, plant leaf length growth was restricted, leaf width narrowed, and leaf number decreased, indicating that its morphological adjustment ability was suppressed. These results revealed the ecological strategy of *V. spinulosa* to adapt to different planting densities by adjusting leaf morphology, thereby optimizing photosynthetic efficiency and resource use efficiency.

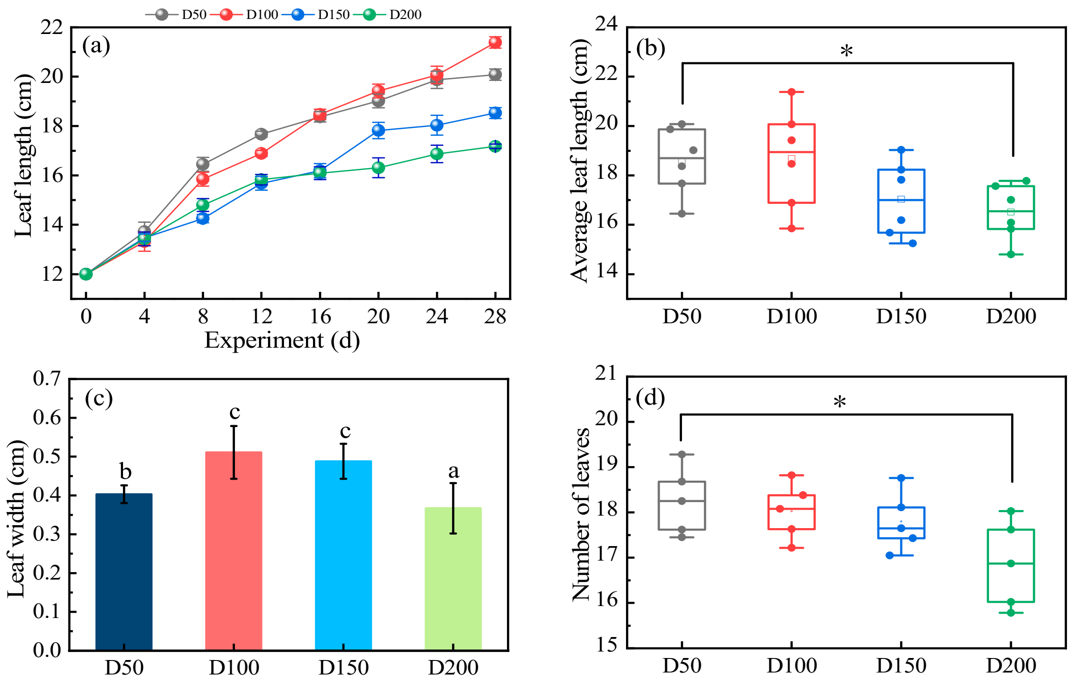

Figure 2. The variation in leaf morphology of *V. spinulosa* under different planting densities: (**a**) leaf length, (**b**) average leaf length, (**c**) leaf width, (**d**) number of leaves. (* represents $p < 0.05$).

The planting density has significant species-specific effects on the growth of submerged macrophytes [17]. Previous studies have shown that submerged macrophytes can adapt to different environmental conditions through morphological variations, and their morphological characteristics usually reflect their resource acquisition and allocation strategies [18]. For instance, research has indicated that when the planting density of *V. spinulosa* increases from 10 plants per square meter to 50 plants, growth parameters such as leaf length, leaf width, maximum root length, and total root length show a positive response; however, when the density exceeds 60 plants per square meter, resource competition among individuals intensifies and growth is restricted, leading to a decline in various morphological indicators [19]. Under high densities, the light transmittance decreases, particularly affecting the photosynthetic efficiency of lower leaves, thereby limiting leaf growth. At the same time, nutrient competition results in limited root development, manifested as shortened maximum root length and total root length [20]. Additionally, studies have suggested that high-density conditions may also inhibit the development of the aerenchyma in *V. spinulosa*, weakening its gas exchange capacity with the water body and further affecting the overall physiological state of the plant [21]. In response to high-density environments, *Vallisneria natans* adopts a vertical expansion strategy, that is, increasing plant height to access light resources while reducing horizontal expansion [22]. However, in this study, it was observed that as density increased, both the leaf length and leaf number of *V. spinulosa* decreased, indicating that it may differ from other submerged macrophytes in its density response strategy. Even under the high-density treatment of 200 plants per square meter, some individuals of *V. spinulosa* survived, suggesting that it possesses a certain degree of individual adaptability and can maintain growth through physiological or morphological adjustment mechanisms in resource-limited environments.

3.1.2. Root Characteristics

After the experiment, four key root parameters of *V. spinulosa*, namely total root length, root diameter, root surface area, and root volume, were measured. As shown in Figure 3a,c,d, with the increase in planting density, the root diameter, root volume, and root surface area generally exhibited a trend of first increasing and then decreasing, reaching their maximum values in the D100 group, which were 1.19 ± 0.11 mm, 4.46 ± 0.37 cm^3, and 49.55 ± 1.49 cm^2, respectively. Further analysis revealed that there was a significant positive correlation between total root length and root surface area with planting density ($p < 0.05$), indicating that density has an important impact on the plastic regulation of root structure. As shown in Figure 3b, the total root length of *V. spinulosa* gradually increased with the increase in planting density, reaching a peak of 208.67 ± 12.96 cm in the D200 group, suggesting that under high-density conditions, *V. spinulosa* may enhance its nutrient and water acquisition ability by increasing root elongation.

