

# **Colonization Characteristics of Bacterial Communities on Plastic Debris Influenced by Environmental Factors and Polymer Types in the Haihe Estuary of Bohai Bay, China**

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## **Supporting Information**

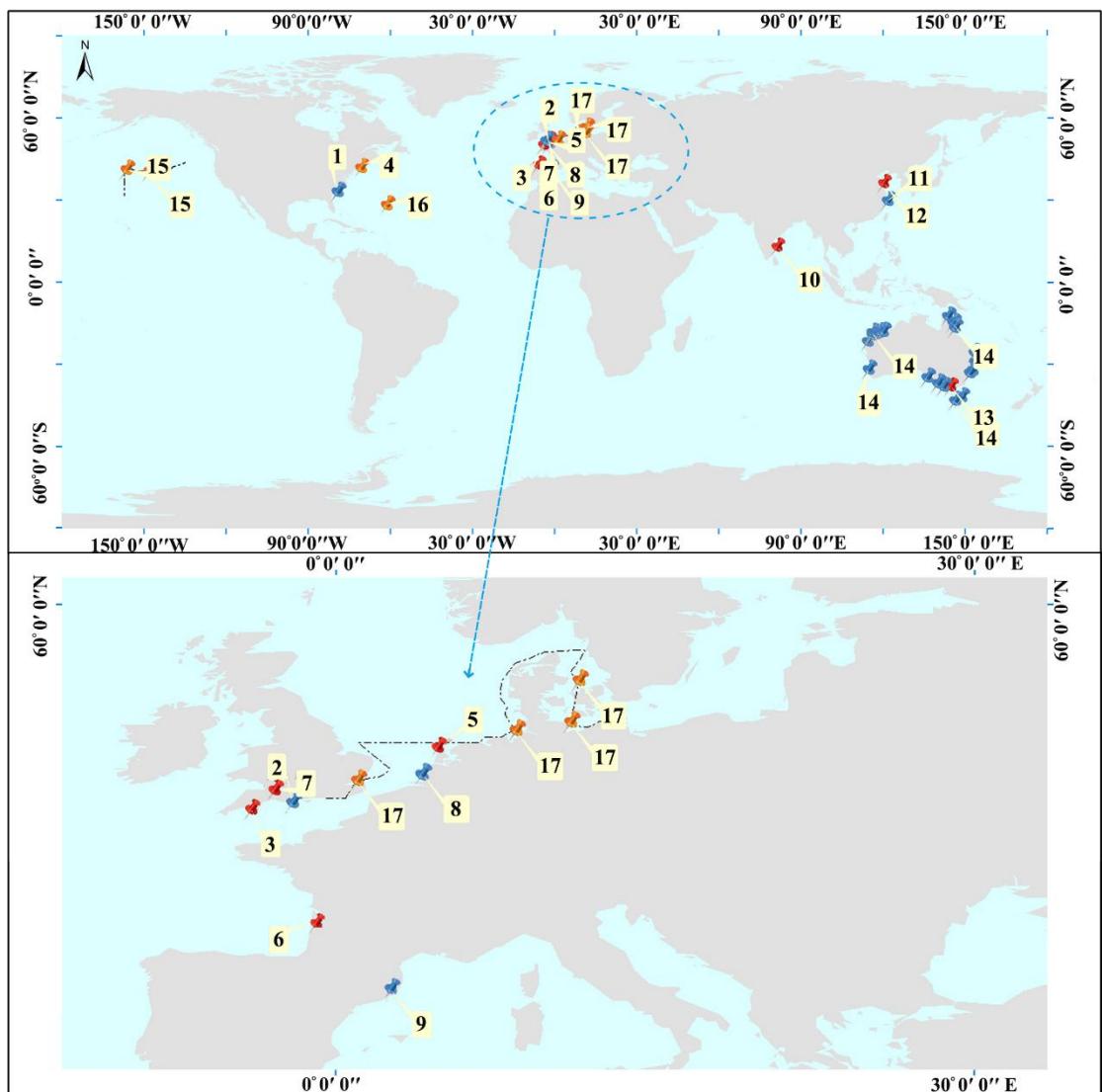
Number of pages: 34

Number of tables: 5

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**Figure S1:** Studies on microbial communities attached to marine plastic debris (PD) in the world. The red mark represented the in situ experiment (ISE), the blue mark represented the sampling experiment (SE), the orange mark represented the route sampling experiment (RSE). 1. SE in Sargasso Sea, western Atlantic Ocean<sup>1</sup> 2. SE in the coastal of Plymouth, UK<sup>2</sup> 3. ISE on intelligent buoy , the English Channel<sup>3</sup> 4. RSE on the route of North Atlantic<sup>4</sup> 5. ISE in Ostend, Belgium<sup>5</sup> 6. ISE in Toulon Bay, France<sup>6</sup> 7. SE in the southern coast of England<sup>7</sup> 8. SE in coast of Belgium<sup>8</sup> 9. SE in coast of Catalonia, Spain<sup>9</sup> 10. ISE in the Bay of Bengal, India<sup>10</sup> 11. ISE in the coast of Qingdao, China East China Sea<sup>11</sup> 12. SE in China Yangtze Estuary<sup>12</sup> 13. ISE in Port Phillip Bay, Australia<sup>13</sup> 14. SE in the coast of Australia<sup>14</sup> 15. SE on the route of Hawaii to Vancouver<sup>15</sup> 16. RSE on the route of Atlantic<sup>16</sup> 17. RES on the route of Britain, Germany and Denmark route<sup>3</sup>

**Table S1:** Studies on degradation of plastic debris (PD) by marine bacterial communities. polyethylene (PE), high density polyethylene (HDPE), low density polyethylene (LDPE), polypropylene (PP), polyethylene terephthalate(PET), polyvinyl chloride polypropylene (PVC)

Experimental type	Experimental site	Plastic types	Plastic degrading bacteria	Degradation time	Degradation efficiency	Reference
In situ experiment	Italy	PE bag		33 days	degradation cracks	<sup>17</sup>
In situ experiment	Mediterranean	PE		12 months	not obvious	<sup>18</sup>
In situ experiment	The Baltic Sea	PE(Containing 8% starch)		12 months	0.6%	
In situ experiment	Netherlands					
In situ experiment	Bay of Bengal	HDPE	<i>Pseudomonas</i> sp.	6 months	1.5% ~ 2.5%	<sup>19</sup>
In situ experiment	India	LDPE	<i>anaerobic</i>	6 months	0.5% ~ 0.8%	
		PP		6 months	0.5% ~ 0.6%	
Laboratory experiment	Bay of Bengal	bisphenol-A-carbonate	<i>Pseudomonas</i> sp.	12 months	9.0%	<sup>20</sup>
Laboratory experiment	India					
Laboratory experiment	Gulf of Mannar	HDPE	<i>Arthrobacter</i> sp.	30 days	12.0%	<sup>21</sup>
Laboratory experiment	India		<i>Pseudomonas</i> sp.	30 days	15.0%	
Laboratory experiment	Arabian Sea	PE	<i>Kocuria palustris</i> M16	30 days	1.0%	<sup>22</sup>
Laboratory experiment	India		<i>Bacillus pumilus</i> M27	30 days	1.5%	
			<i>Bacillus subtilis</i> H1584	30 days	1.8%	
Laboratory experiment	Mangrove	PE	<i>Bacillus cereus</i>	40 days	1.6%	<sup>23</sup>
Laboratory experiment	Malaysia	PET		40 days	6.6%	
		PVC		40 days	7.4%	
Laboratory experiment	Mangrove	PE	<i>Bacillus gottheilii</i>	40 days	6.2%	<sup>23</sup>
Laboratory experiment	Malaysia	PET		40 days	3.0%	
		PP		40 days	3.2%	
		PVC		40 days	5.8%	

Laboratory experiment	Mangrove Malaysia	PP	<i>Bacillus</i> sp. strain 27	40 days	6.4%	<sup>24</sup>
Laboratory experiment	Mangrove Malaysia	PP	<i>Rhodococcus</i> sp. Strain 36	40 days	4.0%	<sup>24</sup>

**Table S2A:** Physical and chemical parameters of the surface water in the Haihe estuarine areas after the two-week exposure in 26 July 2018.

In situ sites	Longitude	Latitude	Temperature (°C)	Salinity(‰)	Dissolved oxygen (mg/L)	pH	Total nitrogen (mg/L)	Total phosphorus (mg/L)
S1	117°42'41"E	38°59'20"N	21.20	8.21	11.12	8.89	3.14	0.442
S2	117°42'53"E	38°58'38"N	21.80	16.22	10.42	8.27	2.26	0.110
S3	117°45'19"E	38°57'34"N	21.60	27.15	9.56	8.29	1.26	0.115
S4	117°50'22"E	38°56'17"N	21.70	31.41	8.13	8.18	0.63	0.073
S5	117°53'5"E	38°58'16"N	20.60	31.56	8.80	8.06	0.25	0.058
S6	117°49'27"E	38°57'36"N	20.20	28.12	9.15	8.12	0.26	0.052
S7	117°45'54"E	38°59'14"N	20.30	30.05	10.20	8.06	0.83	0.091
S8	117°44'4"E	38°58'1"N	19.70	27.89	3.72	7.42	4.05	0.351
S9	117°46'41"E	38°55'55"N	19.40	29.89	10.92	7.56	2.07	0.113

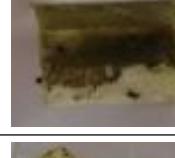
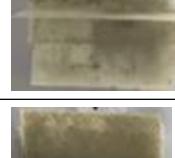
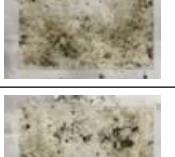
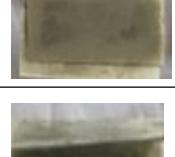
**Table S2B:** Physical and chemical parameters of the surface water in the Haihe estuarine areas after the four-week exposure in 9 august 2018.

