Pol. J. Environ. Stud. Vol. 32, No. 6 (2023), 5691-5707

DOI: *10.15244/pjoes/169838*

ONLINE PUBLICATION DATE: 2023-09-25

Original Research

Effects of Interactions between Light Intensity and Total Sediment Organic Matter Load on the Submerged Macrophyte *Vallisneria natans*

Yahua Li[1, 2], Jianglong Zhu[3], Lu Yao[2], Yao Guo[2], Qianzheng Li[2], Xiangfen Liu[2], Chuan Wang[2, 3, 4]*, Qiaohong Zhou[2], Zhenbin Wu[1, 2]**

[1]School of Environmental Studies, China University of Geosciences, Wuhan 430074, PR China
[2]State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, PR China
[3]Faculty of Resources and Environmental Science, Hubei University, Wuhan 430062, PR China
[4]Hubei Key Laboratory of Regional Development and Environmental Response, Hubei University, Wuhan 430062, PR China

Received: 18 May 2023
Accepted: 20 July 2023

Abstract

Low light intensity and high sediment organic matter (SOM) conditions coexist recurrently in aquatic ecosystems, which affects submerged macrophyte re-habilitation. In this greenhouse study, four light intensity levels (10% [natural light intensity], 30%, 60%, and 100%) and three initial SOM loads (11% [measured as the loss on ignition], 17%, and 25%) were applied to explore the effects of their interactions on the growth of *Vallisneria natans* (*V. natans*). Two-way ANOVA revealed the effects of interactions between light intensity and SOM load on the growth characteristics of *V. natans*. Multiple linear regression models indicate that the dry weights and root lengths exhibited a single maximum as the SOM load increased. The inhibitory effect of the sediment on the growth of *V. natans* could be alleviated by increasing the light intensity in a certain SOM range. However, the inhibited growth of *V. natans* was not alleviated by enhancing the light intensity at a 25% SOM load. We explored a potential mechanism for this phenomenon based on ammonium (NH_4^+) toxicity regulation. Structural equation modeling indicates that enhanced light intensity could directly reduce sediment NH_4^+ contents or reduce them indirectly by decreasing the abundances of bacterial functional genes associated with NH_4^+ formation. Subsequently, lower sediment NH_4^+ content increased the plant dry weight, thereby facilitating the removal of nitrogen and phosphorus from the sediment. Therefore, enhancing the light intensity over a wide range of SOM loads resulted in the restoration of submerged macrophytes,

---

*e-mail: wangchuan@hubu.edu.cn
**e-mail: wuzb@ihb.ac.cn

whereas reducing SOM loads could be performed in high SOM conditions to improve the restoration of submerged macrophytes.

Keywords: light intensity, sediment organic matter, submerged macrophytes, ammonium toxicity

Introduction

Eutrophication is the collective term used by aquatic scientists to describe a series of problems exhibited by nutrient-enriched lakes [1]. Some lakes have been observed to change from a stable clear-water state dominated by submerged macrophytes to an alternate state characterized by turbid phytoplankton overgrowth [2], leading to a decline in aquatic biodiversity and impaired ecological functions [3, 4]. Sustained macrophytes are commonly described as ecological engineers and are essential for maintaining clear water homeostasis and biodiversity in lakes [5-7]. In addition, submerged macrophytes can reduce sediment resuspension [8], absorb nutrients from the surrounding environment [9, 10], provide favorable habitats and food for aquatic animals [11, 12], and maintain the diversity of the lake ecosystem [5]. Therefore, submerged macrophyte restoration is widely used in practical projects to improve lake ecosystems [13, 14]. Although the growth of submerged macrophytes is influenced by various environmental factors [15, 16], the response of submerged macrophytes to environmental factors is not fully understood.

Light intensity and sediment organic matter (SOM) load are environmental factors that affect submerged macrophyte growth. Previous studies have established that light intensity is the primary factor that affects submerged macrophyte growth, as well as water depth, turbidity, number of epiphytes, total suspended matter, and chlorophyll concentration, which indirectly affect plant growth and distribution by influencing the light intensity available to the submerged macrophytes [15, 17-19]. As in terrestrial plants with root systems, the growth of submerged macrophytes is undoubtedly influenced by the physicochemical properties of the sediment. At higher SOM loads, the anoxic environment produced by the decomposition of large amounts of organic matter can lead to the enrichment of potentially phytotoxic substances in the sediment, including phenols and organic acids, reduced iron and manganese, hydrogen sulfide, and NH_4^+, which consequently inhibit the growth of submerged macrophytes [15, 20, 21]. After implementing submerged macrophyte restoration at West Lake (30°14'N, 120°08'E) in Hangzhou City, Zhejiang Province, China, ecological surveys performed from 2013 to 2019 found that high SOM load was a major environmental factor that limited submerged macrophyte restoration [22]. However, previous studies have focused on either the effect of light intensity or SOM load on submerged macrophyte growth, not their combined effects.

The environmental factors affecting submerged macrophyte growth are complex, and a single factor does not independently determine growth. Ersoy et al. found that higher lake water temperatures resulting from climatic warming promoted the growth of submerged macrophytes; however, under eutrophic conditions, growth was inhibited indirectly by the attenuation of underwater light intensity caused by algal growth [18]. Chappuis et al. showed that low light intensity (35% natural light intensity) did not increase mortality in *Isoetes lacustris*; however, low light intensity and high SOM loads collectively increased mortality [23]. These studies established the potential for interactions between multiple environmental factors to affect submerged macrophyte restoration. In lake environments, low light intensities and high SOM loads coexist recurrently. Furthermore, enhancing the light intensity can reduce the inhibition of potentially phytotoxic compounds in submerged macrophytes at high SOM loads [15, 24]. Therefore, further research on the effects of the interactions between light intensity and SOM load on submerged macrophyte growth could optimize and guide the selection of submerged macrophyte restoration sites in practical projects.

Ammonium (NH_4^+) toxicity is a global ecological problem [25]. NH_4^+ is a nitrogen source for amino acid biosynthesis in plant cells; however, its excessive accumulation in cells can lead to carbon-nitrogen imbalances and chloroplast ultrastructure destruction, consequently inhibiting plant growth [26, 27]. Previous studies have reported that SOM enrichment could increase NH_4^+ contents, thereby causing NH_4^+ toxicity in plants [23, 25]. Plants' NH_4^+ tolerance can be increased by high irradiance, which increases the carbon pool and improves NH_4^+ assimilation [24, 26]. However, studies regarding the involvement of NH_4^+ toxicity regulation in the effect of interactions between light intensity and SOM load on submerged macrophyte growth are unavailable. Nitrogen metabolism by microorganisms also directly affects NH_4^+ formation and consumption in sediment [28]. In this study, we aimed to explore the effect of the interactions between light intensity and SOM load on submerged macrophyte growth from the perspective of NH_4^+ toxicity regulation.

We performed a greenhouse study, which aimed to: (1) assess the effect of the interactions between light intensity and SOM load on the growth characteristics of *Vallisneria natans* (*V. natans*); (2) explore the potential associations between light intensity and SOM load in regulating NH_4^+ toxicity to *V. natans*; and (3) evaluate nitrogen (N) and phosphorus (P) removal from the sediment by *V. natans* under different environmental conditions.

Materials and Methods

Sediment and Plant Sources

A continuous multi-year ecological survey was performed in two regions of West Lake (Maojiabu and Xilihu) in Zhejiang Province, China. The SOM load in Maojiabu (7.383±5.860%) was low, and high submerged macrophyte coverage (31.3±21.4%) was maintained after submerged macrophyte restoration measures were implemented at July 2013. Xilihu had a higher SOM load (17.525±7.171%) and maintained lower submerged macrophyte coverage (10.4±4.2%) [22, 29]. Therefore, these regions contain suitable sediments for examining the effects of SOM load on submerged macrophyte restoration under natural conditions. In this study, in order to closely simulate the natural environment, sediments with a lower SOM load (SOM1) were collected from the location with higher submerged macrophyte coverage in Maojiabu, and sediments with higher SOM load (SOM3) were collected from the location with lower submerged macrophyte coverage in Xilihu. Sediment samples were collected using Peterson dredge. The impurities in the sediment were removed using a standard 4-mesh screen. Sediments from Maojiabu and Xilihu were mixed 1:1 by mass to obtain sediments with medium SOM load (SOM2).

V. natans is a common freshwater macrophyte species in the Eastern Plain region of China [6, 30] and has been widely used as an ecological engineering species in aquatic ecosystem restoration, owing to its strong adaptability and rapid growth characteristics [22, 31]. *V. natans* was purchased from a market in May 2021. Healthy plants with similar appearances were selected and pruned to a uniform standard (leaf and root lengths of 10.00 cm and 4.00 cm, respectively) [32]. The dry weights of a randomly selected subset of the plants (n = 10) were also measured. The plants were dried at 85ºC for 36 h to a constant weight and weighed using an electronic balance (absolute accuracy of 0.1 mg) (OHAUS, EX224, USA) to obtain the dry weights. The mean dry weight was 0.16±0.03 g (mean±SD).

Experimental Design

The experiments were conducted in a greenhouse. Four light intensity levels (LI1, LI2, LI3, and LI4) were established using shade nets at 10%, 30%, 60%, and 100% natural light intensity, respectively, based on field measurements and the photosynthetic compensation point of *V. natans* [33]. Before the experiment, sediment sub-samples were collected to characterize the initial sediment conditions (Table 1). Three SOM loads (SOM1, SOM2, and SOM3) were established, with initial SOM loads of 11%, 17%, and 25%, respectively. Twelve treatment groups were set up in this study. Each treatment was conducted with three replicates. For each experimental group, 6 plastic pots (5.6 cm in diameter and 13.6 cm in height) were placed evenly in a plastic case (61 cm in length, 42.5 cm in width, and 35 cm in height). The pots were filled with 7 cm of sediment and then placed slowly into the plastic case, which was filled with tap water. Water was then slowly added to a height of 33 cm above the bottom of the case. After the sediment settled, four plants were transplanted randomly into each pot, stabilized for a week, and covered with a sunshade net to ensure that the light intensity reaching the water surface was the required intensity. The experiments were conducted at room temperature (25-30ºC) for 70 days (sufficient to develop treatment-related growth differences with minimal tissue degradation associated with senescence) between June and September 2021. The evaporated overlying water was replenished weekly to ensure that the appropriate water depth was maintained for *V. natans* growth. For each treatment, three plant pots were removed randomly from three parallel plastic cases after 20, 40, and 70 days. Six plants were then selected randomly from the three pots to measure their growth characteristics [34, 35]. The temperature and pH of the water column were also recorded after each sample collection (Table S1). The growth characteristics included dry weight, root length, and shoot height. After 70 days, the sediment in each pot was homogenized and divided for physicochemical and microbiome analyses (stored at −80ºC until analysis).

