



OPEN ACCESS

EDITED BY

Doru Stelian Bănăduc,
Lucian Blaga University of Sibiu,
Romania

REVIEWED BY

Xinhou Zhang,
Nanjing Normal University, China
Jian Cui,
Institute of Botany, Jiangsu Province
and Chinese Academy of Sciences,
China

*CORRESPONDENCE

Haijun Wang,
wanghaijun@ynu.edu.cn
Weihua Guo,
whguo@sdu.edu.cn

SPECIALTY SECTION

This article was submitted to Freshwater
Science,
a section of the journal
Frontiers in Environmental Science

RECEIVED 21 August 2022

ACCEPTED 23 September 2022

PUBLISHED 10 October 2022

CITATION

Yu Q, Wang H, Liu M, Xu C, Ma Y, Guo W
and Jeppesen E (2022), Interactive
effects of benthivorous fish disturbance
and ammonium loading on two
submersed macrophytes of contrasting
growth forms based on a
mesocosm study.
Front. Environ. Sci. 10:1024524.
doi: 10.3389/fenvs.2022.1024524

COPYRIGHT

© 2022 Yu, Wang, Liu, Xu, Ma, Guo and
Jeppesen. This is an open-access article
distributed under the terms of the
[Creative Commons Attribution License
\(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution or
reproduction in other forums is
permitted, provided the original
author(s) and the copyright owner(s) are
credited and that the original
publication in this journal is cited, in
accordance with accepted academic
practice. No use, distribution or
reproduction is permitted which does
not comply with these terms.

Interactive effects of benthivorous fish disturbance and ammonium loading on two submersed macrophytes of contrasting growth forms based on a mesocosm study

Qing Yu^{1,2}, Haijun Wang^{2,3*}, Miao Liu², Chao Xu², Yu Ma^{2,4},
Weihua Guo^{1*} and Erik Jeppesen^{5,6,7,8,9}

¹Institute of Ecology and Biodiversity, School of Life Sciences, Shandong University, Qingdao, China, ²State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China, ³Institute for Ecological Research and Pollution Control of Plateau Lakes, School of Ecology and Environmental Science, Yunnan University, Kunming, China, ⁴College of Fisheries and Life Science, Dalian Ocean University, Dalian, China, ⁵Department of Bioscience and Arctic Research Centre, Aarhus University, Aarhus, Denmark, ⁶Sino-Danish Centre for Education and Research, Beijing, China, ⁷Limnology Laboratory, Department of Biological Sciences, Middle East Technical University, Ankara, Turkey, ⁸Centre for Ecosystem Research and Implementation (EKOSAM), Middle East Technical University, Ankara, Turkey, ⁹Institute of Marine Sciences, Middle East Technical University, Mersin, Turkey

Benthivorous fish disturbance and nitrogen loading are two important factors that influence the community structure of submersed macrophytes, but their interactive effect is not well elucidated. We conducted an outdoor mesocosm experiment to examine the individual and combined effects of these two factors on the growth of two submersed macrophytes of different growth forms, i.e., the rosette-forming *Vallisneria natans* and the canopy-forming *Myriophyllum spicatum*. The treatments involved two levels of fish (*Carassius auratus*) disturbance crossed with two levels (0 and 12 g NH₄Cl per month) of ammonium (NH₄⁺-N) loading. For *M. spicatum*, we found that maximum height (MH) was reduced by 30.7%, 26.4%, and 51.0% in fish addition alone (F) and nitrogen addition treatments (N, F + N), respectively, compared with the control (C) treatment. The density of *M. spicatum* declined by 20%, 62% and 68.8% in the F, N and F + N treatment. The above-ground biomass (AGB) of *M. spicatum* respectively reduced by 56.7%, 94%, and 96.5% in the F, N and F + N treatments, and the roots/shoots ratio (R/S) increased by 114%, and 176% in N and N + F treatments, respectively. Regarding *V. natans*, only the MH in the N treatment was reduced (71.9%), and the density exhibited a reduction of 59.1% and 64.5% in the N and F + N treatments, respectively. The AGB of *V. natans* was significantly lower in the N (90.3%) and N + F (78.4%) treatments compared with the C treatment, while increased by 60.3% in F treatment. The R/S of *V. natans* increased by 227%, and 74.4% in the N and F + N treatments compared with the C treatment. The interactive effect of fish disturbance and high N on MH and AGB of *V. natans* and density of *M. spicatum* were antagonistic. However, the interactive effect on density and BGB of *V. natans* and AGB of *M. spicatum* were negatively synergistic.

Moreover, fish activity significantly increased the concentration of total suspended solids (TSS) in the water, while total nitrogen (TN), ammonium, total phosphorus (TP), light, pH and salinity were unaffected. When fish and nitrogen were combined, TN, TP, TSS and salinity increased significantly, while pH decreased. Our study reveals that the interactive effects of fish disturbance and high N are synergistic and/or antagonistic, suggesting that the same stressor interaction may vary from synergistic to antagonistic depending on the response variables and growth forms of the macrophytes examined. Our study contributes to the understanding of how different factors can interact with each other and affect submersed macrophytes in aquatic ecosystems. This is timely and relevant knowledge, considering the range of multiple stressors involved in the decline of aquatic ecosystems worldwide at present.

KEYWORDS

growth form, submersed macrophytes, nitrogen loading, fish disturbance, interactive effects

1 Introduction

Due to anthropogenic activities, nitrogen deposition and pollution exert strong stress on aquatic ecosystems worldwide (Greaver et al., 2016), and have adversely affected water quality and human health over the recent decades (Erisman et al., 2013; Hamilton et al., 2016; Ti et al., 2018; Yu et al., 2019). Moreover, the rapid development of agriculture and industrialization have also resulted in massive inputs of nutrient, especially nitrogen (N) to aquatic environments (Ballard et al., 2019; Yu et al., 2019; Ti et al., 2019), with detrimental consequences to the aquatic ecosystem structure, biodiversity and ecological functioning of shallow lakes (Baron et al., 2013; Greaver et al., 2016; Bhagowati and Ahamad, 2019). Submersed macrophytes are key components in freshwater ecosystems and play an important role in maintaining the health of ecosystem structure and function, and they help to maintain clear-water conditions (Jeppesen et al., 1998; Ali et al., 2019; Su et al., 2019a). However, as a result of high nutrient loading and eutrophication, submersed macrophytes in lakes are declining, causing global degradation of aquatic ecosystems (Kosten et al., 2009; Moss et al., 2013; Zhang et al., 2017). Therefore, it is important to explore the role of nitrogen stress on submersed macrophytes for this decline to help guide aquatic ecosystem protection managers.

Presently, several multi-scale experimental studies have revealed the mechanisms of nitrogen (mainly refers to $\text{NH}_4^+\text{-N}$) stress on submersed macrophytes, including 1) physiological toxicity, such as forming oxidative stress (Nimptsch and Pflugmacher, 2007), causing imbalance of nitrogen and carbon metabolism (Zhang et al., 2011) and inhibits photosynthesis (Shi et al., 2020); 2) morphological changes, mainly manifested as decline in shoot height (or leaf length) (Saunkaew et al., 2011), biomass and relative growth rate (Olsen et al., 2015; Zhu et al., 2018; Tan et al., 2019); 3) population decline, negatively affected by longer exposure to high $\text{NH}_4^+\text{-N}$ loading (Yu et al., 2022); 4) combined effect, for example, low light may enhance the toxic effect of $\text{NH}_4^+\text{-N}$ (Cao et al., 2011). In addition to excessive nitrogen (N) loading, fish has also been suggested to contribute to

the recession of macrophytes (Dorenbosch and Bakker, 2012; Nieoczym and Kloskowski, 2014; Badiou and Goldsborough, 2015). For example, fish may 1) cause mechanical damages to submersed macrophytes (e.g., shoot breakage and/or uprooting) by searching for macroinvertebrates (Goldsborough and Wrubleski, 2001); 2) induce low light stress on submersed macrophytes by enhancing water turbidity *via* sediment resuspension (Badiou et al., 2011; Kloskowski, 2011); 3) increase water column nutrient by disturbance and/or prey on zooplankton, promoting the growth of phytoplankton which may result in low-light stress on the submersed macrophytes (Van de Bund and Van Donk, 2004; Badiou and Goldsborough, 2015); 4) directly decrease macrophyte abundance through herbivory (Nurminen et al., 2003). Though the knowledge of nitrogen or fish stressor on water quality and submersed macrophytes is growing, the understanding of the interaction between nitrogen and fish is lacking.

