

Supplementary Materials

Effect and Mechanism of Mixed Aeration-Enhanced *Limnodrilus hoffmeisteri* on Improving nutrients and Organic Matter at the Sediment-Water Interface

Xu Zou^{ab}, Ruihua Cao^{a,b*}, Qiqi Wan^{a,b}, Zhihao Zhang^{a,b}, Qiyang Wang^{a,b}, Tinglin Huang^{a,b,c}, Gang Wen^{a,b,c*}

*

^aKey Laboratory of Northwest Water Resource, Environment and Ecology, MOE, Xi'an University of Architecture and Technology, Xi'an, 710055, PR China.

^bShaanxi Key Laboratory of Environmental Engineering, Xi'an University of Architecture and Technology, Xi'an, 710055, PR China.

^cField Scientific Observation and Research Station for Qinling Water Source Water Quality of Shaanxi Province, Xi'an University of Architecture and Technology, Xi'an 710055, China

*Corresponding author: Tel.: +86-29-82207886; Fax: +86-29-82201354. E-mail address: hitwengang@163.com (Gang Wen) rhcao123@163.com (Ruihua Cao)

Figures:

Fig.S1 Changes in the abundance of dominant benthic fauna species in Shibanyu Reservoir from July 2024 to June 2025.

Fig.S2 Diagram of experimental setup. (Oxygenation using an air pump is performed in a small water tank outside the column, with the oxygen being returned to the experimental apparatus via a peristaltic circulation pump for continued mixing, thereby achieving both mixing and oxygenation. The peristaltic circulation pump operates at a speed of $5 \text{ mL} \cdot \text{min}^{-1}$ to minimize interference from the surface sediment layer; The *L. hoffmeisteri* specimens used in the experiment were all collected from in-situ sediments. During the experiment, only a small number of individuals died, with a mortality rate below 5%, and no significant behavioral abnormalities or mass mortality was observed.)

Fig.S3 The release rates of TN (a), $\text{NH}_4^+\text{-N}$ (b), $\text{NO}_3^-\text{-N}$ (c) and TP(d) in the sediments for each experimental group during the incubation of 23 day at 18°C . (HA, MA, LA and BA represent sediment groups with high- ($15,000 \text{ ind.} \cdot \text{m}^{-2}$), medium- ($10,000 \text{ ind.} \cdot \text{m}^{-2}$), low-density ($5,000 \text{ ind.} \cdot \text{m}^{-2}$) *L. hoffmeister* additions, along with a control group without *L. hoffmeisteri* addition under mixed aeration conditions; H, M, L and B represent sediment groups with high-, medium-, low-density *L. hoffmeisteri* additions, and a control group without *L. hoffmeisteri* addition under non-mixed aeration conditions)

Fig.S4 Vertical variations of TN concentrations (a), TP concentrations (b), mass fraction of organic matter (c) and water content (d) in sediments of each experimental group after the 23 day experiment at 18°C . (HA, MA, LA and BA represent sediment groups with high- ($15,000 \text{ ind.} \cdot \text{m}^{-2}$), medium- ($10,000 \text{ ind.} \cdot \text{m}^{-2}$), low-density ($5,000 \text{ ind.} \cdot \text{m}^{-2}$) *L. hoffmeister* additions, along with a control group without *L. hoffmeisteri*

addition under mixed aeration conditions; H, M, L and B represent sediment groups with high-, medium, low-density *L. hoffmeisteri* additions, and a control group without *L. hoffmeisteri* addition under non-mixed aeration conditions)

Fig. S5 Fig. S5a shows the abundance of nitrogen cycle-related genes in different groups, and Fig. S5b illustrates the metabolic pathways involved in the nitrogen cycle.

Fig. S6 Variations of dissolved oxygen (a) and temperature (b) in Shibianyu Reservoir from July 2024 to June 2025.

Fig. S7 Changes in TN concentration in the surface, middle, and bottom layers of Shibanyu Reservoir from July 2024 to June 2025 (a), and the TN removal predicted by experiment every month (b).

Table S1 Water quality indicators of different groups before and after the experiment.

