Sediments nitrogen cycling influenced by submerged macrophytes growing in winter
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ABSTRACT
Restoration of submerged macrophytes is one of the important measures for ecological treatment of eutrophic lakes. The changes in physical and chemical conditions caused by submerged macrophytes also affect the process of benthic nitrogen cycling. The growth period of *Potamogeton crispus* is mainly in winter. In order to understand the effect of submerged macrophytes growing in winter on nitrification rate and denitrification rate in the process of nitrogen cycling, experiments were carried out from winter to summer with vegetated and non-vegetated treatments. The results showed that the effect of submerged macrophytes on water temperature was not significant in winter. The nitrogen cycling was mainly affected by variables, which were inorganic nitrogen and dissolved oxygen. Submerged macrophytes had little effect on nitrification rate, but had a certain inhibition on denitrification rate by providing oxygen from photosynthesis. In total, submerged macrophytes growing in winter have little effect on nitrogen cycling in sediment. However, submerged macrophytes growing in winter can increase the attachment surface of microbes and inhibit resuspension of sediment, which play a complementary role to submerged macrophytes growing in summer for maintaining stability of eutrophic lakes.

Key words | denitrification, nitrification, submerged macrophytes, winter

HIGHLIGHTS
- In the situation of winter, intensity of nitrogen cycling in sediment is mainly affected by environmental variables, like inorganic nitrogen and DO.
- Environmental variables affected by submerged macrophytes growing in winter showed similar attributes with those growing in summer.
- Temperature had strong effect on nitrogen cycling, as different seasons had different rates of denitrification and nitrification.

INTRODUCTION
It is a great challenge for researchers and managers to control eutrophication so far. The key variables causing water eutrophication are excessive nutrients, such as nitrogen and phosphorus (Paerl & Otten 2013). The deposition of phosphorus increases the internal nutrient load of sediment and water, while nitrogen can be released into atmosphere as gas through nitrogen cycling. Eutrophic lakes caused by nitrogen pollution in China increased from 40% to 85%, from 1980 to 2005 (Liu Zhang & Liu 2010). Different forms of nitrogen, such as nitrate (N-NO₃⁻), ammonium (N-NH₄⁺) or organic nitrogen, can cause higher biomass of cyanobacteria and concentration of microcystins compared with phosphorus under certain conditions (Chaffin et al. 2018). Microcystins are concerned not only in spring and summer, but also in winter when there is less bloom in lakes. A survey found that the maximum value of soluble microcystins in water samples of Lake Furnas was 154.5 µg/L during the

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bloom in winter of 2009 (Santos et al. 2012). Nitrogen cycling in sediment is one of the important pathways to nitrogen removal in freshwater lakes, it can transform inorganic nitrogen to gas, and reduces water bloom and the concentration of microcystins. Therefore it is necessary to explore the impact variables that affect nitrogen cycling significantly, like dissolved oxygen (DO) (Yan et al. 2018), inorganic nitrogen (Holmroos et al. 2015), pH (Mi et al. 2008), and total organic carbon (TOC) (Wang et al. 2018), etc.

As a pathway of permanent removal of nitrogen loading from water, the nitrogen cycling process is directly or indirectly affected by submerged macrophytes (Veraart et al. 2011). Submerged macrophytes can promote nutrient cycling by the combined effects of nitrification and denitrification (Risgaard-Petersen & Jensen 1997; Boerema et al. 2014). Nitrification produces N-NO$_3^-$ under aerobic conditions, while denitrification requires anaerobic conditions, and depends highly on the transport of N-NO$_3^-$ from aerobic areas to anaerobic areas and a change in redox conditions in situ (Seitzinger et al. 2006). Aquatic ecosystems are affected by many variables in the field, such as mixing of water columns and the resuspension of sediment. Therefore, it is difficult to distinguish the changes caused by aquatic macrophytes. In order to accurately evaluate the role of submerged macrophytes in the regulation of nitrogen cycling in sediment, it is necessary to set up treatments in experimental devices to prevent water and sediment from migrating under the influence of wind.

