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Original Research

Effect of *Vallisneria spiralis* on Water Quality and Sediment Nitrogen at Different Growth Stages in Eutrophic Shallow Lake Mesocosms

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Abstract

Submerged macrophytes are commonly highlighted as the key step to restore eutrophic shallow lakes. However, how submerged macrophytes affect water quality and sediment nitrogen at different growth stages remains unclear. In this study, the dynamics of water quality, sediment nitrogen, and denitrification during the four growth periods of *Vallisneria spiralis* (*V. spiralis*), namely, the rapid growth period (stage I), stable growth period (stage II), withering and death period (stage III), and decay and decomposition period (stage IV), were investigated using mesocosms to simulate eutrophic shallow lakes. The results showed that the purification effects of *V. spiralis* on water quality parameters in the four periods clearly differed. Compared with the control group (in the absence of *V. spiralis*), the treatment group presented a lower concentration of Chlorophyll a (Chl.*a*) in stage I; total nitrogen (TN), ammonia nitrogen (NH_4^+-N), and Chl.*a* in stage II; and TN, total phosphorus (TP), chemical oxygen demand (COD), and Chl.*a* in stage III. However, the water quality in stage IV deteriorated for a short-period in the group with *V. spiralis*, especially the increases in NH_4^+-N and Chl.*a*. Moreover, the effect of *V.* spiralis on the forms of nitrogen in sediment primarily occurred from stages II to IV. The content of sediment TN in treatment group was much lower than that in control group, with a final decrease of 40.7%. In addition, the denitrification rate (DNR) differed significantly between the two groups, and the DNR in treatment group increased by 4.8 % compared with that in the control group. The DNR of treatment group decreased in stage I but increased in stages II and IV. Our study provides empirical evidence that re-established submerged macrophytes can contribute differently to eutrophication mitigation in shallow lakes at their various growth stages.

Keywords: eutrophic shallow lake, *Vallisneria spiralis*, growth stages, water quality, sediment nitrogen

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Introduction

Eutrophication has posed a serious threat to lakes worldwide for 50-100 years [1]. Eutrophic lake area in China increased massively since the 1980s, and lake eutrophication has been one of the most important factors that impede sustainable economic development [2-3]. Both the Chinese government and researchers have devoted considerable efforts to combat eutrophication. However, according to the 2018 Ecological and Environmental State Bulletin released by the Ministry of Ecology and Environment of the People's Republic of China, 29% of the lakes in China are still considered to be eutrophic. Therefore, more studies are needed to devise sound solutions for this thorny problem.

The recovery or maintenance of submerged macrophytes has been an important target in the management of shallow lakes [4-5], because research on lake restoration demonstrates that it is possible to change a stable situation with turbid water and dominance by phytoplankton into an 'alternative stable state' with clear water and dominance using submerged macrophytes [6-7]. Previous studies have reported that submerged macrophytes can buffer lake ecosystems against the harmful consequences of anthropogenic eutrophication by suppressing phytoplankton biomass directly via allelopathy, or indirectly, via competition for nutrients or the positive interaction between submerged macrophytes and zooplankton grazing [8-11]. Among the mechanisms of submerged macrophytes in maintaining the clear state of shallow lakes, the one in which submerged macrophytes can influence nutrient dynamics has received a substantial amount of attention [12-14]. Many field experiments and mesocosm experiments highlight the concept that submerged macrophytes can reduce concentrations of nutrients in the water column, especially nitrogen and phosphorus, by absorbing nutrients, promoting sedimentation, stabilizing the sediment, and increasing denitrification [15-17]. Conversely, some studies found that submerged macrophytes could lead to a significant increase in water column nutrients owing to the release of nutrients as they decay [18-19]. Therefore, it is necessary to fully understand how submerged macrophytes affect water quality at their different stages of growth.

We assembled shallow lake mesocosms and chose a widespread species – *Vallisneria spiralis*, which can adapt to eutrophic conditions, to address its effects on water quality and forms of sediment nitrogen at its different stages of growth. According to the growth stages of *V. spiralis* [20, 21], the experimental periods were divided into four periods: the rapid growth period (stage I), stable growth period (stage II), withering and death period (stage III), and decay and decomposition period (stage IV). The objectives of this study are: i) to evaluate the different purification effects of *V. spiralis* on water quality of eutrophic shallow lake at four growth stages; ii) to analyze the changes in sediment nitrogen forms during the four growth stages; and iii) to reveal the role of *V. spiralis* in the denitrification process.

Materials and Methods

Experimental Setup

The experimental system utilized glass vessels, with the length, width and height of 40 * 30 * 60 cm, respectively. A transparent plastic board was placed on the top of each glass vessel to allow sunshine to go through while protecting the system against rain. The treatment and control groups were established, and each group had three replicates.