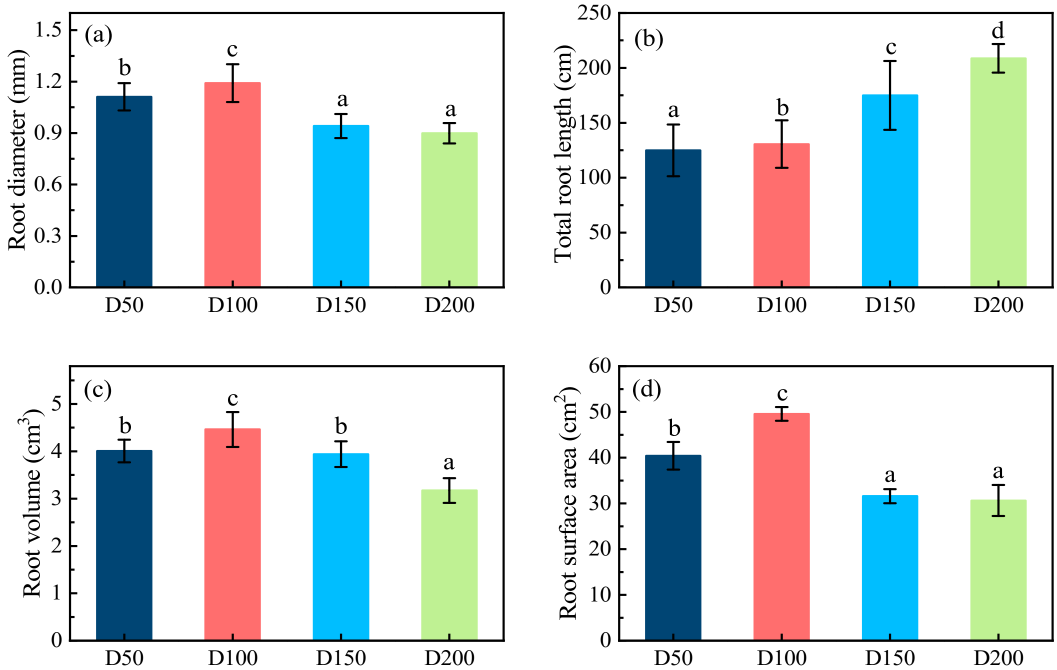

Figure 3. The variation in root morphology of *V. spinulosa* under different planting densities: (**a**) root diameter, (**b**) total root length, (**c**) root volume, (**d**) root surface area (a, b, c and d respectively indicates $p < 0.05$).

However, despite the continuous increase in root length under high-density conditions, the root volume and root surface area showed a downward trend after exceeding the D100 group. This may be due to intensified competition for soil nutrients and spatial resources among individuals under high-density conditions, which inhibits root expansion. Additionally, studies have shown that roots are active organs for absorption and synthesis, and their growth status and activity levels directly affect the nutrient supply to the aerial parts and overall growth [23]. Previous research has indicated that *V. spinulosa* can adapt to density changes by adjusting its root growth pattern. Within the range of 10 to 60 plants per square meter, the root mass, root diameter, root length, root area, and root volume of *V. spinulosa* all decrease as density increases [24]. In this experiment, the root diameter, root volume, and root surface area of *V. spinulosa* also decreased with increasing density. This may be because, under high-density environments, intensified competition for nutrients and space among individuals forces plants to allocate more resources to aerial growth to enhance light competitiveness, thereby reducing investment in root growth. This results in changes in root growth patterns and limits root expansion.

Overall, *V. spinulosa* exhibited significant root growth plasticity under different planting densities ($p < 0.01$). Under low to medium densities, the root structure developed optimally, showing larger root diameter, volume, and surface area, which enhanced water and nutrient absorption efficiency. In high-density conditions, although the plant adapted to resource competition by increasing root length, restricted space and nutrient availability prevented a proportional increase in root volume and surface area. These findings indicate that *V. spinulosa* adjusted its root architecture in response to planting density, optimizing resource acquisition strategies to adapt to varying growth environments.

3.2. The Physiological Characteristics of V. spinulosa

3.2.1. Photosynthesis in Leaves

To cope with adverse conditions caused by density variations, submerged macrophytes typically possess self-regulatory capabilities to mitigate environmental stress [25]. Beyond morphological responses, plants can adapt to harsh environments through physiological changes such as chlorophyll content, antioxidant enzyme activity, and root vigor [26]. Chlorophyll serves as a key physiological indicator of photosynthetic capacity, indirectly reflecting photosynthetic efficiency and demonstrating plant adaptation strategies to varying light conditions. Under different planting densities, the chlorophyll content in *V. spinulosa* leaves showed a significant positive correlation with density. As illustrated in Figure 4a–c, chlorophyll a, chlorophyll b, and total chlorophyll (a + b) exhibited similar trends, all increasing with planting density and peaking at 200 plants/m^2. This phenomenon may be closely linked to photosynthetic regulation mechanisms. In high-density environments, canopy shading significantly reduced underwater light intensity, subjecting plants to prolonged low-light stress. To compensate for the adverse effects of low light on photosynthesis, *V. spinulosa* likely increased chlorophyll content to enhance light capture efficiency [23]. However, sustained low-light conditions still inhibited the formation and function of photosynthetic organs, ultimately reducing overall photosynthetic capacity.

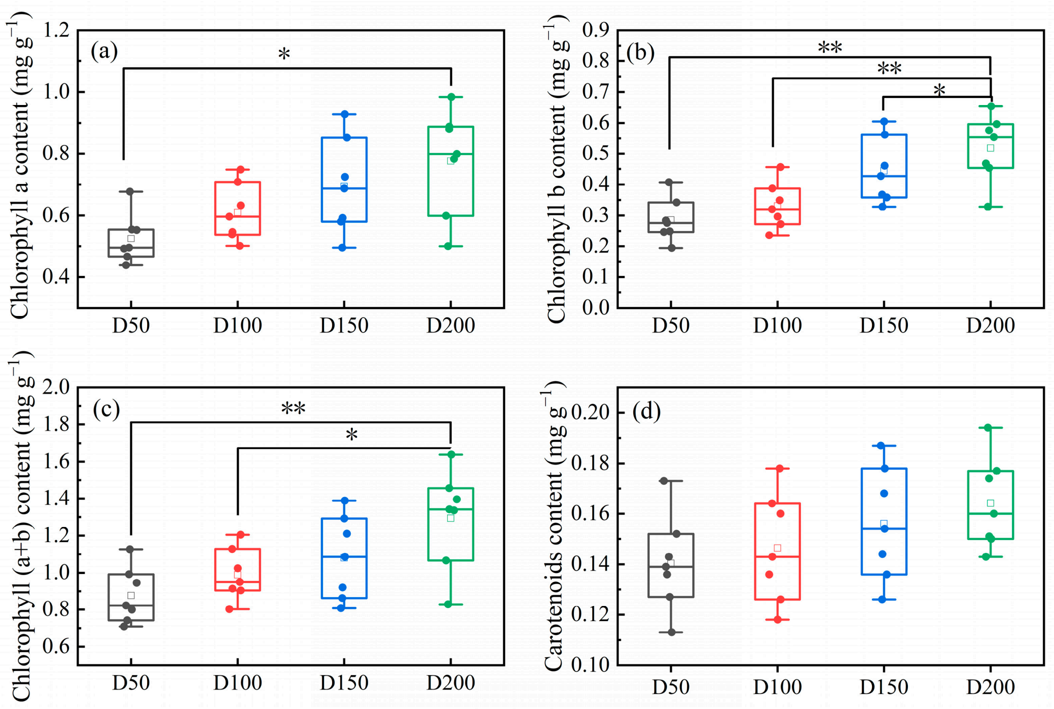

Figure 4. The influence of planting density on the chlorophyll a (**a**), chlorophyll b (**b**), chlorophyll (a + b) (**c**), and carotenoid content (**d**) in *V. spinulosa*. (* represents $p < 0.05$, ** represents $p < 0.01$).

