



Supplement of

Macro constraints and local eutrophication shape sediment nitrogen removal in the Eastern China Plain lakes

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Supplementary Methods

Section S1 Overview of the Eastern China Plain lakes region

The Eastern China Plain lakes region administratively encompasses 19 provincial-level divisions across eastern, central, and southern China, including Jiangxi, Hunan, Hubei, Anhui, Henan, Jiangsu, Shanghai, Shandong, Hebei, Beijing, Tianjin, Zhejiang, Taiwan, Hong Kong, Macau, Hainan, Fujian, Guangdong, and Guangxi. This region hosts 634 lakes with surface areas exceeding 1 km² and notably contains China's five largest freshwater lakes: Poyang, Dongting, Taihu, Hongze, and Chaohu. Limnologically, the lakes in this region are predominantly shallow, with mean depths typically ranging from 1 to 6 m (Ma et al., 2010). The 17 representative lakes selected for this study are primarily distributed across two strategic zones. (1) The Middle and Lower Reaches of the Yangtze River (MLRYR): Comprising 10 lakes, namely Lakes Poyang, Dongting, Taihu, Chaohu, Donghu, Nanyi, and Gucheng, as well as the Fangbian, Zhongshan, and Yaojia Reservoirs. (2) Along the Eastern Route of the South-to-North Water Diversion Project (ER-SNWD): Comprising 7 lakes, namely Lakes Shaobo, Gaoyou, Hongze, Luoma, Nansi, Dongping, and Hengshui.

The study area exhibits distinct climatic gradients. The MLRYR region is characterized by a typical subtropical monsoon climate. The mean annual temperature (MAT) ranges from 14 to 18°C, with the coldest monthly means around 0°C and the warmest between 27 and 28°C. The annual frost-free period lasts 210–270 days. Mean annual precipitation (MAP) exhibits an increasing spatial gradient from the lower reaches (~1,000 mm) to the middle reaches (~1,500 mm) (Guo et al., 2022). While the southern portion of the ER-SNWD region shares similar climatic conditions with the MLRYR, the northernmost lakes—specifically Hengshui, Dongping, and Nansi—are influenced by a temperate monsoon climate, characterized by relatively lower temperatures and precipitation. Specifically, the Hengshui Lake basin records a MAP of 518.9 mm and a MAT of 13.0°C (Zhang et al., 2024). Similarly, the Dongping and Nansi Lake basins exhibit MAP values of 624.4 mm and 685 mm, with MAT values of 13.6°C and 14.2°C, respectively (Wei et al., 2024).

Section S2 Calculation formula for the Comprehensive Trophic Level

Index (TLI)

The TLI is calculated as follows (Wang et al., 2019):

$$TLI(\Sigma) = \sum_{j=1}^m W_j \cdot TLI(j) \quad (1)$$

$$TLI(Chl.a) = 10(2.5 + 1.086 \ln Chl.a) \quad (2)$$

$$TLI(TP) = 10(9.436 + 1.624 \ln TP) \quad (3)$$

$$TLI(TN) = 10(5.453 + 1.694 \ln TN) \quad (4)$$

$$TLI(SD) = 10(5.118 - 1.94 \ln SD) \quad (5)$$

$$TLI(COD_{Mn}) = 10(0.109 + 2.661 \ln COD_{Mn}) \quad (6)$$

where $TLI(\Sigma)$ represents the aggregated comprehensive trophic level index; $TLI(j)$ denotes the individual trophic index of the j -th parameter; and W_j is the weighting factor for the j -th parameter. Specifically, the weights assigned to $Chl.a$, TP , TN , SD , and COD_{Mn} were 0.2663, 0.1879, 0.1790, 0.1834, and 0.1834, respectively (units: $\mu g/L$, mg/L , mg/L , m , and mg/L).

Based on the calculated TLI scores, the trophic status was classified into five categories: oligotrophic ($0 \leq TLI \leq 30$), mesotrophic ($30 < TLI \leq 50$), light eutrophic ($50 < TLI \leq 60$), moderate eutrophic ($60 < TLI \leq 70$), and hyper-eutrophic ($70 < TLI \leq 100$).

Section S3 Land use data sources, preprocessing, and calculations for the Human Activity Intensity (HAI)

Landsat remote sensing images (cloud cover $< 10\%$) from October to November 2024 were acquired from the Geospatial Data Cloud (<http://www.gscloud.cn/>) covering all study lakes and surrounding areas. Following preprocessing steps including radiometric calibration, geometric correction, and cropping, land use was classified into six categories based on the Chinese Academy of Sciences classification system: cultivated land, forest, grassland, water, construction land, and unused land. Interpretation was performed using the Support Vector Machine (SVM) supervised classification method. Post-classification verification confirmed an overall accuracy exceeding 92% (Zhu et al., 2015). Land use data were subsequently extracted for 2 km and 5 km buffer zones

surrounding each lake to quantify human activity intensity.

The HAI is calculated as follows:

$$HAI = \frac{S_{CLE}}{S} \times 100\% \quad (7)$$

$$S_{CLE} = \sum_{i=1}^n (SL_i \cdot CI_i) \quad (8)$$

where HAI represents the human activity intensity; S_{CLE} is the total area of construction land equivalents; S is the total area of the region; SL_i is the area of the i -th land use type; CI_i is the construction land equivalent conversion coefficient for the i -th land use type (Tab. S4); and n is the number of land use types in the region.

Section S4 Detailed protocol for isotope slurry incubations, calculation principles, and formulas

1. Slurry preparation and pre-incubation

Sediment slurries were prepared by mixing homogenized sediment with site water at a mass ratio of 1:7 in 500 mL serum bottles. To eliminate background dissolved oxygen (DO), the slurries were purged with high-purity helium (He) for 30 min. Following purging, the bottles were sealed, wrapped in aluminum foil to maintain darkness, and pre-incubated for 48 h in a constant-temperature water bath set to the in situ temperature. This step was performed to deplete residual background $^{14}\text{NO}_3\text{-N}$ and DO.

2. ^{15}N tracer addition

After pre-incubation, the slurries were vigorously shaken, and a 10 mL aliquot was filtered to determine the background $\text{NO}_3\text{-N}$ concentration. Subsequently, a $\text{Na}^{15}\text{NO}_3$ solution (concentration: 0.4 mg/L) was added to the serum bottles. The slurries were mixed thoroughly, and another 10 mL aliquot was filtered. The actual ^{15}N enrichment in the slurry was calculated based on the difference in $\text{NO}_3\text{-N}$ concentrations between the two filtrations.