The restoration of submerged macrophyte communities is beneficial to the ecological restoration of eutrophic aquatic ecosystems. Recovery of submerged macrophytes in eutrophic aquatic ecosystem, such as Myriophyllum spicatum, Ceratophyllum demersum, Potamogeton malaianus and so on, perform well in summer, and play an important role in preventing sediment from resuspending and providing microbial attachment surfaces. Yet these submerged macrophytes decayed in winter, which resulted in the increase in nitrogen, and may have a negative impact on the stability of macrophytes, especially those recovered in the early stage (Wang et al. 2018). Restoration of submerged macrophytes which sprout in winter, such as Potamogeton crispus and Elodea canadensis, may improve the colonizing conditions of submerged macrophyte communities in eutrophic aquatic ecosystems. In particular, it can improve the adverse effects of the decline of other submerged macrophytes in winter. Potamogeton crispus mainly grows in autumn and winter, and can form a complementary effect with submerged macrophytes growing in summer, and increase the absorption of inorganic nitrogen and dissolved oxygen, providing microorganism attachment in winter (Zhang et al. 2017). In cold climates, Potamogeton crispus with cold tolerance is a more effective and sustainable strategy for removal of organic matter and nitrogen in eutrophic water body (Fan et al. 2016). However, the removal efficiency of nitrogen loading caused by Potamogeton crispus in aquatic ecosystems needs to be further studied, as it may play an important role (Eriksson & Weisner 1997; Fan et al. 2016) or have no significant effect (Madsen & Adams 1988). At present, several researchers have discussed the performance of submerged macrophytes in nitrogen cycling in summer. Few papers have paid attention to submerged macrophytes such as Potamogeton crispus, which perform well in winter, and their performance in nitrogen cycling, such as oxygen supply for microbes, removal of nitrogen and phosphorus, is still unclear, and therefore needs further study (Forshay & Dodson 2011; Fan et al. 2016).

This experiment mainly discusses nitrogen cycling in the sediment and indexes affected by Potamogeton crispus in the water column. The effects of submerged macrophytes on nitrogen cycling in sediment are related to nitrogen removal rate and self-purification capacity of aquatic ecosystem (Forshay & Dodson 2011). The nitrogen cycling process in lake sediments is greatly influenced by water quality and sediment characteristics (Bruesewitz et al. 2011; Yao et al. 2016). The measurement of variables in water will be helpful to understand the nitrogen cycling in sediment. It is hypothesized that submerged macrophytes growing in winter play the role of changing the surrounding environmental variables, like inorganic nitrogen, DO, pH, TOC, total phosphorus (TP) and oxidation-reduction potential (ORP), etc. When other submerged macrophytes decline, submerged macrophytes growing in winter have a significant impact on the nitrogen cycling in sediment. This paper explored the effects of submerged macrophytes growing in winter on the nitrogen cycling process, providing a basis for recovering the role of submerged macrophytes growing in winter.

**MATERIALS AND METHODS**

**Experimental devices and operation mode**

The experimental devices (Figure 1) were built on November 22, 2013. The height of each experimental device is 70 cm, the top and bottom of diameter is 65 and 30 cm, respectively. Bared sediment was gained from West Lake in Hangzhou, China, which is an eutrophication lake in
the city. Homogeneous mixed sediment was tiled on the bottom of experimental devices with a thickness of 10 ± 2 cm thick. Then, 30 ± 2 cm depth of water was added to each device. The water was collected from a Water Supply Plant which is a water source of West Lake. On December 1, 2013, 12 seeds of Potamogeton crispus of similar size were planted in experimental devices numbers 1, 2 and 3. Experimental devices numbers 4, 5, 6 were set as the non-vegetated control. After the experimental devices were established, the initial water conditions were measured. In the vegetated devices, N-NH$_4^+$ was 2.62–2.95 mg/L, N-NO$_2^-$ was 0.0024–0.0038 mg/L, N-NO$_3^-$ was 1.24–1.40 mg/L, TN was 3.50–3.96 mg/L. In the non-vegetated devices, N-NH$_4^+$ was 2.68–3.75 mg/L, N-NO$_2^-$ was 0.0023–0.0024 mg/L, N-NO$_3^-$ was 1.24–1.32 mg/L, TN was 3.79–2.15 mg/L. The timely replenishment of water to keep the depth at 30 ± 2 cm was the daily management of these devices.