Each vessel was filled with 10 cm eutrophic sediment, which had passed through a 2 mm sieve and was thoroughly mixed. The initial sediment samples were collected using a Peterson grab mud sampler from the top 10 cm of the surface sediment in Guanqiao Lake (30°32′N, 114°22′E), which is a typical eutrophic lake and a sub-lake of Donghu Lake in Wuhan City, China. Moreover, a 1 L surface water sample of Guanqiaohu Lake was collected and measured, and the results of water quality were the basis of synthetic water of eutrophication.

The submerged macrophyte *V. spiralis* was purchased from a local aquarium. Plants with good growth conditions, well-preserved stems and leaves, and relatively uniform characteristics were selected for the experiment after 10 days of adaptive culture. *V. spiralis* was cleaned, measured and weighed before use, and the average initial fresh weight and stem height were 6.8±0.39 g and 10±0.3 cm standard error (SE), respectively. Six plants per vessel were planted in the treatment group.

A volume of 48 L of synthetic water was added to each vessel, and the average water depth was 40±0.5 cm SE above the sediment. The ratio of volume of water column to the sediment in each group was 4:1, to mirror the conditions of Guanqiao Lake. The characteristics and components of the synthetic water are shown in Table 1. Synthetic water was added at the beginning of the experiment and then supplemented weekly with sun-exposed tap water.

The experiment was conducted between May 2018 and March 2019. The division of growth cycle of *V. spiralis* was primarily derived from Zhang et al. [21], and four main growth stages were contained in this study. Specifically, the rapid growth period occurred in June 2018, and its major characteristic was that the plants grew rapidly and reached the water surface. The stable growth period, from July to October 2018, was characterized by peak plant coverage and total biomass. The withering and death period occurred from November 2018 to February 2019. The leaves began to thin and turn yellow, and the plants slowly died at this stage. The decay and decomposition period occurred in March 2019. During this stage, most plant litter sunk

Table 1. The initial concentrations of synthetic water in this experiment (mean±standard deviation).

Parameters	Treatment Group	Control Group
TN (mg/L)	3.01±0.18	3.11±0.23
TP (mg/L)	0.54±0.01	0.52±0.02
NH_4^+-N(mg/L)	1.00±0.05	1.09±0.09
NO_3^--N(mg/L)	1.98±0.02	1.98±0.02
COD (mg/L)	43.1±1.80	43.6±1.63
C:N ratio	14.3	14.0

Note: C:N = COD:TN.

into the sediment and quickly decomposed causing the water to become muddy. The seedlings appeared at the end of the experiment. And the water temperature changes of the treatment and control groups in four growth stages are shown in Fig. S1.

Sampling and Analysis

The physicochemical parameters of water of each mesocosm, including the temperature (T), pH, dissolved oxygen (DO), oxidation reduction potential (ORP), salinity (Sal), and conductivity (Cond), were monitored in situ weekly using a YSI probe (Thermo Electron Scientific Company, Madison, WI, USA). Water samples were collected and measured weekly. Water quality indices, including TN (total nitrogen), TP (total phosphorus), NO_3^-N (nitrate nitrogen), and NH_4^+-N (ammonia nitrogen), were determined using standard methods [22]. COD (chemical oxygen demand) was measured using a spectrophotometer (DRB 200, Hach, Loveland, CO, USA). Chl.*a* (chlorophyll a) was determined spectrophotometrically.

Sediment samples were collected six times during the entire experimental period. Since the stable growth period and the withering and death period were comparatively long, sediment collections in these two stages were conducted in the middle period and during the transition period. Thus sampling took place in May 2018, June 2018, August 2018, October 2018, January 2019, and March 2019. Sediment TN was determined by the Kjeldahl method. The NH_4^+-N, NO_3^--N and NO_2^--N of the sediment were extracted with a solution of 2 M KCl and measured using a spectrophotometer (GENESYS 180, ThermoFisher, Waltham, MA, USA) [23]. TIN is the sum of NO_3^--N, NH_4^+-N and NO_2^--N. Sediment NO_2^--N was not shown in this study because the concentrations were too low.

The denitrification rate of sediment was measured using the acetylene inhibition method (AIM) [24]. The AIM is based on the ability of acetylene to inhibit the reduction of N_2O to N_2 and the oxidization of NH_4^+-N to NO_3^--N during the denitrification process [25]. The DNR was measured using the AIM with some modifications [24]. Briefly, approximately 10 g of homogenized sediments from each sample were weighed into a 250 mL customized triangular bottle with 50 mL of incubation solution (final concentrations: 0.1 g/L KNO_3, 0.18 g/L glucose and 1 g/L chloramphenicol). Each bottle was sealed with a stopper and purged with 99.999% high purity N_2 for 5 minutes to remove oxygen and produce anaerobic conditions. Approximately 10% of the gas in bottle was then replaced with an equal volume of acetylene. The bottles were incubated in the dark for 1 h at 30ºC. After incubation, the gas samples were immediately collected using a 50 mL syringe and measured by gas chromatography (Agilent 7890A, Agilent Technologies, Carpinteria, CA, USA).

