# The role of submerged macrophytes in the regulation of internal nitrogen and phosphorus release

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## ABSTRACT

Submerged macrophytes play pivotal roles in the regulation of nitrogen (N) and phosphorus (P) release at the water-sediment interface, however, it remains unclear internal nitrogen and phosphorus release during the growth of submerged macrophytes. This study aims to have a better understanding of N and P release characteristics and their affecting factors during the growth of submerged macrophytes in an ecological microcosm experiment. The results showed that the average N and P release rates at the water-sediment interface in different treatments were negative and their absolute values ranked with the order of V treatment (treatment with *Vallisneria natans*) > M treatment (treatment with *Myriophyllum verticillatum*) > control (without planting submerged macrophytes). Results in this study are useful to understand internal N and P release under the regulation of submerged macrophytes, and provide a theoretical basis for the prevention and control of the internal pollution.

*Keywords*: nitrogen; phosphorus; release; submerged macrophytes; water-sediment interface; ecological microcosm

## 1. INTRODUCTION

As a global environmental issue, eutrophication has been recognized as the most common and severe environmental hazard to water ecosystems (Gao et al., 2017). It is a water degradation process caused by the excessive external input of nitrogen (N) and phosphorus (P) from agricultural run-off, untreated industrial and urban discharges, and ultimately most of them deposited in sediment as an internal source (Ni et al., 2017). In aquatic systems, excessive N and P often leads to rapid production of phytoplankton thus deteriorates the water quality (McGlathery et al., 2007). Reducing the external N and P loading could not always render a prompt recovery from eutrophication (Carey and Rydin, 2011), as in some cases, N and P stored in sediment as the internal source would become main sources for eutrophication (Bai et al., 2012). Such delayed responses typically stem from the positive feedback between internal N and P release, which highlights the importance of internal N and P releasing processes at the water-sediment interface.

Sediment is vital in determining the concentration and vertical transport of N and P (Hou et al., 2013). Internal cycling involves the movement of N and P into or out of the sediment as a result of biological, physical and chemical processes that are affected by factors including temperature, redox conditions, pH, dissolved oxygen, sediment resuspension and benthic biological community (Wilson et al., 2010). Nitrogen and phosphorus deposition may be remineralized as inorganic N and P and released to pore water, which can flux out into overlying water.

Nitrogen and phosphorus can be released from sediment through a variety of mechanisms (Ji et al., 2015). Among several possible processes, N and P release at the water-sediment interface in the regulation of submerged macrophytes is important. Submerged macrophytes play a critical role in the cycling, transfer and preservation of N and P in the ecosystem (Li et al., 2014). Previous studies have provided some perspectives for N and P release at the water-sediment interface affected by submerged macrophytes in some water environments. Riggsbee et al. ( 2012) suggested that vegetation may play an important long-term role in controlling the release of N and P from a dewatered reservoir. Hou et al. ( 2013) indicated that the abilities of submerged macrophytes assimilating $NH_4$-N and absorbing P from sediment were obvious, and submerged macrophytes can effectively inhibited the N and P release from sediment. Submerged macrophytes can directly assimilate N and P in sediment, promote depositing of N and P in overlying water, or indirectly inhibit the release of N and P in sediment by changing the physical and chemical conditions of water-sediment interface (Lu et al., 2018). Therefore, submerged macrophytes has the complex regulation on N and P release at the water-sediment interface, and further research is needed to clarify how N and P release from sediment affected by submerged macrophytes.

In this study, we aim to have a better understanding of sediment N and P release under the growth of submerged macrophytes. In our ecological microcosm experiment, two commonly submerged macrophytes, Vallisneria natans and Myriophyllum verticillatum, were separately planted in glass tanks. Nitrogen and phosphorus release had been studied using sediment and overlying water obtained in the Xinghu Lake in Wuhan University. Temporal variations of N and P in overlying water and sediment were recorded in the ecological microcosm experiment. The release rates of N and P influenced by submerged macrophytes were obtained.