Determination of Growth Characteristics of *V. natans*

The plants were washed gently to remove any residual sediment. The longest root and leaf of each plant were then measured to determine the root length and shoot height, respectively. The plants were dried at 85ºC for 36 h to a constant weight and weighed using an electronic balance (absolute accuracy of 0.1 mg) to obtain the dry weights. The experimental groups each consisted of six replicates.

Sediment Analysis

The sediment was freeze-dried, crushed, sieved through a standard 80-mesh screen, and stored in a desiccator for physicochemical analysis. The organic content was measured as the loss on ignition (5 h at 550ºC). Total phosphorus (TP), inorganic phosphorus (IP), iron and aluminum phosphorus (Fe/Al-P), and calcium-phosphorus (Ca-P) were measured using molybdenum blue spectrophotometry with an ultraviolet spectrophotometer (SHIMADZU, UV-1800, Japan) according to the SMT protocol [36]. Total nitrogen (TN) was measured using alkaline potassium persulfate digestion ultraviolet spectrophotometry [37]. NH_4^+, nitrate (NO_3^-), and nitrite (NO_2^-) were measured using potassium chloride solution extraction spectrophotometry [38].

We measured the potential for N and P nutrient releases from the Maojiabu and Xilihu sediment in

Table 1. Properties of sediment with different SOM loads. SOM1 indicates sediment obtained from Maojiabu; SOM3 indicates sediment obtained from Xilihu; SOM2 indicates mixed SOM1 and SOM3 (1:1 by mass).

	SOM1	SOM2	SOM3	ANOVA
OM (%)	11.289±0.093 ^{c}	17.478±0.025 ^{b}	25.027±0.250 ^{a}	***
TN (mg g^{-1})	1.533±0.093 ^{c}	2.089±0.020 ^{b}	2.226±0.150 ^{a}	***
NO_3-N (mg g^{-1})	0.016 ±0.000 ^{c}	0.022± 0.001 ^{b}	0.026±0.000 ^{a}	***
NO_2-N (mg kg^{-1})	0.019± 0.011 ^{c}	0.041 ±0.019 ^{b}	0.095 ±0.011 ^{a}	**
NH_4^+-N (mg g^{-1})	0.103 ±0.004 ^{c}	0.143 ±0.003 ^{a}	0.112±0.003 ^{b}	***
TP (mg g^{-1})	1.535±0.018 ^{a}	1.450±0.099 ^{a}	1.464±0.004 ^{a}	ns
IP (mg g^{-1})	0.911±0.017 ^{a}	0.811±0.021 ^{b}	0.766±0.125 ^{c}	***
Fe/Al-P(mg g^{-1})	0.615±0.016 ^{a}	0.564±0.012 ^{b}	0.197±0.003 ^{c}	***
Ca-P(mg g^{-1})	0.283±0.009 ^{c}	0.398±0.011 ^{b}	0.489±0.013 ^{a}	***

One-way analysis of variance (ANOVA) was used to test for differences among the sediment types (n = 3), as indicated by ** $p \le 0.01$, *** $p \le 0.001$, ns: ≥0.05. Different letters indicate statistically significant differences.

pre-experiments. The TN content of the water column was measured using alkaline potassium persulfate digestion ultraviolet spectrophotometry [39]. The TP content of the water column was determined using ammonium molybdate spectrophotometry [40]. After the sediment had completely settled, we measured the TP and TN contents in the overlying water every five days. During a period of 30 days, TP was not detected in the overlying water (minimum detection of 0.01 mg L^{-1}), the TN concentration did not change significantly over time, and no significant differences were observed in the TN concentrations of the overlying water between containers with different SOM loads (univariate ANOVA with LSD post hoc test; $p > 0.05$). Therefore, in this experimental system, nutrient releases from the sediment were considered to be negligible in the short term. The decreases in sediment N and P contents were calculated by subtracting the mass concentrations of N and P on day 70 from the initial values. The percentage decreases in the sediment N and P contents were defined as the ratios of the decreases in N and P contents to the original mass concentrations (%).

The bacterial community in the sediment was analyzed using 16S rRNA gene high-throughput sequencing (Shanghai Meiji Biomedical Technology Co., Ltd). Detailed descriptions of the methods are provided in the Supplementary Materials. The standardized operational taxonomic units (OTUs) were input into the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) database to predict the functional abundances of microbiota in the sediment based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) [41]. The nitrogen metabolism pathways and functional genes are available on the KEGG website (https://www.genome.jp/pathway/map00910). Pathways associated with NH_4^+ formation include nitrogen fixation, dissimilatory nitrate reduction, and organic nitrogen conversion to NH_4^+. Pathways related to NH_4^+ consumption include nitrification, complete nitrification, and NH_4^+ transformation to organic nitrogen (Fig. 3a). The 16S rRNA gene high-throughput raw sequence data were uploaded to the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database under accession number PRJNA901230. The raw sequence data are publicly accessible at https://www.ncbi.nlm.nih.gov/.

Data Analysis

Statistical analyses were performed using the R software package (R Project, version 4.1.3). Data were assessed for normality and homogeneity prior to analysis. One-way analysis of variance (ANOVA) with an LSD post-hoc test was performed to evaluate any significant differences in the initial properties of sediment with different SOM loads, as well as significant differences in NH_4^+ contents and abundances of bacterial functional genes associated with NH_4^+ formation and consumption under the respective environmental conditions. Maximum and minimum values were removed from the growth characteristic measurements of *V. natans*. A two-way ANOVA was performed to demonstrate the independent and interactive effects of light intensity and SOM load on the growth of *V. natans*. Multiple regression analysis was used to assess the impacts of light intensity and SOM load on *V. natans* and NH_4^+ content. The results obtained from the above experiments showed that both linear trend and single maximum in response to changes in LI were clear for the growth characteristics of *V. natans* and sediment NH_4^+ contents when the SOM load is fixed. This was also true when LI was fixed (Figs S1−S4), and the interaction of LI and SOM load had a significant effect on the growth characteristics of *V. natans* and sediment

Table 2. Two-way ANOVA results for the growth characteristics of *V. natans* and sediment NH_4^+ contents (n = 4).

		Light intensity		SOM load		Interaction	
		F	*P*	F	*p*	F	*p*
20 days	Dry weight	439.580	<0.001	119.190	<0.001	75.040	<0.001
	Shoot height	76.145	<0.001	4.143	0.024	15.092	<0.001
	Root length	49.145	<0.001	4.728	0.015	6.494	<0.001
40 days	Dry weight	51.919	<0.001	8.516	<0.001	5.949	<0.001
	Shoot height	41.682	<0.001	9.559	<0.001	3.430	0.009
	Root length	103.403	<0.001	31.800	<0.001	8.915	<0.001
70 days	Dry weight	41.600	<0.001	21.160	<0.001	12.160	<0.001
	Shoot height	224.320	<0.001	60.300	<0.001	21.410	<0.001
	Root length	18.080	<0.001	10.760	<0.001	14.220	<0.001
	NH_4^+	263.89	<0.001	354.58	<0.001	31.650	<0.001

NH_4^+ contents (Table 3). After assessing the fit of the models by the coefficient of determination (R_{adj}^2), we selected the best model to explain the variation of the growth characteristics of *V. natans* and sediment NH_4^+ contents with LI and SOM load. The linear model is described as follows:

$$Y = constant + a(LI) + b(SOM) + c(LI^2) + d(SOM^2) + e(LI \times SOM)$$

where Y is the growth characteristics of *V. natans* or the sediment NH_4^+ content, *LI* indicates light intensity, *LI × SOM* indicates the interactions between light intensity and SOM load, *a* is the partial coefficient of the relationship with LI, *b* is the partial coefficient of the relationship with SOM, *c* is the partial coefficient of the relationship with LI^2, *d* is the partial coefficient of the relationship with SOM^2, and *e* is the partial coefficient of the relationship with *LI × SOM*. Hierarchical partitioning was used to estimate the importance of each explanatory variable and group of variables, as well as how well they explained the variabilities of the growth characteristics of *V. natans* and NH_4^+ content. Hierarchical partitioning was performed using the R package “rdacca.hp” [42, 43]. A correlation analysis of the growth characteristics of *V. natans* with NH_4^+ contents and decreases in sediment N and P contents was conducted using linear regression. When the residuals did not achieve normality or homogeneity in the linear regression, *p* values were obtained using the lmorigin function and permutation tests [44, 45]. Structural equation modeling was performed using the “piecewiseSEM” package in R to examine the direct and indirect effects of light intensity and SOM load on the sediment NH_4^+ content, growth characteristics of *V. natans*, and N and P removal from the sediment [46]. The significance level for all tests was set as $p<0.05$.

Results

Interactions between Light Intensity and SOM Load on the Growth of *V. natans*

The plant characteristics after 20, 40, and 70 days are shown in Figs. S1−S3. The results of the two-way ANOVA indicate that both the independent and combined effects of light intensity and SOM load on the growth characteristics of *V. natans* were significant ($p<0.05$) after 20, 40, and 70 days (Table 2).

The hierarchical partitioning indicates that the variable that explained the largest part of the dry weight variability after 70 days was light intensity (R^2 = 0.324), followed by SOM load (R^2 = 0.139) and the interactions between light intensity and SOM load (R^2 = 0.076) (Fig. 1c4). The variable that explained the largest part of the variability in shoot height was light intensity (R^2 = 0.659), followed by SOM load (R^2 = 0.104) and the interactions between light intensity and SOM load (R^2 = 0.036) (Fig. 1c6). The importances of each explanatory variable and group of variables on the variability in root length were consistent with those of the dry weight (Fig. 1c5).After 20 and 40 days, the importances of each explanatory variable and group of variables on the growth characteristics of *V. natans* were essentially the same as those after 70 days (Fig. 1a4-6, Fig. 1b4-6). However, the growth characteristics of *V. natans* exhibited different tendencies in response to the various light intensities and SOM loads.