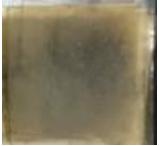
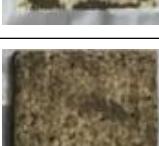
In situ sites	Longitude	Latitude	Temperature (°C)	Salinity(‰)	Dissolved oxygen (mg/L)	pH	Total nitrogen (mg/L)	Total phosphorus (mg/L)
S1	117°42'41"E	38°59'20"N	22.30	10.22	7.98	8.61	3.09	0.40
S2	117°42'53"E	38°58'38"N	22.40	18.54	7.02	7.98	1.96	0.12
S4	117°50'22"E	38°56'17"N	22.70	29.87	5.73	7.81	0.53	0.08
S5	117°53'5"E	38°58'16"N	22.60	30.23	5.46	7.78	0.29	0.05
S6	117°49'27"E	38°57'36"N	21.50	28.61	7.12	7.81	0.25	0.06
S7	117°45'54"E	38°59'14"N	22.30	29.62	7.92	7.68	0.76	0.10
S8	117°44'4"E	38°58'1"N	21.40	27.21	3.14	7.12	3.89	0.30
S9	117°46'41"E	38°55'55"N	21.90	29.89	7.86	7.32	2.01	0.11

**Table S2C:** Physical and chemical parameters of the surface water in the Haihe estuarine areas after the six-week exposure in 23 august 2018.

In situ sites	Longitude	Latitude	Temperature (°C)	Salinity(‰)	Dissolved oxygen (mg/L)	pH	Total nitrogen (mg/L)	Total phosphorus (mg/L)
S1	117°42'41"E	38°59'20"N	24.20	11.12	3.95	8.31	3.21	0.36
S2	117°42'53"E	38°58'38"N	23.90	17.62	5.72	7.44	2.14	0.12
S4	117°50'22"E	38°56'17"N	24.20	29.86	4.53	7.29	0.56	0.08
S5	117°53'5"E	38°58'16"N	24.00	30.02	4.64	7.28	0.31	0.06
S7	117°45'54"E	38°59'14"N	23.10	29.14	4.21	7.26	0.72	0.11
S8	117°44'4"E	38°58'1"N	22.90	27.13	2.64	6.72	3.95	0.32
S9	117°46'41"E	38°55'55"N	23.10	29.34	4.32	6.85	2.14	0.09

**Table S3:** Photos of PD samples.

In situ sites	Plastic types	Two-week samples	Four-week samples	Six-week samples
S1	Polyvinyl chloride			
S1	Polypropylene			
S1	Polyethylene			
S1	Expanded Polystyrene			
S1	Polyurethane			
S2	Polyvinyl chloride			
S2	Polypropylene			
S2	Polyethylene			
S2	Expanded Polystyrene			

S2	Polyurethane			
S3	Polyvinyl chloride			
S3	Polypropylene			
S3	Polyethylene			
S3	Expanded Polystyrene			
S3	Polyurethane			
S4	Polyvinyl chloride			
S4	polypropylene			
S4	Polyethylene			
S4	Expanded Polystyrene			
S4	Polyurethane			

S5	Polyvinyl chloride			
S5	Polypropylene			
S5	Polyethylene			
S5	Expanded Polystyrene			
S5	Polyurethane			
S6	Polyvinyl chloride			
S6	Polypropylene			
S6	Polyethylene			
S6	Expanded Polystyrene			
S6	Polyurethane			
S7	Polyvinyl chloride			

S7	Polypropylene			
S7	Polyethylene			
S7	Expanded Polystyrene			
S7	Polyurethane			
S8	Polyvinyl chloride			
S8	Polypropylene			
S8	Polyethylene			
S8	Expanded Polystyrene			
S8	Polyurethane			
S9	Polyvinyl chloride			
S9	Polypropylene			

S9	Polyethylene			
S9	Expanded Polystyrene			
S9	Polyurethane			

**Table S4A:** Difference analysis of biomass on PD after the six-week exposure based on one-way ANOVA. Group represents a comparison of five plastic debris (PVC, PP, PE, PS, PU). Analysis significant results ( $p < 0.05$ ) are highlighted in bold and Red.

		<i>p</i>			<i>p</i>			<i>p</i>		<i>p</i>		<i>p</i>	
PVC	PP	0.14	PP	PE	0.13	PE	PS	<b>0.01</b>	PS	PU	<b>0.01</b>	Group	<b>0.01</b>
	PE	0.97		PS	<b>0.01</b>		PU	0.06					
	PS	<b>0.01</b>		PU	0.70								
	PU	0.64											

**Table S4B:** Difference analysis of the average growth rate of biofilm on PD at different in situ site based on one-way ANOVA. Group represents a comparison of seven in situ sites (S1, S2, S4, S5, S7, S8, S9). Analysis significant results ( $p < 0.05$ ) are highlighted in bold and Red.

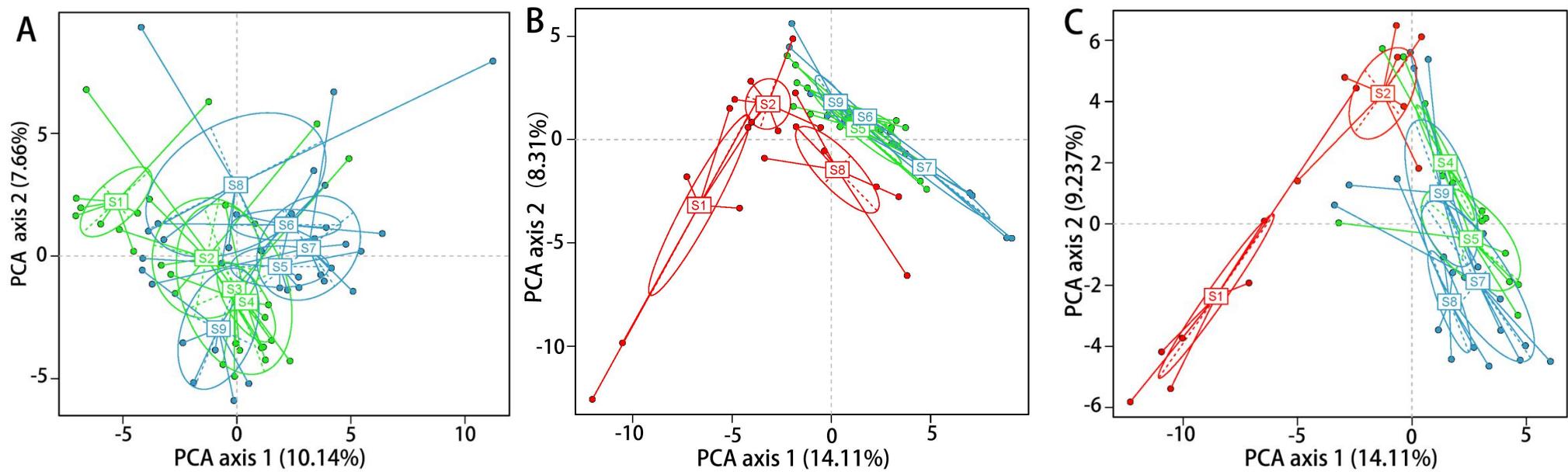
		<i>p</i>			<i>p</i>			<i>p</i>		<i>p</i>		<i>p</i>	
S1	S2	0.42	S2	S4	<b>0.01</b>	S4	S5	0.52	S5	S7	<b>0.01</b>	Group	<b>0.01</b>
	S4	<b>0.01</b>		S5	<b>0.01</b>		S7	<b>0.03</b>		S8	<b>0.01</b>		
	S5	<b>0.01</b>		S7	<b>0.01</b>		S8	<b>0.01</b>		S9	<b>0.01</b>		
	S7	<b>0.01</b>		S8	<b>0.02</b>		S9	<b>0.05</b>					
	S8	0.12		S9	<b>0.01</b>								
	S9	<b>0.01</b>											
S7	S8	<b>0.05</b>	S8	S9	<b>0.03</b>								
	S9	<b>0.03</b>											

**Table S5:** Difference analysis of alpha diversity based on One-way ANOVA. Group represents a comparison of all sites. Analysis significant results ( $p < 0.05$ ) are highlighted in bold and Red. 1\* represents two weeks. 2\* represents four weeks. 3\* represents six weeks.

