Supplementary File for:

Biofilm Bacterial Community Transition under Water Supply Quality Changes in Drinking Water Distribution Systems

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TABLE S1 Pipelines information

	DX	QH	SLJ	HJL
Pipe diameter	DN150	DN100	DN100	DN100
Pipe length	30 m	20 m	20 m	12 m
Pipe age	> 15 a	> 15 a	> 15 a	> 15 a
Pipe material	unlined cast iron	unlined cast iron	unlined cast iron	unlined cast iron
Water supply histories	GW[a]	SW[b]	BW[c]	GW
Flush velocity (m^3/h)	20	15	15	15
Water flow (L/min)	0.400	0.121	0.121	0.090

[a]GW: groundwater
[b]SW: surface water
[c]BW: blend water

TABLE S2 Supply water quality parameter adjustment of different phases

Phase	Adjustment parameter	Operation period/d	Chemicals added
P1	original supply water[a]	36	
P2	SO_4^{2-}	10	Na_2SO_4
P3	Cl^-	9	NaCl
P4	HCO_3^-	12	$NaHCO_3$
P5	pH	12	NaOH
P6	Ca^{2+} and HCO_3^-	11	$Ca(OH)_2$ and CO_2

[a]Original supply water: treated water from a local water treatment plant.

TABLE S3 Primer design of all samples

Sample	Primer sequence	Region	Length	Sequencing platform	Reference
P biofilm	338F: 5'-ACTCCTACGGGAGGCAGCAG-3' 806R: 5'-GGACTACHVGGGTWTCTAAT-3'	V3-V4	468bp	PE300	Xu, et al., 2016
P1-P6 biofilm	515F: 5'-GTGCCAGCMGCCGCGG-3' 907R: 5'-CCGTCAATTCMTTTRAGTTT-3'	V4-V5	392bp	PE250	Yusoff, et al., 2013

Note: The reason for using different primers was that the samples were collected and analyzed at different time and different places although they were treated by the similar methods (attention was paid to avoid possible unreasonable interpretation associated to different primers).

References：

1. Xu N, Tan G, Wang H, et al. Effect of biochar additions to soil on nitrogen leaching, microbial biomass and bacterial community structure[J]. European Journal of Soil Biology, 2016, 74: 1-8.

2. Yusoff M Z M, Hu A, Feng C, et al. Influence of pretreated activated sludge for electricity generation in microbial fuel cell application [J]. Bioresource technology, 2013, 145: 90-96.

TABLE S4 Diversity statistics for P biofilm and P1 biofilm samples

Sample	reads	0.97[a]						
		OTU	Ace	Chao 1	Coverage	Shannon	Simpson	Heip
DX_P	11052	701	758	752	0.990	4.72	0.03	0.16
HJL_P	11052	417	423	430	0.998	5.20	0.01	0.44
QH_P	11052	325	447	401	0.990	2.47	0.23	0.03
SLJ_P	11052	516	535	530	0.996	3.69	0.16	0.08
DX_P1	11052	125	149	144	0.997	2.73	0.12	0.12
HJL_P1	11052	186	202	201	0.997	3.18	0.12	0.12
QH_P1	11052	202	219	219	0.997	3.19	0.11	0.12
SLJ_P1	11052	197	211	215	0.998	3.18	0.11	0.12

[a]0.97: equivalent to 97% similarity

TABLE S5 Diversity statistics for all samples

Sample ID	reads	0.97[a]						
		OTU	Ace	Chao 1	Coverage	Shannon	Simpson	Heip
P1	46321	426	462	464	0.999	3.34	0.10	0.06
P2	46321	407	482	494	0.998	3.80	0.05	0.11
P3	46321	454	530	522	0.998	4.10	0.03	0.13
P4	46321	392	430	422	0.999	3.98	0.04	0.13
P5	46321	289	375	370	0.998	1.06	0.70	0.01
P6	46321	375	432	467.5	0.998	3.40	0.08	0.08

[a]0.97: equivalent to 97% similarity

TABLE S6 Relative abundance of differences species (the top 15 abundance species at genus level) in each pipe biofilm (%)*