Submersed macrophytes with different growth forms respond differently to stress induced by high ammonium ($\text{NH}_4\text{-N}$) loading and low light conditions. For example, canopy-forming species are more tolerant to high $\text{NH}_4\text{-N}$ ($<7\text{ mg L}^{-1}$) than rosette-forming species (Yu et al., 2022), because the former can form a canopy on the water surface, whereas rosette-forming species disperse under water through stolons (Xiao et al., 2006; Li, 2014; Angove et al., 2020). The canopy-forming species can, therefore, capture sufficient light, and plenty of light may relieve plants from NH_4 -related physiological stress, as suggested in previous mesocosm experiments (Cao et al., 2011; Yuan et al., 2016). Moreover, it has been found that fish disturbance did not affect the relative growth rate (RGR) of canopy-forming *H. verticillate* (Chen et al., 2020a) but hindered the growth of rosette-forming *V. natans* (Gu et al., 2018). Therefore, we hypothesised that 1) low light conditions caused by fish disturbance might aggravate NH_4 -related stress on the population growth of submersed macrophytes, in other words create a negative synergistic

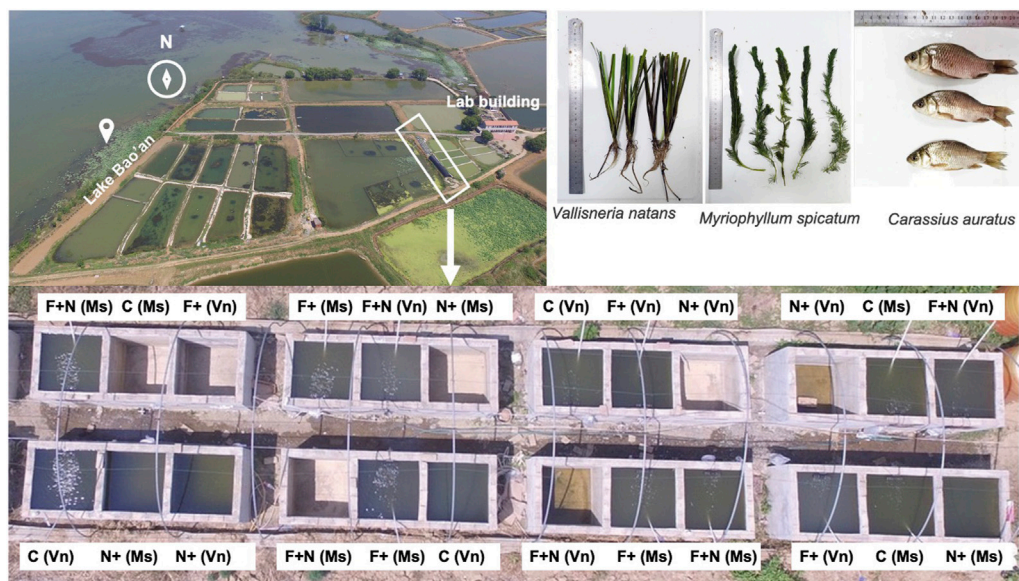


FIGURE 1

The mesocosm experimental systems. C, F+, N+, and F + N represent the following treatments: control, fish introduction alone, ammonium loading alone and both fish introduction and ammonium loading, respectively. Vn, *Vallisneria natans*; Ms, *Myriophyllum spicatum*.

effect; 2) the strategies of different growth forms of submersed macrophyte might have different tolerance levels to the combined effect of high $\text{NH}_4\text{-N}$ stress and fish disturbance.

For our experiment, we selected the canopy-forming species *Myriophyllum spicatum* and the rosette-forming species *Vallisneria natans*, two common submersed macrophytes worldwide representing plants with contrasting growth forms. We then evaluated the effect of *Carassius auratus* (fish) and ammonium ($\text{NH}_4^+\text{-N}$) loading on water quality and the growth of these two submersed macrophytes. The objectives of this study were 1) to examine the interactive effect between fish disturbance and $\text{NH}_4\text{-N}$ loading on two growth forms of submersed macrophytes, 2) to explore the strategies of two growth forms of submersed macrophytes to combined effects of fish disturbance and $\text{NH}_4\text{-N}$ loading.

2 Materials and methods

2.1 Research area and experimental materials

The duration of the experiment was from 15 August to 25 December 2017 (132 days) in cement tanks located to the northeast of Lake Bao'an (N $30^\circ 17' 17''$, E $114^\circ 43' 45''$) (Figure 1) on the south bank of the middle Yangtze River. *Vallisneria natans* and *Myriophyllum spicatum* were cultured in 24 outdoor cement tanks (729 L, length 90 cm, width 90 cm, height 90 cm height). A

10 cm layer of lake sediment and 70 cm of lake water were added to each tank. The sediment (original contents: total nitrogen 1.83 mg g^{-1} , total phosphorus 0.69 mg g^{-1} , organic matter 34.8 mg g^{-1}) was collected from a nearby pond and fully mixed before the experiment to ensure homogeneity. The seedlings/shoots of two submersed macrophytes were collected from the Lake Xi'liang in Xianning, Hubei Province. They were similar in size and healthy in appearance. For *M. spicatum*, the apical shoots were 15 cm in length and had a fresh weight of $5.09 \pm 3.49 \text{ g}$ (mean \pm SD), with no flower. For *V. natans*, intact seedlings (containing roots) were 15 cm in length, with a fresh weight of $4.08 \pm 1.73 \text{ g}$ leaves and 0.57 ± 0.23 roots. The seedlings/shoots were carefully washed to remove attachments before plantation in tanks.

Individuals of crucian carp *Carassius auratus*, a common benthivorous fish widely found in the mid-lower Yangtze lakes (Gao et al., 2014; Yu et al., 2016). Being an omni-benthivorous fish, crucian carp can affect the light intensity by sediment disturbance leading to resuspension and more phytoplankton due to sediment nutrient release, which in turn may result in light limitation of the submersed macrophyte growth (Richardson et al., 1995; Bernes et al., 2015). Therefore, crucian carp is suitable for revealing fish disturbance effects on water quality and submersed macrophytes and it is, moreover, a common fish species in eutrophic shallow Chinese lakes (Liu et al., 2007). The crucian carp *Carassius auratus* in the experiment were captured from the water bodies around Lake Bao'an by a fisherman. The individuals selected were of similar size ($14.3 \pm 6.2 \text{ cm}$ in length and $79.9 \pm 20.8 \text{ g}$ in weight) and cultured in tap water for a week before the experiment.

2.2 Experimental design

At the beginning of the experiment, 25 shoots of *M. spicatum* and 25 seedlings of *V. natans* were planted in 12 tanks respectively, and there were 24 tanks in totally. Then lake water was added to all tanks to a depth of 70 cm. Seven days after the planting, fish were added to the fish treatment tanks. The tanks were randomly assigned to two fish levels and two NH_4^+ -N loading levels, and each treatment was replicated three times: (C) controls (no fish and no NH_4^+ -N addition); (F) three fish per treatment; (N) 12 g NH_4Cl each treatment; (F + N) three fish and 12 g NH_4Cl addition in treatment. The NH_4Cl addition of 12 g per month was to maintain the target NH_4^+ concentration of 7 mg/L, because this NH_4^+ concentration was the threshold of NH_4^+ toxicity according to our earlier pond experiments (Yu et al., 2017, Yu et al., 2018, Yu et al., 2022). Tap water was added weekly to maintain a constant water level of 70 cm. During the experiment, the water temperature ranged from 10.3°C to 28.1°C, and the average temperature in all tanks was 16.4°C.

2.3 Sampling and measurement

Environmental variables were measured once every month, from 10:00 to 11:00 a.m. Water temperature, dissolved oxygen (DO), pH and conductivity (Cond) were measured *in situ* at half water depth with a YSI ProPlus (Yellow spring Inc. OH, Yellow Springs, United States). Photosynthetic photon flux density (PPFD) was measured by an illuminometer (KONICA MINOLTA, T-10, China) at the air-water interface (just above the water surface) and 10 cm and 50 cm below the water surface. Approximately 1 L of overlying water just below the water surface was collected once a month at 10:00–10:30 a.m. to determine total nitrogen (TN), ammonium ($\text{NH}_4\text{-N}$), total phosphorus (TP), total suspended solids (TSS) and phytoplankton biomass (Chl *a*). TN was determined following an alkaline potassium persulphate digestion-UV spectrophotometric method (PERSEE, TU-1810, Beijing, China) and TP following an ammonium molybdate-ultraviolet spectrophotometric method after digestion with $\text{K}_2\text{S}_2\text{O}_8$ solution (Huang et al., 1999). For analysis of ammonium ($\text{NH}_4\text{-N}$), water was filtered with an acetate fiber microporous membrane (0.45 μm pore diameter, Shanghai Xingya Purification Material Factory, Shanghai, China) and then $\text{NH}_4\text{-N}$ was measured with an automatic chemical analyzer (SYSTE A S.p.A, Easychem plus, Italy). TSS was measured by passing a known volume of water through pre-dried and pre-weighed Whatman GF/C filter paper, dried for 24 h at 70°C and then weighed. Phytoplankton chlorophyll *a* (Chl *a*) was extracted using 90% acetone (at 4°C for 24 h) after filtration through GF/C filters (Whatman, GE Healthcare United Kingdom Limited, Buckinghamshire, United Kingdom), and absorbance was then

read at 665 nm and 750 nm both before and after acidification with 10% HCl using a spectrophotometer.