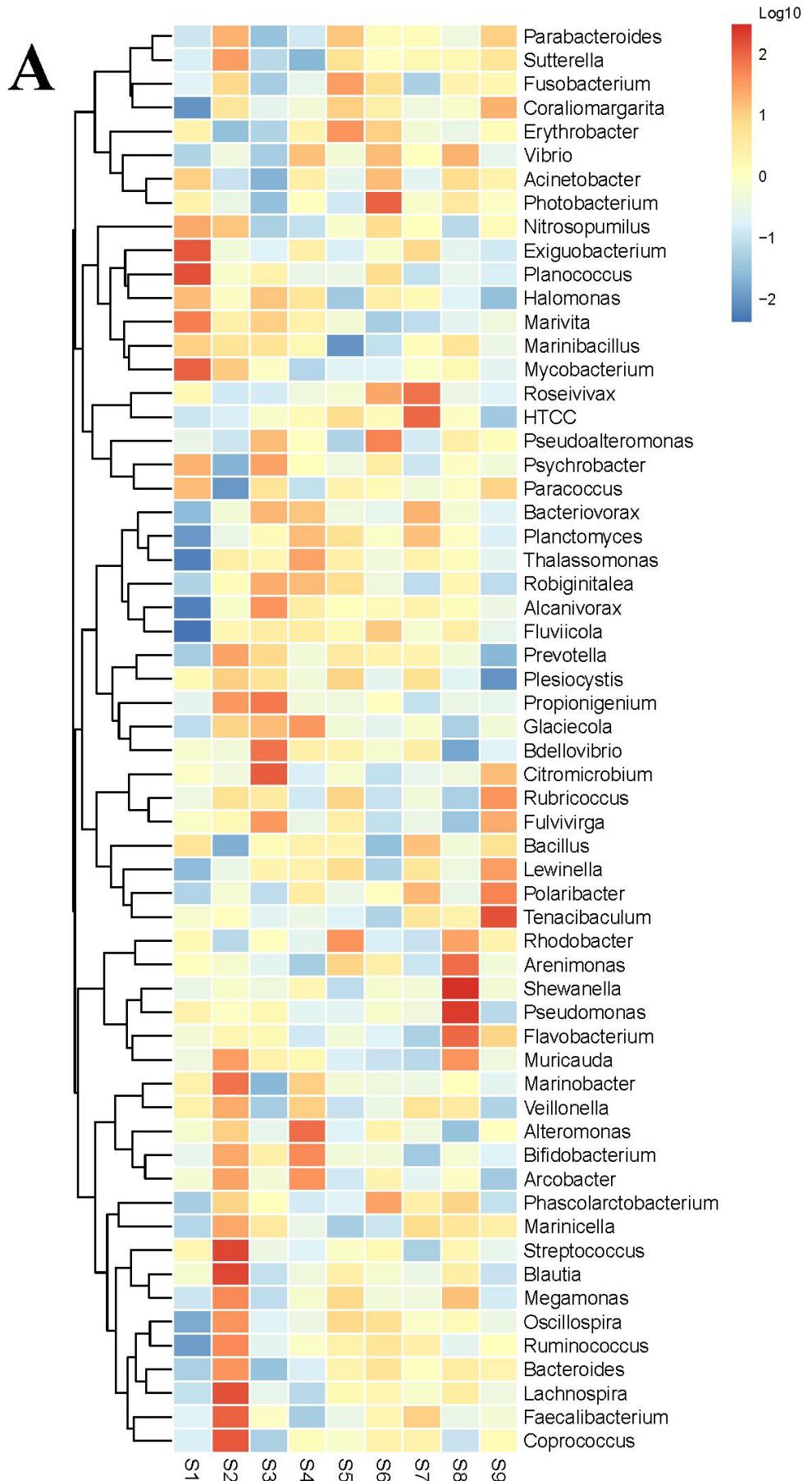
Chao 1 index <sup>1*</sup>		<i>p</i>	Shannon index <sup>1*</sup>		<i>p</i>	Simpson index <sup>1*</sup>		<i>p</i>
Group <sup>1*</sup>		<b>0.01</b>	Group <sup>1*</sup>		<b>0.01</b>	Group <sup>1*</sup>		<b>0.01</b>
S1	S2	0.10	S1	S2	<b>0.02</b>	S1	S2	<b>0.01</b>
	S3	<b>0.01</b>		S3	<b>0.01</b>		S3	<b>0.01</b>
	S4	<b>0.01</b>		S4	<b>0.01</b>		S4	<b>0.01</b>
	S5	<b>0.01</b>		S5	<b>0.01</b>		S5	<b>0.01</b>
	S6	<b>0.01</b>		S6	<b>0.01</b>		S6	<b>0.01</b>
	S7	<b>0.01</b>		S7	<b>0.01</b>		S7	<b>0.01</b>
	S8	<b>0.01</b>		S8	<b>0.01</b>		S8	<b>0.01</b>
	S9	<b>0.01</b>		S9	<b>0.01</b>		S9	<b>0.01</b>
S2	S3	0.18	S2	S3	0.12	S2	S3	0.12
	S4	0.09		S4	<b>0.01</b>		S4	0.06
	S5	<b>0.01</b>		S5	<b>0.01</b>		S5	0.08
	S6	<b>0.02</b>		S6	0.09		S6	0.60
	S7	<b>0.01</b>		S7	<b>0.01</b>		S7	0.08
	S8	0.12		S8	0.07		S8	0.85
	S9	0.39		S9	<b>0.05</b>		S9	0.08
S3	S4	0.70	S3	S4	0.13	S3	S4	0.74
	S5	0.18		S5	0.32		S5	0.82
	S6	0.28		S6	0.85		S6	0.30
	S7	<b>0.02</b>		S7	<b>0.02</b>		S7	0.82
	S8	0.81		S8	0.78		S8	0.17
	S9	0.63		S9	0.62		S9	0.83
S4	S5	0.33	S4	S5	0.58	S4	S5	0.92
	S6	0.48		S6	0.18		S6	0.18
	S7	0.06		S7	0.39		S7	0.92
	S8	0.89		S8	0.21		S8	0.09
	S9	0.39		S9	0.30		S9	0.91
S5	S6	0.78	S5	S6	0.42	S5	S6	0.21
	S7	0.33		S7	0.16		S7	0.92
	S8	0.27		S8	0.47		S8	0.11
	S9	0.07		S9	0.61		S9	0.93
S6	S7	0.21	S6	S7	<b>0.03</b>	S6	S7	0.21
	S8	0.40		S8	0.92		S8	0.74
	S9	0.12		S9	0.75		S9	0.21
S7	S8	<b>0.04</b>	S7	S8	<b>0.04</b>	S7	S8	0.11
	S9	<b>0.01</b>		S9	0.06		S9	0.94
S8	S9	0.47	S8	S9	0.83	S8	S9	0.12

