Supplementary Materials

The impact of extreme weather events on bacterial communities and opportunistic pathogens in a drinking water treatment plant

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The SI contains 12 pages, which has 7 tables and 4 Figures.

Table S1. Construction of major treatment facilities in the DWTP.

Treatment facility		Size of each tank (m)	Effective depth (m)
Coagulation tank		19.5 × 8.5	5.0
Horizontal flow sedimentation tank		112.2 × 8.5	3.6
V-type filtration tank		15.0 × 7.0	2.4
Multiflo sedimentation tank [1]	Coagulation	6.7× 6.7	6.5
	Flocculation	15.0 × 6.0	7.5
	Sedimentation	15.0 × 15.0	7.1
TGV filtration tank [2]		16.4 × 6.1	3.2
Ozonation contact reactor		20.2 × 22.4	7.5
Biological activated carbon (BAC) tank		17.5 × 8.0	4.2

[1] Multiflo sedimentation tank has been developed by Veolia Water Technologies, which contains three parts (coagulation, flocculation and sedimentation) for partial, normal or advanced removal of suspended solids, carbonaceous pollutants and phosphorous.
[2] TGV filtration tank, a high-rate filtration tank developed by Veolia Water Technologies, employs the familiar basic principle of rapid gravitational filtration of settled water through a granular media. The filtering bed is composed of single, dual or triple media layers.

Table S2. Physiochemical parameters of source water samples collected at different timepoints.

Sample [1]	pH	Temp (℃)	Turbidity (NTU)	NO$_3$-N (mg/L)	NH$_3$-N (mg/L)	DOC (mg/L)
S1_Aut	7.32	20.1	48.3	7.0	0.13	2.29
S2_Aut	7.71	20.8	23.3	2.7	0.03	2.26
S3_Aut	7.85	20.3	17.1	3.5	0.14	2.13
S4_Aut	7.29	19.5	18.3	5.0	0.11	1.96
S1_Win	8.01	8.7	27.0	4.5	0.29	2.30
S2_Win	7.95	8.5	18.8	5.0	0.42	2.97
S1_Spr	8.03	17.4	16.8	2.8	0.06	1.69
S2_Spr	7.74	18.0	14.1	2.5	0.04	1.59
S1_Sum	7.69	24.2	26.1	5.2	0.06	2.21
S2_Sum	7.71	25.6	40.2	4.0	0.10	2.31
S3_Sum	7.77	26.0	26.4	4.9	0.12	2.26
S4_Sum	7.77	25.4	25.8	5.7	0.06	2.33
S1_Sum_R	7.80	25.2	23.6	3.6	0.20	2.53
S2_Sum_R	7.73	25.6	28.4	4.9	/	2.35
S3_Sum_R	7.64	25.1	19.1	3.5	/	3.91
S4_Sum_R	7.69	25.0	19.8	3.8	0.47	3.40

[1] Different timepoints. Aut = autumn; Win = winter; Spr = spring; Sum = summer; Sum_R = after summer rainfall.

Table S3. Physiochemical parameters of treated water samples collected at different timepoints.