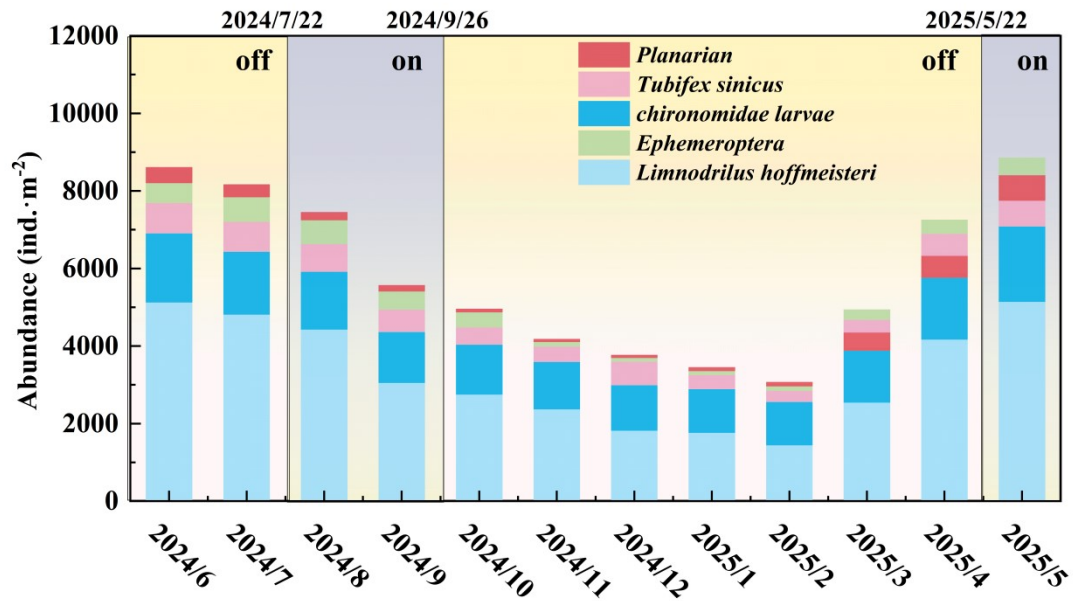


Fig. S1 Changes in the abundance of dominant benthic fauna species in Shibanyu Reservoir from July 2024 to June 2025.

