Supplementary Material

Nanobubble can modulate microbial communities and sedimentary ecosystem in pond water treatment

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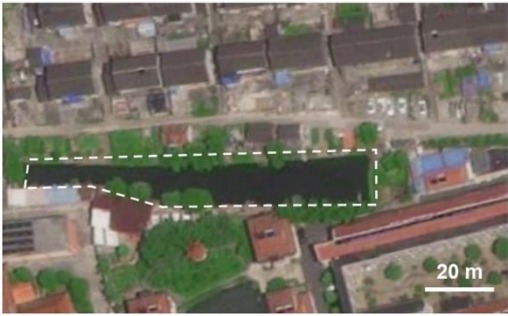[4]Shanghai University of Medicine & Health Sciences, Shanghai 201318, China

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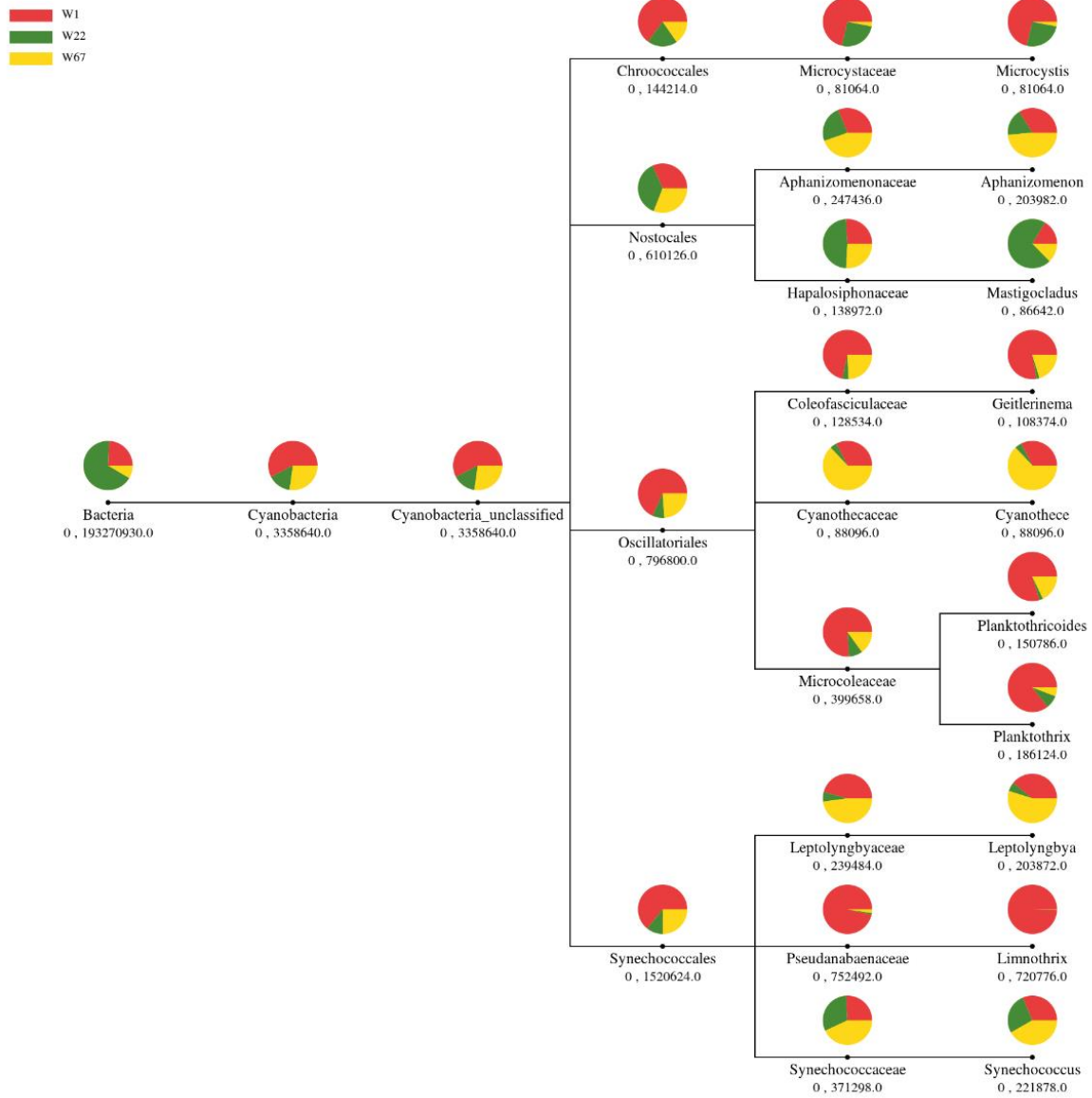
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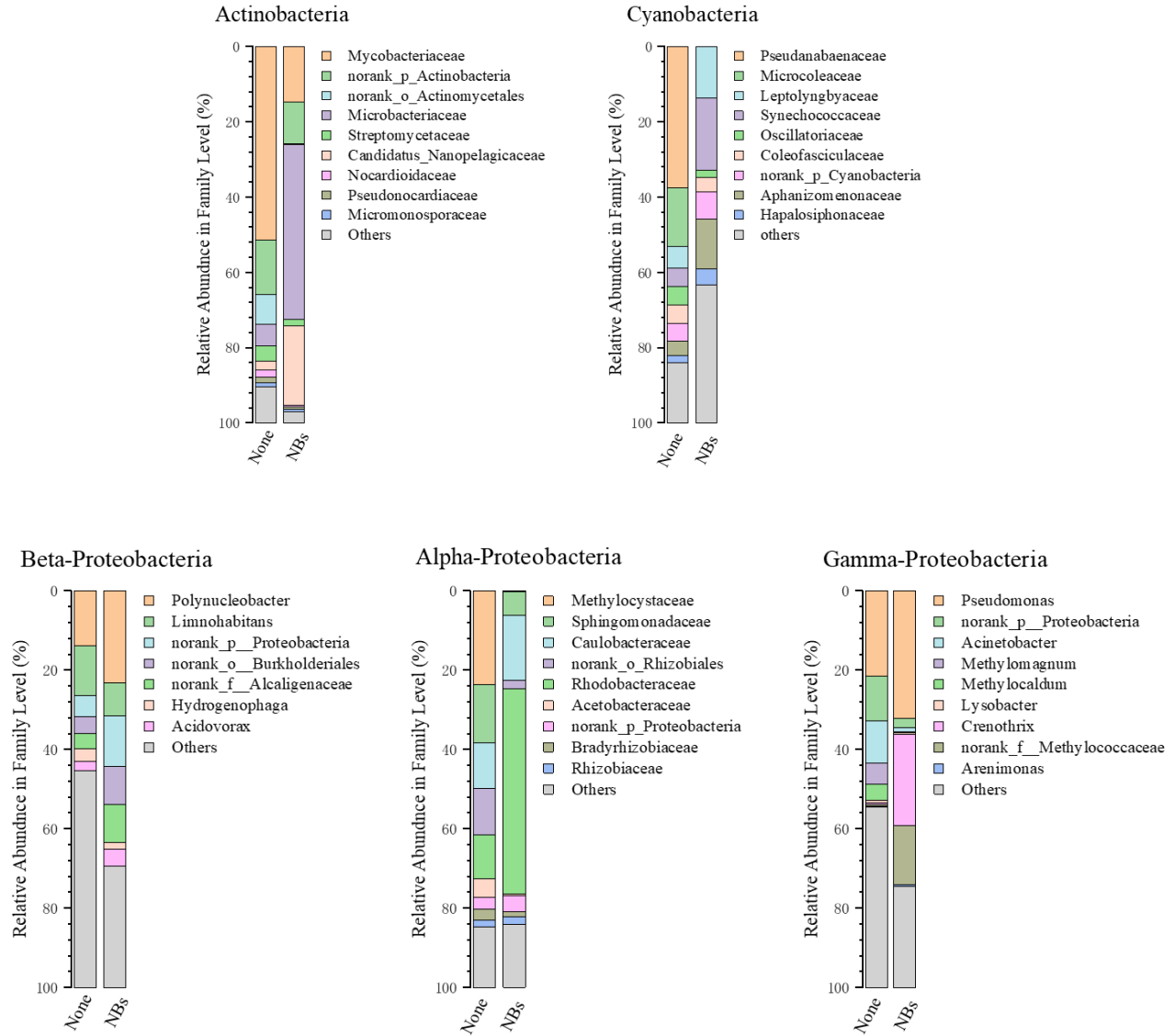
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1. Supplementary Figures

