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Effects of ammonium stress on the growth of two submerged macrophytes (*Vallisneria natans* and *Hydrilla verticillata*) and phosphorus dynamics in sediments

PUMING LI, YU BAI, YUN CAI, XIUMEI SU, QIANJIA ZHANG, GANG SUN, AND HONG SU*

ABSTRACT

Eutrophication caused by excessive nitrogen inputs threatens freshwater ecosystems by degrading water quality and altering sediment nutrient dynamics. Submerged macrophytes may mitigate these effects through nutrient uptake and sediment stabilization, but ammonium stress can limit their performance. We tested how elevated ammonium concentrations affected the growth of *Vallisneria natans* and *Hydrilla verticillata* and their influence on sediment phosphorus dynamics. In a 56-d mesocosm experiment, moderate ammonium enrichment (0.5 mg L^{-1}) promoted plant growth and was associated with stronger reductions in sediment total phosphorus, inorganic phosphorus, and Fe/Al-bound phosphorus. High ammonium enrichment (1.0 mg L^{-1}) inhibited growth, increased oxidative stress, and weakened plant-mediated phosphorus regulation. Both species followed similar overall response patterns, although *V. natans* accumulated less malondialdehyde under high ammonium and appeared more tolerant than *H. verticillata*. These findings indicate that ammonium conditions can determine whether submerged-macrophyte restoration improves water quality and limits internal phosphorus loading.

Key words: phosphorus fractions, sediment, submerged macrophytes.

INTRODUCTION

Lake eutrophication caused by excessive nitrogen (N) and phosphorus (P) inputs is one of the major environmental challenges facing freshwater ecosystems worldwide (Camargo and Alonso 2006, Adhikari et al. 2015). Eutrophication degrades water quality, reduces biodiversity, and alters ecosystem functioning, thereby affecting both aquatic organisms and human uses of freshwater systems (Zhang et al. 2015, Li et al. 2021a). Although management efforts have long focused on reducing external nutrient inputs from agricultural runoff, wastewater discharge, and atmospheric deposition, effective restoration is often constrained by internal nutrient loading from sediments (Dittrich et al. 2011, Zamparas and Zacharias 2014, Li et al. 2021b). Sediments can act as long-term reservoirs of N and P and may continue to release nutrients to the overlying water under changing environmental conditions, such as oxygen depletion, temperature shifts, and hydrodynamic disturbance (Jarvie et al. 2006, Withers and Jarvie 2008). Therefore, understanding how to control internal nutrient loading is essential for the long-term recovery of eutrophic lakes.

Submerged macrophytes are widely recognized as important components of lake restoration because they can stabilize sediments and take up nutrients from both the water column and sediment porewater (Horppila and Nurminen 2005, Nixon 2009, Su et al. 2019a). Through rhizosphere oxidation and sediment stabilization, submerged plants can influence sediment biogeochemistry and reduce the mobility of phosphorus, especially iron- and aluminum-bound forms, thereby limiting phosphorus release to the overlying water (Jaynes and Carpenter 1986, Koch 2001). Their ecological functions, however, are not constant and may vary with environmental conditions such as nutrient availability, light, and temperature (Chen et al. 2022, Zhu et al. 2023).

Among the environmental factors affecting submerged macrophytes, ammonium is particularly important because it is a dominant form of inorganic nitrogen in many eutrophic waters and a common product of anthropogenic N loading (Dodds et al. 1998, Cao et al. 2011, Eliašová et al. 2021). Ammonium can directly impair plant growth and metabolism through toxic effects, including disturbance of nitrogen metabolism and inhibition of biomass accumulation (Cao et al. 2007, Gao et al. 2016). Previous studies have also shown that ammonium stress induces oxidative damage in submerged plants, reflected by changes in hydrogen peroxide (H_2O_2), malondialdehyde (MDA), and antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) (Cao et al. 2007, Wang et al. 2010, Gao et al. 2019). Although these physiological responses are increasingly well documented, less is known about how ammonium stress influences the ability of submerged macrophytes to regulate sediment phosphorus fractions.

Vallisneria natans and *Hydrilla verticillata* are common submerged macrophytes in eutrophic freshwater ecosystems, and they represent contrasting growth forms and restoration-relevant management functions. *Vallisneria natans* is a

*All authors: School of Resources and Chemical Engineering, Sanming University, Sanming, Fujian 365004, China. Sixth and seventh authors: Fujian Provincial Key Laboratory of Resources and Environment Monitoring & Sustainable Management and Utilisation, Sanming University, Sanming 365004, China. Corresponding author's E-mail: 20190112@fjsmu.edu.cn and sungang@nenu.edu.cn. Received for publication November 19, 2025 and in revised form July 1, 2026.

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rosette-forming species frequently used in restoration projects because of its sediment-stabilizing growth form, whereas *H. verticillata* is a canopy-forming species characterized by rapid biomass accumulation and strong nutrient uptake (Chambers and Kalff 1987, Chen et al. 2020). These morphological and functional differences suggest that the two species may differ in ammonium tolerance and in their influence on sediment phosphorus dynamics. In this study, we used a mesocosm experiment with controlled ammonium gradients to examine the effects of ammonium stress on the growth of *V. natans* and *H. verticillata* and on sediment phosphorus fractions. We measured plant growth, overlying water quality, chlorophyll a (Chl a), oxidative stress indicators (H_2O_2 and MDA), antioxidant enzyme activities (SOD, POD, and CAT), plant TP content, and sediment phosphorus fractions including TP, IP, Fe/Al-P, Ca-P, and OP. Based on previous studies on ammonium toxicity, plant stoichiometry, and phosphorus release from sediments (Cao et al. 2007, Ma et al. 2018, Su et al. 2019b), we proposed the following hypotheses: 1) moderate ammonium enrichment (0.5 mg L^{-1}) would stimulate plant growth and phosphorus uptake, whereas high ammonium loading (1.0 mg L^{-1}) would inhibit growth and increase oxidative stress; 2) both submerged macrophytes would reduce sediment phosphorus content, particularly labile fractions such as IP and Fe/Al-P, but this effect would weaken under high ammonium stress; and 3) due to their contrasting growth forms and ecological strategies, *V. natans* and *H. verticillata* would differ in ammonium tolerance and in their effects on sediment phosphorus dynamics.