## 2. MATERIALS AND METHODS

### 2.1 Microcosms and Experimental Design

To have a better understanding of N and P release processes under the growth of submerged macrophytes, N and P release simulation was conducted in microcosms. Two commonly used submerged macrophytes in eutrophicated water purification in China, V. natans and M. verticillatum, were selected as the experimental plants (Wu et al., 2017). Sediment and overlying water used in the ecological microcosm experiment were collected from Xinghu Lake in Wuhan University, China (30°31'47″N, 114°21'10″E).

Filling 72 L 1.2-cm-thick glass tanks with 20-cm-thick sediment. Black cloths were wrapped around the glass tanks to exclude light from the tanks' sides. The collected water was fully mixed and slowly added to the glass tanks until the water depth reached 35 cm, respectively. Leave the microcosms for 48 hours to be stabilized before planting. Before transplanted into glass tanks, plants of these two submerged macrophytes were washed carefully with water to remove impurities and then transplanted in glass tanks, separately. The average height of these submerged macrophytes was about 20 cm. The ecological microcosm experiment was conducted with and without submerged macrophytes. Three treatments were designed which included the control (without planting submerged macrophytes), V treatment (treatment with V. natans) and M treatment (treatment with M. verticillatum). Each treatment had three replicates.

To simulate outdoor temperature, the ecological microcosm experiment was conducted under laboratory conditions without any artificial heat preservation. Glass tanks were placed in the greenhouse of the College of Resources and Environmental Science, Wuhan University, China.

### 2.2 Sample Collection and Analysis

Water samples were collected at intervals of 5 cm, 15 cm and 30 cm at equal volume (10 mL). The water samples were taken back to the laboratory for measurements. Total nitrogen (TN) and total phosphorus (TP) in overlying water were measured three times with a FLOWSYS continuous flow analyzer (Systea Inc., ITA). Once samples were collected, pH, water temperature (T), dissolved oxygen (DO) and oxidation-reduction potential (ORP) were measured immediately with a YSI EXO2 Multiparameter Water Quality Sonde (YSI Inc., USA).

In order to prevent the sediment disturbance, sediment samples were collected by a sediment core sampler with 2-cm-diameter cross section. After air-dried at room temperature, the sediment samples were separated from the plant roots and fully mixed after 80 meshes sieving. Polyethylene bags were used to store sediment samples. TN concentrations in sediment were analyzed by automatic Kjeldahl analyzer (Shanghai Fiber Inspection Instrument Co. Ltd., KDN-103F) after digesting samples with concentrated sulfuric acid/Kjeldahl catalyst (sodium sulfate and selenium) on a heating block. TP concentrations in sediment were measured by ultraviolet and visible spectrophotometer (Shanghai Unocal Instrument Co. Ltd., UV-4802) after digesting samples through sodium hydroxide fusion method (Wu et al., 2017). TN or TP concentrations in sediment were expressed as N or P mass per g dry sediment, respectively.

The average height of V. natan and M. verticillatum was measured by tape when water and sediment sampled. In order to avoid disturbing the sediment, we didn't measure the biomass of submerged macrophytes at each sampling time.

### 2.3 Nitrogen and Phosphorus Release Rates Calculations

N and P release rates ($R_N$ and $R_P$) at the water-sediment interface can be calculated by the equation Stephen et al., 1997):

$$\text{release rate } (\mathrm{mg \cdot m^{-2} \cdot d^{-1}}) = \frac{C * H * 1000}{i} \quad (1)$$

where C is N or P concentration differences in overlying water during the ecological microcosm experiment, mg/L; H is the height of overlying water, m; i is the ecological microcosm experimental time, d.

### 2.4 Statistical Analysis

Most data shown in this present study are given as means with an associated standard deviation of three replicates. The significance of differences in the factors of different treatments was tested by one-way ANOVA. Significance was accepted when $P < 0.05$. Spearman correlation analysis (2-tailed test) was used to analyze the relationship between RN and RP and the affecting factors in different treatments. Differences were considered as significant at $P < 0.05$ and extremely significant at $P < 0.01$. The major factors affecting RN and RP were selected by the principal component analysis method. All statistical analysis made in the study was performed using IBM SPSS 21.0 software.