Genus	P1	P2	P3	P4	P5	P6
Rhizobacter	28.26	10.15				
Sphingomonas	8.15	2.90				
Rhizobacter	28.26		9.68			
Parvularcula	0.02		4.97			
Rhizobacter	28.26			3.40		
Sphingobium	0.38			11.56		
Phreatobacter	0.88			9.38		
Rhodobacter	6.02			1.91		
Hyphomicrobium	6.44			0.38		
Sphingopyxis	5.16			0.37		
Burkholderia	0				83.53	
Rhizobacter	28.26				0.07	
Sphingomonas	8.15				0.25	
Porphyrobacter	7.53				0.55	
Hyphomicrobium	6.44				0.17	
Rhodobacter	6.02				0.65	
Sphingopyxis	5.16				0.12	
Bosea	1.75				0.11	
Bradyrhizobium	0.37				1.60	
Burkholderia	0					18.51
Sporosarcina	0.04					6.10

*The blank cells mean “0”.

TABLE S7 ANOSIM statistics for P1-P6 biofilm samples

Method	Statistic	*p* value	Permutation_number
ANOSIM	0.8891	0.001	999

TABLE S8 Relative abundance of potential corrosive bacteria at genus level in biofilms with two different water sources (%)

	Genus	P				P1			
		DX_P	QH_P	SLJ_P	HJL_P	DX_P1	QH_P1	SLJ_P1	HJL_P1
IOB	*Acidovorax*	0.072	0.018	0.127	0.217	0	0	0	0
	Bradyrhizobium	0.968	0.010	0.299	0.416	0.027	0.371	0.398	0.624
	Aquabacterium	1.692	0.271	0.416	2.226	0	0	0	0
	Sediminibacterium	0.009	0	0.109	0.407	0.009	0.389	0.118	0.172
	Sideroxydans	2.172	0.036	0.145	0	0.027	0.063	0.036	0.181
IRB	*Bacillus*	1.837	1.629	1.004	0.090	0.072	0.407	0.145	0.244
	Geothrix	0.018	0.036	0.054	0	0	0.036	0.072	0.018
	Pseudomonas	0.127	0.081	0.986	1.773	0.036	0.072	0.009	0.172
SOB	*Sulfuricella*	0.299	0.081	0	0	0	0	0	0
	Thiobacillus	0	0	0	0	0.009	0.389	0.226	0
SRB	*Desulfovibrio*	0.253	0.136	0.072	0	0	0.054	0	0

TABLE S9 COG data of different samples

	P1 (%)	P2 (%)	P3 (%)	P4 (%)	P5 (%)	P6 (%)	Description
COG 0175	0.083	0.069	0.07	0.077	0.047	0.057	Reduction of activated sulfate into sulfite
COG 4114	0.0004	0.0005	0.0007	0.00003	0.014	0.011	ferric iron reductase

TABLE S10 Relative abundance of potential opportunistic pathogens at genus level in biofilms with two different water sources (%)

	P				P1			
Genus	DX_P	QH_P	SLJ_P	HJL_P	DX_P1	QH_P1	SLJ_P1	HJL_P1
Acinetobacter	0.344	0.452	2.117	11.039	0	0.054	0.027	0.009
Pseudomonas	0.127	0.081	0.986	1.773	0.036	0.072	0.009	0.172
Mycobacterium	7.818	1.077	0.814	2.018	0.163	0.308	0.335	0.733
Ralstonia	1.321	0.452	0.226	1.077	0.271	0.217	0.109	0.525
Kocuria	0.172	43.739	0.516	0.443	0	0	0	0