MATERIALS AND METHODS

Experimental site and plant materials

Sediment was collected near a sewage outfall of the Shaxi River (26°16′20″N, 117°38′14″E) in Sanming City, China. Overlying water was collected from the same river and contained 0.78 mg L^{-1} total nitrogen (TN), 0.16 mg L^{-1} ammonium nitrogen, and 0.059 mg L^{-1} total phosphorus (TP). The initial TN:TP mass ratio was 13.2, indicating relatively low N availability relative to P and possible slight N limitation (Olde Venterink et al. 2003). Shoots of *V. natans* and *H. verticillata* were obtained from the Honghu Aquatic Products shop. Healthy plants were acclimated for 2 wk in aquaria containing sediment and overlying water from the Shaxi River. Acclimation conditions were 25 ± 2 C, a 12-h light:12-h dark photoperiod, and approximately 100 µmol m^{-2} s^{-1} light intensity. Shoots were trimmed to approximately 20 cm. Initial shoot length and fresh mass were 19.51 ± 0.17 cm and 2.14 ± 0.07 g for *V. natans* and 19.84 ± 1.30 cm and 1.89 ± 0.21 g for *H. verticillata*. The single 56-d mesocosm experiment was conducted from 25 September to 20 November 2019 and was not repeated in time.

Experimental design

The experimental setup employed transparent glass tanks measuring 50 cm in length, 50 cm in width, and 80 cm in height. Each tank was filled with 10 cm of sediment collected from the Shaxi River and then overlaid with river water to a depth of 50 cm. Distilled water was added throughout the experiment to compensate for evaporation and sampling losses, thereby maintaining a constant water depth.

The ammonium factor comprised three levels: 1) a control using Shaxi River water (background NH_4^+-N concentration: 0.16 mg L^{-1}) without ammonium addition; 2) a moderate ammonium treatment with a target final NH_4^+-N concentration of 0.5 mg L^{-1}; and 3) a high ammonium treatment with a target final NH_4^+-N concentration of 1.0 mg L^{-1}. Because these values refer to final NH_4^+-N concentrations rather than addition amounts, the corresponding nominal TN concentrations were estimated as 0.78, 1.12, and 1.62 mg L^{-1} for the control, 0.5 mg L^{-1}, and 1.0 mg L^{-1} treatments, respectively. These values correspond to TN:TP mass ratios of approximately 13.2, 19.0, and 27.5, thereby providing a broader stoichiometric context for the experimental nutrient conditions (Olde Venterink et al. 2003).

The plant-treatment factor also comprised three levels: 1) tanks planted exclusively with *H. verticillata*, 2) tanks planted exclusively with *V. natans*, and 3) tanks without plants, which served as the unplanted control. Six shoots were evenly distributed within each tank, approximately 10 cm from the tank walls, and inserted about 3 cm into the sediment. Each ammonium × plant treatment combination was replicated three times.

To maintain the target nitrogen concentrations, NH_4Cl (>99.5% purity; Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) was dissolved in distilled water and added to the tanks by syringe. The tanks remained unmixed, and no phosphorus fertilizer was added. NH_4Cl was added on days 8, 25, and 43 of the experiment to maintain the target ammonium concentrations. After 56 d, all plants were removed, cleaned, drained, and measured for fresh mass and ramet number.

Sampling

Sediment and plant phosphorus. The concentrations of various phosphorus (P) fractions in the sediment were assessed through the sequential extraction scheme proposed by the European Commission, following the Standard Measurements and Testing protocols (Ruban et al. 2001). The analyzed P fractions encompassed Fe/Al-P (phosphorus bound to aluminum, iron, and manganese oxides and hydroxides), Ca-P (phosphorus associated with calcium), organic P (OP), inorganic P (IP), and total P (TP). The quantification of each P form was conducted using the ammonium molybdate spectrophotometric technique. At the end of the experiment, harvested plants were thoroughly rinsed with deionized water to remove attached sediment and debris, blotted dry, and separated into roots, stems, and leaves. Each plant part was oven-dried at 65 C to constant weight, ground into fine powder, and digested for TP determination. The TP contents of roots, stems, and leaves were measured using the ammonium molybdate ascorbic acid method (Sparks et al. 1996).

Water-quality measurements. Water quality was monitored on 16 occasions at intervals of 3 or 4 d. Measurements included pH, dissolved oxygen (DO), total nitrogen (TN), total phosphorus (TP), and phytoplankton chlorophyll a (Chl a). A 100-ml composite sample was collected from five randomly selected locations in each tank at middepth. TN and TP were measured from well-mixed samples according

Table 1. Two-way ANOVA of overlying-water variables.

