

Supplementary. The Effect of the Effluent from a Small-Scale Conventional Wastewater Treatment Plant Treating Municipal Wastewater on the Composition and Abundance of the Microbial Community, Antibiotic Resistome, and Pathogens in the Sediment and Water of a Receiving Stream

Table S1. Characteristics of qPCR primer pairs and programs. LOQ – limit of quantification per qPCR reaction.

Primers	Sequence 5'-3'	Target gene	Amplicon size (bp)	Primers concentration (μ M)	qPCR program	LOQ	Reference	Amplification efficiency (standard) ²	Amplification efficiency (samples) ²
Arc519F	CAGYCGCCRCGGTAA	Archaeal 16S rRNA	393	0.6	50°C 2 min, 95°C 10 min; 35 cycles: 95°C 15 s; 56°C 30 s; 72°C 30s	1000	[1]	1.74 \pm 0.04	1.93 \pm 0.07
Arch910R	GAATWGGCGGGGGRGC								
Bact517F	GCCAGCAGCCGCGGTAA	Bacterial 16S rRNA	530	0.6	50°C 2 min, 95°C 10 min; 35 cycles: 95°C 30 s; 60°C 45 s; 72°C 45s	1000	[2] [3]	1.88 \pm 0.02	1.85 \pm 0.05
Bact1028R	CGACARCCATGCASCACCT								
aadA-F	GCTGGCCGTRCATTGTAC	<i>aadA</i> ¹	479	0.8	50°C 2 min, 95°C 10 min; 35 cycles: 95°C 15 s, 59°C 30 s, 72°C 30 s	25	[4]	1.72 \pm 0.05	1.71 \pm 0.07
aadA-R	AGCACTACATTTCGCTCATCG								
acrB-F	ATATCTACGATTGCACCGC	<i>acrB</i>	160	0.8	50°C 2 min, 95°C 10 min; 40 cycles: 95°C 15 s, 58°C 30 s, 72°C 30 s	12	[5]	1.87 \pm 0.10	1.84 \pm 0.08
acrB-R	GGTACCCGTGGAGTCACTGT								
catQ-F	AGGTGCACTTACAGTATGACTGC	<i>catQ</i>	262	0.8	50°C 2 min, 95°C 10 min; 40 cycles: 95°C 15 s, 57°C 30 s, 72°C 30 s	12	[6]	1.90 \pm 0.13	1.98 \pm 0.07
catQ-R	AACGTGGGAAGTTCTCGTCATAC								
tetA F2	TCAATTTCTGACGGGCTG	<i>tetA</i>	96	0.8	50°C 2 min, 95°C 10 min; 45 cycles: 95°C 15 s, 58°C 30 s, 72°C 30 s	25	[7]	1.93 \pm 0.14	1.90 \pm 0.12
tetA R2	GAAGCGAGCGGGTTGAGAG								
tetB-F1	AGTGCCTTTGGATGCTG	<i>tetB</i>	101	0.8	50°C 2 min, 95°C 10 min; 45 cycles: 95°C 15 s, 58°C 30 s, 72°C 30 s	25	[7]	1.91 \pm 0.08	1.94 \pm 0.08
tetB-R1	TGAGGTGGTATCGGCAATGA								
tetQ-F	GCTCACATTGATGCAGGAAA	<i>tetQ</i>	153	0.8	50°C 2 min, 95°C 10 min; 35 cycles: 95°C 15 s, 58°C 30 s, 72°C 30 s	25	Modified from [5]	1.88 \pm 0.08	2.00 \pm 0.10
tetQ-R	CGTAGAAGCCCGRACAGTAA								
tetW-FC	GGGAAATTGTTCCGGACAGAC	<i>tetW</i>	549	0.8	50°C 2 min, 95°C 10 min; 35 cycles: 95°C 15 s, 57°C 30 s, 72°C 30 s	25	[8]	1.83 \pm 0.05	1.94 \pm 0.10
tetW-RC	AACGGATACCATCCCTGACA								
sul1-F1	CTGAACGATATCCAAGGATTYCC	<i>sul1</i>	239	0.8	50°C 2 min, 95°C 10 min; 45 cycles: 95°C 15 s, 54°C 30 s, 72°C 30 s	25	[9]	1.81 \pm 0.04	1.81 \pm 0.06
sul1-R1	AAAAATCCCATCCCCGGRTC								
sul2-F	CTCAATGATATTCGCGGTTTYCC	<i>sul2</i>	245	0.6	50°C 2 min, 95°C 10 min; 45 cycles: 95°C 15 s, 58°C 30 s, 72°C 30 s	6	[10]	1.69 \pm 0.06	1.84 \pm 0.09
sul2-R	AAAAACCCCATGCCGGGRTC								
UP	ACCAAYGATATYCGGGTGAT	<i>blaCTX-M</i>	101	0.6	50°C 2min, 95°C 10min; 45 cycles: 95°C 15s, 56°C 30s, 72°C 30s	12	Modified from [11]	1.77 \pm 0.07	1.62 \pm 0.06
LP	ACATCGCGRCGGCKYTCT								
blaTEM1-F	CATTTCGTGTCGCCCTTAT	<i>blaTEM1</i>	167	0.6	50°C 2 min, 95°C 10 min; 45 cycles: 95°C 15 s, 57°C 30 s, 72°C 30 s	50	[5]	1.88 \pm 0.06	1.93 \pm 0.07
blaTEM1-R	GGGCGAAAACCTCAAGGAT								
blaOXA2F	TCTTCGCGATACTTTYCTCCA	<i>blaOXA2</i>	177	0.8	50°C 2 min, 95°C 10 min; 35 cycles: 95°C 15 s, 55°C 30 s, 72°C 30 s	12	[5]	1.91 \pm 0.05	2.02 \pm 0.10
blaOXA2R	ATCGCACAGGATCAAAAACC								
mexF-F1	CAGGACAAGCARTACCTGGTGGC	<i>mexF</i>	566	0.8	50°C 2 min, 95°C 10 min; 40 cycles: 95°C 15 s, 60°C 30 s, 72°C 30 s	12	This study	1.75 \pm 0.04	1.80 \pm 0.10
mexF-R2	AGGTARAYCTGCAGGGTGTCTG								
QnrS-Ru	AAACACCTCGACTTAAGTCT	<i>qnrS</i>	169	0.4	50°C 2 min, 95°C 10 min; 45 cycles: 95°C 15 s, 52°C 30 s, 72°C 30 s	1000	[12]	1.90 \pm 0.07	1.81 \pm 0.07
QnrS-Fu	GTGAGTAATCGTATGTACTTTTGC								

¹ The primers amplify all *aadA* gene variants (*aadA1-aadA25*) except *aadA4*, *aadA5*, *aadA9*, and *aadA14*.

² Amplification efficiencies derived from LinRegPCR program are presented in the range of one to two, where one corresponds to no amplification (0% efficiency) and two corresponds to doubling of amplification product in each cycle (100% efficiency).

Table S2. The numbers of total reads and reads after quality trimming, coverage and diversity metrics, the proportions of classified reads (%) and the numbers of contigs in metagenomes of studied samples. E is the effluent of the wastewater treatment plant (WWTP), SW and RW are water samples, and SS and RS are sediment samples of the stream and river, respectively. SW_U and SS_U are the samples taken upstream of the WWTP discharge point and RW_U and RS_U are the samples taken from the river upstream of the stream inflow point. The number in the subscript of the sample code shows the distance (km) of the sampling point downstream from the WWTP discharge point.