Carotenoids play a crucial role in photosynthesis by absorbing light energy from spectral regions that chlorophyll cannot utilize and transferring it to chlorophyll, thereby improving light utilization efficiency. Additionally, they reduce damage to the photosynthetic apparatus caused by excessive light through photoprotection mechanisms. As shown in Figure 4d, planting density significantly affected the carotenoid content, with a trend similar to that of chlorophyll a, showing an increase with increasing density. This suggests that under high-density conditions, *V. spinulosa* may enhance its light energy utilization capacity and alleviate oxidative stress by increasing carotenoid synthesis in order to adapt to fluctuations in the light environment resulting from density changes [27].

3.2.2. Antioxidant Enzyme Activity in Leaves

Submerged plants demonstrate physiological and ecological adaptability through the synergistic action of antioxidant enzyme activities at different planting densities [28]. As shown in Figure 5a, the SOD activity in the leaves of *V. spinulosa* exhibited a significant upward trend with increasing planting density, with the SOD activity in the D150 and D200 groups reaching 159.4 ± 12.8 and 165.5 ± 15.6 U g^{-1}, respectively, which were significantly higher than those in the low-density group ($p < 0.05$). This phenomenon may be related to the accumulation of reactive oxygen species (ROS) within *V. spinulosa* cells under high-density conditions [29]. At higher densities, the ROS levels remained within the clearance capacity of SOD, so *V. spinulosa* effectively scavenged excess ROS by short-term enhancement of SOD activity to maintain cellular homeostasis and membrane system stability. Additionally, as shown in Figure 5b, there was no significant difference in POD activity under different planting densities. However, during the experiment, the POD activity in all density groups showed a trend of first increasing and then decreasing, which may be related to the oxidative stress induced by density stress [30]. In the early stages of density stress, *V. spinulosa* activated POD to participate in ROS scavenging to enhance antioxidant defense capabilities. However, as the density further increased or the stress persisted in the later stages, the antioxidant system became overloaded, leading to a decrease in POD activity. This trend differs from previous research findings, indicating that the antioxidant enzyme activity in *V. spinulosa* has a protective effect within a certain range of stress intensities, but beyond this range, the defense system function may fail.

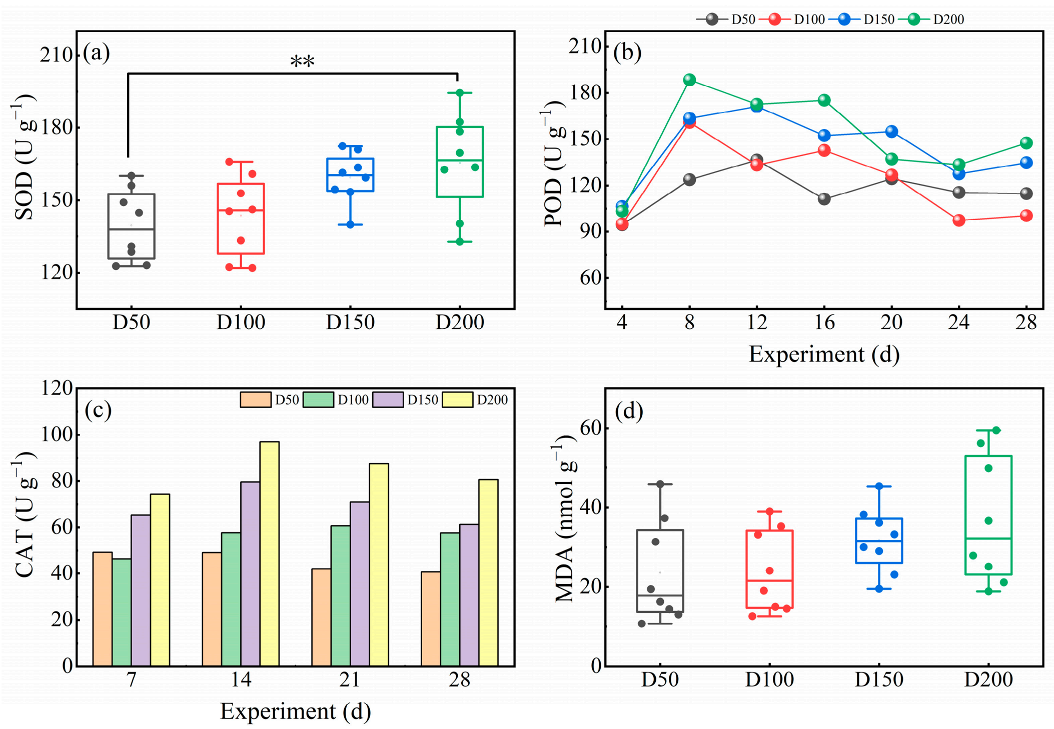

Figure 5. The effect of planting density on the antioxidant enzyme activity of *V. spinulosa* leaves: (**a**) SOD activity, (**b**) POD activity, (**c**) CAT activity, (**d**) MDA activity. (** represents $p < 0.01$).

