

Article

Response of *Vallisneria natans* to Increasing Nitrogen Loading Depends on Sediment Nutrient Characteristics

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Abstract: High nitrogen (N) loading may contribute to recession of submerged macrophytes in shallow lakes; yet, its influences vary depending on environmental conditions. In August 2013, we conducted a 28-day factorial-designed field mesocosm experiment in Lake Taihu at the Taihu Laboratory for Lake Ecosystem Research (TLLER) to examine the effects of high N loading on the growth of *Vallisneria natans* in systems with contrasting sediment types. We ran the experiments with two levels of nutrient loading—present-day external nutrient loading (average P: $5 \mu\text{g}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$, N: $130 \mu\text{g}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$) and P: $5 \mu\text{g}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$, and with three times higher N loading (N: $390 \mu\text{g}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$) and used sediment with two contrasting nutrient levels. *V. natans* growth decreased significantly with increasing N loading, the effect being dependent, however, on the nutrient status of the sediment. In low nutrient sediment, relative growth rates, leaf biomass and root biomass decreased by 11.9%, 18.2% and 23.3%, respectively, at high rather than low N loading, while the decline was larger (44.0%, 32.7% and 41.8%, respectively) when using high nutrient sediment. The larger effect in the nutrient-rich sediment may reflect an observed higher shading of phytoplankton and excess nutrient accumulation in plant tissue, though potential toxic effects of the high-nutrient sediment may also have contributed. Our study confirms the occurrence of a negative effect of increasing N loading on submerged plant growth in shallow nutrient-enriched lakes and further shows that this effect is augmented when the plants grow in nutrient-rich sediment. External N control may, therefore, help to protect or restore submerged macrophytes, especially when the sediment is enriched with nutrients and organic matter.

Keywords: nitrogen loading; sediments; *Vallisneria natans*; growth

1. Introduction

Submerged macrophytes have strong effects on the trophic dynamics and water clarity of shallow lakes [1,2]. However, worldwide, many shallow lakes are affected by accelerating eutrophication [3], and loss of submerged macrophytes and increasing turbidity have occurred accordingly. Elevated input of phosphorus is commonly accepted as an underlying cause [4–6], but also the role of nitrogen has recently received attention [7,8]. Lower species diversity of submerged macrophytes has been observed with high nitrogen loading in natural eutrophic shallow lakes [9] and in experiments [10]. Also, reduced coverage of submerged plants has been recorded [11,12], but the underlying mechanisms are subject to debate. Enhanced shading by phytoplankton and periphyton are often considered to be key factors [13]. An alternative explanation is that ammonia toxicity triggers a macrophyte decline in eutrophic lakes [14,15].

The effect of nitrogen on plant growth can be influenced by environmental conditions such as phosphorus concentrations in the water, water level fluctuations and fish density [16,17]. However, nutrient-rich sediment may be another contributory factor [18,19]. Excessive nutrients in the sediment may facilitate nutrient accumulation in submerged macrophyte tissue, which, when in excess, may lead to decreased macrophyte growth [20]. Moreover, phosphorus release from fertile sediment into the lake water [21] may interact synergistically with N, stimulating phytoplankton and periphyton growth, and thus enhance the shading of macrophytes [22]. However, whether or not the fertile sediment will accelerate the decline of submerged macrophytes with high nitrogen loading has not been fully elucidated.

A decline in submerged macrophyte coverage has occurred in recent decades in Gonghu Bay of Taihu Lake, a subtropical shallow lake located in Wuxi City, China. The lake is eutrophic with frequent cyanobacterial blooms [23,24]. A recent survey has shown that the TN (total nitrogen) concentration in Gonghu Bay increased by 60% from 2003 to 2010 [25]. Furthermore, submerged macrophyte coverage has declined, the rate of decline being dependent on the sediment type in the bay [26]. Whether the decline of submerged macrophytes was related to the high N concentration and influenced by sediment nutrients is, however, unclear.