Sample collection

Samples of sediment and water were collected eight times on December 2, 2013, December 15, 2013, January 15, 2014, February 25, 2014, March 25, 2014, April 16, 2014, May 20, 2014 and June 28, 2014, then named initial, Dec, Jan, Feb, Mar, Apr, May and Jun, respectively. The winter of this study area was from December to February, spring was from March to May, samples for summer in this paper were only collected in June. The surface layer less than 10 cm of sediment in each device was collected by a 50 ml sampling bottle, and then stored at −20 °C for the latter detection of sediment samples. The water sample of each device was collected with a 500 ml sampling bottle under the water surface, stored at 4 °C, and then tested after 3 days.

Testing methods

Variables of overlying water

DO, temperature (T), pH, ORP, conductivity in overlying water were measured using an ORION 5-star multi-function analyzer, USA. Total nitrogen (TN), TP, ammonia (N-NH$_4^+$), nitrate (N-NO$_3^-$) and nitrite (N-NO$_2^-$) were measured by a standard Chinese method (Standard Method for the Examination of Water and Wastewater Editorial Board 2002).

Variables of sediment

Total organic carbon. Pretreatment: Sufficient concentrated hydrochloric acid was added into the wet sediment and weighing more than 100 g, then stirred until there were no bubbles, so that the organic matter in sample was fully ashed. The addition of concentrated hydrochloric acid depended on the content of organic matter in the wet soil. If there were bubbles in the wet soil when concentrated hydrochloric acid was added into the wet soil, this indicated that it still contained organic matter, so it was necessary to continue the addition of concentrated hydrochloric acid. The ashed samples were air dried for the next step.

The prepared samples with 29.00–32.00 mg were added to the total organic carbon analyzer (Elementar, vario TOC, Germany) for measurement.

Nitrification rate (N$r$). Sediment with 5 g was weighed in three 50 mL centrifugal tubes and mixed with 4 mL of 0.9 mM 2,4-dinitrophenol (2,4-DNP) solution, 1 mL of 25 mM KNO$_3$ solution and 5 mL of distilled water (control treatment was replaced by distilled water). Two of the centrifuge tubes were placed in a thermostat with 25 °C and the third one was placed at −20 °C for 24 h. Then 10 mL 4 M KCl was added into each tube and mixed evenly. Filter of 5 mL was added to 5 mL pH = 8.50 19 M ammonium chloride buffer and 1 mL color developer of nitrite, and then mixed evenly. Colorimetry at 520 nm was operated after 15 minutes (Abdelmagid & Tabatabai 1987).

Denitrification rate (D$e$). Sediment with 15 g was taken in 50 mL centrifugal tube and 40 mL of denitrification solution was added (KNO$_3$ 2.0 g, glucose 3.0 g, K$_2$HPO$_4$ 0.27 g, KH$_2$PO$_4$ 0.09 g in denitrification solution per liter, pH 7.5). The initial concentration of nitrate was 1.23 g/L. The tube was sealed up immediately after 3 minutes of nitrogen aeration. Samples were cultured at a constant temperature on a shaking bed at 28 °C, 150 rpm for 20 hours (using distilled water instead of sediment as control). After filtration, the supernatant was taken to measure concentration of TN. The difference in TN concentration

Figure 1 | Diagram of experimental devices. Vegetated represents the devices with Potamogeton crispus, while non-vegetated represents the devices without Potamogeton crispus.
between sample and control was the denitrification rate (Yu et al. 2008).

**Data analysis**

One-way analysis of variance (one-way ANOVA) was used to analyze the data of overlying water and sediment. Indexes of overlying water and sediment were performed in PAST3 for principal component analysis (PCA). Pearson’s correlation coefficients among variables were determined using SPSS.

**RESULTS**

**The physicochemical indexes of water and sediment in experimental devices**

As shown in Figure 2, the physicochemical indexes of water between vegetated and non-vegetated treatments showed significant differences after April, at which time *Potamogeton crispus* usually reached their highest biomass. Then summer followed and *Potamogeton crispus* decayed gradually, there was a convergence of the physicochemical indexes between vegetated and non-vegetated treatments. Indexes of ORP, DO and pH for vegetated treatments were higher than those of non-vegetated treatments, especially in April and May. Concentrations of DO in vegetated treatments were 3.16 mg/L and 2.31 mg/L higher than that of non-vegetated treatments in April and May respectively. In vegetated treatments, pH was 0.5–1.2 higher than that of non-vegetated treatments in April and May. While values of ORP in vegetated treatments were 61.53 and 27.00 mV higher than that of non-vegetated treatments in April and May respectively. Temperature and electric conductivity in non-vegetated treatments were higher than those in vegetated treatments, especially in April. Conductivity of non-vegetated treatments was 202.33 μs/cm higher than that of vegetated treatments in April, namely 1.7 times higher than that of vegetated treatments.

