

## Supplementary Information

### **Cyanobacterial bloom significantly boosts hypolimnetic anammox bacterial abundance in a subtropical stratified reservoir**

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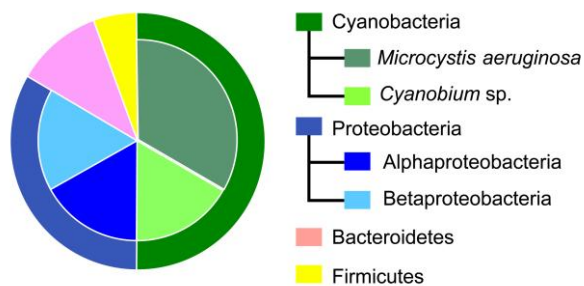
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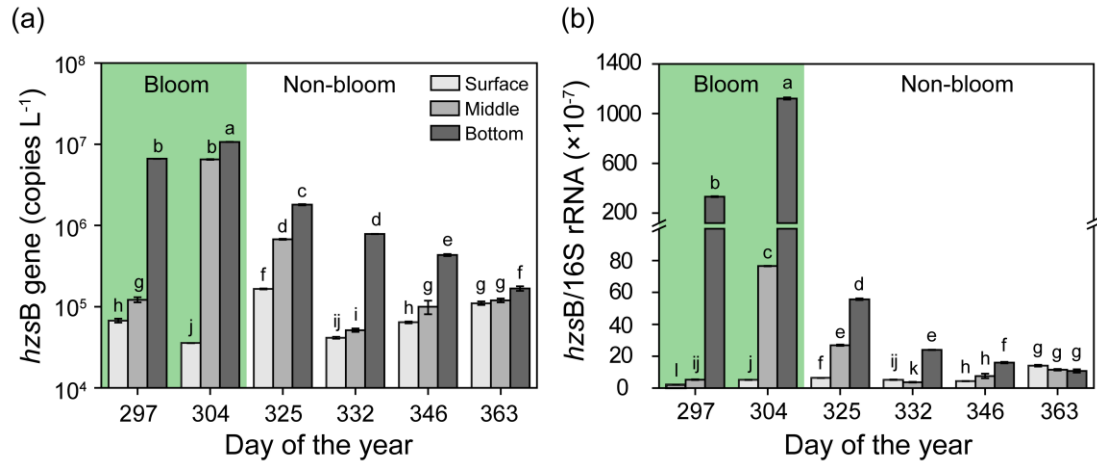
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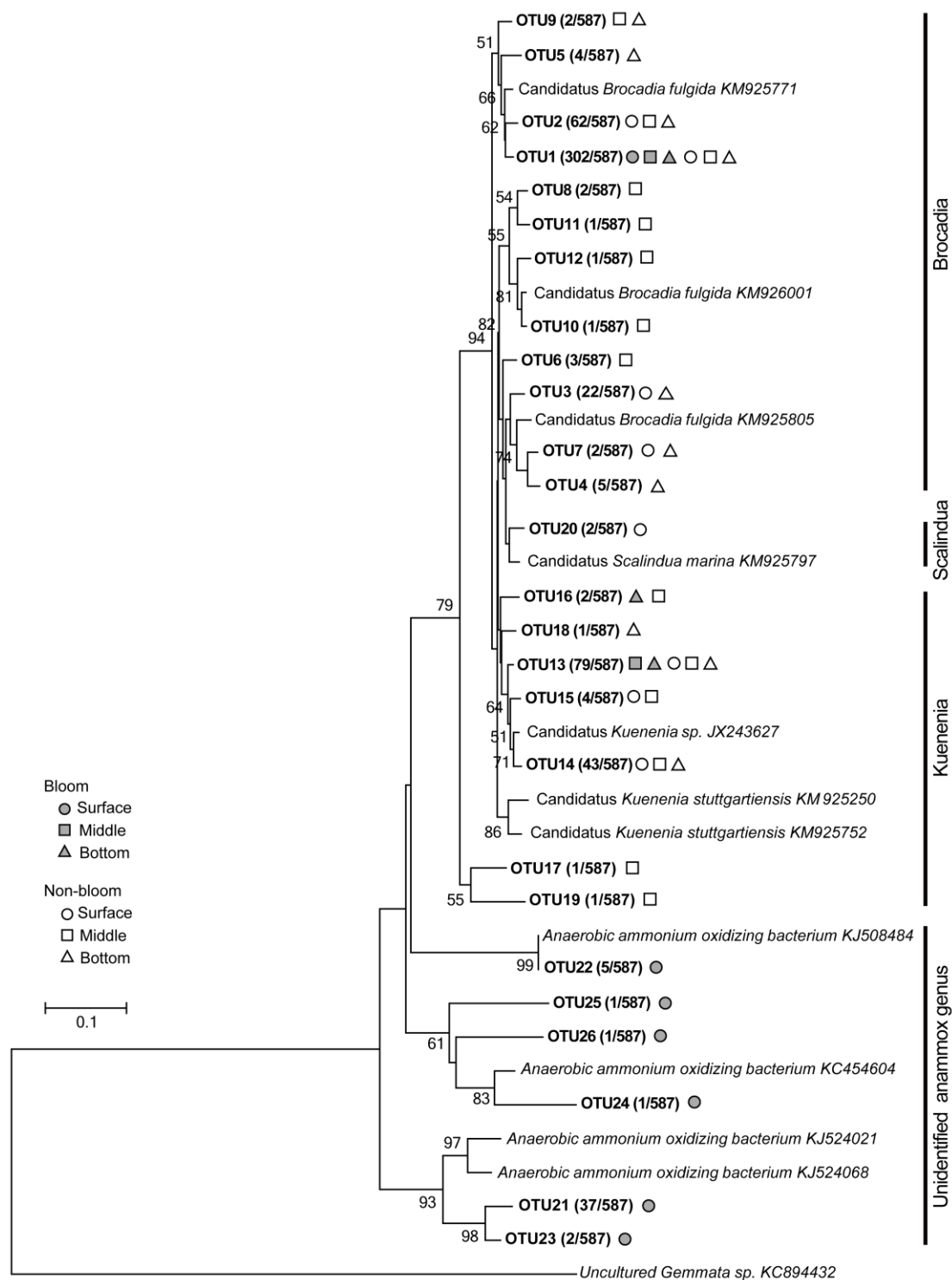
Number of Tables: 5