At the end of the experiment, all plants were harvested and washed carefully with tap water for further measurement. In each tank, the total number of ramets were counted for each species. A shoot that rose from the base was considered a ramet for *M. spicatum*. The maximum shoot length for *M. spicatum* and the maximum leaf length for *V. natans* were considered as the plant maximum height. *M. spicatum* is a typical canopy macrophyte, the branch number and the height of the lowest branch are vital for canopy formation and influence their adaptation to the environment. Thus, we counted the total branch number and measured the height of the lowest branch for each *M. spicatum* individual. All *V. natans* were divided into above-ground and below-ground parts, dried with bibulous paper and weighed, then oven-dried at 75°C to constant weight and weighed again to determine the dry weight. For *M. spicatum*, 4–7 individuals were randomly selected to determine the dry weight of their leaves and stems, and the rest values were calculated according to their relative proportions. The total biomass of each species in each tank was the sum of the dry weight of all plant organs.

The relative growth rates of the above-ground biomass (RGR-AGB, $\text{g g}^{-1} \text{d}^{-1}$) in each tank was calculated by the following formula:

$$\text{RGR} - \text{AGB} = \ln (DW_t / DW_0) / T$$

where DW_t and DW_0 are the plant dry weight at the end and beginning of the experiment, respectively, and T is the duration of the experimental period. To assess the stressor interaction type, the interaction statistic ρ was calculated following the equation in Turschwell et al. (2022):

$$\rho = -\ln \left(\frac{Y_{FN} / Y_C}{Y_F Y_N / Y_C^2} \right)$$

The interaction is synergistic when ρ is positive, additive when ρ is zero and antagonistic when ρ is negative. Y_F , Y_N , Y_C , and Y_{FN} are biomass of the treatment with stressor fish (F) only, stressor nitrogen (N) only, no stressors (control—C), and both stressors (F and N), respectively. The metric ρ was used to assess whether the change rate of the response is greater with both stressors present than the multiplication with individual stressors present.

2.4 Statistical analyses

Origin 2021, StataSE 15 and Microsoft Excel 2019 were used for all statistical analyses. Values are expressed as the mean \pm standard deviation (SD). Shapiro-Wilk test was used to test the model residual normality. Levene homogeneity test of variance was used to test the equality of variance. One way analysis of variance (ANOVA) was used to test the statistical significance of

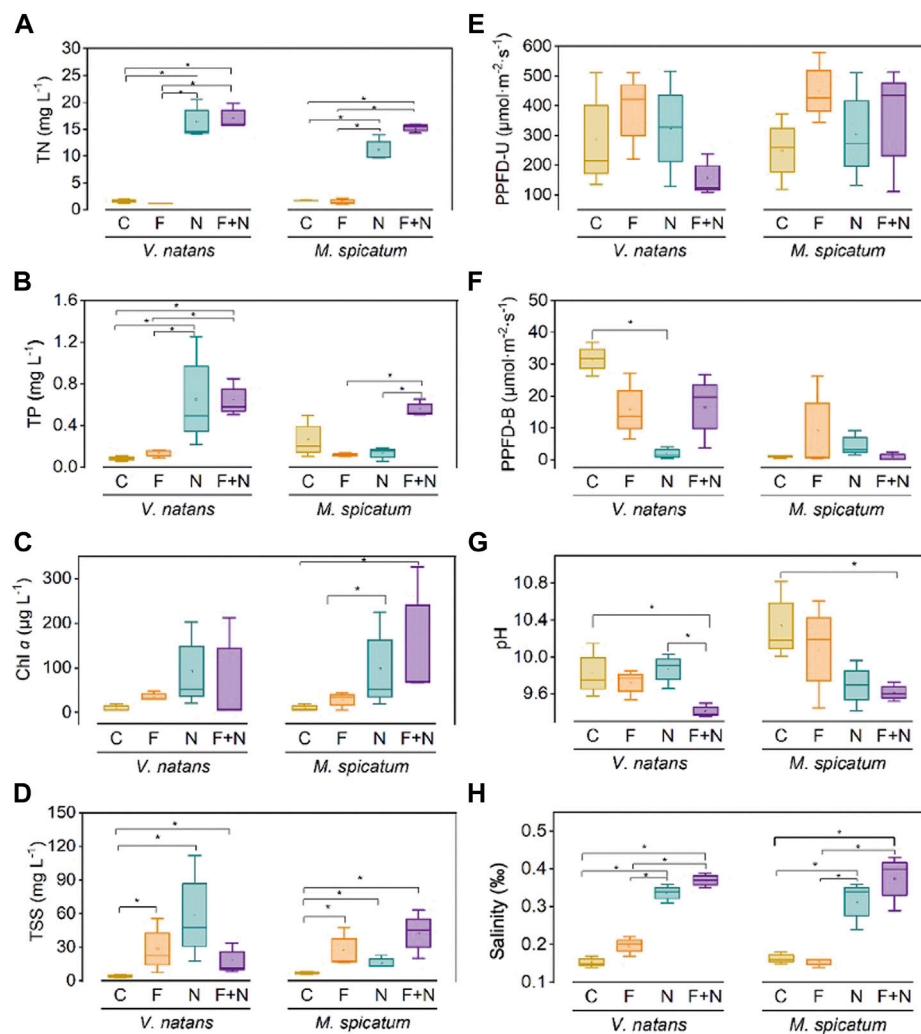


FIGURE 2

Physico-chemical water variables (mean \pm SD) including total nitrogen (TN, mg L^{-1}) (A), total phosphorus (mg L^{-1}) (B), phytoplankton chlorophyll a (Chl a , $\mu\text{g L}^{-1}$) (C), total suspended solids (SS, mg L^{-1}) (D), photosynthetic photon flux density in the upper layer of water with 10 cm water depth (PPFD-U, $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) (E), photosynthetic photon flux density in the bottom layer of water with 60 cm water depth (PPFD-B, $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) (F), pH (G), and salinity (%) (H) in the water column at the end of the experiment. See Figure 1 for the meaning of the various treatments. ** indicates significant differences at the 0.05 significance level determined by least significance difference test (LSD).

plant growth, morphological and water quality indices. 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 (at the 0.05 significance level). Effect sizes allowed us to compare the observed effects of individual and combined stressors. Effect sizes for individual stressors were calculated based on pairwise comparisons between each experimental condition and the control ($n = 3$). Cohen's d effect sizes and 95% confidence intervals (CI) were calculated by StataSE 15.0. Given the fact that the effect sizes were large (>0.8 ; Cohen, 2013) and their CIs did not touch the zero line, they were considered meaningful.