**Figure 2** | The physicochemical variables of water in experimental devices. The symbol ‘*’ in the figure represents the significant difference at $p < 0.05$. 

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Table 1 shows the variation in indexes in water and sediment from December to June. The difference between vegetated and non-vegetated treatments was analyzed for each index. Different forms of nitrogen showed a decreasing trend with concentrations from the beginning to the end of the experiment. TN and N-NH$_4^+$ levels were significantly different between vegetated and non-vegetated treatments since March, while N-NO$_2$ and TP were significantly different between the two treatments from December. The concentration of TP was generally low, ranging from 0.02 to 0.15 mg/L. TOC and Nr were significantly different between vegetated and non-vegetated treatments in May and April, respectively. The content of TOC in the experimental devices ranged from 6.1% to 10.5%. There was no significant difference between vegetated and non-vegetated treatments in denitrification intensity in different months. The De in vegetated treatments ranged from 0.01–1.65 μg g$^{-1}$ d$^{-1}$, the De in non-vegetated treatments ranged from 0.10–2.01 μg g$^{-1}$ d$^{-1}$.

**PCA and Pearson’s correlation coefficients among variables**

As shown in Figure 3, N-NH$_4^+$, N-NO$_3^-$, TN, TP, TOC, De and Nr were compared between vegetated and non-vegetated treatments. N-NH$_4^+$, N-NO$_3^-$, TN and TP had significant differences between vegetated and non-vegetated treatments. The larger difference between vegetated and non-vegetated treatments was revealed in index of De. Meanwhile, there had little different between vegetated and non-vegetated treatments with indexes of TOC and Nr.

Table 2 showed that De of both vegetated and non-vegetated treatments had extremely significant

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**Table 1 | Physicochemical variables of the water and sediment in experimental devices**

| Sampling time | N-NH$_4^+$ (mg L$^{-1}$) | N-NO$_2$ (mg L$^{-1}$) | N-NO$_3^-$ (mg L$^{-1}$) | TN (mg L$^{-1}$) |
|---------------|--------------------------|------------------------|--------------------------|-----------------|
|               | Vegetated | Non-vegetated | Vegetated | Non-vegetated | Vegetated | Non-vegetated | Vegetated | Non-vegetated |
| Initial       | 2.64 ± 0.03 | 3.74 ± 0.40 | 0.0034 ± 0.0004 | 0.0024 ± 0.0001 | 1.34 ± 0.09 | 1.29 ± 0.04 | 3.70 ± 0.23 | 4.59 ± 0.72 |
| Dec           | 0.54 ± 0.08 | 1.67 ± 0.25 | 0.0038 ± 0.0004 | 0.0053 ± 0.0005 | 1.73 ± 0.26 | 0.41 ± 0.26 | 2.76 ± 0.17 | 3.47 ± 0.46 |
| Jan           | 0.30 ± 0.02 | 0.34 ± 0.03 | 0.0020 ± 0.0000 | 0.0020 ± 0.0000 | 0.30 ± 0.18 | 1.19 ± 0.04 | 0.58 ± 0.03 | 1.47 ± 0.50 |
| Feb           | 0.44 ± 0.06 | 0.67 ± 0.07 | 0.0010 ± 0.0000 | 0.0010 ± 0.0000 | 0.31 ± 0.07 | 0.61 ± 0.25 | 0.58 ± 0.13 | 0.92 ± 0.35 |
| Mar           | 0.27 ± 0.02 | 0.24 ± 0.01 | 0.0001 ± 0.0000 | 0.0003 ± 0.0003 | 1.05 ± 0.11 | 0.38 ± 0.02 | 0.95 ± 0.08 | 0.34 ± 0.01 |
| Apr           | 0.31 ± 0.03 | 0.36 ± 0.02 | 0.0001 ± 0.0000 | 0.0023 ± 0.0006 | 0.18 ± 0.02 | 0.29 ± 0.18 | 1.00 ± 0.08 | 1.23 ± 0.24 |
| May           | 0.05 ± 0.01 | 0.04 ± 0.01 | 0.0001 ± 0.0000 | 0.0031 ± 0.0000 | 0.43 ± 0.01 | 0.95 ± 0.24 | 0.74 ± 0.10 | 1.31 ± 0.34 |
| Jun           | 0.18 ± 0.01 | 0.29 ± 0.01 | 0.0001 ± 0.0000 | 0.0001 ± 0.0000 | 0.08 ± 0.01 | 0.10 ± 0.01 | 0.94 ± 0.29 | 0.70 ± 0.18 |