The multiple linear regression modeling results indicate that after 70 days, as the SOM load increased, the dry weights and root lengths of *V. natans* increased then decreased, exhibiting a single maximum. When the SOM load was 11% or 17%, an increase in light intensity increased the dry weights and root lengths of *V. natans*; however, increasing the light intensity at a

Table 3. Results of equations with the best fit for the growth characteristics of *V. natans* (dry weight, shoot height, and root length) and sediment NH_4^+ contents (n = 4).

	Equations									
	Characteristics	Constant	a (LI)	b (LI^2)	c (SOM)	d (SOM^2)	e (LI × SOM)	R_{adj}^2	F	*p*
20 days	Dry weight	-	1.49E-03	-	1.86E-02	-5.70E-04	-	0.648	18.30	1.34E-09
	Height	3.83E+01	5.61E-01	-5.42E-03	-	-	-	0.481	9.72	3.21E-06
	Root length	-	1.68E-01	-8.61E-04	-	-	-	0.624	16.60	4.88E-09
40 days	Dry weight	-	-	3.42E-03	-	-	-1.13E-04	0.753	29.70	8.90E-13
	Height	3.16E+01	8.16E-01	-7.59E-03	-	-	-	0.685	21.40	1.38E-10
	Root length	-1.55E+01	2.07E-01	-	2.31E+00	-5.75E-02	-4.18E-03	0.837	49.40	2.20E-16
70 days	Dry weight	-	5.29E-03	-	4.38E-02	-1.22E-03	-	0.540	12.00	2.98E-07
	Height	-	1.26E+00	-1.11E-02	6.27E+00	-1.74E-01	-	0.799	38.40	1.28E-14
	Root length	6.38E+00	7.34E-02	-	-	-	-	0.340	5.84	3.49E-04
	NH_4^+	1.82E-02	-1.54E-04	1.36E-06	-8.53E-04	4.72E-05	-5.41E-06	0.883	71.82	< 2.2E-16

LI: light intensity; LI × SOM: interaction between LI and SOM load. The model parameters of the equation used for the species were as follows: a = partial coefficient for the relationship with LI; b = partial coefficient for the relationship with LI^2 (quadratic term); c = partial coefficient for the relationship with SOM; d = partial coefficient for the relationship with SOM^2 (quadratic term); and e = partial coefficient for the relationship with LI×SOM. Only significant coefficients are presented.

25% SOM load failed to increase the dry weights and root lengths further (Fig. 1c1, c2). The shoot heights of *V. natans* varied negligibly with increasing SOM loads, but exhibited a single maximum with increasing light intensity. The shoot height reached a maximum at a 60% light intensity and 17% SOM load (Fig. 1c3). After 20 and 40 days, the responses of the growth characteristics of *V. natans* to changes in light intensity and SOM load were the same as those after 70 days (Fig. 1a1-3, b1-3).

Variability in NH_4^+ Contents and Bacterial Functional Gene Abundances

The sediment NH_4^+ contents after 70 days are shown in Fig. S4. Based on hierarchical partitioning, the variable that explained most of the variability in NH_4^+ content was the SOM load (R^2 = 0.435), followed by light intensity (R^2 = 0.323) and the interactions between light intensity and SOM load (R^2 = 0.126) (Fig. 2b). The response of the NH_4^+ content to changes in light intensity and SOM load was approximately opposite of those of the dry weights and root lengths (Fig. 2a). As the SOM load increased, the NH_4^+ content increased significantly ($p<0.001$); however, when the light intensity increased, the NH_4^+ content decreased significantly ($p<0.001$) (Fig. 2c, d). Furthermore, the linear regression analysis indicates that dry weight and root length were negatively correlated with NH_4^+ content (R^2 = 0.243–0.426, $p<0.001$) (Fig. 2e, f).

The one-way ANOVA showed that the abundances of bacterial functional genes associated with NH_4^+ formation in sediment did not differ significantly among the LI2, LI3, and LI4 groups ($p>0.05$), but were significantly lower than that of the LI1 group ($p<0.05$) (Fig. 3b). The abundances of bacterial functional genes associated with NH_4^+ consumption in the sediment of the different LI groups did not differ significantly ($p>0.05$) (Fig. 3c) There were no significant differences between the abundances of functional genes associated with NH_4^+ formation among the different SOM loads ($p>0.05$) (Fig. 3d). In addition, no significant differences were observed in the abundances of functional genes associated with NH_4^+ consumption between the SOM2 and SOM3 groups ($p>0.05$); however, the abundances were lower than those in the SOM1 group ($p<0.05$) (Fig. 3e).

Relationship between the Growth Characteristics of *V. natans* and Decreases in Sediment N and P Contents

Submerged macrophytes have the ecological function of removing N and P from the sediment. After 70 days of growth, the sediment TN contents decreased by 0.22-20.58% (Fig. 4a) and the Fe/Al-P contents decreased by 5.08-51.86% (Fig. 4c). The linear regression results indicate that the decrease in sediment TN content was positively correlated with shoot height (R_{adj}^2 = 0.272, $p<0.001$) (Fig. 4b), and that the decrease in Fe/Al-P content was positively correlated with dry weight (R_{adj}^2 = 0.112, p = 0.015) (Fig. 4d).

Synthetic Effects Illustrated by Structural Equation Modeling

According to the structural equation modeling results, light intensity and SOM load could affect the NH_4^+ content of the sediment both directly and indirectly, which affected the growth characteristics of *V. natans*, as well as N and P removal from the sediment (Fig. 5). For the direct effects, light intensity and NH_4^+ content were negatively correlated (standard path coefficient = −0.418, $p<0.001$), whereas the SOM load was positively correlated with NH_4^+ content (standard path coefficient = 0.581, $p<0.001$). Indirectly, the light intensity and SOM load altered the abundances of functional genes associated with NH_4^+ formation (standard path coefficient = −0.575, $p<0.001$) and NH_4^+ consumption (standard path coefficient = −0.630, $p<0.001$). The abundance of functional genes associated with NH_4^+ formation was positively correlated with NH_4^+ content (standard path coefficient 0.354, $p<0.001$). NH_4^+ content was negatively correlated with dry weight (standard path coefficient = −0.662, $p<0.001$) and shoot height (standard path coefficient = −0.180, $p>0.05$). Dry weight and shoot height were positively correlated with the removal of sediment Fe/Al-P (standard path coefficient = 0.320, $p<0.05$) and TN (standard path coefficient = 0.508, $p<0.001$), respectively.

Discussion

Understanding the responses of submerged macrophytes to environmental factors is essential for their successful restoration. However, previous studies have insufficiently revealed the effects of the interactions between multiple environmental factors [15, 17-21]. In this study, we investigated the interactions and the mechanisms by which light intensity and SOM load affect submerged macrophyte growth. The results indicate that light intensity and SOM load significantly affected the growth of *V. natans*. Enhanced light intensity decreased the sediment NH_4^+ content, thereby alleviating the NH_4^+ toxicity to *V. natans* and improving its growth and N and P removal from the sediment.

Interactions between Light Intensity and SOM Load Affected *V. natans* Growth

In this study, we collected sediment from West Lake to closely simulate the natural environment. The results indicate that the dry weight of *V. natans* exhibited a single maximum with increasing SOM loads. Both low and high SOM loads were unfavorable for *V. natans* growth. Silveira and Thomaz observed a single maximum in the growth characteristics of *Hydrilla verticillata*s with increasing SOM loads [21],

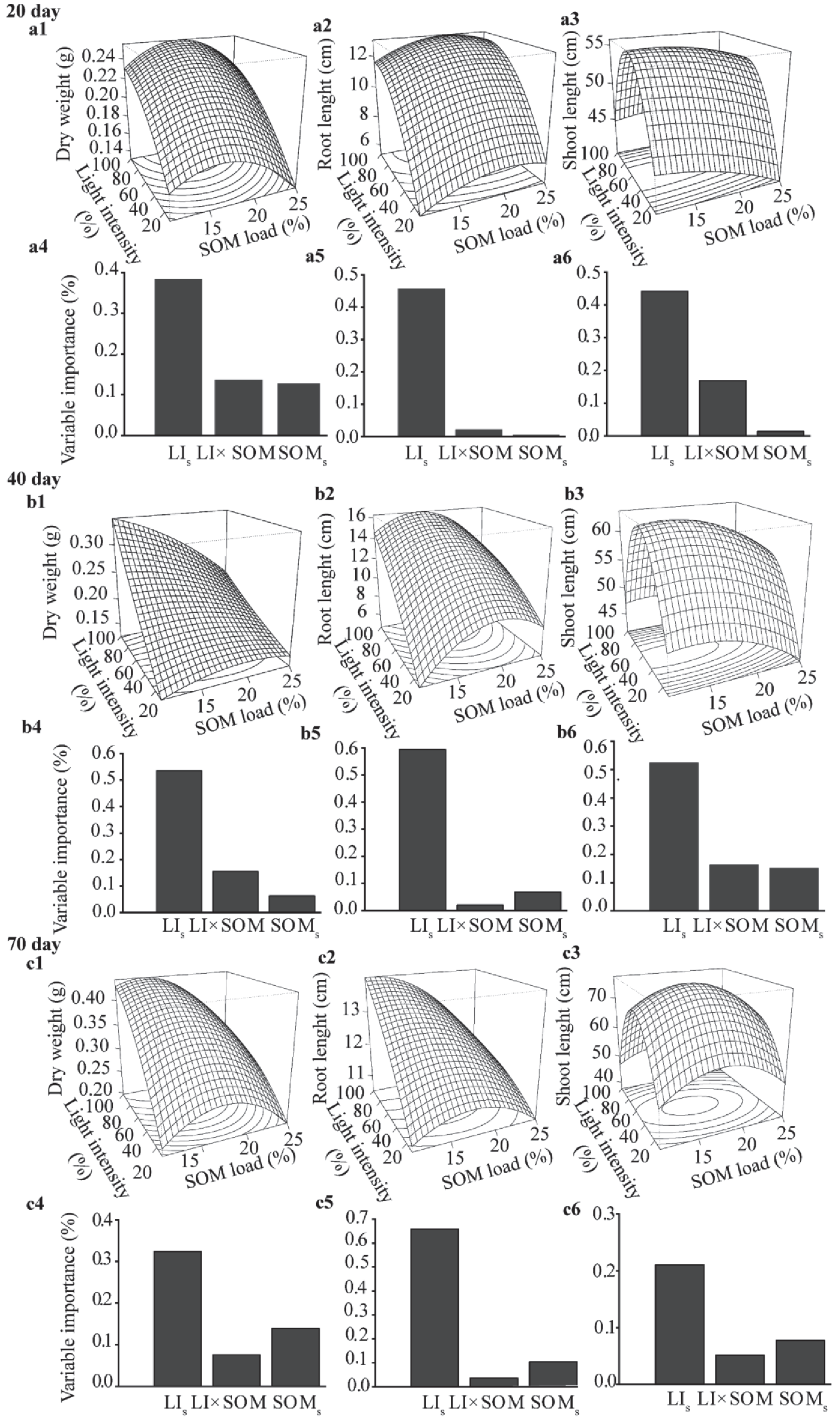


Fig. 1. Effects of the interactions between light intensity and sediment organic matter (SOM) load on the growth characteristics of *V. natans*, including dry weight (a1, b1, c1), root length (a2, b2, c2), and shoot height (a3, b3, c3) at different times (n = 4). The importance of each explanatory variable and group of variables for the dry weight (a4, b4, c4), root length (a5, b5, c5), and shoot height (a6, b6, c6) variabilities at different times are also shown. (LI: light intensity; LI × SOM: interactions between light intensity and SOM load. LIs include LI and LI^2; SOMs include SOM and SOM^2.).