## 3. RESULTS AND DISCUSSION

### 3.1 Submerged Macrophytes Growth

Temporal variations of the average height of the two submerged macrophytes during the ecological microcosm experiment were shown in Figure 1. The results of one-way ANOVA test showed that the differences of the average height between V. natans and M. verticillatum were not significant ($P > 0.05$). The average height of V. natans in the V treatment ranged between 18.2 cm and 48.4 cm, and that in the M treatment ranged between 19.2 cm and 62.8 cm. The average growth rate of M. verticillatum was 1.29 cm/d, larger than that of V. natans (0.86 cm/d). M. verticillatum had a relatively better growth than V. natans in this ecological microcosm experiment, which is in accordance with the results reported by Duan et al. (2011).

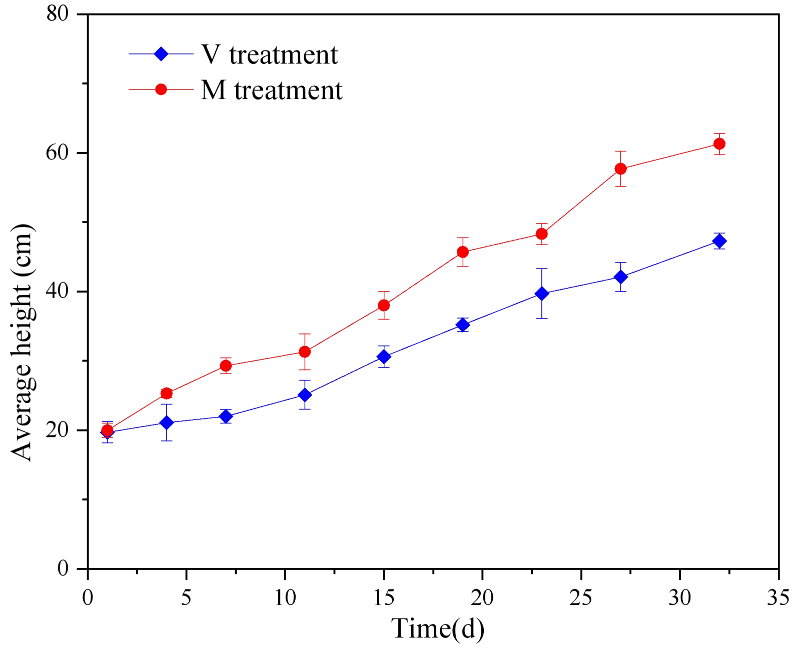


Figure 1. Temporal variations of the average height of submerged macrophytes during the ecological microcosm experiment (mean ± SD, n=3). V treatment represents treatment with V. natans; M treatment represents treatment with M. verticillatum.

### 3.2 Environmental indicators in Overlying Water under the Effect of Submerged Macrophytes

Temporal variations of pH, ORP, DO and T in overlying water in different treatments were shown in Figure 2. pH and DO were significantly influenced by the treatments ($P < 0.05$), while ORP showed no significant differences. The pH values in the control were higher than that in the V and M treatments at each sampling time. Generally, the pH values in the three treatments showed an upward trend. The average ORP values in the control were lower than that in the V and M treatments. There was no significant difference between the ORP values in the V and M treatments. DO concentrations in the control were lower than that in the V and M treatments at each sampling time. DO concentrations in the V treatment were higher than values in the M treatment at each sampling time. DO concentrations peaked at the end of the ecological microcosm experiment in the V and M treatments. Water temperature in both treatments had neither significant difference with each other nor to the outdoor temperature.