**Table 2 | Denitrification intensity**

| Sampling time | TP (mg L$^{-1}$) | TOC (%) | Nr (μg g$^{-1}$ d$^{-1}$) | Denitrification intensity (μg g$^{-1}$ d$^{-1}$) |
|---------------|-----------------|--------|--------------------------|---------------------------------------------|
|               | Vegetated | Non-vegetated | Vegetated | Non-vegetated | Vegetated | Non-vegetated | Vegetated | Non-vegetated |
| Initial       | 0.12 ± 0.03 | 0.08 ± 0.02 | 10.0 ± 0.3 | 8.5 ± 1.7 | 16.92 ± 1.08 | 18.32 ± 0.36 | 1.65 ± 0.10 | 1.90 ± 0.09 |
| Dec           | 0.08 ± 0.01 | 0.11 ± 0.01 | 8.5 ± 0.9 | 8.1 ± 1.7 | 16.35 ± 3.06 | 19.34 ± 0.22 | 1.59 ± 0.16 | 2.01 ± 0.10 |
| Jan           | 0.06 ± 0.01 | 0.10 ± 0.01 | 7.3 ± 1.6 | 9.1 ± 2.1 | 0.12 ± 0.11 | 0.53 ± 0.19 | 0.01 ± 0.00 | 0.10 ± 0.04 |
| Feb           | 0.04 ± 0.01 | 0.05 ± 0.01 | 8.1 ± 0.6 | 8.3 ± 1.1 | 0.96 ± 0.51 | 0.37 ± 0.07 | 0.26 ± 0.15 | 0.71 ± 0.17 |
| Mar           | 0.03 ± 0.00 | 0.05 ± 0.01 | 7.3 ± 0.6 | 8.0 ± 1.4 | 9.66 ± 0.07 | 9.66 ± 0.01 | 0.60 ± 0.06 | 0.86 ± 0.11 |
| Apr           | 0.02 ± 0.01 | 0.05 ± 0.01 | 7.3 ± 1.4 | 7.7 ± 1.8 | 12.54 ± 0.70 | 17.86 ± 0.15 | 0.84 ± 0.05 | 1.297 ± 0.07 |
| May           | 0.02 ± 0.00 | 0.05 ± 0.01 | 7.1 ± 0.7 | 9.2 ± 1.2 | 14.17 ± 2.67 | 17.18 ± 1.02 | 0.97 ± 0.18 | 1.21 ± 0.08 |
| Jun           | 0.04 ± 0.01 | 0.05 ± 0.01 | 7.8 ± 1.3 | 7.2 ± 1.7 | 3.95 ± 0.71 | 5.21 ± 4.48 | 0.16 ± 0.03 | 0.44 ± 0.05 |

Significant comparison was made between vegetated and non-vegetated groups. The symbol ‘*’ represents the significant difference between vegetated and non-vegetated groups (p < 0.05). Vegetated and non-vegetated refer to groups with Potamogeton crispus or not. Initial: December 2, 2013; Dec: December 15, 2013; Jan: January 15, 2014; Feb: February 25, 2014; Mar: March 25, 2014; Apr: April 16, 2014; May: May 20, 2014; Jun: June 28, 2014.
correlation with N-NH4, N-NO2, TN, DO and Nr. De had significant correlation with N-NO3, TP, pH and TOC in vegetated treatments, while De had no significant correlation with these indexes in non-vegetated treatments. Nr had significant correlation with N-NO3, ORP in vegetated treatments, while Nr had an extremely significant correlation with TN, DO in both vegetated and non-vegetated treatments.