To further elucidate the causes further, we conducted a 28-day dosing experiment using two concentrations of nitrogen loading and two types of sediment to investigate how the nitrogen and sediment nutrient content affected the growth of *Vallisneria natans*. *V. natans* is one of the dominant macrophyte species in Gonghu Bay of Taihu Lake, but its abundance has gradually decreased in recent years [26]. Our hypothesis is that an expected inhibitive effect of high N on plant growth is further aggravated in nutrient-rich sediments.

2. Materials and Methods

2.1. Experimental Design

A 4-week outdoor mesocosm experiment was conducted from 29 July to 26 August 2013 in Lake Taihu at the Taihu Laboratory for Lake Ecosystem Research (TLLER) station. Sixteen polyethylene tanks with a volume of 85 L (height 56 cm, upper diameter 50 cm, bottom diameter 38 cm) were placed in a pond in order to ensure relatively small temperature differences of the overlying water. Each tank held 10 cm sediment and 60 L water. Two types of sediments were collected from Lake Taihu and their different initial chemical properties are shown in Table 1. Prior to the experiment, the sediment was sieved through a 0.5 cm sieve to remove coarse debris and then thoroughly mixed to ensure uniformity. The water collected from the lake initially contained 0.81 mg·L^{−1} total nitrogen (TN) and 0.05 mg·L^{−1} total phosphorus (TP) and was filtered through a 112-μm mesh plankton net to remove larger organisms and inorganic particles.

Table 1. The initial content of total nitrogen, total phosphorus and organic matter in two sediment types and significance level (*t*-test) of differences between them (mean \pm SD, *n* = 4) *.

Sediment Type	Total Nitrogen (mg·g ⁻¹)	Total Phosphorus (mg·g ⁻¹)	Organic Matter (%)
High nutrient sediment	4.36 \pm 0.09	1.6 \pm 0.06	10.68 \pm 0.03
Low nutrient sediment	1.09 \pm 0.05	0.40 \pm 0.04	5.39 \pm 0.04
<i>p</i>	<0.01	<0.01	<0.01

Note: * Based on sediment dry mass.

Specimens of the submerged macrophyte *V. natans* were collected from Lake Taihu and pre-cultivated in outdoor polyethylene tanks filled with lake water and sediment for ten days before initiation of the experiment. To each polyethylene tank we added 10 young plants with similar length (approximately 24 ± 0.28 cm), biomass (approximately 24 ± 0.05 g in wet weight) and shoot number to achieve an initial plant density of $200 \text{ g} \cdot \text{m}^{-2}$, which is consistent with the average density in macrophyte-covered areas in Lake Taihu [27]. Four 8×10 cm size polyethylene (PE) boards were installed at 20 cm water depth in each tank to monitor the biomass of periphyton on *V. natans*. The boards were attached to a string crossing the floating frame and stretched through the water column by a metal anchor.

A 2×2 factorial experiment was carried out with four replicates per treatment, including two types of sediment (low nutrient sediment; high nutrient sediment) and two levels of nitrogen loading (low N loading; high N loading). Nitrogen (N: $130 \text{ } \mu\text{g} \cdot \text{L}^{-1} \cdot \text{day}^{-1}$) was added as potassium nitrate (KNO_3) every day at 8 a.m. local time (after sampling, if any) as nitrate is the dominant form of inorganic N in the lake [28]. Low N loading corresponded to the average level of external nutrient loading of Lake Taihu (P: $5 \text{ } \mu\text{g} \cdot \text{L}^{-1} \cdot \text{day}^{-1}$, N: $130 \text{ } \mu\text{g} \cdot \text{L}^{-1} \cdot \text{day}^{-1}$); high N loading corresponded to a three times higher N loading (P: $5 \text{ } \mu\text{g} \cdot \text{L}^{-1} \cdot \text{day}^{-1}$, N: $390 \text{ } \mu\text{g} \cdot \text{L}^{-1} \cdot \text{day}^{-1}$). These additions reflected nitrogen concentrations similar to the mean values recorded in Lake Taihu surface waters or in occasional peak seasons. After sampling, tap water with relatively constant nutrient concentrations (TN: $0.22 \pm 0.08 \text{ mg} \cdot \text{L}^{-1}$; TP: $0.034 \pm 0.01 \text{ mg} \cdot \text{L}^{-1}$) was added to guarantee a stable water level. Relative to the total water volume in each tank, nutrients added with tap water were insignificant.

