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Three-year Survey of Nitrogen Dynamics in a Stratified Reservoir of the North China Plain

Xiaopeng Qiu[1, a], Tinglin Huang[2, *], Mingzheng Zeng[2, b] and Shilei Zhou[2, c]

[1]State Key Laboratory of Eco-hydraulics in Northwest Arid Region of China, Xi'an University of Technology, Xi'an 710048, China
[2]Key Laboratory of Northwest Water Resource, Environment and Ecology, MOE, Xi'an University of Architecture and Technology, Xi'an 710055, China

*Corresponding author e-mail: huangtinglin@xauat.edu.cn, [a]qxp2015@163.com, [b]254507774 @qq.com, [c]744896496@qq.com

Abstract. Freshwater ecosystem health and water supply safety are under severe threat due to the recent increase in anthropogenic nitrogen. Therefore, this study investigated the seasonal variation and vertical distribution of nitrogen and other environmental factors from April 2012 to April 2015 in the Zhoucun Reservoir. The results show that nitrate was the primary nitrogen species in the entire water column except the hypolimnion, where ammonium dominated. Total nitrogen fluctuations were larger in near-surface water, with a range of 0.38–3.11 mg/L. Nitrate concentrations were homogeneous throughout the water column and experienced a clear decline in spring. In contrast, ammonium was in stratification from May to October. Surface ammonium reached a maximum in the high flow period during summer, while bottom ammonium remained high throughout stratification. Our results suggest that aerobic denitrification and nitrogen load from inflow rivers were the main processes that affected surface nitrogen content, while bottom nitrogen content was primarily affected by anaerobic denitrification and nutrient release from sediment. Of the measured environmental factors, water temperature, water residence time, dissolved oxygen, rainfall, and total phosphorus were the most critical in affecting nitrogen content. Our field surveys show the effects of complex process and environmental factors on the dynamics of nitrogen in the lacustrine zone of the reservoir, helping people understand and predict nitrogen dynamics in temperate, stratified lentic waters. Based on our results, the water resource managers can select suitable measures to control the eutrophication.

1. Introduction

Recently, levels of anthropogenic nitrogen have risen in lentic water, due to the increasing frequency of agricultural and industrial activities [1]. Thus, although phosphorus was conventionally regarded as the key factor to control eutrophication and algal blooms, nitrogen is now receiving greater attention [2]. Nitrogen content and the ratio of nitrogen to phosphorus considerably affects phytoplankton communities and dynamics [3]. Therefore, understanding nitrogen dynamics in lakes and reservoirs is of great importance for eutrophication control and water supply safety [4].

Nitrogen content in lentic water is primarily determined through complex interactions between nitrogen enrichment and loss. Nitrogen enrichment is mainly the result of exogenous nitrate input from inflow rivers [5] and endogenous release of ammonium from sediments under anaerobic conditions [6]. Nitrogen loss mainly consists of denitrification, assimilation by aquatic plants, and sedimentation [7]. Of these, denitrification is regarded as the major source of nitrogen loss [8]. The process reduces NO_x into N_2, lowering nitrogen concentrations in water [9].

In addition to these general processes that influence lentic water nitrogen concentrations, site-specific environmental conditions also play a role in determining nitrogen content. Common factors include water temperature, dissolved oxygen, water residence time, rainfall, nitrogen load, phosphorus load, and algal abundance. For example, high water temperature and low dissolved oxygen concentrations specifically promote sediment release and denitrification while high dissolved oxygen increase nitrification rate [10]. Algal blooms can alter nitrogen composition and provide favourable conditions for denitrification [11]. Finally, water residence time and phosphorus load are often positively correlated with nitrogen loss in freshwater systems [12].

The importance of nitrogen as an indicator of water quality has resulted in numerous recent studies examining nitrogen circulation and transformation in lentic water. Research has focused on nitrogen loss [8, 12-15], the effects of exogenous input [16-18], and sediment release [19], but few have included systematic, comprehensive analyses of the entire nitrogen cycle. Even fewer studies have done so in reservoirs and lakes. Incomplete information on the processes of the nitrogen cycle can potentially result in contradictory conclusions regarding these ecosystems and difficulty in predicting nitrogen dynamics.