Table 2 | Pearson’s correlation coefficients among variables, nitrate reductase activity (Nr) and denitrification intensity (De) in sediment

| Variables   | Correlation coefficients (Nr) | Correlation coefficients (De) |
|-------------|--------------------------------|--------------------------------|
|             | Vegetated                      | Non-vegetated                  | Vegetated                      | Non-vegetated                  |
| N-NH4þ      | 0.44*                          | 0.46                          | 0.63**                          | 0.71**                         |
| N-NO2       | 0.27                           | 0.59**                        | 0.53**                          | 0.65**                         |
| N-NO3       | 0.62**                         | 0.10                          | 0.71**                          | 0.16                           |
| TN          | 0.68**                         | 0.56**                        | 0.84**                          | 0.74**                         |
| TP          | 0.26                           | 0.08                          | 0.48*                           | 0.18                           |
| T           | 0.09                           | 0.16                          | –0.15                           | –0.17                          |
| DO          | 0.73**                         | 0.72**                        | 0.59**                          | 0.61**                         |
| conductivity | 0.10                           | 0.23                          | 0.03                            | 0.03                           |
| pH          | –0.25                          | –0.33                         | –0.44                           | –0.39                          |
| ORP         | 0.41*                          | 0.34                          | 0.49*                           | 0.52**                         |
| TOC         | 0.23                           | –0.06                         | 0.45*                           | –0.05                          |
| Nr/De       | 0.93**                         | 0.87**                        | 0.93**                          | 0.87**                         |

The symbol ‘*’ in the figure represents the significant difference at $p < 0.05$. The symbol ‘**’ in the figure represents the significant difference at $p < 0.01$.

**DISCUSSION**

Changes in physical and chemical conditions caused by submerged macrophytes growing in winter

Submerged macrophytes are widely used for restoration of aquatic ecosystems, especially in shallow freshwater ecosystems. Submerged macrophytes affect nitrogen cycling processes in different ways. The impact of submerged macrophytes on environmental variables in aquatic ecosystems shows similar characteristics whether growing in winter or summer. Submerged macrophytes can increase the area of biofilms, which include nitrobacteria and denitrifying bacteria (Eriksson & Weisner 1999). They have the function of resuspension inhibition, and then reduce the release of nitrogen and phosphorus at the water–sediment interface (Zhou et al. 2018). Environmental variables such as nutrient concentration, dissolved oxygen (DO), pH and organic carbon in water and sediment changed by submerged macrophytes can affect nitrogen cycling (Carpenter & Lodge 1986; Weisner et al. 1994; Forshay & Dodson 2011; Paranychianakis et al. 2016). For example, submerged macrophytes can provide oxygen and organic carbon, which increase the dissolved oxygen and carbon resources in water and sediment (Caffrey & Kemp 1992; Weisner et al. 1994; Chimney et al. 2006; Reeder 2011). They need inorganic nitrogen as nutrient resources for growing, submerged macrophytes take up inorganic nitrogen, especially in growing periods (Weisner et al. 1994). Thus nutrient concentrations usually decrease with the growth of submerged macrophytes. Submerged macrophytes
can enhance electron acceptors, causing a higher charge transfer resistance and a lower rate of electron transfer, showed by the increase in ORP (Timmers et al. 2012; Xu et al. 2019). According to Table 1, Figures 2 and 3, this paper is consistent with these studies that Potamogeton crispus increased the concentration of DO and ORP in water. Meanwhile, it reduced the concentration of TN, TP, N-NO₃ and N-NH₄ in water.

The significant difference in the environment between submerged macrophytes growing in winter and summer is temperature. The different temperature caused by seasonal variation, showed a lower temperature in winter, which means lower enzyme activity and DO in winter. Macrophytes reduce water temperature by providing shelter (Rose & Crumpton 1996). In larger lakes, the temperature in areas with aquatic macrophytes differ from those in open water (Murkin et al. 1992). This phenomenon is more obvious in summer other than in winter. In this paper, the lower temperature in vegetated treatments happened in spring, not in winter or summer. Under the low temperature of winter, submerged macrophytes can barely change the temperature of overlying water. The largest biomass of submerged macrophytes is seen in the spring, and it had the lower temperature of vegetated treatments compared with non-vegetated treatments for providing shade. With decaying of the submerged macrophytes in summer, macrophytes had little impact on water temperature. As shown in Figure 2, the pH value increased with growth of Potamogeton crispus, and reached the highest value in April. This is consistent with previous studies. The growth of Potamogeton crispus was accompanied by the increase in pH, and the pH value could reach 9.91 ± 0.04 at the sampling point with Potamogeton crispus (Mi et al. 2008).

