Supplementary Information (SI) for Environmental Science: Processes & Impacts.

Supplementary Information

Study on the Mechanism of Phoslock's Impact on Nitrogen Migration at the Sediment-Water Interface

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Materials and methods

Overview of the study area

Lake Xuanwu is located in Nanjing, Jiangsu Province, and is a famous urban landscape lake in China. The lake covers an area of 3.78 km^2 with a shoreline of about 10 km. Due to its unique geographical location and rich ecological resources, Lake Xuanwu has become an important natural landscape and ecological barrier in Nanjing. However, since the 1970s, Lake Xuanwu has faced challenges related to eutrophication. From the 1990s onward, measures such as dredging, pollution interception, relocation of surrounding enterprises, and water diversion have been taken, leading to a significant improvement in water quality and a reduction in the degree of eutrophication. Nevertheless, recent monitoring data show that total nitrogen in Lake Xuanwu remains one of the primary factors exceeding regulatory standards.

Lake Li, an inland lake spanning from Meiliang Bay into Wuxi City, exhibits both relative independence and connectivity to Lake Taihu. The water area is 8.6 km^2, with a length of 6 km from east to west, and a width of 0.3-1.2 km from north to south, and a circumference of about 21 km around the lake. In recent years, various environmental remediation projects have been implemented, resulting in the effective control of external pollution and a significant improvement inthe water quality. However, in some areas, especially in the estuaries of Lake Dongli where sediment has not been dredged, or in some bays of the lake, the water quality is still at levels IV to V, and the issue of water body eutrophication remains one of the major environmental issues faced by Lake Li.

Analysis of overlying water and interstitial water indexes and microbial functional genes

The indicators of overlying water temperature (T), pH, DO , salinity (Sal), oxidation-reduction potential (ORP), and electrical conductivity (EC) were measured using Manta multiparameter water quality sonde (Eureka, U. S.); NO_3^- and NH_4^+ were determined using the ultraviolet spectrophotometry method (HJ/T 346-2007) and Nessler's reagent colorimetric method (GB 7479-87), respectively, while the determination of NO_3^- and NH_4^+ in interstitial water was conducted using the micro-colorimetric method.

Total microbial DNA was extracted from the surface sediment of Lake Li using the E.Z.N.A.® DNA Kit (Omega Bio-tek, Norcross, GA, U.S.). Metagenomic sequencing libraries were constructed at Shanghai Biozeron Biotechnology Co., Ltd., and sequencing of nitrification (AOA, AOB) and denitrification functional genes (*nirK, nirS, nosZ*) was performed using NGS instruments in a paired-end 150bp (PE 150) mode. The raw data from the sequencing were processed for quality control, concatenation, assembly, prediction, and annotation. Based on the annotation results, the target genes were extracted and categorized. Finally, the TPM abundance values of the target genes were calculated using the salmon software, with TPM calculated using the following formula[1]:

$$TPM = \frac{\frac{N_i}{L_i} * 10^6}{sum(\frac{N_1}{L_1} + \frac{N_2}{L2} + \ldots + \frac{N_n}{L_n})} \qquad (1)$$

Where, N_i is the number of reads to the i gene, L_i is the length of the i gene, and sum ($N_1/L_1+N_2/L_2$ +... + Nn/Ln) is the sum of the values of all (n) genes normalized by length.

Study on key processes of sediment nitrogen migration

Ability of sediment to replenish bioavailable nitrogen to interstitial water

The concentration changes of bioavailable nitrogen at the SWI were determined using the DGT device. The specific operation is as follows: the day before collecting the cylindrical samples, the rectangular DGT device was slowly and vertically inserted into the sediment, with 3-5 cm of overlying water retained. After placing it for 24 hours, it was removed, the position of the SWI was marked, the water temperature was recorded, the device's surface was rinsed with pure water to remove attached matter, and finally, it was sealed and preserved in a self-sealing bag with a small amount of deionized water for moisture retention. In the laboratory, the gel inside the DGT was cut into 1 mm intervals using a ceramic blade and placed in centrifuge tubes. Then, 0.4 mL of 1 mol/L NaCl solution was added to extract at room temperature for 16 hours. The concentrations of NO_3^- and NH_4^+ in the extracts of each slice were determined using the micro-colorimetric method. The specific calculation method [2] is as follows:

$$M = \frac{C_e(V_e + V_g)}{f_e} \qquad (2)$$

Where, M is the accumulation of effective state elements in the fixed film (μg), Ce is the concentration of the extraction solution (mg/L); Ve and V_g are the extraction agent and gel volumes (mL), respectively; f_e is the extraction rate corresponding to the target ions on the fixed film. C_{DGT} is calculated by the following equation [3]:

$$C_{DGT} = \frac{M \triangle_g}{DAt} \quad (3)$$

Where, C_{DGT} is the mean concentration of the bioactive state in the sediment profile (mg/L), M is the accumulation of the effective state elements in the fixed film (μg), △g is the thickness of the diffusion layer (cm), D is the diffusion coefficient of the effective state elements in the diffusion film (cm^2/s), and A is the area of each film (cm^2), t is the placement time (s) of the DGT device. Based on the available nitrogen content of sediments and nitrogen content of interstitial water obtained by DGT technology, combined with the DIFS model [4], the ability of sediments to recharge available nitrogen to interstitial water can be calculated.

$$R = \frac{C_{DGT}}{C} \quad (0 < R < 1) \quad (4)$$

Where, C_{DGT} is the mean concentration of available state in sediment profile (μg /L), C is the nitrogen content in interstitial water (μg /L), and R value reflects the ability of sediment to replenish available state nitrogen to interstitial water, which can be divided into complete replenishment type ($0.95<R<1$), partial replenishment type ($0.1<R\leq0.95$) and unidirectional diffusion type ($R< 0.1$).