	Ammonium Treatment			Plant Treatment			Ammonium Treatment × Plant Treatment		
Water Indices	df	F	Pr > F	df	F	Pr > F	df	F	Pr > F
TP	2	71.9	**	2	48.2	**	4	3.5	*
TN	2	1831.1	**	2	91.7	**	4	17.6	**
pH	2	1.5	n.s.	2	8.1	**	4	0.7	n.s.
DO	2	4.4	*	2	14.5	**	4	2.2	n.s.
Chl a	2	262.0	**	2	126.1	**	4	7.6	**

*$P < 0.05$; **$P < 0.01$; n.s., not significant ($P > 0.05$).

to Xie and Wang (1998). For Chl a determination, 500 ml of water was filtered through a glass-fiber filter. Retained particulate material was extracted in 90% acetone for 24 h in darkness and analyzed spectrophotometrically.

Physiological measurements and antioxidant enzyme activities. For H_2O_2 and malondialdehyde (MDA) measurements, approximately 0.5 g of fresh leaf tissue was ground in liquid nitrogen and homogenized in 3 ml of 50 mM sodium phosphate-buffered saline (PBS, pH 6.5) following Wang et al. (2010). After centrifugation at 8,000 × g for 20 min, the appropriate volume of supernatant was mixed with 2.5 m of 0.1% titanium sulfate in 20% (v/v) H_2SO_4. The mixture was centrifuged at 10,000 × g for 5 min, and absorbance was measured at 410 nm against an H_2O_2 standard curve. For MDA determination, tissue was extracted with 5 ml of 1% trichloroacetic acid (TCA) and centrifuged at 15,000 × g for 10 min. A 0.5-ml aliquot of supernatant was mixed with 2 ml of 20% TCA containing 0.5% thiobarbituric acid, heated at 96 C for 30 min, cooled on ice, and centrifuged at 10,000 × g for 5 min. Absorbance was measured at 532 and 600 nm.

For enzyme extraction, 1 g of fresh leaf tissue was frozen in liquid nitrogen and ground into a fine powder, then homogenized in 10 ml of extraction buffer containing 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, and 1% polyvinylpyrrolidone (PVP). After centrifugation at 15,000 × g for 20 min at 4 C, the supernatant was used for enzyme activity assays. SOD, CAT, and POD activities were determined using the method previously described (Wang et al. 2011).

Statistical analyses

The IBM SPSS 19.0 program was used for all statistical analyses. Prior to analysis, data were checked for normality and homogeneity. When necessary, data were log10(x)-transformed to meet the assumptions of normality and homoscedasticity before further analysis. Mean and standard deviation (SD) were used to represent the data. To assess the statistical significance of water indices, plant morphological and physiological indices, sediment P fractions, and plant tissue TP content, one-way ANOVA was used. A two-way ANOVA was employed to examine the effects of ammonium treatment, submerged macrophyte species, and their interactions on water indices, plant growth indices, plant antioxidant system and sediment P fractions. Three-way ANOVA was employed to examine the effects of ammonium treatment, submerged macrophyte species, and plant part on plant TP content. All results were considered significant at the $P < 0.05$ level, and *post hoc* pairwise comparisons of means to determine significance were performed using the LSD test.

RESULTS AND DISCUSSION

Overlying-water properties and chlorophyll a

The physicochemical properties of the overlying water varied with ammonium addition and submerged macrophyte treatment during the 56-d experiment. Ammonium treatment, plant treatment, and their interaction affected TP, TN, and Chl a concentrations (all $P < 0.01$; Table 1). Plant treatment affected DO and pH (both $P < 0.01$), and ammonium treatment affected DO ($P < 0.05$). The ammonium × plant interaction did not affect DO or pH ($P > 0.05$).

The planted treatments exhibited better water quality than the plant-free control (Figure 1). Both *V. natans* and *H. verticillata* reduced TP and Chl a concentrations in the overlying water, suggesting submerged macrophytes suppressed phytoplankton development and contributed to nutrient removal from the water column. This pattern is consistent with the reported role of submerged macrophytes in competing with phytoplankton for nutrients and improving water clarity (Horppila and Nurminen 2005). The two species did not exhibit identical effects under all ammonium levels. Under 0.5 mg L^{-1} ammonium, *V. natans* reduced TP more strongly than *H. verticillata*, whereas no difference between species was observed under 1.0 mg L^{-1} ammonium. In addition, both species increased DO under the high-ammonium treatment, suggesting that they still contributed to oxygenation of the water column under stress conditions.

Macrophyte growth responses to ammonium stress

Ammonium concentration affected the growth of both submerged macrophytes. Ammonium treatment affected total mass, ramet number, root mass, stem mass, leaf mass, and the proportional allocation of root, stem, and leaf mass (all $P < 0.01$; Table 2). Species identity affected total mass, ramet number, stem mass, and stem mass proportion (all $P < 0.01$), as well as root mass, leaf mass, and leaf mass proportion (all $P < 0.05$), but did not affect root mass proportion ($P > 0.05$). The ammonium × species interaction affected total mass, stem mass, leaf mass, stem mass proportion, and leaf mass proportion (all $P < 0.05$), but not ramet number, root mass, or root mass proportion ($P > 0.05$). Both species showed a similar response pattern, with growth promoted at 0.5 mg L^{-1}

