Water Supply

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Water Supply Vol 22 No 3, 2440 doi: 10.2166/ws.2021.375

Comparing the effect of tailwater replenishment at different time intervals on eutrophic surface freshwater: a mesocosm simulation study

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ABSTRACT

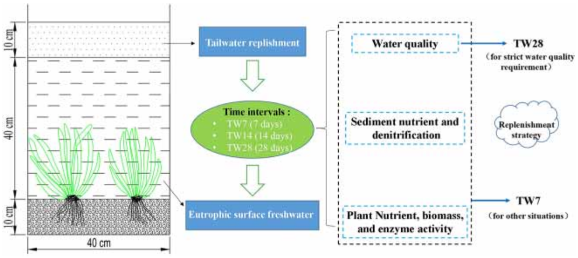
Tailwater is becoming the main water source supplied to surface freshwater worldwide. However, few studies have compared the effect of tailwater replenishment at different time intervals on eutrophic surface freshwater. In this study, we investigated the changes of water quality, sediment, and submerged macrophytes in eutrophic water in an outdoor mesocosm in response to different tailwater replenishment time intervals [every 7 days (TW7), 14 days (TW14) and 28 days (TW28)]. An 84-day simulation experiment demonstrated that there were only significant differences in the total nitrogen (TN) concentration of the overlying water, with the lowest mean value occurring in TW28. Nevertheless, the sediment TN was lowest in TW7 with a denitrification rate of 102.9 μmol/m^2/h. Tailwater replenishment also increased the nitrogen content and total biomass of *Vallisneria spiralis*, and TW7 had the highest total biomass of 20.19 g. Additionally, tailwater replenishment also affected plant enzyme activity, causing an increase in superoxide dismutase, peroxidase, and catalase, coupled with a decrease in malondialdehyde concentration in leaves. Overall, TW28 can be adopted as a tailwater replenishment strategy to ensure water quality, whereas TW7 can be applied without a strict water quality requirement for TN.

Key words: antioxidant enzyme, replenishment time intervals, sediment denitrification, tailwater, *Vallisneria spiralis*, water quality

HIGHLIGHTS

- Tailwater replenishment strategy with three different application time intervals was tested in eutrophic water bodies.
- Replenishment with tailwater every 28 days can be adopted as a tailwater replenishment strategy to ensure water quality.
- Replenishment with tailwater every 7 days can be applied without a strict water quality requirement for TN.

GRAPHICAL ABSTRACT



INTRODUCTION

Eutrophication (i.e. excessive nutrient enrichment) threatens global water resources globally, and has therefore received widespread attention (Schindler *et al.* 2008). In China, the eutrophication of aquatic ecosystems began in the 1960s, but has become very serious in recent years. According to China's Eco-environmental Status Bulletin, issued by the Ministry of Ecology and Environment in 2019, eutrophic surface freshwater still accounts for 28% of the 107 most important lakes (reservoirs) in China. Further, recent studies have shown that global warming increases the growth of algae and aggravates eutrophication (Verbeek *et al.* 2018).

Ecological water replenishment entails the implementation of engineering or non-engineering measures to reincorporate water into ecosystems damaged due to water scarcity, thereby alleviating the loss of ecosystem structure and function and gradually restoring the original self-regulating capacity of ecosystems. This strategy is an effective eutrophication control measure and has been widely used in many surface freshwater ecosystems (Hu *et al.* 2010). However, with the growing water resource scarcity and the deterioration of water quality in recent decades, available clean water sources have sharply decreased (Sun *et al.* 2016). As a result, tailwater from sewage treatment plants is becoming an important alternative water source for many urban lakes and rivers.

In China, the number of urban sewage treatment plants had reached 5,027 as of June 2018, with a daily treatment capacity of approximately 188 million tons (Li *et al.* 2019). Zhang *et al.* (2016) suggested that the annual increase in tailwater volumes from sewage treatment plants could ensure the long-term recharge of most urban rivers and lakes in the country. Moreover, the total amount of reused water that is required is much lower than the amount of tailwater discharged, which leaves ample room for the development of tailwater utilization strategies (Yang & Abbaspour 2007). Nevertheless, the nutrient content of tailwater, particularly total nitrogen (TN), can still be higher than that of eutrophic lakes.

Although there are few reported cases on the use of tailwater to replenish rivers and lakes, tailwater replenishment can improve water quality and inhibit algal growth (Barr *et al.* 2020). Nevertheless, many researchers have pointed out the potential risks associated with the use of reuse water in freshwater ecosystems (Vargas-González *et al.* 2014). In most of these cases, the tailwater replenishment time interval examined was either daily or applied once a time. The water replenishment time interval is one of the most critical parameters determining the success of ecological water replenishment efforts, and a suitable water replenishment time interval could avoid deteriorating the water quality of the receiving water body (Li *et al.* 2011). However, few studies have focused on determining the optimal tailwater replenishment time intervals for eutrophic freshwaters. Furthermore, water replenishment alone cannot improve water quality (Jin *et al.* 2015). Aquatic plants play important and varied roles in maintaining the structure and function of aquatic ecosystems, including the improvement of water quality (Søndergaard *et al.* 2010), and therefore the rehabilitation of submerged macrophytes has become an essential step in the conservation and restoration of many freshwater ecosystems, particularly shallow lakes (van Donk *et al.* 1993). Nevertheless, most previous studies have largely focused on the effects of tailwater on water quality and microorganisms (Vaquer-Sunyer *et al.* 2016), whereas the combined effects of tailwater on water quality, sediment, and macrophytes in freshwater environments have rarely been reported.