3. Incubation procedure

Following isotope addition, the headspace was purged with high-purity He for 5 min to ensure anaerobic conditions. Under continuous He flow, the slurries were transferred via a peristaltic pump into 12 mL gas-tight glass vials (Labco Exetainer, UK), ensuring

no headspace remained. Microbial activity in the initial samples (0 h) was immediately terminated by injecting 0.2 mL of saturated ZnCl₂ solution (50% w/v). The remaining vials were wrapped in aluminum foil and incubated in the dark at *in situ* temperature for 4 h. At the end of the incubation, ZnCl₂ was added to terminate the reaction. All treatments were performed in triplicate.

4. Calculation Principles and formulas

The two N atoms required for anammox to produce N₂ are supplied by NO₃-N and NH₃-N, respectively. Since NH₃-N was not isotopically labeled in this experiment, anammox utilized only ¹⁴NO₃-N and ¹⁵NO₃-N to generate ²⁸N₂ and ²⁹N₂, respectively. Conversely, denitrification produces ²⁸N₂, ²⁹N₂, and ³⁰N₂ through the addition of Na¹⁵NO₃. The rates were calculated as follows:

$$V_{\text{den}} = (C_{30}^* - C_{30}) \times \frac{k}{4} / F_n^2 \times 1000 \quad (9)$$

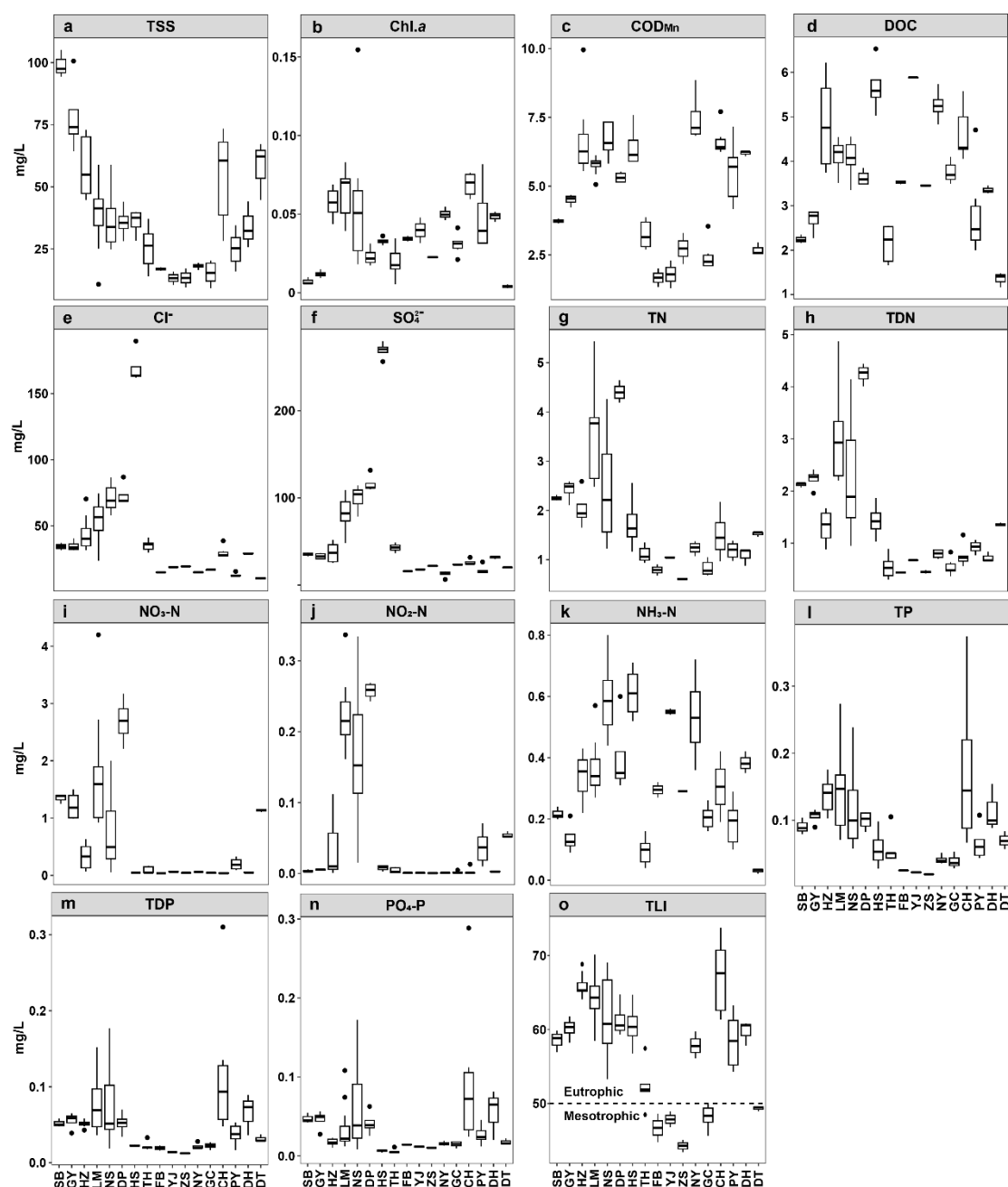
$$V_{\text{ana}} = [(C_{29}^* - C_{29}) + 2 \times (1 - 1/F_n) \times (C_{30}^* - C_{30})] \times \frac{k}{4} / F_n \times 1000 \quad (10)$$

Where: V_{den} and V_{ana} are the rates of denitrification and anammox (nmol·g⁻¹·h⁻¹), respectively; C_{30}^* and C_{30} are the concentrations of ³⁰N₂ (μmol/L) at 4 h and 0 h, respectively (analogous for C_{29}^* and C_{29}); k represents the ratio of slurry volume to sediment mass in the vial; and F_n is the ¹⁵N labeling fraction of the nitrate pool.

Total nitrogen removal rate was defined as the sum of V_{den} and V_{ana} . The relative contribution of anammox (Ra) was calculated as:

$$Ra = V_{\text{ana}} / (V_{\text{den}} + V_{\text{ana}}) \quad (11)$$

145 **Supplementary Figures**



147 **Figure S1 Water quality parameters of the Eastern China Plain Lakes**

148 Note: Total suspended solids (TSS), chlorophyll a (Chl.a), permanganate index
 149 (COD_{Mn}), dissolved organic carbon (DOC), chloride ion (Cl⁻), sulfate ion (SO₄²⁻), total
 150 nitrogen (TN), total dissolved nitrogen (TDN), nitrate nitrogen (NO₃-N), nitrite
 151 nitrogen (NO₂-N), ammonia nitrogen (NH₃-N), total phosphorus (TP), total dissolved
 152 phosphorus (TDP), orthophosphate (PO₄-P), and Comprehensive Trophic Level Index
 153 (TLI).