Sample name	No. of reads	No. of reads after quality trimming	Coverage	Diversity	Classified reads (%)	No. of contigs
E	20911340	19655893	0.567	21.18	68.5	674253 (579158545 bp)
SW _U	18416530	17956649	0.503	21.49	68.0	593902 (454892072 bp)
SW _{0.3}	18563693	17523811	0.410	22.04	69.1	551181 (447254012 bp)
SS _U	22332423	21039728	0.162	23.63	70.8	325383 (207848632 bp)
SS _{0.3}	20180178	19025365	0.389	22.24	69.8	495233 (409619263 bp)
SS _{2.7}	22187376	20838296	0.168	23.57	72.3	257362 (152933903 bp)
SS _{3.2}	20827890	19390049	0.159	23.57	73.5	251018 (152254693 bp)
RS _U	23196456	21597354	0.156	23.72	73.2	302471 (180816447 bp)
RS _{3.7}	20940559	19430683	0.145	23.63	71.5	243151 (143192010 bp)

Table S3. The means and standard deviations (in parentheses) of physicochemical parameters in influent (IN) and effluent (E) of wastewater treatment plant (A), as well as values of individual measurements of physicochemical parameters of IN and E (B) over the 11-month period. ORP - oxidation-reduction potential; N_{tot} – content of total nitrogen; P_{tot} – content of total phosphorous; C_{tot} – content of total carbon; TOC – content of total organic carbon; DOC – content of dissolved organic carbon; TIC – content of total inorganic carbon, NM – not measured, NA – not applicable.

A – Means and standard deviations of physicochemical parameters													
Sample	Temp (°C)	O₂ (mg/L)	pH	ORP (mV)	N_{tot} (mg/L)	NH₄-N (mg/L)	NO₃-N (mg/L)	P_{tot} (mg/L)	PO₄³⁻-P (mg/L)	C_{tot} (mg/L)	TOC (mg/L)	DOC (mg/L)	TIC (mg/L)
IN (n=2)	11.9 (2.3)	4.1 (1.1)	7.76 (0.12)	121 ±21	119 ±26	44.8 ±16.8	39.0 ± 51.7	14.5 ±3.5	13.2 ±3.0	333 ±50	249 ±51	130.00	83.50 ±2.12
E (n=3)	15.7 (2.3)	6.1 (0.8)	7.25 (0.28)	128 ±37	8.9 ±3.8	1.82 ±0.93	7.4 ±7.8	6.2 ±5.9	5.4 ±4.8	95 ±37	40 ±28	21.5 ±12.0	43.5 ±9.2
B – Values of measured physicochemical parameters of individual samples													
IN (05.12.2012)	10.2	4.9	7.84	136	137	32.9	75.5	17.0	15.3	368	285	NM	82
IN (20.11.2013)	13.5	3.3	7.67	107	100	56.7	2.4	12.0	11.0	298	213	130	85
E (05.12.2012)	13.4	6.8	7.18	164	10.0	1.16	16.5	3.0	2.7	69	32	NM	37
E (26.09.2013)	18.0	6.4	7.55	91	4.6	1.42	3.1	2.7	2.6	NA	17	13	NM
E (20.11.2013)	15.8	5.2	7.01	129	12.0	2.89	2.7	13.0	11.0	121	71	30	50

Table S4A. The means and standard deviations (in parentheses) of the target gene abundances and relative abundances in prokaryotic community of wastewater treatment plant influent (IN; n=3) and effluent (E; n=4) over a 11-month period. 16S_{tot} – abundance of total prokaryotic community; B16S – abundance of bacterial 16S rRNA gene; A16S – abundance of archaeal 16S rRNA gene.

Gene	Abundances of genes (copies/mL)		Relative abundances of genes (%)	
	IN	E	IN	E
16S_{tot}	1.54x10 ⁸ (2.00x10 ⁸)	9.47x10 ⁷ (5.89x10 ⁷)	x	x
B16S	1.53x10 ⁸ (1.97x10 ⁸)	8.81x10 ⁷ (5.48x10 ⁷)	99.3 (0.4)	90.7 (7.6)
A16S	1.50x10 ⁶ (2.38x10 ⁶)	6.69x10 ⁶ (5.81x10 ⁶)	0.7 (0.4)	9.3 (7.6)
<i>aadA</i>	1.20x10 ⁶ (1.61x10 ⁶)	3.25x10 ⁵ (3.14x10 ⁵)	1.18 (0.89)	0.36 (0.23)
<i>acrB</i>	8.41x10 ³ (5.50x10 ³)	2.21x10 ⁴ (2.14x10 ⁴)	0.017 (0.016)	0.021 (0.017)
<i>bla_{CTX-M}</i>	7.96x10 ³ (1.14x10 ⁴)	3.61x10 ⁴ (2.18x10 ⁴)	0.005 (0.002)	0.041 (0.004)
<i>bla_{OXA2}</i>	4.39x10 ⁴ (7.42x10 ⁴)	1.67x10 ³ (1.52x10 ³)	0.015 (0.017)	0.002 (0.002)
<i>bla_{TEM1}</i>	9.59x10 ³ (1.33x10 ⁴)	1.26x10 ⁴ (5.29x10 ³)	0.008 (0.005)	0.019 (0.012)
<i>catQ</i>	7.93x10 ³ (5.87x10 ³)	2.38x10 ³ (3.10x10 ³)	0.012 (0.008)	0.003 (0.003)
<i>mexF</i>	9.70x10 ⁴ (1.28x10 ⁵)	2.69x10 ⁴ (2.62x10 ⁴)	0.058 (0.008)	0.029 (0.012)
<i>qnrS</i>	2.38x10 ⁶ (2.07x10 ⁶)	4.27x10 ⁴ (1.84x10 ⁴)	3.38 (2.46)	0.088 (0.088)
<i>sul1</i>	1.55x10 ⁶ (1.94x10 ⁶)	1.41x10 ⁶ (1.35x10 ⁶)	0.99 (0.13)	1.33 (0.56)
<i>sul2</i>	1.15x10 ³ (1.94x10 ³)	1.24x10 ³ (4.67x10 ²)	0.0004 (0.0004)	0.0020 (0.0013)
<i>tetA</i>	2.47x10 ⁵ (2.08x10 ⁵)	1.21x10 ⁴ (8.33x10 ³)	0.242 (0.244)	0.013 (0.006)
<i>tetB</i>	4.09x10 ³ (2.02x10 ³)	1.80x10 ⁴ (1.16x10 ⁴)	0.025 (0.040)	0.017 (0.010)
<i>tetQ</i>	7.20x10 ⁵ (9.11x10 ⁵)	5.66x10 ⁴ (8.89x10 ⁴)	0.557 (0.154)	0.080 (0.107)
<i>tetW</i>	3.52x10 ⁵ (4.49x10 ⁵)	3.30x10 ³ (2.01x10 ³)	0.263 (0.063)	0.004 (0.002)

Table S4B. The target gene abundances (copies/mL) and relative abundances in prokaryotic community (% , given in parentheses) of individual samples of wastewater treatment plant influent (IN) and effluent (E) over a 11-month period. 16S_{tot} – abundance of total prokaryotic community; B16S – abundance of bacterial 16S rRNA gene; A16S – abundance of archaeal 16S rRNA gene.