This study aimed to evaluate the feasibility of using tailwater from sewage treatment plants as a source of replenishment water for eutrophic surface freshwaters undergoing phytoremediation. To this end, we (1) compared the effects of tailwater discharge at different replenishment time intervals on water quality, as well as the denitrification rate of the sediment; and (2) explored the possible impact of tailwater discharge on the physiological characters of submerged macrophytes. We planted *Vallisneria spiralis* (*V. spiralis*) in our model systems because it is a widespread and highly conspicuous species in freshwater ecosystems and has also been widely used as a common pioneer plant in many restoration projects of eutrophic water bodies in China.

MATERIALS AND METHODS

Experimental setup

An outdoor mesocosm simulation system was constructed on the roof of a temperature control building in the Institute of Hydrobiology, Chinese Academy of Sciences (30°32′N, 114°21′E), with a total of 12 glass aquaria (length × width × height: 40 × 30 × 60 cm) to simulate the tailwater replenishment processes for eutrophic water bodies. Each aquarium was filled with 12 L of eutrophic sediment collected from Guanqiao Lake and 48 L of synthetic eutrophic water, which was prepared based on the water quality parameters of the overlying water of Guanqiao Lake at the beginning of the experiment. The ratio of water column volume to sediment volume was 4:1. All the aquaria were exposed to natural sunlight in an open room with a transparent roof. Three experimental groups and one control group were set up, with three replicates per group. These groups were classified according to the tailwater replenishment time intervals used: replenishment with tailwater every 7 days (TW7), every 14 days (TW14), every 28 days (TW28), and no replenishment (CK).

The experimental sediment used in each aquarium was collected using a Peterson grab mud sampler from the top 10 cm of the surface sediment of Guanqiao Lake (30°32′N, 114°22′E), a typical eutrophic lake which is a sub-lake of Donghu Lake in Wuhan City, China. The sediment was passed through a 2 mm sieve and mixed thoroughly before use. A 1 L surface freshwater sample was also collected and measured from Guanqiao Lake, and synthetic eutrophic water was prepared based on the water quality results. The experimental tailwater was artificially synthesized, and the water quality of the tailwater was classified as Level 1A standard according to the Discharge Standard of Pollutants for Municipal Wastewater Treatment Plant (GB18918-2002): i.e. TN: 15 mg/L, total phosphorus (TP): 0.5 mg/L, chemical oxygen demand (COD): 50 mg/L. Sodium nitrate ($NaNO_3$) and ammonium chloride (NH_4Cl) were used as nitrogen sources, potassium dihydrogen phosphate (KH_2PO_4) as phosphorus source, and glucose ($C_6H_{12}O_6$) as a carbon source for the synthetic overlay water in the experimental and control groups. We purchased the *V. spiralis* plants from a nearby local aquatic planting base. After two weeks incubation, all the *V. spiralis* grew very well. Plants with similar morphology and size were selected and carefully cleaned to wash away sediments and epiphytons as much as possible from the surfaces of the roots and leaves. In each aquarium six plants were planted and their average initial height was approximately 10 cm.

The water quality of the synthetic eutrophic water and tailwater (before the mixture) are shown in Table 1. The date of the first tailwater replenishment was recorded as day 0. The experimental period lasted 84 days (from June to August 2019), with 13, 7 and 4 tailwater replenishments for TW7, TW14 and TW28, respectively.

The single make-up water volume of the tailwater of the three experimental groups was 12 L. The height of the water level before the tailwater replenishment was 40 cm, and increased to 50 cm after the replenishment. Prior to each replenishment, the overlying water in the aquaria was drained to a height of 40 cm. To ensure a relatively uniform mixing after the replenishment and to reduce the risk of floating of the sediment caused by the replenishment, a plastic hose with an inner diameter of 8 mm was used to slowly siphon 12 L of tailwater into each aquarium in the experimental groups. Tap water was used to fill the control aquaria after exposure to sunlight to replenish the water lost due to sampling and evaporation.

Sampling and analysis

The physicochemical characteristics of the overlying water of each group including the temperature (T), pH, dissolved oxygen (DO), oxidation reduction potential (ORP), conductivity, total dissolved solids (TDS), and salinity were measured weekly on-site using a portable multimeter (Thermo Electron Scientific Company, USA). Collection and measurement of water samples were conducted before tailwater replenishment on a weekly basis. Water quality variables including TN, TP, nitrate nitrogen (NO_3-N), and ammonia nitrogen (NH_3-N) were measured according to national standard methods. COD was determined using a spectrophotometer (DRB 200, Hach, USA).

Sediment samples were collected once per month. Simplified sediment collection aquaria were constructed using 550 mL mineral water bottles and surface sediment was collected from the bottom 0–5 cm of the aquaria. Three parallel samples of each treatment group were collected at a time(i.e. one from each aquarium), and each sediment collection volume (wet weight) was approximately 100 g. The samples were mixed and stored in sealed bags. Rocks, animal remains, and plant tissues were removed, after which the samples were freeze-dried and ground into a powder. The powder was then sieved through a 0.15-μm mesh for further analysis. TN and TP in the sediment were first digested with sulfuric acid/perchloric acid and then measured via spectrophotometry. The inorganic nitrogen content of the sediment samples was analyzed using the standard methods with some modifications, as described by Chi *et al.* (2015). Briefly, NO_3-N and NH_3-N were extracted with 2 M KCl and shaken for 1 hour at 30 °C and shaken at 150 rpm in a shaker. The extracts were filtered through 0.45 μm membrane filters and measured using a spectrophotometer (GENESYS 180, ThermoFisher, USA). The denitrification rate of the sediment was measured using the acetylene inhibition method with some modifications as described by Groffman *et al.* (2006). Briefly, approximately 10 g of homogenized sediment from each sample was weighed and transferred to a 250 mL customized triangular bottle with 50 mL incubation solution (final concentrations: 0.1 g/L KNO_3, 0.18 g/L glucose, and