The variations caused by submerged macrophytes growing in winter have different effects on nitrogen cycling. For example, with the decrease in temperature, the enzyme activity is lower, causing nitrification and denitrification rate both to decrease. The increase in DO can increase the nitrification rate, and decrease the denitrification rate. The needs for inorganic nitrogen for growing makes submerged macrophytes compete with nitrogen cycling process for the source of N-NH₄, N-NO₃, N-NO₂. Under the comprehensive conditions, the nitrification rate is higher than the denitrification rate as the result.

Effects of submerged macrophytes on denitrification in winter

Denitrification rate of sediments varies under different environmental conditions. The denitrification rate of woodchip bioreactors is 192 μg g⁻¹ d⁻¹ (Hathaway et al. 2017). The highest denitrification intensity of sediment along the reservoir shoreline was 58 μg g⁻¹ d⁻¹, with an average of 9.2 μg g⁻¹ d⁻¹ (Yu et al. 2010). The denitrification intensity of sediment in the Seine River Basin increased gradually from upstream to downstream, namely 79.2 to 218.4 μg g⁻¹ d⁻¹ (Garnier et al. 2010). Meanwhile, the denitrification intensity in this paper was 0.01–1.65 μg g⁻¹ d⁻¹ in vegetated treatments, and 0.10–2.01 μg g⁻¹ d⁻¹ in non-vegetated treatments. The denitrification intensity in this experiment was lower than the reported values. This can be explained because the resources for denitrification processes are limited in the small-scale experimental devices, which limits the denitrification rate. As shown in Table 1, the organic carbon source and inorganic nitrogen required by denitrification process decreased gradually with the experiment. In the absence of carbon and nitrogen sources, the denitrification intensity would also decrease. At the same time, some studies showed that, compared with epiphytic denitrifying bacteria that attached to submerged macrophytes, the richness of rhizosphere denitrifying bacteria in the sediment is low, which leads to the lower denitrification rate in the sediment (Eriksson & Weisner 1997; Holmroos et al. 2015; Pang et al. 2016). The biofilm on the leaves of submerged macrophytes may change the pH and DO to be conducive to nitrification and denitrification in water (Dong et al. 2014; Ji et al. 2015). Season has a strong impact on the nitrogen cycle of the lake sediment, and the highest rate of denitrification is usually in December (Yao et al. 2018). However, the main growing season of submerged macrophytes in the lake is summer. The findings reported in this paper are consistent with this result. It is possible that the removal process of nitrogen in the lake is mainly controlled by abiotic variables, rather than the diversity and abundance of denitrifying bacteria and submerged macrophytes (Liu et al. 2018b).

Table 1 shows that there was no significant difference in denitrification rate between the vegetated and non-vegetated treatments for each month. These results may be caused by the low rate of denitrification. However, according to Figure 3 and Table 1, in the whole period of the experiment, macrophytes had a negative effect on denitrification significantly. Several studies have shown that the abundance of nitrogen cycling microbes in lakes is generally higher in the areas with macrophytes than areas without macrophytes. However, due to the influence of environmental conditions such as water mixing and resuspension, there may be no significant difference in nitrification rate and denitrification rate between the areas with and without macrophytes (Huang et al. 2016;
Dynamics of nitrification process under submerged macrophytes situation in winter

Generally, the highest rate of nitrification is in September (Yao et al. 2018). However, this paper describes the result that the nitrification rate in winter was higher than that in summer, which may be caused due to the following reasons. According to Table 1, the main growing season of submerged macrophytes in this experiment was in the winter, and DO in winter was not lower than that in the summer. With the consumption of inorganic nitrogen, TOC and other resources in the experimental equipment, not only the nitrification rate, but also the denitrification rate, was significantly reduced. Clearly, the effect of resource consumption on nitrification and denitrification had a much greater impact than that of DO and T. Therefore, under the comprehensive effect, submerged macrophytes growing in winter may have had little effect on nitrification, as shown in Figure 3.