**Figure S1.** The relative abundance of bacterial 16S rRNA gene sequences showing the dominance of *Microcystis aeruginosa* in surface waters of the Xidong Reservoir during cyanobacterial bloom (day 297). At 97% sequence similarity level, the bacterial sequences were mostly affiliated to *Microcystis aeruginosa* (33.3%, 2 OTUs). One representative sequence for each OTU affiliated to *Microcystis aeruginosa* was stored in GenBank under accession numbers KY285028 and KY285029.



**Figure S2.** Patterns of *hzsB* gene abundance of anammox bacteria (a) and ratio of anammox bacteria to total bacteria (b) over space and time. Data are shown as means  $\pm$  standard error ( $n = 3$ ). Different letters represent significant differences among samples at  $P < 0.05$  level (one-way ANOVA).



**Figure S3.** Neighbor-joining tree of phylogenetic analysis of the anammox bacterial 16S rRNA gene sequences using Kimura two-parameter distance in the MEGA 6.0 package. Sequences of the reference known anammox bacteria are indicated in italics, followed by accession numbers. The sequences obtained in the present study are indicated in boldface and are identified by “OTU” followed by a number. The number in parentheses represents the number of sequences in each OTU and the total number of sequences. Bootstrap values (%) were generated from 1000 replicates, and values of > 50% are shown.

**Table S1.** Phytoplankton parameters for the Xidong Reservoir from 24 October 2014 (day 297) to 29 December 2014 (day 363).