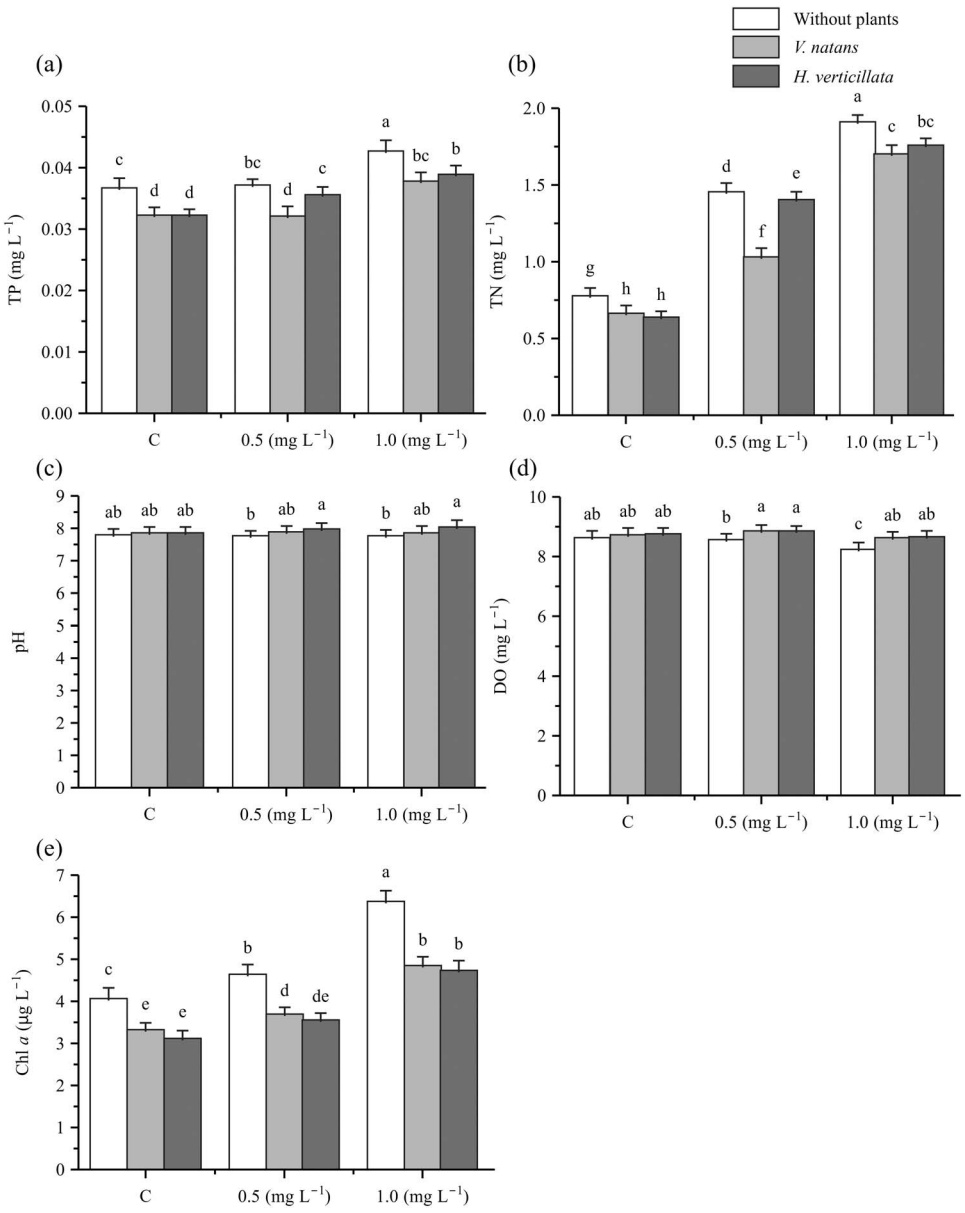

Figure 1. Mean values of (a) total phosphorus (TP), (b) total nitrogen (TN), (c) pH, (d) dissolved oxygen (DO), and (e) chlorophyll a (Chl a) in the overlying water during the experiment.

ammonium and inhibited at 1.0 mg L^{-1} ammonium (Figure 2). In *V. natans*, total mass and ramet number were highest at 0.5 mg L^{-1}. At 1.0 mg L^{-1}, total mass was 20.9% lower than the control and 34.8% lower than the 0.5 mg L^{-1} treatment. *H. verticillata* followed the same trend, with greater total mass and ramet number at 0.5 mg L^{-1} than in the other treatments (LSD test, $P < 0.05$; Figure 2). Root, stem, and leaf biomass of both species were highest at 0.5 mg L^{-1} and lowest at 1.0 mg L^{-1}.

These results suggest moderate ammonium enrichment provided an available nitrogen source that supported plant growth, whereas high ammonium-imposed stress and reduced biomass accumulation. This pattern agrees with previous studies reporting that low to moderate ammonium can promote the growth of submerged plants, while excessive ammonium becomes inhibitory or toxic (Gao et al. 2016, Hao et al. 2020). In the present study, the reduced biomass under 1.0 mg L^{-1} ammonium indicates that the beneficial effect of nitrogen enrichment was offset by ammonium stress. In addition to the direct physiological effects of ammonium (Cao et al. 2007, Yuan et al. 2023), indirect effects mediated by algae may also have contributed to the reduced performance of submerged macrophytes under the highest ammonium treatment (Ren et al. 2022). In our experiment, the 1.0 mg L^{-1} ammonium treatment corresponded to the highest Chl a concentration in the overlying water, suggesting enhanced phytoplankton growth. Increased phytoplankton biomass, and possibly epiphytic algae, may have reduced underwater light availability to the plants (Yang et al. 2020), thereby suppressing growth

Table 2. Two-way ANOVA of plant growth indices.

Plant Growth Indices	Ammonium Treatment			Species			Ammonium Treatment × Species		
	df	F	Pr > F	df	F	Pr > F	df	F	Pr > F
Total mass	2	12.8	**	1	45.7	**	2	5.9	*
Ramet number	2	32.6	**	1	9.0	**	2	0.11	n.s.
Root mass	2	56.2	**	1	4	*	2	0.8	n.s.
Stem mass	2	621.1	**	1	31.9	**	2	17.0	**
Leaf mass	2	13.9	**	1	6.1	*	2	4	*
Root mass proportion	2	57.1	**	1	0.4	n.s.	2	0.6	n.s.
Stem mass proportion	2	155.5	**	1	12.8	**	2	10.3	**
Leaf mass proportion	2	13.9	**	1	6.1	*	2	4	*

*$P < 0.05$; **$P < 0.01$; n.s., not significant ($P > 0.05$).