2.2. Sampling

Samplings were conducted weekly on days 0 (before the first addition of nutrients), 7, 14, 21 and 28 of the experiment with a 1-L sampler at middle depth in each mesocosm for analysis of water chemistry and phytoplankton (measured as chlorophyll *a*, Chl *a*). Care was taken to avoid disturbance of sediments and plants. Total phosphorus (TP) and total dissolved phosphorus (TDP) concentrations were determined following the ammonium molybdate spectrophotometric method after digestion with a $\text{K}_2\text{S}_2\text{O}_8$ solution; total nitrogen (TN) and total dissolved nitrogen (TDN) were measured using an alkaline potassium persulfate digestion-ultraviolet (UV) spectrophotometric method. Ammonium ($\text{NH}_4\text{-N}$) was measured by the Nessler's reagent colorimetric method with potassium, mercury and iodine to obtain yellow-brownish coloration [29]. Chl *a* was measured spectrophotometrically from matter retained on a GF/C filter over 24 h and extracted in a 90% (*v/v*) acetone/water solution, and values were calculated without correction for phaeophytin interference [30].

Starting with the second sampling event, the pre-installed PE board was collected from each mesocosm once a week and subsequently stored in a zip-lock bag for periphyton biomass analyses. The periphyton attached on the PE boards was brushed into a beaker filled with distilled water, and water subsamples of 100–200 mL were filtered through pre-weighed glass fibre filters (GF/C, Whatman International Ltd., Maidstone, UK), which had been pre-combusted at 450°C for 4 h and weighed. The used filters were then dried in the oven at 105°C for 2 h, cooled in a desiccator for 30 min and weighed to determine periphyton biomass.

At the end of the experiment, all *V. natans* were harvested from all mesocosms for relevant ecological index measurement. The plants were washed and cleaned thoroughly with deionized

water and then weighed to estimate the total biomass, root biomass (including roots and tubers) and leaf biomass of each tank. To determine the TN and TP contents of *V. natans*, all leaves in the *V. natans* samples were dried at 70 °C for 48 h and then ground to fine powder for further analysis. Plant N contents were determined by salicylate-nitro-prusside colorimetry and plant P contents by ammonium molybdate colorimetry after digestion with sulphuric acid and hydrogen peroxide using a zinc catalyst [31]. The relative growth rate (RGR) of the plants in each mesocosm was calculated using the following equation: $RGR (mg \cdot g^{-1} \cdot day^{-1}) = 1000 \times \ln (W_f/W_i)/days$, where W_f (g) and W_i (g) were the final and initial total wet weight of plants per tank, respectively.

The sediment samples were air-dried and sieved through a 100 mesh net after collection. The initial contents of total nitrogen and total phosphorus were measured using colorimetry [32]. Organic matter content was estimated as weight loss after ignition at 550 °C for 5 h [29].

2.3. Statistical Analyses

Repeated measures analysis of variance (rANOVA) was used to analyse time series data including nutrient concentrations in water, Chl *a* and periphyton biomass. Two-way ANOVA was used to evaluate the effects of sediment type and N loading on *V. natans* characteristics, including identifying the main effects and the interaction term. In case of a significant interaction term, a further simple test (Bonferroni procedure) using the sediment as a categorical factor and N loading as a quantitative factor was performed to determine where the differences lay. Log or square-root transformations were applied to data if they did not fit the assumptions for ANOVA. Paired *t*-tests were used to assess the differences between the two types of sediment. Statistical analyses were performed using the SPSS 16.0 with a level of significance of $p < 0.05$.

3. Results

3.1. Sediments

There were significant differences between the two types of sediment ($p < 0.01$, Table 1). The TN, TP and organic matter contents of the high nutrient sediment were all significantly higher than in the low nutrient sediment.