3 Results

3.1 Growing environments

TN concentrations were significantly higher in the 2 N addition treatments (N and F + N) (ca. 0.6 mg/L) than in those without N addition (C and F) for both macrophyte species (Figure 2A). The differences were not significant between N and F + N, nor were they between C and F. During the experiment, TN showed increasing trends in N and F + N and stayed relative stable in C and F for both species (Supplementary Figures S1A,B). The result of NH_4^+

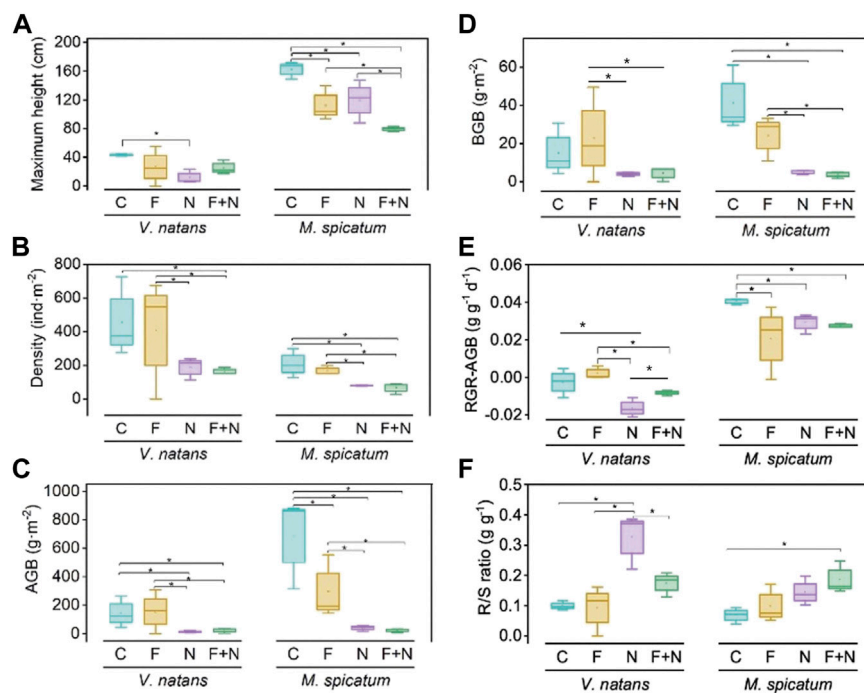


FIGURE 3

The maximum height (A), density (B), above-ground biomass (AGB) (C), below-ground biomass (BGB) (D), relative growth rate of above-ground biomass (RGR-AGB) (E) and root biomass to shoot biomass ratio (R/S ratio) (F) of *Vallisneria natans* and *Myriophyllum spicatum* in each treatment at the end of experiment. See Figure 1 for the meaning of various treatments. “*” indicates significant differences at the 0.05 significance level determined by least significance difference test (LSD).

concentration was consistent with that of TN (Supplementary Figure S2).

TP concentrations were significantly higher in the combined F addition and N addition treatments (F + N) (ca. 0.2–0.6 mg/L) than in the treatments without N addition (C and F) (ca. 0.08–0.27 mg/L) for both macrophyte species (Figure 2B). In the N treatment, the TP concentration with *V. natans* was significantly higher in the C treatment, while this not for *M. spicatum*. During the experiment, TP showed a declining trend in C, F and N for both species but remained relatively stable in C and F for both species (Supplementary Figures S1C,D).

Chl *a* concentration in the F and N addition treatments (F, N, F + N) were not significantly different from the C treatment for *V. natans* species (Figure 2C). However, for *M. spicatum*, Chl *a* level in the 2 N addition treatments (N and F + N) were significantly higher than in those without N addition. During the experiment, Chl *a* in the two N-addition treatments (N and F + N) with *V. natans* showed a tendency to rise at first and then decrease, while it decreased at first and then increased for *M. spicatum* (Supplementary Figures S1E,F).

There were significant differences in TSS concentrations in both the F and N addition treatments (F, N and F + N) compared

with the C treatment (Figure 2D). For both species, the TSS concentration showed a declining trend during the experiment, except in the N treatment with *V. natans* and the F + N treatment with *M. spicatum* (Supplementary Figures S1G,H). The concentration of TSS ranged between ca. 24.8 and 54.1 g L⁻¹ among all treatments.

The PPFD in the upper layer of water (PPFD-U) showed no difference among most treatments (Figure 2E). However, the PPFD at 50 cm depth (PPFD-B) was lower in both the F and N addition treatments (F, N and F + N) with *V. natans*, while no differences appeared among all treatments with *M. spicatum*. (Figure 2F).

Other variables in the water column changed with combined F and N addition. pH was significantly lower in the N + F treatment than in the C treatment (Figure 2G). During the experiment, pH was lower in the 2 N addition treatments (N and F + N) for both species (Supplementary Figures S1I,J). The salinity in the 2 N addition treatments (N and F + N) were significantly higher than that in the two treatments without N addition (C and F) for both species (Figure 2H). During the experiment, salinity in the 2 N addition treatments showed an increasing trend, while it remained relatively stable in C and F for both species (Supplementary Figures S1K,L).

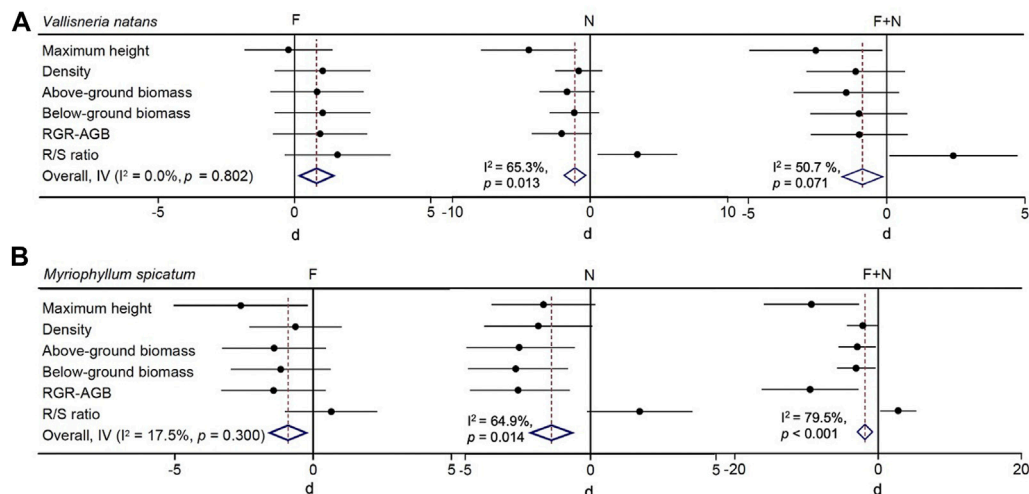


FIGURE 4

Effect sizes (Cohen's $d \pm 95\%$ confidence interval) of individual and combined stressor effects on the growth of *Vallisneria natans* (A) and *Myriophyllum spicatum* (B) at the end of the experiment. No significant effects are inferred when confidence intervals cross the zero line. Diamond indicates the overall effect. The diamond on the left-hand side of the zero line indicates a negative effect and a positive effect on the right-hand side. See Figure 1 for the meaning of the various treatments.

3.2 Growth of submersed macrophytes

For *V. natans*, the maximum height (MH) in the N treatment was significantly lower than in the C treatment (Figure 3A) where it was reduced by 71.9%. However, in the 2 F addition (F and F + N) treatments, MH was not significantly different from that in the C treatment. Regarding *M. spicatum*, MH in the F addition and N addition treatments (F, N and F + N) were all significantly lower (Figure 3A), and reduced by 30.7%, 26.4%, and 51.0% in F, N, and F + N compared with the C treatment. In all treatments, *M. spicatum* exhibited significantly higher MH than *V. natans*.

V. natans density was significantly lower in the 2 N addition treatments (F + N and N) compared with the C treatment (Figure 3B), exhibiting a reduction of 59.1% and 64.5% in the N and F + N treatments respectively. For *M. spicatum*, the density declined by 20%, 62%, and 68.8% in the F, N and F + N treatment compared to the C treatment (Figure 3B). Though the density of both species demonstrated a decreasing trend with fish and/or nitrogen addition, the density of *V. natans* was higher than that of *M. spicatum* in all treatments.

The above-ground biomass (AGB) of *V. natans* was significantly lower in the N (90.3%) and N + F (78.4%) treatments compared with the C treatment (Figure 3C). The results for *M. spicatum* AGB were similar to those for *V. natans* in the N and F + N treatments (Figure 3C), showing a reduction of, respectively, 94% and 96.5% in the N and F + N treatments compared to the C treatment. However, the AGB of *V. natans* and *M. spicatum* contrasted each other in the F treatment. Thus, the AGB of *V. natans* increased by 60.3%,

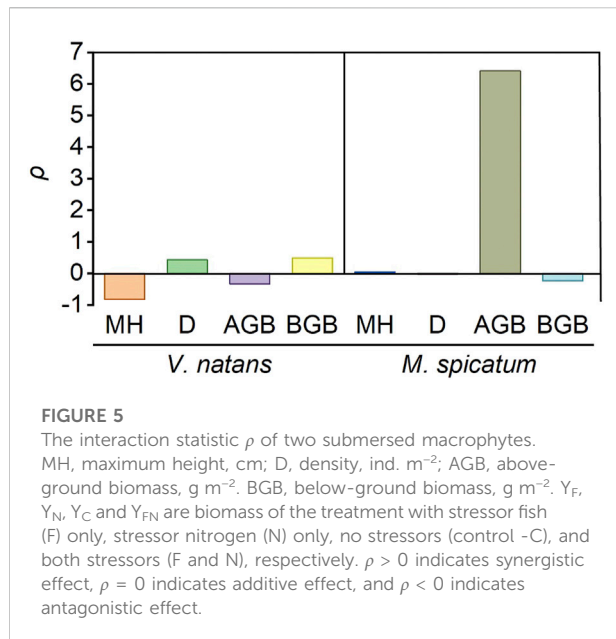
while it declined by 56.7% for *M. spicatum* compared with the C treatment. The below-ground biomass (BGB) of both species exhibited similar fluctuations as with AGB (Figure 3D).