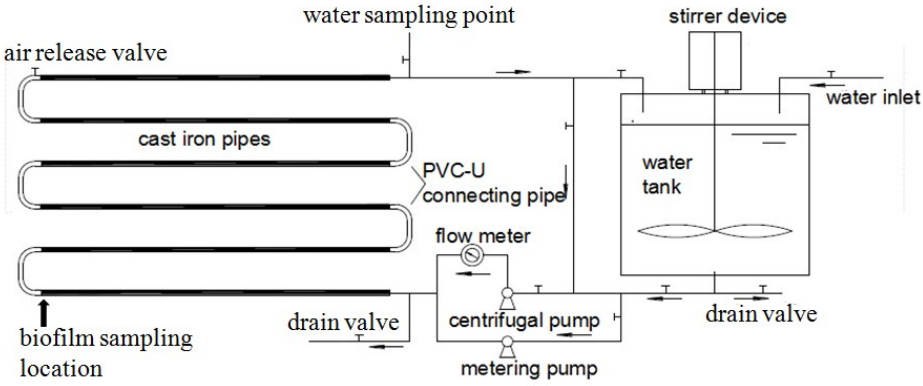
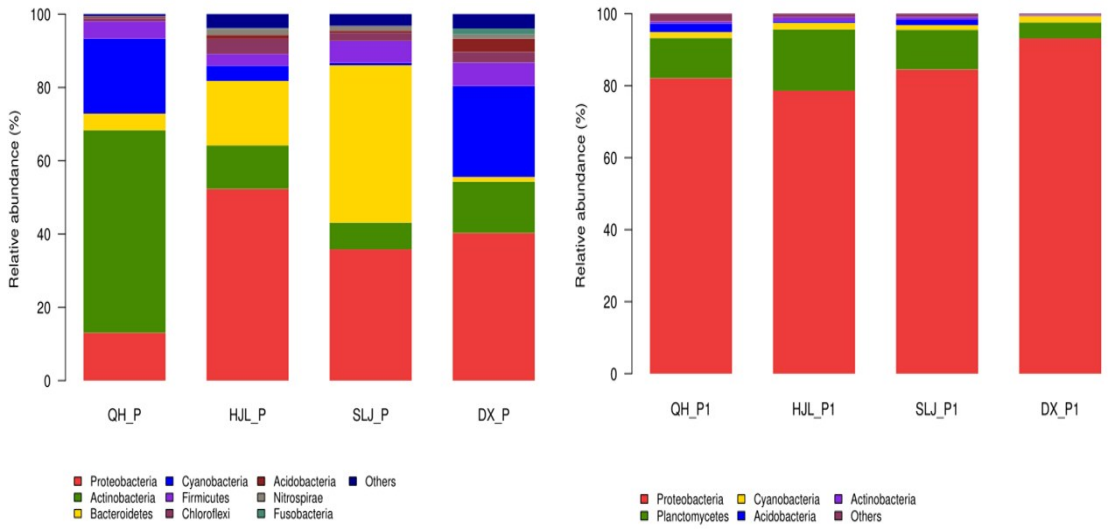
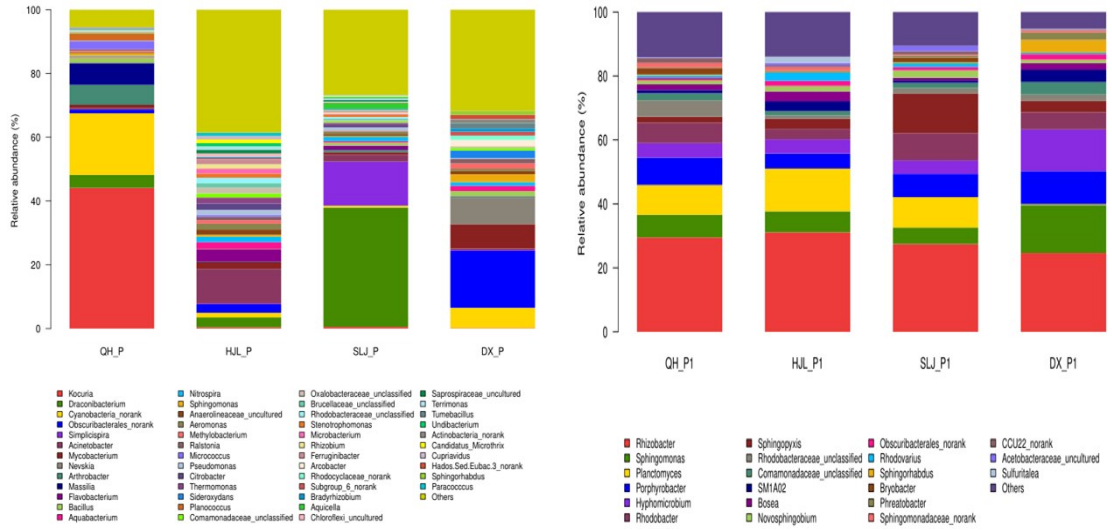