Sample ID	Depth (m)	Date (d/m/y)	Day of year	Fv/Fm			Yield			Chlorophyll <i>a</i> ( $\mu\text{g L}^{-1}$ )				IK (Semi-light saturation point)			TSIc
				(Photosynthesis efficiency)			Bl	Gr	Br	Bl	Gr	Br	Total	Bl	Gr	Br	
				Bl	Gr	Br											
297S	0.5	24/10/14	297	0.23	0.00	0.32	0.27	0.00	0.20	36.81	0.00	12.60	46.28	541.47	0.00	399.43	62.2
297M	12	24/10/14	297	0.36	0.00	0.46	0.34	0.00	0.31	20.02	0.00	7.64	26.54	727.30	0.00	503.23	-
297B	25	24/10/14	297	0.20	0.00	0.30	0.24	0.00	0.23	19.74	0.00	7.25	25.54	503.20	0.00	582.80	-
304S	0.5	31/10/14	304	0.00	0.00	0.20	0.00	0.00	0.12	27.83	0.00	5.41	29.58	1316.37	0.00	510.07	61.3
304M	14	31/10/14	304	0.03	0.00	0.29	0.05	0.00	0.14	15.38	0.00	3.70	17.33	1805.87	0.00	525.27	-
304B	25	31/10/14	304	0.02	0.00	0.19	0.02	0.00	0.09	8.26	0.00	3.91	11.88	641.60	0.00	825.30	-
325S	0.5	21/11/14	325	0.00	0.57	0.38	0.00	0.39	0.26	0.25	4.13	1.77	6.69	4828.03	521.57	461.90	48.6
325M	17	21/11/14	325	0.00	0.63	0.54	0.00	0.39	0.39	0.32	3.79	2.21	6.99	3456.13	568.90	604.43	-
325B	25	21/11/14	325	0.00	0.53	0.31	0.00	0.31	0.27	0.13	3.60	1.49	5.69	959.83	544.77	703.53	-
332S	0.5	28/11/14	332	0.00	0.68	0.55	0.26	0.00	0.43	0.79	1.02	9.92	14.87	889.13	31.03	811.67	52.4
332M	17	28/11/14	332	0.00	0.50	0.52	0.00	0.31	0.40	0.25	1.90	4.22	7.72	0.00	1347.70	664.97	-
332B	25	28/11/14	332	0.00	0.75	0.42	0.23	0.46	0.33	0.53	0.68	4.19	6.69	681.57	1927.30	770.53	-
346S	0.5	12/12/14	346	0.00	0.77	0.39	0.00	0.52	0.27	0.42	0.64	4.92	7.53	774.83	831.37	507.03	51.5
346M	20	12/12/14	346	0.00	0.00	0.49	0.00	0.54	0.32	0.46	0.00	4.84	6.80	783.33	1645.40	567.93	-
346B	25	12/12/14	346	0.00	0.44	0.33	0.00	0.31	0.27	0.00	1.88	2.71	5.53	0.00	2004.27	549.53	-
363S	0.5	29/12/14	363	0.00	0.62	0.36	0.00	0.33	0.28	0.36	2.49	2.55	6.18	3617.57	471.43	642.50	46.1
363M	18	29/12/14	363	0.00	0.60	0.47	0.00	0.34	0.41	0.23	2.39	2.98	6.54	2248.00	545.40	604.10	-
363B	25	29/12/14	363	0.00	0.66	0.49	0.00	0.32	0.35	0.25	2.30	2.99	6.49	2504.30	541.33	572.00	-

Dark grey background denotes sampling dates during cyanobacterial bloom. Light grey background denotes sampling dates during non-bloom period. White background denotes water mixing and non-bloom period. The letters S, M and B represent surface, middle and bottom waters, respectively. The letters Bl, Gr and Br represent cyanobacteria, green algae and diatoms/dinoflagellates, respectively. TSIc denotes comprehensive trophic state index.