For *V. natans*, the relative growth rate of the above-ground biomass (RGR-AGB) was significantly lower in the two N-addition treatments (N and F + N) than in the fish-alone treatments (F) (Figure 3E). Regarding *M. spicatum*, RGR-AGB was significantly lower in the F, N and F + N treatments than in the C treatment. (Figure 3E). The RGR-AGB of *M. spicatum* was higher than that of *V. natans* in the C, N and F + N treatments.

The root to shoot biomass ratio (R/S ratio) of *V. natans* increased by 227% and 74.4% in the N and F + N treatments compared with the C treatment (Figure 3F). For *M. spicatum*, R/S was significantly higher in the N and N + F treatments than in the C treatment, exhibiting an increase of 114% and 176%, respectively (Figure 3F).

3.3 Integrative assessment of the stress effects

The individual effect of fish on *V. natans* was not significant for the growth variables studied, and the overall effect of fish was positive (Figure 4A). The individual effect of N was significantly negative on MH and RGR-AGB, while positive on R/S, with a significantly negative overall effect ($p = 0.01$). Though the combined effect of fish and N was significantly positive on the R/S ratio, the combined effect on the other growth variables of *V.*



natans was negative. The overall effect of fish and N on *V. natans*. Was negative for all growth variables.

The individual effect of fish on the MH of *M. spicatum* was significantly negative. Unlike *V. natans*, all variables of *M. spicatum* were negatively affected by fish (Figure 4B). Also, the individual effect of N on the AGB, BGB and RGR-AGB of *M. spicatum* was significantly negative, and the overall effect of N was significantly negative for all variables of *M. spicatum* ($p = 0.014$). The observed combined effects of fish and N on MH, density, AGB, BGB and RGR-AGB were negative, while the combined effect on R/S was positive. The overall effect of fish and N was significantly negative for all variables of *M. spicatum* ($p < 0.001$).

According to the results of the interaction metric ρ , the combined fish and N has antagonistic effect on MH, AGB of *V. natans* and BGB of *M. spicatum* ($p < 0$), while synergistic on D, BGB of *V. natans* and AGB of *M. spicatum* ($\rho > 0$) (Figure 5). However, the interaction metric ρ of MH ($\rho = 0.04$) and D ($\rho = -0.02$) of *M. spicatum* were close to zero, implying an additive effect.

4 Discussion

4.1 Effects of fish disturbance and ammonium loading on water quality

We found no effect of fish alone on the nutrient concentrations. These contrasts the findings in several other studies (e.g., Badiou and Goldsborough, 2015; Chen et al., 2020a) but it may reflect the high biomass of macrophytes,

buffering the effect of fish disturbance and nutrient excretion (Kaldy, 2014). Previous studies have showed that fish disturbance has positive (He et al., 2017; Gu et al., 2018), neutral (Parkos et al., 2003; Chen et al., 2020b) and negative effects (Wahl et al., 2011; Badiou and Goldsborough, 2015) on the content of Chl *a* in the water column. In our study, the content of Chl *a* was not affected by fish disturbance, which may be explained by the following mechanisms: 1) the rather dense submersed macrophytes may control phytoplankton by producing allelochemicals that suppress phytoplankton growth rates (Vanderstukken, et al., 2014), and 2) submersed macrophytes may control phytoplankton by competing for nutrients (Jeppesen et al., 1998; Declerck et al., 2007). Benthivorous fish disturbance did increase the content of TSS, consistent with previous findings that fish disturbance causes resuspension of sediment (Badiou and Goldsborough, 2015). However, the resuspension of fish alone had only a weak effect on other water physical and chemical parameters, such as PPFD, salinity and pH.

We found that NH_4-N alone or the combined effect of nitrogen and fish significantly changed and the physical-chemical variables in the water and led to higher biomass of phytoplankton, likely as a result of the increasing N loading (Badiou and Goldsborough, 2015; Olsen et al., 2015) combined with N-induced sediment phosphorus release (Ma et al., 2018). The significantly higher salinity in the mesocosms of N treatment can be explained by the Cl^- being added with the NH_4Cl caused higher salinity.

4.2 Effects of fish disturbance and ammonium loading on submersed macrophytes

The responses of *V. natans* and *M. spicatum* to nitrogen and fish, both individually and in combination, were tested in the 5-month mesocosm experiment. Overall, the results showed that fish alone had no effects on the growth of *V. natans* but negatively affected the height and biomass of *M. spicatum*. To some extent, nitrogen alone or combined with fish disturbance negatively influenced the growth of both species.

Both macrophyte species survived in fish alone treatment (F) during the experiment, although the height and biomass of *M. spicatum* were negatively affected by fish disturbance. *V. natans* produced more ramets than *M. spicatum* as indicated by the high density of *V. natans*. However, adult plants of *M. spicatum* could maintain a higher RGR-AGB and MH than *V. natans* in the fish treatment. A possible explanation for this is that the clonal growth of *V. natans* is interconnected by stolons to help stabilise the sediment. Moreover, the striped leaves of *V. natans* may provide sufficient substrate for the suspended solids and have buffering effect. In addition, the large number of ramets (high density) produced by *V. natans* may be an advantage for further population reproduction and expansion.

Our results are different from those of previous studies showing that fish disturbance inhibited the growth of *V. natans* (Gu et al., 2018). However, as a canopy-forming species, the leaves of *M. spicatum* are more abundant in the upper layer of the water column and sparse in the lower part. Therefore, the ability of *M. spicatum* to compensate for the light reduction due to resuspension is weak, and this species is therefore more vulnerable to fish disturbance.

Although both macrophyte species survived in ammonium loading alone treatment (N) in the 5-month experiment, their growth was negatively affected, as indicated by the changes in maximum height, density, biomass and RGR-AGB of both populations. We found that *M. spicatum* could maintain higher RGR-AGB and MH than *V. natans* in N treatment. The higher density of *V. natans* at high N is an advantage for further population reproduction and expansion. The growth strategies of *M. spicatum* and *V. natans* may be an advantage when facing long-term stress, as also indicated by the higher R/S of the two species than in the C treatment. The maximum height and RGR-AGB of *V. natans* were significantly higher in the N treatment than for *M. spicatum*, which might reflect that rosette-forming macrophytes 1) usually exhibit a weaker ability to increase plant height in N stress environments, as demonstrated for *V. natans* by Yu et al. (2017; 2022), and 2) mainly grow by making physiological adjustments to tolerate stress conditions, such as adjusting the metabolisms of carbon and nitrogen (Yuan et al., 2016; Chen et al., 2020b), rather than by increasing plant height. Canopy-forming macrophytes, by contrast, usually elongate their shoots towards the water surface to capture light for growing (Yu et al., 2018; Angove et al., 2020). Consistent with previous studies, the high maximum height in our experiment enabled the shoots of *M. spicatum* to easily reach the water surface where they formed a canopy allowing them to maintain a competitive advantage in the presence of F and N stressors. In addition, the fact that the MH of *M. spicatum* were higher than *V. natans* in all treatments indicated that *M. spicatum* better tolerate stressors by shoot elongation.

Ecological responses to multiple stressors have been reported, and the interaction types include additive effect, antagonism and synergism which can vary between specific stressor as well as intraspecifically and interspecifically (Birk et al., 2020; Simmons et al., 2021; Turschwell et al., 2022). In our study, the joint effects of fish disturbance and high N on AGB of *M. spicatum* were negatively synergistic, probably with fish disturbance aggravating high ammonium stress. The joint effects on MH and density of *M. spicatum* were additive. This is probably because plants under high N stress become more vulnerable when at low light conditions (Gao et al., 2020), which was induced by fish disturbance in this study. The extra energy and carbohydrates are key components for the detoxication process (Netten et al., 2013; Gao et al., 2020), while photosynthesis as a key production process of energy and carbohydrate is inhibited under low light (Cao et al., 2009,

2011; Yuan et al., 2013, 2016). Differently, the joint effect was antagonistic to BGB of *M. spicatum*, suggesting that though their joint effects may form stress on submersed macrophytes as showed in Figures 3, 4, the consequence is opposite on the below-ground biomass. This implies that fish disturbance may, to some extent, weaken the effect of high N on the below-ground biomass. The above indicated that joint effects from the same stressors may vary from antagonistic, to additive and synergistic depending on the response variables examined. For rosette-forming *V. natans*, the interactive effects on MH and AGB was antagonistic while synergistic on density and BGB. On average, both macrophytes exhibited reduced growth in response to fish disturbance and N stress, but the interactive effect type was highly species-specific and response variable dependent.