Table 1 | The water quality of the synthetic eutrophic water and tailwater (mean ± standard deviation)

Parameters	Eutrophic water	Tailwater
TN (mg/L)	2.88 ± 0.16	14.33 ± 0.23
TP (mg/L)	0.52 ± 0.02	0.48 ± 0.05
NO_3-N (mg/L)	1.98 ± 0.18	12.72 ± 0.32
NH_3-N (mg/L)	0.49 ± 0.11	1.44 ± 0.07
COD (mg/L)	44.17 ± 2.35	41.23 ± 4.23

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. Then, approximately 10% of the gas in the bottle was replaced with an equal volume of acetylene. The bottles were incubated in the dark for 1 hour at 30 °C. Gas samples were collected immediately after incubation using a 50 mL syringe and measured using a gas chromatograph (Agilent 7890A, Agilent Technologies, USA).

V. Spiralis samples were collected at the end of the experiment and carefully washed to remove anything attached to the leaves and roots before measurement. The TN and TP concentrations of the *V. Spiralis* were analyzed as described by Bao (2000). Sample supernatants were used for enzyme activity determination. Superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and malondialdehyde (MDA) concentrations were determined according to kits provided by the Nanjing Jiancheng Institute of Biological Engineering. Plant homogenates for enzyme activity analysis were prepared following Wang *et al.* (2017). At the end of the experiment, we removed all the plants from the devices, washed them and absorbed the water from the plant surface with absorbent paper. Then, the above-ground and below-ground tissues were separated and weighed with an electronic balance to get the fresh weight of the plants. A part of the plants was selected and dried in an oven at 60 °C until constant weight, thus the dry weight of this part was obtained. Finally, the final biomass (dry weight) of *V. spiralis* in each group was obtained by calculation. All plant samples were carefully cleaned, and then measured to ensure the accuracy of the data.

Statistical analyses

Statistical analyses were performed using R version 4.0.2 (R Development Core Team, Austria). Graphs were generated using Origin Pro 2016 (OriginLab Corporation, USA). The differences in water quality, sediment, and plant variables among the groups were evaluated via repeated-measures analysis of variance (ANOVA) with least significant difference (LSD) for multiple comparisons. The data obey normal distribution by the Shapiro-Wilk normality test ($P > 0.05$). Correlation analysis was used to explore the relationship between tailwater replenishment time intervals and water quality parameters. Differences were deemed significant when $P < 0.05$ and highly significant when $P < 0.01$.

RESULTS

Physicochemical characteristics of the water column

TN concentrations exhibited a similar increasing and then decreasing trend in both the experimental and control groups. However, for most of the experiment, the TN concentration in the experimental groups was higher than that in the control (Figure 1). A relatively small difference in NO_3-N and NH_3-N was observed among the groups (Figure 1(b) and 1(c)). Similar to TN, TP and COD concentrations in all systems showed a similar trend (i.e., an increase-decrease-increase trend was found) (Figure 1(d)). There was no observable regularity for the N:P ratio, which was higher in the experimental groups than in the control at the end of the experiment (Figure 1(f)).

Repeated-measures ANOVA indicated that the physicochemical variables of the water column changed significantly with time ($P < 0.05$, Table 2). Specifically, there were significant differences in the TN, COD, conductivity, TDS, salinity, pH, and ORP among the groups.

The LSD post-hoc test revealed that tailwater replenishment significantly affected all physicochemical factors of the water except TP and temperature (Table 3). Specifically, tailwater discharge increased TN, NO_3-N, NH_3-N, COD, DO, and ORP, while decreasing TDS, conductivity, and salinity. The average TN concentrations increased gradually as the replenishment time intervals became shorter, with the highest values observed in TW7. The mean concentration of TN in TW7 was 1.22 mg/L, which was 0.18 mg/L higher than that in the control.

Correlation analysis indicated that the concentrations of TN, TDS, and salinity in the water columns were strongly correlated with the increase in the replenishment time intervals (Figure 2). The relationship between TN concentration and replenishment time intervals was negative, whereas TDS and salinity were positively correlated.

Nutrient and potential denitrification rates in the sediment

Sediment TN concentration in all systems significantly decreased in June, but then subsequently increased at different degrees (Figure 3(a)). In contrast, the variation in TP during the first month showed the opposite trend (Figure 3(b)). At the end of the experiment, there was no significant difference in sediment TN and TP content between any experimental group and the control ($P > 0.05$). Sediment NO_3-N in all groups showed a decreasing trend in all groups. For NO_3-N, its trend in TW7 differed