Bioavailable nitrogen diffusion flux at the SWI

The spatial distribution of different forms of nitrogen at the SWI obtained by DGT device conforms to Fick's first diffusion law. The diffusion flux of nitrogen at the SWI is calculated according to the following formula [5]:

$$F = \varphi \cdot D_s \frac{\partial_c}{\partial_x}|_{x=0} \quad (5)$$

Where, F is the ion diffusion flux at the SWI (mg/ ($m^2 \cdot d$)); φ is the surface sediment porosity; $\frac{\partial_c}{\partial_x}|_{x=0}$ is the concentration gradient (mg/L) of ions at the sediment water interface in unit distance. Ds is the diffusion coefficient of ions in aqueous solution (cm^2/s), which is generally calculated by the following equation [6]:

$$D_s = \varphi D_0 \ (\varphi < 0.7) \quad (6)$$

$$D_s = \varphi^2 D_0 \ (\varphi > 0.7) \quad (7)$$

Where, D_0 represents the ideal diffusion coefficient of ions, that is, the ionic diffusion coefficient under infinite dilution of the solution.

Table S1

Characteristics of changes in overlying water basic indexes

The characteristics of changes in pH and DO of the overlying water during the experimental period are shown in Table S1. The pH of the overlying water in Lake Xuanwu Lake Li ranged from 7.93 to 8.67, while in Lake Li, it ranged from 7.98 to 8.48, respectively, indicating that both lakes are weakly alkaline. Except for the 30°C Lake Li Phoslock aerobic group, where the overlying water pH was similar to that of the control group, the pH of the overlying water in the other experimental groups was consistently lower than that of the control group. The addition of Phoslock under anaerobic conditions resulted in a more pronounced decrease in pH. The DO in the overlying water at 30°C under the same treatment was significantly lower than at 15°C ($p < 0.05$), which can be attributed to the different saturation of DO and microbial activity in the water at different temperatures. Additionally, the DO levels in the overlying water of the experimental groups were generally higher than those in the control groups, suggesting that the addition of Phoslock can increase the content of DO in the overlying water. The ORP (Oxidation-Reduction Potential) of the overlying water in Xuanwu Lake and Li Lake are in the ranges of 193.63~332.24 mV and 197.55~342.33 mV, respectively. The EC (Electrical Conductivity) is between 508.9 $\mu s \cdot cm^{-1}$ and 698.7 $\mu s \cdot cm^{-1}$ for Xuanwu Lake, and between 684.3 $\mu s \cdot cm^{-1}$ and 1039.9 $\mu s \cdot cm^{-1}$ for Li Lake. The Salinity (Sal) ranges from 0.243 PSU to 0.340 PSU for Xuanwu Lake and from 0.330 PSU to 0.514 PSU for Li Lake. Under different temperature and dissolved oxygen conditions, there are no significant patterns of change in EC and Sal in the overlying water of both the experimental and control groups.

Table S1 Characteristics of changes in overlying water basic indexes

T /(℃)	**Group**	**pH**	**DO (mg•L^{-1})**	**ORP (mV)**	**EC (μs•cm^{-1})**	**Sal (PSU)**
30	X_1	8.67±0.04	5.88±0.08	194.5±4.0	572.3±24.2	0.273±0.012
	X_2	8.53±0.02	6.07±0.11	195.4±0.8	508.9±44.2	0.243±0.021
	X_3	8.29±0.18	3.37±0.37	326.5±12.6	648.5±19.8	0.310±0.008
	X_4	7.93±0.23	2.98±0.23	332.2±13.4	644.3±19.3	0.307±0.013
15	X_1	8.51±0.09	7.52±0.12	195.4±1.0	698.7±5.8	0.340±0.001
	X_2	8.45±0.02	8.15±0.08	202.6±0.7	563.5±37.4	0.269±0.022
	X_3	8.27±0.11	6.77±0.22	194.9±2.2	627.8±46.4	0.302±0.025
	X_4	8.11±0.06	6.85±0.26	193.6±1.0	625.8±3.8	0.300±0.001

30	L_1	8.35±0.05	5.53±0.25	209.4±6.3	886.1±7.9	0.427±0.005
	L_2	8.35±0.07	5.80±0.27	201.0±1.8	848.2±78.8	0.413±0.038
	L_3	8.08±0.11	3.51±0.15	299.0±8.0	815.0±173.8	0.391±0.089
	L_4	7.98±0.05	3.80±0.19	342.3±13.2	945.6±17.6	0.457±0.01
15	L_1	8.28±0.12	8.07±0.25	198.5±2.1	822.7±149.9	0.400±0.079
	L_2	8.25±0.10	8.15±0.27	198.1±3.5	943.3±275.3	0.465±0.143
	L_3	8.48±0.10	7.28±0.15	197.6±0.4	684.3±17.3	0.330±0.01
	L_4	8.25±0.03	7.96±0.19	200.5±0.7	1039.9±30.3	0.514±0.013

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