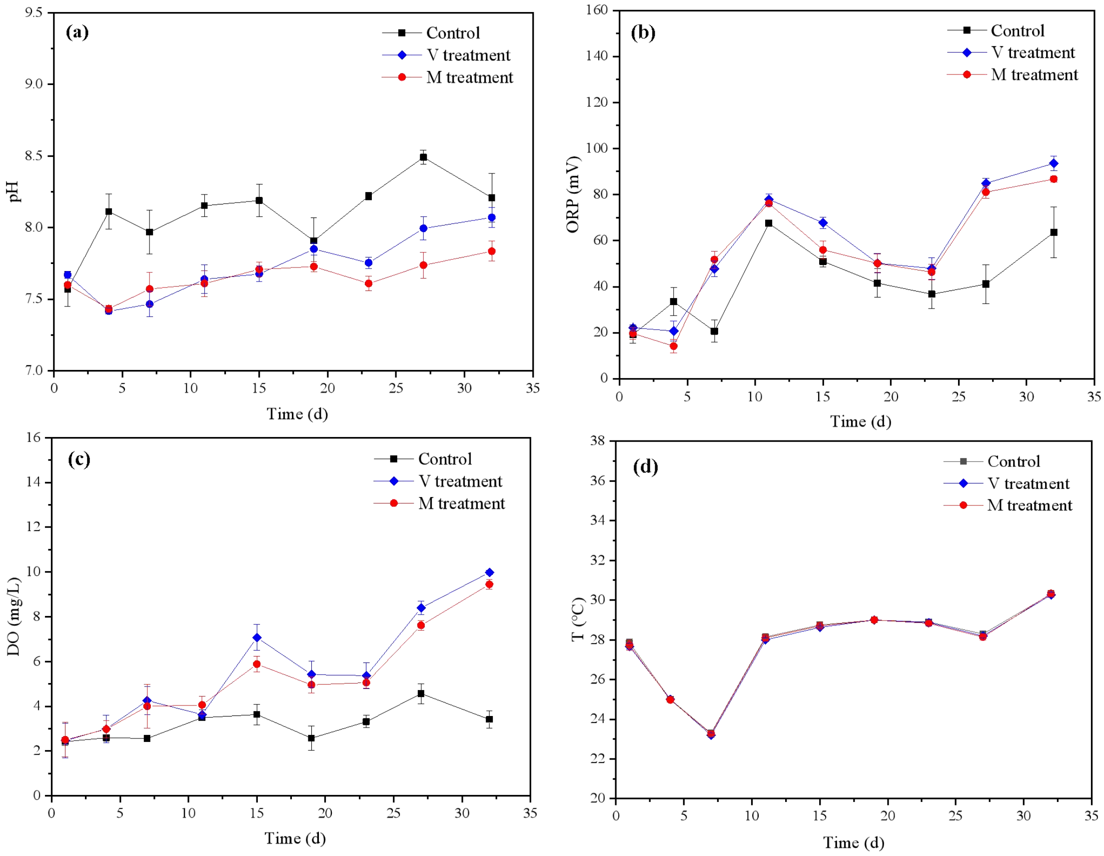


Figure 2. Temporal variations of (a) pH, (b) oxidation-reduction potential (ORP), (c) dissolved oxygen (DO) and (d) water temperature (T) in overlying water during the ecological microcosm experiment (mean ± SD, n=3). Control represents treatment without planting submerged macrophytes; V treatment represents treatment with V. natans; M treatment represents treatment with M. verticillatum.

## 3.3 Nitrogen and Phosphorus Release under the Effect of Submerged Macrophytes

### *3.3.1 Nitrogen and Phosphorus in Overlying Water*

Temporal variations of TN and TP concentrations in overlying water (TNw and TPw) in different treatments were shown in Figure 3. TNw were significantly influenced by the treatments ($P < 0.05$), while TPw showed no significant differences. In the first 4 days, TNw in different treatments gradually increased, and then varied in a wave pattern. TNw in the control were higher than that in the V and M treatments. TNw in the V treatment at the end of the ecological microcosm experiment were lower than that in the M treatment. TNw was reduced at the presence of V. natans and M. verticillatum. TPw in the control fluctuated, and that in the V and M treatments increased in the first 4 days and then decreased rapidly. TPw were significantly reduced at the effect of submerged macrophytes.