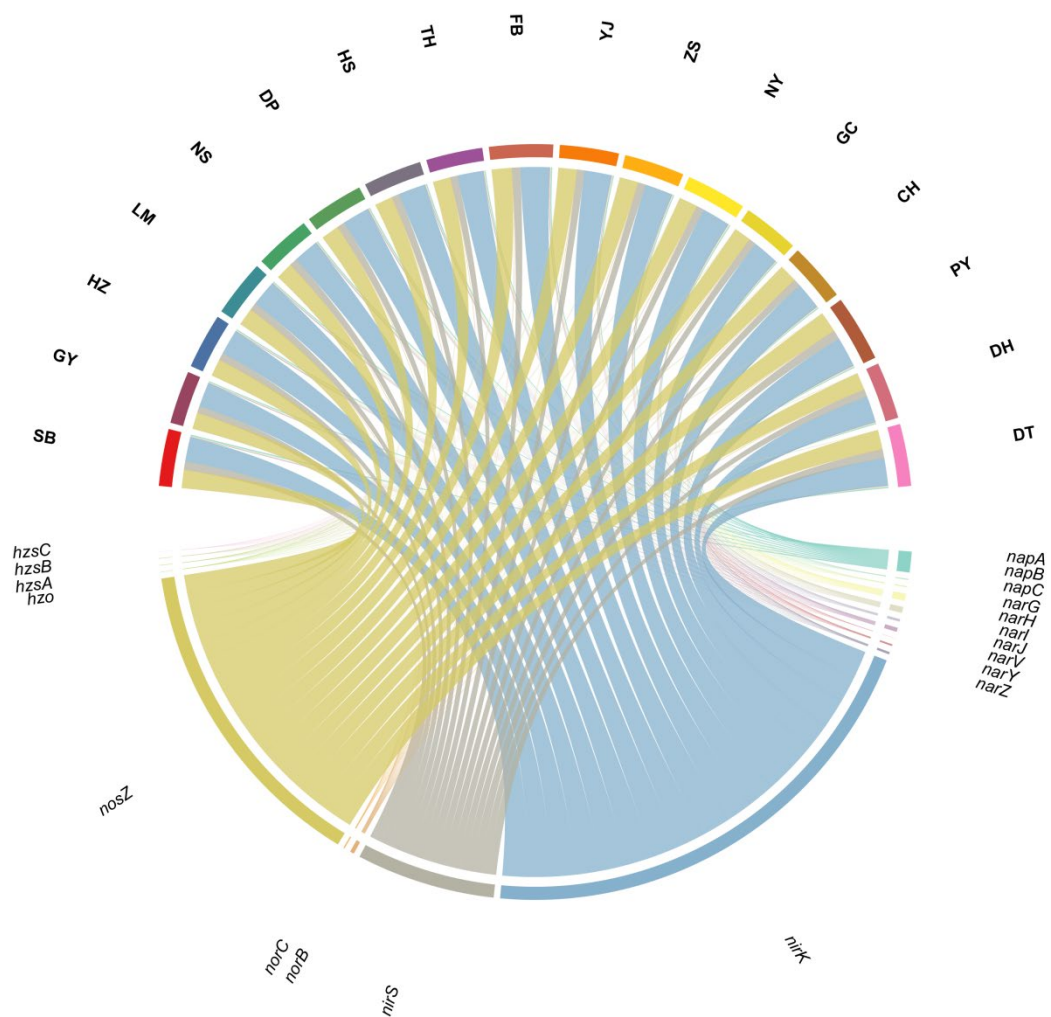


Figure S2 Relative abundance of nitrogen removal functional genes in sediments of the Eastern China Plain Lakes