which is consistent with the results of the present study. The inhibitory effect of SOM on the growth of *V. natans* could be alleviated by increasing the light intensity under a certain SOM range; however, when the SOM load reached 25%, the inhibition could not be alleviated effectively by enhancing the light intensity. Under low light intensity and high SOM load conditions (e.g., LI1_SOM3), decreasing the SOM load was more effective than increasing the light intensity. Therefore, enhancing the light intensity ensured submerged

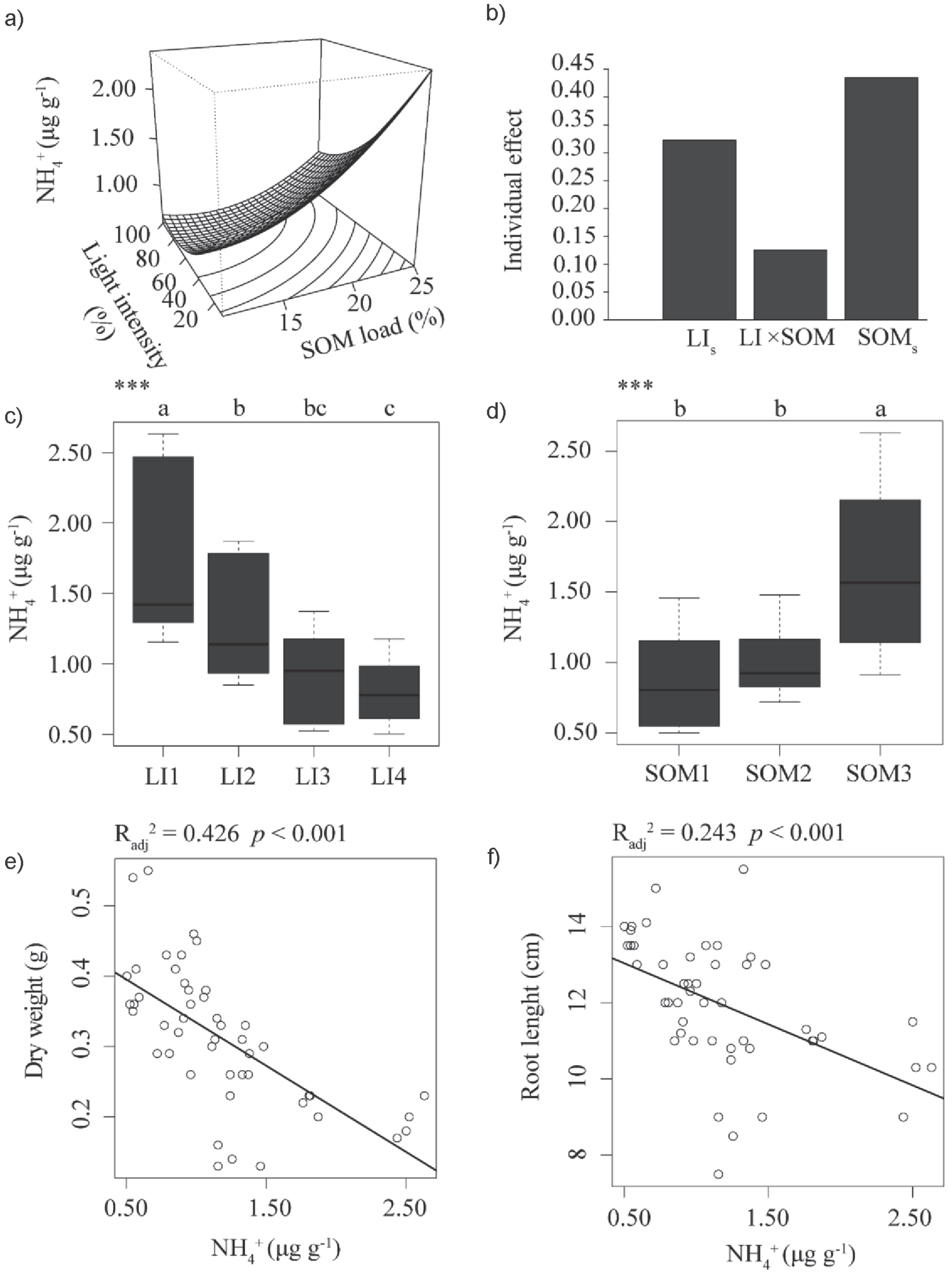


Fig. 2. Relationships between sediment NH_4^+ content and light intensity, SOM load, and growth characteristics of *V. natans* (n = 4). a. Effects of the interactions between light intensity and SOM on NH_4^+ content. b. Importance of each explanatory variable and group of variables on the variability in NH_4^+ content. c, d. NH_4^+ contents under various light intensity levels and SOM loads. e, f. Relationships between NH_4^+ content and the growth characteristics of *V. natans*. (LI: light intensity; LI × SOM: interactions between light intensity and SOM load. LIs include LI and LI^2; SOMs include SOM and SOM^2. LI1, LI2, LI3, and LI4 indicate 10%, 30%, 60%, and 100% natural light intensities, respectively. SOM1, SOM2, and SOM3 had initial SOM loads of 11%, 17%, and 25%, respectively.).

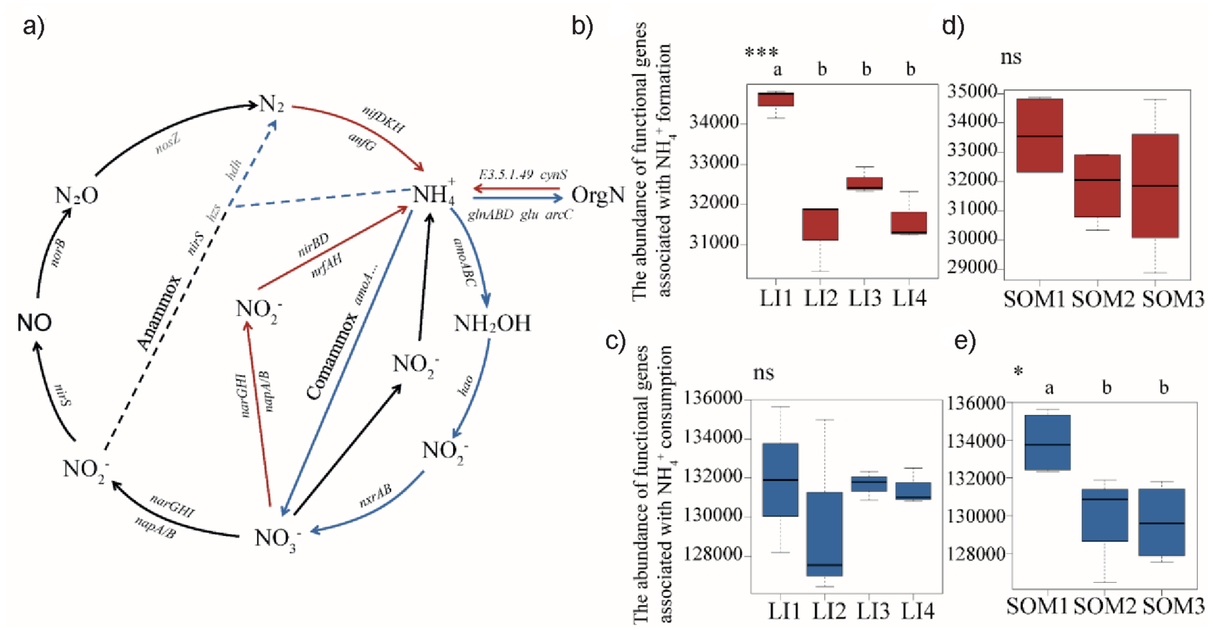


Fig. 3. Abundances of bacterial functional genes associated with NH_4^+ formation and consumption at different light intensity levels (n = 3) and SOM loads (n = 4). (LI1, LI2, LI3, and LI4: 10%, 30%, 60%, and 100% natural light intensity, respectively. SOM1, SOM2, and SOM3 had initial SOM loads of 11%, 17%, and 25%, respectively.).

macrophyte restoration over a wide range of SOM loads; however, improving both the SOM load and light intensity were necessary at a 25% SOM load. Decreasing the SOM load is a cost-efficient option. Under low SOM load and low light intensity conditions, submerged macrophytes could not obtain sufficient nutrients; consequently, their growth was impaired. Enhancing the light intensity improved the photosynthetic capacity of the plants, resulting in the production of more carbohydrates. A SOM load of 25% produced an anoxic environment, which can enrich potentially phytotoxic substances in the sediment, including phenols and organic acids, reduced iron and manganese, hydrogen sulfide, and NH_4^+, and inhibit plant growth [15, 20, 21]. In this study, the inhibition of *V. natans* growth by high SOM loads was alleviated by enhancing the light intensity. A potential explanation for this phenomenon is that the enhanced light intensity promotes photosynthesis in the plants, thereby promoting the release of oxygen outwards from the root system, ensuring aerobic plant metabolism in low-oxygen sediment, and counteracting phytotoxins [47].