Sample [1]	pH	Temp (℃)	Turbidity (NTU)	NO_3-N (mg/L)	NH_3-N (mg/L)	DOC (mg/L)	Total Cl_2 (mg/L)
Raw water_Aut	7.64	20.1	29.8	5.0	0.08	2.14	0.14
Sedimentation_Aut	7.48	20.3	1.16	0.7	<0.01	2.27	0.02
Multiflo_Aut	7.20	20.3	1.73	0.7	0.05	2.06	<0.01
Filtration_Aut	7.56	20.4	0.11	1.1	0.01	1.79	0.36
TGV_Aut	6.97	22.9	0.11	1.3	<0.01	2.02	0.22
O3-BAC_Aut	7.58	20.4	0.08	1.5	0.02	1.50	<0.01
Disinfection_Aut	7.39	22.9	0.01	1.4	0.04	1.45	0.66
Raw water_Win	7.79	8.4	29.0	5.5	0.15	2.22	0.19
Sedimentation_Win	7.72	8.3	1.51	2.2	0.22	1.85	0.59
Multiflo_Win	7.68	8.4	1.12	2.0	0.19	1.78	0.63
Filtration_Win	8.20	8.4	0.11	1.8	0.01	1.76	0.36
TGV_Win	8.14	8.4	0.17	2.4	0.01	1.71	0.58
O3-BAC_Win	8.15	8.4	0.08	1.6	0.18	1.40	<0.01
Disinfection_Win	8.14	9.1	0.08	1.9	<0.01	1.45	0.44
Raw water_Spr	7.91	18.0	27.0	3.4	0.05	1.59	0.11
Sedimentation_Spr	7.85	20.3	0.86	1.1	<0.01	1.46	0.24
Multiflo_Spr	7.88	18.1	1.0	0.7	0.01	1.40	0.17
Filtration_Spr	7.56	17.6	0.06	1.0	<0.01	1.05	0.71
TGV_Spr	7.72	18.3	0.04	1.0	<0.01	1.08	0.66
O3-BAC_Spr	7.74	18.6	0.04	0.4	<0.01	1.13	0.04
Disinfection_Spr	7.90	18.3	0.09	0.7	<0.01	1.05	0.74
Raw water_Sum	7.90	26.5	51.0	8.3	0.07	1.94	0.39
Sedimentation_Sum	7.73	30.7	0.93	1.0	<0.01	2.01	0.14
Multiflo_Sum	7.82	26.4	1.60	1.4	<0.01	1.90	0.15
Filtration_Sum	7.79	26.5	0.06	0.9	<0.01	1.53	0.69
TGV_Sum	7.80	26.4	0.15	1.5	0.10	1.56	0.71
O3-BAC_Sum	7.73	26.5	0.08	1.6	<0.01	1.67	0.03
Disinfection_Sum	7.75	26.4	0.11	1.0	<0.01	1.57	0.71
Raw water_Sum_R	7.77	25.8	23.2	7.9	0.07	2.30	0.35
Sedimentation_Sum_R	7.87	30.2	1.11	2.4	<0.01	2.12	0.14
Multiflo_Sum_R	7.69	26.6	1.72	1.5	<0.01	2.04	0.15
Filtration_Sum_R	7.65	26.7	0.09	1.3	<0.01	1.62	0.86
TGV_Sum_R	7.69	26.7	0.24	2.3	<0.01	1.64	0.91
O3-BAC_Sum_R	7.61	26.0	0.07	1.3	0.02	1.57	0.01
Disinfection_Sum_R	7.63	26.4	0.11	1.6	<0.01	1.59	0.79

[1] Same abbreviation as **Table S2**.

Table S4. Physiochemical parameters of water samples collected during typhoon period.

Sample [1]	pH	Temp (℃)	Turbidity (NTU)	NO_3-N (mg/L)	NH_3-N (mg/L)	Total Cl_2 (mg/L)
Before SW	8.11	30.6	31.2	3.8	0.05	
BeforeRW	8.01	30.6	32.8	5.0	0.07	0.12
Before FW	7.76	30.3	0.18	1.2	0.01	0.66
During SW	8.06	29.8	41.6	3.6	0.05	
DuringRW	7.96	29.5	41.4	7.4	0.04	0.26
During FW	7.73	29.3	0.20	1.5	0.02	0.88
After1RW	7.98	29.1	39.1	2.1	0.05	0.37
After1 FW	7.76	29.1	0.16	4.3	0.01	0.73
After2 SW	8.13	28.9	35.1	1.7	0.04	
After2RW	8.03	28.8	38.3	2.9	0.02	0.25
After2 FW	7.74	28.7	0.09	0.9	0.01	0.66
After3 SW	8.16	29.6	33.5	3.0	0.05	
After3RW	8.06	29.5	35.9	4.0	0.09	0.34
After3 FW	7.73	29.2	0.08	1.7	0.03	0.71

[1] SW = source water; RW = raw water; FW = finished water; After1, 2, and 3 = after one, two and three days of the typhoon event; Before = before the typhoon event.