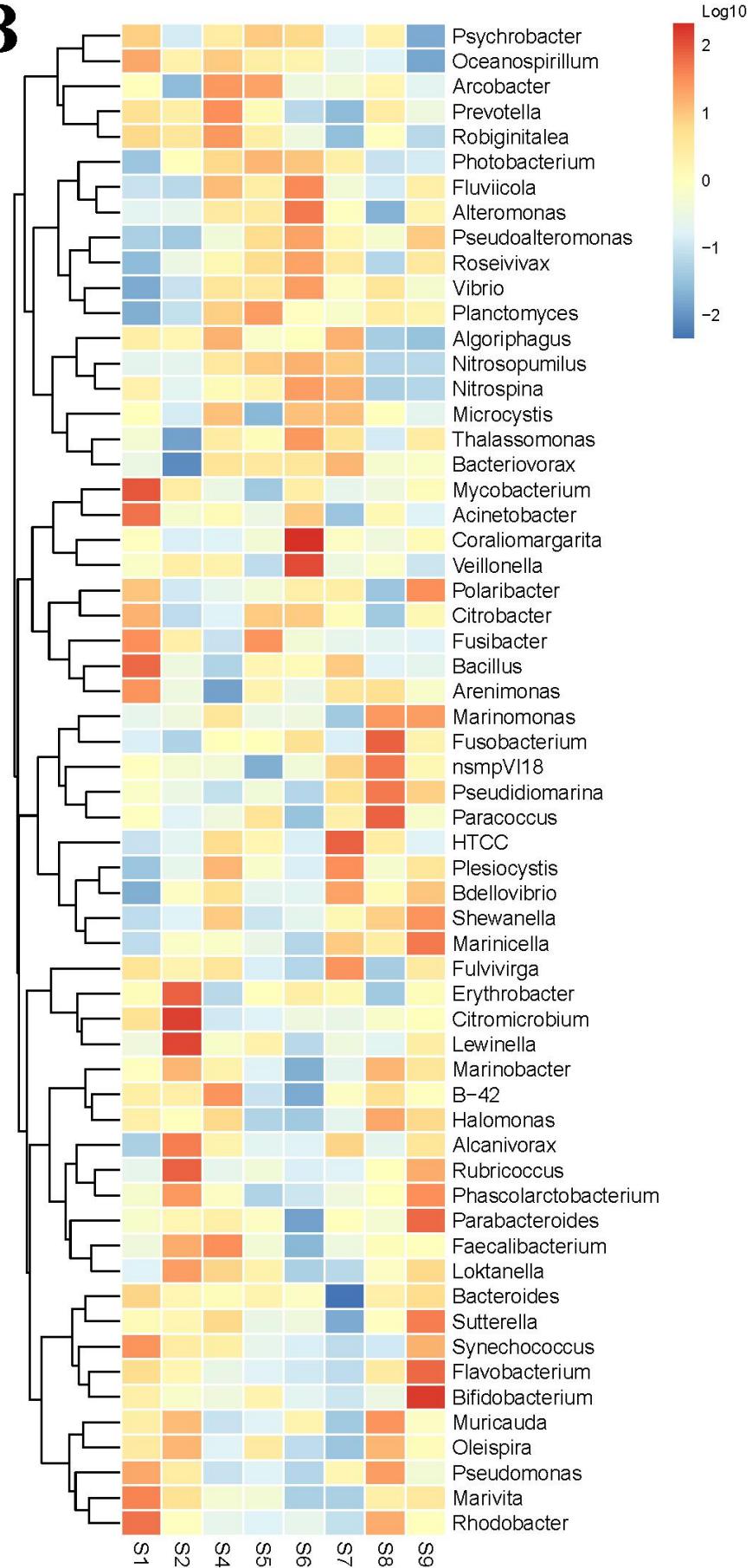
Chao 1 index <sup>2*</sup>	<i>p</i>	Shannon Index <sup>2*</sup>	<i>p</i>	Simpson Index <sup>2*</sup>	<i>p</i>
Group <sup>2*</sup>	<b>0.01</b>	Group <sup>2*</sup>	0.09	Group <sup>2*</sup>	0.61
S1	S2	0.45	S1	S2	0.89
	S4	<b>0.01</b>		S4	<b>0.03</b>
	S5	<b>0.01</b>		S5	0.75
	S6	<b>0.01</b>		S6	0.73
	S7	<b>0.01</b>		S7	<b>0.03</b>
	S8	<b>0.01</b>		S8	0.91
	S9	<b>0.01</b>		S9	0.44
S2	S4	<b>0.01</b>	S2	S4	<b>0.04</b>
	S5	<b>0.01</b>		S5	0.26
	S6	<b>0.03</b>		S6	0.97
	S7	<b>0.01</b>		S7	<b>0.05</b>
	S8	<b>0.01</b>		S8	0.73
	S9	<b>0.01</b>		S9	0.45
S4	S5	0.72	S4	S5	0.35
	S6	0.11		S6	<b>0.04</b>
	S7	0.30		S7	0.95
	S8	0.72		S8	<b>0.02</b>
	S9	0.16		S9	0.19
S5	S6	0.21	S5	S6	0.24
	S7	0.16		S7	0.39
	S8	0.48		S8	0.14
	S9	0.29		S9	0.70
S6	S7	<b>0.01</b>	S6	S7	<b>0.05</b>
	S8	0.06		S8	0.76
	S9	0.84		S9	0.43
S7	S8	0.48	S7	S8	<b>0.02</b>
	S9	<b>0.02</b>		S9	0.21
S8	S9	0.08	S8	S9	0.28
Chao 1 index <sup>3*</sup>	<i>p</i>	Shannon Index <sup>3*</sup>	<i>p</i>	Simpson Index <sup>3*</sup>	<i>p</i>
Group <sup>3*</sup>	<b>0.01</b>	Group <sup>3*</sup>	0.06	Group <sup>3*</sup>	0.21
S1	S2	0.21	S1	S2	<b>0.05</b>
	S4	<b>0.01</b>		S4	0.25
	S5	<b>0.01</b>		S5	0.45
	S7	<b>0.01</b>		S7	0.40
	S8	<b>0.01</b>		S8	0.72
	S9	<b>0.01</b>		S9	0.66
S2	S4	<b>0.01</b>	S2	S4	<b>0.01</b>
	S5	<b>0.01</b>		S5	<b>0.01</b>
	S7	<b>0.01</b>		S7	<b>0.01</b>
	S8	<b>0.01</b>		S8	0.11

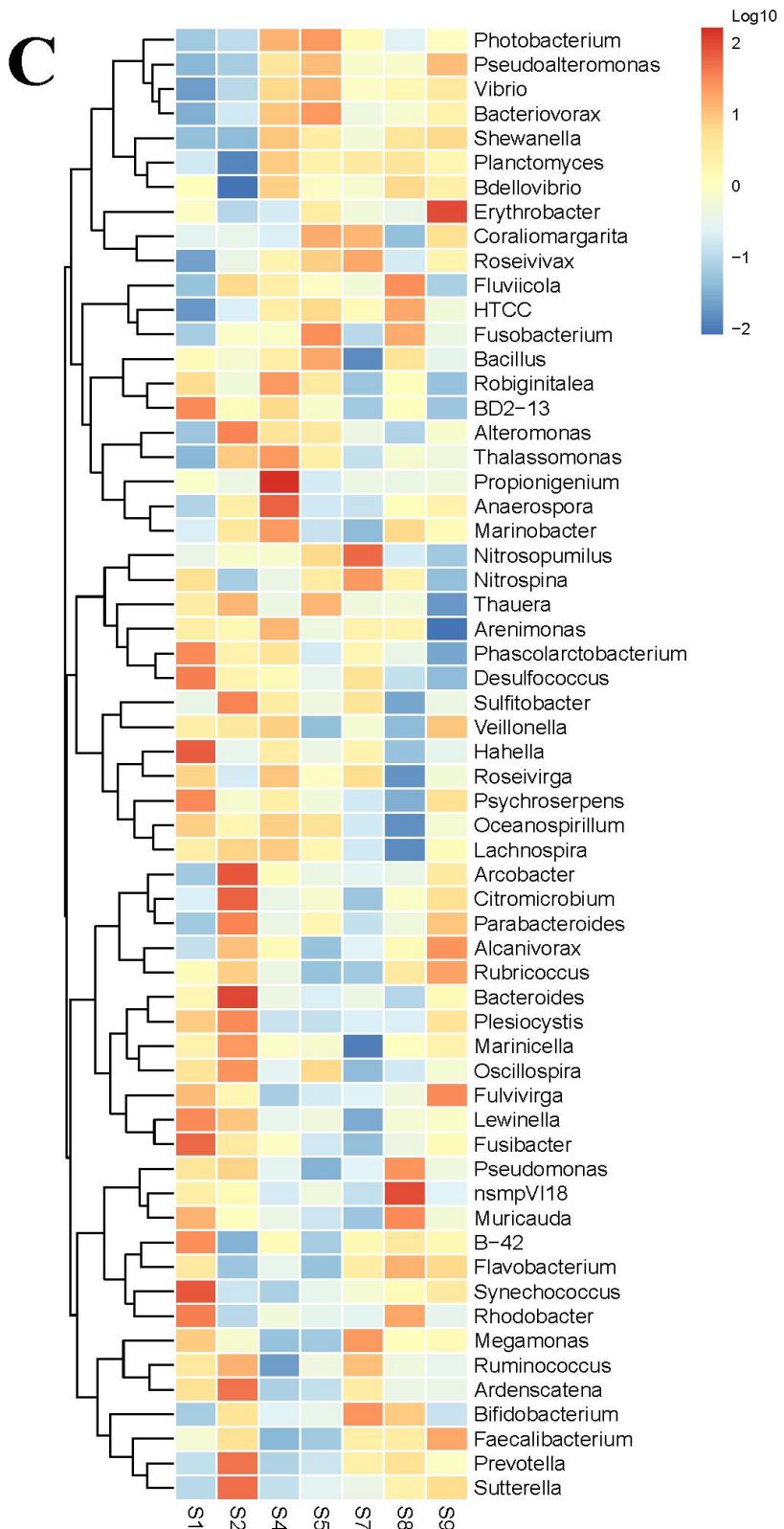
	S9	0.08		S9	<b>0.02</b>		S9	0.50
S4	S5	0.89	S4	S5	0.70	S4	S5	0.47
	S7	0.47		S7	0.75		S7	0.56
	S8	0.47		S8	0.14		S8	<b>0.01</b>
	S9	0.17		S9	0.48		S9	0.28
S5	S7	0.56	S5	S7	0.94	S5	S7	0.89
	S8	0.55		S8	0.27		S8	0.06
	S9	0.13		S9	0.75		S9	0.72
S7	S8	0.93	S7	S8	0.24	S7	S8	<b>0.05</b>
	S9	<b>0.04</b>		S9	0.69		S9	0.62
S8	S9	<b>0.04</b>	S8	S9	0.42	S8	S9	0.13