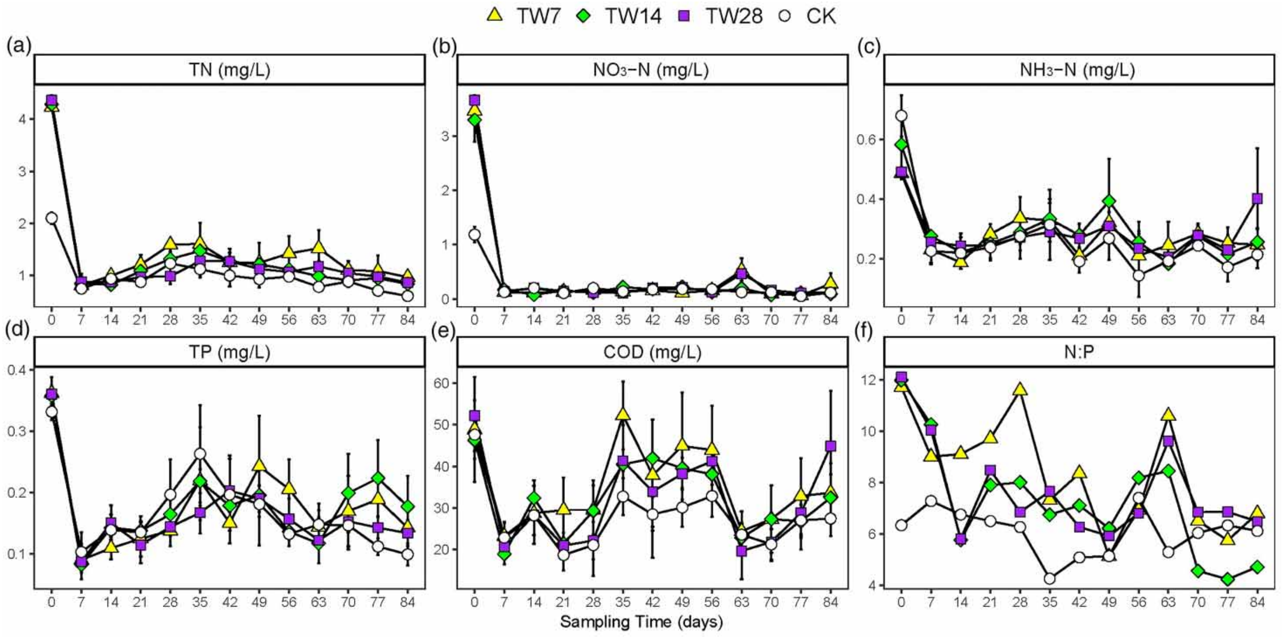


Figure 1 | Temporal variations in TN, NO_3-N, NH_3-N, TP, and COD concentrations and the N:P ratios in the water column of the experimental (TW7, TW14, TW28) and control (CK) groups. All data are reported as means ± SD ($n = 3$).

Table 2 | Repeated-measures ANOVA results for the data reported in Figure 1, showing the effects of group and time on the water quality indices of the water columns ($n = 144$)

	Time		**Group*Time**		**Group**	
Variables	**F**	***P***	**F**	***P***	**F**	***P***
TN	11.75	**0.001**	1.43	0.095	13.11	**0.002**
TP	9.982	**0.001**	1.681	**0.028**	0.726	0.565
NO_3-N	15.136	**0.001**	3.469	**0.001**	1.502	0.286
NH_3-N	5.335	**0.001**	1.060	0.403	2.234	0.162
COD	13.527	**0.001**	0.954	0.547	16.3	**0.001**
Temperature	2805.16	**0.001**	2.309	**0.001**	1.391	0.314
Conductivity	52.197	**0.018**	2.064	**0.004**	4.085	**0.049**
TDS	15.280	**0.001**	2.489	**0.001**	5.24	**0.027**
Salinity	13.370	**0.001**	2.581	**0.001**	4.751	**0.034**
DO	41.011	**0.001**	2.146	**0.002**	3.983	0.052
pH	75.069	**0.001**	5.341	**0.001**	8.548	**0.007**
ORP	809.62	**0.001**	12.36	**0.001**	8.967	**0.006**

Significant differences at $P < 0.05$ are displayed in bold.

from that of the other two experimental groups. As for NH_3-N, a similar trend was observed across the three experimental groups (Figure 3(c) and 3(d)).

Sediment denitrification rate (DNR) presented the same trend in all systems. Specifically, they decreased first and then increased, with the lowest value recorded in July (Figure 4). At the end of the experiment, there were significant differences in the DNR between TW7 and the control, which was consistent with the results of sediment NO_3-N. Among the three experimental groups, TW7 had the highest DNR of 102.9 μmol/m^2/h, which was 3.17 times higher than that of the control group.

Table 3 | Multiple comparisons (LSD) of all physicochemical variables of the water columns for the data reported in Figure 1 ($n = 144$)

Variables	TW7	TW14	TW28	CK
TN (mg/L)	1.22a	1.07b	1.04b	0.92c
TP (mg/L)	0.16a	0.16a	0.15a	0.15a
NO_3-N (mg/L)	0.20a	0.18ab	0.20a	0.16b
NH_3-N (mg/L)	0.27a	0.27a	0.27a	0.23b
COD (mg/L)	31.5a	30.9a	30.1ab	26.2c
Temperature (°C)	27.3a	27.4a	27.4a	27.2a
Conductivity (ms/cm)	0.34a	0.33a	0.35b	0.37c
TDS (g/L)	0.21a	0.21ab	0.22a	0.23c
Salinity (ppt)	0.15a	0.15ab	0.16a	0.17c
DO (mg/L)	5.80a	5.25b	5.05b	4.38c
pH	8.95a	9.24b	9.25b	9.05c
ORP (mv)	97.5a	88.5b	86.5b	88.6b

Lowercase letters indicate significant differences at $P < 0.05$.