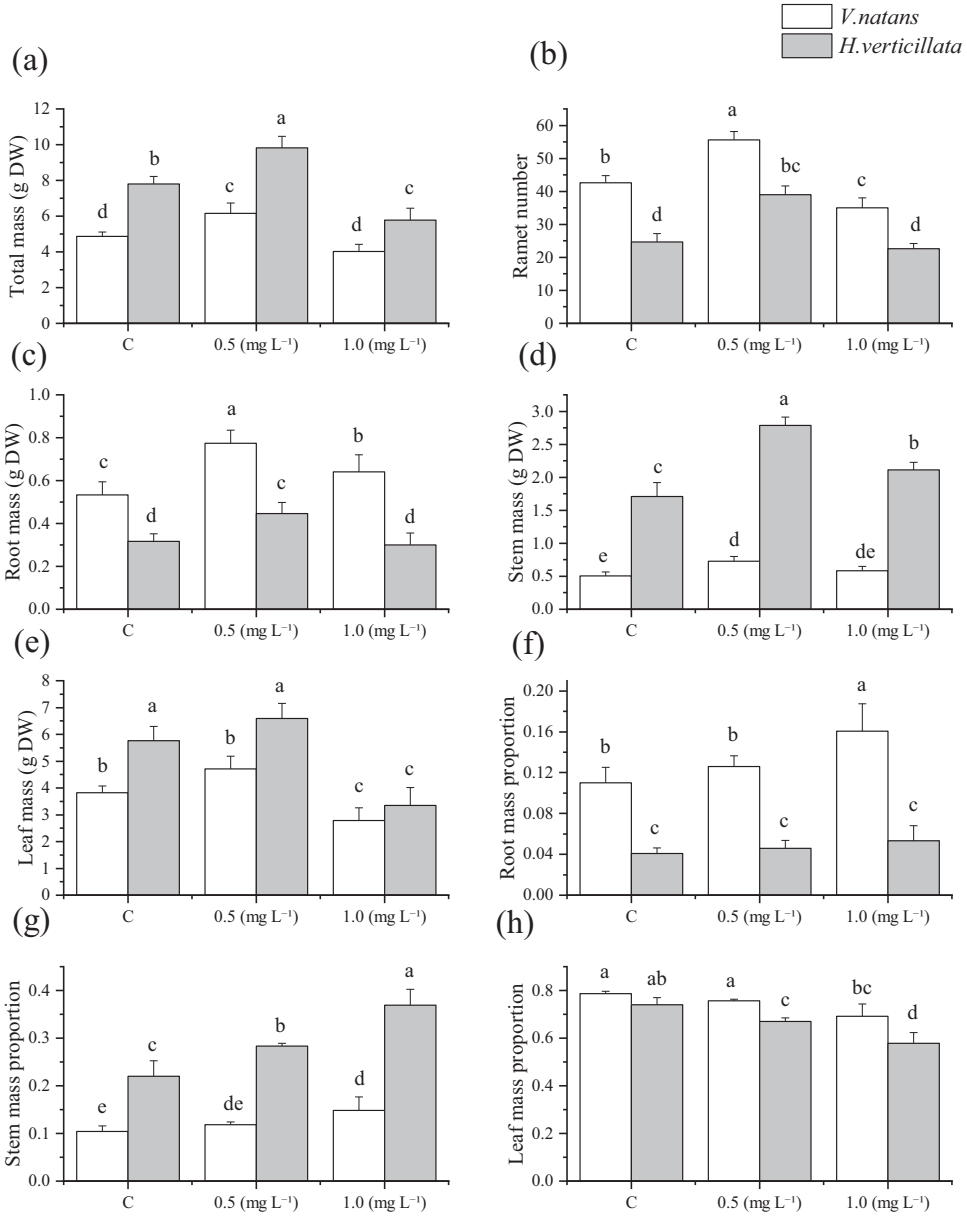

Figure 2. Growth indices of *V. natans* and *H. verticillata* (mean ± SD).

Table 3. Two-way ANOVA of oxidative stress indicators and antioxidant enzyme activities.

	Ammonium Treatment			Plant species			Ammonium Treatment × Plant Species		
Antioxidant System	df	F	Pr > F	df	F	Pr > F	df	F	Pr > F
H_2O_2	2	402.0	**	1	11.0	**	2	48.2	**
CAT	2	282.5	**	1	4.2	n.s.	2	24.0	**
POD	2	958.4	**	1	207.0	**	2	45.0	**
MDA	2	97.7	**	1	39.0	**	2	0.3	n.s.
SOD	2	191.1	**	1	2.6	n.s.	2	0.4	n.s.

*$P < 0.05$; **$P < 0.01$; n.s., not significant ($P > 0.05$).

and potentially exacerbating oxidative stress. Although the two species responded similarly, species and ammonium × species effects for several biomass indices indicate differences in growth strategy and biomass allocation under ammonium enrichment (Wu et al. 2021).