**Table S2.** Phylogenetic affiliations of anammox bacterial 16S rRNA gene sequences revealed by cloning sequence.

OTU <sub>0.03</sub> (clone number)	Best match sequences from GeneBank	Similarity (%)	Accession number	Source
OTU1(302)	<i>Ca. Brocadia fulgida</i>	98	KM 925771	Coastal sediment of China
OTU2 (62)	<i>Ca. Brocadia fulgida</i>	98	KM 925771	Coastal sediment of China
OTU3 (22)	<i>Ca. Brocadia fulgida</i>	97	KM 925805	Coastal sediment of China
OTU4 (5)	<i>Ca. Brocadia fulgida</i>	95	KM 925805	Coastal sediment of China
OTU5 (4)	<i>Ca. Brocadia fulgida</i>	98	KM 925771	Coastal sediment of China
OTU6 (3)	<i>Ca. Brocadia fulgida</i>	98	JX 243636	Marsh sediment of the Yangtze estuary
OTU7 (2)	<i>Ca. Brocadia fulgida</i>	97	KM 925805	Coastal sediment of China
OTU8 (2)	<i>Ca. Brocadia fulgida</i>	97	KM 926001	Coastal sediment of China
OTU9 (2)	<i>Ca. Brocadia fulgida</i>	96	KM 925771	Coastal sediment of China
OTU10 (1)	<i>Ca. Brocadia fulgida</i>	99	KM 926001	Coastal sediment of China
OTU11 (1)	<i>Ca. Brocadia fulgida</i>	97	KM 926001	Coastal sediment of China
OTU12 (1)	<i>Ca. Brocadia fulgida</i>	95	KM 926001	Coastal sediment of China
OTU13 (79)	<i>Ca. Kuenenia stuttgartiensis</i>	94	KM 925752	Coastal sediment of China
OTU14 (43)	<i>Ca. Kuenenia stuttgartiensis</i>	95	KM 925250	Coastal sediment of China
OTU15 (4)	<i>Ca. Kuenenia stuttgartiensis</i>	94	KM 925250	Coastal sediment of China
OTU16 (2)	<i>Ca. Kuenenia stuttgartiensis</i>	94	KM 925752	Coastal sediment of China
OTU17 (1)	<i>Ca. Kuenenia stuttgartiensis</i>	97	KM 925752	Coastal sediment of China
OTU18 (1)	<i>Ca. Kuenenia stuttgartiensis</i>	94	KM 925752	Coastal sediment of China
OTU19 (1)	<i>Ca. Kuenenia sp.</i>	98	JX 243627	Marsh sediment of the Yangtze estuary
OTU20 (2)	<i>Ca. Scalindua marina</i>	96	KM 925797	Coastal sediment of China
OTU21 (37)	Uncultured anammox bacteria	88	KJ 524021	Rice rhizosphere
OTU22 (5)	Uncultured anammox bacteria	100	KJ 508484	Paddy soil
OTU23 (2)	Uncultured anammox bacteria	90	KJ 524068	Rice rhizosphere
OTU24 (1)	Uncultured anammox bacteria	89	KC 454604	Terrestrial aquatic ecosystem
OTU25 (1)	Uncultured anammox bacteria	85	KC 454604	Terrestrial aquatic ecosystem
OTU26 (1)	Uncultured anammox bacteria	85	KC 454604	Terrestrial aquatic ecosystem
OTU27 (2)	Uncultured Planctomycete	97	JQ 958789	Cyanobacterial aggregates in eutrophic lake
OTU28 (1)	Uncultured Planctomycete	98	KM 095339	Rhizosphere sediment
OTU29 (1)	Uncultured Planctomycete	93	HQ 162757	Sediment
OTU30 (1)	Uncultured Planctomycete	91	HQ 162757	Sediment
OTU31 (1)	Uncultured Planctomycete	89	JQ 701675	Water sample
OTU32 (1)	Uncultured Planctomycete	88	JN 003005	Water-rock interactions area
OTU33 (1)	Planctomycete bacteria	90	JF 488158	Surface water sample
OTU34 (6)	Uncultured bacteria	88	AB 656421	Paddy soil
OTU35 (1)	Uncultured bacteria	90	AB 656421	Paddy soil
OTU36 (1)	Uncultured bacteria	96	FJ 447723	Mesotrophic lake

Note: For analysis of anammox bacterial community and diversity, unidentified bacteria from OTU27 to OTU36 (i.e. Planctomycete and uncultured bacteria) were excluded in this study.