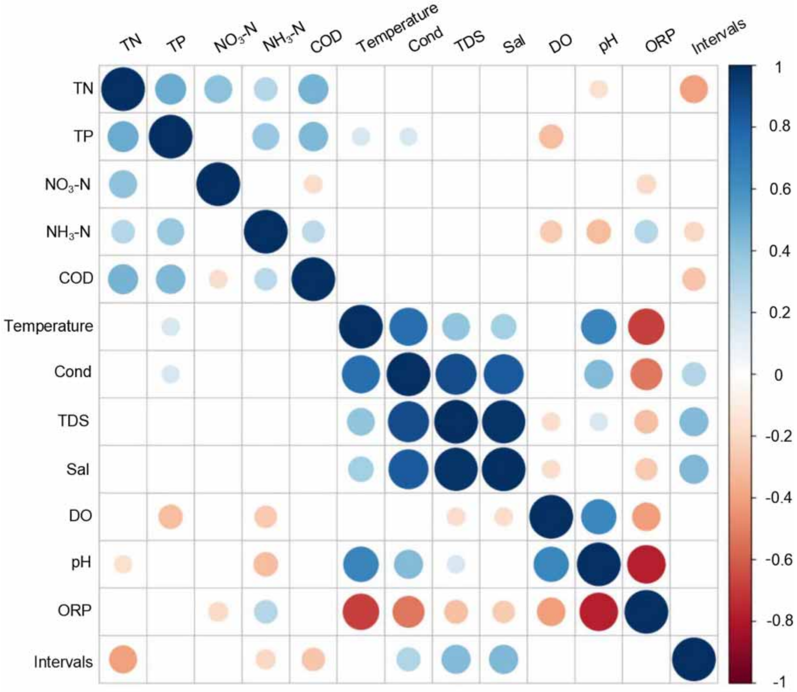


Figure 2 | Correlation analysis between tailwater replenishment time intervals and water quality parameters (the dots indicate a significant difference between the two variables (P value <0.05), and the size of the dot represents the correlation. The larger the area of the circle, the higher the correlation coefficient. Blue indicates a positive correlation, whereas red indicates a negative correlation). Please refer to the online version of this paper to see this figure in colour: http://dx.doi.10.2166/ws.2021.375.

Nutrient, biomass, and enzyme activity of *V. spiralis*

During *V. spiralis* growth, the nitrogen concentration in the leaves and the phosphorus concentration in the roots increased significantly (Figure 5). Different effects on the accumulation of nitrogen and phosphorus in *V. spiralis* were found after tailwater supplementation. Shorter replenishment time intervals resulted in a significant nitrogen accumulation in the leaves and roots. Hence, nitrogen concentration in *V. spiralis* leaves and roots was significantly higher in TW7 and TW14 than in the control ($P < 0.05$). Among the three experimental groups, the nitrogen concentrations in the leaves and roots of TW7

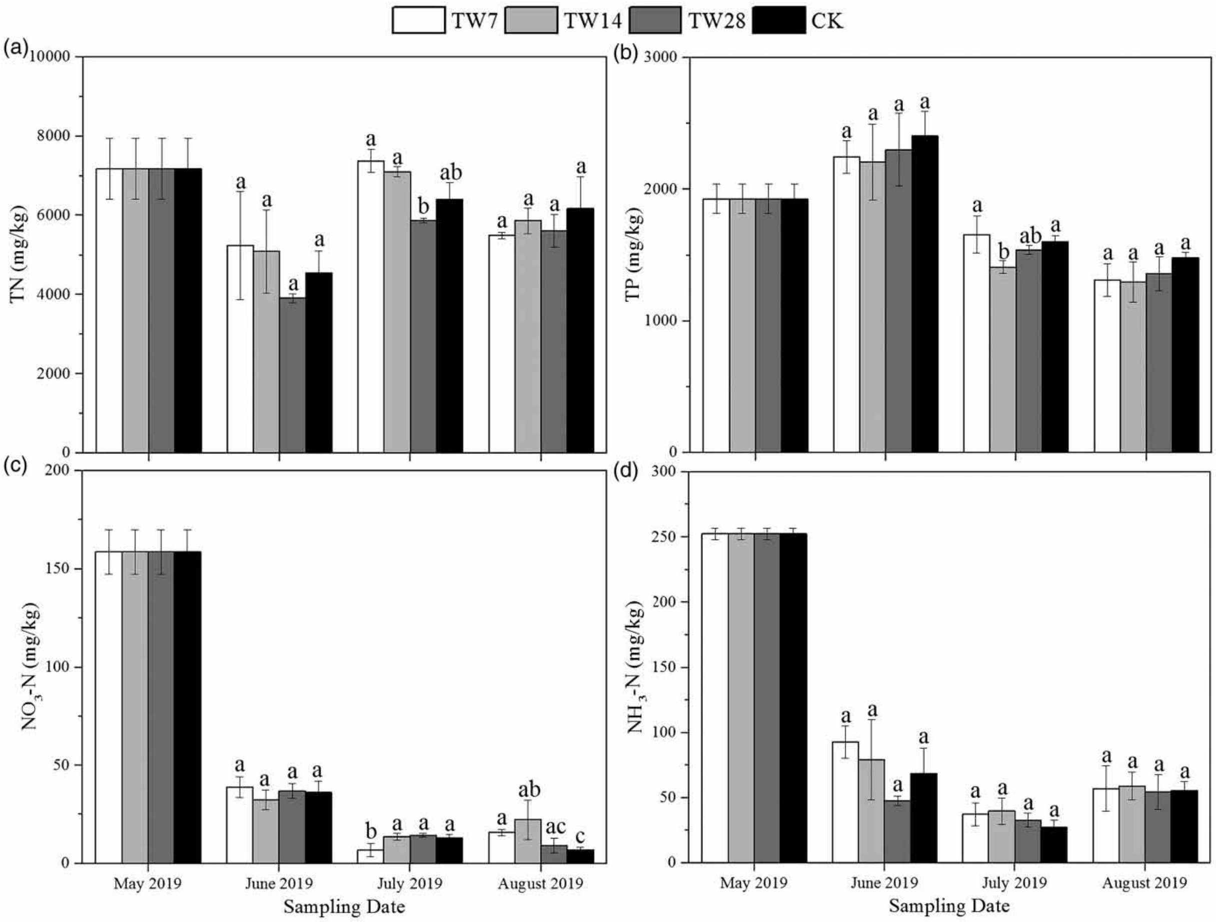


Figure 3 | Concentration variations in sediment nutrients between the experimental groups (TW7, TW14, TW28) and the control group (CK). Lowercase letters a-c represent the results of multiple comparisons (LSD) among groups, with different letters indicating significant differences between the two groups ($P < 0.05$).