Gene	IN (05.12.2012)	IN (10.06.2013)	IN (20.11.2013)	E (05.12.2012)	E (10.06.2013)	E (26.09.2013)	E (20.11.2013)
16S_{tot}	3.82x10 ⁸	7.46x10 ⁶	7.40x10 ⁷	8.38x10 ⁷	1.20x10 ⁸	1.57x10 ⁸	1.85x10 ⁷
B16S	3.78x10 ⁸ (98.89)	7.42x10 ⁶ (99.39)	7.38x10 ⁷ (99.72)	8.08x10 ⁷ (96.45)	1.15x10 ⁸ (96.01)	1.41x10 ⁸ (90.22)	1.48x10 ⁷ (80.16)
A16S	4.25x10 ⁶ (1.11)	4.56x10 ⁴ (0.61)	2.10x10 ⁵ (0.28)	2.97x10 ⁶ (3.55)	4.78x10 ⁶ (3.99)	1.53x10 ⁷ (9.78)	3.67x10 ⁶ (19.84)
<i>aadA</i>	3.06x10 ⁶ (0.81)	1.62x10 ⁵ (2.19)	3.92x10 ⁵ (0.53)	3.14x10 ⁵ (0.39)	7.66x10 ⁵ (0.66)	1.76x10 ⁵ (0.11)	4.24x10 ⁴ (0.29)
<i>acrB</i>	1.35x10 ⁴ (0.004)	2.61x10 ³ (0.035)	9.08x10 ³ (0.012)	1.50x10 ⁴ (0.019)	5.18x10 ⁴ (0.045)	2.04x10 ⁴ (0.013)	1.11x10 ³ (0.008)
<i>blaCTX-M</i>	2.11x10 ⁴ (0.006)	4.62x10 ² (0.006)	2.33x10 ³ (0.003)	3.33x10 ⁴ (0.041)	4.75x10 ⁴ (0.041)	5.67x10 ⁴ (0.036)	6.87x10 ³ (0.046)
<i>blaOXA2</i>	1.30x10 ⁵ (0.034)	5.67x10 ² (0.008)	1.63x10 ³ (0.002)	3.80x10 ³ (0.005)	1.19x10 ³ (0.001)	1.47x10 ³ (0.001)	2.18x10 ² (0.002)
<i>blaTEM1</i>	2.49x10 ⁴ (0.007)	1.04x10 ³ (0.014)	2.84x10 ³ (0.004)	1.22x10 ⁴ (0.015)	1.73x10 ⁴ (0.015)	1.55x10 ⁴ (0.010)	5.34x10 ³ (0.036)
<i>catQ</i>	1.27x10 ⁴ (0.003)	1.38x10 ³ (0.019)	9.68x10 ³ (0.013)	6.97x10 ³ (0.009)	8.49x10 ² (0.001)	1.47x10 ³ (0.001)	2.45x10 ² (0.002)
<i>mexF</i>	2.43x10 ⁵ (0.064)	3.58x10 ³ (0.048)	4.45x10 ⁴ (0.060)	2.26x10 ⁴ (0.028)	1.57x10 ⁴ (0.014)	6.47x10 ⁴ (0.041)	4.77x10 ³ (0.032)
<i>qnrS</i>	4.57x10 ⁶ (1.21)	4.48x10 ⁵ (6.05)	2.13x10 ⁶ (2.88)	6.96x10 ⁴ (0.086)	3.01x10 ⁴ (0.026)	3.92x10 ⁴ (0.025)	3.18x10 ⁴ (0.215)
<i>sul1</i>	3.75x10 ⁶ (0.99)	6.37x10 ⁴ (0.86)	8.32x10 ⁵ (1.13)	5.89x10 ⁵ (0.73)	1.70x10 ⁶ (1.47)	3.19x10 ⁶ (2.04)	1.58x10 ⁵ (1.07)
<i>sul2</i>	3.38x10 ³ (0.0009)	1.70x10 ¹ (0.0002)	4.00x10 ¹ (0.0001)	1.50x10 ³ (0.0019)	1.58x10 ³ (0.0014)	1.31x10 ³ (0.0008)	5.58x10 ² (0.0038)
<i>tetA</i>	3.46x10 ⁵ (0.092)	8.19x10 ³ (0.110)	3.87x10 ⁵ (0.525)	1.68x10 ⁴ (0.021)	1.02x10 ⁴ (0.009)	2.02x10 ⁴ (0.013)	1.27x10 ³ (0.009)
<i>tetB</i>	5.23x10 ³ (0.001)	5.28x10 ³ (0.071)	1.76x10 ³ (0.002)	2.30x10 ⁴ (0.028)	2.54x10 ⁴ (0.022)	2.30x10 ⁴ (0.015)	6.83x10 ² (0.005)
<i>tetQ</i>	1.76x10 ⁶ (0.466)	5.45x10 ⁴ (0.735)	3.48x10 ⁵ (0.472)	1.90x10 ⁵ (0.235)	9.04x10 ³ (0.008)	1.76x10 ⁴ (0.011)	1.00x10 ⁴ (0.068)
<i>tetW</i>	8.64x10 ⁵ (0.229)	2.50x10 ⁴ (0.337)	1.66x10 ⁵ (0.225)	5.30x10 ³ (0.007)	2.67x10 ³ (0.002)	4.47x10 ³ (0.003)	7.72x10 ² (0.005)

Table S5. The proportions of the most abundant bacterial genera ($\geq 1\%$ at least in one sample) in the bacterial communities of studied samples. E is the effluent of the wastewater treatment plant (WWTP), SW and RW are water samples, and SS and RS are sediment samples of the stream and river, respectively. SW_U and SS_U are the samples taken upstream of the WWTP discharge point and RW_U and RS_U are the samples taken from the river upstream of the stream inflow point. The number in the subscript of the sample code shows the distance (km) of the sampling point downstream from the WWTP discharge point.

Genera	Proportions of genera (%)								
	Water samples			Sediment samples					
	E	SW _U	SW _{0.3}	SS _U	SS _{0.3}	SS _{2.7}	SS _{3.2}	RS _U	RS _{3.7}
<i>Acidimicrobium</i>	0.014	2.711	0.123	0.028	0.016	0.063	0.050	0.042	0.031
<i>Acidovorax</i>	0.807	2.437	0.601	0.309	0.335	0.364	0.506	0.279	0.316
<i>Anaeromyxobacter</i>	0.090	0.084	0.136	0.902	0.202	0.459	0.581	0.767	1.567
<i>Aquabacterium</i>	1.106	0.729	0.779	0.335	0.373	0.502	0.646	0.351	0.435
<i>Bradyrhizobium</i>	0.514	0.336	0.633	2.009	0.651	1.741	1.525	2.343	1.277
<i>Ca. Accumulibacter</i>	2.413	0.174	4.314	0.595	1.445	0.424	0.520	0.511	0.489
<i>Ca. Nanopelagicus</i>	0.001	7.134	0.284	0.002	0.001	0.001	0.001	0.002	0.001
<i>Ca. Planktophila</i>	0.003	7.834	0.344	0.005	0.004	0.006	0.005	0.008	0.005
<i>Chitinophaga</i>	0.659	0.224	0.698	0.348	1.008	0.360	0.380	0.318	0.392
<i>Curvibacter</i>	0.575	1.254	0.392	0.279	0.186	0.254	0.406	0.232	0.333
<i>Dechloromonas</i>	2.247	0.434	1.876	1.393	1.016	0.252	0.556	0.319	0.407
<i>Flavobacterium</i>	1.444	2.386	1.518	0.840	2.254	0.650	0.898	0.729	0.922
<i>Geobacter</i>	0.142	0.162	0.262	2.020	0.570	0.395	1.152	1.017	1.840
<i>Geothrix</i>	2.696	0.017	2.624	0.133	3.323	0.078	0.077	0.075	0.110
<i>Gordonia</i>	4.441	0.118	1.042	0.084	1.182	0.109	0.087	0.101	0.084
<i>Haliscomenobacter</i>	0.656	0.056	0.711	0.034	1.115	0.073	0.107	0.045	0.052
<i>Hydrogenophaga</i>	0.461	1.283	0.577	0.329	0.390	0.388	0.624	0.297	0.312
<i>Ideonella</i>	1.115	0.411	1.131	0.495	0.878	0.816	0.996	0.588	0.771
<i>Limnohabitans</i>	0.485	7.800	0.628	0.236	0.207	0.290	0.374	0.246	0.267
<i>Mycobacterium</i>	0.651	1.703	0.599	0.649	0.494	0.871	0.613	0.773	0.582
<i>Nannocystis</i>	1.247	0.035	0.446	0.059	0.079	0.078	0.081	0.064	0.062
<i>Nitrospira</i>	1.223	0.303	1.284	2.022	1.116	3.151	2.035	2.022	1.932
<i>Novosphingobium</i>	0.827	1.565	0.809	0.289	0.440	1.307	1.541	0.526	0.526
<i>Pedobacter</i>	0.709	0.462	0.763	0.395	1.156	0.348	0.392	0.330	0.433
<i>Polynucleobacter</i>	0.084	3.988	0.230	0.099	0.057	0.102	0.107	0.121	0.094
<i>Pseudomonas</i>	1.569	1.618	1.255	1.260	1.046	1.363	1.441	1.330	1.259
<i>Rhodoferax</i>	2.262	1.728	1.210	0.541	0.537	0.626	0.826	0.431	0.564
<i>Rhodoluna</i>	0.003	1.706	0.068	0.003	0.003	0.004	0.004	0.013	0.005
<i>Rubrivivax</i>	0.853	0.535	0.979	0.645	0.783	0.941	1.250	0.713	0.972
<i>Sphingomonas</i>	0.678	1.065	0.723	0.562	0.708	0.932	1.062	0.653	0.593
<i>Streptomyces</i>	1.566	1.186	1.681	2.414	1.663	2.604	2.032	2.765	2.411
<i>Syntrophorhabdus</i>	0.024	0.040	0.148	1.306	0.451	0.087	0.126	0.556	0.980
<i>Tetrasphaera</i>	1.239	0.066	1.689	0.061	1.209	0.103	0.084	0.073	0.077
<i>Variovorax</i>	0.794	0.741	0.750	0.772	0.562	1.108	1.225	0.944	0.872