**Supplementary Figure 1.** Micro/nano-bubble treated pond aquatic ecosystem mentioned in this study.

Supplementary Figure 2. Taxonomy analysis tree of microorganisms in water at 1st, 22nd and 67th days of NBs' treatment.

Supplementary Figure 3. The effect of NBs on the three most abundant phyla (Actinobacteria, Proteobacteria and Cyanobacteria) at the genus level.
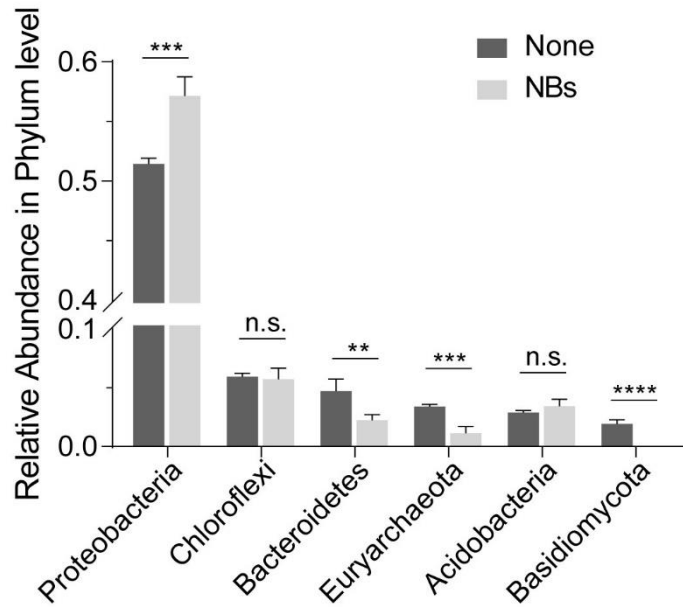
Supplementary Figure 4. Significance test on six most abundant phyla in sediment before and after NBs treatment. *T*-test was carried out and four replicates each group. ****: $P<0.0001$; ***: $P<0.001$; **: $P<0.01$; *: $P<0.05$; n.s.: no significance.
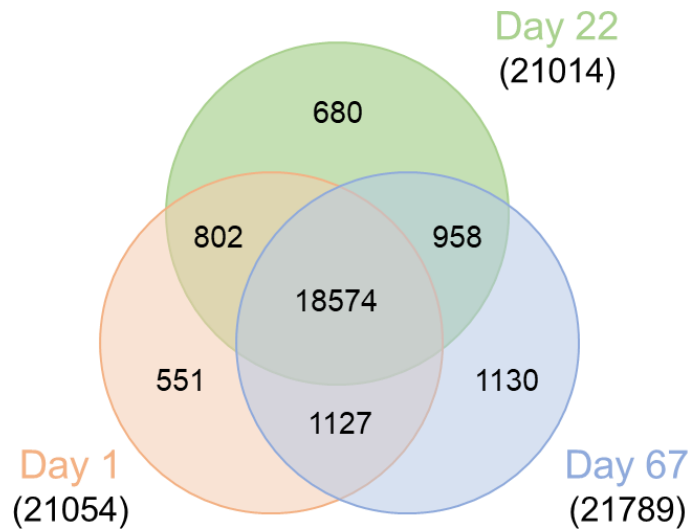
Supplementary Figure 5. The Venn diagram of microbial communities in sediment under different NBs treatment days.