**Table S3.** Physicochemical parameters for the Xidong Reservoir from 24 October 2014 (day 297) to 29 December 2014 (day 363).

Sample ID	Depth (m)	Date (m/d/y)	Day of year	Temperature (°C)	EC ( $\mu\text{S cm}^{-1}$ )	Turbidity (NTU)	pH	ORP (mV)	DO	TC	TOC	TN	Nutrients ( $\text{mg L}^{-1}$ )					TN:TP	Transparency (m)
													NH <sub>4</sub> -N	NO <sub>3</sub> -N	NO <sub>2</sub> -N	TP	PO <sub>4</sub> -P		
297S	0.5	24/10/14	297	25.52	24.9	19.5	8.06	170	7.01	6.631	3.513	0.779	0.020	0.209	0.002	0.022	0.002	35.45	1.09
297M	12	24/10/14	297	25.23	24.2	4.3	7.34	277	3.55	5.639	2.573	0.641	0.015	0.202	0.002	0.018	0.002	35.56	-
297B	25	24/10/14	297	17.13	81.9	6.1	7.57	29	0.00	5.637	1.460	0.995	0.519	0.200	0.004	0.048	0.017	20.83	-
304S	0.5	31/10/14	304	25.19	26.2	34.4	7.86	385	7.40	6.546	3.472	0.872	0.083	0.239	0.001	0.032	0.006	27.19	0.95
304M	14	31/10/14	304	23.80	20.9	4.6	7.00	410	1.57	5.621	2.365	0.656	0.056	0.239	0.001	0.026	0.004	25.38	-
304B	25	31/10/14	304	17.18	82.0	5.5	7.57	42	0.00	5.841	0.796	1.257	0.838	0.114	0.004	0.069	0.039	18.26	-
325S	0.5	21/11/14	325	22.04	25.9	5.0	6.47	548	5.22	4.224	0.951	0.738	0.196	0.275	0.019	0.024	0.002	30.83	2.50
325M	17	21/11/14	325	21.55	29.2	14.6	6.07	563	1.10	4.229	0.758	0.697	0.212	0.224	0.018	0.024	0.004	29.17	-
325B	25	21/11/14	325	17.20	88.2	5.4	6.53	49	0.00	5.434	0.493	1.272	0.865	0.211	0.014	0.071	0.028	17.89	-
332S	0.5	28/11/14	332	22.46	26.8	3.5	6.82	275	8.27	4.245	1.082	0.707	0.053	0.318	0.006	0.028	0.002	25.36	3.00
332M	17	28/11/14	332	21.46	29.6	7.8	5.97	326	0.50	4.311	0.823	0.748	0.267	0.245	0.002	0.020	0.004	37.50	-
332B	25	28/11/14	332	17.22	99.3	5.4	6.53	85	0.00	4.599	0.399	1.098	0.772	0.008	0.005	0.048	0.030	22.92	-
346S	0.5	12/12/14	346	19.79	29.0	6.4	6.19	483	6.33	4.078	0.713	0.810	0.218	0.284	0.001	0.024	0.004	33.75	1.48
346M	20	12/12/14	346	19.62	30.6	24.0	6.21	460	1.86	3.958	0.429	0.913	0.245	0.275	0.000	0.020	0.002	45.50	-
346B	25	12/12/14	346	17.23	110.6	8.0	6.79	67	0.00	4.907	0.378	1.282	0.777	0.155	0.004	0.059	0.022	21.69	-
363S	0.5	29/12/14	363	16.88	26.6	4.7	6.27	413	8.22	3.743	0.563	0.697	0.174	0.318	0.018	0.016	0.004	43.75	3.27
363M	18	29/12/14	363	16.81	25.9	4.8	6.59	403	8.18	3.625	0.368	0.677	0.190	0.318	0.018	0.016	0.004	42.50	-
363B	25	29/12/14	363	16.80	26.3	5.1	6.60	403	8.17	3.579	0.293	0.697	0.174	0.318	0.018	0.016	0.002	43.75	-

Dark grey background denotes sampling dates during cyanobacterial bloom. Light grey background denotes sampling dates during non-bloom period. White background denotes water mixing and non-bloom period. The letters S, M and B represent surface, middle and bottom waters, respectively. EC, electrical conductivity; ORP, oxidation reduction potential; DO, dissolved oxygen; TC, total carbon; TOC, total organic carbon; TN, total nitrogen; NH<sub>4</sub>-N, ammonium nitrogen; NO<sub>3</sub>-N, nitrate nitrogen; NO<sub>2</sub>-N, nitrite nitrogen; TP, total phosphorous; PO<sub>4</sub>-P, phosphate phosphorus.