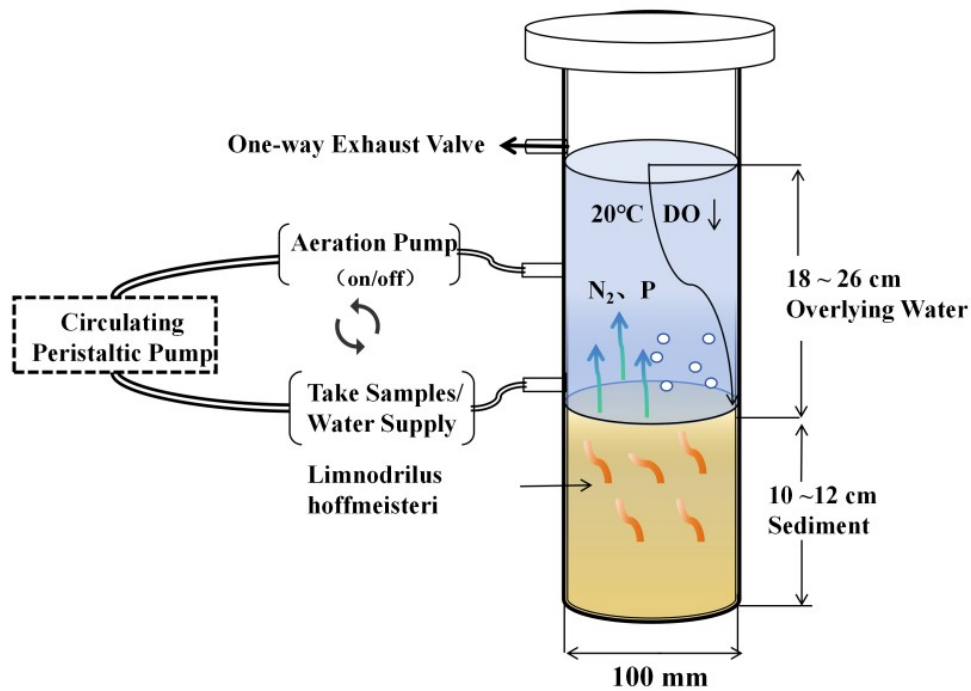


Fig. S2 Diagram of experimental setup. (Surface sediments (0–12 cm) collected from the reservoir were homogenized before the experiment. Thereafter, in-situ water was incorporated into the sediment, followed by a 21-day pre-incubation to recover microbial activity and guarantee the representativeness of the indigenous functional microbial community. Oxygenation using an air pump is performed in a small water tank outside the column, with the oxygen being returned to the experimental apparatus via a peristaltic circulation pump for continued mixing, thereby achieving both mixing and oxygenation. The peristaltic circulation pump operates at a speed of $5 \text{ mL} \cdot \text{min}^{-1}$ to minimize interference from the surface sediment layer; The *L. hoffmeisteri* specimens used in the experiment were all collected from in-situ sediments. During the experiment, only a small number of individuals died, with a mortality rate below 5%, and no significant behavioral abnormalities or mass mortality was observed.)