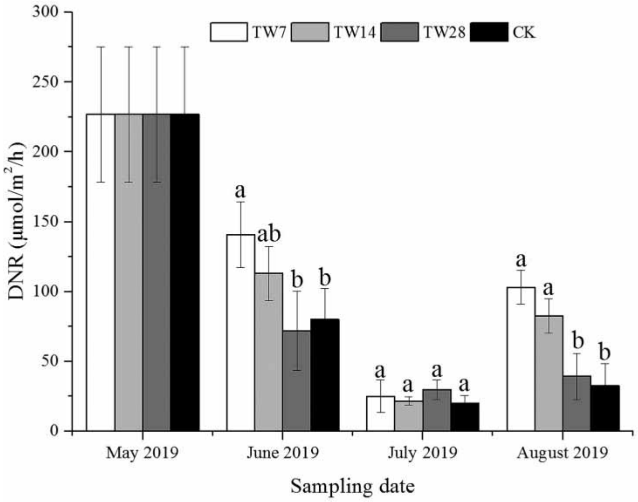


Figure 4 | Monthly variation in sediment denitrification rates (DNR) in the experimental (TW7, TW14, TW28) and control (CK) groups. Lowercase letters a-b represent the results of multiple comparisons (LSD) among groups, with different letters indicating significant differences between the two groups ($P < 0.05$).

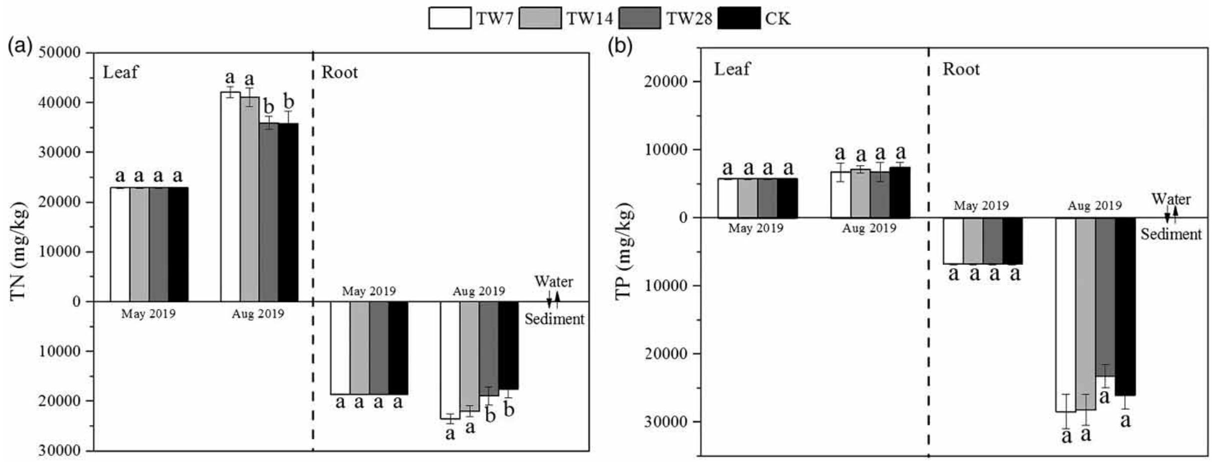


Figure 5 | Nitrogen and phosphorus concentrations in *V. spiralis* leaves and roots in the experimental (TW7, TW14, TW28) and control (CK) groups before and after the experiment (May and August 2019, respectively). Lowercase letters a-b represent the results of multiple comparisons (LSD) among groups, with different letters indicating significant differences between the two groups ($P < 0.05$).

were the highest. However, there were no significant differences in the phosphorus concentrations of the plant tissues, either between the experimental and control groups or among the three experimental groups. Additionally, sediment nitrogen and phosphorus concentrations decreased in the experimental groups compared with the control. In contrast, the nitrogen content in leaves and roots of *V. spiralis* significantly increased.

A comparison of the total biomass of *V. spiralis* before and after the experiment indicated that the total biomass increased significantly over time in each experimental group. *V. spiralis* biomass increased the most in TW7, with a post-experiment biomass being 4.19 times higher than its initial value. Nevertheless, there was no statistically significant difference in the total biomass between the experimental groups and the control. In TW7 and TW14, the above-ground biomass increased whereas the below-ground biomass decreased compared with the control group (Table 4). There were no significant differences in the biomass of the plant components ($P > 0.05$) among the three experimental groups, and the above-ground, below-ground, and total biomass of TW7 were the highest.

Tailwater replenishment also affected plant enzyme activity. Specifically, it increased SOD, POD, and CAT while decreasing MDA concentrations in the leaves of *V. spiralis* were detected (Figure 6). Among the three experimental groups, only TW7 and the control group were not significantly different in terms of SOD, POD, CAT, and MDA concentrations ($P > 0.05$).

DISCUSSION

Water quality is one of the biggest concerns for the use of tailwater as a source of water recharge. Differences in the water quality of the receiving water bodies may lead to variations in water replenishment effects. In the experiments in this study, we took the water quality of Guanqiao Lake, an urban eutrophic lake, as the research object. Although the total amount of tailwater added in the three experimental groups with different replenishment time intervals was different, the research

Table 4 | Changes in *V. spiralis* biomass (dry weight) in each group before and after the experiment (May and August 2019, respectively) in the experimental (TW7, TW14, TW28) and control (CK) groups

	May 2019		**August 2019**	
Groups	**Initial biomass/g**	**Above-ground biomass (leaf)/g**	**Below-ground biomass (root and stem)/g**	**Total biomass/g**
CK	3.96 ± 0.10a	14.82 ± 0.85a	4.84 ± 0.59a	19.67 ± 0.97a
TW7	3.89 ± 0.13a	15.97 ± 0.71a	4.22 ± 0.36ab	20.19 ± 1.32a
TW14	4.05 ± 0.12a	15.65 ± 1.25a	4.09 ± 0.35ab	19.75 ± 0.51a
TW28	3.98 ± 0.06a	14.52 ± 0.77a	4.02 ± 0.31b	18.54 ± 0.52a

Lowercase letters (under experimental conditions) indicate significant differences at $P < 0.05$.