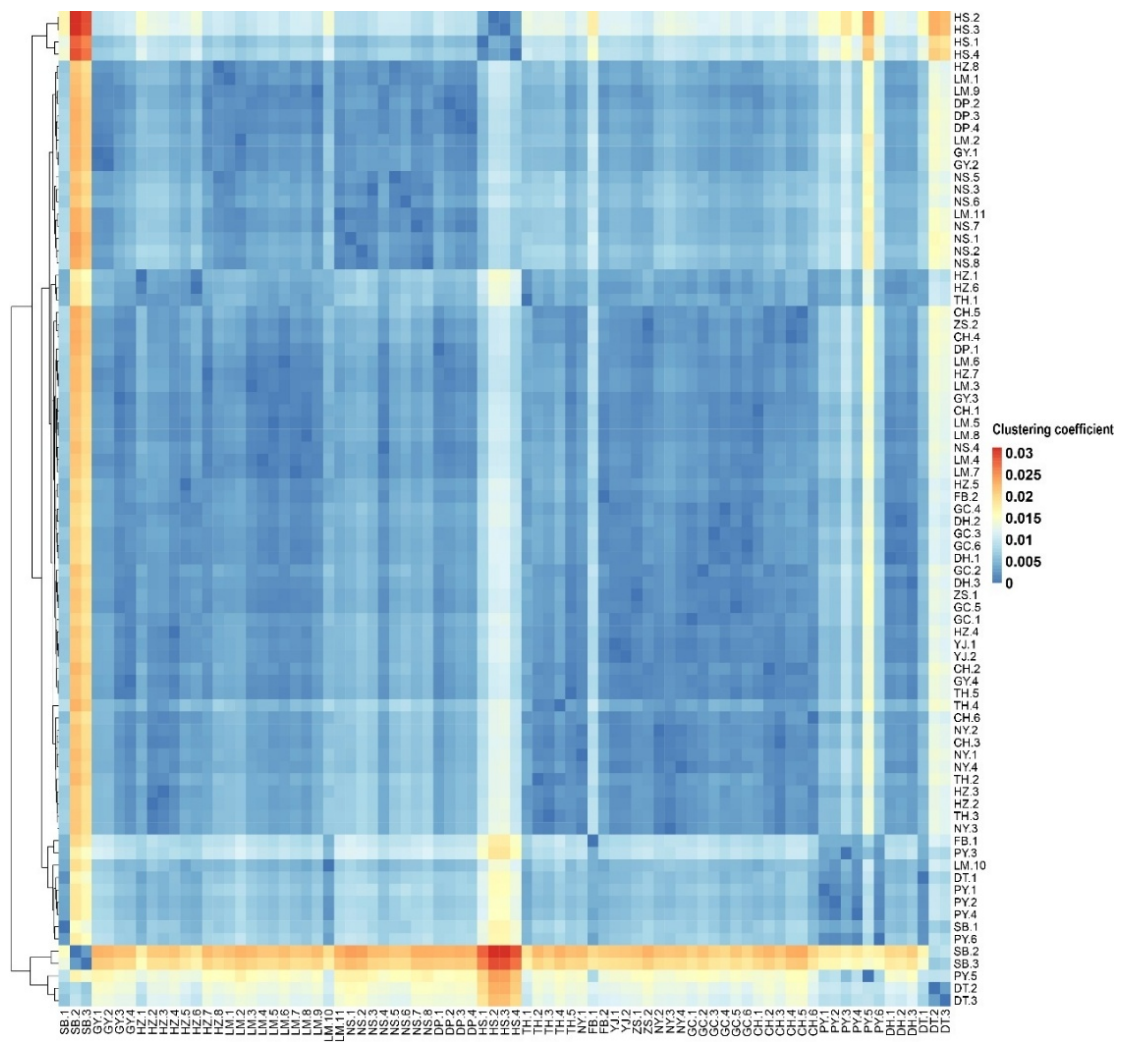


Figure S3 Similarity matrix of nitrogen removal functional genes in sediments of the Eastern China Plain Lakes and hierarchical clustering of sampling sites

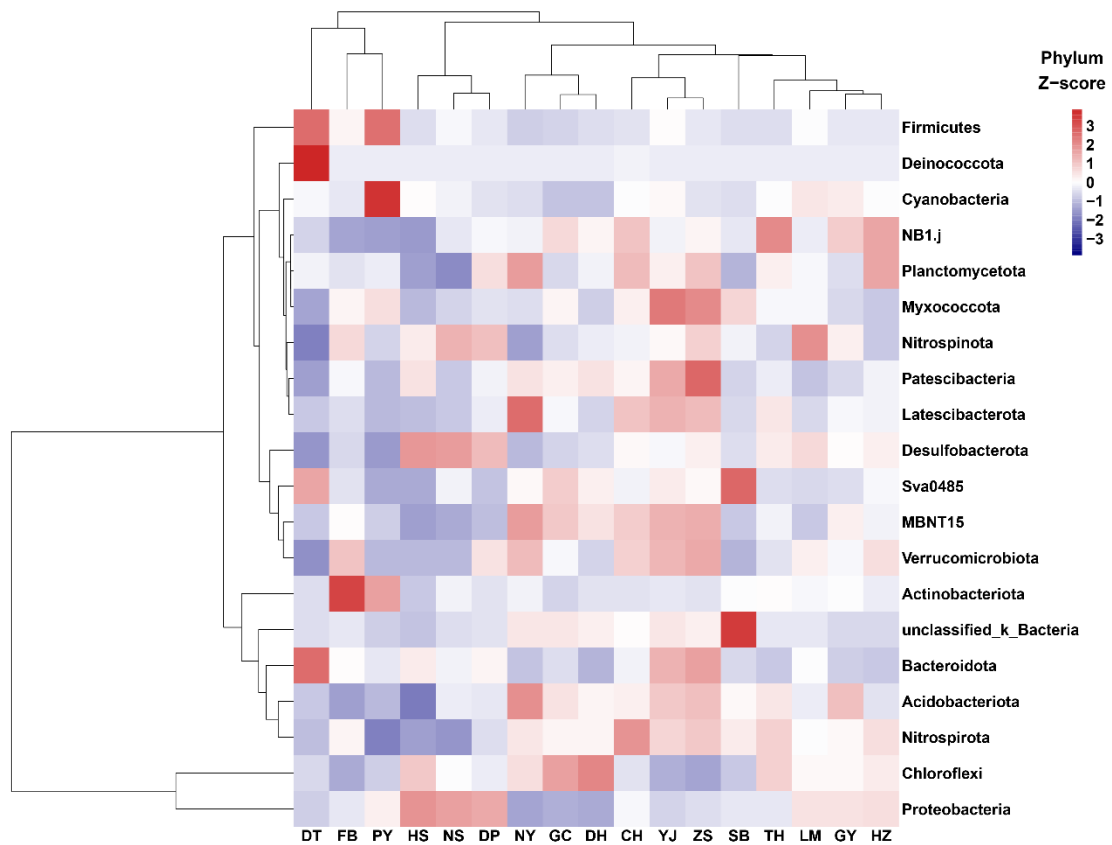


Figure S4 Relative abundance cluster analysis of the Top 20 phylum microbial in sediments of the Eastern China Plain Lakes