Enhanced Light Intensity Alleviated NH_4^+ Toxicity in *V. natans*

Previous studies of the effects of NH_4^+ on submerged macrophytes have primarily investigated NH_4^+ in the water column [25, 27]. Sediment serves as the basis for submerged macrophyte root systems, subsequently influencing their growth. However, few studies have investigated the effects of NH_4^+ in sediment on submerged macrophytes. The sediment NH_4^+ content was negatively correlated with the dry weight of *V. natans* in the present study (Fig. 2e). By adding ammonium carbonate to the sediment, Zhang et al. observed that the limiting NH_4^+ content in the initial sediment was 0.01 mg g^{-1} [48]. Higher NH_4^+ contents resulted in increased growth inhibition. In this study, the initial NH_4^+ contents were 0.103–0.143 mg g^{-1} (Table 1), which is sufficient to inhibit the growth of *V. natans*. After 70 days, the NH_4^+ contents were only 0.005-0.026 mg g^{-1} (Fig. 2a), which is still capable of inhibiting growth. This observed inhibitory effect is due to increased NH_4^+ absorption by plants at rates higher than the conversion of NH_4^+ into amino acids and amides at high sediment NH_4^+ contents. NH_4^+ accumulates in plant tissues, causing C–N imbalances and damage to the chloroplast ultrastructure [26, 27].

The NH_4^+ content increased significantly with increasing SOM loads after 70 days, but decreased with enhanced light intensities. SOM enrichment results in higher NH_4^+ contents, which causes NH_4^+ toxicity in plants [23, 25]. The results of this study indicate that the abundances of functional genes associated with NH_4^+ consumption in the SOM2 and SOM3 groups were significantly lower than those in the SOM1 group (Fig. 3e). Therefore, the sediment NH_4^+ enrichment phenomenon observed at high SOM loads may be due to the inhibition of bacterial nitrification reactions in the anaerobic environment. Further experimental verification is required to verify this hypothesis. Higher tricarboxylic acid cycle activity after enhancing the light intensity supports the production of more amino acids and proteins by plants, which can improve NH_4^+ assimilation by plants and decrease the sediment NH_4^+

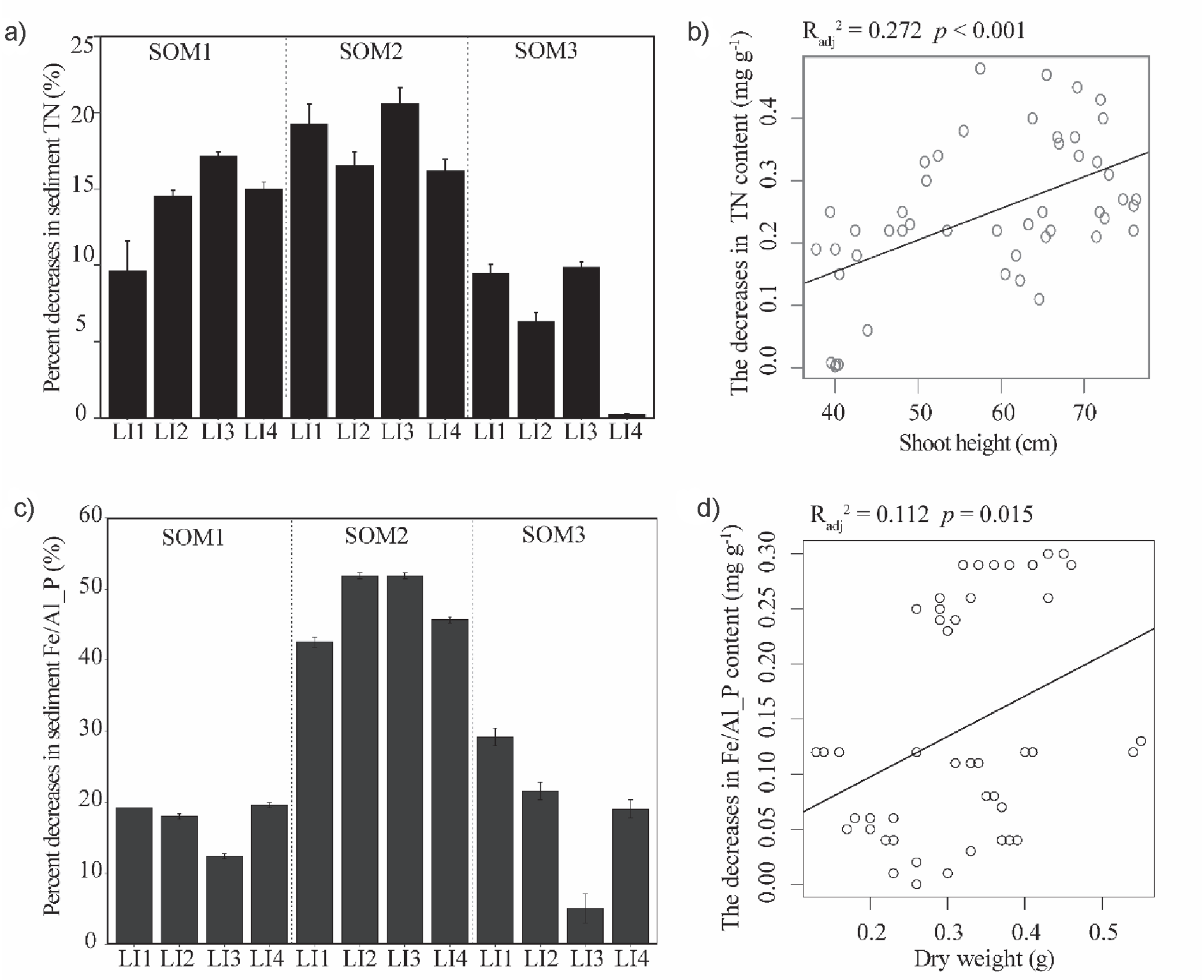


Fig. 4. Percent decreases in sediment total nitrogen (TN) and iron and aluminum phosphorus (Fe/Al-P) contents under different environmental conditions after 70 days and the relationships between TN and Fe/Al-P removal and the growth characteristics of *V. natans* (n = 4). (LI1, LI2, LI3, and LI4: 10%, 30%, 60%, and 100% natural light intensity, respectively. SOM1, SOM2, and SOM3 had initial SOM loads of 11%, 17%, and 25%, respectively.).

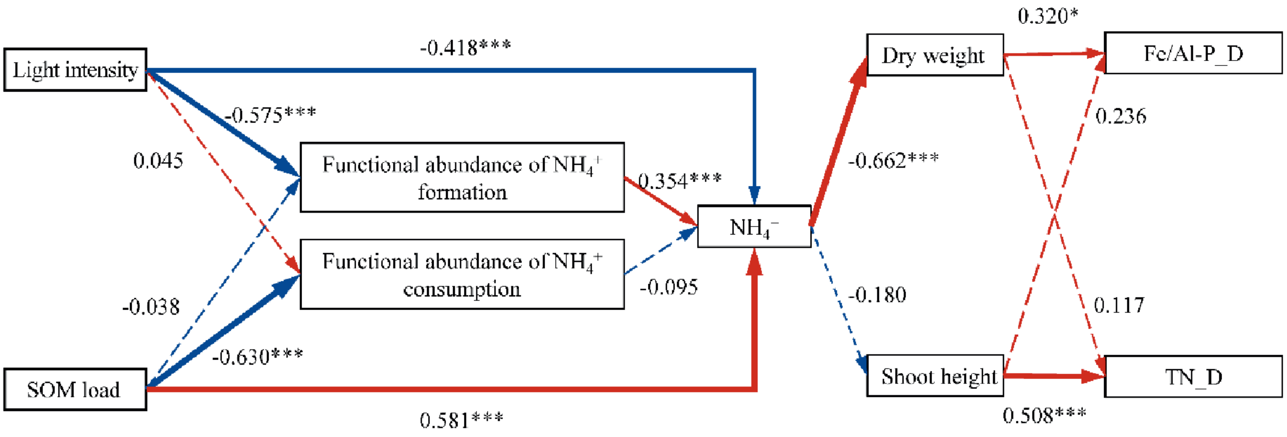


Fig. 5. Structural equation modeling representing the relationship between light intensity, SOM load, abundances of bacteria functional genes associated with NH_4^+ formation and consumption, growth characteristics of *V. natans*, and decreases in sediment TN and Fe/Al-P contents. Solid lines indicate significant paths (n = 4; N = 48; * $p<0.05$; ** $p<0.01$; *** $p<0.001$). Dashed lines indicate non-significant paths ($p>0.05$). Red and blue colors indicate positive and negative path intensities, respectively. Numbers on the arrows are the normalized path coefficients, which indicate the strength of the influence of one factor on another. Arrow widths are proportional to the standard path coefficients denoted by the adjacent numbers.

content [24, 26]. Collectively, the findings of previous studies are consistent with the results of this study. Evidently, SOM enrichment could subject submerged macrophytes to NH_4^+ toxicity, but enhancing the light intensity alleviated the resulting toxicity.

The abundances of functional genes associated with NH_4^+ formation in the sediment of the LI2–LI4 groups were significantly lower than those in the LI1 group (Fig. 3a). Enhanced light intensity resulted in higher photosynthetic capacity of the plants, which supports the release of more oxygen from the roots [15, 47]. Allen et al. found that oxygen released by wetland plants is the main oxygen supply to the root zone, contributing up to 90% of the total [49]. Increased oxygen release shifted the sediment toward a more oxidizing environment, which led to the inhibition of denitrifying bacterial growth and a lower NH_4^+ formation rate [25, 50]. Therefore, light intensity enhancement can mitigate NH_4^+ toxicity to *V. natans* in two ways: by improving NH_4^+ assimilation and reducing the bacterial NH_4^+ formation process.

Growth Characteristics of *V. natans* Directly Affected its N and P Removal Capacity

Sediment plays a critical role as both a sink and source in the N and P dynamics of lakes [51, 52]. Therefore, in addition to reducing exogenous pollution inputs, controlling lake eutrophication by reducing N and P loads in sediment is considered an important ecological restoration method. The results of this study indicate that after 70 days of *V. natans* growth, the TN and Fe/Al-P contents of the sediment decreased by 0.22–20.58% and 5.08–51.86%, respectively. The observed decreases were also positively correlated with the dry weight and shoot height of *V. natans*. Previous studies have indicated that the absorption of N and P from sediment by submerged macrophytes is necessary to reduce endogenous pollution in lakes [53-55]. The absorption capacity observed in *V. natans* was higher than that in other submerged macrophytes, owing to its highly developed root tissues [54]. In this study, the growth characteristics of *V. natans* were positively correlated with Fe/Al-P removal, but not with TP removal. A potential reason for this is that TP also contains organic phosphorus (OP) and Ca-P, which plants cannot utilize directly. Compared with other forms of phosphorus, Fe/Al-P is more bioavailable, and can be absorbed and used by plants [31]. Furthermore, previous studies have determined that Fe/Al-P removal by *V. natans* is higher than its OP and Ca-P removal [54, 55]. Therefore, it is crucial to ensure the growth of appropriate submerged macrophytes to maintain the ecological function of sediment N and P removal.