Table S6. The proportions of the most abundant archaeal genera ($\geq 1\%$ at least in one sample) in the archaeal communities of studied samples. E is the effluent of the wastewater treatment plant (WWTP), SW and RW are water samples, and SS and RS are sediment samples of the stream and river, respectively. SW_U and SS_U are the samples taken upstream of the WWTP discharge point and RW_U and RS_U are the samples taken from the river upstream of the stream inflow point. The number in the subscript of the sample code shows the distance (km) of the sampling point downstream from the WWTP discharge point.

Genera	Proportions of genera (%)								
	Water samples			Sediment samples					
	E	SW _U	SW _{0.3}	SS _U	SS _{0.3}	SS _{2.7}	SS _{3.2}	RS _U	RS _{3.7}
<i>Ca. Methanofastidiosum</i>	1.195	0.943	1.131	0.651	1.685	0.696	0.863	0.787	0.744
<i>Ca. Methanoperedens</i>	2.499	1.202	1.018	1.327	1.095	2.376	2.404	5.201	1.765
<i>Ca. Nitrosocosmicus</i>	0.530	0.982	0.274	0.915	0.333	4.712	2.852	3.230	0.815
<i>Ca. Nitrosopelagicus</i>	0.167	2.326	0.072	0.026	0.043	0.075	0.106	0.100	0.036
<i>Ca. Nitrosotenuis</i>	0.502	0.896	0.190	0.281	0.197	1.267	3.006	1.126	0.355
<i>Halobellus</i>	0.803	1.030	0.321	0.304	0.212	0.940	0.914	0.569	0.272
<i>Halococcus</i>	1.070	0.959	0.366	0.350	0.216	1.124	1.109	0.620	0.341
<i>Haloferax</i>	1.830	1.375	0.606	0.587	0.464	1.803	1.695	1.141	0.535
<i>Halorubrum</i>	3.660	3.379	1.350	1.097	0.933	3.617	3.448	2.000	1.067
<i>Halorussus</i>	1.023	0.951	0.387	0.382	0.318	1.241	1.161	0.682	0.353
<i>Methanobacterium</i>	3.785	3.364	2.262	5.315	2.686	3.256	3.913	3.761	2.707
<i>Methanobrevibacter</i>	2.585	2.491	0.900	0.690	0.951	1.040	1.126	0.863	0.740
<i>Methanocella</i>	2.103	1.595	1.154	1.806	1.061	1.743	2.068	1.893	1.499
<i>Methanococcus</i>	0.655	1.187	0.319	0.269	0.264	0.375	0.360	0.331	0.288
<i>Methanocorpusculum</i>	1.864	0.362	0.760	0.318	0.670	0.246	0.202	0.212	0.294
<i>Methanoculleus</i>	2.767	2.106	2.368	3.421	2.668	2.617	2.855	3.080	3.339
<i>Methanofollis</i>	1.257	0.778	1.079	1.478	1.152	1.078	1.168	1.305	1.425
<i>Methanolinea</i>	0.621	0.464	0.998	1.993	1.384	0.728	0.613	1.207	1.822
<i>Methanolobus</i>	2.647	2.036	1.323	1.367	1.181	1.734	2.208	1.848	1.412
<i>Methanomassiliicoccus</i>	1.042	1.163	2.977	2.341	2.564	2.370	1.281	1.459	1.816
<i>Methanomethylovorans</i>	0.793	0.644	0.504	0.628	0.450	0.768	1.120	0.779	0.584
<i>Methanoregula</i>	2.595	3.977	17.909	20.061	21.570	3.543	3.883	9.595	20.182
<i>Methanosarcina</i>	10.035	6.995	7.542	6.257	7.121	7.828	8.563	7.340	4.919
<i>Methanosphaerula</i>	0.745	0.550	1.160	1.479	1.363	0.745	0.729	0.994	1.402
<i>Methanospirillum</i>	2.470	1.250	2.472	2.059	3.250	1.238	1.349	1.445	1.879
<i>Methanothrix</i>	9.614	14.249	32.502	25.738	31.902	9.046	9.826	17.168	30.144
<i>Nitrosarchaeum</i>	0.378	0.912	0.140	0.106	0.179	1.167	0.702	0.319	0.186
<i>Nitrosopumilus</i>	0.951	2.334	0.358	0.282	0.301	0.883	1.065	0.633	0.344
<i>Nitrososphaera</i>	0.683	0.534	0.228	0.613	0.337	4.176	2.291	2.672	0.762
<i>Salinigranum</i>	1.085	0.707	0.353	0.306	0.278	1.038	0.942	0.564	0.270
<i>Thermococcus</i>	2.031	1.847	0.979	0.941	0.786	1.622	1.712	1.459	1.010

Table S7. The abundances of bacterial and archaeal 16S rRNA genes (B16S and A16S, respectively) and antibiotic resistance genes (ARGs) in the studied water and sediment samples (A and B, respectively). E is the effluent of the wastewater treatment plant (WWTP), SW and RW are water samples, and SS and RS are sediment samples of the stream and river, respectively. SW_U and SS_U are the samples taken upstream of the WWTP discharge point and RW_U and RS_U are the samples taken from the river upstream of the stream inflow point. The number in the subscript of the sample code shows the distance (km) of the sampling point downstream from the WWTP discharge point. <LOQ denotes values below the limit of quantification.