Statistical Analysis

Statistical analyses were performed using SPSS 18.0. The graph drawings were accomplished by Origin Pro 2016. The differences between the treatment and control groups in the water quality indices (e.g., TN, TP, NH_4^+-N, NO_3-N, COD, Chl.*a*, T, Cond, TDS, Sal, DO, and pH) at various growth stages were evaluated using a one-way analysis of variance (ANOVA). The relationships between DNR and environment variables (e.g., TN, TP, and COD of the water and TN, TIN, NH_4^+-N, and NO_3^--N of the sediment) were tested with a Pearson correlation analysis. Differences were considered significant when $P<0.05$ and highly significant when $P<0.01$.

Results

Physiochemical Characteristics of the Water Column at Different Growth Stages of *V. spiralis*

V. spiralis improved the water quality in eutrophic shallow lake mesocosms (Fig. 1). The concentrations of TN, TP, and COD in treatment and control groups showed the same trend, first decreasing, then increasing and decreasing again, with the highest concentrations observed in stage II. The NH_4^+-N and NO_3^--N of two groups decreased rapidly at the beginning of the experiment and then fluctuated slightly. The Chl.*a* increased first and then decreased gradually in both groups, and its concentration was the highest in July. The average concentrations of TN, TP, NH_4^+-N, NO_3^--N, COD, and Chl.*a* in the treatment group were 1.65 mg/L, 0.56 mg/L, 0.36 mg/L, 0.22 mg/L, 47.5 mg/L, and 28.6 μg/L, respectively, which those in the control group were 2.24 mg/L, 0.81 mg/L, 0.37 mg/L, 0.20 mg/L, 56.9 mg/L, and 61.0 μg/L, respectively. The changes in purification of *V. spiralis* were 26.3 %, 31.0 %, 2.7 %, -10 %, 16.5 %, and 53.1 %, respectively.

The curves of TN and NH_4^+-N between the treatment and control groups were very close in stage I, but separated from stages II to IV (Fig. 1a, c). The change trend of TP was similar to that of TN during the first three stages (Fig. 1b). There was little difference in NO_3^--N between the two groups, with the exception of stage IV (Fig. 1d). COD in two groups first decreased and then increased (Fig. 1e). Chl.*a* in the treatment group was obviously different than that in the control (Fig. 1f).

The one-way ANOVA results suggested that there was no significant difference in the water quality parameters between the treatment and control groups in stage I, with the exception of Chl.*a* (Table 2). The TN, NH_4^+-N, Chl.*a*, Cond, TDS, Sal, and pH differed significantly in stage II, and the TN, TP, COD, Chl.*a*, Cond, TDS, Sal, and DO were distinct in stage III. However, The NH_4^+-N, Chl.*a,* Cond, TDS, Sal, DO, and pH differed during stage IV. Overall, *V. spiralis* primarily purified the water in stages II and III.

Nitrogen in Sediment at Different Growth Stages of *V. spiralis*

The TN, TIN, NH_4^+-N, and NO_3^--N decreased gradually with time in the control group, while a decrease during the first three periods and an increase in stage IV were observed in the treatment group (Fig. 2). At the end of the experiment, the removal rates of TN, TIN, NH_4^+-N, and NO_3^--N in the treatment group were 78.8%, 83.8%, 84.3%, and 83.3%, respectively, which those in the control group were 64.2%, 87.0%, 87.1%, and 86.9%, respectively. *V. spiralis* was successful in reducing the amount of sediment nitrogen, and the final content of TN in the treatment group was 386.9 mg/kg, which was 40.7% lower than that in the control group.

Sediment TN in the treatment group was lower than that in the control group during the four periods (Fig. 2a). There were no significant differences in sediment TIN between the two groups in stage I. During stage II, TIN in the treatment group was higher in August and lower in October than that in the control group. In addition, it continued to be lower in stage III, while increasing in stage IV (Fig. 2b).

Sediment NH_4^+-N in the treatment group was higher in stage I but lower in stages II and III compared with the control group. During the stage IV, sediment NH_4^+-N was higher in the treatment group (Fig. 2c), while the change in NO_3^--N in sediment was similar to that in TIN (Fig. 2d).

The analysis of differences between the control and treatment groups showed that the influences of *V. spiralis* on sediment nitrogen forms primarily occurred from stages II to IV. According to the analysis of variance within the treatment group, sediment nitrogen forms differed significantly in four growth periods of *V. spiralis*. This was particularly true for the TN.

To study the effects of *V. spiralis* on the denitrification process, simulation experiments were conducted. Fig. 3 showed that both groups decreased during the first three periods and increased in stage IV. *V. spiralis* changed the process of sediment denitrification in four stages. The DNR in the treatment group was lower in stage I and higher in stages II and IV than control group. Furthermore, as indicated in Table 3, a correlation analysis showed that the DNR was significantly related to the TN, TIN, NH_4^+-N, and NO_3^--N in the sediment and TN, TP, NO_3^--N, Chl.*a*, T, Cond in the water column ($P<0.05$).