FIG S1 Simulated distribution systems



(a1) P samples (a2) P1 samples

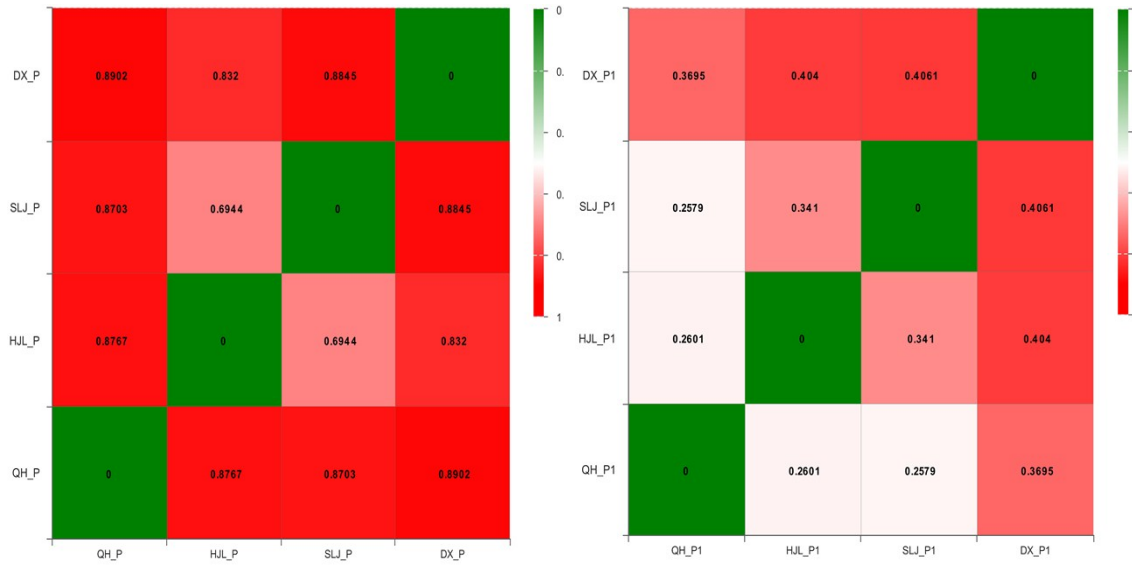
(a) phylum



(b1) P samples (b2) P1 samples

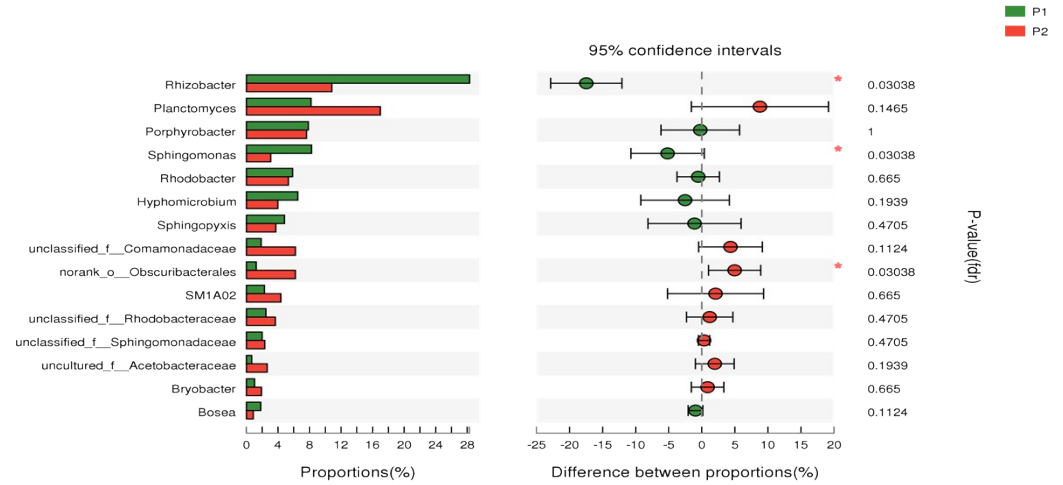
(b) genus