Oxidative status and antioxidant responses

Oxidative stress indicators and antioxidant enzyme activities were measured at the end of the experiment to clarify the physiological basis of growth inhibition under high

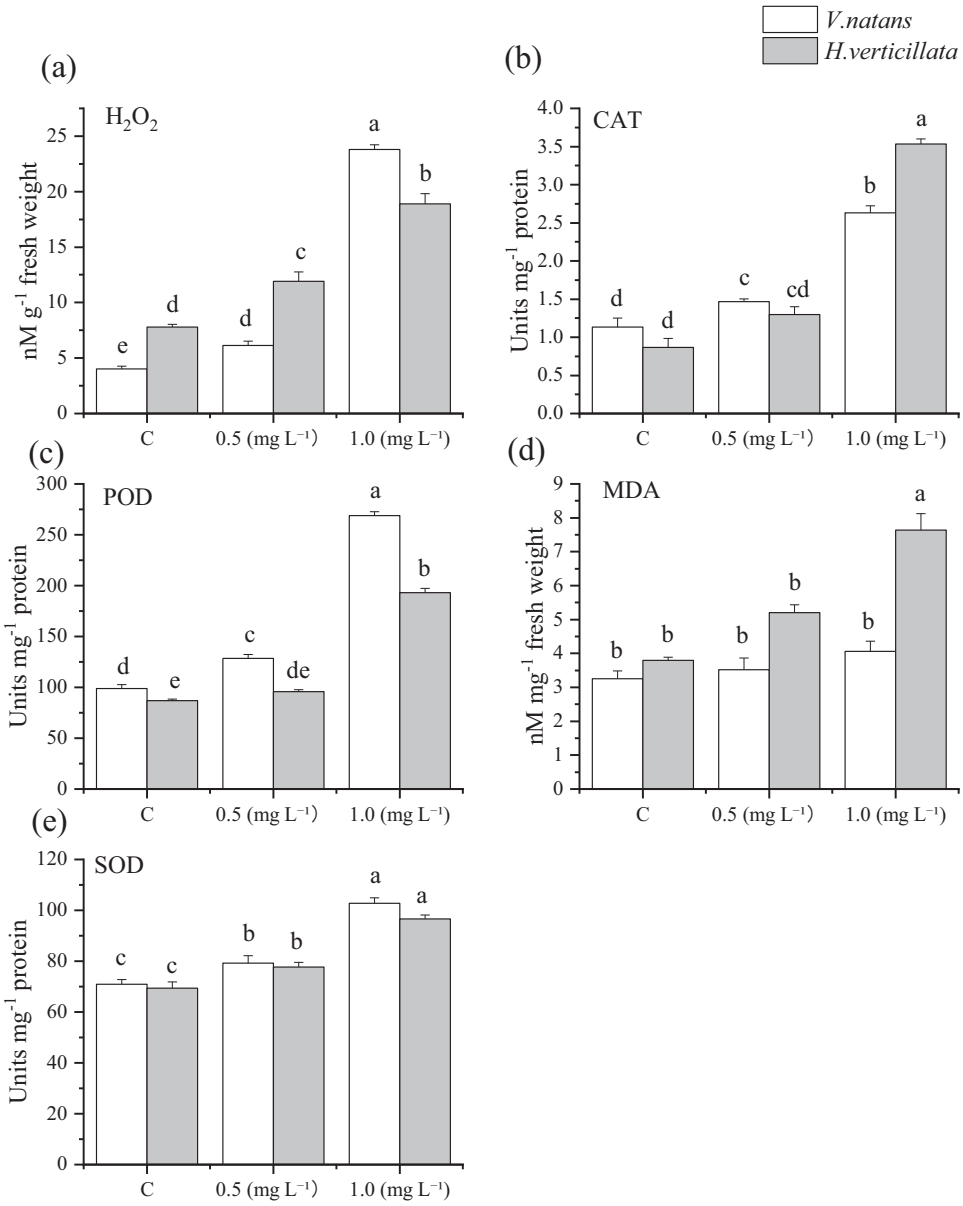

Figure 3. Effects of ammonium concentration on (a) H_2O_2 content, (b) CAT activity, (c) POD activity, (d) MDA content, and (e) SOD activity on day 56 (mean ± SE).

Table 4. Two-way ANOVA of sediment phosphorus fractions at the end of the experiment.

	Treatment			Species			Treatment × Species		
Sediment P Fractions	df	F	Pr > F	df	F	Pr > F	df	F	Pr > F
TP	2	84.83	**	1	13.89	**	2	0.79	n.s.
IP	2	166.20	**	1	31.71	**	2	2.31	n.s.
Fe/Al-P	2	70.16	**	1	21.32	**	2	3.01	n.s.
Ca-P	2	3.68	n.s.	1	3.04	n.s.	2	0.58	n.s.
OP	2	6.59	*	1	1.58	n.s.	2	0.02	n.s.

*$P < 0.05$; **$P < 0.01$; n.s., not significant ($P > 0.05$).

ammonium (Nimptsch and Pflugmacher 2007). Ammonium treatment affected H_2O_2, MDA, SOD, CAT, and POD (all $P < 0.01$; Table 3). Species affected H_2O_2, POD, and MDA (all $P < 0.01$), and the ammonium × species interaction affected H_2O_2, CAT, and POD (all $P < 0.01$). H_2O_2 increased under 1.0 mg L^{-1} ammonium in both species (LSD test, $P < 0.05$; Figure 3a). In *V. natans*, H_2O_2 content at 1.0 mg L^{-1} was 5.91 times the control value and 3.88 times the value at 0.5 mg L^{-1}. In *H. verticillata*, H_2O_2 content at 1.0 mg L^{-1} was 2.43 times the control value. These increases indicate that elevated ammonium promoted reactive oxygen species accumulation (Yuan et al. 2015, Zhu et al. 2025). SOD, CAT, and POD activities were also higher at 1.0 mg L^{-1} than at the lower ammonium concentrations (LSD test, $P < 0.05$; Figures 3c–e), indicating activation of antioxidant defenses (Cao et al. 2007).