3.2. Nutrients

Both sediment and N loading significantly increased TN and TDN concentrations during the experiment ($p < 0.01$) (Figure 1) and the effects of the time-loading interactions were significant ($p < 0.01$). At the end of the experiment, the mean TN and TDN concentrations at high N loading were 42.3% and 27.4% higher than at low N loading in the low nutrient sediment regime, while they were 57.6% and 48.3% higher, respectively, in the high nutrient sediment regime. Only sediment nutrient characteristics had a significant effect on NH_4 -N ($p < 0.05$), but N loading and its interaction with sediment nutrient characteristics were insignificant ($p > 0.05$). Changes in NH_4 -N were not significant throughout our experiment.

The effects of N loading on TP and TDP concentrations were insignificant ($p > 0.05$), whereas high nutrient sediment significantly promoted TP and TDP concentrations compared with the low nutrient sediment ($p < 0.01$) (Figure 1). The interactions were also significant ($p < 0.05$). In the low nutrient sediment regime, TP and TDP concentrations exhibited no significant differences between low and high N loading ($p > 0.05$), while in the high nutrient sediment treatment, TP and TDP concentrations were 13.4% and 34.7% higher at low N loading than at high N loading ($p < 0.01$).

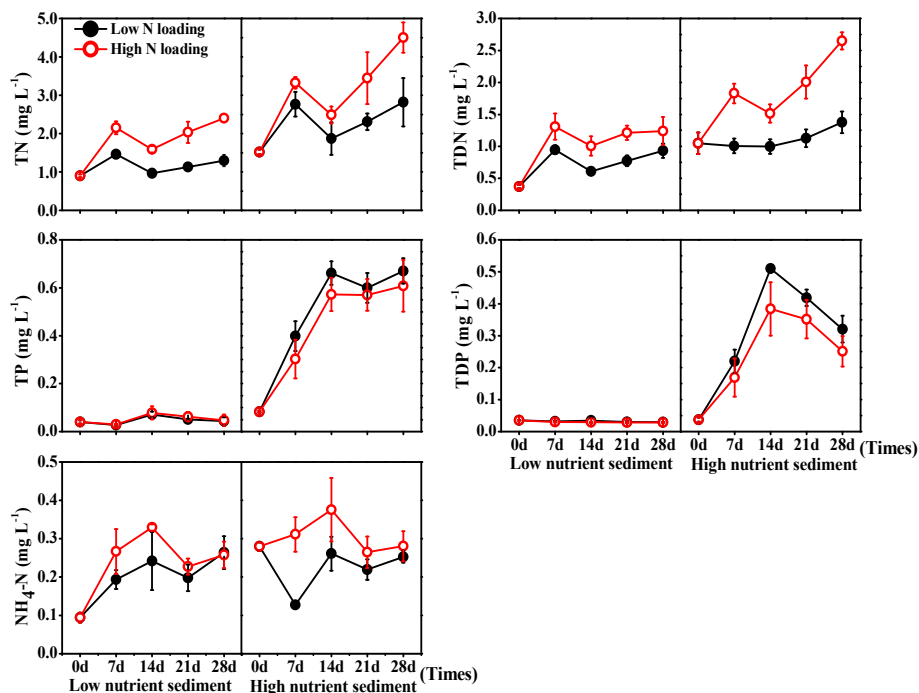


Figure 1. Time series of TN, TP, TDN, TDP and $\text{NH}_4\text{-N}$ in different treatments during the experiment. TN, total nitrogen; TDN, total dissolved nitrogen; TP, total phosphorus and TDP, total dissolved phosphorus; $\text{NH}_4\text{-N}$, ammonia nitrogen. Factors are sediment type and external nitrogen loading. Solid circle: low N loading; empty circle: high N loading. Values are mean \pm SD, $n = 4$, and the bars show standard deviation.