Therefore, the aim of this study is to investigate the nitrogen dynamics of a stratified and eutrophic reservoir in the North China Plain. We examined the nutrient load, sediment release, denitrification, and phytoplankton assimilation to understand the dominant factors that influence nitrogen content and composition at different depths and across seasons.

2. Methods

2.1. Study area
Built in 1960 and located in the North China Plain (34°56′38.74″N, 117°41′14.13″E), the Zhoucun Reservoir is one of the main water sources of Zaozhuang City. The reservoir has a total storage capacity of 84.04 million m^3, a surface area of 6.5 km^2, a mean depth of 13 m, and a maximum depth of 18 m. The catchment area is 121 km^2, primarily occupied by villages and agricultural land. Three rivers flow into the reservoir: the Xijia, the Xiashi, and the Xuwa. Of the three, the Xiashi and Xuwa Rivers are fairly polluted due to the presence of chicken farms along their banks. The climate of the reservoir is sub-humid continental monsoon. The annual average temperature is 13.9 °C and the annual average rainfall is approximately 847 mm. The reservoir experiences severe sediment pollution, owing to the water cage fish culture that operated from the 1970s to 2008. The atmospheric N deposition in the North China Plain is approximately 28.0 $kg/hm^2.year^{-1}$) [20]. Differences in annual rainfall was most marked during the summer (particularly July and August), with 2012 and 2013 experiencing heavier precipitation than 2014. Precipitation in 2014 was more evenly distributed. Water residence times ranged from 45 days to 1307 days and generally exhibited the opposite trend from precipitation, being of shorter durations in summer and longer durations in spring and winter.

2.2. Sampling and analysis
The field survey lasted from April 2012 to April 2015. The inflow rivers were sampled monthly and the sampling sites were near the entrance of the reservoir. The lacustrine zone of the reservoir was sampled weekly (in spring, summer, and autumn) or biweekly (in winter). Water samples in the lacustrine zone of the reservoir were collected at 2.5-m intervals, using a 2.5-L Van Dorn Bottle. Samples were then stored in acid-washed polyethylene bottles and preserved at 4°C until nutrient chemical analyses.

Vertical distributions of water temperature (T), dissolved oxygen (DO), and electrical conductivity (Cond) were measured *in situ*, using Hydrolab DS5 (HACH, Colorado, America). Chemical analyses for total nitrogen (TN), ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), and total phosphorus (TP) were undertaken within 24 hours post-collection, according to standard water quality analysis methods [21]. Samples for NH_4^+, NO_3^-, and NO_2^- analyses were filtered with GF/C glass-fibre filters (0.45-μm pore size). Samples for phytoplankton analysis were fixed using Lugol's solution *in situ*. Phytoplankton was counted using an Olympus microscope at ×400 magnification, following previously established methods [22].Rainfall, discharge, and water level data were supplied by the Bureau of Water Resources of Zaozhuang Municipality.Water residence time was the ratio of monthly average volume to discharge [8, 23]. Inflow nitrogen load was the product of nitrogen concentration and inflow volumes of the inflow rivers [24].

2.3. Statistical analysis
Significant correlations between monthly mean values of nitrogen and environmental factors were tested in SPSS 19.0 (IBM, Inc., New York). We used Spearman's correlations because most variables did not have a normal distribution. Significance was set at $P < 0.05$.