Discussion

Effects of *V. spiralis* on Water Quality at Different Growth Stages

In this study, *V. spiralis* showed distinct effects on water quality at its four different growth stages, and the purification primarily occurred during stages II and III. Previous studies have reported that aquatic plants have a strong effect on water TN, TP, NH_4^+-N, and NO_3^--N via absorption during stage I [26, 27]. However, in this study, only a significantly lower concentration of Chl.*a* was measured in the treatment group compared with that of the control group. This may be related to the way in which plants use nutrients. *V. spiralis* primarily absorbs nutrients from the sediment through its root [28]. Therefore, the concentration of TN in the treatment group was lower than that in the control group at this stage.

V. spiralis reduced TN, NH_4^+-N and Chl.*a* in stage II. We found no significant differences in TP between the treatment and control groups. This lack of a change could possibly be owing to the fact that although *V. spiralis* also had a good purification effect on TP [21], the water TP was affected by both the initial phosphorus concentration and the temperature of sediment [29]. A previous study showed that high temperatures weakened the fixation effect of roots on TP [30], causing the migration of sediment TP to upper water in the treatment group, just as in the control group. In addition, there was still a significant difference in Chl.*a* between the two groups during this stage. Submerged plants can effectively reduce the content of chlorophyll and maintain the state of clean water [31].

Water quality during stage III was improved in the experimental and control groups compared with that during stage II. TN, TP, COD, and Chl.*a* in the treatment group were significantly lower than those in the control group. The migration and transformation of nitrogen in sediment weakened with the decrease in environmental temperature, but root microorganisms could still perform nitrification and denitrification [32], which could be the reason why TN in the treatment group was lower than that in the control group. Phosphorus in sediment and water column can be absorbed by roots, stems and leaves [33]. TP in the treatment group was lower than that in the control group, possibly because the absorption of TP by roots was enhanced as the temperature dropped, and plants settled to the surface