One limitation of this study is that we focused on the effects of the interactions between SOM load and light intensity on submerged macrophyte growth, and did not consider the effects of nutrients released from the sediment into the overlying water column. However, this does not exclude the problem of endogenous pollution caused by long-term releases of nutrient salts from the sediment to the overlying water column in lake ecosystems.

Conclusions

In this study, we determined the effects of the interactions between light intensity and SOM load on the growth of *V. natans*. The growth of *V. natans* exhibited a single maximum with increasing SOM loads. Within a certain range of SOM loads, enhancing the light intensity could alleviate stress caused by the sediment on the growth of *V. natans*; however, this stress persisted with increasing light intensities at a 25% SOM load. Enhanced light intensity may alleviate NH_4^+ toxicity to *V. natans* by increasing its NH_4^+ assimilation capacity and decreasing the bacterial functions related to NH_4^+ formation, thereby promoting plant growth and sediment N and P removal by *V. natans*. In the future, long-term *in situ* ecological enclosure experiments should be designed to consider the combined effects of more environmental factors on submerged macrophyte growth. In addition, quantitative real-time polymerase chain reaction and metabolomic analyses should be applied to analyze the responses of microbial functional genes and metabolites associated with N and P metabolism. The results of this study indicate that enhancing the light intensity could achieve submerged macrophyte restoration and sediment N and P removal over a broad range of SOM loads. However, under low light intensity and high SOM load conditions, submerged macrophyte restoration is dependent on improving the environmental factors (i.e., reducing the SOM load, which is more cost-efficient). This study contributes to the optimization of submerged macrophyte restoration measures in lakes.

Acknowledgments

We thank Qingjun Fang and Ze Hu for their assistance with sediment collection from West Lake in Hangzhou. This work was supported by the National Natural Science Foundation of China (Nos. 51809257 and 32173027), and the Wuhan Knowledge Innovation Project (No. 2022020801020332).

Conflict of Interest

The authors declare no conflict of interest.

References

1. SCHINDLER D.W., HECKY R.E., FINDLAY D.L., STAINTON M.P., PARKER B.R., PATERSON M.J., BEATY K.G., LYNG M., KASIAN S.E.M. Eutrophication

of lakes cannot be controlled by reducing nitrogen input: results of a 37-year whole-ecosystem experiment. Proceedings of The National Academy of Sciences of The United States of America. **105**, 11254, **2008**.

2. SCHEFFER M. Alternative stable states in eutrophic, shallow freshwater systems: a minimal model. Aquatic Ecology. **23**, 73, **1989**.
3. BLINDOW I. Deline of charophytes during eutrophication: comparison with angiosperms. Freshwater Biology. **28**, 9, **1992**.
4. SMITH V.H., SCHINDLER D.W. Eutrophication science: where do we go from here? Trends in Ecology & Evolution. **24**, 201, **2009**.
5. LAW A., BAKER A., SAYER C., FOSTER G., GUNN I.D.M., TAYLOR P., PATTISON Z., BLAIKIE J., WILLBY N.J. The effectiveness of aquatic plants as surrogates for wider biodiversity in standing fresh waters. Freshwater Biology. **64**, 1664, **2019**.
6. LIU H., ZHOU W., LI X.W., CHU Q.S., TANG N., SHU B.Z., LIU G.H., XING W. How many submerged macrophyte species are needed to improve water clarity and quality in Yangtze floodplain lakes? Science of The Total Environment. **724**, 138267, **2020**.
7. ZHANG X.K., ZHANG J.W., LI Z.F., WANG G.J., LIU Y., WANG H.L., XIE J. Optimal submerged macrophyte coverage for improving water quality in a temperate lake in China. Ecological Engineering. **162**, 106177, **2021**.
8. ROLLAND D., HAURY J., MARMONIER P., LAGADEUC Y. Effect of macrophytes on flow conditions and deposition of suspended particles in small streams: an experimental study using artificial vegetation (French). Revue des Sciences de I Education. **28**, 231, **2015**.
9. GRANÉLI W., SOLANDER D. Influence of aquatic macrophytes on phosphorus cycling in lakes. Hydrobiologia. **170**, 245, **1988**.
10. LIU Z.S., BAI G.L., LIU Y.L., ZOU Y.L.Y., DING Z.M., WANG R., CHEN D., KONG L., WANG C., LIU L., LIU B., ZHOU Q., HE F., WU Z., ZHANG Y. Long-term study of ecological restoration in a typical shallow urban lake. Science of The Total Environment. **846**, 157505, **2022**.
11. ENGELHARDT K.A.M., RITCHIE M.E. Effects of macrophyte species richness on wetland ecosystem functioning and services. Nature. **411**, 687, **2001**.
12. WOOD K.A., O'HARE M.T., MCDONALD C., SEARLE K.R., DAUNT F., STILLMAN R.A. Herbivore regulation of plant abundance in aquatic ecosystems. Biological Reviews. **92**, 1128, **2017**.
13. BIRK S., BONNE W., BORJA A., BRUCET S., COURRAT A., POIKANE S., SOLIMINI A., VAN DE BUND W., ZAMPOUKAS N., HERING D. Three hundred ways to assess Europe's surface waters: an almost complete overview of biological methods to implement the Water Framework Directive. Ecological Indicators. **18**, 31, **2012**.
14. SONG Y.L., LIEW J.H., SIM D.Z.H., MOWE M.A.D., MITROVIC S.M., TAN H.T.W., YEO D.C.J. Effects of macrophytes on lake-water quality across latitudes: a meta-analysis. Oikos. **128**, 468, **2019**.
15. KOCH E.M. Beyond light: physical, geological, and geochemical parameters as possible submersed aquatic vegetation habitat requirements. Estuaries. **24**, 1, **2001**.
16. BORNETTE G., PUIJALON S. Response of aquatic plants to abiotic factors: a review. Aquatic Sciences. **73**, 1, **2011**.
17. HE L., ZHU T.S., WU Y., LI W., ZHANG H., ZHANG X.L., CAO T., NI L.Y., HILT S. Littoral slope, water depth and alternative response strategies to light attenuation shape the distribution of submerged macrophytes in a mesotrophic lake. Frontiers in Plant Science. **10**, 169, **2019**.
18. ERSOY Z., SCHARFENBERGER U., BAHO D.L., BUCAK T., FELDMANN T., HEJZLAR J., LEVI E.E., MAHDY A., NÕGES T., PAPASTERGIADOU E., STEFANIDIS K., ŠORF M., SØNDERGAARD M., TRIGAL C., JEPPESEN E., BEKLIOĞLU M. Impact of nutrients and water level changes on submerged macrophytes along a temperature gradient: a pan-European mesocosm experiment. Global Change Biology. **26**, 6831, **2020**.
19. FENG M., ZHANG P., CHENG H., FRENKEN T., XU J., ZHANG M. Interactive effects of light and snail herbivory rather than nutrient loading determine early establishment of submerged macrophytes. Evolutionary Ecology. **12**, e9070, **2022**.
20. SOANA E., NALDI M., BARTOLI M. Effects of increasing organic matter loads on pore water features of vegetated (*Vallisneria spiralis* L.) and plant-free sediments. Ecological Engineering. **47**, 141, **2012**.
21. SILVEIRA M.J., THOMAZ S.M. Growth of a native versus an invasive submerged aquatic macrophyte differs in relation to mud and organic matter concentrations in sediment. Aquatic Botany. **124**, 85, **2015**.
22. BAI G.L., ZHANG Y., YAN P., YAN W.H., KONG L.W., WANG L., WANG C., LIU Z.S., LIU B.Y., MA J.M., ZUO J.C., LI J., BAO J., XIA S.B., ZHOU Q.H., XU D., HE F., WU Z.B. Spatial and seasonal variation of water parameters, sediment properties, and submerged macrophytes after ecological restoration in a long-term (6 year) study in Hangzhou west lake in China: submerged macrophyte distribution influenced by environmental variables. Water Research. **186**, 116379, **2020**.
23. CHAPPUIS E., LUMBRERAS A., BALLESTEROS E., GACIA E. Deleterious interaction of light impairment and organic matter enrichment on *Isoetes lacustris* (Lycopodiophyta, Isoetales). Hydrobiologia. **760**, 145, **2015**.
24. SETIÉN I., FUERTES-MENDIZABAL T., GONZÁLEZ A., APARICIO-TEJO P.M., GONZÁLEZ-MURUA C., GONZÁLEZ-MORO M.B., ESTAVILLO J.M. High irradiance improves ammonium tolerance in wheat plants by increasing N assimilation. Journal of Plant Physiology. **170**, 758, **2013**.
25. BRITTO D.T., KRONZUCKER H.J. NH_4^+ toxicity in higher plants: a critical review. Journal of Plant Physiology. **159**, 567, **2002**.
26. BITTSÁNSZKY A., PILINSZKY K., GYULAI G., KOMIVES T. Overcoming ammonium toxicity. Plant Science. **231**, 184, **2015**.
27. SHI D.L., ZHUANG K., CHEN Y.H., XU F.L., HU Z.B., SHEN Z.G. Effects of excess ammoniacal nitrogen (NH_3-N) on pigments, photosynthetic rates, chloroplast ultrastructure, proteomics, formation of reactive oxygen species and enzymatic activity in submerged plant *Hydrilla verticillata* (L.f.) Royle. Aquatic Toxicology. **226**, 105585, **2020**.
28. REIS C.R.G., NARDOTO G.B., OLIVEIRA R.S. Global overview on nitrogen dynamics in mangroves and consequences of increasing nitrogen availability for these systems. Plant and Soil. **410**, 1, **2017**.
29. BAI G.L., LIU Y.L., LIU Z.S., KONG L.W., TANG Y.D., DING Z.M., ZOU Y.L.Y., WANG C., ZHANG C., CHEN D.S., LIU L., LIU B.Y., RAO L.H., XIA S.B., ZHOU Q.H., XU D., HE F., WU Z.B., ZHANG Y. Effects of lake geo-engineering on plankton in a typical shallow urban lake:

evidence based on 10-year data. ACS ES&T Engineering. **3**, 105, **2022**.