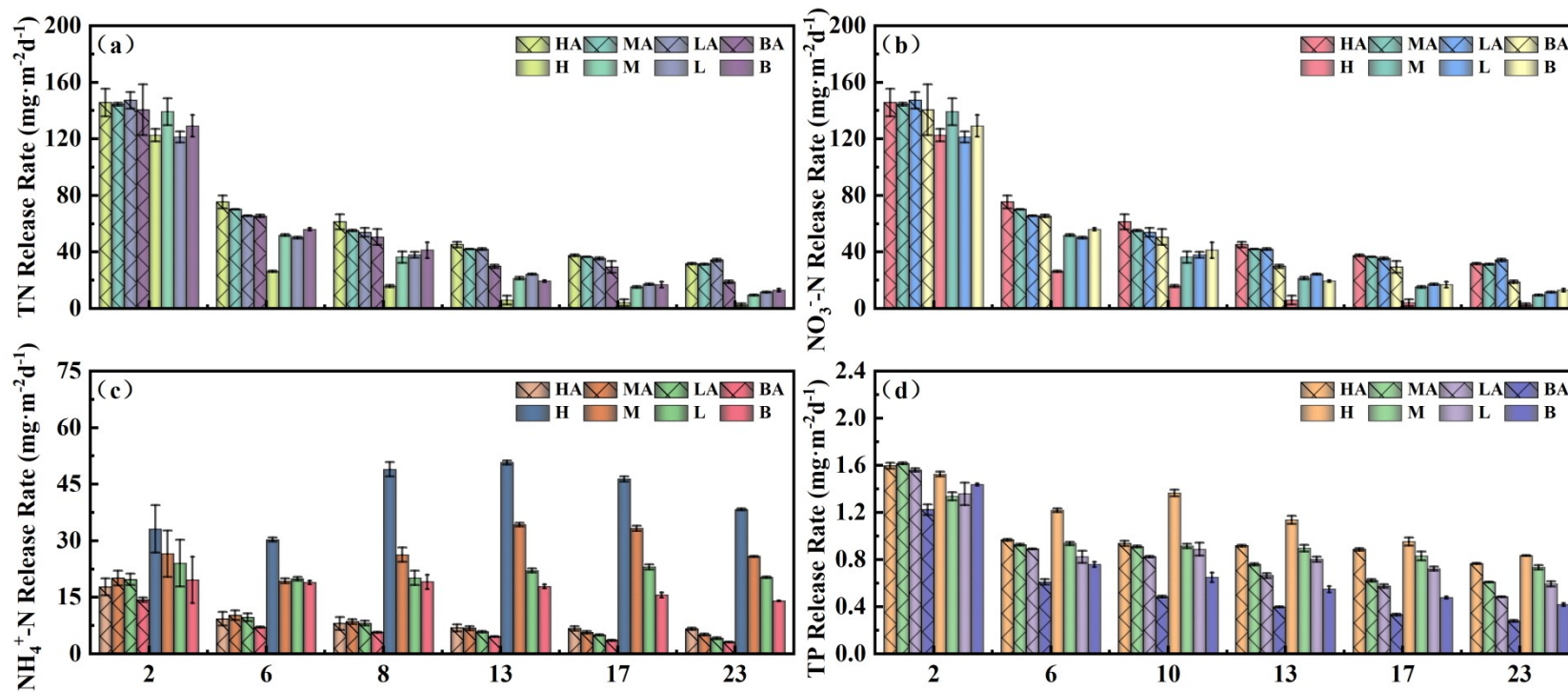


Fig. S3 The release rates of TN (a), $\text{NH}_4^+\text{-N}$ (b), $\text{NO}_3^-\text{-N}$ (c) and TP(d) in the sediments for each experimental group during the incubation of 23 day at 18°C. (HA, MA, LA and BA represent sediment groups with high- (15,000 ind. $\cdot\text{m}^{-2}$), medium- (10,000 ind. $\cdot\text{m}^{-2}$), low-density (5,000 ind. $\cdot\text{m}^{-2}$) *L. hoffmeister* additions, along with a control group without *L. hoffmeisteri* addition under mixed aeration conditions; H, M, L and B represent sediment groups with high-, medium-, low-density *L. hoffmeisteri* additions, and a control group without *L. hoffmeisteri* addition under non-mixed aeration conditions)