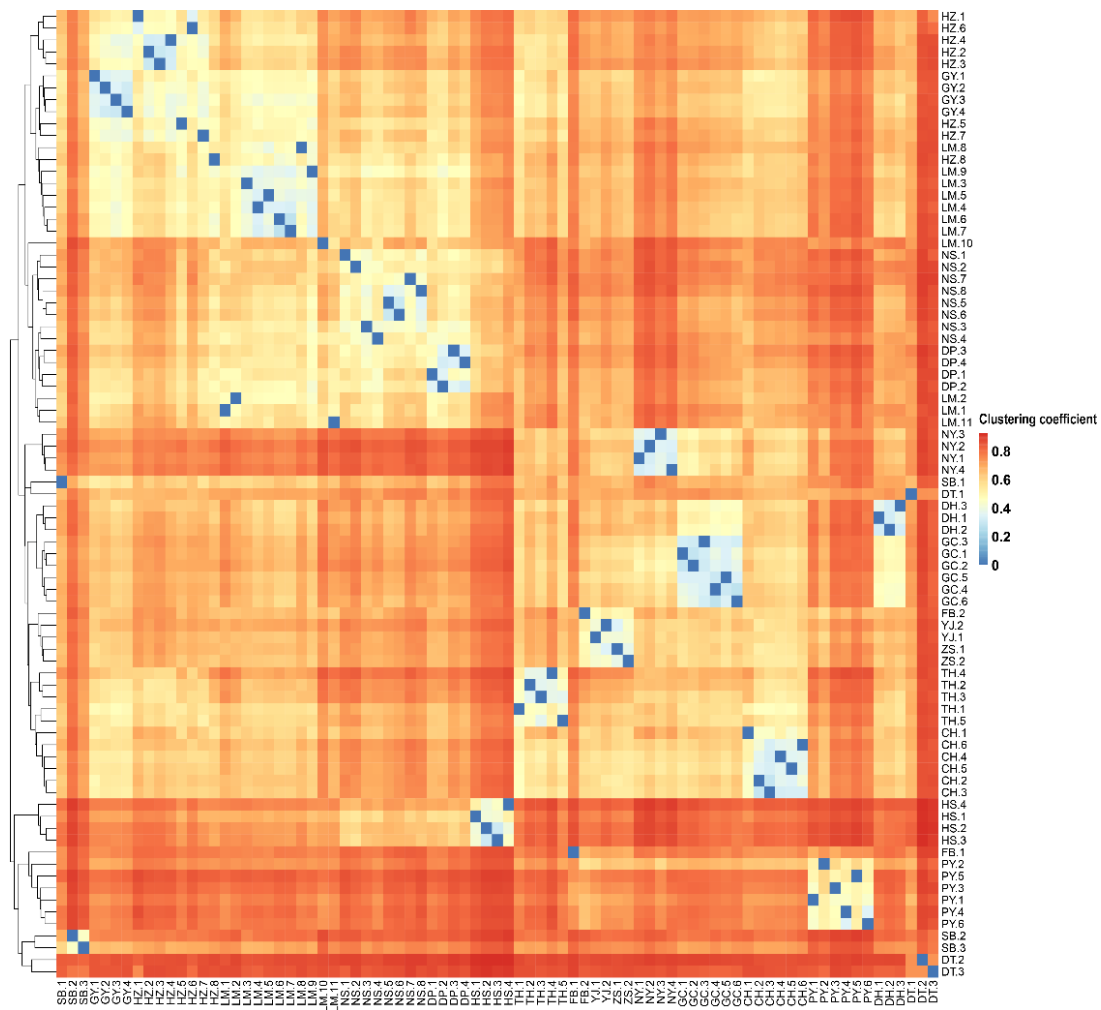


Figure S5 Similarity matrix of microbial communities in sediments of the Eastern China Plain Lakes and hierarchical clustering of sampling sites

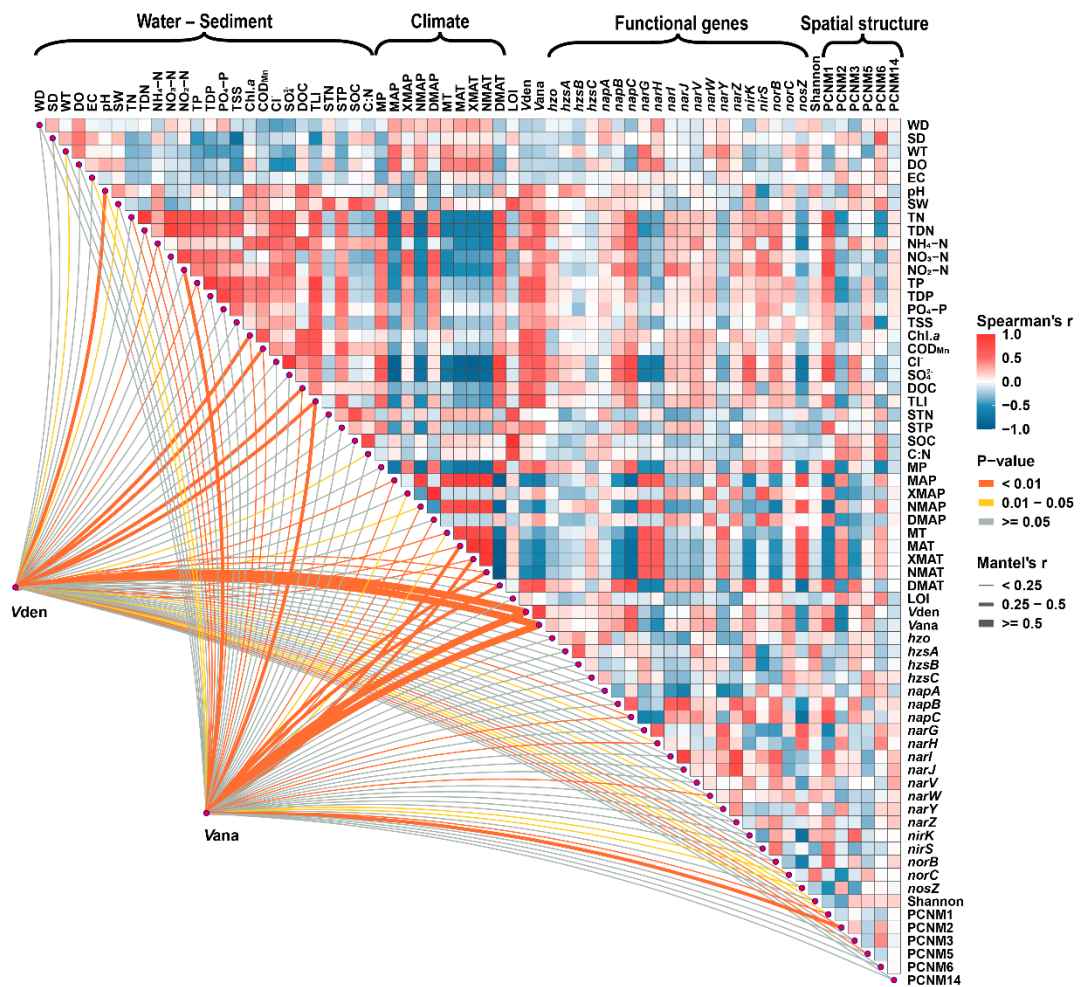


Figure S6 Network of interactions linking nitrogen removal rates with environmental factors, functional genes, and microbial communities, revealed by Spearman's correlations and Mantel tests