3.3. Phytoplankton and Periphyton

High nutrient sediment and high N loading led to markedly elevated Chl *a* concentrations compared with low nutrient sediment and low N loading ($p < 0.01$), and their interactions were significant ($p < 0.01$) (Figure 2). In the low nutrient sediment regime, the Chl *a* at high N loading was $39.3 \pm 6.5 \mu\text{g}\cdot\text{L}^{-1}$, which was 35.8% higher than in the low N loading treatment. However, in the high nutrient sediment regime, Chl *a* was $240.1 \pm 35.0 \mu\text{g}\cdot\text{L}^{-1}$ at high N loading, being 62.1% higher than at low N loading. Both sediment type and N loading effects on periphyton biomass were significant ($p < 0.01$), whereas the interactions were not significant ($p > 0.05$). High N loading significantly increased periphyton biomass compared with low N loading in both types of sediment ($p < 0.01$); however, periphyton biomass was slightly higher in the low nutrient than in the high nutrient sediment, i.e., contrasting the Chl *a* pattern.

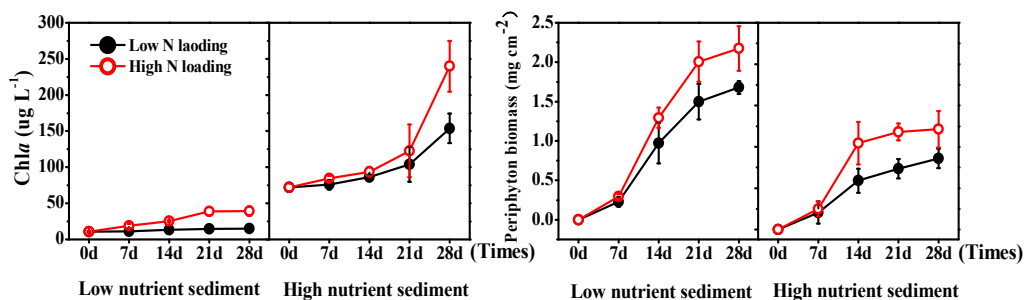


Figure 2. Time series of chlorophyll *a* (Chl *a*) concentrations and periphyton biomass in different treatments during the experiment. Factors are sediment type and external nitrogen loading. Solid circle: low N loading; empty circle: high N loading. Values are mean \pm SD, $n = 4$, and the bars show standard deviation.

3.4. Macrophytes

High nutrient sediment and high N loading pronouncedly decreased the relative growth rate, leaf mass and root mass of *V. natans* compared with the low nutrient sediment and low N loading ($p < 0.01$) (Figure 3, Table 2). Interactions were significant for relative growth rates and leaf mass ($p < 0.05$) but not for root mass ($p > 0.05$). In the low nutrient sediment regime, the relative growth rate, leaf mass and root mass with high N loading were 11.9%, 18.2% and 23.3% lower than at low N loading but as high as 44.0%, 32.7% and 41.8%, respectively, in the high nutrient regime.

The plant N and P concentrations were higher in the high than in the low nutrient sediment ($p < 0.01$) (Figure 4, Table 2), whereas high N loading only significantly increased the plant N concentrations ($p < 0.01$) but had no effect on the plant P concentrations ($p > 0.05$). Interactions were recorded for both the plant N and P concentrations ($p < 0.05$). Plant N and P concentrations were always highest at high N loading and high nutrient sediment and lowest for the low N loading and low nutrient sediment.