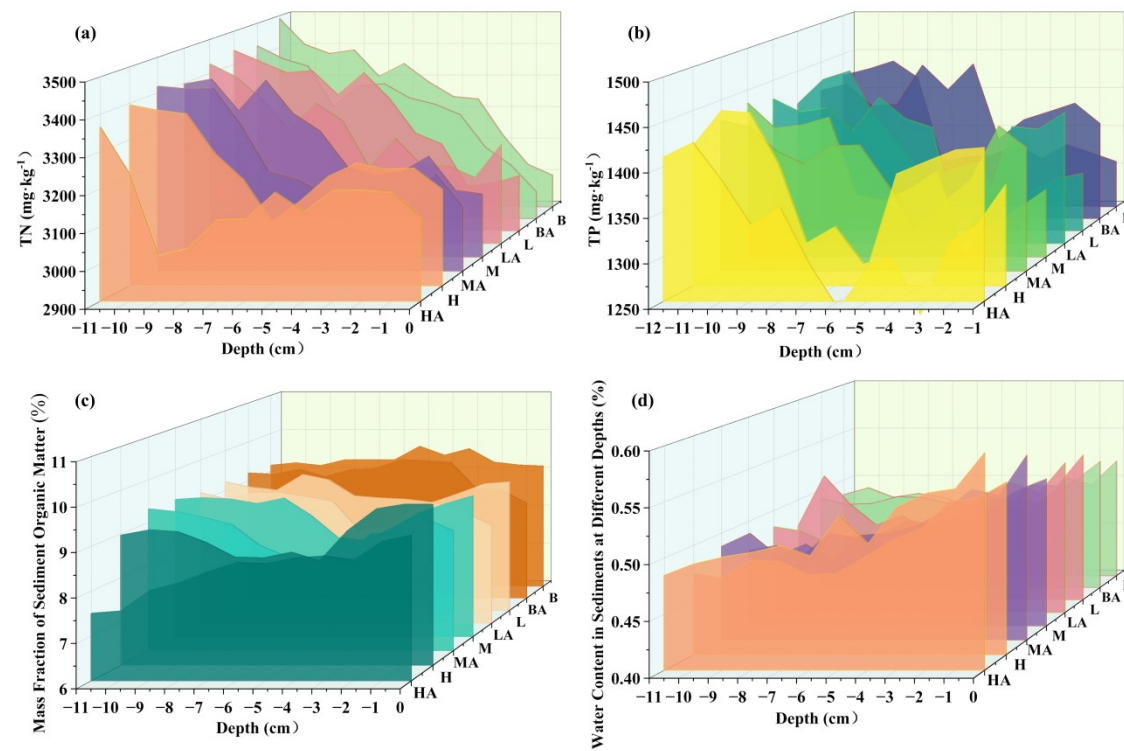


Fig. S4 Vertical variations of TN concentrations (a), TP concentrations (b), mass fraction of organic matter (c) and water content (d) in sediments of each experimental group after the 23 day experiment at 18°C. (HA, MA, LA and BA represent sediment groups with high- (15,000 ind.·m⁻²), medium- (10,000 ind.·m⁻²), low-density (5,000 ind.·m⁻²) *L. hoffmeister* additions, along with a control group without *L. hoffmeisteri* addition under mixed aeration conditions; H, M, L and B represent sediment groups with high-, medium, low-density *L. hoffmeisteri* additions, and a control group without *L. hoffmeisteri* addition under non-mixed aeration conditions)

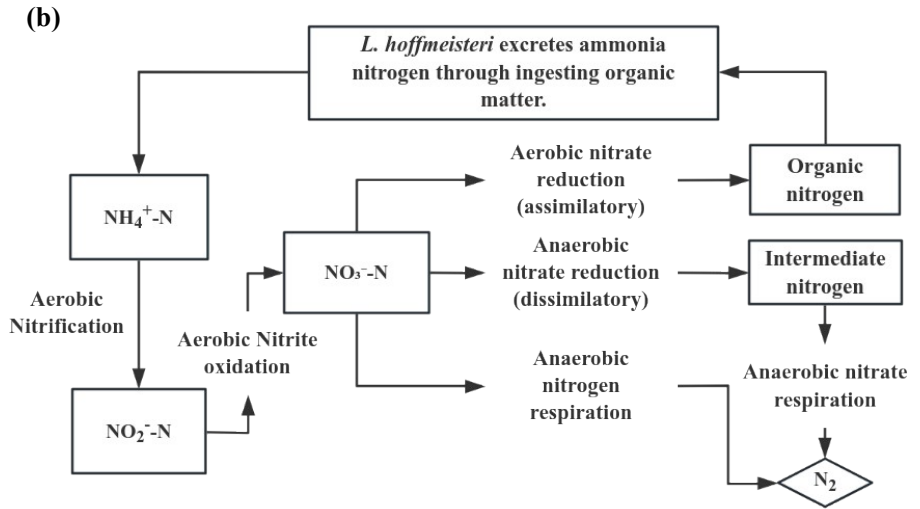
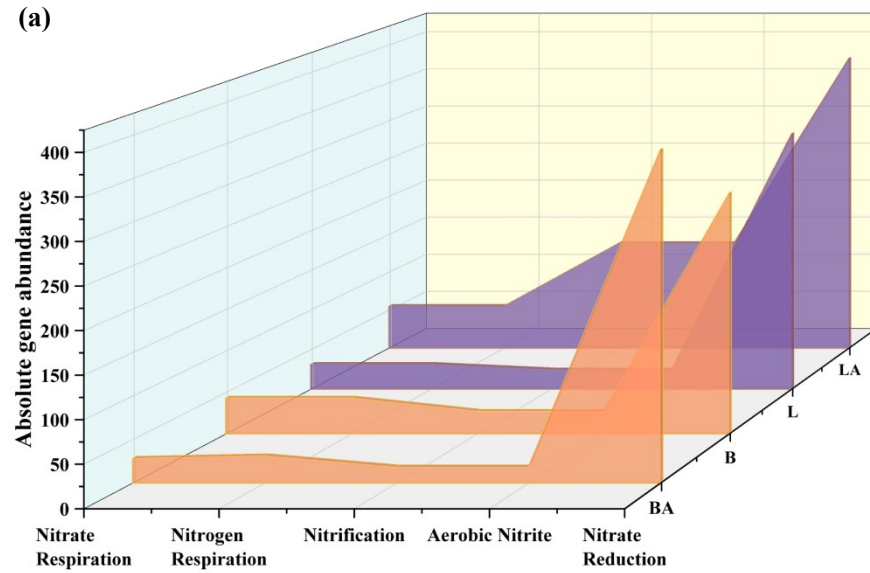


Fig. S5 Fig. S5a shows the abundance of nitrogen cycle-related genes in different groups, and Fig. S5b illustrates the metabolic pathways involved in the nitrogen cycle.