N and P concentrations in overlying water in V and M treatments changed across time course, which was probably related to N and P release from sediment and assimilation of submerged macrophytes. In the early stage of the ecological microcosm experiment (the first 4 days), N and P were released from sediment to overlying water, and submerged macrophytes barely grew. Thus, TNw and TPw increased initially at the early stage, which possibly due to N and P released from sediment. After a certain time, submerged macrophytes grew fast and started to uptake a large amount of N and P in both overlying water and sediment. At the same time, N and P deposition in overlying water would occur due to the reduction of N and P in sediment, resulting in the gradual decrease of N and P concentrations in overlying water.

Considering the whole experimental period, the N:P (the ratio of TN concentrations and TP concentrations in overlying water) varied from 17.9 to 19.5 in the control, from 18.6 to 48.2 in the V treatment, and from 19.4 to 75.4 in the M treatment. Submerged macrophytes have a strong enhancive impact on the N:P ratio of overlying water. Due to the differential effect of macrophytes on sediment N and P, N:P ratio of overlying water during the growth of submerged macrophytes is considerably higher. An increased N:P ratio in the remaining Cabomba and Hydrilla litter with longer rewetting periods was also found by Lu et al. (2018).

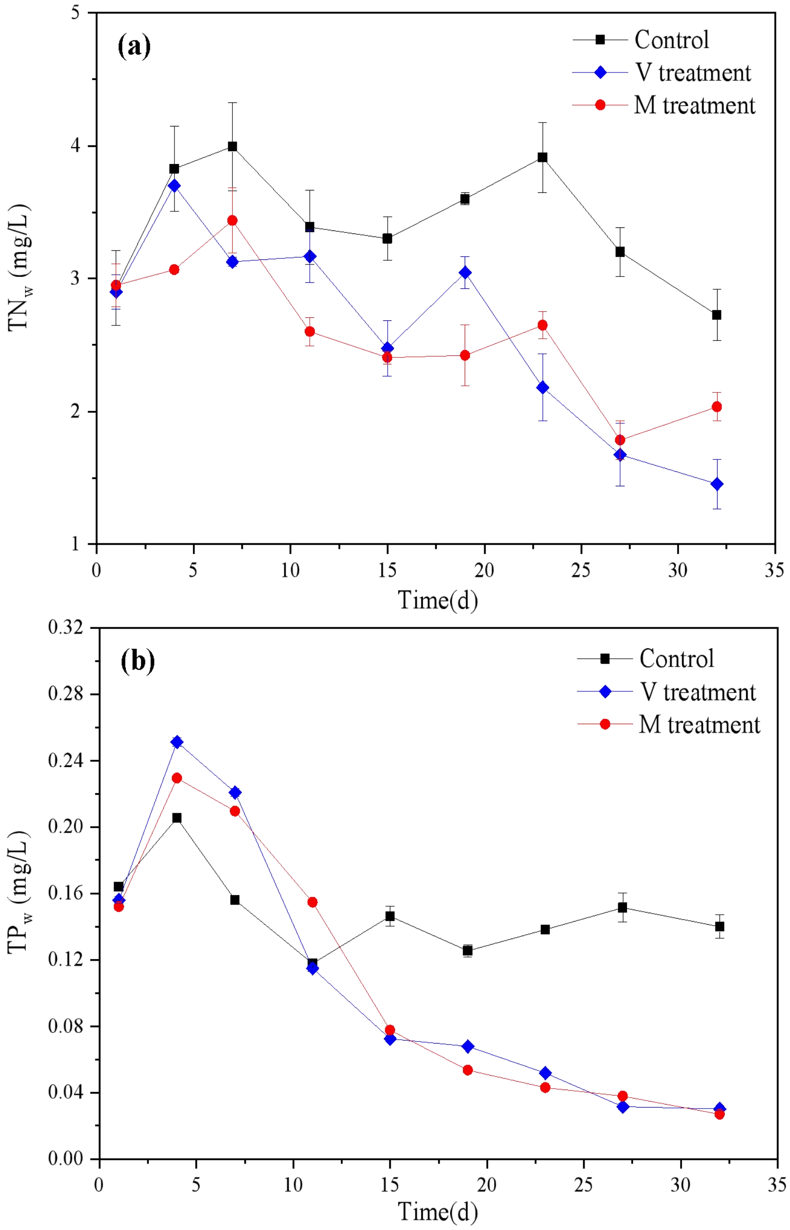


Figure 3. Temporal variations of (a) total nitrogen concentrations in overlying water (TNw) and (b) total phosphorus concentrations in overlying water (TPw) during the ecological microcosm experiment (mean ± SD, n=3).