**Table S4.** Comparisons of anammox bacterial *hzsB* gene abundance and *hzsB* gene/bacterial 16S rRNA gene ratio across depth and time.

		<i>hzsB</i> gene	<i>hzsB</i> /16S rRNA
		<i>P</i> -value	<i>P</i> -value
<i>Bloom stratification</i>			
	Surface vs. Middle	<b>0.002</b>	<b>0.015</b>
	Surface vs. Bottom	<b>0.002</b>	<b>0.002</b>
	Middle vs. Bottom	<b>0.002</b>	<b>0.002</b>
<i>Non-bloom stratification</i>			
Depth	Surface vs. Middle	0.258	0.387
	Surface vs. Bottom	<b>0.000</b>	<b>0.000</b>
	Middle vs. Bottom	<b>0.004</b>	<b>0.050</b>
<i>Non-bloom mixing</i>			
	Surface vs. Middle	0.400	0.100
	Surface vs. Bottom	0.100	0.100
	Middle vs. Bottom	0.100	0.700
<i>Surface</i>			
	Bloom stratification vs. Non-bloom stratification	0.181	0.113
	Bloom stratification vs. Non-bloom mixing	<b>0.024</b>	<b>0.024</b>
	Non-bloom stratification vs. Non-bloom mixing	0.482	<b>0.009</b>
<i>Middle</i>			
Time	Bloom stratification vs. Non-bloom stratification	0.066	0.328
	Bloom stratification vs. Non-bloom mixing	0.262	1.000
	Non-bloom stratification vs. Non-bloom mixing	0.864	0.482
<i>Bottom</i>			
	Bloom stratification vs. Non-bloom stratification	<b>0.000</b>	<b>0.000</b>
	Bloom stratification vs. Non-bloom mixing	<b>0.024</b>	<b>0.024</b>
	Non-bloom stratification vs. Non-bloom mixing	<b>0.009</b>	<b>0.009</b>

Boldface indicates the *P*-values < 0.05. Statistical test is nonparametric Mann-Whitney *U* test.



**Table S5.** Spearman rank correlation between environmental factors and anammox bacteria.

Environmental variables	OTUs	Chao 1	Shannon-Wiener	Simpson	Pielou's evenness
Temperature	0.171	0.151	-0.050	-0.383	-0.360
Electrical conductivity	0.068	0.084	0.400	0.600	0.209
Turbidity	0.624	0.698*	0.550	0.417	-0.477
pH	-0.222	-0.134	-0.317	-0.483	0.050
ORP	0.333	0.202	0.267	0.100	-0.293
Dissolved oxygen	0.087	0.077	-0.119	-0.186	-0.179
Total carbon	-0.305	-0.165	-0.494	-0.695*	-0.084
Total organic carbon	-0.171	-0.126	-0.617	-0.917**	-0.285
Total nitrogen	-0.026	0.025	0.317	0.617	0.335
Ammonium nitrogen	-0.073	-0.038	0.234	0.586	0.332
Nitrate nitrogen	0.430	0.362	0.205	0.274	-0.313
Nitrite nitrogen	0.147	0.047	0.160	-0.160	-0.241
Total phosphorous	-0.165	-0.154	0.153	0.356	0.272
Phosphate phosphorus	-0.350	-0.275	-0.017	0.315	0.395
TN:TP	0.222	0.202	0.033	-0.067	-0.176
Chlorophyll <i>a</i>	-0.248	-0.143	-0.667*	-0.767*	-0.167

\* $P < 0.05$ , \*\* $P < 0.01$ . ORP, Oxidation reduction potential.