3. Results
The range of the surface water temperature was 13–25°C in spring, 25–31°C in summer, 11–25°C in autumn, and 0–10°C in winter (Fig. 1). The bottom water temperature showed no seasonal variation from early spring to early fall. Bottom water temperatures ranged from 8 to 14°C in spring and summer. The reservoir possessed a stratified period and a mixing period, with the former lasting from mid-April to early November. During stratification, the epilimnion was between 0 m and 5 m, the thermocline was between 5 m and 10 m, and the hypolimnion was between 10 m and the bottom.
DO also experienced seasonal stratification and was similar to water temperature in vertical distribution. Beginning from early April of every year, DO in the bottom water decreased gradually. The bottom of the reservoir remained anaerobic (DO = 0 mg/L) until November, when the thermal structure altered. During stratification, the water below 5 m was anaerobic and the water above 5 m was aerobic. During mixing periods, DO concentrations throughout the water column remained high.
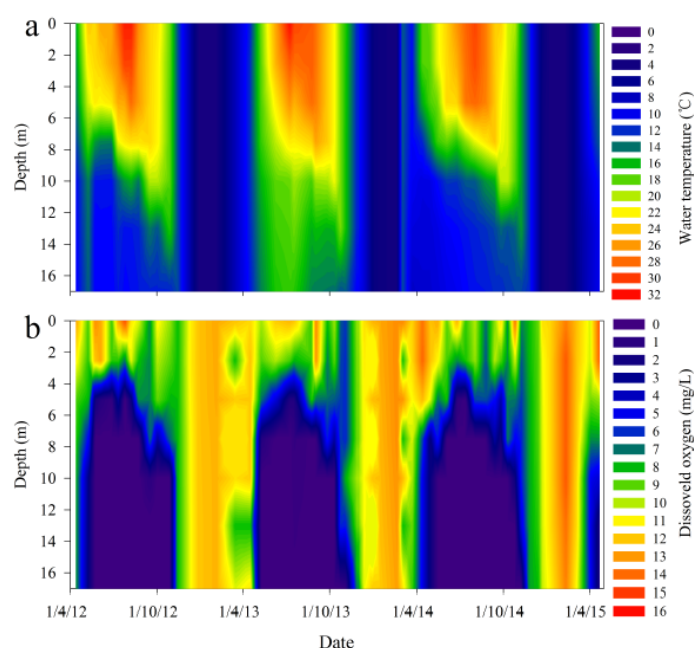

Figure 1. Temporal and vertical variation of water temperature and dissolved oxygen in the lacustrine zone of the Zhoucun Reservoir (2012–2015).

Analyses of algal abundance revealed that the Zhoucun Reservoir experienced a 'clear-water phase' during spring (Fig. 2). The lowest surface water algal concentrations in 2012, 2013, and 2014 were 65×10^4 cells/L (9 May), 119×10^4 cells/L (16 April), and 420×10^4 cells/L (20 April), respectively. Algal blooms occurred during summer 2012 and 2013, reaching a maximum surface abundance of 1.94×10^8 cells/L (29 August) and 7.30×10^7 cells/L (19 August), respectively.
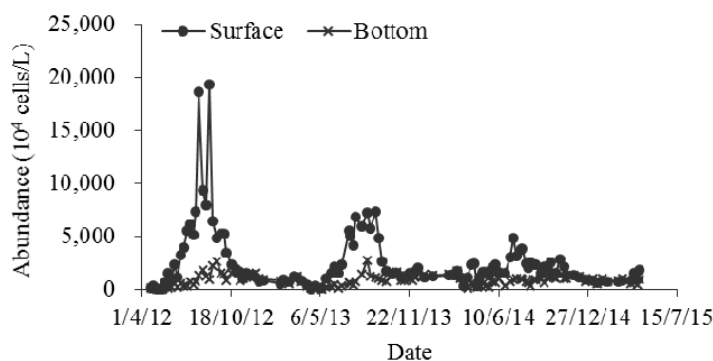

Figure 2. Seasonal variations of algal abundance in the lacustrine zone of the Zhoucun Reservoir.

Analysis of inflow water nitrogen load revealed that nitrate was the primary nitrogen species, followed by ammonium, with minimal contribution from other nitrogen species (Fig. 3). Generally, nitrogen load was high in the summer and low during winter and spring. Variations in nitrogen load were similar between 2012 and 2013, with nitrate and ammonium contributions peaking in the summer. However, in 2014, the inflow nitrogen load was much lower during the summer and was evenly distributed from May to November.
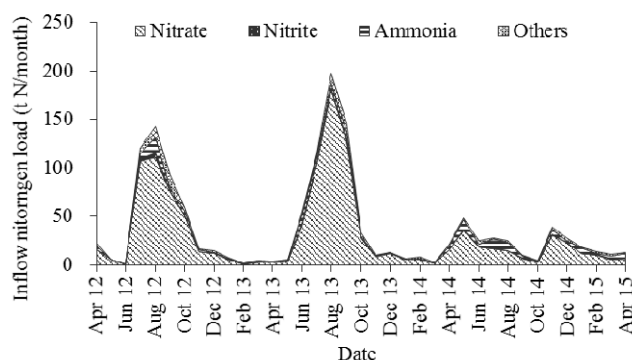

Figure 3. Seasonal variations of inflow nitrogen load in the lacustrine zone of the Zhoucun Reservoir.