Table S5. PCR primers, probes, and annealing temperatures used in this study. [1]

Targeted organism	Targeted gene	Sequences (5'-3')	Annealing temperature	Reference
Total Bacteria	16S rRNA	1368F:CGGTGAATACGTTCYCGG 1492R:GGWTACCTTGTTACGACTT	55℃	[1]
Legionella spp.	23S rRNA	Leg23SF: CCCATGAAGCCCGTTGAA Leg23SR:ACAATCAGCCAATTAGTACGAGTTAC Probe: FAM-TCCACACCTCGCCTATCAACGTCGTAGT	58.5℃	[2]
L. pneumophila	*mip*	LmipF:AAAGGCATGCAAGACGCTATG LmipR: GAAACTTGTTAAGAACGTCTTTCATTTG Probe: FAM-TGGCGCTCAATTGGCTTTAACCGA	60℃	[2]
Mycobacterium spp.	16S rRNA	110F: CCTGGGAAACTGGGTCTAAT I571R: CGCACGCTCACAGTTA Probe: FAM-TTTCACGAACAACGCGACAAACT	56℃	[3]
M. avium	16S rRNA	MycavF: AGAGTTTGATCCTGGCTCAG MycavR: ACCAGAAGACATGCGTCTTG	64℃	[4]

[1] DNA standards were obtained by amplifying target genes cloned to plasmids (TOPO® TA cloning® kit, Carlsbad, CA) using vector-specific primer M13F and M13R. M13 PCR products were quantified and then diluted to obtain a series of 10-fold DNA standards.

Table S6. The numbers of gene copies of *Mycobacterium* spp., *Legionella* spp. and *Legionella pneumophila* in biofilm from the top (B1), middle (B2) and bottom (B3) of the reactor. These samples were collected in the fourth, fifth and sixth months after the start of operation of BAC filtration in the water treatment plant.

Target pathogens	Sample	Concentration (copies/g)		
		4th month	5th month	6th month
Legionella spp.	B1	1.40×10^5	3.84×10^6	4.37×10^4
	B2	2.53×10^5	2.81×10^6	2.46×10^4
	B3	1.76×10^5	/	2.07×10^4
L. pneumophila	B1	/	7.22×10^3	/
	B2	/	1.54×10^4	/
	B3	/	/	/
Mycobacterium spp.	B1	1.96×10^3	8.03×10^3	3.48×10^3
	B2	4.34×10^3	/	1.64×10^3
	B3	/	2.73×10^3	2.13×10^3

Table S7. Correlations between water quality parameters of source water and gene copies of bacteria and opportunistic pathogens.

	pH	Temp (℃)	Turbidity (NTU)	NO₃-N (mg/L)	NH₄-N (mg/L)	DOC (mg/L)
Total bacteria	0.10	0.10	-0.08	0.09	**0.84** **	**0.66** **
Mycobacterium spp.	-0.05	0.29	-0.16	-0.12	**0.70** **	**0.73** **
Legionella spp.	-0.11	0.43	-0.07	-0.09	0.63	**0.77** **
L. pneumophila	-0.05	0.45	-0.28	-0.34	**0.83** **	**0.90** **