Nitrification rate is related to many environmental variables. Studies have shown that Nr is positively correlated with water temperature and concentration of N-NH_4^+ (Yao et al. 2018). This means that the higher water temperature, the decay of submerged macrophytes and the increase in N-NH_4^+ in the sediment determine the Nr of the lake (Forshay & Dodson 2011). N-NH_4^+ into N-NO_3^-, showed a positive correlation with N-NH_4^+ and TN as seen in Table 2. The product of the nitrification is N-NO_3^-, which is a substrate of denitrification (Holmes et al. 1996). According to Table 2, Nr has an extremely significant positive correlation with TN, N-NO_3^-, and has a significant positive correlation with N-NH_4^+. However, Nr has a significant positive correlation with DO as shown in this study. As Nr is higher than Dc, shown in Table 1, the reason for this result may be the relatively higher concentration of DO, which benefited the nitrification process and was disadvantageous to the denitrification process. In this paper, the water depth of experimental devices was about 30 ± 2 cm, and the surface DO of the sediment is high relatively, existing under barely anaerobic conditions.

CONCLUSIONS

(1) The effect of submerged macrophytes on the physical and chemical properties of overlying water and sediment is significant when Potamogeton crispus reaches its largest biomass in spring.

(2) At low temperatures in winter, submerged macrophytes had little effect on the water temperature for providing shade, while the temperature of water where submerged macrophytes grow in the summer is generally lower than that of areas without submerged macrophytes significantly. Thus the difference in temperature caused by submerged macrophytes on nitrogen cycling is more significant in summer, rather than winter.

Vila-Costa et al. 2016; Liu et al. 2018a, 2018b). Submerged macrophytes growing in summer or winter also have little impact on denitrification rate. Based on the sampling in winter in Liangzi Lake, which is located in Wuhan, it was found that there was no significant difference in the abundance of anammox and denitrifying bacteria between vegetated and non-vegetated areas. The vegetated areas grow Ceratophyllum demersum, Hydrilla verticillata and Potamogeton crispus, of which two grow in summer and one grows in winter (Yin et al. 2018). Therefore, there was no significant difference in denitrification rate between the vegetated and non-vegetated areas in large lakes, except for the small-scale experimental devices.

It is widely believed that the denitrification rate is mainly affected by abiotic variables, and submerged macrophytes that have little effect on denitrification. In this paper, denitrification rate was positively correlated with N-NO_3^- and TOC in vegetated treatments. The denitrification rate was positively correlated with N-NH_4^+, TN in both vegetated and non-vegetated treatments. In the denitrification process, N-NO_3^- is the final electron acceptor, while organic carbon is the electron donor of the denitrification process. Therefore, N-NO_3^- and TOC in water and sediment are important variables affecting denitrification (Bruesewitz et al. 2011). The rate-limiting factors of denitrification are the availability of nitrogen oxides, followed by temperature and organic matter of sediment (Holmroos et al. 2015; Yin et al. 2018). The influence of organic carbon on the denitrification rate is direct or indirect through changing the absorption of inorganic nitrogen by aquatic macrophytes or affecting the abundance of denitrifying bacteria. Higher levels of organic matter can affect submerged macrophytes and reduce nitrogen uptake by macrophytes (Soana et al. 2015). The rhizosphere of submerged macrophytes can secrete organic matter, which provides organic carbon sources for denitrifying bacteria, and has a positive influence on the denitrification process. Organic matter was correlated with denitrification intensity in regions with macrophytes (Forshay & Dodson 2011). They change the denitrification process in the sediment by releasing oxygen from the rhizosphere, changing organic carbon levels in the sediment and trapping organic matter in water (Alldred & Baines 2016).
Denitrification is inhibited to some extent by the growth of Potamogeton crispus. Although the submerged macrophytes growing in winter had little effect on the nitrogen cycling process of sediment, they can form complementary effects with the submerged macrophytes growing in summer, which can make up for the adverse conditions of sediment resuspension or microbial attachment area reduction after the submerged macrophytes that grew in summer then decayed in winter.

**DATA AVAILABILITY STATEMENT**

All relevant data are included in the paper or its Supplementary Information.

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