### *3.3.2 Nitrogen and Phosphorus in Sediment*

Temporal variations of N and P concentrations in sediment (TNs and TPs) in different treatments were shown in Figure 4. TNs and TPs were significantly influenced by the treatments ($P < 0.05$). TNs and TPs were reduced with the presence of V. natans and M. verticillatum, whose values were arranged as M treatment < V treatment < control. TNs and TPs in the V and M treatments had a gradually decreased trends over time. The reduction of TNs and TPs in the V treatment was 2.83 mg N/g and 0.41 mg P/g, accounting for 17.2 % and 30.4 % of the initial amounts, respectively. Compared to the initial amounts, TNs and TPs in the M treatment were 21.8% (3.61 mg N/g ) and 38.8% (0.52 mg P/g ) lower, respectively.

Submerged macrophytes can uptake and excrete N and P by roots and shoots (Diepens et al., 2014), whereas the impact of healthy shoots during the growing season is usually insignificant (Chambers et al., 1989). Wigand et al. (2001) revealed that porewater nutrients were depleted by the plant roots demand in the lab experiments. Gudimov et al. (2015) also showed submerged macrophytes could obtain P both from water column and sediment, but nutrient uptake from sediment dominates under normal P concentrations. Chambers et al. (1989) demonstrated that nutrient uptake by aquatic macrophytes is largely affected by the roots. Thus, N and P uptake by roots of V. natans and M. verticillatum is responsible for loss of N and P from the surficial sediment in the ecological microcosm experiment.