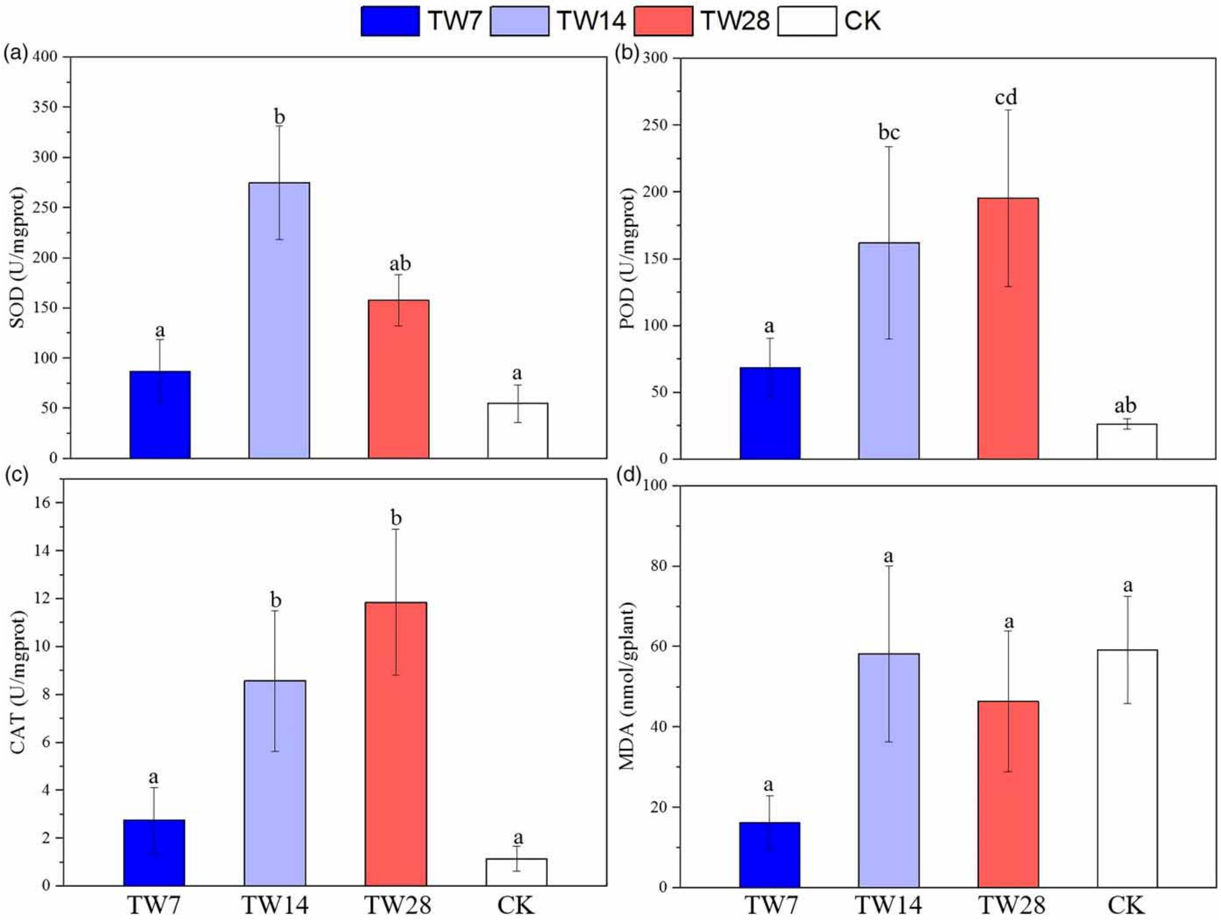


Figure 6 | Antioxidant enzyme activities in the leaves of *V. spiralis* in the experimental (TW7, TW14, TW28) and control (CK) groups. The data are presented as means ± SE ($n = 5$). SOD, POD, CAT, and MDA represent superoxide dismutase, peroxidase, catalase, and malondialdehyde, respectively. Lowercase letters a-c represent the results of multiple comparisons (LSD) among groups, with different letters indicating significant differences between the two groups ($P < 0.05$).