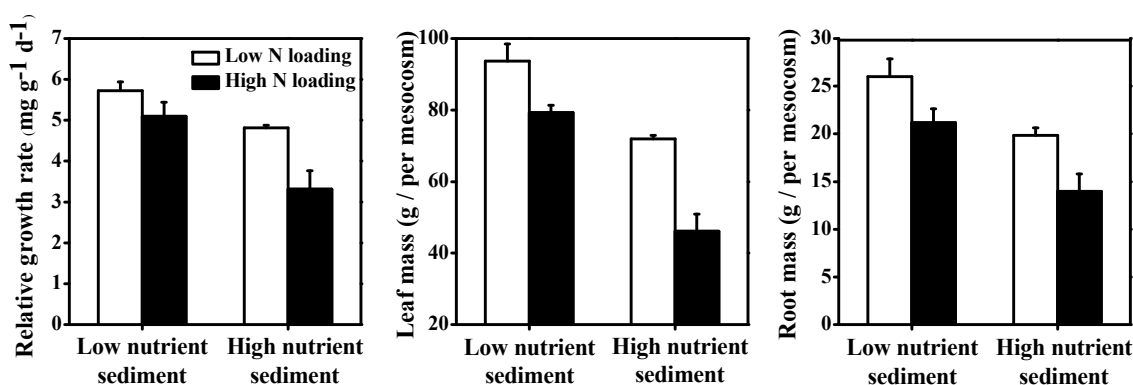


Figure 3. Relative growth rate, leaf mass and root mass of *V. natans* grown in two nutrient sediments (low and high nutrient sediment) and with two levels of nitrogen loading (low and high N loading). Blank column represents low N loading; black column represents high N loading. Values are mean \pm SD, $n = 4$, and the bars show standard deviation.

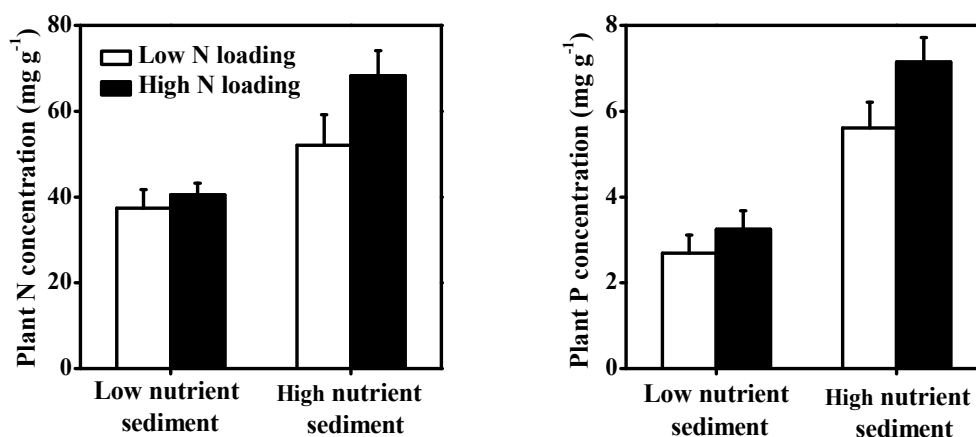


Figure 4. Plant N and P of *V. natans* grown in two nutrient sediments (low and high nutrient sediment) and with two levels of nitrogen loading (low and high N loading). Blank column represents low N loading; black column represents high N loading. Values are mean \pm SD, $n = 4$ and the bars show standard deviation.

Table 2. Analysis of two-way ANOVA results for relative growth rate, leaf mass, root mass and plant nutrient contents of *V. natans* grown in two types of sediment and with two levels of external nitrogen loading at the end of experiment.

Dependent Variables	Sediment Type (S)		Nitrogen Loading (N)		S × N	
	P	SS (%)	P	SS (%)	P	SS (%)
Relative growth rate	<0.001	53.27%	<0.001	33.02%	0.013	5.70%
Leaf mass	<0.001	32.53%	<0.001	60.67%	0.018	2.65%
Root mass	<0.001	55.33%	<0.001	35.50%	ns	0.30%
Plant N content	<0.001	67.37%	0.003	13.86%	0.028	6.40%
Plant P content	<0.001	84.58%	ns	7.95%	0.030	5.32%

Note: ns = not significant ($p > 0.05$).

4. Discussion

We found that high N loading inhibited *V. natans* growth, not least in nutrient-rich sediment. The two-way ANOVA analysis suggests that the relative growth rate of *V. natans* and root biomass were particularly affected by differences in the sediment nutrient characteristics and leaf biomass by the differences in the water nutrient (Table 2).