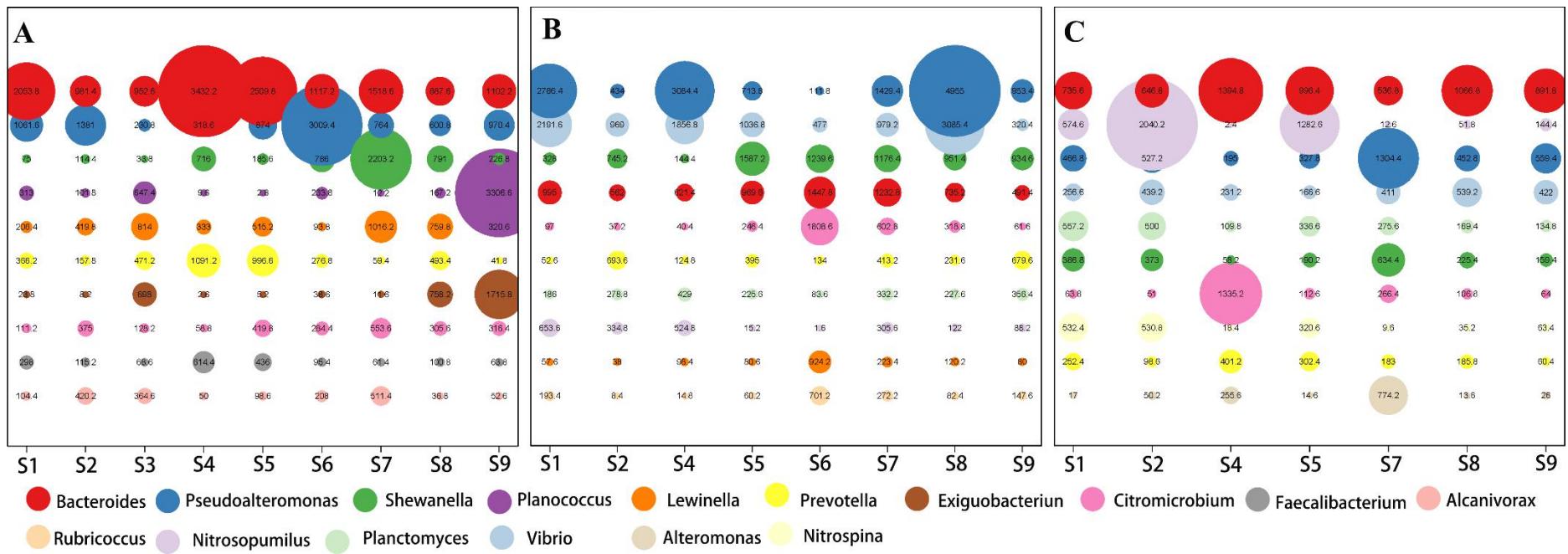
**Figure S2:** PCA profile of pairwise community dissimilarity indices (Bray–Curtis), calculated from marker genera of the bacteria communities on PD at different sites ( $n = 9$ ), ovals indicated the 95% confidence intervals for each sample type. Green circles represent the interaction zones between the freshwater and seawater (F-S zones). Blue circles represent special zones (Sp zones). Red circles represent the in situ sites with significant difference (ANOVA,  $p < 0.05$ ). (A) PCA profile after the two-week exposure. (B) PCA profile after the four-week exposure. (C) PCA profile after the six-week exposure.



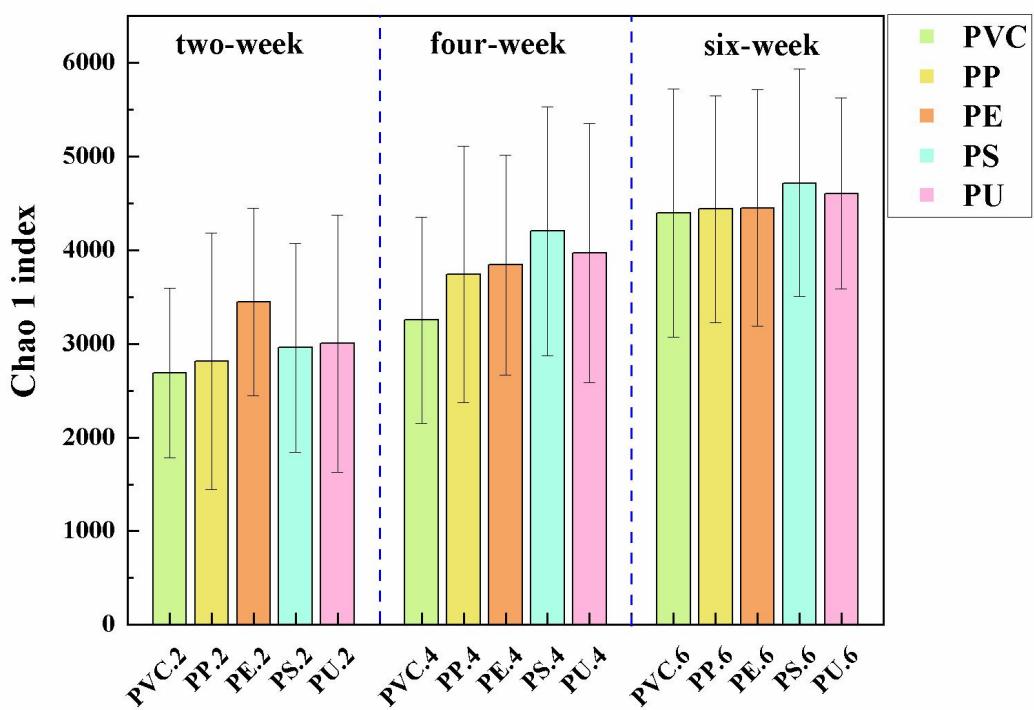
**B**



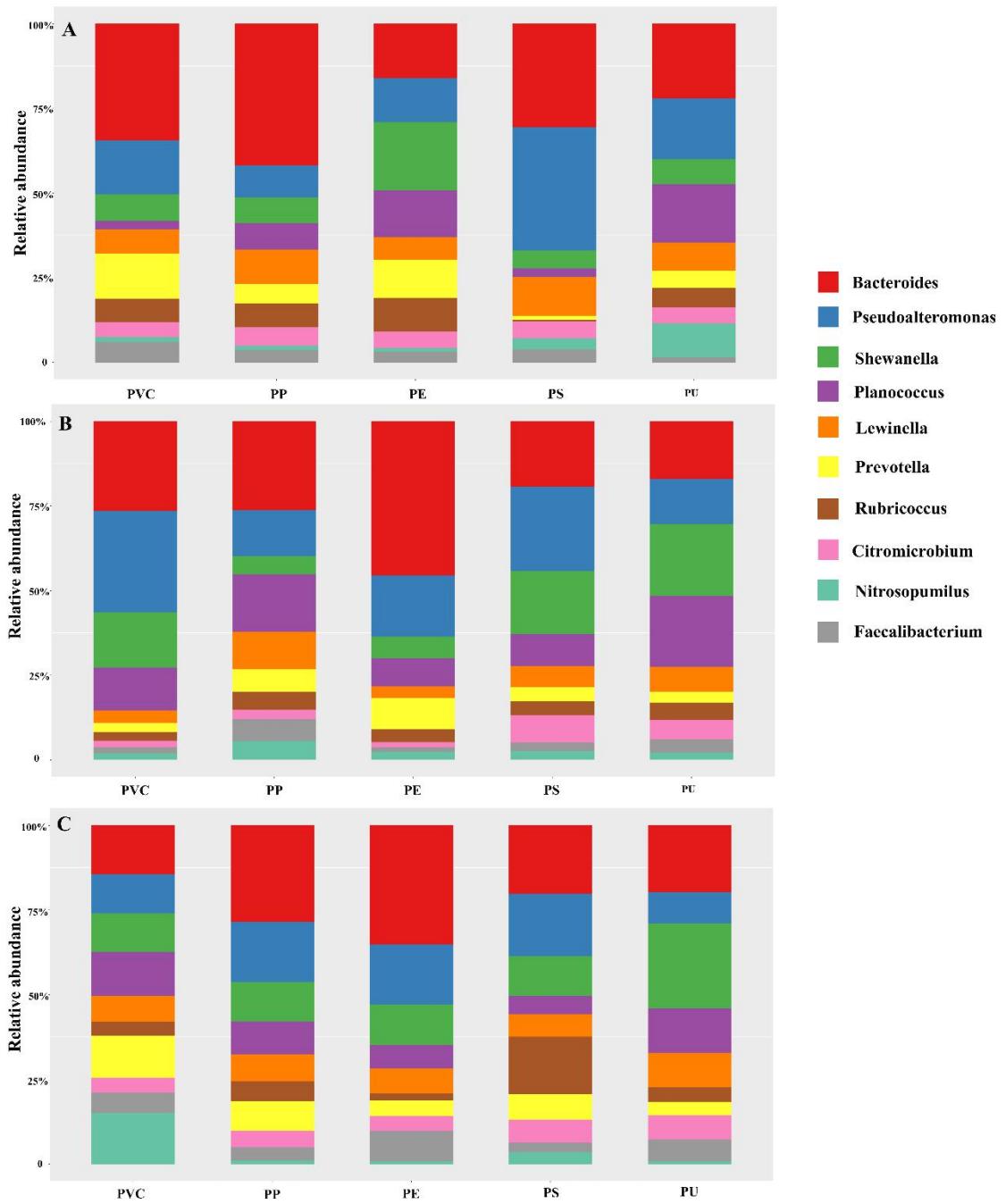
**Figure S3:** Heatmap of marker genera on PD (n = 5) at different in situ sites (n = 9).  
 (A) Heatmap after the two-week exposure. (B) Heatmap after the four-week exposure.  
 (C) Heatmap after the six-week exposure.