Sample	Abundances of target genes															
	B16S	A16S	<i>aadA</i>	<i>acrB</i>	<i>bla</i> <i>CTX-M</i>	<i>bla</i> <i>OXA2</i>	<i>bla</i> <i>TEM1</i>	<i>catQ</i>	<i>mexF</i>	<i>qnrS</i>	<i>sul1</i>	<i>sul2</i>	<i>tetA</i>	<i>tetB</i>	<i>tetQ</i>	<i>tetW</i>
A (copies/mL)																
E	1.41x10 ⁸	1.53x10 ⁷	1.76x10 ⁵	2.04x10 ⁴	5.67x10 ⁴	1.47x10 ³	1.55x10 ⁴	1.47x10 ³	6.47x10 ⁴	3.92x10 ⁴	3.19x10 ⁶	1.31x10 ³	2.02x10 ⁴	2.30x10 ⁴	1.76x10 ⁴	4.47x10 ³
SW _U	3.58x10 ⁵	2.25x10 ⁴	<LOQ	1.27x10 ²	2.91x10 ²	<LOQ	5.53x10 ³	<LOQ	5.75x10 ²	<LOQ	<LOQ	<LOQ	1.28x10 ³	1.36x10 ³	<LOQ	5.00x10 ¹
SW _{0.3}	5.94x10 ⁶	1.30x10 ⁶	2.91x10 ³	1.31x10 ³	1.03x10 ⁴	3.63x10 ¹	6.14x10 ³	2.36x10 ²	1.61x10 ⁴	<LOQ	3.68x10 ⁴	3.80x10 ²	3.50x10 ³	3.34x10 ³	5.05x10 ²	9.23x10 ²
SW _{2.7}	4.80x10 ⁵	6.46x10 ⁴	<LOQ	1.77x10 ²	8.16x10 ²	<LOQ	6.19x10 ³	<LOQ	1.13x10 ³	<LOQ	3.25x10 ¹	2.12x10 ¹	2.24x10 ³	2.20x10 ³	<LOQ	4.02x10 ¹
SW _{3.2}	6.67x10 ⁵	4.73x10 ⁴	<LOQ	1.31x10 ²	1.18x10 ³	<LOQ	5.92x10 ³	<LOQ	1.19x10 ³	<LOQ	4.72x10 ¹	1.73x10 ¹	2.16x10 ³	2.64x10 ³	<LOQ	3.14x10 ¹
RW _U	3.12x10 ⁵	2.32x10 ⁴	<LOQ	1.01x10 ²	2.45x10 ²	<LOQ	4.31x10 ³	<LOQ	2.63x10 ²	<LOQ	<LOQ	<LOQ	1.44x10 ³	1.52x10 ³	<LOQ	4.00x10 ¹
RW _{3.7}	6.26x10 ⁵	3.12x10 ⁴	<LOQ	5.83x10 ¹	3.54x10 ²	<LOQ	6.06x10 ³	<LOQ	5.71x10 ²	<LOQ	<LOQ	<LOQ	4.29x10 ³	2.96x10 ³	<LOQ	5.70x10 ¹
B (copies/g dw)																
SS _U	1.42x10 ¹⁰	1.13x10 ⁹	2.44x10 ⁵	3.85x10 ⁷	9.17x10 ⁷	9.49x10 ⁴	2.59x10 ⁶	<LOQ	3.50x10 ⁷	<LOQ	3.11x10 ⁵	4.42x10 ⁵	2.41x10 ⁷	7.80x10 ⁵	<LOQ	9.81x10 ⁵
SS _{0.3}	2.60x10 ¹⁰	8.02x10 ⁹	1.73x10 ⁷	7.71x10 ⁷	1.02x10 ⁸	8.41x10 ⁵	3.07x10 ⁶	3.92x10 ⁶	7.78x10 ⁷	<LOQ	9.25x10 ⁷	1.84x10 ⁶	1.84x10 ⁷	1.93x10 ⁵	6.70x10 ⁶	2.86x10 ⁶
SS _{2.7}	2.10x10 ⁹	2.37x10 ⁸	2.00x10 ⁵	1.16x10 ⁷	2.15x10 ⁷	2.86x10 ⁴	1.17x10 ⁶	<LOQ	1.21x10 ⁷	<LOQ	4.39x10 ⁵	1.07x10 ⁵	9.31x10 ⁶	2.94x10 ⁵	<LOQ	1.43x10 ⁵
SS _{3.2}	1.22x10 ¹⁰	1.01x10 ⁹	1.22x10 ⁶	4.61x10 ⁷	7.25x10 ⁷	1.90x10 ⁵	1.65x10 ⁶	<LOQ	8.94x10 ⁷	<LOQ	2.82x10 ⁶	4.85x10 ⁵	1.59x10 ⁷	4.51x10 ⁵	<LOQ	9.36x10 ⁵
RS _U	8.67x10 ⁹	1.37x10 ⁹	2.91x10 ⁵	3.01x10 ⁷	5.94x10 ⁷	9.05x10 ⁴	2.07x10 ⁶	<LOQ	5.76x10 ⁷	<LOQ	1.58x10 ⁵	3.97x10 ⁵	2.07x10 ⁷	4.50x10 ⁵	<LOQ	9.30x10 ⁵
RS _{3.7}	3.80x10 ¹⁰	8.89x10 ⁹	2.03x10 ⁶	1.08x10 ⁸	2.15x10 ⁸	3.66x10 ⁵	4.09x10 ⁶	<LOQ	1.69x10 ⁸	<LOQ	1.31x10 ⁷	1.52x10 ⁶	4.34x10 ⁷	1.03x10 ⁶	<LOQ	3.20x10 ⁶

Table S8. The relative abundances of archaea (arc%) and antibiotic resistance genes (ARGs) in prokaryotic communities of water and sediment samples (A and B, respectively). E is the effluent of the wastewater treatment plant (WWTP), SW and RW are water samples, and SS and RS are sediment samples of the stream and river, respectively. SW_U and SS_U are the samples taken upstream of the WWTP discharge point and RW_U and RS_U are the samples taken from the river upstream of the stream inflow point. The number in the subscript of the sample code shows the distance (km) of the sampling point downstream from the WWTP discharge point. <LOQ denotes values below the limit of quantification.