A potential mechanism behind the lower growth rate is higher shading by phytoplankton. In a previous experiment, Gonzales et al. [13] found only a minor effect of high TN loading on phytoplankton growth at low TP concentrations ($<0.1 \text{ mg}\cdot\text{L}^{-1}$) but a marked effect at higher TP ($\geq 0.1\text{--}0.2 \text{ mg}\cdot\text{L}^{-1}$). Concurrently, in our experiment with low nutrient sediment, where TP and TDP were consistently $\leq 0.1 \text{ mg}\cdot\text{L}^{-1}$, Chl *a* showed only a modest increase at high N loading. However, in the mesocosms with high nutrient sediment, where release from the sediment resulted in much higher TDP and TP ($\geq 0.2 \text{ mg}\cdot\text{L}^{-1}$), Chl *a* was substantially higher at high TN loading, and shading of *V. natans* was thus more severe. Periphyton biomass also increased at high N, but we found no significant differences among the different sediment treatments, perhaps due to increased competition for light with phytoplankton in the mesocosms holding high nutrient sediment where Chl *a* was much higher. In addition, some studies have shown that $\text{NH}_4\text{-N}$ levels $>1 \text{ mg}\cdot\text{L}^{-1}$ may have toxic effects and lead to a decline in macrophyte growth in eutrophic lakes [33]. However, in our experiment, $\text{NH}_4\text{-N}$ concentrations (ranging from 0.09 to $0.37 \text{ mg}\cdot\text{L}^{-1}$) were lower than this threshold, suggesting that toxicity of ammonia at high N loading was unimportant.

Fertile sediments reinforced the reduction of plant growth as indicated by the particularly strong effect of high-nutrient sediment on the biomass of roots and the overall growth rate of the plants in the high nutrient scenario (Table 1). Cao et al. [14] also showed reduced growth of *V. natans* in experiments with fertile sediment (TP = $1.5 \text{ mg}\cdot\text{g}^{-1}$ DW, TN = $4.1 \text{ mg}\cdot\text{g}^{-1}$ DW, organic matter = 7.7% DW) with, respectively, 6.3%, 6.0% and 27.9% lower TP, TN and organic matter concentrations than in our high-nutrient sediment. Fertile sediments with high organic matter content typically have low oxygen concentrations and redox levels and may contain various byproducts of anaerobic microbial metabolism, some of which may be toxic to plant growth. Ni et al. [20], for example, demonstrated enhanced peroxidase activity in plants induced by phytotoxins when nutrient and organic contents in the sediment supplied to the plants were high (TN = 4.8%, TP = 3.2%, organic content = 11.2% of dry weight in their study). Silveira [34] showed reduced growth of submerged plants with high concentrations of organic matter (5.3% DW) in the sediment due to oxygen deficiency in the roots. Thus, we speculated that organic matter concentrations up to 10.7% in the high nutrient sediments of our study limited plant, especially root, growth. Although submerged macrophytes, to a certain extent, are capable of adapting to anaerobic conditions by transporting photosynthetically originated oxygen to their roots [35], this mechanism seems to be less effective in fertile sediments [36].

Via luxury consumption, submerged macrophytes can accumulate excess nutrients in their tissue under eutrophic conditions [37]. We also found that high N loading facilitated higher nutrient accumulation in plants when growing in the high nutrient sediment ($68.2 \text{ mg}\cdot\text{N}\cdot\text{g}^{-1}$ DW, $7.1 \text{ mg}\cdot\text{P}\cdot\text{g}^{-1}$