FIG S2 Bacterial community of P and P1 biofilms of the 4 systems

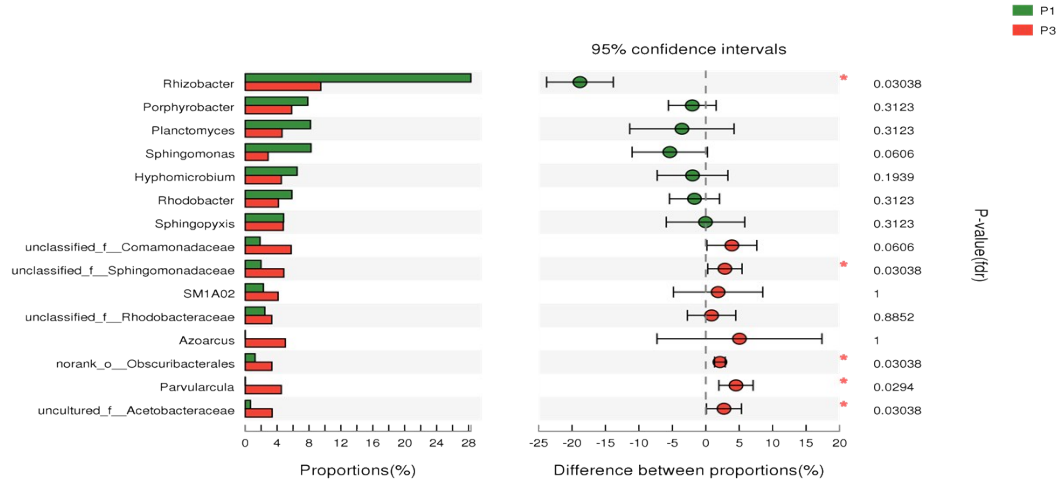


P biofilm P1 biofilm

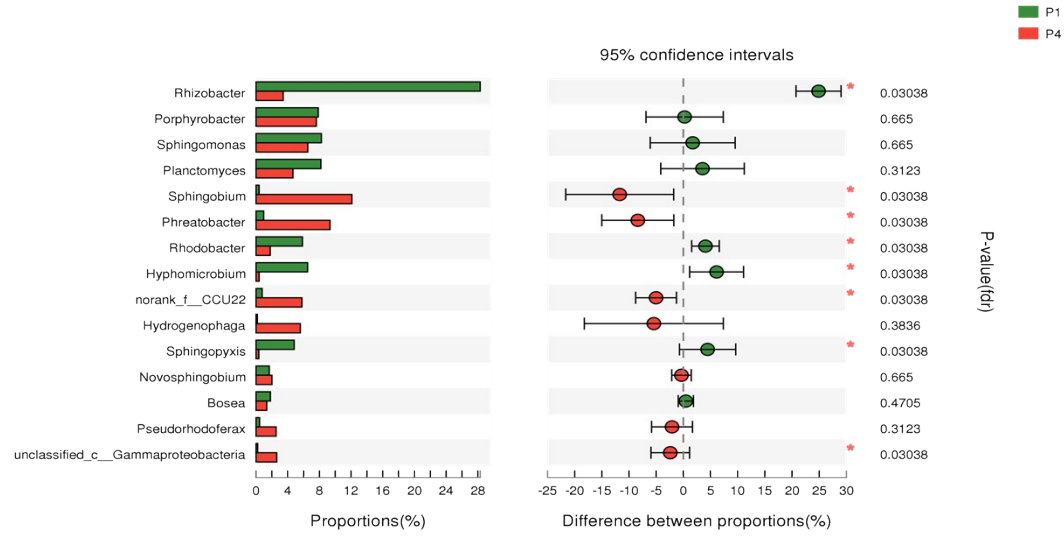
FIG S3 Samples distances heatmap at OTU level