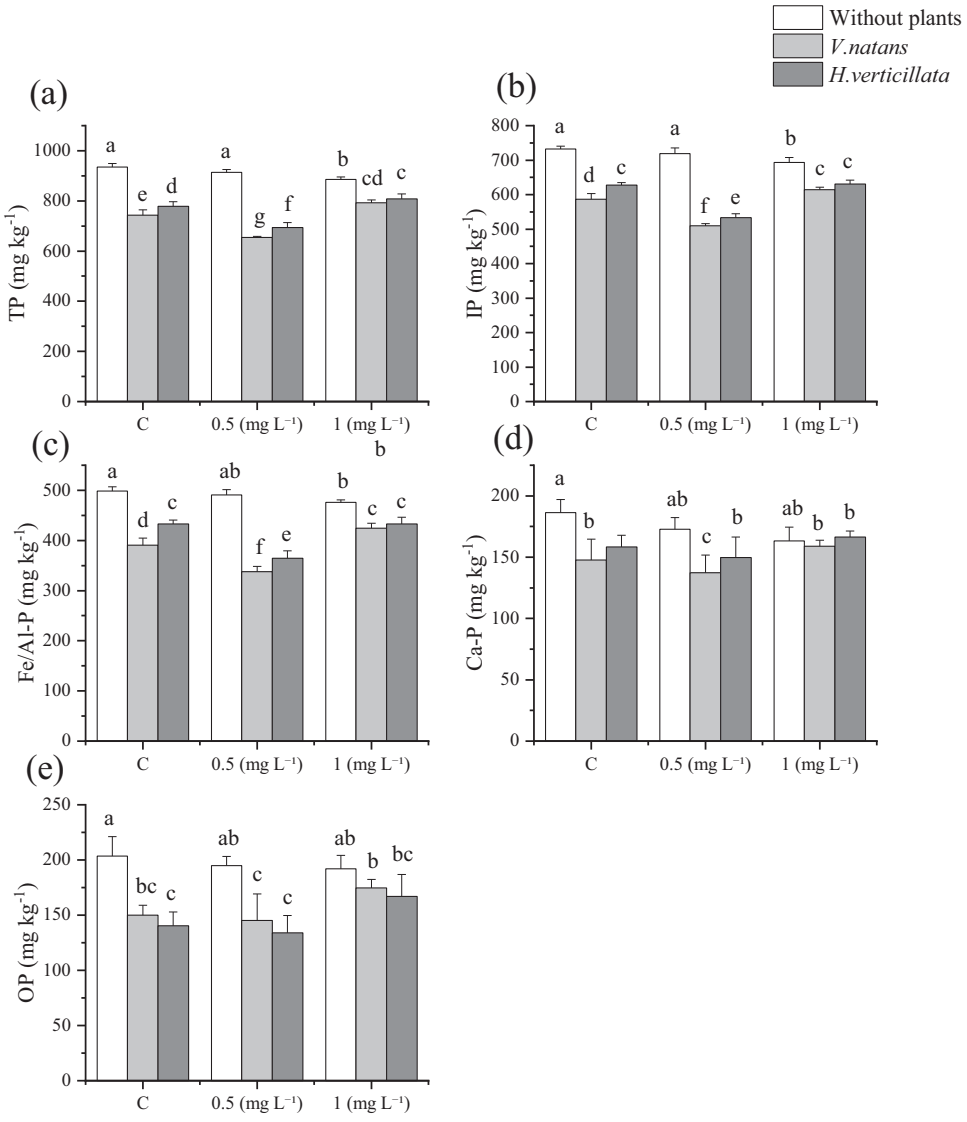

Figure 4. Sediment phosphorus fractions under the experimental treatments on day 56.

Table 5. Three-way ANOVA of plant TP content at the end of the experiment.

	df	F	Pr > F
Ammonium treatment (A)	2	14.2	**
Species (B)	1	146.2	**
Plant part (C)	2	220.9	**
A × B	2	5.2	*
B × C	2	1.2	n.s.
A × C	4	3.8	*
A × B × C	4	2.5	n.s.

*$P < 0.05$; **$P < 0.01$; n.s., not significant ($P > 0.05$).

The two species differed in the extent of oxidative damage. MDA content in *V. natans* did not differ among ammonium treatments ($P > 0.05$; Figure 3b), despite increased H_2O_2 content and antioxidant enzyme activities. In contrast, MDA content in *H. verticillata* was higher at 1.0 mg L^{-1} than at the lower concentrations (LSD test, $P < 0.05$; Figure 3b). Because MDA reflects membrane lipid peroxidation (Jampeetong et al. 2012), high ammonium caused greater cellular damage in *H. verticillata* than in *V. natans*. Both species experienced oxidative stress under high ammonium, but *V. natans* maintained lower membrane damage under the same conditions.

Sediment and plant phosphorus dynamics

Sediment phosphorus was dominated by Fe/Al-bound phosphorus (Fe/Al-P), followed by organic phosphorus (OP) and calcium-bound phosphorus (Ca-P). Sediment TP, inorganic phosphorus (IP), and Fe/Al-P were affected by ammonium treatment and submerged macrophyte species (all $P < 0.01$; Table 4), whereas the ammonium × species interaction did not affect these fractions ($P > 0.05$). Both macrophytes reduced sediment TP, IP, and Fe/Al-P relative to the corresponding plant-free treatments (Figure 4). This pattern is consistent with the capacity of submerged macrophytes to take up sediment phosphorus and modify nutrient cycling (Carignan and Kalff 1980, Su et al. 2023). Reductions were greatest at 0.5 mg L^{-1} ammonium. Under this treatment, sediment TP was 28.4% lower with *V. natans* and 24.1% lower with *H. verticillata* than in the plant-free treatment. At 1.0 mg L^{-1} ammonium, the corresponding reductions were 10.5% and 8.8%.