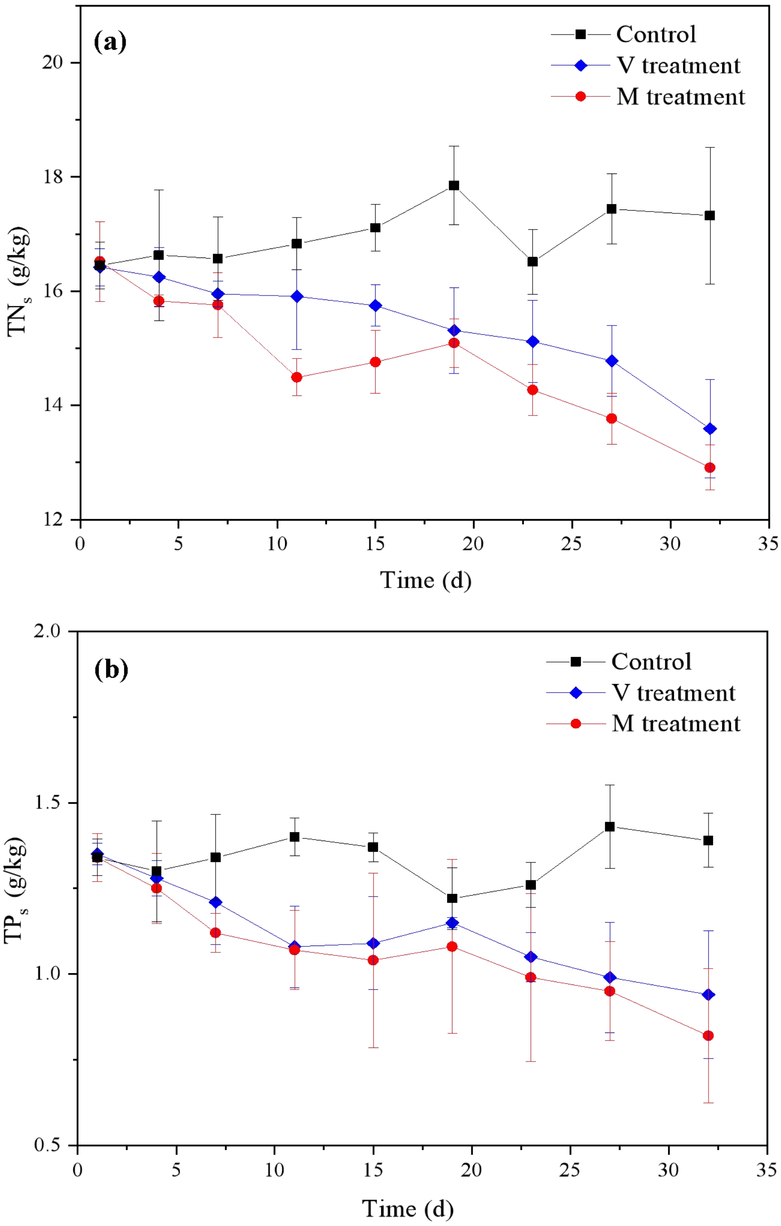


Figure 4. Temporal variations of (a) total nitrogen concentrations in sediment (TNs) and (b) total phosphorus concentrations in sediment (TPs) during the ecological microcosm experiment (mean ± SD, n=3).

### *3.3.3 Nitrogen and Phosphorus Release Rates at the Water-Sediment Interface*

N and P release rates at the water-sediment interface ($R_N$ and $R_P$) in different treatments were shown in Table 1. The average release rates of TN and TP at the water-sediment interface in the three treatments were negative indicating the N and P deposition from overlying water to sediment is predominant during the ecological microcosm experiment. The absolute values of $R_N$ and $R_P$ in the V and M treatments were obviously larger than that in the control, and arranged in the order of V treatment > M treatment > control. Thus, V. natans and M. verticillatum which are mainly wetland plants can accelerate the deposition of N and P in overlying water, and have differences in controlling internal N and P release. Sediment with and without submerged macrophytes has different microecology as that influence N and P release in different ways. N and P retention and reduction in sediment may be promoted by submerged macrophyte growth. N and P lost from sediment without submerged macrophytes may be larger than that from sediment with submerged macrophytes.

**Table 1.** The average N and P release rates at the water-sediment interface during the ecological microcosm experiment.

| Treatment | Variable | Average release rate ($mg \cdot m^{-2} \cdot d^{-1}$) |
|---|---|---|
| Control | $R_N$ | -2.25 |
| | $R_P$ | -0.26 |
| V treatment | $R_N$ | -15.81 |
| | $R_P$ | -1.38 |
| M treatment | $R_N$ | -10.02 |
| | $R_P$ | -1.37 |

## 4. CONCLUSIONS

In this study, N and P internal release into overlying water under the growth of submerged macrophytes were simulated in ecological microcosms. Results indicated that M. verticillatum had a relatively larger average growth rate than V. natans. After planting submerged macrophytes, the ORP values and DO concentrations in overlying water increased, while pH decreased. TN and TP concentrations in overlying water and sediment decreased under the effect of V. natans and M. verticillatum. The study revealed that the decreased release rates of N and P would be presented under the growing of submerged macrophytes. The $R_N$ and $R_P$ in all treatments were negative, and the descending order of their absolute values was V treatment, M treatment and control. Interactions between macrophytes and environmental conditions are in non-linear relationships, and modelling the interactions is important for the understanding of feedback dynamics and ecosystem resilience. In the future, predictive models will be developed based on the results of this present study to simulate internal N and P flux affected by macrophytes in lakes.


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