Plant biomass storage and its allocation reflect ecosystem productivity and adaption to different environments (Poorter et al., 2012). We found that R/S increased with N alone and the combination of N and F, indicating a biomass allocation favouring root growth rather than stolon and leaf growth. Our results indicate that *M. spicatum* and *V. natans* responded to combined fish and nitrogen stress by changing their allocation patterns. Moreover, R/S of *V. natans* was the highest in the N treatment. *V. natans* thus allocates more biomass to the below-ground part than to the above-ground part when facing high N stress compared with *M. spicatum*. This supports our hypothesis that the strategies of different growth forms of submersed macrophyte might have different tolerance levels to the combined effect of high $\text{NH}_4\text{-N}$ stress and fish disturbance. Previous studies have shown the R/S ratio to be markedly reduced by N addition, as also seen for most herbaceous terrestrial plants (Müller et al., 2000; Poorter et al., 2012; Li et al., 2015; Jing et al., 2020). For aquatic plants, the allocation pattern of above-ground biomass and under-ground biomass depends on the type of resource limitation, when sediment nutrients are limiting, plants allocate more resources to the growth of roots, resulting in higher R/S ratio (Wang et al., 2008; Xie et al., 2013). Our results are inconsistent with the terrestrial plants but supports allocation pattern found for aquatic plants. It reveals that biomass allocation of aquatic plants (high R/S ratio under stress) is not only related to resource limitation, but also a strategy adopted by submersed macrophytes under adverse conditions.

5 Conclusion

Our study demonstrates a combination of fish disturbance and ammonium loading may have negative but variable effects on submersed macrophytes with different growth forms. The interactive effect on height and above-ground biomass of rosette-forming *V. natans* was antagonistic while being

negatively synergistic to density and below-ground biomass. However, the interactive effect on above-ground biomass, density and height of canopy-forming *M. spicatum* was negatively synergistic and has antagonistic effect on below-ground biomass. These results suggest that the interactive effect type may vary from antagonism to synergism depending on the response variables and growth forms of macrophytes which are examined. This emphasizes the necessity of conducting experiments that include the multiple response indicators to both individual and combined stressors. In contrast to most other studies, we found no negative effect of the fish on the water quality, which likely reflect a high macrophyte coverage in our experiment. We also found that the plants allocated more biomass to roots under multiple stress. Our study contributes to the understanding of how different environmental factors can interact with each other and affect submersed macrophytes in aquatic ecosystems. This is timely and relevant knowledge, considering the range of multiple stressors involved in the decline of aquatic ecosystems worldwide. Further research that explores the interactive mechanisms among different growth traits and growth forms may provide a greater understanding for the ecological responses to multiple stressors.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Author contributions

QY: Validation, conceptualization, investigation, methodology, formal analysis, data curation, writing—original draft, writing—review and editing. HW: Conceptualization, methodology, writing—review, supervision, resources, project administration, funding acquisition. ML: Validation, investigation, data curation. CX: Validation, investigation. YM: Validation, investigation. WG: Writing—review and editing, conceptualization, methodology, supervision, and project administration. EJ: Writing—review and editing.

References

Ali, F., Jilani, G., Fahim, R., Bai, L., Wang, C., Tian, L., et al. (2019). Functional and structural roles of wiry and sturdy rooted emerged macrophytes root functional traits in the abatement of nutrients and metals. *J. Environ. Manag.* 249, 109330. doi:10.1016/j.jenvman.2019.109330

Angove, C., Norkko, A., and Gustafsson, C. (2020). The fight to capture light: Functional diversity is related to aquatic plant community productivity likely by enhancing light capture. *Front. Mar. Sci.* 7, 140. doi:10.3389/fmars.2020.00140

Funding

This work was supported by Major Science and Technology Program for Water Pollution Control and Treatment of China (2017ZX07302-002), National Natural Science Foundation of China (31970347), National Key Research and Development Program of China (2018YFD0900805), State Key Laboratory of Freshwater Ecology and Biotechnology (2019FBZ01), Natural Science Foundation of Shandong Province (ZR2021QC081), and Yunnan Provincial Department of Science and Technology (202001BB050078; 202103AC100001). HW was supported by the Youth Innovation Association of Chinese Academy of Sciences as an excellent member (Y201859). EJ was supported by the TÜBITAK program BIDEB2232 (project 118C250) and ANAEE-Denmark.

Acknowledgments

We thank Anne Mette Poulsen for editing the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fenvs.2022.1024524/full#supplementary-material>

Badiou, P., Goldsborough, L. G., and Wrubleski, D. (2011). "Impacts of the common carp (*Cyprinus carpio*) on freshwater ecosystems: A review (chapter 4)," in *Carp: Habitat management and diseases*. Editors J. D. Sanders and S. B. Peterson (New York: Nova Science Publishers), 121–146.

Badiou, P. H. J., and Goldsborough, L. G. (2015). Ecological impacts of an exotic benthivorous fish, the common carp (*Cyprinus carpio* L.), on water quality, sedimentation, and submerged macrophyte biomass in wetland mesocosms. *Hydrobiologia* 755 (1), 107–121. doi:10.1007/s10750-015-2220-6