30. YANG G.S. Report of Lake Survey in China. Science Press. Beijing, 97, **2019** [In Chinese].
31. LIU Y.L., BAI G.L., ZOU Y.L.Y., DING Z.M., TANG Y.D., WANG R., LIU Z.S., ZHOU Q.H., WU Z.B., ZHANG Y. Combined remediation mechanism of bentonite and submerged plants on lake sediments by DGT technique. Chemosphere. **298**, 134236, **2022**.
32. BAI G.L., LUO F., ZOU Y.L.Y., LIU Y., WANG R., YANG H., LIU Z.S., CHANG J.J., WU Z.B., ZHANG Y. Effects of vermiculite on the growth process of submerged macrophyte *Vallisneria spiralis* and sediment microecological environment. Journal of Environmental Sciences-China. **118**, 130, **2022**.
33. VAN T.K., HALLER W.T., BOWES G. Comparison of photosynthetic characteristics of three submersed aquatic plants. Plant Physiology. **58**, 761, **1976**.
34. CHEN L., HAO Z.H., LI K.K., SHA Y., WANG E.T., SUI X.H., MI G.H., TIAN C.F., CHEN W.X. Effects of growth-promoting rhizobacteria on maize growth and rhizosphere microbial community under conservation tillage in Northeast China. Microbial Biotechnology. **14**, 535, **2020**.
35. YANG X., ZHANG Y., LAI J.L., LUO X.G., HAN M.W., ZHAO S.P., ZHU Y.B. Analysis of the biodegradation and phytotoxicity mechanism of TNT, RDX, HMX in alfalfa (*Medicago sativa*). Chemosphere. **281**, 130842, **2021**.
36. RUBAN V., LÓPEZ-SÁNCHEZ J.F., PARDO P., RAURET G., MUNTAU H., QUEVAUVILLER P. Harmonized protocol and certified reference material for the determination of extractable contents of phosphorus in freshwater sediments: a synthesis of recent works. Fresenius Journal of Analytical Chemistry. **370**, 224, **2001**.
37. LIN Q., PENG X., LIU B., MIN F., ZHANG Y., ZHOU Q., MA J., WU Z. Aluminum distribution heterogeneity and relationship with nitrogen, phosphorus and humic acid content in the eutrophic lake sediment. Environmental Pollution. **253**, 516, **2019**.
38. STATE EPA OF CHINA. Soil – Determination of ammonium, nitrite and nitrate by extraction with potassium chloride solution-spectrophotometric methods. China Environmental Science Press, Beijing, **2012** [In Chinese].
39. STATE EPA OF CHINA. Water quality – Determination of total nitrogen – Alkaline potassium persulfate digestion UV spectrophotometric method. China Environmental Science Press, Beijing (in Chinese), **2012**.
40. STATE EPA OF CHINA. Water quality – Determination of total phosphorus – Ammonium molybdate spectrophotometric method. China Environmental Science Press, Beijing, **1990** [In Chinese].
41. YU H.W., QI W.X., CAO X.F., HU J.W., LI Y., PENG J.F., HU C.Z., QU J.H. Microplastic residues in wetland ecosystems: do they truly threaten the plant-microbe-soil system? Environment International. **156**, 106708, **2021**.
42. LAI J.S., ZOU Y., ZHANG J.L., PERES-NETO P.R. Generalizing hierarchical and variation partitioning in multiple regression and canonical analyses using the rdacca.hp R package. Methods in Ecology and Evolution. **13**, 782, **2022**.
43. PECUCHET L., JØRGENSEN L.L., DOLGOV A.V., ERIKSEN E., HUSSON B., SKERN-MAURITZEN M., PRIMICERIO R. Spatio-temporal turnover and drivers of bentho-demersal community and food web structure in a high-latitude marine ecosystem. Diversity and Distributions. **28**, 2503, **2022**.
44. LEGENDRE P., DESDEVISES Y. Independent contrasts and regression through the origin. Journal of Theoretical Biology. **259**, 727, **2009**.
45. ZHANG Y., ZHANG H.C., LIU Q., DUAN L.Z., ZHOU Q.C. Total nitrogen and community turnover determine phosphorus use efficiency of phytoplankton along nutrient gradients in plateau lakes. Journal of Environmental Sciences. **124**, 699, **2023**.
46. LEFCHECK J.S. piecewiseSEM: Piecewise structural equation modeling in R for ecology, evolution, and systematics. Methods in Ecology and Evolution. **7**, 573, **2016**.
47. BRODERSEN K.E., NIELSEN D.A., RALPH P.J., KÜHL M. Oxic microshield and local pH enhancement protects *Zostera muelleri* from sediment derived hydrogen sulphide. New Phytologist. **205**, 1264, **2015**.
48. ZHANG J. The effect of sediment characters to the growth of submerged macrophytes. Master's Thesis. College of Environmental and Engineering, HoHai University, Nanjing, **2006** [In Chinese].
49. ALLEN W.C., HOOK P.B., BIEDERMAN J.A., STEIN O.R. Temperature and wetland plant species effects on wastewater treatment and root zone oxidation. Journal of Environmental Quality. **31**, 1010, **2002**.
50. RACCHETTI E., BARTOLI M., RIBAUDO C., LONGHI D., BRITO L.E.Q., NALDI M., IACUMIN P., VIAROLI P. Short term changes in pore water chemistry in river sediments during the early colonization by *Vallisneria spiralis*. Hydrobiologia. **652**, 127, **2010**.
51. SMITH V.H., TILMAN G.D., NEKOLA J.C. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environmental Pollution. **100**, 179, **1999**.
52. YAO Y., WANG P.F., WANG C., HOU J., MIAO L.Z., YUAN Y., WANG T., LIU C. Assessment of mobilization of labile phosphorus and iron across sediment-water interface in a shallow lake (Hongze) based on in situ high-resolution measurement. Environmental Pollution. **219**, 873, **2016**.
53. RACCHETTI E., LONGHI D., RIBAUDO C., SOANA E., BARTOLI M. Nitrogen uptake and coupled nitrification-denitrification in riverine sediments with benthic microalgae and rooted macrophytes. Aquatic Sciences. **79**, 487, **2017**.
54. WANG C., LIU Z.S., ZHANG Y., LIU B.Y., ZHOU Q.H., ZENG L., HE F., WU Z.B. Synergistic removal effect of P in sediment of all fractions by combining the modified bentonite granules and submerged macrophyte. Science of The Total Environment. **626**, 458, **2018**.
55. LIU Z.S., ZHANG Y., YAN P., LUO J., KONG L.W., CHANG J.J., LIU B.Y., XU D., HE F., WU Z.B. Synergistic control of internal phosphorus loading from eutrophic lake sediment using MMF coupled with submerged macrophytes. Science of The Total Environment. **731**, 138697, **2020**.

Supplementary Material

Materials and Methods

16S rRNA Gene High Throughput Sequencing Technique

Briefly, the V3–V4 hypervariable regions of bacteria 16S rRNA gene were amplified with primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') [1]. Polymerase chain reaction was performed using the following setup: 3 min of denaturation at 95ºC, 27 cycles of 30 s at 95ºC, 30 s for annealing at 55ºC, and 45 s for elongation at 72ºC, and a final extension at 72ºC for 10 min. The raw sequenced reads of the 16S rRNA gene were quality-filtered by fastp version 0.20.0 [2] and merged by FLASH version 1.2.7 [3] with the following criteria: (i) the 300 bp reads were truncated at any site receiving an average quality score of <20 over a 50 bp sliding window, and the truncated reads shorter than 50 bp were discarded, reads containing ambiguous characters were also discarded; (ii) only overlapping sequences longer than 10 bp were assembled according to their overlapped sequence. The maximum mismatch ratio of the overlap region is 0.2. Reads that could not be assembled were discarded; (iii) Samples were distinguished according to the barcode and primers, and the sequence direction was adjusted to exact barcode matching and two nucleotide mismatch in primer matching. UPARSE (version 7.1 http://drive5.com/uparse/) was used to cluster operational taxonomic units (OTUs) with 97% similarity [4, 5]. The resampled OTU table was used for subsequent community analysis. The taxonomy of each OTU representative sequence was analyzed by RDP Classifier version 2.2 against the 16S rRNA database (Silva v138) using confidence threshold of 0.7 [6]. All of the OTUs belonged to bacteria.

Table S1. Temperature and pH of the overlying water at 20, 40 and 70 days (mean ± SD, n = 3). LI1, LI2, LI3, and LI4 indicate 10%, 30%, 60%, and 100% natural light intensity, respectively. SOM1, SOM2, and SOM3 had initial SOM loads of 11%, 17%, and 25%, respectively.

		T (ºC)	pH
20 days	LI1_SOM1	22.30±0.10	8.00±0.05
	LI1_SOM2	22.27±0.12	7.90±0.08
	LI1_SOM3	22.23±0.06	7.85±0.04
	LI2_SOM1	22.27±0.38	7.90±0.01
	LI2_SOM2	22.03±0.06	7.87±0.02
	LI2_SOM3	22.13±0.06	8.02±0.05
	LI3_SOM1	22.19±0.11	8.12±0.08
	LI3_SOM2	22.10±0.09	8.15±0.07
	LI3_SOM3	22.30±0.15	8.07±0.04
	LI4_SOM1	22.32±0.06	8.13±0.04
	LI4_SOM2	22.40±0.16	8.05±0.09
	LI4_SOM3	22.32±0.12	8.10±0.05
40 days	LI1_SOM1	23.30±0.06	8.13±0.04
	LI1_SOM2	23.27±0.08	8.15±0.02
	LI1_SOM3	23.23±0.10	8.12±0.08
	LI2_SOM1	23.27±0.06	8.15±0.06
	LI2_SOM2	23.03±0.09	8.26±0.04
	LI2_SOM3	23.13±0.10	8.14±0.01
	LI3_SOM1	23.19±0.11	8.23±0.01
	LI3_SOM2	23.10±0.12	8.20±0.03
	LI3_SOM3	23.30±0.12	8.19±0.05
	LI4_SOM1	23.32±0.15	8.27±0.10
	LI4_SOM2	23.40±0.16	8.24±0.04
	LI4_SOM3	23.32±0.38	8.15±0.04
70 days	LI1_SOM1	24.30±0.08	8.51±0.05
	LI1_SOM2	24.27±0.16	8.42±0.09
	LI1_SOM3	24.23±0.15	8.56±0.03
	LI2_SOM1	24.27±0.09	8.4±0.06
	LI2_SOM2	24.03±0.12	8.41±0.12
	LI2_SOM3	24.13±0.11	8.42±0.01
	LI3_SOM1	24.19±0.10	8.5±0.03
	LI3_SOM2	24.10±0.09	8.64±0.14
	LI3_SOM3	24.30±0.11	8.57±0.09
	LI4_SOM1	24.32±0.06	8.54±0.04
	LI4_SOM2	24.40±0.08	8.49±0.05
	LI4_SOM3	24.32±0.06	8.57±0.17