Changes in nitrogen patterns across the seasons are shown in Fig. 4. NO_3^- exhibited a wide variation, ranging from 0 to 2.5 mg/L. Surface and bottom NO_3^- variation occurred almost synchronously, except during the summers of 2012 and 2013, which experienced heavy rainfall. Yearly trends in NO_3^- variation were very similar, with NO_3^- consistently and sharply decreasing from April to July, reaching the lowest concentration directly before the concentrated precipitation. The lower levels of summer rainfall in 2014 correspond with lower NO_3^- levels during that year.

Surface NH_4^+ ranged from 0.01 to 0.76 mg/L and bottom NH_4^+ ranged from 0.02 to 2.36 mg/L. Surface NH_4^+ concentrations showed a clear peak during the summers of 2012 and 2013, but changed little in the summer of 2014. In contrast, bottom NH_4^+ began to accumulate during April of every year and remained at high levels during stratification. NO_2^- ranged between 0 mg/L and 0.22 mg/L. No clear difference was observed in surface versus bottom NO_2^-.

Lastly, TN ranged between 0.38 mg/L and 3.11 mg/L in surface waters, and between 0.76 mg/L and 3.30 mg/L in bottom waters. Because surface TN is composed primarily of NO_3^-, variation in TN patterns agreed with changes in NO_3^- patterns. Annual minimum TN in surface waters occurred during early summer. In bottom waters, TN levels were high throughout the year, consisting mainly of NH_4^+ during the stratified period and NO_3^- during the mixing period.
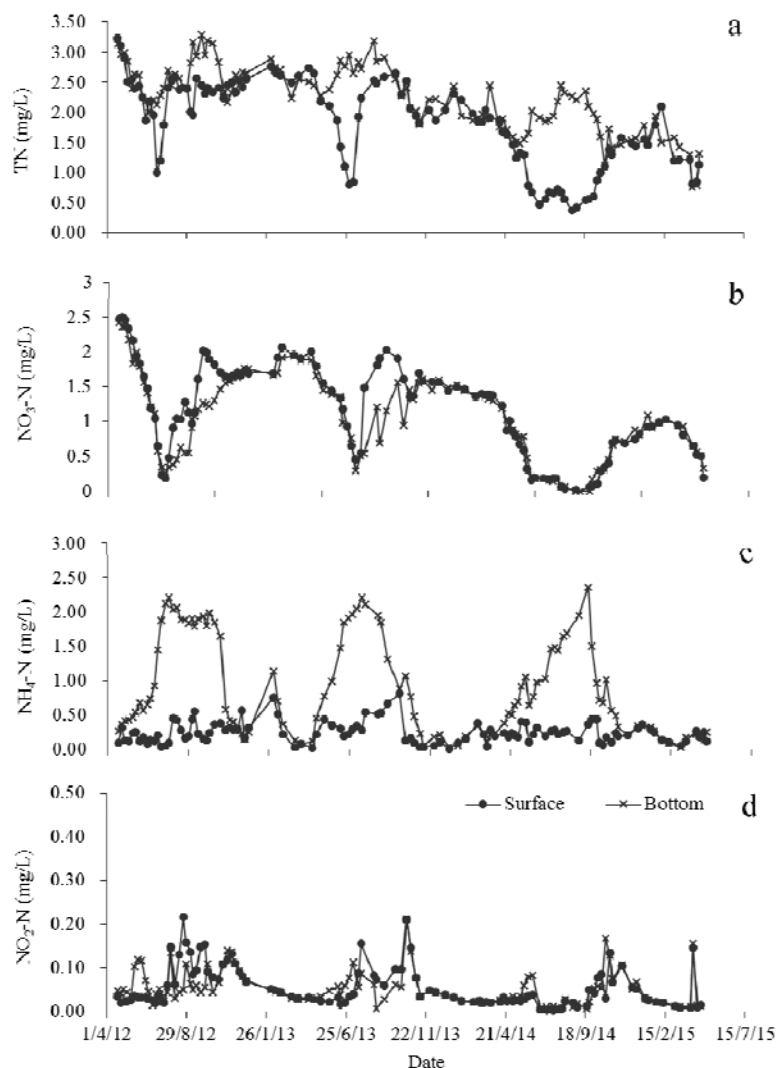

Figure 4. Seasonal variations of nitrogen concentration in the lacustrine zone of Zhoucun Reservoir. (a) Total nitrogen, (b) Nitrate, (c) Ammonium, (d) Nitrite.