As shown in Figure 5c, CAT activity exhibited a significant trend of first increasing and then slowly decreasing as planting density increased. On the 14th day of the experiment, the CAT activity in the D200 group reached a peak of 96.9 ± 6.8 U g^{-1}, significantly higher than that in other density groups ($p < 0.01$). In the initial stages of high-density environments, *V. spinulosa* induced an antioxidant response to alleviate oxidative stress caused by ROS accumulation. However, in the later stages of the experiment, under long-term high-density stress, the antioxidant defense capacity of *V. spinulosa* gradually decreased, leading to cumulative oxidative damage to the plant. Additionally, the CAT activity in the low-density (D50) and medium-to-low-density treatment groups (D100) remained stable at around 50.4 ± 3.7 U g^{-1} with no significant changes during the experiment, indicating that *V. spinulosa* can maintain stable antioxidant capacity and adapt to environmental stress within the range of medium-to-low densities [28]. In this study, the degree of membrane lipid peroxidation in *V. spinulosa* leaves was also measured. As shown in Figure 5d, the MDA content in the D200 group was significantly higher than that in other treatment groups. MDA content is an important indicator of membrane lipid peroxidation. Under high-density conditions, competition among plant individuals intensifies, leading to increased ROS accumulation and subsequently elevated membrane lipid peroxidation levels, suggesting that the plant is unable to effectively mitigate high-intensity oxidative stress, ultimately inhibiting its growth, development, and ecological adaptability [31].

Previous studies have indicated that under high-density stress conditions, the activities of antioxidant enzymes such as SOD, POD, and CAT in plant leaves generally increase gradually with increasing density to maintain ROS balance [30]. The results of this study partially support this view, but the POD activity of *V. spinulosa* showed a decreasing trend in the later stages, suggesting that the defensive function of the antioxidant enzyme system may malfunction under severe stress conditions, leading to increased membrane lipid peroxidation, manifested as a significant increase in MDA content [31]. Overall, under high-density stress conditions, *V. spinulosa* responds to oxidative stress by increasing antioxidant enzyme activity. However, when the stress intensity exceeds its tolerance range, the function of the plant's antioxidant system is impaired, limiting the ecological adaptability and growth performance of *V. spinulosa*.

3.2.3. Leaf Secretions

Soluble sugars serve as an important energy source for plant growth and are fundamental to plant metabolism. Changes in their content can reflect a plant's resistance under stressful conditions. Research by Zhang Jing et al. showed that within a planting density range of 15 to 75 plants per square meter, the soluble sugar content of *V. spinulosa* peaked at 30 to 45 plants per square meter [32]. However, in this experiment, the soluble sugar content of *V. spinulosa* showed a gradual increase with increasing planting density and reached its highest value in the D200 group, as shown in Figure 6a. This may be related to the reduction in photosynthetic carbon assimilation caused by the canopy shading effect, prompting plants to quickly release soluble sugars by degrading starch to maintain osmotic balance [33]. Additionally, plants exhibit different metabolic responses to density stress under different experimental conditions. Under high-density conditions, plant metabolism accelerates to meet energy demands and initiate stress responses, accompanied by enhanced protein degradation and significant consumption of soluble sugars.

Soluble proteins play a crucial role in the growth, reproduction, and metabolism of *V. spinulosa*, serving as an important indicator of its developmental processes and stress response mechanisms. As shown in Figure 6b, the soluble protein content of *V. spinulosa* exhibited a significant decrease with increasing planting density, and the impact of density on its content reached a significant level. Among them, the D50 group had the highest soluble protein content, at 8.6 ± 1.3 mg g^{-1}, indicating that a low-density environment can enhance the metabolism of *V. spinulosa*, which is more conducive to the synthesis of soluble proteins for adapting to environmental changes.

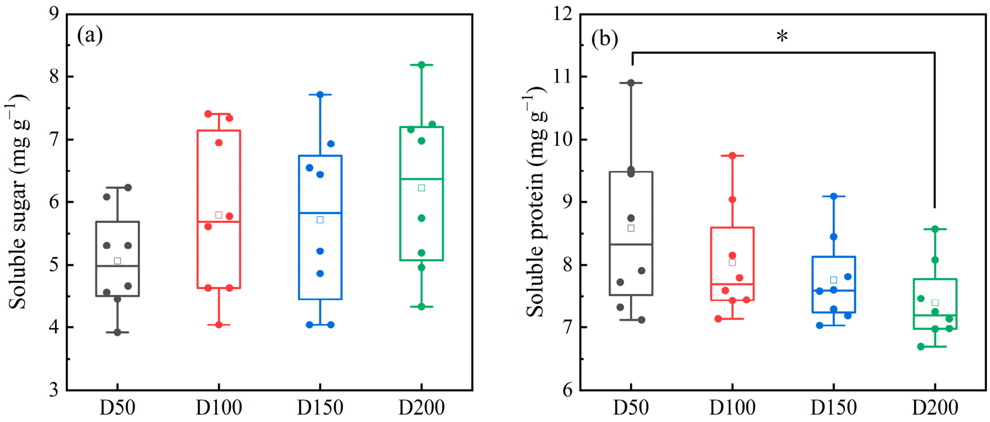

Figure 6. Planting density on the influence of soluble sugar (**a**) and soluble protein (**b**) in *V. spinulosa* (* represents $p < 0.05$).

3.3. The Water Restoration Effectiveness of the System

3.3.1. The Physicochemical Environment of the Water Body

As shown in Figure 7a, the water temperature remained stable between 31 and 36.5 °C during the experimental period. According to Figure 7b,c, as the density of *V. spinulosa* increased, both DO ($p < 0.01$) and pH values ($p < 0.05$) showed a significant increasing trend. There were significant differences in DO among different planting densities, which was consistent with the trend in photosynthesis changes in *V. spinulosa* leaves. This indicated that high-density planting intensified competition for light resources, leading to increased canopy shading among plants, reduced photosynthetic efficiency of leaves, decreased oxygen release flux, and simultaneously increased oxygen consumption due to root respiration, further exacerbating DO consumption [34]. Under high-density planting conditions, the photosynthesis intensity of *V. spinulosa* significantly increased, resulting in the absorption of a large amount of CO_2 and thus enhancing the alkalinity of the water body. As shown in Figure 7d, electrical conductivity (EC) significantly decreased with increasing density of *V. spinulosa* ($p < 0.05$). This may be due to increased nutrient absorption by *V. spinulosa* in high-density environments and enhanced decomposition of organics, which reduced the concentration of dissolved ions in the water body. This result suggests that high-density planting has a significant impact on the chemical environment of the water body and helps improve water quality.