Sample	Relative abundances of target genes (%)														
	arc%	<i>aadA</i>	<i>acrB</i>	<i>bla</i> <i>CTX-M</i>	<i>bla</i> <i>OXA2</i>	<i>bla</i> <i>TEM1</i>	<i>catQ</i>	<i>mexF</i>	<i>qnrS</i>	<i>sul1</i>	<i>sul2</i>	<i>tetA</i>	<i>tetB</i>	<i>tetQ</i>	<i>tetW</i>
A															
E	9.78	0.1125	0.0130	0.0362	0.0009	0.0099	0.0009	0.0413	0.0250	2.0368	0.0008	0.0129	0.0147	0.0112	0.0029
SW _U	5.92	<LOQ	0.0335	0.0765	<LOQ	1.4543	<LOQ	0.1513	<LOQ	<LOQ	<LOQ	0.3368	0.3580	<LOQ	0.0132
SW _{0.3}	18.00	0.0401	0.0181	0.1424	0.0005	0.0848	0.0033	0.2217	<LOQ	0.5074	0.0052	0.0483	0.0461	0.0070	0.0127
SW _{2.7}	11.87	<LOQ	0.0326	0.1498	<LOQ	1.1370	<LOQ	0.2077	<LOQ	0.0060	0.0039	0.4114	0.4046	<LOQ	0.0074
SW _{3.2}	6.62	<LOQ	0.0183	0.1652	<LOQ	0.8285	<LOQ	0.1667	<LOQ	0.0066	0.0024	0.3026	0.3696	<LOQ	0.0044
RW _U	6.93	<LOQ	0.0302	0.0731	<LOQ	1.2868	<LOQ	0.0786	<LOQ	<LOQ	<LOQ	0.4288	0.4555	<LOQ	0.0120
RW _{3.7}	4.75	<LOQ	0.0089	0.0539	<LOQ	0.9212	<LOQ	0.0869	<LOQ	<LOQ	<LOQ	0.6527	0.4497	<LOQ	0.0087
B															
SS _U	7.39	0.0016	0.2507	0.5976	0.0006	0.0169	<LOQ	0.2281	<LOQ	0.0020	0.0029	0.1571	0.0051	<LOQ	0.0064
SS _{0.3}	23.54	0.0509	0.2264	0.3001	0.0025	0.0090	0.0115	0.2285	<LOQ	0.2718	0.0054	0.0541	0.0006	0.0197	0.0084
SS _{2.7}	10.12	0.0085	0.4947	0.9176	0.0012	0.0498	<LOQ	0.5184	<LOQ	0.0188	0.0046	0.3977	0.0126	<LOQ	0.0061
SS _{3.2}	7.66	0.0092	0.3487	0.5476	0.0014	0.0125	<LOQ	0.6757	<LOQ	0.0213	0.0037	0.1199	0.0034	<LOQ	0.0071
RS _U	13.67	0.0029	0.2999	0.5910	0.0009	0.0206	<LOQ	0.5731	<LOQ	0.0016	0.0039	0.2056	0.0045	<LOQ	0.0093
RS _{3.7}	18.96	0.0043	0.2303	0.4581	0.0008	0.0087	<LOQ	0.3607	<LOQ	0.0279	0.0032	0.0926	0.0022	<LOQ	0.0068

Table S9. Proportions of antibiotic resistance genes (ARGs, per 16S rRNA reads) in the metagenomes of studied samples. E is the effluent of the wastewater treatment plant (WWTP), SW and RW are water samples, and SS and RS are sediment samples of the stream and river, respectively. SW_U and SS_U are the samples taken upstream of the WWTP discharge point and RW_U and RS_U are the samples taken from the river upstream of the stream inflow point. The number in the subscript of the sample code shows the distance (km) of the sampling point downstream from the WWTP discharge point. ARGs subjected to the qPCR quantification are marked in bold. ABC – ATP-binding cassette type efflux complex; BAC – bacitracin; CHL – chloramphenicol; F – Fosfomycin; FOS – fosmidomycin; K – kasugamycin; MATE – multidrug and toxic compound extrusion type efflux complex; MFS – major facilitator superfamily efflux complex; MLS – macrolide-lincosamide-streptogramin; P – puromycin; Q – quinolone; R – rifamycin; RND – resistance-nodulation-division superfamily efflux complex; SMR – small multidrug resistance transporter complex; SUL – sulfonamide; TMP – trimethoprim.

Antibiotic resistance		Proportion of genes (%)									
Resistance type		Resistance mechanism	Water			Stream sediment					
Resistance subtype (ARG)			E	SW _U	SW _{0.3}	SS _U	SS _{0.3}	SS _{2.7}	SS _{3.2}	RS _U	RS _{3.7}
Aminoglycoside	<i>aac(2')-I</i>	Inactivation	0	0	0	0	0.035	0.043	0	0.021	0
	<i>aac(3)-I</i>	Inactivation	0.066	0.009	0.014	0.100	0.027	0.092	0.017	0.063	0.057
	<i>aac(3)-IIIa</i>	Inactivation	0.011	0	0	0	0	0	0.033	0	0
	<i>aac(3)-IV</i>	Inactivation	0	0	0	0.010	0	0.023	0.021	0.032	0.024
	<i>aac(6')-3I</i>	Inactivation	0.034	0	0.022	0	0	0	0	0	0.015
	<i>aac(6')-I</i>	Inactivation	0.015	0.012	0	0	0	0	0	0	0.017
	<i>aac(6')-II</i>	Inactivation	0.121	0.082	0.103	0.020	0.028	0.196	0.113	0.125	0
	<i>aad(6)</i>	Inactivation	0.022	0	0	0	0	0	0	0	0
	<i>aadA</i>	Inactivation	1.168	0	0.601	0	0.247	0.016	0.039	0	0.025
	<i>aadE</i>	Inactivation	0.011	0	0.014	0	0.009	0	0.015	0	0
	<i>ant(2'')-I</i>	Inactivation	0.018	0	0.029	0	0	0	0	0	0
	<i>ant(9)-I</i>	Inactivation	0	0	0	0	0.009	0	0	0	0
	<i>aph(3''')-III</i>	Inactivation	0	0	0.015	0	0	0	0	0	0
	<i>aph(3'')-I</i>	Inactivation	0.142	0	0.193	0	0.042	0	0	0	0
	<i>aph(3')-I</i>	Inactivation	0	0.045	0	0	0	0	0	0	0
	<i>aph(6)-I</i>	Inactivation	0.174	0	0.267	0	0.137	0	0	0	0
B A	<i>bacA</i>	Target alteration	6.203	6.912	5.386	4.033	3.226	5.665	7.484	4.962	4.002

Antibiotic resistance			Proportion of genes (%)								
Resistance type		Resistance mechanism	Water			Stream sediment					
Resistance subtype (ARG)			E	SW _U	SW _{0.3}	SS _U	SS _{0.3}	SS _{2.7}	SS _{3.2}	RS _U	RS _{3.7}
	<i>bcrA</i>	Efflux: ABC	0	0.427	0.033	0.011	0.015	0.021	0.026	0.043	0.035
β-lactam	<i>AER-1</i>	Inactivation	0.040	0	0.100	0	0.028	0	0	0	0
	<i>BEL-2</i>	Inactivation	0.062	0	0.041	0	0	0	0	0	0
	<i>BEL-3</i>	Inactivation	0.098	0	0.041	0	0	0	0	0	0
	<i>CfxA2</i>	Inactivation	0.047	0	0.012	0	0	0	0	0	0
	<i>FEZ-1</i>	Inactivation	0	0	0	0	0.044	0	0.015	0	0
	<i>IMP-5</i>	Inactivation	0.440	0	0.114	0	0	0	0.017	0	0
	<i>IMP-13</i>	Inactivation	0.135	0	0	0	0	0	0	0	0
	<i>IMP-15</i>	Inactivation	0.050	0	0	0	0	0	0	0	0
	<i>IMP-16</i>	Inactivation	0	0	0.016	0	0	0	0	0	0
	<i>IMP-24</i>	Inactivation	0.010	0	0	0	0	0	0	0	0
	<i>IMP-28</i>	Inactivation	0.156	0	0	0	0	0	0	0	0
	<i>IMP-29</i>	Inactivation	0.985	0	0.150	0	0	0	0	0	0
	<i>IMP-33</i>	Inactivation	0	0	0	0	0	0	0.017	0	0
	<i>IMP-37</i>	Inactivation	0.443	0	0	0	0	0	0	0	0
	<i>JOHN-1</i>	Inactivation	0	0.010	0.008	0	0	0.036	0.011	0	0
	<i>KHM-1</i>	Inactivation	0	0.010	0	0	0	0.012	0	0	0
	<i>LCR-1</i>	Inactivation	0	0	0.046	0	0	0	0	0	0
	<i>LEN-21</i>	Inactivation	0	0	0.014	0	0	0	0	0	0
	<i>LRA-1</i>	Inactivation	0	0.008	0	0	0	0	0.014	0	0
	<i>LRA-2</i>	Inactivation	0	0	0.012	0	0.021	0.059	0.185	0	0.086
	<i>LRA-3</i>	Inactivation	0	0	0	0	0	0.027	0	0.012	0
<i>LRA-5</i>	Inactivation	0	0	0	0	0	0.013	0	0	0	
<i>LRA-8</i>	Inactivation	0	0	0	0	0	0.008	0	0.013	0.013	
<i>LRA-9</i>	Inactivation	0	0	0	0	0	0	0.028	0	0	
<i>LRA-12</i>	Inactivation	0.010	0	0.067	0	0.069	0.030	0.042	0	0.026	