- Ballard, T. C., Sinha, E., and Michalak, A. M. (2019). Long-term changes in precipitation and temperature have already impacted nitrogen loading. *Environ. Sci. Technol.* 53, 5080–5090. doi:10.1021/acs.est.8b06898
- Baron, J. S., Hall, E. K., Nolan, B. T., Finlay, J. C., Bernhardt, E. S., Harrison, J. A., et al. (2013). The interactive effects of excess reactive nitrogen and climate change on aquatic ecosystems and water resources of the United States. *Biogeochemistry* 114 (1–3), 71–92. doi:10.1007/s10533-012-9788-y
- Bernes, C., Carpenter, S. R., Gårdmark, A., Larsson, P., Persson, L., Skov, C., et al. (2015). What is the influence of a reduction of planktivorous and benthivorous fish on water quality in temperate eutrophic lakes? A systematic review. *Environ. Evid.* 4, 7–28. doi:10.1186/s13750-015-0032-9
- Bhagwati, B., and Ahamad, K. U. (2019). A review on lake eutrophication dynamics and recent developments in lake modeling. *Ecolohydrol. Hydrobiology* 19 (1), 155–166. doi:10.1016/j.ecohyd.2018.03.002
- Birk, S., Chapman, D., Carvalho, L., Spears, B. M., Andersen, H. E., Argillier, C., et al. (2020). Impacts of multiple stressors on freshwater biota across spatial scales and ecosystems. *Nat. Ecol. Evol.* 4, 1060–1068. doi:10.1038/s41559-020-1216-4
- Cao, T., Ni, L. Y., Xie, P., Xu, J., and Zhang, M. (2011). Effects of moderate ammonium enrichment on three submersed macrophytes under contrasting light availability. *Freshw. Biol.* 56, 1620–1629. doi:10.1111/j.1365-2427.2011.02601.x
- Cao, T., Xie, P., Li, Z. Q., Ni, L. Y., Zhang, M., and Xu, J. (2009). Physiological stress of high NH_4^+ concentration in water column on the submersed macrophyte *Vallisneria spiralis*. *Bull. Environ. Contam. Toxicol.* 82, 296–299. doi:10.1007/s00128-008-9531-5
- Chen, J., Liu, Z., Xiao, S., Chen, R., Luo, C., Zhu, T., et al. (2020b). Effects of benthivorous fish disturbance on chlorophyll *a* contents in water and the growth of two submersed macrophytes with different growth forms under two light regimes. *Sci. Total Environ.* 704, 135269. doi:10.1016/j.scitotenv.2019.135269
- Chen, J., Su, H., Zhou, G., Dai, Y., Hu, J., Zhao, Y., et al. (2020a). Effects of benthivorous fish disturbance and snail herbivory on water quality and two submersed macrophytes. *Sci. Total Environ.* 713, 136734. doi:10.1016/j.scitotenv.2020.136734
- Declerck, S., Vanderstukken, M., Pals, A., Muylaert, K., and De Meester, L. (2007). Plankton biodiversity along a gradient of productivity and its mediation by macrophytes. *Ecology* 88, 2199–2210. doi:10.1890/07-0048.1
- Dorenbosch, M., and Bakker, E. S. (2012). Effects of contrasting omnivorous fish on submersed macrophyte biomass in temperate lakes: A mesocosm experiment. *Freshw. Biol.* 57 (7), 1360–1372. doi:10.1111/j.1365-2427.2012.02790.x
- Erismann, J. W., Galloway, J. N., Seitzinger, S., Bleeker, A., Dise, N. B., Petrescu, A. M., et al. (2013). Consequences of human modification of the global nitrogen cycle. *Phil. Trans. R. Soc. B* 368 (1621), 20130116. doi:10.1098/rstb.2013.0116
- Gao, J., Liu, L., Ma, N., Yang, J., Dong, Z., Zhang, J., et al. (2020). Effect of ammonia stress on carbon metabolism in tolerant aquatic plant *Myriophyllum aquaticum*. *Environ. Pollut.* 263, 114412. doi:10.1016/j.envpol.2020.114412
- Gao, J., Liu, Z. W., and Jeppesen, E. (2014). Fish community assemblages changed but biomass remained similar after lake restoration by biomanipulation in a Chinese tropical eutrophic lake. *Hydrobiologia* 724 (1), 127–140. doi:10.1007/s10750-013-1729-9
- Goldsborough, L. G., and Wrubleski, D. A. (2001). “The decline of Delta Marsh, an internationally significant wetland in southcentral Manitoba,” in *6th prairie conservation and endangered species conference* (Winnipeg, MB (CD-ROM)).
- Greaver, T. L., Clark, C. M., Compton, J. E., Vallano, D., Talhelm, A. F., Weaver, C. P., et al. (2016). Key ecological responses to nitrogen are altered by climate change. *Nat. Clim. Chang.* 6 (9), 836–843. doi:10.1038/nclimate3088
- Gu, J., He, H., Jin, H., Yu, J., Jeppesen, E., Nairn, R. W., et al. (2018). Synergistic negative effects of small-sized benthivorous fish and nitrogen loading on the growth of submersed macrophytes – relevance for shallow lake restoration. *Sci. Total Environ.* 610–611, 1572–1580. doi:10.1016/j.scitotenv.2017.06.119
- Hamilton, D. P., Salmaso, N., and Paerl, H. W. (2016). Mitigating harmful cyanobacterial blooms: Strategies for control of nitrogen and phosphorus loads. *Aquat. Ecol.* 50, 351–366. doi:10.1007/s10452-016-9594-z
- He, H., Hu, E., Yu, J., Luo, X., Li, K., Jeppesen, E., et al. (2017). Does turbidity induced by *Carassius auratus* limit phytoplankton growth? A mesocosm study. *Environ. Sci. Pollut. Res.* 24, 5012–5018. doi:10.1007/s11356-016-8247-z
- Huang, X. F., Chen, W. M., and Cai, Q. M. (1999). *Standard methods for observation and analysis in Chinese ecosystem research network-survey, Observation and Analysis of Lake Ecology*. Beijing: Standards Press of China.
- Jeppesen, E., Brucet, S., Naselli-Flores, L., Papastergiadou, E., Stefanidis, K., Nöges, T., et al. (2015). Ecological impacts of global warming and water abstraction on lakes and reservoirs due to changes in water level and related changes in salinity. *Hydrobiologia* 750 (1), 201–227. doi:10.1007/s10750-014-2169-x
- Jeppesen, E., Søndergaard, M., Søndergaard, M., and Christoffersen, K. (1998). “The structuring role of submersed macrophytes in lakes,” in *Ecological studies* (New York: Springer-Verlag).
- Jing, M., Jia, X., Zhang, Y., Cao, J., Zhou, W., Wang, J., et al. (2020). Effects of long-term nitrogen addition on community aboveground and belowground biomass and their ratio in a typical steppe of Inner Mongolia. *Chin. J. Ecol.* 39, 3185–3193. (in Chinese).
- Kaldy, J. E. (2014). Effect of temperature and nutrient manipulations on eelgrass *Zostera marina* L. from the Pacific Northwest, USA. *J. Exp. Mar. Biol. Ecol.* 453, 108–115. doi:10.1016/j.jembe.2013.12.020
- Kloskowski, J. (2011). Impact of common carp *Cyprinus carpio* on aquatic communities: Direct trophic effects versus habitat deterioration. *Fundam. Appl. Limnol.* 178, 245–255. doi:10.1127/1863-9135/2011/0178-0245
- Kosten, S., Kamarainen, A. M. Y., Jeppesen, E., Van Nes, E. H., Peeters, E. T. H. M., Mazzeo, N., et al. (2009). Climate-related differences in the dominance of submersed macrophytes in shallow lakes. *Glob. Change Biol.* 15 (10), 2503–2517. doi:10.1111/j.1365-2486.2009.01969.x
- Li, W. (2014). Environmental opportunities and constraints in the reproduction and dispersal of aquatic plants. *Aquat. Bot.* 118, 62–70. doi:10.1016/j.aquabot.2014.07.008
- Li, W., Jin, C., Guan, D., Wang, Q., Wang, A., Yuan, F., et al. (2015). The effects of simulated nitrogen deposition on plant root traits: A meta-analysis. *Soil Biol. Biochem.* 82, 112–118. doi:10.1016/j.soilbio.2015.01.001
- Liu, E. S., Liu, Z. W., and Bao, C. H. (2007). The changes of catches of *Carassius auratus* and relationship with environment in Lake Taihu, China. *J. Lake Sci.* 19 (3), 345–350. (in Chinese). doi:10.18307/2007.0318
- Ma, S. N., Wang, H. J., Wang, H. Z., Li, Y., Liu, M., Liang, X. M., et al. (2018). High ammonium loading can increase alkaline phosphatase activity and promote sediment phosphorus release: A two-month mesocosm experiment. *Water Res.* 145, 388–397. doi:10.1016/j.watres.2018.08.043
- Moss, B., Jeppesen, E., Søndergaard, M., Lauridsen, T. L., and Liu, Z. W. (2013). Nitrogen, macrophytes, shallow lakes and nutrient limitation: Resolution of a current controversy? *Hydrobiologia* 710 (1), 3–21. doi:10.1007/s10750-012-1033-0
- Müller, I., Schmid, B., and Weiner, J. (2000). The effect of nutrient availability on biomass allocation patterns in 27 species of herbaceous plants. *Perspect. Plant Ecol. Evol. Syst.* 3, 115–127. doi:10.1078/1433-8319-00007
- Netten, J. J. C., van der Heide, T., and Smolders, A. J. P. (2013). Interactive effects of pH, temperature and light during ammonia toxicity events in *Elodea canadensis*. *Chem. Ecol.* 29, 448–458. doi:10.1080/02757540.2013.769971
- Nieoczym, M., and Kloskowski, J. (2014). The role of body size in the impact of common carp *Cyprinus carpio* on water quality, zooplankton, and macrobenthos in ponds. *Int. Rev. Hydrobiol.* 99, 212–221. doi:10.1002/iroh.201301644
- Nimptsch, J., and Pflugmacher, S. (2007). Ammonia triggers the promotion of oxidative stress in the aquatic macrophyte *Myriophyllum matogrossense*. *Chemosphere* 66, 708–714. doi:10.1016/j.chemosphere.2006.07.064
- Nurminen, L., Horppila, J., Lappalainen, J., and Malinen, T. (2003). Implications of Rudd (*Scardinius erythrophthalmus*) herbivory on submersed macrophytes in a shallow eutrophic lake. *Hydrobiologia* 506, 511–518. doi:10.1023/b:hydr.0000008577.16934.a9
- Olsen, S., Chan, F., Li, W., Zhao, S., Søndergaard, M., and Jeppesen, E. (2015). Strong impact of nitrogen loading on submersed macrophytes and algae: A long-term mesocosm experiment in a shallow Chinese lake. *Freshw. Biol.* 60, 1525–1536. doi:10.1111/fwb.12585
- Parkos, J., Santucci, V. J., Jr., and Wahl, D. H. (2003). Effects of adult common carp (*Cyprinus carpio*) on multiple trophic levels in shallow mesocosms. *Can. J. Fish. Aquat. Sci.* 60, 182–192. doi:10.1139/f03-011
- Poorter, H., Niklas, K. J., Reich, P. B., Oleksyn, J., Poot, P., and Mommer, L. (2012). Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytol.* 193 (1), 30–50. doi:10.1111/j.1469-8137.2011.03952.x
- Richardson, M. J., Whoriskey, F. G., and Roy, L. H. (1995). Turbidity generation and biological impacts of an exotic fish *Carassius auratus*, introduced into shallow seasonally anoxic ponds. *J. Fish Biol.* 47 (4), 576–585. doi:10.1111/j.1095-8649.1995.tb01924.x
- Saunkaew, P., Wangpakapattanawong, P., and Jampeetong, A. (2011). Growth, morphology, ammonium uptake and nutrient allocation of *Myriophyllum brasiliense* Cambess. under high NH_4^+ concentrations. *Ecotoxicology* 20 (8), 2011–2018. doi:10.1007/s10646-011-0744-8
- Shi, D., Zhuang, K., Chen, Y., Xu, F., Hu, Z., and Shen, Z. (2020). Effects of excess ammoniacal nitrogen ($\text{NH}_4^+\text{-N}$) on pigments, photosynthetic rates, chloroplast ultrastructure, proteomics, formation of reactive oxygen species and enzymatic activity in submersed plant *Hydrilla verticillata* (L.f.) Royle. *Aquat. Toxicol.* 226, 105585. doi:10.1016/j.aquatox.2020.105585