3.3.2. Phosphorus Removal from Water Bodies

Research by Li et al. indicated that planting density has a significant impact on the efficiency of total phosphorus removal by *V. spinulosa* from water bodies [35]. At lower planting densities, the limited number of plants results in a lower overall biomass, which restricts the phosphorus absorption capacity and leads to a lower total phosphorus removal rate. As planting density increases, the biomass of *V. spinulosa* significantly improves, enhancing its phosphorus absorption and immobilization, thereby significantly increasing the total phosphorus removal load. However, when planting density further increases, removal efficiency tends to plateau due to limitations in light exposure, competition among root secretions, and the self-purification capacity of the water body. The results of this experiment are consistent with the aforementioned research conclusions, showing that as planting density increases, the removal load of total phosphorus and phosphate first rises and then stabilizes, as illustrated in Figure 8. In the low-density groups, the limited number of plants results in a lower overall biomass and limited phosphorus removal capacity, leading to a lower total phosphorus removal load. When the planting density is increased to the range of 100–200 plants per square meter, the plant biomass increases, enhancing phosphorus absorption and immobilization, and significantly increasing the total phosphorus removal load. Among them, the D100 group had the highest total phosphorus and phosphate removal loads, which may be related to optimal plant growth conditions, suitable hydrodynamic conditions, and higher biomass. Additionally, higher planting densities can increase the dissolved oxygen content and redox potential of the water body, promoting phosphorus settlement, adsorption, and chemical precipitation, thereby enhancing the effective removal efficiency of phosphorus in the water body [36]. Meanwhile, the abundance of cyanobacteria in the experimental system was high, utilizing dissolved inorganic phosphorus and organic phosphorus in the water as nutrients for growth, further reducing phosphorus concentrations in the water and synergistically promoting the purification effect of the water body [37].

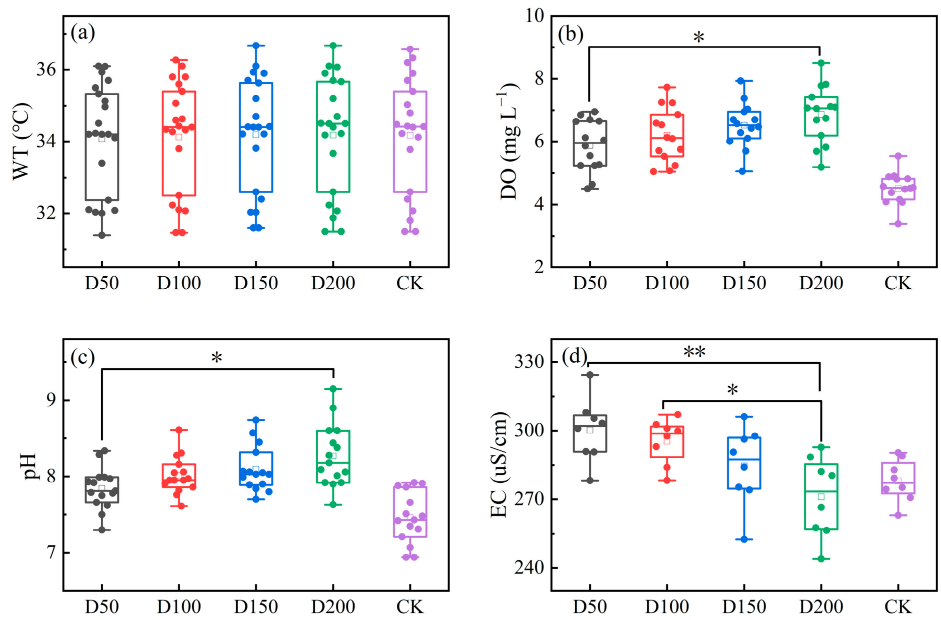

Figure 7. Differences of WT (**a**), DO (**b**), pH (**c**) and EC (**d**) among different planting density groups. (* represents $p < 0.05$, ** represents $p < 0.01$).

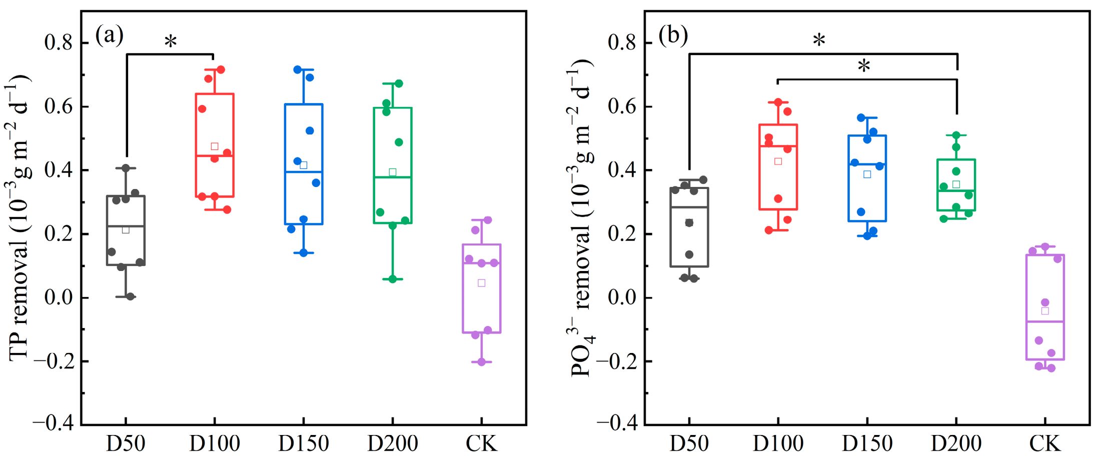

Figure 8. Difference of TP (**a**) and PO_4^{3-}-P (**b**) removal load among different planting density groups. (* represents $p < 0.05$).