Table 1 summarizes the results of our Spearman's correlations between nitrogen and a host of environmental factors. In surface waters, NO_3^- was negatively correlated with rainfall, TP, and algal abundance ($P < 0.05$), while NH_4^+ was positively correlated with rainfall ($P < 0.05$). In water near the bottom, NO_3^- was positively correlated with DO ($P < 0.05$) and negatively correlated with both rainfall and TP ($P < 0.01$). NH_4^+ was negatively correlated with DO ($P < 0.01$) and positively correlated with T ($P < 0.05$). Lastly, TN was positively correlated with NO_3^- and negatively correlated with NH_4^+ ($P < 0.01$).

Table 1. Spearman's correlation analysis of nitrogen and environmental factors

	Surface			Bottom		
	TN	NO_3^-	NH_4^+	TN	NO_3^-	NH_4^+
TN	1			1		
NO_3^-	0.912[**]	1		0.315	1	
NH_4^+	-0.023	-0.122	1	0.462[**]	-0.529[**]	1
T	-0.155	-0.275	0.222	0.395[*]	-0.18	0.571[**]
DO	-0.076	-0.013	-0.302	-0.431[*]	0.421[*]	-0.833[**]
Rain	-0.204	-0.392[*]	0.350[*]	0.199	-0.465[**]	0.655[**]
TP	-0.296	-0.397[*]	0.301	0.222	-0.581[**]	0.716[**]
Algae	-0.245	-0.383[*]	0.264	0.088	0.005	0.01
Cond	0.175	0.307	-0.237	0.265	0.216	0.145

[*]Correlation is significant at the 0.05 level; [**]Correlation is significant at the 0.01 level.

4. Discussion

NO_3^- was the primary nitrogen species in the Zhoucun Reservoir. We noticed a considerable NO_3^- decrease in the hypolimnion when the reservoir was stratified. Numerous previous studies have demonstrated that denitrification—rather than phytoplankton assimilation or sedimentation—is the primary means of nitrate removal in lentic waters [15, 25]. We consider it likely that the nitrate decrease observed in this study was also due to denitrification, for the following reasons. First, denitrification occurs mainly on the sediment surface [26] and generally under anaerobic conditions (DO < 0.2 mg/L) [27]. In the Zhoucun Reservoir, the hypolimnion was anaerobic from May to October due to stratification, thus favouring the denitrification process. Moreover, assimilation by aquatic plants is not very probable, due to the lack of light at the reservoir bottom.

Surface NO_3^- declined sharply in the Zhoucun Reservoir during spring. However, anaerobic denitrification responsible for bottom NO_3^- decline is unlikely to be the cause, since the thermal stratification that occurred in April hinders the vertical migration of dissolved matters [28]. In addition, high surface DO levels also indicate that surface anaerobic denitrification did not occur. Although high DO concentrations are typically thought to suppress denitrification in aquatic environments [29], several studies have demonstrated that lakes and reservoirs exhibit substantial denitrification under high DO conditions [30-31]. This phenomenon is explained by recent reports of aerobic denitrifiers in freshwater systems, such as lakes, reservoirs [32], ponds, and canals [33]. These aerobic denitrifying bacteria can simultaneously use oxygen and nitrate as electron acceptors [32]. The aerobic denitrifying bacteria in the Zhoucun Reservoir have been shown to remove a substantial percentage of nitrate (~30%) after 48 hours [34]. We therefore suggest that aerobic denitrification is probably the main cause of surface NO_3^- decrease in the Zhoucun Reservoir.

Another other possible explanation for surface NO_3^- decrease is phytoplankton assimilation, but the Zhoucun Reservoir experienced a 'clear-water phase' with low algal abundance during the spring, with low algal abundance during the spring, implying that phytoplankton uptake contributed little to NO_3^- removal. Moreover, the presence of NH_4^+ (~0.2 mg/L) indicates that algal absorption of NO_3^- is negligible [35], as the less energetically costly NH_4^+ is preferentially assimilated by phytoplankton . Denitrification is promoted by long water residence times [8], which increases the contact period between the water and denitrifiers [36]. In this study, the lowest average water residence time was 45 d, indicating that the hydraulic conditions in the Zhoucun Reservoir provided the prerequisite for denitrification [37].