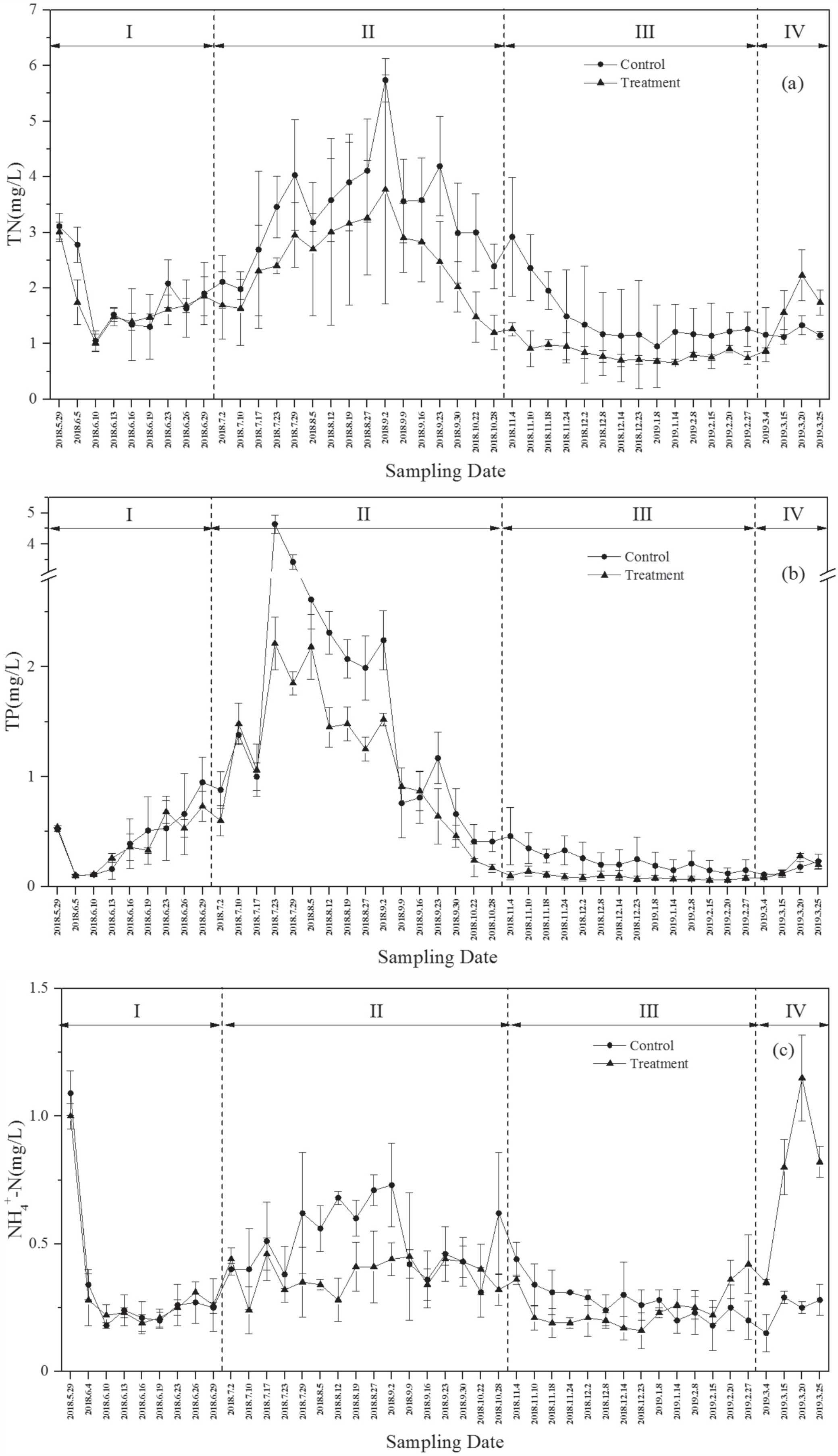

Fig.1. Temporal variations in the concentrations of TN, TP, NH_4^+-N, NO_3^--N, COD, and Chl.*a* of the water column in treatment and control groups. All values are means±SD (n = 3).

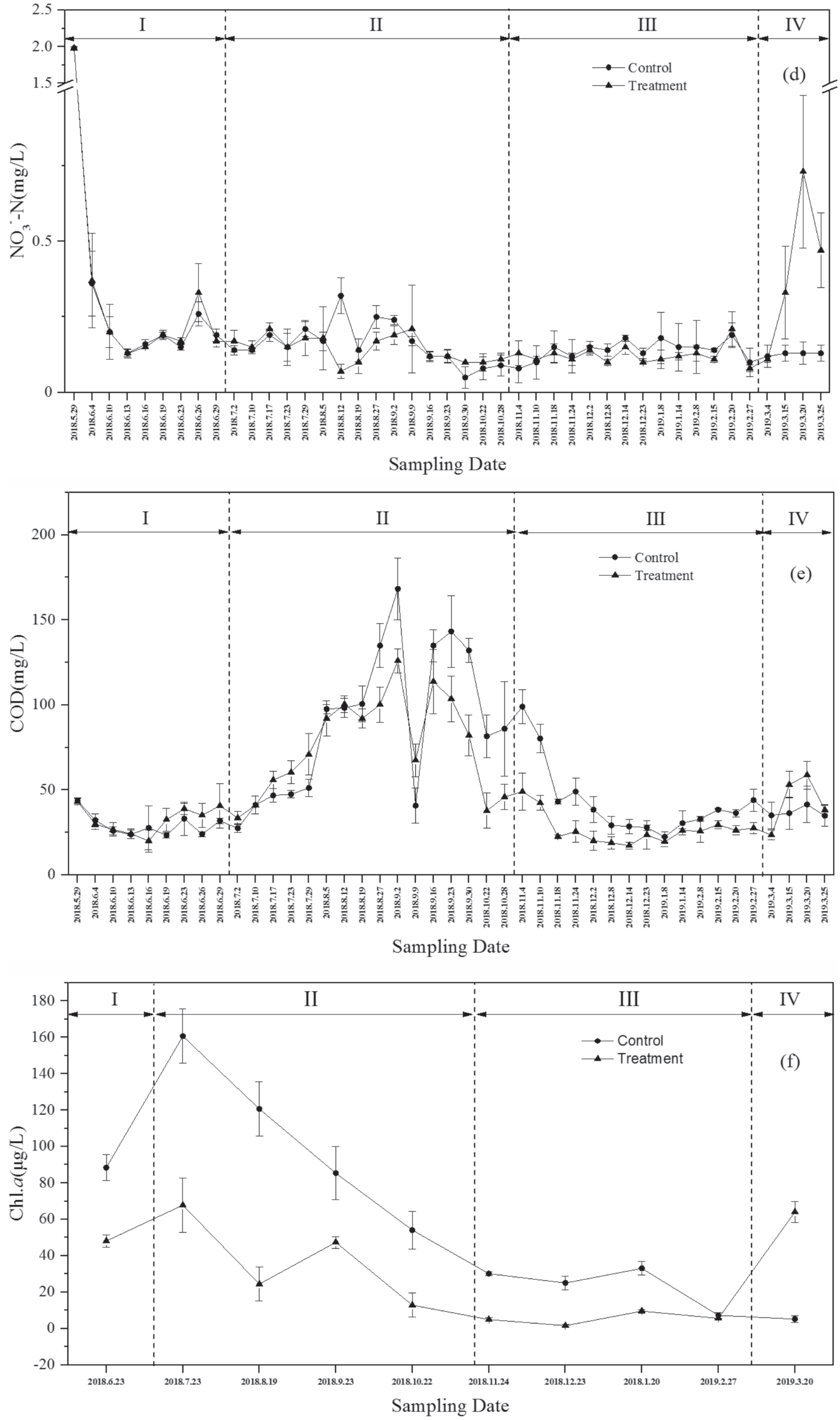

Fig.1. Continued.

Table 2. The results of One-way ANOVA on water variables at different growth stages.