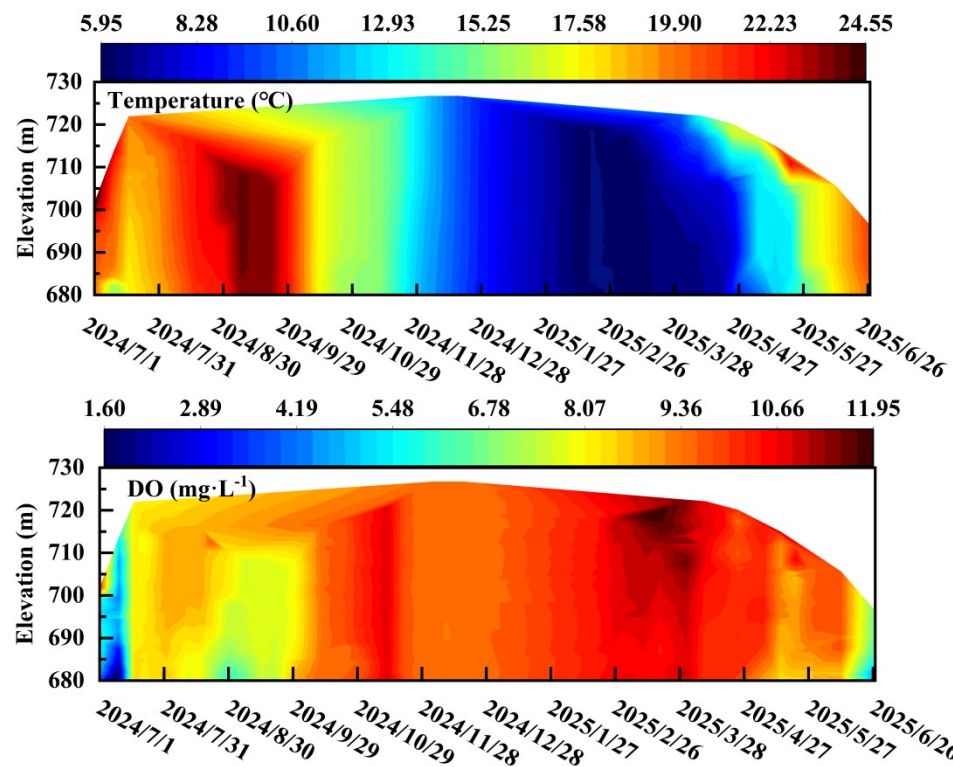


Fig. S6 Variations of dissolved oxygen (a) and temperature (b) in Shibianyu Reservoir from July 2024 to June 2025.