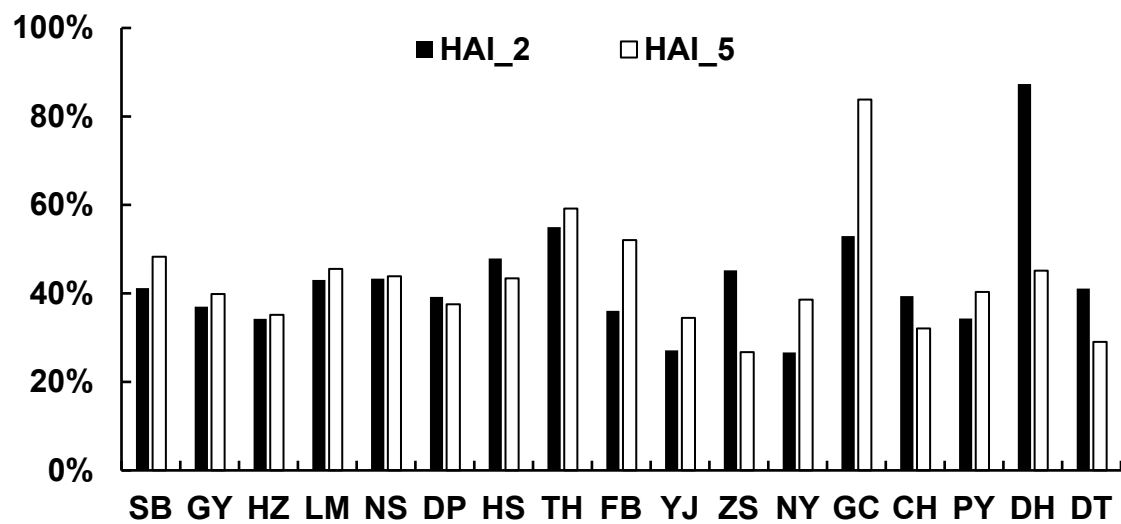


Figure S7 Human activity intensity within 2-km and 5-km buffer zones surrounding the Eastern China Plain Lakes

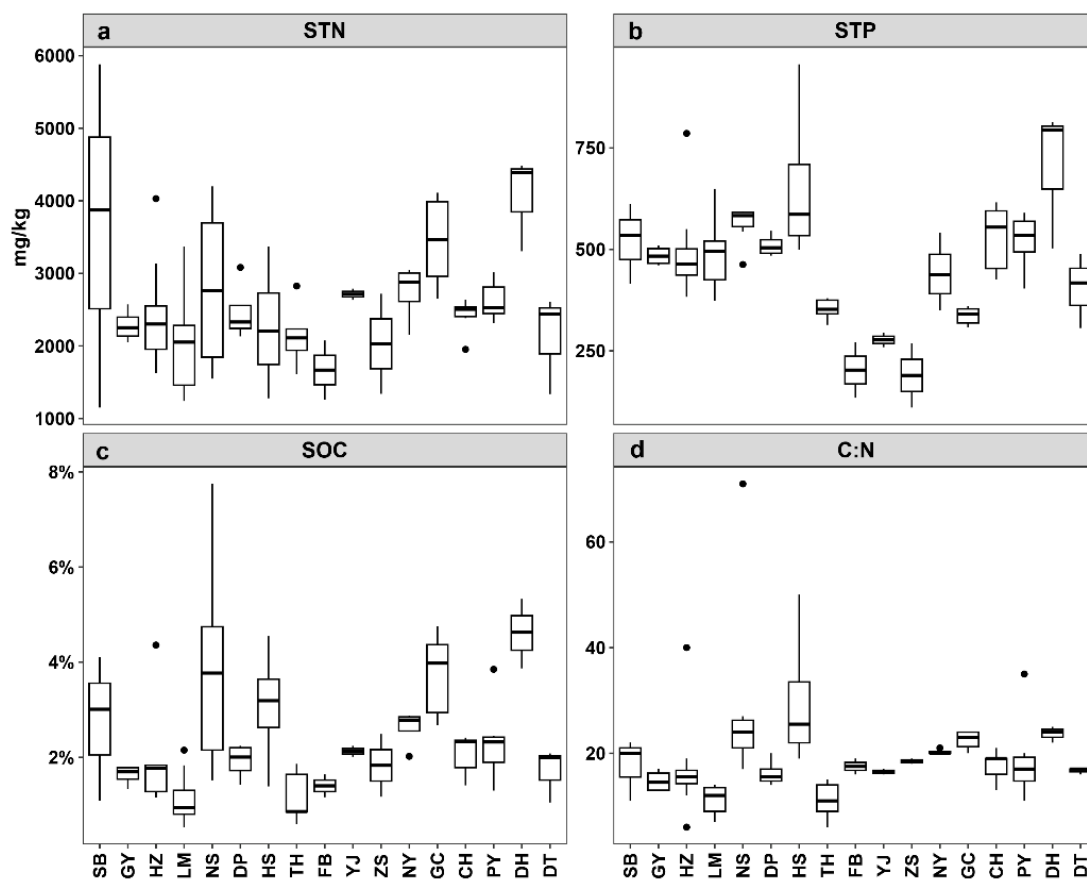


Figure S8 Sediment parameters of the Eastern China Plain Lakes

Note: Total nitrogen in sediments (STN), total phosphorus in sediments (STP), total organic carbon in sediments (SOC), and calculating the C:N ratio of sediments based on the ratio of STN to SOC.

180 **Supplementary Tables**

181 **Table S1 Location of lakes and number of sampling sites**

No.	Lake name	Name abbreviation	Longitude/°	Latitude/°	Area /km ²	Mean water depth /m	Number of sampling sites
1	Lake Shaobo	SB	119.493333	32.523056	63.2	1.9	3
2	Lake Gaoyou	GY	119.193334	32.753485	760.7	1.5	4
3	Lake Hongze	HZ	118.814649	33.320416	1775.0	1.8	7
4	Lake Luoma	LM	118.113251	34.133221	290.0	3.3	11
5	Lake Nansi	NS	116.623005	35.257664	1266.0	2.6	8
6	Lake Dongping	DP	116.230180	35.936334	124.0	2.5	4
7	Lake Hengshui	HS	115.628872	37.639519	75.0	1.7	4
8	Lake Taihu	TH	120.192694	31.409092	2338.1	1.9	5
9	Fangbian reservoir	FB	119.131537	31.688846	4.9	3.6	2
10	Yaojia reservoir	YJ	119.104672	31.562082	1.7	6.3	2
11	Zhongshan reservoir	ZS	119.069223	31.640595	3.2	5.5	2
12	Lake Nanyi	NY	118.889108	31.108874	200.0	2.2	4
13	Lake Gucheng	GC	118.889237	31.308475	37.0	4.2	6
14	Lake Chaohu	CH	117.362960	31.682970	780.0	3.0	6
15	Lake Poyang	PY	116.140549	29.578348	3950.0	8.4	6
16	Lake Donghu	DH	114.369986	30.548226	33.0	2.5	3
17	Lake Dongting	DT	113.009083	29.342002	2625.0	6.7	3

Note: The longitude and latitude listed in the table represent only a specific site for each lake (including reservoir).