Antibiotic resistance		Proportion of genes (%)									
Resistance type	Resistance subtype (ARG)	Resistance mechanism	Water			Stream sediment					
			E	SW _U	SW _{0.3}	SS _U	SS _{0.3}	SS _{2.7}	SS _{3.2}	RS _U	RS _{3.7}
	<i>LRA-17</i>	Inactivation	0	0	0	0	0	0.010	0.011	0	0
	<i>LRA-19</i>	Inactivation	0.020	0	0	0	0.044	0	0.027	0	0
	<i>MOX-6</i>	Inactivation	0	0	0.010	0	0	0	0	0	0
	<i>OXA-2</i>	Inactivation	0.152	0.048	0.108	0	0.079	0	0.039	0.015	0
	<i>OXA-3</i>	Inactivation	0.011	0	0	0	0	0	0	0.011	0
	<i>OXA-5</i>	Inactivation	0.202	0	0.058	0	0.063	0.017	0.016	0	0
	<i>OXA-9</i>	Inactivation	0.419	0	0.089	0	0.012	0	0	0	0
	<i>OXA-10</i>	Inactivation	0.222	0	0.090	0	0.026	0	0.017	0	0
	<i>OXA-12</i>	Inactivation	0	0.029	0	0	0	0	0	0	0
	<i>OXA-13</i>	Inactivation	0.023	0	0	0	0	0	0	0	0
	<i>OXA-17</i>	Inactivation	0	0	0.015	0	0	0	0	0	0
	<i>OXA-20</i>	Inactivation	0.175	0.009	0.104	0	0.013	0	0.016	0	0
	<i>OXA-21</i>	Inactivation	0.021	0	0.035	0	0.018	0	0	0	0
	<i>OXA-34</i>	Inactivation	0	0	0.015	0.013	0	0	0.015	0	0
	<i>OXA-36</i>	Inactivation	0	0	0	0.010	0	0	0	0	0
	<i>OXA-37</i>	Inactivation	0.069	0	0.074	0	0	0.017	0	0	0
	<i>OXA-46</i>	Inactivation	0.045	0	0.015	0	0.020	0	0	0	0
	<i>OXA-47</i>	Inactivation	0.011	0	0	0	0	0	0	0	0
	<i>OXA-53</i>	Inactivation	0.044	0	0	0	0	0	0	0	0
	<i>OXA-54</i>	Inactivation	0.011	0	0	0	0	0	0	0	0
	<i>OXA-118</i>	Inactivation	0.031	0.010	0	0	0.048	0	0	0	0
	<i>OXA-119</i>	Inactivation	0.033	0	0.059	0	0.025	0	0.016	0	0.015
	<i>OXA-129</i>	Inactivation	0.094	0	0.055	0	0.051	0	0.015	0	0
	<i>OXA-141</i>	Inactivation	0	0	0	0	0	0	0.015	0.015	0
	<i>OXA-142</i>	Inactivation	0.011	0	0	0	0.013	0	0	0	0
	<i>OXA-147</i>	Inactivation	0	0	0.015	0	0	0	0	0	0

Antibiotic resistance		Proportion of genes (%)										
Resistance type	Resistance subtype (ARG)	Resistance mechanism	Water			Stream sediment						
			E	SW _U	SW _{0.3}	SS _U	SS _{0.3}	SS _{2.7}	SS _{3.2}	RS _U	RS _{3.7}	
	<i>OXA-183</i>	Inactivation	0.009	0	0	0	0	0	0	0	0	0
	<i>OXA-205</i>	Inactivation	0.081	0	0.088	0	0	0	0.013	0.012	0	0
	<i>OXA-209</i>	Inactivation	0.022	0.066	0	0	0.061	0	0	0.015	0.014	0
	<i>OXA-226</i>	Inactivation	0.022	0	0	0	0	0.016	0	0	0	0
	<i>OXA-334</i>	Inactivation	0.011	0	0	0	0	0	0	0	0	0
	<i>PBP-1A</i>	Target alteration	0	0	0	0	0.005	0	0	0	0	0
	<i>PER-1</i>	Inactivation	0	0.009	0	0	0	0	0	0	0	0
	<i>PER-6</i>	Inactivation	0	0.009	0	0	0	0	0	0	0	0
	<i>SIM-1</i>	Inactivation	0	0.010	0	0	0	0	0	0	0	0
	<i>TEM-1</i>	Inactivation	0.017	2.163	0.041	0.013	0.077	0	0	0	0.029	0
	<i>TEM-6</i>	Inactivation	0	0.401	0	0	0.012	0	0	0	0	0
	<i>TEM-29</i>	Inactivation	0	0.027	0	0	0	0	0	0	0	0
	<i>TEM-43</i>	Inactivation	0	0.036	0	0	0	0	0	0	0	0
	<i>TEM-63</i>	Inactivation	0	0.197	0	0	0	0	0	0	0	0
	<i>TEM-75</i>	Inactivation	0.012	0.330	0	0	0	0	0	0	0	0
	<i>TEM-89</i>	Inactivation	0	0.144	0	0	0	0	0	0	0	0
	<i>TEM-91</i>	Inactivation	0	0.531	0.010	0.017	0.012	0.009	0	0	0	0
	<i>TEM-95</i>	Inactivation	0	0.009	0	0	0	0	0	0	0	0
	<i>TEM-106</i>	Inactivation	0	0.049	0	0	0	0	0	0	0	0
	<i>TEM-117</i>	Inactivation	0	1.785	0.031	0.015	0.014	0.018	0	0	0	0
	<i>TEM-118</i>	Inactivation	0	0.798	0.015	0	0.026	0.017	0	0	0	0
	<i>TEM-123</i>	Inactivation	0	0.018	0	0	0	0	0	0	0	0
	<i>TEM-154</i>	Inactivation	0	0.055	0	0.013	0	0	0	0	0.014	0
	<i>TEM-156</i>	Inactivation	0	0.009	0	0	0	0	0	0	0	0
	<i>TEM-157</i>	Inactivation	0.011	1.113	0.028	0	0.036	0.031	0.044	0	0	0
	<i>TEM-171</i>	Inactivation	0	0.035	0.056	0	0	0	0	0	0	0