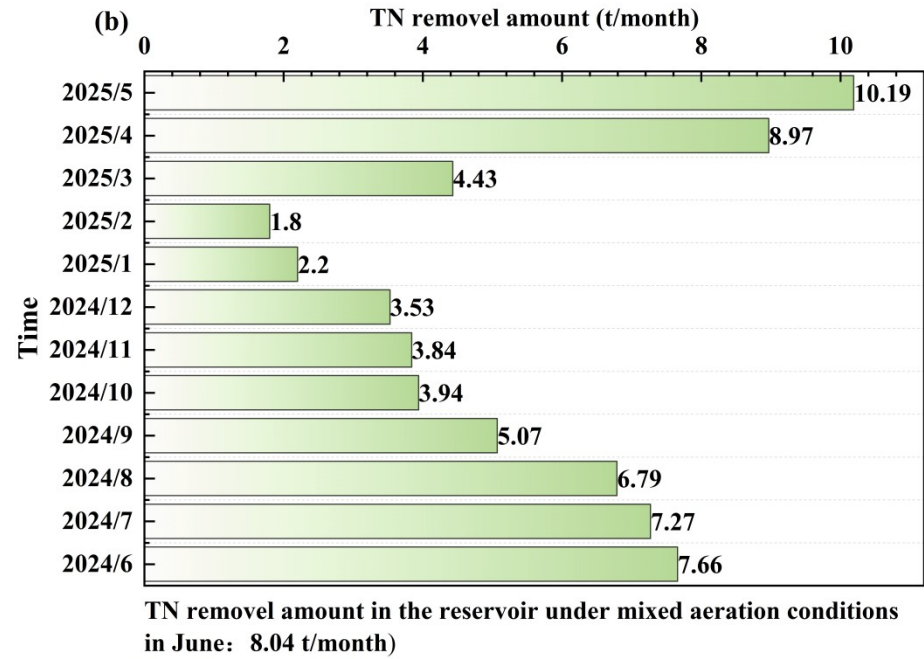
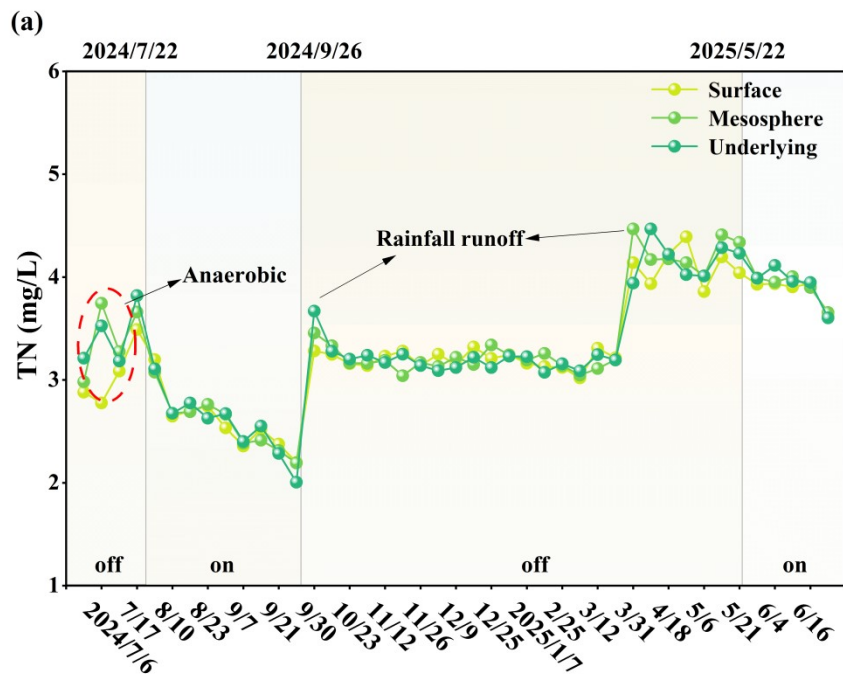


Fig. S7 Changes in TN concentration in the surface, middle, and bottom layers of Shibanyu Reservoir from July 2024 to June 2025 (a), and the TN removal predicted by experiment every month (b).

Table.S1 Water quality indicators of different groups before and after the experiment

Group	HA		MA		LA		H		M		L		B		BA	
Time(d)	0	23	0	23	0	23	0	23	0	23	0	23	0	23	0	23
DO	7.793	8.234	8.493	8.284	8.219	8.322	4.769	0.222	5.779	1.182	4.829	4.418	6.811	5.119	7.851	8.321
pH	7.933	8.134	7.893	7.994	7.989	8.242	7.542	7.459	7.569	7.672	7.779	7.685	8.021	7.577	8.241	8.331
NO₃⁻-N	1.848	3.178	1.805	3.143	1.833	3.430	1.703	0.262	1.784	0.956	1.660	1.161	1.738	1.303	1.822	1.881
NH₄⁺-N	0.193	0.663	0.235	0.510	0.232	0.412	0.360	3.833	0.233	2.585	0.225	2.034	0.178	1.404	0.173	0.312
TN	2.361	3.972	2.239	3.740	2.186	3.623	2.232	4.371	2.201	3.971	2.174	3.820	2.181	3.150	2.249	2.988
TP	0.016	0.077	0.016	0.061	0.017	0.048	0.015	0.083	0.015	0.073	0.015	0.059	0.014	0.042	0.014	0.028