results showed that the main difference in the water quality variables among the experimental groups was TN concentration. Therefore, we concluded that TN is the most critical factor for tailwater replenishment in Guanqiao Lake and other similar eutrophic water bodies, which is consistent with the conclusions of previous studies (Hu *et al.* 2010). The rapid decreases in the concentrations of water quality variables in the early stage of the experiment may be related to the adsorption of sediment (Geng *et al.* 2015). Shortening the water replenishment time interval in summer could reduce the risk of eutrophication in water bodies. In this study, the lowest and highest TN concentrations were found in TW28 and TW7, respectively. Tailwater with high NH_3-N would lead to an increase in NH_3-N in the water column after supplementation. However, NH_3-N concentrations in this study were very low in both the experimental groups and the control, and no significant differences were identified among the three experimental groups. Moreover, although the NO_3-N concentration of the tailwater used in this experiment was approximately 12 mg/L (accounting for more than 80% of the TN), the NO_3-N content in the three experimental groups was all less than 0.5 mg/L at the end of the experiment, which was likely due to the presence of aquatic plants, epiphyton and rhizosphere microorganisms (Eriksson & Weisner 2015; Racchetti *et al.* 2017). The nitrogen forms in the overlying water of the experimental and control groups were mainly organic nitrogen, and their concentrations were very low. No studies have shown that low concentrations of organic nitrogen are harmful to eutrophic water bodies. Generally, there is a risk of an increase for TP in the receiving waters due to tailwater discharge. However, in this study, there was no significant difference in the TP concentration between the experimental systems and the control in this study regardless of the replenishment time intervals. This is probably due to the growth of *V. spiralis*, which has a strong removal capacity for phosphorus in overlying water (Liu *et al.* 2016). DO and ORP in the water column increased after tailwater replenishment, with TW7 showing the highest increases in this experiment. High DO facilitates the complexation of phosphate with metal ions (e.g., Fe^{3+}) and promotes the migration of phosphate into the sediment (Lehtoranta *et al.* 2004; Cooke *et al.* 2005). High ORP also promoted phosphorus absorption by the sediments. These might be the reasons for the small differences in the TP concentration of the overlying water between the experimental and control groups. At the same time, tailwater replenishment promoted DO increases, which was also observed in the Taihu Lake water transfer project (Hu *et al.* 2010). Moreover, algal blooms were not observed in any system throughout the experiment, which may be related to the low N:P ratio and the presence of submerged plants (Klausmeier *et al.* 2004). Although the N:P mass ratio of the replenished tailwater was extremely high (~25.8) in both the experimental and control systems, the N:P mass ratios in the three experimental groups tended to decrease and all of them were below 7 by the end of the experiment. This caused nitrogen to be the limiting factor, as the optimal N:P mass ratio for algal growth is 7.2 (Klausmeier *et al.* 2004). Further, submerged macrophytes are widely known to directly inhibit algal growth by competing for resources (nutrients and light), excreting allelopathic substances (which are harmful to algal growth), and altering hydraulic conditions, particularly when the macrophytes are in the rapid growth period (van Donk *et al.* 1993; Lv *et al.* 2018). This also indicated that the growth of submerged plants could reduce the risk of water bloom outbreaks after tailwater replenishment.

No TN or TP accumulation was observed in the sediments at the end of the experiment. In contrast, TN increased in the leaves and roots of *V. spiralis* in TW7 and TW14, with the TN concentration in TW7 being higher than that in TW14. Additionally, an increase in the sediment DNR was observed in TW7 (June and August) and TW14 (August), which most likely explains the reduction in TN in the sediment (Racchetti *et al.* 2017). The N transfer from the sediment to the overlying water may be another reason for the aforementioned TN reduction (Liang *et al.* 2015). According to a study by Racchetti *et al.* (2017), higher NO_3-N availability in the water column likely stimulates nitrate uptake from the roots of *V. spiralis*. High availability of water NO_3-N also stimulates NO_3-N reductase activity, thus enhancing leaf NO_3-N uptake (Konnerup & Brix 2010). This might explain why the root nitrogen content of *V. spiralis* gradually increased as the tailwater replenishment time interval was shortened. High nitrate-rich tailwater input altered the nitrogen forms in the water and sediment, which affected the absorption patterns of the nutrients. It has been found that *V. spiralis* effectively utilizes nitrate concentrations below 25 mg/L (Ma *et al.* 2007). Increasing concentration of NO_3-N can promote the growth of *V. spiralis* when NH_3-N concentration in the water column is low. In the present study, NH_3-N was low both in the water and the sediment of all experimental groups. Tailwater recharge also altered the above-ground and below-ground biomass of *V. spiralis*. TW7 and TW14 both exhibited higher above-ground biomass but lower below-ground biomass than the control. As the available nitrogen in the water column of the control was limited, nutrients were mainly absorbed by the roots, leading to an increase in the below-ground biomass in the control. This is consistent with the study by Rattray *et al.* (1991). Additionally, the POD, SOD, and CAT activities increased and the MDA concentrations decreased in the three experimental groups, which might suggest that tailwater replenishment had no significant adverse effect on plant growth (Nimptsch & Pflugmacher 2007). Our study and previous studies demonstrate that submerged macrophytes can effectively remove high nutrient concentrations in tailwater and alleviate the deterioration of water quality caused by tailwater replenishment (Zhou *et al.* 2016). To be clear, during the experiment there was no obvious attachment of epiphyton on the surface of *V. spiralis*. Meanwhile, the plants were repeatedly washed before the determination. Hence, we assumed no significant effect for the results from the epiphyton in our study. The nitrogen and phosphorus content of tailwater can promote the growth of *V. spiralis* without altering its physiology, which in turn inhibits the growth of algae in the waters due to nutrient and resource competition. Therefore, submerged plants may play an extremely important role in the process of tailwater replenishment to eutrophic water bodies.

CONCLUSIONS

1. Among the water quality parameters measured, significant differences were only observed in TN concentration among the three experimental groups, with the TW28 concentration being the lowest.
2. Tailwater replenishment had no significant effect on sediment TN and TP, and TW7 had the lowest concentration and highest DNR. It also promoted an increase in the total biomass and nitrogen uptake of *V. spiralis*, with the highest values observed in TW7 among the three experimental groups.
3. TW7 and TW28 can be adopted as suitable tailwater replenishment time intervals for eutrophic surface freshwaters with and without a strict requirement for TN, respectively.

ACKNOWLEDGEMENTS

This study was supported by the Key Research and Development Project of Zhejiang Province (2019C03110).

DECLARATION OF COMPETING INTEREST

The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

DATA AVAILABILITY STATEMENT

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

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First received 26 January 2021; accepted in revised form 18 October 2021. Available online 29 October 2021