P1
P2
95% confidence intervals
Rhizobacter
Planctomyces
Porphyrobacter
Sphingomonas
Rhodobacter
Hyphomicrobium
Sphingopyxis
unclassified_f__Comamonadaceae
norank_o__Obscuribacterales
SM1A02
unclassified_f__Rhodobacteraceae
unclassified_f__Sphingomonadaceae
uncultured_f__Acetobacteraceae
Bryobacter
Bosea
0.03038
0.1465
1
0.03038
0.665
0.1939
0.4705
0.1124
0.03038
0.665
0.4705
0.4705
0.1939
0.665
0.1124
P-value(fdr)
0 4 8 12 16 20 24 28
Proportions(%)
-25 -20 -15 -10 -5 0 5 10 15 20
Difference between proportions(%)

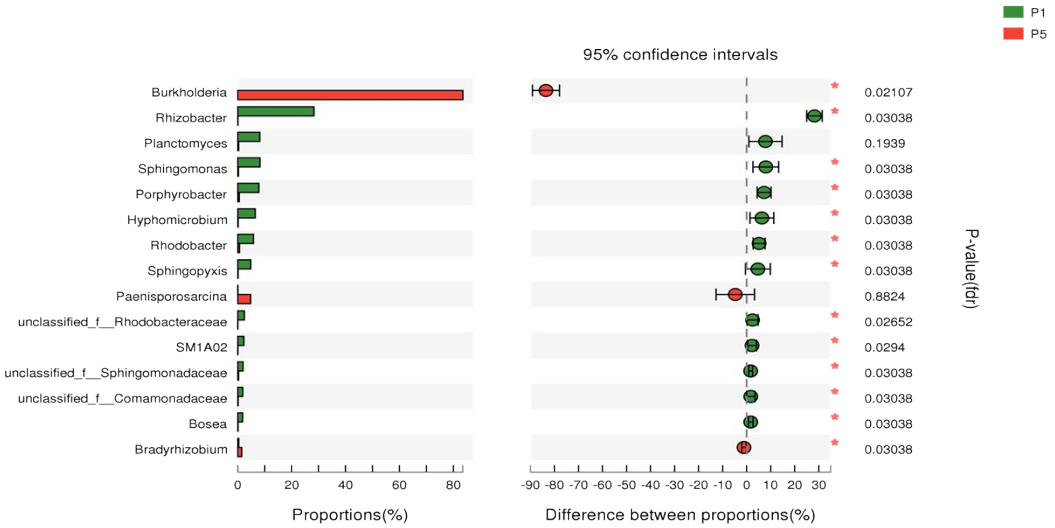
a


P1
P3
95% confidence intervals
Rhizobacter
Porphyrobacter
Planctomyces
Sphingomonas
Hyphomicrobium
Rhodobacter
Sphingopyxis
unclassified_f__Comamonadaceae
unclassified_f__Sphingomonadaceae
SM1A02
unclassified_f__Rhodobacteraceae
Azoarcus
norank_o__Obscuribacterales
Parvularcula
uncultured_f__Acetobacteraceae
0.03038
0.3123
0.3123
0.0606
0.1939
0.3123
0.3123
0.0606
0.03038
1
0.8852
1
0.03038
0.0294
0.03038
P-value(fdr)
0 4 8 12 16 20 24 28
Proportions(%)
-25 -20 -15 -10 -5 0 5 10 15 20
Difference between proportions(%)