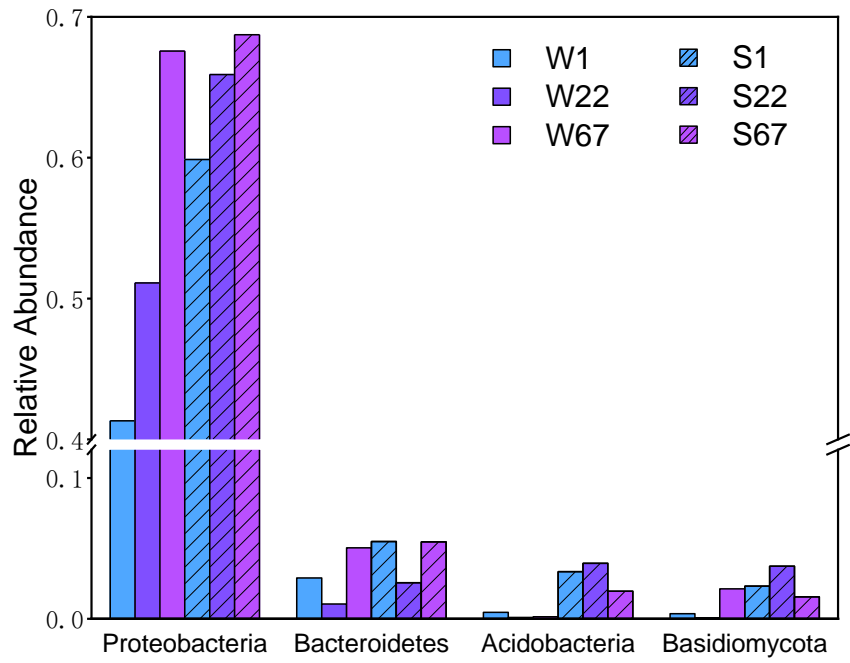
Supplementary Figure 6. Variation of the relative abundance of bacteria shared by pond water and sediments over time. The colors indicate different processing days (1, 22 and 67 days). The left three columns of each bacteria represent the water and the right three ones represents the sediment.