IP and Fe/Al-P followed a similar pattern, indicating that moderate ammonium favored plant-mediated phosphorus removal, whereas high ammonium weakened this effect. Fe/Al-P is a relatively active sediment phosphorus fraction and can contribute to internal phosphorus loading when redox conditions change (Rydin 2000, Søndergaard et al. 2003). Its reduction is therefore relevant to internal-load management. Better plant growth under moderate ammonium likely enhanced phosphorus uptake and sediment regulation, whereas high ammonium reduced plant biomass and physiological performance (Madsen et al. 2001, Wang et al. 2008). Changes in Ca-P and OP were smaller than those in TP, IP, and Fe/Al-P. Ca-P was not affected by ammonium treatment, species, or their interaction ($P > 0.05$), whereas OP was affected only by ammonium treatment ($P < 0.05$; Table 4). Plant effects were therefore more evident for reactive phosphorus fractions than for less labile fractions during the experiment (Ruban et al. 2001, Hupfer and Lewandowski 2008).

Ammonium treatment also affected phosphorus accumulation in plant tissues (Table 5; Figure 5), consistent with the well-established influence of external nutrient conditions on phosphorus uptake and tissue nutrient status in submerged macrophytes (Britto and Kronzucker 2002, Yuan et al. 2023). In *V. natans*, leaf TP increased with ammonium concentration, whereas stem and root TP showed little variation among treatments. In *H. verticillata*, leaf TP was highest at 0.5 mg L^{-1} ammonium, while stem TP increased with increasing ammonium concentration.

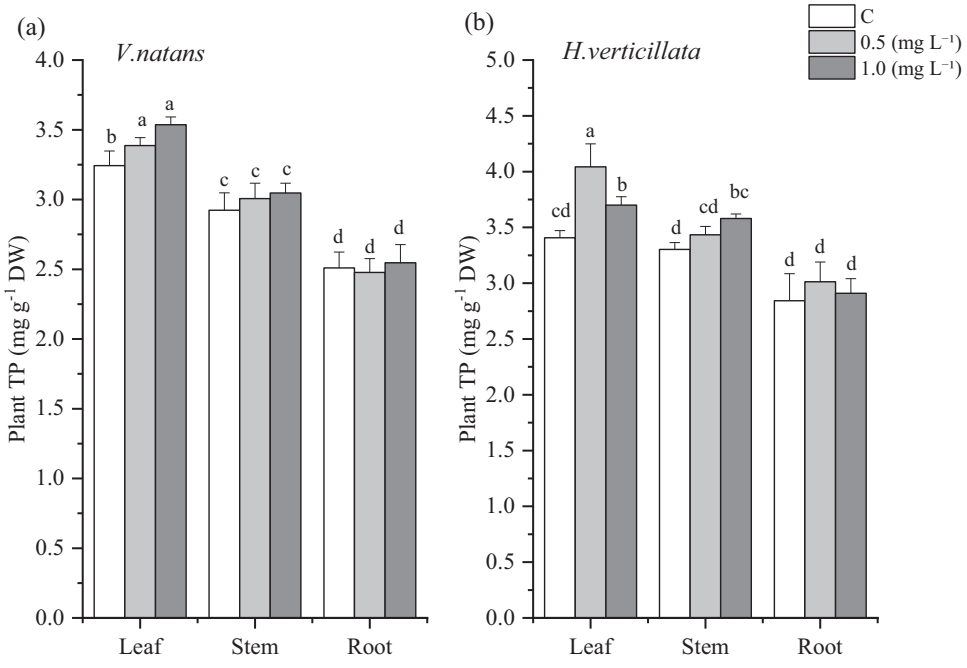

Figure 5. TP content of leaves, stems, and roots of *V. natans* and *H. verticillata* under the experimental treatments on day 56.

These results indicate that phosphorus allocation differed between the two species under ammonium enrichment, in agreement with previous studies showing that aquatic macrophytes often exhibit species-specific nutrient concentration patterns and allocation strategies (Yuan et al. 2015, Rao et al. 2023).

In conclusion, both submerged macrophytes improved overlying water quality and reduced sediment phosphorus, but their performance depended strongly on ammonium concentration. Moderate ammonium enrichment (0.5 mg L^{-1}) promoted plant growth and was associated with stronger reductions in sediment TP, IP, and Fe/Al-P. In contrast, high ammonium enrichment (1.0 mg L^{-1}) suppressed growth, increased oxidative stress, and weakened the ability of both species to regulate sediment phosphorus dynamics. Although the two species showed similar overall trends, *V. natans* accumulated less MDA under high ammonium and therefore appeared more tolerant than *H. verticillata* under the conditions of this study.

These findings have practical significance for aquatic resource managers involved in submerged vegetation restoration, eutrophication control, and conservation of clear-water habitats. Managers should consider ammonium conditions when evaluating whether planted macrophytes can improve water quality and regulate internal phosphorus loading. Ammonium concentrations should be monitored before and during restoration, planting should be prioritized where ammonium stress is not excessive, and tolerant species such as *V. natans* may be preferable where ammonium loading recurs. Because high ammonium weakened plant performance and phosphorus regulation, macrophyte restoration should be combined with external nitrogen control, wastewater reduction, sediment management, and postplanting monitoring. This integrated approach may reduce restoration failure and improve the capacity of restored macrophyte beds to stabilize sediments, suppress phytoplankton, and improve water quality. As this study was based on a single 56-d mesocosm experiment, field studies across seasons and water bodies would help determine how broadly these responses apply.

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