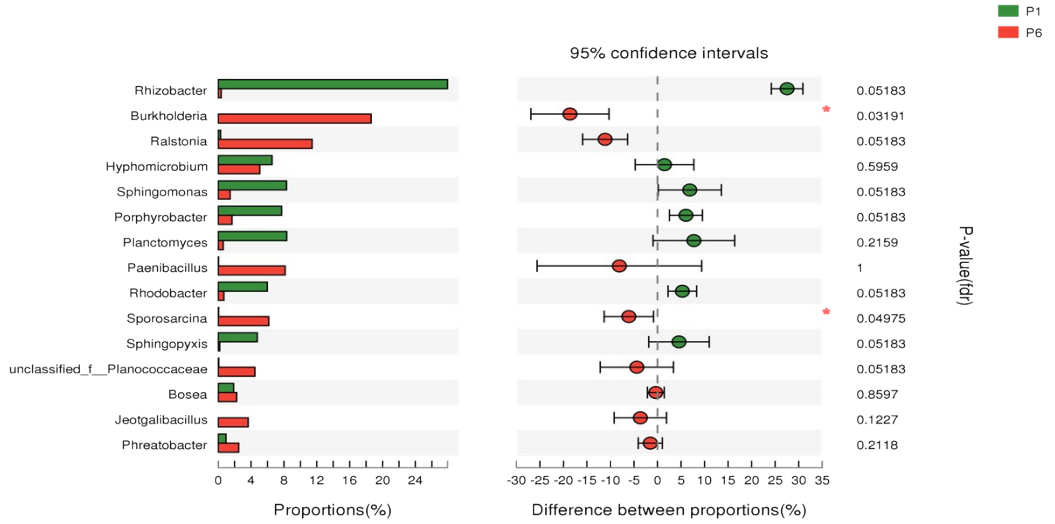
b


P1
P4
95% confidence intervals
Rhizobacter
Porphyrobacter
Sphingomonas
Planctomyces
Sphingobium
Phreatobacter
Rhodobacter
Hyphomicrobium
norank_f__CCU22
Hydrogenophaga
Sphingopyxis
Novosphingobium
Bosea
Pseudorhodoferax
unclassified_c__Gammaproteobacteria
0.03038
0.665
0.665
0.3123
0.03038
0.03038
0.03038
0.03038
0.03038
0.3836
0.03038
0.665
0.4705
0.3123
0.03038
P-value(fdr)
0 4 8 12 16 20 24 28
Proportions(%)
-25 -20 -15 -10 -5 0 5 10 15 20 25 30
Difference between proportions(%)

c



d



e

FIG S4 Wilcoxon rank-sum test bar plot on Genus level among P1 and P2, P3, P4, P5, P6 biofilm

There were two significantly different species (*Rhizobacter*, *Sphingomonas*) (only display a clear classification status of the species, the same below) between pipe wall biofilm P1 and P2 (Fig. S5a). Several *Rhizobacter* have been found in biofilms of polluted rivers [1] and most remain uncultured so far. The relative abundance of *Rhizobacter* has a different degree of reduced in high SO_4^{2-}, Cl^-, HCO_3^- supply water and disappeared under high pH supply water. *Sphingomonas* isolated from many different land and water habitats, are widely distributed in natureas well as in certain toxic environment. Many *Sphingomonas* have been isolated from environments contaminated with toxic compounds, where they display the ability to utilize the contaminants as nutrients .[2] The relative abundance of *Sphingomonas* of pipe wall biofilm decreased when increasing the SO_4^{2-} of supply water, correspond with the relative abundance of *Sphingomonas* of tap water is also decreased. There were two significantly different species, *Rhizobacter* and *Parvularcula*, between pipe wall