Water parameters	Stage I	Stage II	Stage III	Stage IV
TN	0.593a	0.004b	0.000b	0.201a
TP	0.794a	0.129a	0.000b	0.855a
NH_4^+-N	0.939a	0.001b	0.314a	0.018b
NO_3^--N	0.978a	0.450a	0.179a	0.073a
COD	0.413a	0.328a	0.015b	0.453a
Chl.*a*	0.015b	0.000b	0.014b	0.000b
T	0.852a	0.936a	0.946a	0.989a
Cond	0.772a	0.000b	0.000b	0.000b
TDS	0.706a	0.000b	0.000b	0.001b
Sal	0.842a	0.000b	0.000b	0.000b
DO	0.638a	0.933a	0.005b	0.005b
pH	0.777a	0.034b	0.755b	0.000b

Values with different letters are significantly different, $P<0.05$.

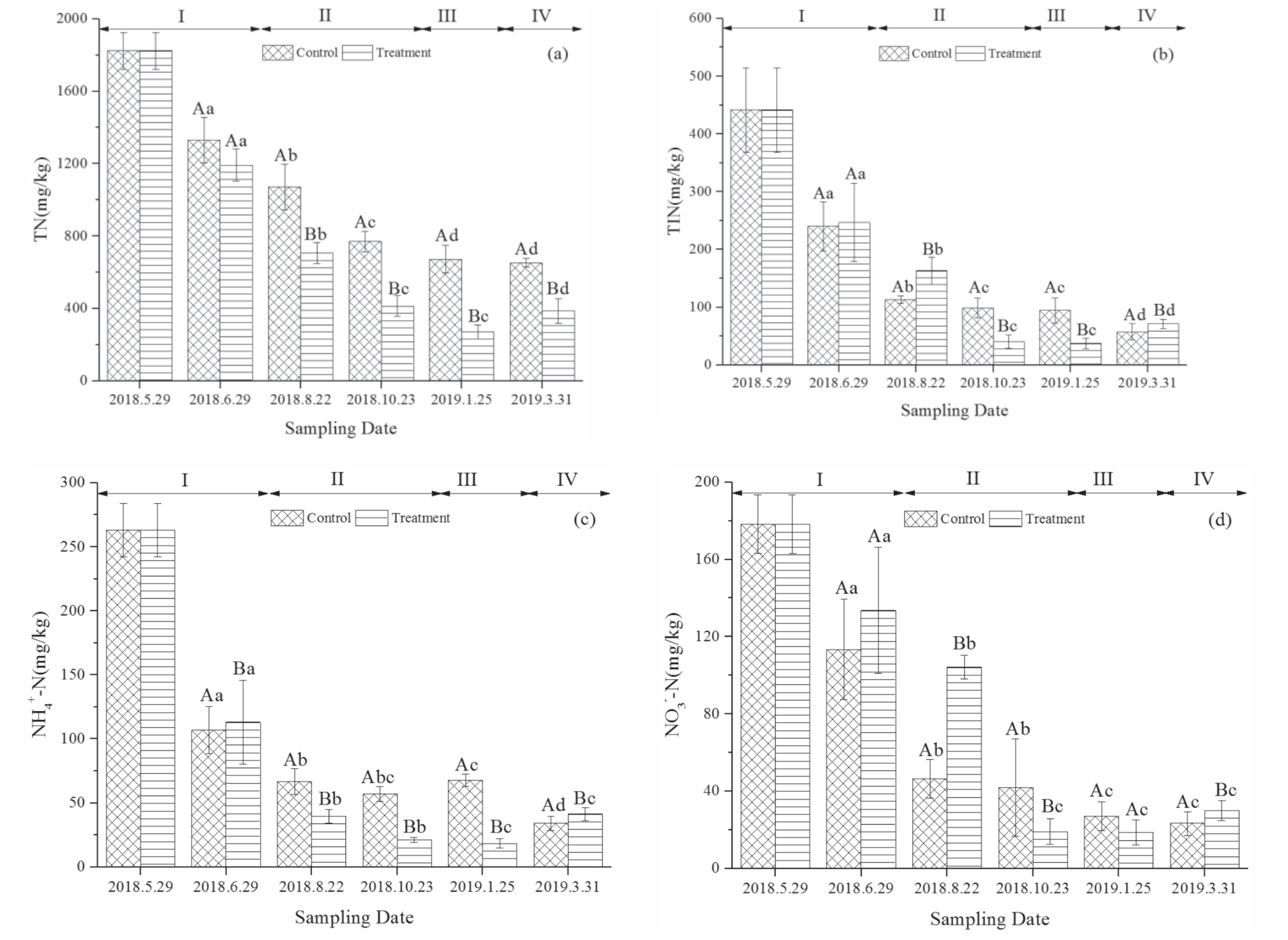

Fig. 2. Variations in the concentrations of TN, TIN, NH_4^+-N and NO_3-N in sediment between the control and treatment groups. Letters a-d and A-D indicate significant differences ($P<0.05$) among the different stages of each group and between the two groups at the same stage, respectively.

Table 3. Pearson's correlation coefficient between sediment denitrification rates (DNR) and the physiochemical parameters of water column and sediment.