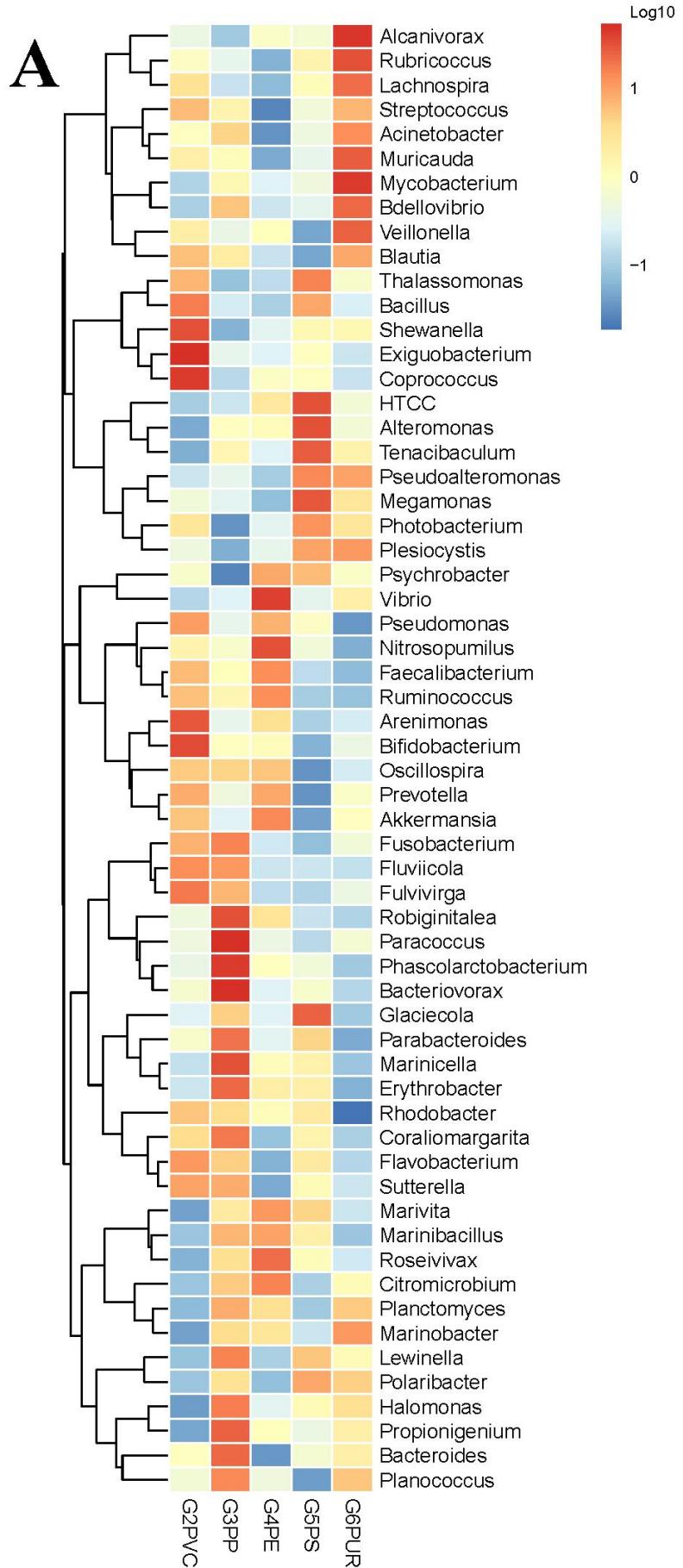
**Figure S4:** Relative abundance of top 10 bacterial genera on PD at different in situ sites. (A) Relative abundance of top 10 bacterial genera on PD after the two-week exposure. (B) Relative abundance of top 10 bacterial genera on PD after the four-week exposure. (C) Relative abundance of top 10 bacterial genera on PD after the six-week exposure.

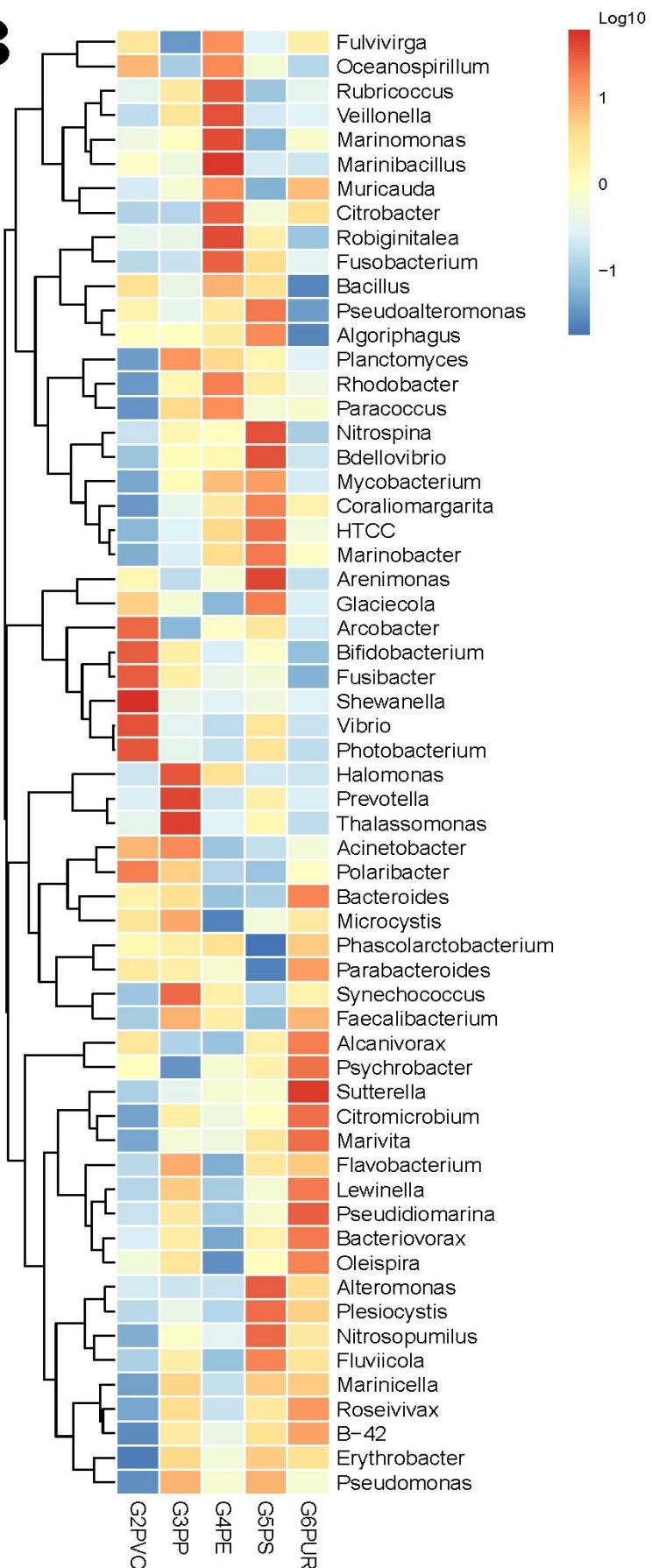


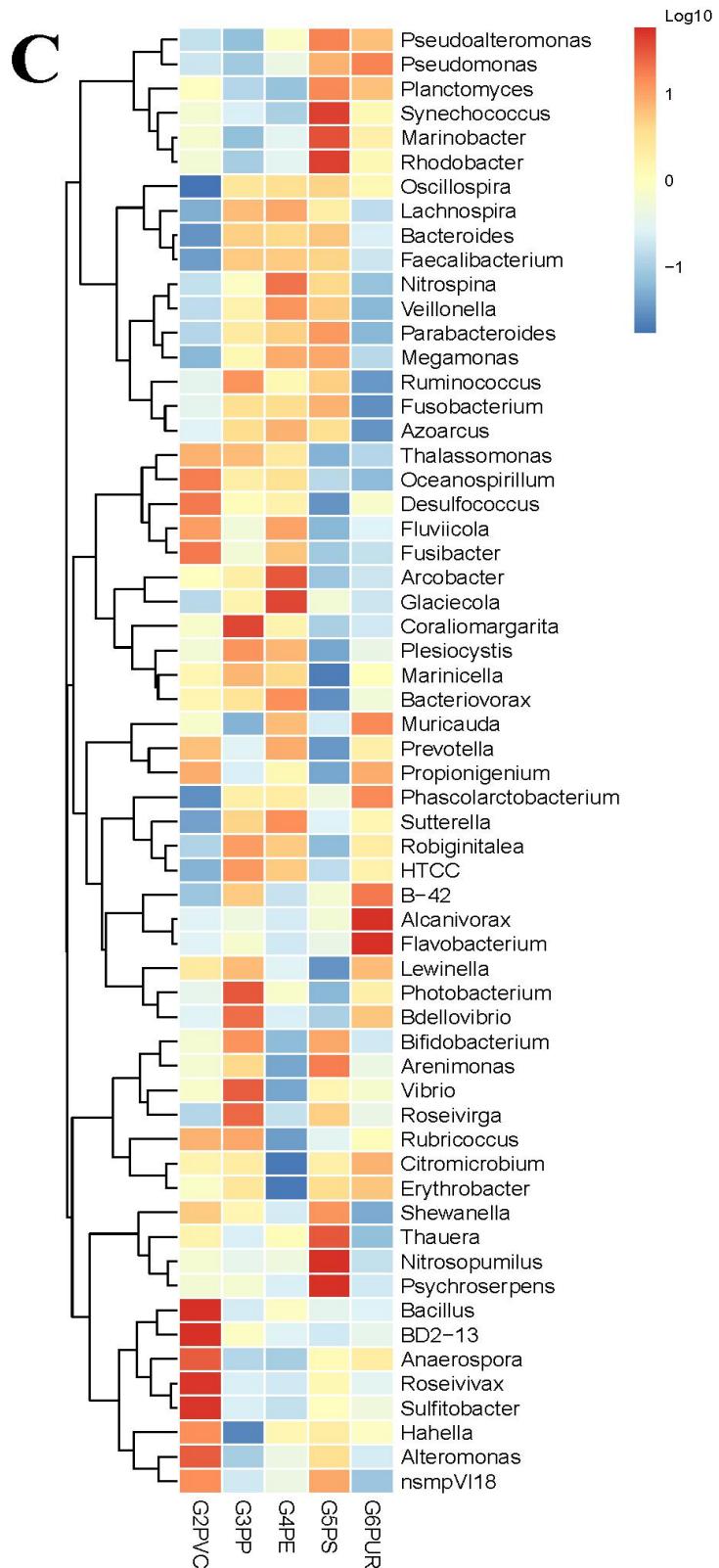
**Figure S5:** The Chao 1 index of different types of PD in three-time periods.