* $p < 0.05$
** $p < 0.01$
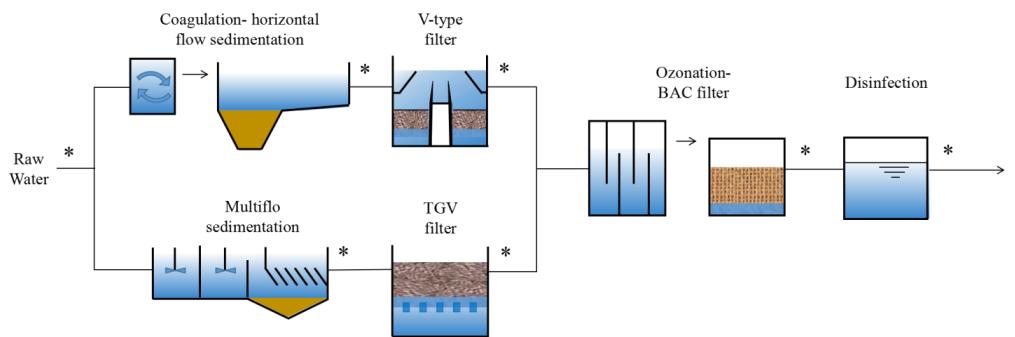
Figure S1. Schematic diagram of the drinking water treatment plant with two trains of treatment processes. Water samples were taken at the locations indicated by an asterisk (*).
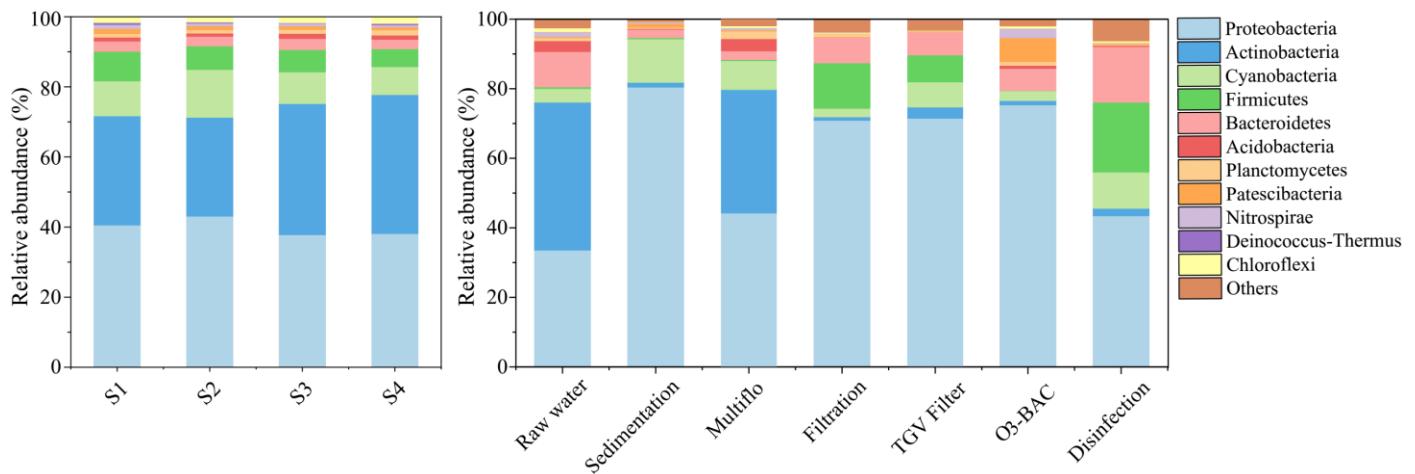
Figure S2. The annual average relative abundance of dominant taxa at the phylum level (**a**) in source water samples from four different sampling sites and (**b**) in water samples at each treatment step. The legend shows the most abundant phyla in these samples. The height of the color bar represents the percentage of each taxon in the microbial community.
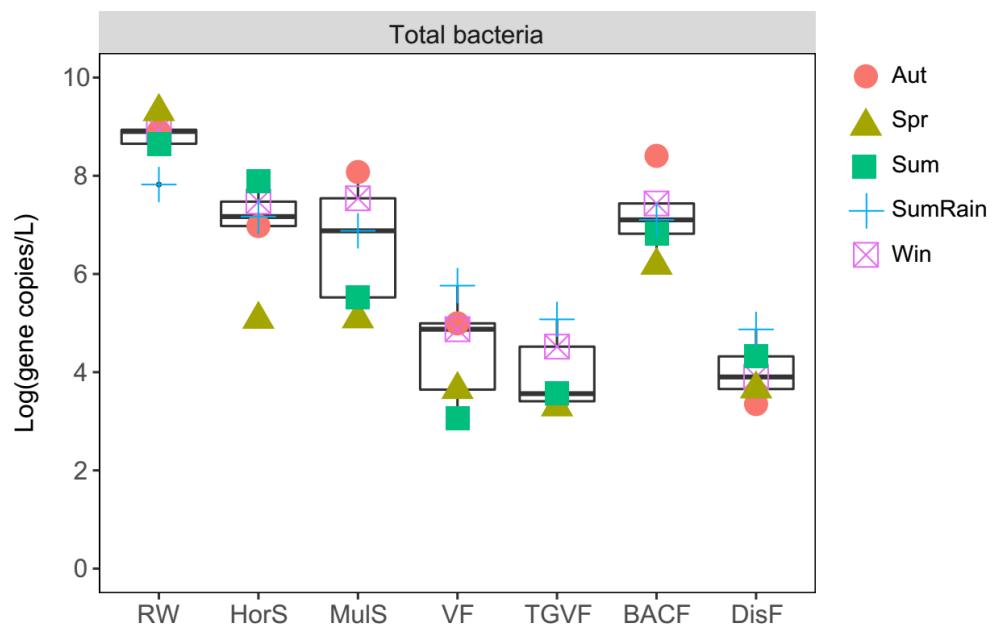
Figure S3. Total 16S rRNA gene copy number at various stages of the water treatment process at different time points. RW, HorS, MulS, VF, TGVF, BACF, and DisF was the abbreviation of raw water, horizontal flow sedimentation, Multiflo sedimentation, V-type filtration, TGV filtration, O3-BAC filtration, and disinfection, respectively. The different colors and shapes represent different seasons. The results of summer rainfall sample were removed due to insufficient DNA volume which can not be used to verify the primary qPCR results.