182 **Table S2 Environmental physicochemical parameters and determination methods**

Parameter	Unit	Measurement method
Water Depth (WD)	cm	Speedtech SM-5 depth sounder
Secchi depth (SD)	cm	Secchi disk
Water temperature (WT)	°C	portable multi-parameter water quality meter (YSI Professional Plus, USA)
Dissolved oxygen (DO)	mg/L	
Electrical conductivity (EC)	μS/cm	
pH	/	
Total nitrogen (TN)	mg/L	Alkaline Potassium Persulfate Digestion—UV Spectrophotometry
Total dissolved nitrogen (TDN)	mg/L	
Ammonia nitrogen (NH ₃ -N)	mg/L	Nessler's Reagent—UV Spectrophotometry
Nitrate nitrogen (NO ₃ -N)	mg/L	UV Spectrophotometry
Nitrite nitrogen (NO ₂ -N)	mg/L	
Total phosphorus (TP)	mg/L	Potassium Persulfate Digestion Ammonium Molybdate—UV Spectrophotometry
Total dissolved phosphorus (TDP)	mg/L	
Orthophosphate (PO ₄ -P)	mg/L	Ammonium Molybdate—UV Spectrophotometry
Total suspended solids (TSS)	mg/L	Filter Membrane Drying and Weighing Method
Permanganate index (COD _{Mn})	mg/L	Potassium Permanganate Titration
Chloride ion (Cl ⁻)	mg/L	Silver Nitrate Titration
Sulfate ion (SO ₄ ²⁻)	mg/L	Barium Chromate—UV Spectrophotometry
Chlorophyll a (Chl. <i>a</i>)	ug/L	Alcohol (95%) Extraction—UV Spectrophotometry
Dissolved organic carbon (DOC)	mg/L	Tekmar Torch Total Organic Carbon Analyzer
Water content in sediments (SW, %)	/	Weight Method
Total nitrogen in sediments (STN)	mg/kg	Alkaline Potassium Persulfate Digestion—UV Spectrophotometry
Total phosphorus in sediments (STP)	mg/kg	Potassium Persulfate Digestion Ammonium Molybdate—UV Spectrophotometry
Total organic carbon in sediments (SOC, %)	/	Euro Vector EA3000 Fully Automated Elemental Analyzer

Note: All colorimetric assays were performed using a UV-visible spectrophotometer (UV-1102 II, Techcomp, China).

184 **Table S3 Climate data for the Eastern China Plain lakes**

Lake	MP /mm	MAP /mm	XMAP /mm	NMAP /mm	DMAP /mm	MT /°C	MAT /°C	XMAT /°C	NMAT /°C	DMAT /°C
SB	338.9	99.7	453.5	2.5	451	30	16.8	29.4	3.6	25.8
GY	338.9	99.7	453.5	2.5	451	30	16.8	29.4	3.6	25.8
HZ	162	93.2	444.6	2.9	441.6	29.8	16.6	29.2	3.1	26.2
LM	191.4	89.1	450.3	1.9	448.4	29.8	16.3	29	2	27
NS	189.7	77.6	442.7	0.8	441.9	29.3	16.1	28.9	1.3	27.6
DP	185.8	64.3	364.3	0.4	363.8	28.9	15.9	28.5	0.8	27.8
HS	202.5	50.3	248.5	0.5	248	28.4	15.3	28.4	-0.6	29
TH	108	106.6	301.1	10.2	290.9	30.2	17.4	29.7	5	24.7
FB	118.4	108.1	372.9	5.2	367.7	30.4	17.3	29.8	4.5	25.3
YJ	118.4	108.1	372.9	5.2	367.7	30.4	17.3	29.8	4.5	25.3
ZS	118.4	108.1	372.9	5.2	367.7	30.4	17.3	29.8	4.5	25.3
NY	134.3	115.2	331.9	8.1	323.8	30.7	17.7	30	4.9	25.1
GC	134.3	115.2	331.9	8.1	323.8	30.7	17.7	30	4.9	25.1
CH	160.9	99.5	318.1	5.4	312.6	30.8	17.7	30	4.7	25.4
PY	133.6	150.6	467	4	463	31.8	19.3	31.1	6.9	24.2
DH	31.5	104	316.2	4.4	311.8	31.1	18.3	30.5	5.5	25
DT	54.3	113.1	386.4	6.1	380.3	26.3	18.8	30.6	6.5	24.1

Note: The monthly mean precipitation (MP) and mean temperature (MT) were extracted for the sampling months, mean annual precipitation (MAP), maximum (XMAP) and minimum (NMAP) monthly precipitation, difference between XMAP and NMAP (DMAP), mean annual temperature (MAT), maximum (XMAT) and minimum (NMAT) monthly temperatures, and difference between XMAT and NMAT (DMAT).

Table S4 Conversion index (*CI*) of construction land equivalent of different land use types

Land use type	Characteristic indicator description	<i>CI</i>
cultivated land	Surface natural vegetation altered—cultivated with annual crops	0.2
forest	Surface natural vegetation altered—cultivated with perennial plants	0.133
grassland	Surface natural vegetation unchanged but utilized	0.067
water	Surface natural vegetation unchanged and unused	0
construction land	Surface layer with artificial barrier, impeding water, nutrient, air, and heat exchange	1
unused land	Surface natural cover unchanged and unused	0

189 **Table S5 Nitrogen removal rates in sediments of the Eastern China**
190 **Plain Lakes**