- Simmons, B. I., Blyth, P. S. A., Blanchard, J. L., Clegg, T., Delmas, E., Garnier, A., et al. (2021). Refocusing multiple stressor research around the targets and scales of ecological impacts. *Nat. Ecol. Evol.* 5, 1478–1489. doi:10.1038/s41559-021-01547-4
- Su, H., Chen, J., Wu, Y., Chen, J., Guo, X., Yan, Z., et al. (2019a). Morphological traits of submerged macrophytes reveal specific positive feedbacks to water clarity in freshwater ecosystems. *Sci. Total Environ.* 684, 578–586. doi:10.1016/j.scitotenv.2019.05.267
- Su, H., Wu, Y., Xia, W., Yang, L., Chen, J., Han, W., et al. (2019b). Stoichiometric mechanisms of regime shifts in freshwater ecosystem. *Water Res.* 149, 302–310. doi:10.1016/j.watres.2018.11.024
- Tan, X., Yuan, G., Fu, H., Peng, H., Ge, D., Lou, Q., et al. (2019). Effects of ammonium pulse on the growth of three submerged macrophytes. *PLoS One* 14, e0219161. doi:10.1371/journal.pone.0219161
- Ti, C., Gao, B., Luo, Y., Wang, S., Chang, S. X., and Yan, X. (2018). Dry deposition of N has a major impact on surface water quality in the Taihu Lake region in southeast China. *Atmos. Environ.* 190, 1–9. doi:10.1016/j.atmosenv.2018.07.017
- Ti, C., Xia, L., Chang, S. X., and Yan, X. (2019). Potential for mitigating global agricultural ammonia emission: A meta-analysis. *Environ. Pollut.* 245, 141–148. doi:10.1016/j.envpol.2018.10.124
- Turschwell, M. P., Connolly, S. R., Schäfer, R. B., De Laender, F., Campbell, M. D., Mantyka-Pringle, C., et al. (2022). Interactive effects of multiple stressors vary with consumer interactions, stressor dynamics and magnitude. *Ecol. Lett.* 25, 1483–1496. doi:10.1111/ele.14013
- Van de Bund, W. J., and Van Donk, E. (2004). Effects of fish and nutrient additions on food-web stability in a Charophyte-dominated lake. *Freshw. Biol.* 49, 1565–1573. doi:10.1111/j.1365-2427.2004.01301.x
- Vanderstukken, M., Declerck, S. A. J., Decaestecker, E., and Muylaert, K. (2014). Long-term allelopathic control of phytoplankton by the submerged macrophyte *Elodea nuttallii*. *Freshw. Biol.* 59 (5), 930–941. doi:10.1111/fwb.12316
- Wahl, D. H., Wolfe, M. D., Santucci, V. J., and Freedman, J. A. (2011). Invasive carp and prey community composition disrupt trophic cascades in eutrophic ponds. *Hydrobiologia* 678, 49–63. doi:10.1007/s10750-011-0820-3
- Wang, J., Yu, D., and Wang, Q. (2008). Growth, biomass allocation, and autofragmentation responses to root and shoot competition in *Myriophyllum spicatum* as a function of sediment nutrient supply. *Aquat. Bot.* 89, 357–364. doi:10.1016/j.aquabot.2008.04.001
- Xiao, K. Y., Yu, D., and Wang, J. W. (2006). Habitat selection in spatially heterogeneous environments: A test of foraging behaviour in the clonal submerged macrophyte *Vallisneria spiralis*. *Freshw. Biol.* 51, 1552–1559. doi:10.1111/j.1365-2427.2006.01590.x
- Xie, D., Yu, D., You, W., and Wang, L. (2013). Morphological and physiological responses to sediment nutrients in the submerged macrophyte *Myriophyllum spicatum*. *Wetlands* 33, 1095–1102. doi:10.1007/s13157-013-0465-2
- Yu, C., Huang, X., Chen, H., Godfray, H. C. J., Wright, J. S., Hall, J. W., et al. (2019a). Managing nitrogen to restore water quality in China. *Nature* 567 (7749), 516–520. doi:10.1038/s41586-019-1001-1
- Yu, G., Jia, Y., He, N., Zhu, J., Chen, Z., Wang, Q., et al. (2019b). Stabilization of atmospheric nitrogen deposition in China over the past decade. *Nat. Geosci.* 12 (6), 424–429. doi:10.1038/s41561-019-0352-4
- Yu, J. L., Liu, Z. W., He, H., Zhen, W., Guan, B., Chen, F., et al. (2016). Submerged macrophytes facilitate dominance of omnivorous fish in a subtropical shallow lake: Implications for lake restoration. *Hydrobiologia* 775 (1), 97–107. doi:10.1007/s10750-016-2717-7
- Yu, Q., Wang, H. J., Wang, H. Z., Li, Y., Liang, X. M., Xu, C., et al. (2017). Does the responses of *Vallisneria natans* (Lour.) Hara to high nitrogen loading differ between the summer high-growth season and the low-growth season? *Sci. Total Environ.* 601–602, 1513–1521. doi:10.1016/j.scitotenv.2017.05.268
- Yu, Q., Wang, H., Wang, H., Xu, C., Liu, M., Ma, Y., et al. (2022). Effects of High ammonium loading on two submersed macrophytes of different growth form based on an 18-month pond experiment. *Front. Plant Sci.* 13, 939589. doi:10.3389/fpls.2022.939589
- Yu, Q., Wang, H. Z., Xu, C., Li, Y., Ma, S. N., Liang, X. M., et al. (2018). Higher tolerance of canopy-forming *Potamogeton crispus* than rosette-forming *Vallisneria natans* to high nitrogen concentration as evidenced from experiments in 10 ponds with contrasting nitrogen levels. *Front. Plant Sci.* 9, 1845. doi:10.3389/fpls.2018.01845
- Yuan, G., Fu, H., Zhong, J., Qian, L., Ni, L., and Cao, T. (2016). Growth and C/N metabolism of three submersed macrophytes in response to water depths. *Environ. Exp. Bot.* 122, 94–99. doi:10.1016/j.envexpbot.2015.09.009
- Yuan, G. X., Cao, T., Fu, H., Ni, L. Y., Zhang, X. L., Li, W., et al. (2013). Linking carbon and nitrogen metabolism to depth distribution of submersed macrophytes using high ammonium dosing tests and a lake survey. *Freshw. Biol.* 58, 2532–2540. doi:10.1111/fwb.12230
- Zhang, M., Wang, Z., Xu, J., Liu, Y., Ni, L. Y., Cao, T., et al. (2011). Ammonium, microcystins, and hypoxia of blooms in eutrophic water cause oxidative stress and C–N imbalance in submersed and floating-leaved aquatic plants in Lake Taihu, China. *Chemosphere* 82, 329–339. doi:10.1016/j.chemosphere.2010.10.038
- Zhang, Y., Jeppesen, E., Liu, X., Qin, B., Shi, K., Zhou, Y., et al. (2017). Global loss of aquatic vegetation in lakes. *Earth. Sci. Rev.* 173, 259–265. doi:10.1016/j.earscirev.2017.08.013
- Zhu, Z., Song, S., Yan, Y., Li, P., Jeelani, N., Wang, P., et al. (2018). Combined effects of light reduction and ammonia nitrogen enrichment on the submerged macrophyte *Vallisneria natans*. *Mar. Freshw. Res.* 69 (5), 764–770. doi:10.1071/mf17146