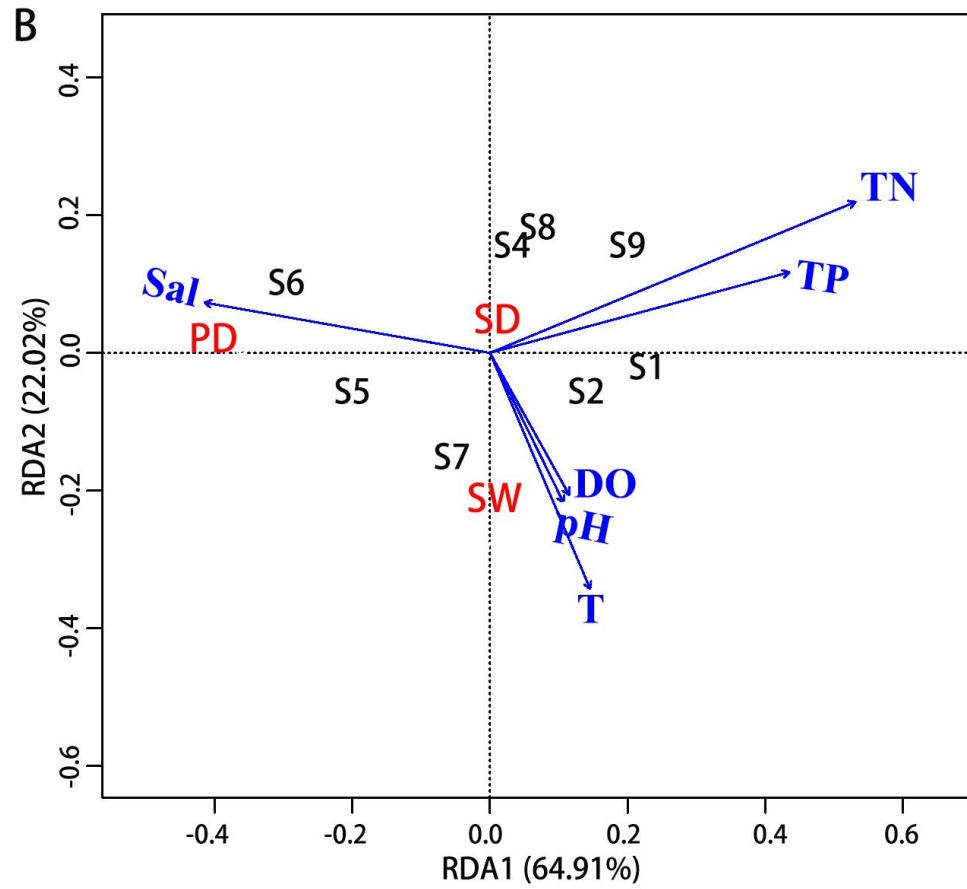
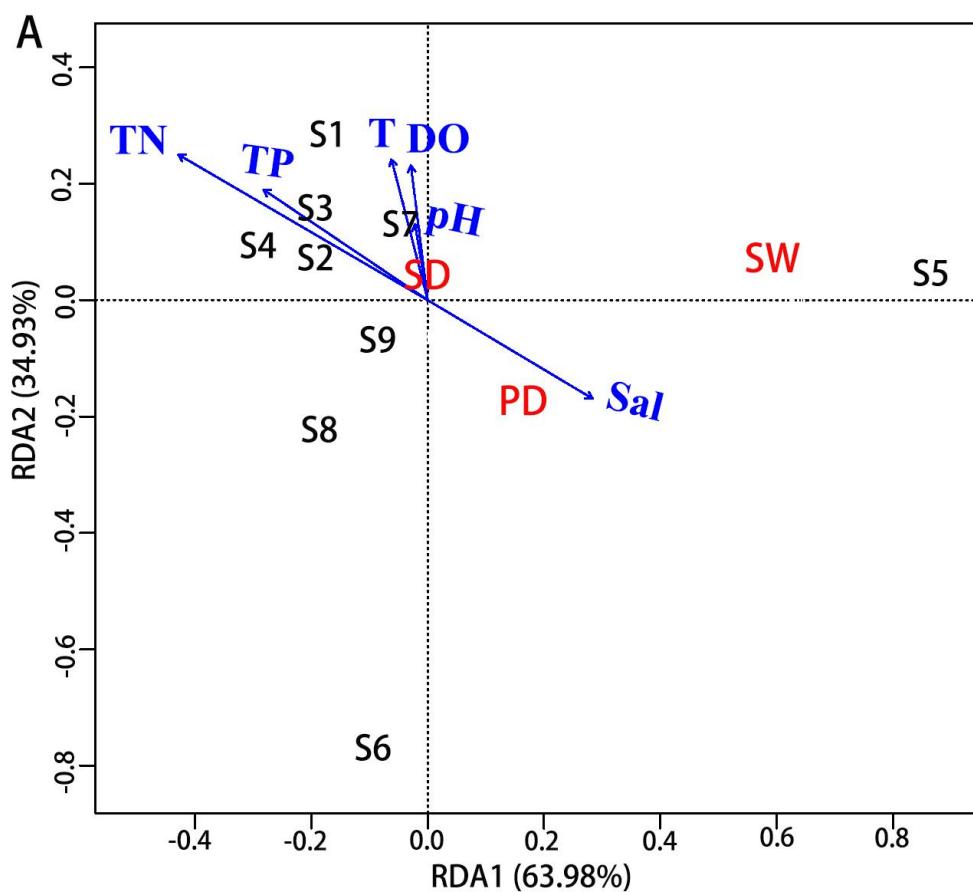
**Figure S6:** The major genera of bacterial communities (top 10) on different PD types in three-time periods. (A) The major genera of bacterial communities (top 10) on different PD after the two-week exposure. (B) The major genera of bacterial communities (top 10) on different PD after the four-week exposure. (C) The major genera of bacterial communities (top 10) on different PD after the six-week exposure.