Lake	V_{den} (mean $\pm$ SE)	V_{ana} (mean $\pm$ SE)	$Ra/\%$ (mean $\pm$ SE)
SB	66.98 $\pm$ 7.49	14.93 $\pm$ 1.74	22.33 $\pm$ 1.94
GY	64.55 $\pm$ 5.20	14.48 $\pm$ 2.56	22.34 $\pm$ 2.85
HZ	117.54 $\pm$ 17.95	31.54 $\pm$ 8.67	26.73 $\pm$ 6.43
LM	101.18 $\pm$ 10.28	30.27 $\pm$ 6.19	29.96 $\pm$ 5.46
NS	111.97 $\pm$ 15.69	28.47 $\pm$ 5.14	25.46 $\pm$ 3.48
DP	71.61 $\pm$ 8.77	24.67 $\pm$ 4.69	34.34 $\pm$ 4.38
HS	78.39 $\pm$ 12.22	13.56 $\pm$ 4.45	17.03 $\pm$ 3.98
TH	47.42 $\pm$ 12.48	6.01 $\pm$ 3.06	12.64 $\pm$ 5.00
FB	46.96 $\pm$ 2.89	4.16 $\pm$ 1.93	9.00 $\pm$ 4.66
YJ	57.76 $\pm$ 1.43	11.63 $\pm$ 0.53	20.15 $\pm$ 1.42
ZS	57.42 $\pm$ 10.72	4.55 $\pm$ 3.07	8.58 $\pm$ 6.95
GC	75.06 $\pm$ 7.55	11.25 $\pm$ 4.02	15.19 $\pm$ 5.67
NY	76.19 $\pm$ 5.33	13.67 $\pm$ 1.45	18.00 $\pm$ 2.29
CH	112.23 $\pm$ 27.78	19.67 $\pm$ 8.26	17.64 $\pm$ 5.19
PY	40.64 $\pm$ 12.98	6.75 $\pm$ 2.97	16.37 $\pm$ 4.13
DH	93.52 $\pm$ 9.49	17.73 $\pm$ 1.58	18.99 $\pm$ 0.87
DT	26.58 $\pm$ 4.84	2.50 $\pm$ 0.90	9.28 $\pm$ 2.65

Note: The units for V_{den} and V_{ana} are $\text{nmol N} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$.

191

192 **Table S6 Physical parameters of water and sediments in Eastern China**

193 **Plain lakes (mean $\pm$ SE)**

Lake	WD/m	SD/cm	WT/°C	DO/(mg/L)	EC/(μ S/cm)	pH	SW/%
SB	4.2 $\pm$ 0.6	11.7 $\pm$ 2.9	31.0 $\pm$ 0.2	5.3 $\pm$ 0.4	160.2 $\pm$ 0.3	8.5 $\pm$ 0.1	43.0 $\pm$ 12.3
GY	3.7 $\pm$ 0.2	17.8 $\pm$ 2.1	31.3 $\pm$ 0.8	5.1 $\pm$ 0.3	170.4 $\pm$ 221.7	8.5 $\pm$ 0.1	50.7 $\pm$ 6.8
HZ	2.5 $\pm$ 0.3	22.5 $\pm$ 4.1	31.8 $\pm$ 0.4	5.8 $\pm$ 0.9	208.4 $\pm$ 276.6	8.8 $\pm$ 0.2	53.9 $\pm$ 9.1
LM	5.5 $\pm$ 3.9	45.5 $\pm$ 13.1	31.3 $\pm$ 0.3	7.2 $\pm$ 1.7	237.8 $\pm$ 249.3	8.9 $\pm$ 0.3	52.4 $\pm$ 9.7
NS	3.0 $\pm$ 0.9	65.0 $\pm$ 41.0	30.8 $\pm$ 0.6	4.9 $\pm$ 1.2	236.2 $\pm$ 324.5	8.7 $\pm$ 0.3	62.3 $\pm$ 12.3
DP	4.6 $\pm$ 2.9	46.5 $\pm$ 18.9	31.0 $\pm$ 0.2	7.1 $\pm$ 0.3	229.5 $\pm$ 368.6	8.8 $\pm$ 0.2	57.3 $\pm$ 3.8
HS	1.7 $\pm$ 0.3	24.0 $\pm$ 1.2	30.5 $\pm$ 0.9	3.8 $\pm$ 0.9	215.1 $\pm$ 195.7	8.7 $\pm$ 0.2	54.2 $\pm$ 15.6
TH	2.3 $\pm$ 0.1	38.4 $\pm$ 6.1	32.1 $\pm$ 0.9	5.3 $\pm$ 0.8	359.4 $\pm$ 36.9	8.8 $\pm$ 0.3	52.6 $\pm$ 6.3
FB	3.6 $\pm$ 0.3	52.5 $\pm$ 6.4	31.3 $\pm$ 0.0	6.0 $\pm$ 0.7	259.1 $\pm$ 0.4	7.9 $\pm$ 0.2	43.5 $\pm$ 10.6
YJ	6.3 $\pm$ 0.2	52.0 $\pm$ 7.1	32.0 $\pm$ 0.2	8.9 $\pm$ 0.1	282.6 $\pm$ 0.6	8.8 $\pm$ 0.0	62.5 $\pm$ 2.1
ZS	5.5 $\pm$ 2.8	93.5 $\pm$ 6.4	31.8 $\pm$ 0.0	8.2 $\pm$ 0.2	326.2 $\pm$ 0.1	8.8 $\pm$ 0.0	55.0 $\pm$ 11.3
NY	3.4 $\pm$ 0.5	55.2 $\pm$ 5.7	35.3 $\pm$ 0.6	11.3 $\pm$ 1.3	202.9 $\pm$ 5.9	9.3 $\pm$ 0.1	64.0 $\pm$ 5.9
GC	4.2 $\pm$ 0.6	79.0 $\pm$ 15.1	32.0 $\pm$ 0.3	8.0 $\pm$ 0.5	276.2 $\pm$ 5.3	8.7 $\pm$ 0.1	69.5 $\pm$ 6.2
CH	4.1 $\pm$ 0.6	16.7 $\pm$ 8.8	29.2 $\pm$ 0.3	6.2 $\pm$ 0.6	151.5 $\pm$ 147.3	9.3 $\pm$ 0.2	64.2 $\pm$ 5.7
PY	6.8 $\pm$ 5.2	37.5 $\pm$ 5.2	32.7 $\pm$ 0.7	7.6 $\pm$ 0.4	117.9 $\pm$ 18.8	7.2 $\pm$ 0.4	49.5 $\pm$ 9.5
DH	2.8 $\pm$ 0.2	53.3 $\pm$ 16.1	28.9 $\pm$ 0.8	6.8 $\pm$ 0.6	401.8 $\pm$ 5.0	8.2 $\pm$ 0.3	74.0 $\pm$ 3.0
DT	6.7 $\pm$ 2.9	35.0 $\pm$ 0.0	23.6 $\pm$ 0.6	6.9 $\pm$ 0.1	283.2 $\pm$ 43.7	7.4 $\pm$ 0.1	30.7 $\pm$ 3.8

Note: Water depth (WD), secchi depth (SD), water temperature (WT), dissolved oxygen (DO), electrical conductivity (EC), sediment water content (SW).

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