biofilm P1 and P3 (Fig. S5b). *Parvularcula* mainly were isolated from the seawater ,[3] the Cl^- may be has a positive effect on it. There were six significant differences species (*Rhizobacter*, *Sphingobium*, *Phreatobacter*, *Rhodobacter*, *Hyphomicrobium*, *Sphingopyxis*) between pipe wall biofilm P1 and P4 (Fig. S5c). And there were nine significantly different species (*Burkholderia*, *Rhizobacter*, *Sphingomonas*, *Porphyrobacter*, *Hyphomicrobium*, *Rhodobacter*, *Sphingopyxis*, *Bosea*, *Bradyrhizobium*) between pipe wall biofilm P1 and P5 (Fig. S5d). After increasing pH of supply water, most of bacteria (such as *Rhizobacter*, *Sphingomonas*, *Porphyrobacter*, *Hyphomicrobium*, *Rhodobacter*, *Sphingopyxis*, *Bosea*) were disappear except *Burkholderia*. This result indicated that pH had a significant impact on bacterial community structure. There were two significantly different species (*Burkholderia*, *Sporosarcina*) between pipe wall biofilm P1 and P6 (Fig. S5e). It is noteworthy that *Burkholderia* is known as a opportunistic pathogen whose pathogenic members include *Burkholderia cepacia*, an important pathogen of pulmonary infections in people with cystic fibrosis .[4]

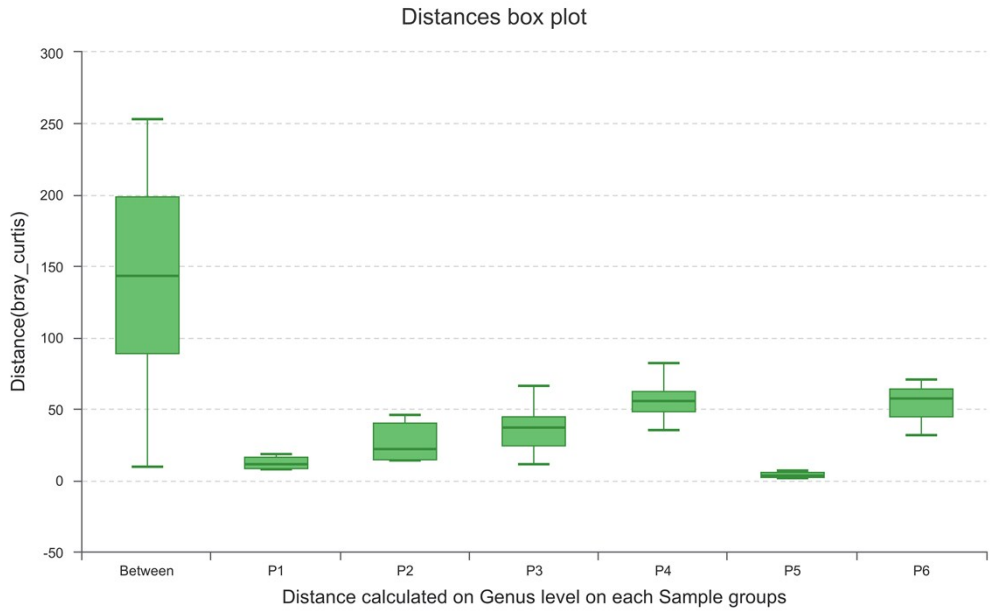


FIG S5 Distances calculated on genus level on each sample group

1. I. H. M. Brümmer, A. Felske and I. WagnerDöbler, Diversity and Seasonal Variability of β-Proteobacteria in Biofilms of Polluted Rivers: Analysis by Temperature Gradient Gel Electrophoresis and Cloning, *Applied & Environmental Microbiology*, 2003, **69** (8), 4463-4473.

2. K. Nilgiriwala, A. Alahari and A. Rao, Sk, Cloning and overexpression of alkaline phosphatase PhoK from Sphingomonas sp strain BSAR-1 for bioprecipitation of uranium from alkaline solutions, *Applied & Environmental Microbiology*, 2008, **74** (17), 5516-5523.

3. X. Q. Zhang, Y. H. Wu, X. Zhou, X. Zhang, X. W. Xu and M. Wu, Parvularcula flava sp. nov., an alphaproteobacterium isolated from surface seawater of the South China Sea, *International Journal of Systematic & Evolutionary Microbiology*, 2016, **66** (9).

4. D. E. Woods and P. A. Sokol, *The Genus Burkholderia*, Springer New York, 2006.