Water depth did not appear to affect denitrification rate in the Zhoucun Reservoir. Most of the physicochemical factors such as T, DO, and pH varied with depth, confounding attempts to identify major factors that affect denitrification rates. However, it is likely that multiple interacting variables

resulted in the homogenous denitrification rates across differing depths in the reservoir, rather than one primary factor.

Correlation analysis showed that TP was significantly and negatively correlated with NO_3^-, in accordance with past reports [38]. A TP increase promotes algal blooms, which enhance nitrogen uptake, decrease DO, and provide carbon and nitrogen sources for denitrifying bacteria [39].

While denitrification was the main source of nitrogen loss in the Zhoucun Reservoir, inflow nitrogen load was a major factor in surface nitrogen increase. The catchment area is 121 km^2, much larger than the surface area of the reservoir (6.5 km^2). Therefore, exogenous nitrogen input from inflow river contributes much more to nitrogen load than inflow water that comes direct rainfall. Consistent with previous studies [40], nitrate was the primary nitrogen species in the inflow river. The summers of 2012 and 2013 experienced greater rainfall than summer 2014, resulting in larger inflow volumes during those 2 years. Since the temperature of the runoff was higher than the bottom water, inflow mainly diffused in the epilimnion, leading to higher surface nitrate concentrations than bottom nitrate concentrations during summer 2012 and 2013.

In addition to exogenous input, surface NH_4^+ concentrations are also affected by phytoplankton assimilation and sediment release. Given the similar thermal structure and sediment properties among the 3 years in the Zhoucun Reservoir, sediment release contributed little to the difference in surface NH_4^+ concentrations. Next, more algae were present in the epilimnion during summer 2012 and 2013, implying more phytoplankton assimilation in those two years than in 2014. However, surface NH_4^+ concentrations were actually higher in the summers of 2012 and 2013, allowing us to conclude that phytoplankton assimilation plays only a small role in NH_4^+ removal. Therefore, we conclude that runoff had the largest effect on surface NH_4^+. As rainfall in East Asia is forecasted to increase during the 21st century [41], surface nitrogen content in the Zhoucun Reservoir will probably also increase in the future.

Finally, the serious polluted sediment discharged large amounts of nitrogen especially NH_4^+ during stratification, which resulted in the transformation of dominant nitrogen species from NO_3^- to NH_4^+. Our results indicated that bottom NH_4^+ concentrations in the bottom were higher than surface concentrations during stratification. This is likely because NH_4^+ is easily released from sediment under anaerobic conditions [6], such as those found in the bottom of the stratified reservoir. Previous research in the Zhoucun Reservoir supports the release of NH_4^+ by lake sediment under anaerobic conditions [42]. The NH_4^+ concentration is 16.82–20.08 mg/L in the interstitial water of the sediment during stratification, much higher than the NH_4^+ concentration in bottom water. Therefore, the accumulated NH_4^+ in bottom water mainly comes from sediment release. Moreover, another previous study suggests that NO_3^- concentrations greater than 1 mg/L can suppress the release of reduced pollutants from sediment, such as ammonia, iron, phosphorus and manganese [28]. Therefore, denitrification in the sediment during stratification, which reduces NO_3^- concentration, can facilitate NH_4^+ release from sediment.

5. Conclusion

We conclude that seasonal TN variations in the Zhoucun Reservoir are the cumulative result of changes in NO_3^- and NH_4^+ concentrations, which were driven primarily by inflow nitrogen load, sediment nutrient release, and aerobic/anaerobic denitrification. In water near the surface, nitrate was the primary nitrogen species. Factors that increased surface nitrate levels in this study included heavy summer rains, long water residence time, and high phosphorus concentration, all of which favour aerobic denitrification. In water near the bottom, NO_3^- was the primary nitrogen species during the mixing period, and NH_4^+ was the primary nitrogen species during the stratified period. Anaerobic conditions in the hypolimnion promoted both NH_4^+ release and anaerobic denitrification at the reservoir bottom. Lastly, although denitrification clearly decreases nitrogen levels, we suggest that effective eutrophication control in the reservoir still requires proper management of catchment area nutrient loads and efforts to reduce sediment pollution. Our field survey and theoretical analysis on

nitrogen dynamics of the reservoir can provide valuable information for understanding and predicting the seasonal and vertical changes of nitrogen in temperate, stratified lentic waters.

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