Variables	Denitrification rate
	Correlation Coefficient
TN	0.525**
TP	0.632**
NO_3^--N	0.604**
NH_3-N	0.249
COD	0.275
Chl.*a*	0.577**
C/N	-0.317
T	0.900**
pH	0.136
TDS	-0.240
DO	-0.222
Cond	0.382*
TN (sediment)	0.844**
TIN (sediment)	0.906**
NO_3^--N (sediment)	0.900*
NH_4^+-N (sediment)	0.797**

*P<0.05;**P<0.01.

of sediment as a covering layer after withering [34], which could also reduce the release of phosphorus from sediment to water column. Moreover, the decrease in water COD may be caused by the withering and settlement of *V. spiralis* with the absorption of some organic particles from the water column.

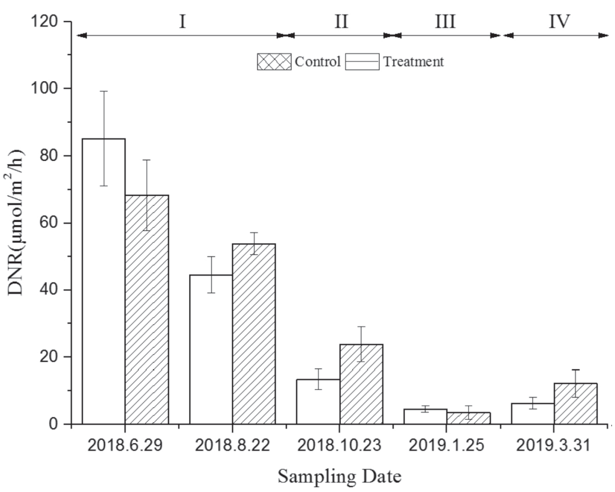

Fig. 3. Sediment denitrification rates at different stages in the treatment and control groups.

A previous study showed that the microbial activity was enhanced and the rate of decomposition of the plants increased with the increase in temperature at stage IV [35]. The process of decomposition caused the sediment-water interface to be anoxic and released a large amount of nitrogen and phosphorus into the water column, leading to the deterioration in water quality [36]. In this study, NH_4^+-N and Chl.*a* in the treatment group increased significantly, but there was no significant difference in the other indicators of water quality. The residues of *V. spiralis* released various forms of nitrogen, leading to the increases in TN, NH_4^+-N and NO_3^--N in the water column in treatment group. In contrast, decomposition produced an anaerobic environment that increased denitrification and the removal of NO_3^--N and TN, which may be the reason why there was no significant difference for NO_3^--N and TN between the two groups. The decomposition also led to the increase in Chl.*a*, which was consistent with the results of previous studies [20].

Effects of *V. spiralis* on Nitrogen Forms and Denitrification of Sediment

All forms of nitrogen in sediment of the treatment and control groups tended to decrease in this study, and the effects of *V. spiralis* on sediment nitrogen forms primarily occurred from stages II to IV. At stage I, the sediment TN in treatment group was lower than that in the control group, and a large amount of nitrogen in sediment and water was absorbed and assimilated by *V. spiralis* in this stage [20]. However, NH_4^+-N and NO_3^--N in the treatment group were higher than those in the control group, possibly owing to the secretion of oxygen by plant roots [37]. This factor was conducive to the mineralization of organic nitrogen in sediment to produce NH_4^+-N, and also accelerated the transformation of NH_4^+-N to NO_3^--N through nitrification, leading to higher contents of NH_4^+-N and NO_3^--N in the treatment group. Compared with the sediment NO_3^--N, the decrease rate of NH_4^+-N in the treatment group was obvious higher, indicating that *V. spiralis* was beneficial to the transformation of NH_4^+-N, which could be accelerated by ammonia oxidation or plant absorption [38].

Various forms of nitrogen in sediment decreased further during stage II. Some of these forms were transformed into N_2, N_2O, or other gases through denitrification, and others were transferred to the water column or assimilated by *V. spiralis*, resulting in an increase in nitrogen in the water column [39]. In this stage, the plants continued to absorb TIN, especially NH_4^+-N from the water column [40]. The existence of NH_4^+-N could also inhibit the absorption of NO_3^--N [41]. The plants used more NO_3^-N instead with the decrease in sediment NH_4^+-N. Therefore, sediment NH_4^+-N in the treatment group was significantly lower than that in the control group, while NO_3^--N in the treatment group was higher in August and lower in October than control group.

Sediment TN in two groups continued to decrease during stage III, significantly more slowly than in stage II. The possible reason maybe that microbial activity and amount of denitrification bacteria decreased [32]. While *V. spiralis* slowly released nutrients into the water, so NH_4^+-N in water column increased at this stage.

When *V. spiralis* decomposed, nitrogen would first deposit into sediment and then be released into the water column during stage IV [39], leading to the increase of nitrogen in both sediment and water, especially NH_4^+-N. Decomposition consumed a large amount of dissolved oxygen, resulting in an anoxic or anaerobic state, which was conducive to denitrification [42], so that sediment TN in the treatment group decreased.