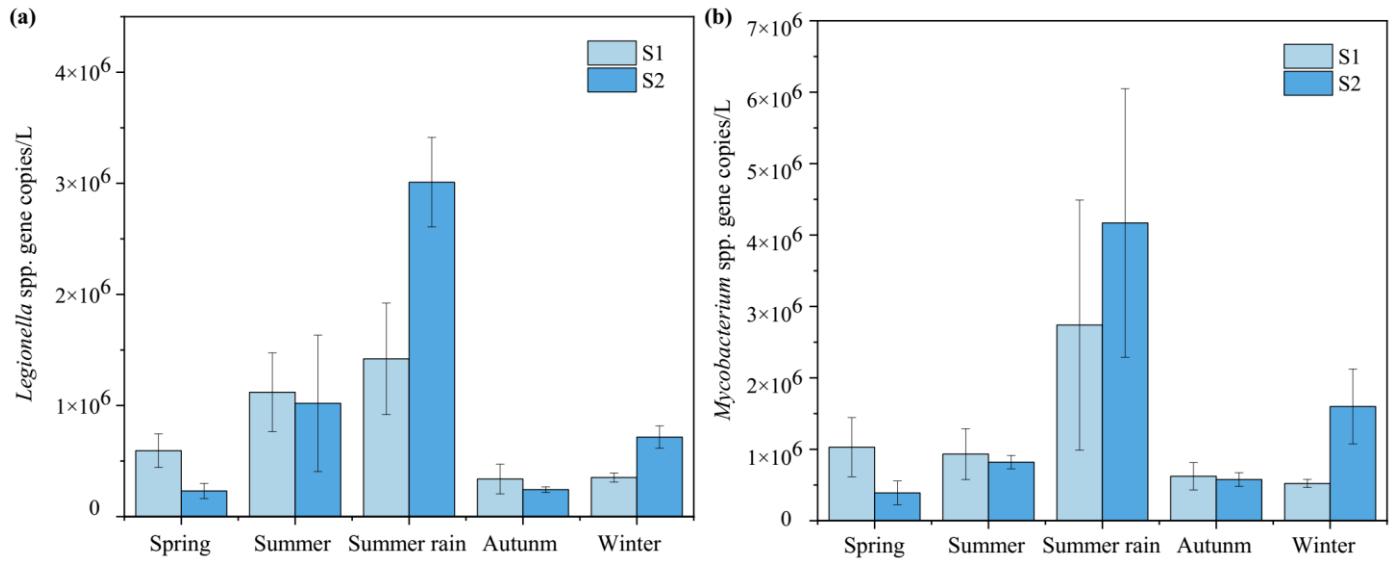
Figure S4. (**a**) *Legionella* spp. and (**b**) *Mycobacterium* spp. in water samples from the intake (S1) and alternative intake (S2) of source water at different time points.

References

1. Suzuki, M.T.; Taylor, L.T.; DeLong, E.F. Quantitative analysis of small-subunit rRNA genes in mixed microbial populations via 5 '-nuclease assays. *Appl. Environ. Microbiol.* **2000**, *66*, 4605–4614. DOI: 10.1128/AEM.66.11.4605-4614.2000
2. Nazanian, E.J.; Bopp, D.J.; Saylors, A.; Limberger, R.J.; Musser, K.A. Design and implementation of a protocol for the detection of *Legionella* in clinical and environmental samples. *Diagn. Microbiol. Infect. Dis.* **2008**, *62*, 125–132. DOI: 10.1016/j.diagmicrobio.2008.05.004
3. Radomski, N.; Lucas, F.S.; Moilleron, R.; Cambau, E.; Haenn, S.; Moulin, L. Development of a real-time qPCR method for detection and enumeration of *Mycobacterium* spp. in surface water. *Appl. Environ. Microbiol.* **2010**, *76*, 7348–7351. DOI: 10.1128/AEM.00942-10
4. Wilton, S.; Cousins, D. Detection and identification of multiple mycobacterial pathogens by DNA amplification in a single tube. *Genome Res.* **1992**, *1*, 269–273. DOI: 10.1101/gr.1.4.269