3.3.3. Nitrogen Removal from Water Bodies

According to Figure 9a,b, there were significant differences in total nitrogen removal loads among different planting density groups, and as planting density increased, the removal loads of total nitrogen, nitrate nitrogen, and ammonia nitrogen all exhibited a trend of first increasing and then decreasing. Among them, the D100 group had the highest total nitrogen removal load, possibly due to the enhanced photosynthesis of *V. spinulosa* at an optimal planting density, which improved the plant's absorption and utilization capacity for nitrogen nutrients in the water body, thereby promoting the removal of total nitrogen and nitrate nitrogen. This result is consistent with research by Mo et al., who found that the higher the planting density of *V. spinulosa*, the higher the removal efficiency of total nitrogen, mainly attributed to the direct absorption of nutrient nitrogen by submerged plants, microbial nitrification-denitrification, and substrate adsorption and precipitation. However, when planting density was further increased, the removal loads of total nitrogen and nitrate nitrogen decreased instead. This may be due to excessive algae growth caused by excessively high plant density. The proliferation of algae may affect the nitrogen cycle and biodegradation processes in the water body, thereby reducing nitrogen removal efficiency. Further analysis revealed that the nitrate nitrogen removal load was significantly positively correlated with growth parameters such as leaf length, root length, root surface area, and underground biomass of *V. spinulosa*, indicating that nitrate nitrogen removal is mainly influenced by the combined effects of plant absorption and assimilation and denitrification by rhizosphere microorganisms. *V. spinulosa* with developed roots can not only directly absorb nitrate nitrogen but also promote the conversion and removal of nitrate nitrogen through nitrification–denitrification by rhizosphere microorganisms. As ammonia nitrogen is the most easily absorbed and utilized form of nitrogen by plants and microorganisms, its removal is greatly influenced by plant growth status and rhizosphere microbial activity. As shown in Figure 9c, different planting densities had no significant impact on ammonia nitrogen removal loads, with the mean ammonia nitrogen removal load stabilizing between $5.95 \pm 1.28 \times 10^{-3}$ and $7.48 \pm 1.73 \times 10^{-3}$ $g \cdot m^{-2} \cdot d^{-1}$ for all experimental groups. This may be due to the high utilization rate of ammonia nitrogen by rhizosphere microorganisms and the plants themselves during the growth of *V. spinulosa*, which kept the ammonia nitrogen removal load at a consistently high level, and there was no significant difference among different planting densities.

3.3.4. Correlation Between Planting Density and Water Restoration Efficiency

As shown in Figure 10, it is worth noting that there is a positive correlation between plant density and TN, NO_3^--N, NH_4^+-N, TP, PO_4^{3-}-P and turbidity removal loads. The analysis suggests that the increase in plant density can enhance nitrogen, phosphorus, and SS removal. The significant positive correlation between TN removal load and NO_3^--N, NH_4^+-N removal loads reveals that the enhancement of nitrogen removal by the increase in plant density is derived from both NO_3^--N and NH_4^+-N removal. Similarly, the enhancement of phosphorus removal by the increase in plant density is mainly derived from PO_4^{3-}-P removal. In addition, there is a significant positive correlation between plant density and DO and pH, suggesting that the increase in plant density can elevate DO levels and pH values by the enhancement of plant photosynthesis. The significant negative correlation between plant density and EC indicates the increase in ion removal by plants.

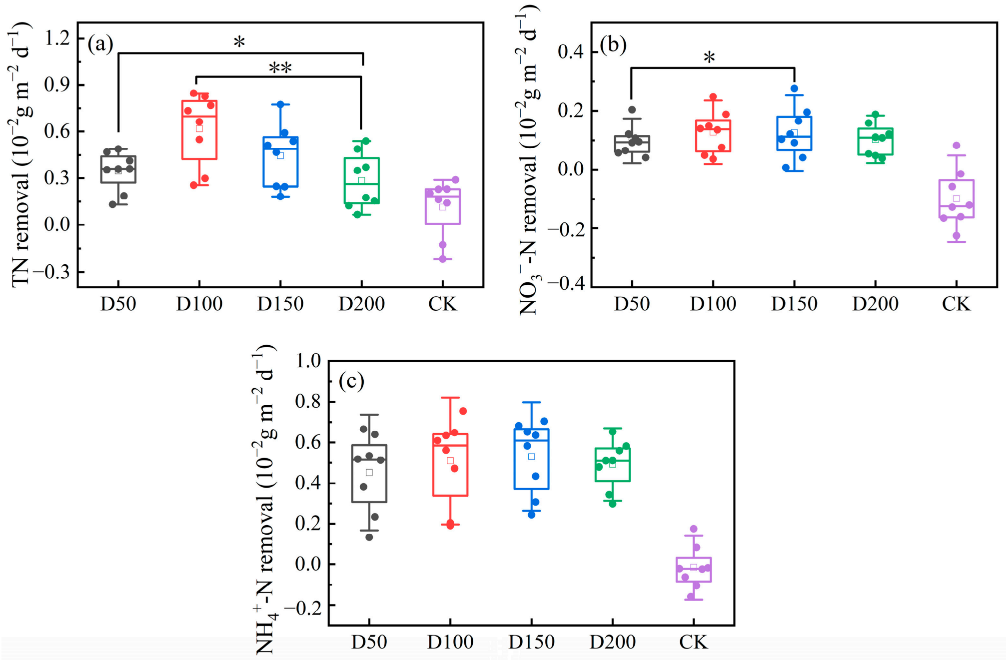

Figure 9. The difference of TN (**a**), NO_3^--N (**b**) and NH_4^+-N (**c**) removal load among different planting density groups. (* represents $p < 0.05$, ** represents $p < 0.01$).

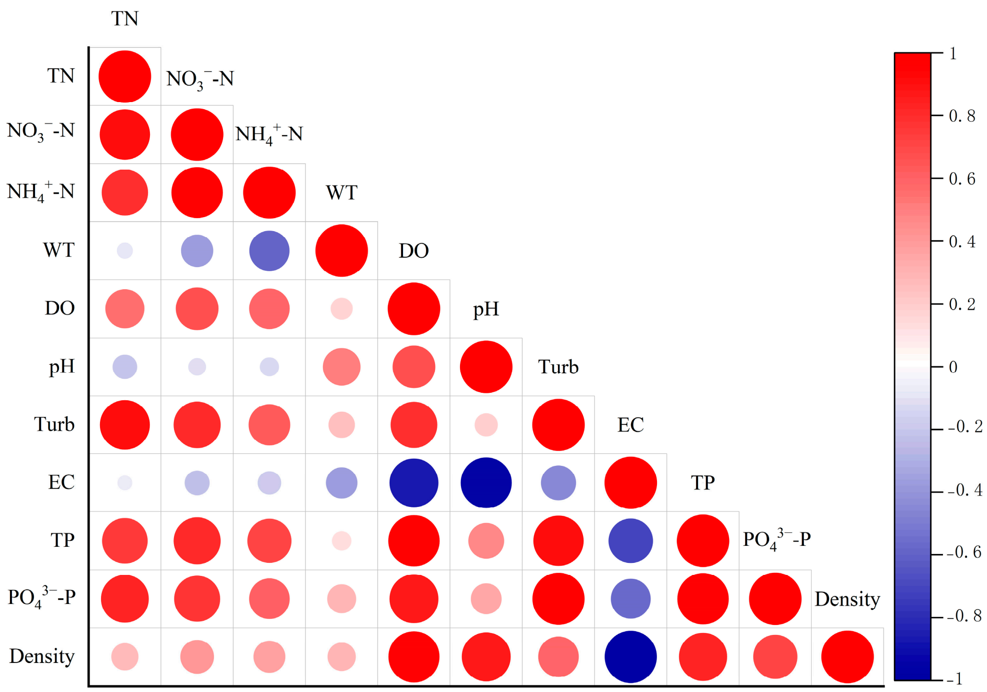

Figure 10. Correlation between planting density and water restoration.