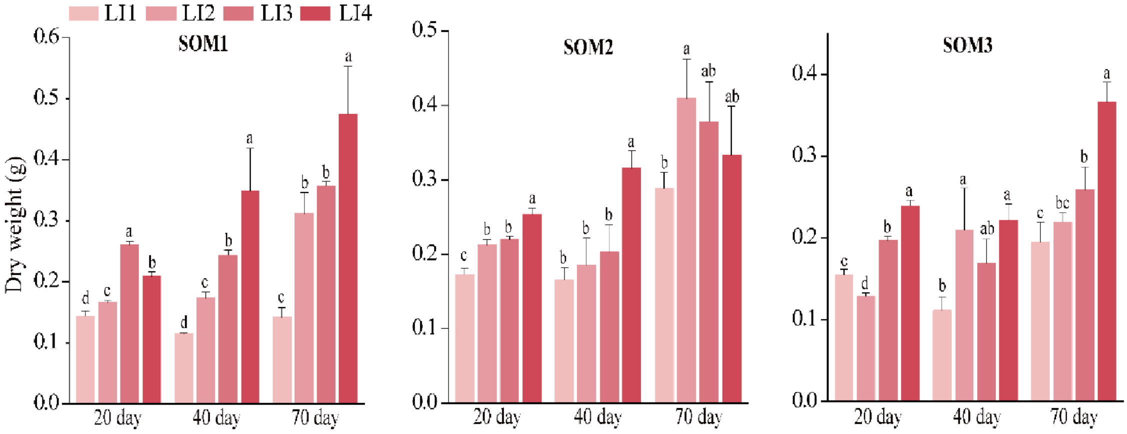


Fig. S1. The dry weight of *V. natans* after 20, 40 and 70 days (n = 4). (LI1, LI2, LI3, and LI4 indicate 10%, 30%, 60%, and 100% natural light intensity, respectively. SOM1, SOM2, and SOM3 had initial SOM loads of 11%, 17%, and 25%, respectively.)

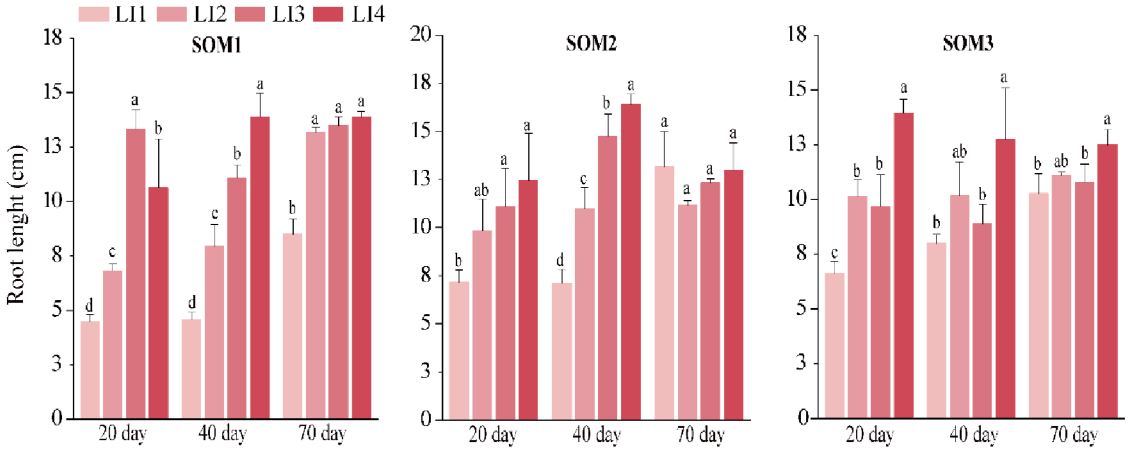


Fig. S2. The root lenght of *V. natans* after 20, 40 and 70 days (n = 4). (LI1, LI2, LI3, and LI4 indicate 10%, 30%, 60%, and 100% natural light intensity, respectively. SOM1, SOM2, and SOM3 had initial SOM loads of 11%, 17%, and 25%, respectively.)

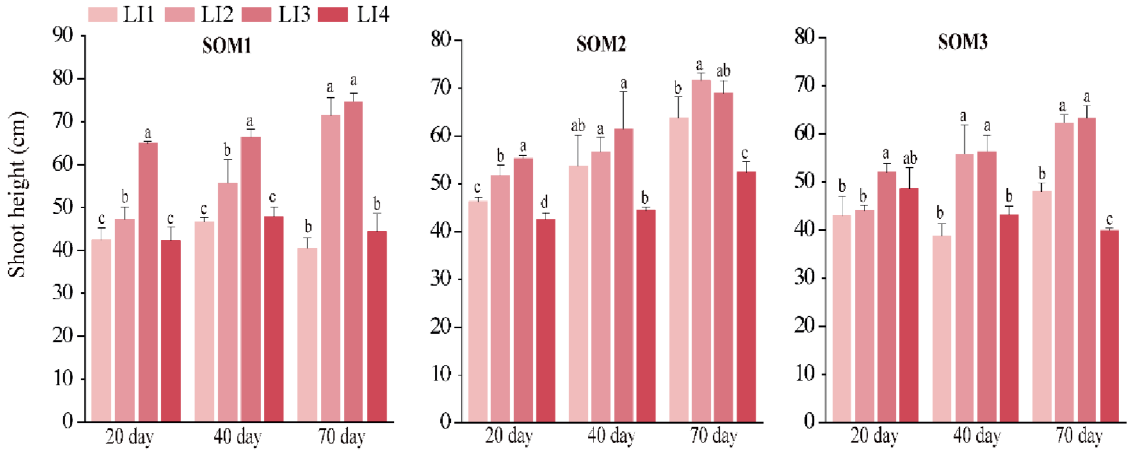
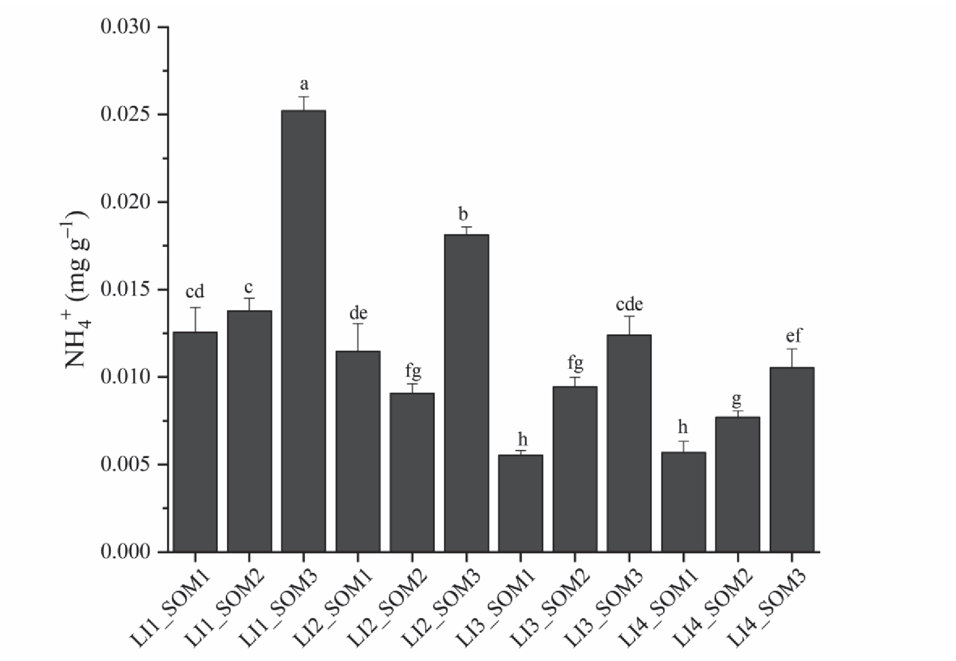


Fig. S3. The shoot height of *V. natans* after 20, 40 and 70 days (n = 4). (LI1, LI2, LI3, and LI4 indicate 10%, 30%, 60%, and 100% natural light intensity, respectively. SOM1, SOM2, and SOM3 had initial SOM loads of 11%, 17%, and 25%, respectively.)



Fig, S4. The sediment NH_4^+ contents after 70 days (n = 4). (LI1, LI2, LI3, and LI4 indicate 10%, 30%, 60%, and 100% natural light intensity, respectively. SOM1, SOM2, and SOM3 had initial SOM loads of 11%, 17%, and 25%, respectively.)

References

1. YANG H., WU J., HUANG X., ZHOU Y., ZHANG Y., LIU M., LIU Q., KE S., HE M., FU H., FANG S., XIONG X., JIANG H., CHEN Z., WU Z., GONG H., TONG X., HUANG Y., MA J., GAO J., CHARLIER C., COPPIETERS W., SHAGAM L., ZHANG Z., AI H., YANG B., GEORGES M., CHEN C., HUANG L. ABO genotype alters the gut microbiota by regulating GalNAc levels in pigs. Nature. **606**, 358, **2022**.
2. CHEN S.F., ZHOU Y.Q., CHEN Y.R., GU J. FASTQ: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics. **34** (17), 884, **2018**.
3. MAGOČ T., SALZBERG S.L. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics. **27** (21), 2957, **2011**.
4. STACKEBRANDT E., GOEBEL B.M. A place for DNA-DNA reassociation and 16S ribosomal-RNA sequence-analysis in the present species definition in bacteriology. International Journal of Systematic and Evolutionary Microbiology. **44** (4), 846, **1994**.
5. EDGAR R.C. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature Methods. **10** (10), 996, **2013**.
6. WANG J., YU D. Influence of sediment fertility on morphological variability of *Vallisneria spiralis* L. Aquatic Botany. **87** (2), 127, **2007**.