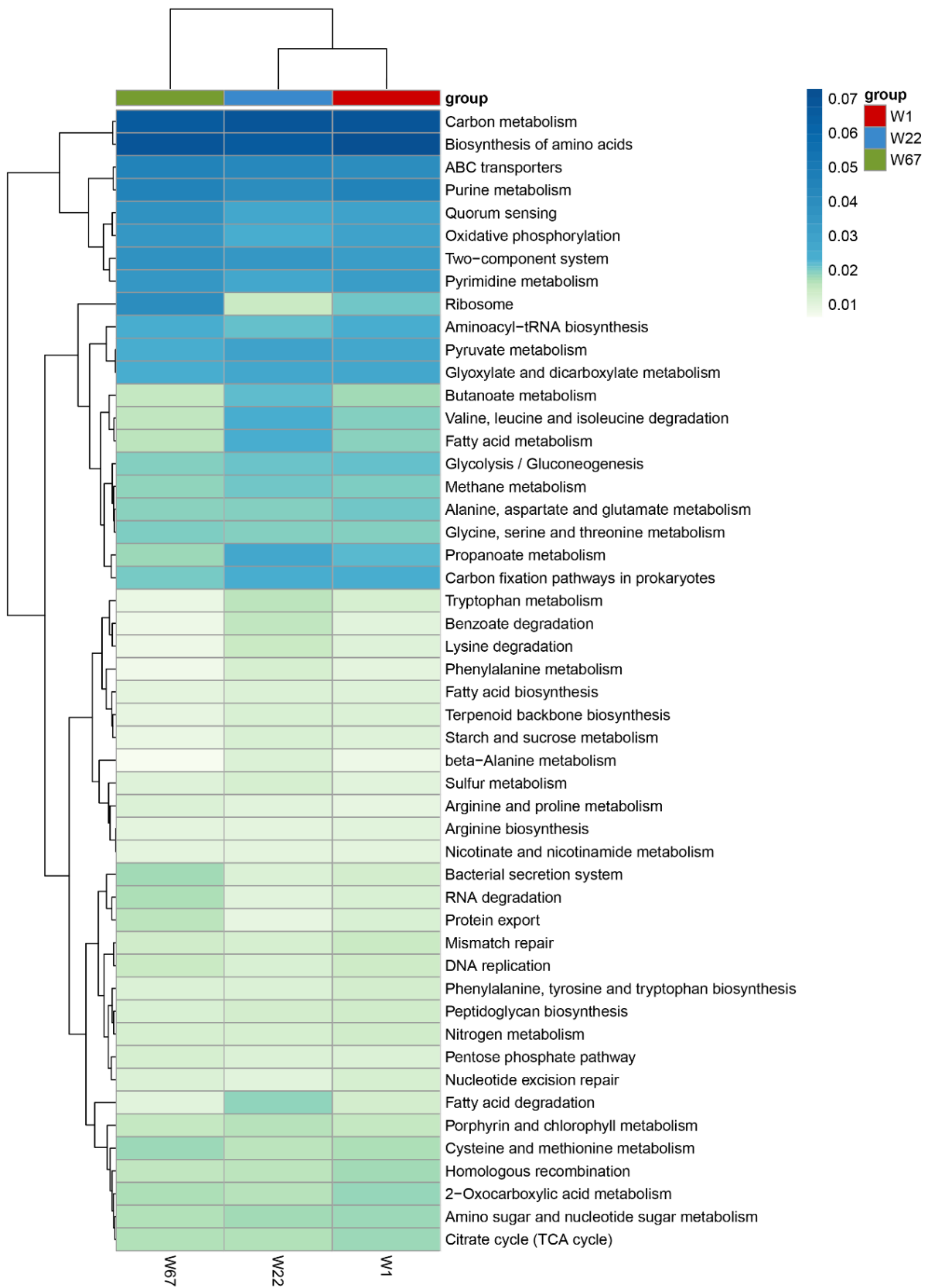
Supplementary Figure 7. Heatmap of KEGG pathway of microorganisms in pond water.
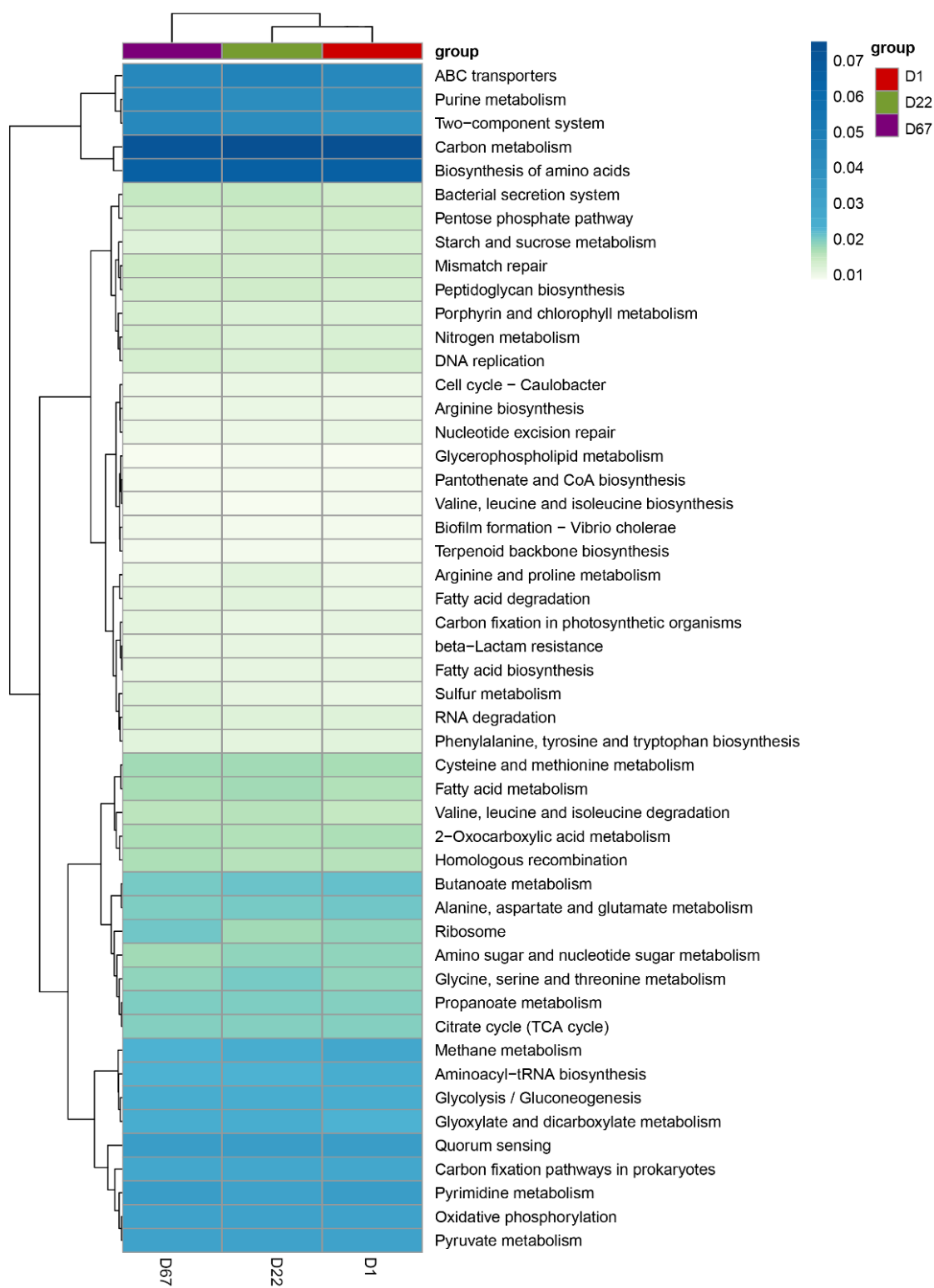
Supplementary Figure 8. Heatmap of KEGG pathway of microorganisms in pond sediment.
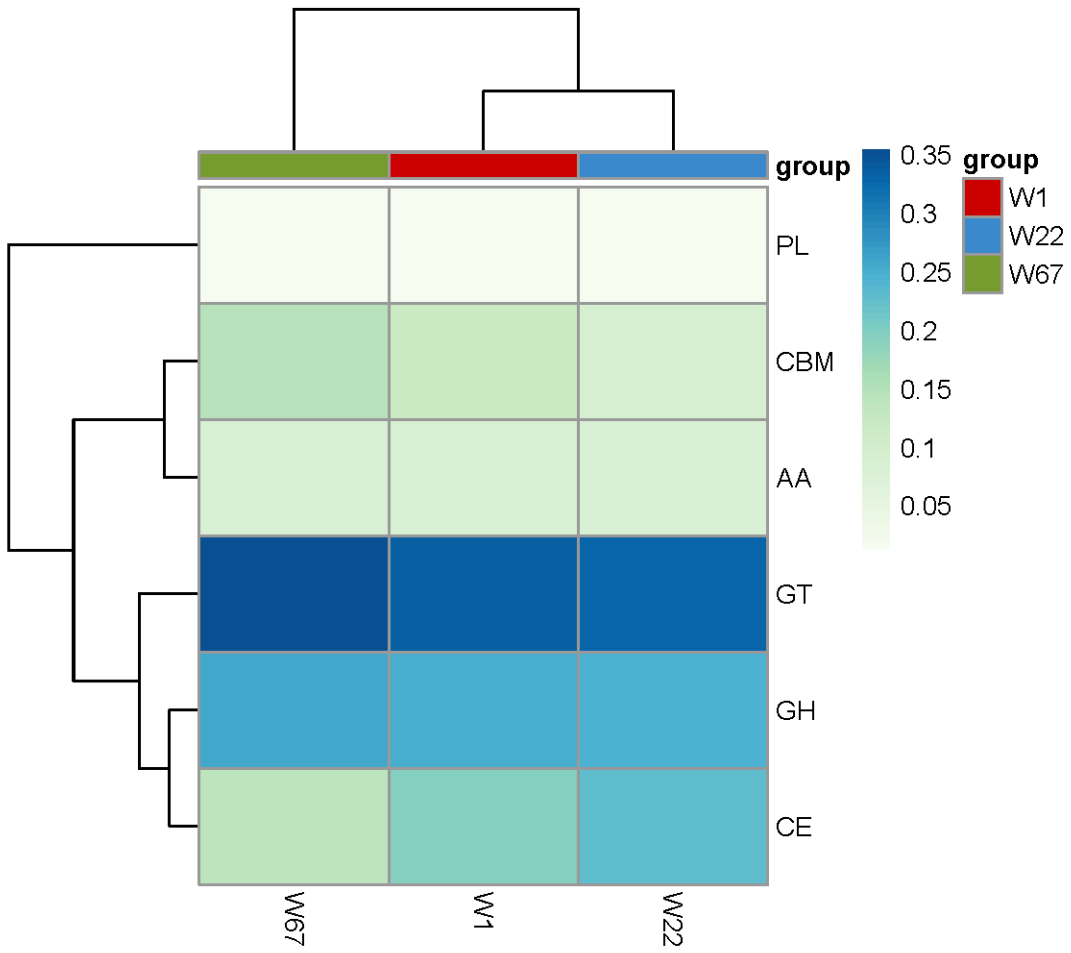
Supplementary Figure 9. Heatmap of Carbohydrate-active enzyme annotation in Water.