The denitrification process was affected by temperature, dissolved oxygen, nitrogen, organic matter [43]. Submerged plants influenced the denitrification process by providing organic carbon and changing the concentrations of dissolved oxygen [44]. In this study, a correlation analysis showed that DNR was primarily related to TN, TIN, NH_4^+-N, NO_3^--N in the sediment and TN, TP, NO_3^--N, Chl.*a*, T, Cond in the water column (P<0.05). Among them, sediment NO_3^--N correlated significantly with denitrification, which was consistent with previous studies [45-46]. Yao et al. [47] showed that NH_4^+-N in sediment positively correlated with nitrification process. Zhong et al. [48] found that the DNR gradually increased from January to March in Taihu Lake, but this study found that the DNR increased only in the treatment group. The TIN in the sediment of the control group was too low and caused decreases in the DNR. However, for the treatment group, plant decomposition provided a substrate for denitrification, resulting in an increase in the DNR. It was apparent that the aquatic plant had a substantial impact on the denitrification process, and ignoring it in actual studies may lead to biased results.

Conclusions

(1) *V. spiralis* could improve the water quality of eutrophic shallow lake, and contribute to a significant decrease in TN in sediment.

(2) The effects of *V. spiralis* on water quality clearly differed during the four growth stages. The treatment group exhibited a lower concentration of Chl.*a* in stage I; and TN, NH_4+-N, Chl.*a* in stage II; and TN, TP, COD, Chl.*a* in stage III. Water quality deterioration was appeared for a short time in stage IV, especially the increases in NH_4+-N and Chl.*a*.

(3) The effect of *V. spiralis* on nitrogen forms in sediment primarily occurred from stages II to IV.

(4) *V. spiralis* had a clear effect on the sediment denitrification process in its life cycle, and the denitrification experiments showed that the DNR was reduced in stage I, increased in stages II and IV, and was barely affected in stage III.

Acknowledgements

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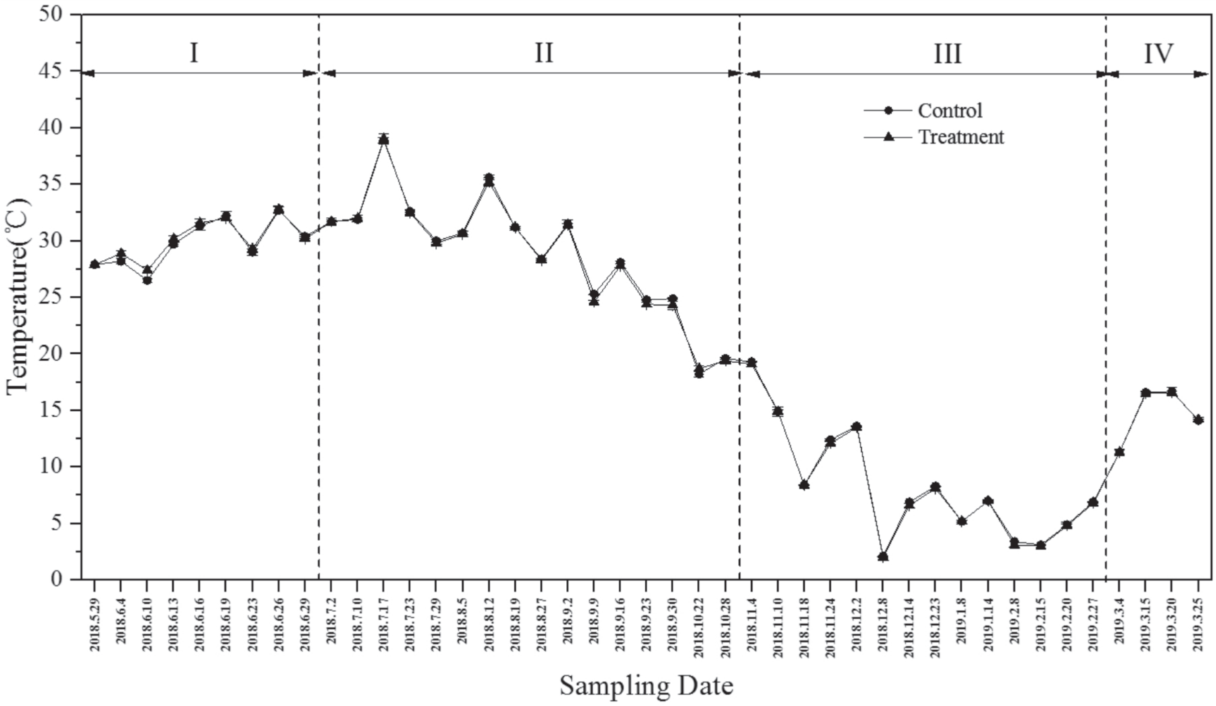

Fig. S1. Temporal variations of water temperature in the treatment and control groups. All the values are means ± SD (n = 3).

Conflict of Interest

The authors declare no conflict of interest.

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