DW) than in the low nutrient sediment ($40.5 \text{ mg}\cdot\text{N}\cdot\text{g}^{-1} \text{ DW}$, $3.2 \text{ mg}\cdot\text{P}\cdot\text{g}^{-1} \text{ DW}$). These values are far above the critical N and P levels reported for submerged macrophyte growth in general (about $13 \text{ mg}\cdot\text{N}\cdot\text{g}^{-1} \text{ DW}$ and $3 \text{ mg}\cdot\text{P}\cdot\text{g}^{-1} \text{ DW}$, respectively). Despite sufficiently high concentrations of these nutrients in both sediment treatments, we found lower growth of plants in the high nutrient sediment, which may be attributed physiological stress of the plants [38]. Li et al. [39] found the critical levels for growth inhibition of *Vallisneria* to be $36 \text{ mg}\cdot\text{N}\cdot\text{g}^{-1} \text{ DW}$ and $4 \text{ mg}\cdot\text{P}\cdot\text{g}^{-1} \text{ DW}$, respectively, i.e., lower than those recorded in the high N loading and high nutrient sediment treatment in our study. Physiological stress may be due to a shift towards smaller cell size with thicker walls [20] or to alteration of the normal physiological metabolism (i.e., the decreasing carbohydrate reserves in storage organs and increasing free amino acids in tissues) [40]; however, lack of physiological data from our experiment prevents further evaluation of the mechanisms behind the reduced growth observed in the high N loading and high nutrient sediment treatment.

Field data support our findings. The coverage of macrophytes in Gonghu Bay (Lake Taihu) has gradually decreased since 2003, and in 2007 they almost completely vanished [27]. TN was considered the major responsive factor due to its rapidly increasing concentrations to $3.09 \text{ mg}\cdot\text{L}^{-1}$ in 2008 (TP was $0.11 \text{ mg}\cdot\text{L}^{-1}$), which correspond well with the results of enclosure experiments conducted in Danish lakes indicating high probability of reduced submerged macrophytes abundance when TN exceeds $1.2\text{--}2 \text{ mg}\cdot\text{L}^{-1}$ and TP ranges between 0.1 and $0.2 \text{ mg}\cdot\text{L}^{-1}$ [13]. Moreover, sediment TN and TP concentrations in Gonghu Bay increased up to $1877 \text{ mg}\cdot\text{kg}^{-1}$ and $814 \text{ mg}\cdot\text{kg}^{-1}$ in 2012 [41], suggesting serious nutrient pollution of the sediment [42]. Fertile sediment can continuously release nutrients to lake water, not least P. Søndergaard et al. [21] suggested that internal nutrient release in summer may be intense and persist a long time after an external nutrient loading reduction, consequently preventing any major improvement of water quality. In correspondence with this, the concentrations of TN and TP were much higher in the high nutrient sediment than in the low nutrient sediment treatment in our experiment. Moreover, in Gonghu Bay, wind- or fish-induced sediment resuspension occurs frequently, further enhancing the nutrient release from sediment [43,44]. Hu et al. [45] estimated the annual average internal loading of total nitrogen and phosphorus in Lake Taihu to 1149 t and 564 t, potentially causing algae blooms for at least five years, even when the external loading is completely cut off [46].

V. natans is often the dominant macrophyte in eutrophic Chinese lakes and was selected for use in our experiment as it is among the last plants to disappear when eutrophication leads to complete loss of submerged macrophyte [16]. Other plant species may be even more sensitive and fragile as has been demonstrated in experiments with *Myriophyllum spicatum* [47] and *Potamogeton crispus* [48]. Our small-scale experiment has limitations since plant-nutrient interactions may be stronger in mesocosms than in natural lakes, but the observed direction of change is supported by findings in both experiments at larger scale and in the field [26,42].

In conclusion, in our experiment a higher N concentration led to declined abundance of *V. natans*, the decline being most pronounced for plants grown in high-nutrient sediment. This suggests that under nutrient-rich conditions, external N control may help protect and restore submerged macrophytes, especially when the sediment is enriched with nutrients and organic matter. Removal of fertile sediment or increasing the sediment's sorption capacity by addition of iron or alum or similar P-binding substances may support macrophyte establishment if plants respond only slowly to an external N loading reduction.

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Author Contributions: Kuanyi Li, Jiao Gu designed the study, Zenghong Xu, Hui Jin and Xiaoyu Ning conducted the sampling, Jiao Gu, Kuanyi Li, Hu He, Jinlei Yu and Erik Jeppesen conducted the data analyses and wrote the paper.

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

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