2. Supplementary Tables

Supplementary Table 1. Base mass distribution of sequence

Source	Day	Q20 (%)	Q30 (%)	CG(%)
	1	98.69	95.95	60.81
Sediment	22	98.01	94.63	61.74
	67	97.47	92.82	59.26
	1	98.21	94.97	52.33
Water	22	97.97	94.69	60.4
	67	96.83	91.67	49.05

Q20(%): the percentage of bases with a quality score of more than 20; Q30(%): the percentage of bases with a quality score of more than 30; CG(%): the percentage of CG base pairs to total base pairs.

Supplementary Table 2. Obtained and processed genetic data

Source	Day	Raw reads	Total bases (bp)	Clean reads	Total bases (bp)	Contigs	ORFs	Non-redundant
Sediment	1	130036994	19505549100	130011426	19477734092	565798	972510	
	22	172444976	25866746400	172397638	25830172658	942956	1675547	5738386
	67	100481522	15072228300	100474528	15057616428	583451	999815	
Water	1	167706948	25156042200	167682312	25101077476	713353	1193498	
	22	258667604	38800140600	258615468	38745501439	615377	1214061	2665795
	67	85807464	12871119600	85802430	12848391138	276521	551746	

Clean reads: filtered data; Contigs: spliced sequence; ORFs: open reading frames predicted by the comparison database; Non-redundant: non-redundant gene set.

Supplementary Table 3. Paired *t*-test at the phylum level based on the family abundance along with NBs' treatment for 1, 22 and 67 days

	Phyla	Day 1 vs. Day 22	Day 22 vs. Day 67	Day 1 vs. Day 67
	Actinobacteria	****	****	****
Water	Cyanobacteria	****	**	n.s.
	Proteobacteria	****	****	****
	Proteobacteria	n.s.	n.s.	***
	Chloroflexi	n.s.	**	****
Sediment	Bacteroidetes	****	****	n.s.
	Euryarchaeota	****	**	****
	Acidobacteria	****	****	n.s.
	Planctomycetes	*	**	**

****: $P<0.0001$; ***: $P<0.001$; **: $P<0.01$; *: $P<0.05$; n.s.: no significance

Supplementary Table 4. The relative abundance of most abundant classes of Proteobacteria in total community in water samples

Day	1	22	67
Alpha-Proteobacteria	9.72%	22.92%	19.10%
Beta-Proteobacteria	25.05%	20.10%	29.44%
Gamma-Proteobacteria	5.34%	7.29%	17.66%

Supplementary Table 5. The Shannon diversity index of microbial species in pond water after continuously NBs' treatment for 1, 22 and 67 days

Source	Day	Shannon Index
	1	6.83
Water	22	5.94
	67	6.60