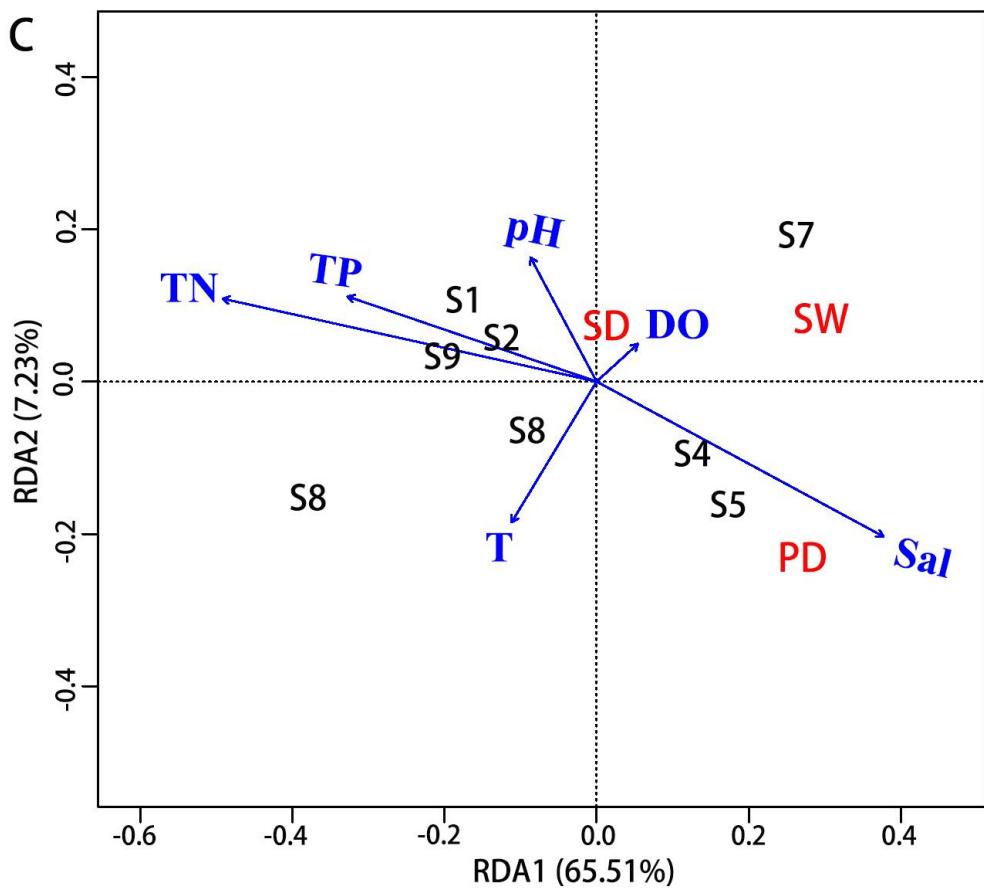


**B**

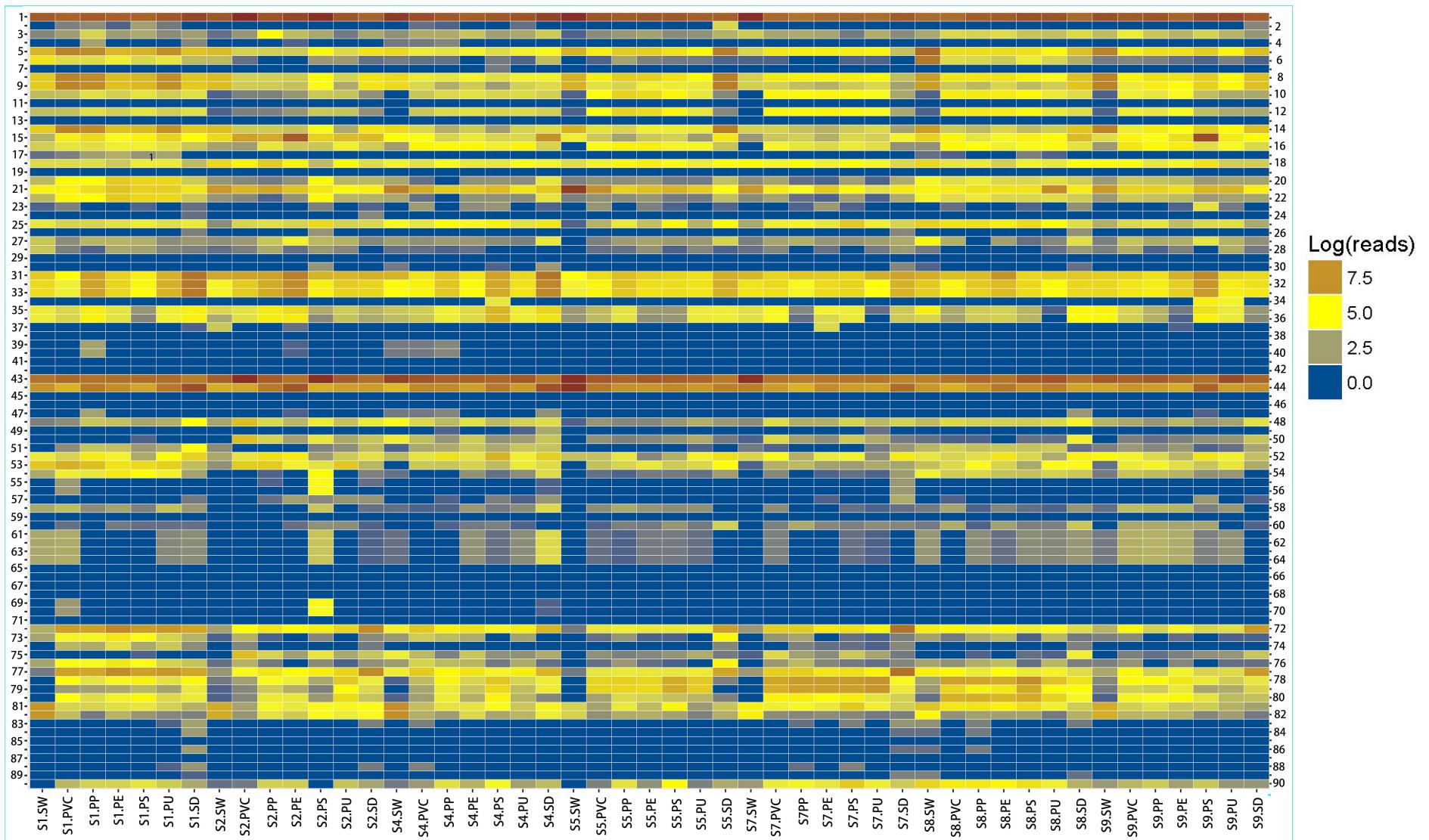


**Figure S7:** Heatmap of marker genus on different PD types ( $n = 5$ ), the data for each PD is derived from the sum of the same PD at all in situ sites ( $n = 9$ ). (A) Heatmap after the two-week exposure. (B) Heatmap after the four-week exposure. (C) Heatmap after the six-week exposure.





**Figure S8:** Redundancy analysis (RDA) identifying the correlation between the relative abundance of conditional pathogen *Vibrio* on different substrates (SW, SD and PD) and environmental factors, including T(temperature), Sal (salinity), DO (dissolved oxygen), pH, TN (total nitrogen) and TP (total phosphorus). (A) RDA after the two-week exposure. (B) RDA after the four-week exposure. (C) RDA after the six-week exposure.



**Figure S9:** Heatmap of metabolic functions of bacteria genera on SW, SD and PD samples after the six-week exposure based on FAPROTAX Software. Metabolic functions are shown in the table below.

1	chemoheterotrophy	31	animal_parasites_or_symbionts	61	denitrification
2	reductive_acetogenesis	32	mammal_gut	62	nitrous_oxide_denitrification
3	ureolysis	33	human_gut	63	nitrite_denitrification
4	plastic_degradation	34	fish_parasites	64	nitrate_denitrification
5	phototrophy	35	human_pathogens_all	65	anammox
6	photoheterotrophy	36	human_pathogens_diarrhea	66	dissimilatory_arsenite_oxidation
7	aerobic_anoxigenic_phototrophy	37	human_pathogens_gastroenteritis	67	arsenite_oxidation_energy_yielding
8	photoautotrophy	38	human_pathogens_meningitis	68	arsenite_oxidation_detoxification
9	oxygenic_photoautotrophy	39	human_pathogens_nosocomia	69	dissimilatory_arsenate_reduction
10	anoxygenic_photoautotrophy	40	human_pathogens_pneumonia	70	arsenate_respiration
11	anoxygenic_photoautotrophy_Fe_oxidizing	41	human_pathogens_septicemia	71	arsenate_detoxification
12	anoxygenic_photoautotrophy_S_oxidizing	42	invertebrate_parasites	72	respiration_of_sulfur_compounds
13	anoxygenic_photoautotrophy_H2_oxidizing	43	aerobic_chemoheterotrophy	73	thiosulfate_respiration
14	cyanobacteria	44	fermentation	74	sulfite_respiration
15	chloroplasts	45	ligninolysis	75	dark_sulfite_oxidation
16	predatory_or_exoparasitic	46	manganese_respiration	76	sulfur_respiration
17	chlorate_reducers	47	manganese_oxidation	77	sulfate_respiration
18	intracellular_parasites	48	dark_oxidation_of_sulfur_compounds	78	nitrification

19	fumarate_respiration	49	dark_thiosulfate_oxidation	79	aerobic_nitrite_oxidation
20	nitrogen_respiration	50	dark_sulfur_oxidation	80	aerobic_ammonia_oxidation
21	nitrate_reduction	51	dark_sulfide_oxidation	81	methylotrophy
22	nitrate_respiration	52	xylanolysis	82	methanol_oxidation
23	iron_respiration	53	cellulolysis	83	methanogenesis
24	dark_iron_oxidation	54	nitrite_respiration	84	hydrogenotrophic_methanogenesis
25	hydrocarbon_degradation	55	nitrite_ammonification	85	methanogenesis_by_reduction_of_methyl_compounds_with_H2
26	aliphatic_non_methane_hydrocarbon_degradation	56	nitrate_ammonification	86	methanogenesis_by_CO2_reduction_with_H2
27	aromatic_compound_degradation	57	nitrogen_fixation	87	methanogenesis_using_formate
28	aromatic_hydrocarbon_degradation	58	dark_hydrogen_oxidation	88	methanogenesis_by_disproportionation_of_methyl_groups
29	oil_bioremediation	59	knallgas_bacteria	89	acetoclastic_methanogenesis
30	plant_pathogen	60	chitinolysis	90	methanotrophy

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