4. Conclusions and Prospect

This study investigated the growth and physiological characteristics of *V. spinulosa* planted in enclosed plots at different planting densities, as well as the purification effects of its growth system on water bodies. The main conclusions obtained are as follows:

(1) In terms of growth characteristics, *V. spinulosa* exhibited significant density-responsive features. Low densities (D50 and D100 groups) favored leaf elongation and expansion, demonstrating stronger light resource acquisition ability and morphological plasticity. However, under high densities (D150 and D200 groups), leaf growth was re-

stricted, indicating suppression due to resource stress. In terms of root structure, medium to low densities (D100 group) promoted the growth of root diameter, surface area, and volume, enhancing nutrient absorption efficiency, while high-density environments inhibited root development.

(2) Regarding physiological characteristics, the content of photosynthetic pigments (Chl-a, Chl-b, Car) increased with density, indicating a certain level of light adaptation and regulation ability. At high densities, the activities of SOD and CAT increased, helping to alleviate oxidative stress. However, POD activity decreased in the later stages, and MDA increased significantly, reflecting the functional limits of the antioxidant system. The increase in soluble sugar content demonstrated a stress-response energy storage mechanism, while high protein content at low densities indicated stronger metabolic activity.

(3) In terms of water purification, an appropriate density (D100 group) had significant effects on increasing DO, regulating pH, and reducing EC, enhancing the redox and ion balance of the water body. In terms of nutrients, the D100 group performed optimally in the removal of TP, PO_4^{3-}, TN, and NO_3^-, while the efficiency of high-density groups tended to saturate.

Based on this study, the construction of a submerged plant community by an enclosure system in water bodies with high water turbidity is feasible. It is suggested to conduct relevant studies to determine the optimum conditions for these systems before application, including plant density, size and area of enclosure size, influent cycle, etc. In addition, only *V. spinulosa* and one kind of water body were studied, and future works should focus on more kinds of submerged plants and water bodies with different water quality.

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References

1. Gomi, T.; Moore, R.; Hassan, M.A. Suspended sediment dynamics in small forest streams of the Pacific Northwest. *J. Am. Water Resour. Assoc.* **2010**, *41*, 877–898. [CrossRef]
2. Walling, D.E.; Fang, D. Recent trends in the suspended sediment loads of the world's rivers. *Glob. Planet. Change* **2003**, *39*, 111–126. [CrossRef]
3. Barko, J.W.; Gunnison, D.; Carpenter, S.R. Sediment interactions with submersed macrophyte growth and community dynamics. *Aquat. Bot.* **1991**, *41*, 41–65. [CrossRef]
4. Hu, S.; Chen, X.; Huang, X.; Wu, C. Submerged Macrophyte Restoration in Enclosure: A Proper Way for Ecological Remediation of Shallow Lakes? *Water* **2023**, *15*, 1317. [CrossRef]
5. Hu, S.; Zhao, D.; He, R.; Sun, X.; Zeng, J. Enclosure restoration regulates epiphytic microbial communities involved in carbon sequestration in a restored urban lake: A new insight from the stability of dissolved organic matter. *J. Clean. Prod.* **2025**, *501*, 145295. [CrossRef]
6. Nan, J.; Yang, C.; Yu, H.; Shi, X.; Huang, Q.; Huang, J.; Li, J. Enclosure culture ban and its effects on water quality and phytoplankton community in a subtropical shallow lake. *Aquac. Res.* **2022**, *53*, 221–231. [CrossRef]

7. ILEC (International Lake Environment Committee). *World Lake Vision Report 2021: Pathways for Sustainable Lake Management*; ILEC Foundation: Shiga-ken, Japan, 2021.
8. Chen, K.N.; Bag, X.M.; Shi, L.X.; Chen, W.M. Ecological restoration engineering in Lake Wuli, Lake Taihu: A large enclosure experiment. *J. Lake Sci.* **2006**, *18*, 417–424.
9. Chinese Academy of Environmental Sciences. *Assessment Report on Ecological Restoration Technology of Chinese Lakes (2022)*; Ministry of Ecology and Environment: Beijing, China, 2023.
10. Sondergaard, M.; Jeppesen, E.; Lauridsen, T.L.; Skov, C.; Vannes, E.H.; Roijackers, R.; Lammens, E.; Portielje, R. Lake restoration: Successes, failures and long-term effects. *J. Appl. Ecol.* **2010**, *44*, 1095–1105. [CrossRef]
11. Lin, X.; Wu, X.; Chao, J.; Ge, X.; Tan, L.; Liu, W.; Sun, Z.; Hou, J. Effects of combined ecological restoration measures on water quality and underwater light environment of Qingshan Lake, an urban eutrophic lake in China. *Ecol. Indic.* **2024**, *163*, 112107. [CrossRef]
12. Li, Q.; Wan, P.; Han, C.; Dai, X.; Hua, X.; Wang, Y.; Zhang, K.; Cai, S.; Tian, X. Ecological engineering in a eutrophic lake: A case study of large aquatic macrophyte enclosures in Baima Lake, China. *Limnologica* **2021**, *90*, 125907. [CrossRef]
13. Yin, Y.; Gao, M.; Cao, X.; Wei, J.; Zhong, X.; Li, S.; Peng, K.; Gao, J.; Gong, Z.; Cai, Y. Restore polder and aquaculture enclosure to the lake: Balancing environmental protection and economic growth for sustainable development. *Sci. Total Environ.* **2024**, *933*, 173036. [CrossRef] [PubMed]
14. Chao, C.; Wang, L.; Li, Y.; Yan, Z.W.; Liu, H.M.; Yu, D.; Liu, C.H. Response of sediment and water microbial communities to submerged vegetations restoration in a shallow eutrophic lake. *Sci. Total Environ.* **2025**, *801*, 149634. [CrossRef] [PubMed]
15. Ferreira, T.; Finkler, R.; Crossetti, L.O.; David, M.L.; Cardoso, L.; Fragoso, C.R.; Egbert, H. The structuring role of submerged macrophytes in a large subtropical shallow lake: Clear effects on water chemistry and phytoplankton structure community along a vegetated-pelagic gradient. *Limnologica* **2018**, *69*, 142–154. [CrossRef]
16. Xiao, L.T.; Wang, S.G. *Experimental Techniques in Plant Physiology*; China Agriculture Press: Beijing, China, 2023.
17. Jia, J.; Wang, Y. Exploring cohesive sediment flocculation with surface heterogeneity: A theoretical Lagrangian-type flocculation model. *Water Res.* **2024**, *250*, 121045.
18. Davies, R.J.; Smith, D.G. Turbidity, suspended sediment, and water clarity: A review. *J. Am. Water Resour. Assoc.* **2001**, *37*, 1085–1101. [CrossRef]
19. Horppila, J.; Nurminen, L. Effects of submerged macrophytes on sediment resuspension and internal phosphorus loading in Lake Hiidenvesi. *Hydrobiologia* **2003**, *506*, 217–224. [CrossRef]
20. Yang, J.Q. Solute flow and particle transport in aquatic ecosystems: A review on the effect of emergent and rigid vegetation. *Environ. Sci. Ecotechnol.* **2024**, *21*, 100412. [CrossRef]
21. Nepf, H.M. Flow and transport in regions with aquatic vegetation. *Annu. Rev. Fluid Mech.* **2012**, *44*, 123–142. [CrossRef]
22. Liu, S.W.; Li, D.X.; Liu, D.C.; Zhang, X.F.; Wang, Z.L. Characteristics of sedimentation and sediment trapping efficiency in the Three Gorges Reservoir, China. *Catena* **2022**, *208*, 105715. [CrossRef]
23. Yusuf, A.; Lei, D.; Sun, Y.Q.; Duan, S.; Zhang, Y.Z. Nitrogen transformation and microbial community interactions in hydrodynamic heterogeneous hyporheic zone sediment: Insights for ecosystem sustainability. *Limnologica* **2025**, *112*, 126143. [CrossRef]
24. Xiao, Y.; Yin, X.J.; Chen, L.J.; Wang, J.; Wang, Y.C.; Liu, G.L.; Hua, Y.M.; Wan, X.Q.; Xiao, N.D.; Zhao, J.W.; et al. Effects of illumination on nirS denitrifying and anammox bacteria in the rhizosphere of submerged macrophytes. *Sci. Total Environ.* **2020**, *760*, 143420. [CrossRef] [PubMed]
25. Wang, S.; Li, L.; Zhou, D.W. Root morphological responses to population density vary with soil conditions and growth stages: The complexity of density effects. *Ecol. Evol.* **2021**, *11*, 10590–10599. [CrossRef]
26. Chen, J.; Zhang, M.; Xie, P. Alterations in biomass allocation indicate the adaptation of submersed macrophytes to low-light stress. *Ecol. Indic.* **2020**, *113*, 106235. [CrossRef]
27. Li, Y.; Wang, L.G.; Chao, C.X.; Yu, H.W.; Yu, D.; Liu, C.H. Submerged macrophytes successfully restored a subtropical aquacultural lake by controlling its internal phosphorus loading. *Environ. Pollut.* **2020**, *267*, 115494. [CrossRef] [PubMed]
28. Bledsoe, B.P.; Shear, T.H. Vegetation along hydrologic and edaphic gradients in a North Carolina coastal plain creek bottom and implications for restoration. *Wetlands* **2000**, *20*, 126–147. [CrossRef]
29. Wan, L.L.; Chen, X.Y.; Deng, Q.H.; Yang, L.; Li, X.W.; Zhang, J.Y.; Song, C.L.; Zhou, Y.Y.; Cao, X.Y. Phosphorus strategy in bloom-forming cyanobacteria (*Dolichospermum* and *Microcystis*) and its role in their succession. *Harmful. Algae* **2019**, *84*, 46–55. [CrossRef]
30. Tóth, V.R. Vertical optical complexity shaped by submerged macrophytes. *Sci. Rep.* **2025**, *14*, 5100. [CrossRef]
31. Lu, J.; Wang, Z.X.; Xing, W.; Liu, G.H. Effects of substrate and shading on the growth of two submerged macrophytes. *Hydrobiologia* **2013**, *700*, 157–167. [CrossRef]
32. Yan, X.; Yu, D.; Wang, H.Y.; Wang, J.W. Response of submerged plant (*Vallisneria spinulosa*) clones to lead stress in the heterogenous soil. *Chemosphere* **2006**, *63*, 1459–1465. [CrossRef]

33. Huang, Z.J.; Liu, Q.Q.; An, B.; Wu, X.J.; Sun, L.J.; Wu, P.F.; Liu, B.; Ma, X.Q. Effects of planting density on morphological and photosynthetic characteristics of leaves in different positions on Cunninghamia lanceolata saplings. *Forests* **2021**, *12*, 853. [CrossRef]
34. Song, Y.Z.; Kong, F.F.; Xue, Y.; Qin, B.Q. Responses of chlorophyll and MDA of Vallisneria natans to nitrogen and phosphorus availability and epiphytic algae. *J. Freshw. Ecol.* **2015**, *30*, 103–110. [CrossRef]
35. Szabó, S.; Csizmár, A.; Koleszár, G.; Oláh, V.; Birk, S.; Peeters, E.T.H.M. Density-dependent facilitation and inhibition between submerged and free–floating plants. *Hydrobiologia* **2024**, *851*, 2749–2760. [CrossRef]
36. Xie, D.; Yu, D.; You, W.H.; Wang, G.L. Morphological and physiological responses to sediment nutrients in the submerged macrophyte *Myriophyllum spicatum*. *Wetlands* **2013**, *33*, 1095–1102. [CrossRef]
37. Courbier, S.; Pierik, R. Canopy light quality modulates stress responses in plants. *iScience* **2019**, *22*, 441–452. [CrossRef] [PubMed]