Antibiotic resistance			Proportion of genes (%)								
Resistance type	Resistance subtype (ARG)	Resistance mechanism	Water			Stream sediment					
			E	SW _U	SW _{0.3}	SS _U	SS _{0.3}	SS _{2.7}	SS _{3.2}	RS _U	RS _{3.7}
	<i>TEM-177</i>	Inactivation	0	0.318	0.028	0	0.012	0	0	0	0
	<i>TEM-178</i>	Inactivation	0	0.322	0	0	0	0	0	0	0
	<i>TEM-183</i>	Inactivation	0	0.009	0	0	0	0	0	0	0
	<i>TEM-184</i>	Inactivation	0	0.018	0	0	0	0	0	0	0
	<i>TEM-185</i>	Inactivation	0	0.009	0	0	0	0	0	0	0
	<i>TEM-187</i>	Inactivation	0	0.824	0	0	0.033	0	0.015	0	0.014
	<i>TEM-193</i>	Inactivation	0	0.009	0	0	0	0	0	0	0
	<i>TEM-195</i>	Inactivation	0	0.118	0	0	0	0	0	0	0
	<i>TEM-197</i>	Inactivation	0	0.009	0	0	0	0	0	0	0
	<i>TEM-205</i>	Inactivation	0.011	0.323	0.014	0	0	0	0	0	0
	<i>TEM-209</i>	Inactivation	0	0.132	0.014	0	0	0	0	0	0
	<i>THIN-B</i>	Inactivation	0	0	0	0.006	0	0	0.021	0.025	0.012
	<i>VEB-1</i>	Inactivation	0	0	0	0	0.051	0	0	0	0
	<i>ampC</i>	Inactivation	0.006	0	0	0	0	0	0	0	0
	<i>penA</i>	Target alteration	0	0	0	0	0	0	0.005	0	0
	<i>Class A β-lactamase</i>	Inactivation	0.251	0	0.249	0	0.098	0.029	0.014	0	0
	<i>Class B β-lactamase</i>	Inactivation	0.012	0.080	0.016	0	0	0	0	0	0
	<i>Class C β-lactamase</i>	Inactivation	0.015	0.030	0.053	0	0.022	0	0.009	0	0
	<i>metallo-β-lactamase</i>	Inactivation	0.058	0	0.012	0	0.012	0.015	0	0	0
CHL	<i>catA</i>	Inactivation	0	0	0.010	0	0	0	0	0	0
	<i>catB</i>	Inactivation	0.015	0	0	0	0	0	0.011	0	0
	<i>Chloramphenicol acetyltransferase cat</i>	Inactivation	0.015	0	0	0	0.056	0	0.014	0.027	0.018
	<i>Chloramphenicol exporter</i>	Efflux: MFS	0.031	0	0.020	0	0	0.011	0	0	0
	<i>floR</i>	Efflux: MFS	0.038	0	0.056	0	0	0	0	0	0
F	<i>fosX</i>	Inactivation	0.018	0	0.022	0.019	0	0	0	0.016	0.033
F O	<i>rosA</i>	Efflux: MFS	0.243	0.143	0.403	0.354	0.321	0.566	0.324	0.360	0.259

Antibiotic resistance			Proportion of genes (%)								
Resistance type		Resistance mechanism	Water			Stream sediment					
	Resistance subtype (ARG)		E	SW _U	SW _{0.3}	SS _U	SS _{0.3}	SS _{2.7}	SS _{3.2}	RS _U	RS _{3.7}
	<i>rosB</i>	Efflux: MFS	0.586	0.248	0.497	0.505	0.365	0.692	0.890	0.601	0.311
K	<i>ksgA</i>	Inactivation	0.019	0.038	0.014	0	0.008	0.046	0	0	0
Macrolide-lincosamide-streptogramin (MLS)	<i>ereA</i>	Inactivation	0.294	0	0.072	0	0.008	0	0	0	0
	<i>erm(35)</i>	Target alteration	0.033	0	0.036	0	0.041	0	0	0	0
	<i>erm(38)</i>	Target alteration	0	0	0	0	0	0.011	0	0	0
	<i>erm(39)</i>	Target alteration	0	0	0.016	0	0	0.018	0	0	0
	<i>ermB</i>	Target alteration	0.048	0	0.016	0	0.091	0	0	0	0
	<i>ermF</i>	Target alteration	0.315	0	0.220	0	0.086	0	0	0	0
	<i>ermX</i>	Target alteration	0	0	0	0	0.013	0	0	0	0
	<i>lsa</i>	Target protection	0	0	0.007	0.008	0.041	0	0.008	0	0
	<i>macA</i>	Efflux: ABC	0.122	0.068	0.116	0.015	0.039	0.036	0.111	0.052	0.045
	<i>macB</i>	Efflux: ABC	0.999	0.455	1.036	1.512	0.977	1.767	1.690	1.829	1.543
	<i>mefA</i>	Efflux: MFS	0.038	0	0.020	0	0.017	0	0	0	0.009
	<i>mphA</i>	Inactivation	0.058	0	0.126	0	0.195	0	0	0	0
	<i>msrC</i>	Target protection	0.004	0	0.004	0	0	0	0	0	0
	<i>tlcC</i>	Target protection	0.003	0	0.009	0	0	0	0	0	0
	<i>vatA</i>	Inactivation	0.008	0	0.034	0	0	0	0	0	0
	<i>vatB</i>	Inactivation	0	0	0.037	0	0.032	0.042	0.014	0	0
ABC	<i>ABC transporter</i>	Efflux: ABC	0.311	0.206	0.347	0.412	0.257	0.712	0.576	0.618	0.371
	<i>bcr</i>	Efflux: ABC	0	0	0.010	0	0.017	0	0	0	0
RND	<i>tolC</i>	Regulation	0	0.040	0.041	0	0.008	0	0	0	0
	<i>acrA</i>	Efflux: RND	0	0.004	0.026	0.043	0.014	0.006	0.009	0.015	0.069
	<i>acrB</i>	Efflux: RND	0.348	0.278	0.553	0.847	0.398	0.391	0.665	0.629	0.591
	<i>acrF</i>	Efflux: RND	0.009	0.010	0.015	0.029	0.019	0.013	0.032	0.012	0.019
	<i>adeB</i>	Efflux: RND	0.018	0.057	0.046	0.013	0.023	0.004	0.012	0.016	0.023
	<i>adeC</i>	Efflux: RND	0	0	0.004	0	0	0	0	0	0

Antibiotic resistance		Proportion of genes (%)									
Resistance type	Resistance subtype (ARG)	Resistance mechanism	Water			Stream sediment					
			E	SW _U	SW _{0.3}	SS _U	SS _{0.3}	SS _{2.7}	SS _{3.2}	RS _U	RS _{3.7}
	<i>adeJ</i>	Efflux: RND	0.006	0.016	0.028	0.025	0.013	0.008	0.018	0.021	0.029
	<i>adeK</i>	Efflux: RND	0.007	0	0	0	0	0	0	0	0
	<i>amrB</i>	Efflux: RND	0.081	0.058	0.078	0.054	0.043	0.087	0.065	0.031	0.040
	<i>bpeF</i>	Efflux: RND	0.008	0.029	0.030	0.006	0.021	0.067	0.061	0.030	0.032
	<i>ceoB</i>	Efflux: RND	0.009	0.070	0.036	0.038	0.032	0.115	0.070	0.051	0.036
	<i>cmeB</i>	Efflux: RND	0.085	0.017	0.031	0.027	0.013	0.035	0.037	0.016	0.033
	<i>mdtA</i>	Efflux: RND	0.007	0.028	0	0	0.004	0	0.005	0	0
	<i>mdtB</i>	Efflux: RND	0.431	0.500	0.618	0.689	0.523	1.308	0.936	1.034	0.677
	<i>mdtC</i>	Efflux: RND	0.278	0.239	0.315	0.321	0.270	0.641	0.490	0.469	0.319
	<i>mdtD</i>	Efflux: RND	0.013	0.016	0	0	0	0	0	0	0
	<i>mdtE</i>	Efflux: RND	0	0.014	0	0	0	0	0	0	0
	<i>mdtF</i>	Efflux: RND	0.057	0.032	0.047	0.024	0.005	0.028	0.047	0.023	0.028
	<i>mexA</i>	Efflux: RND	0	0.051	0.019	0.037	0.006	0.012	0.041	0.020	0.043
	<i>mexB</i>	Efflux: RND	0.116	0.094	0.173	0.165	0.094	0.075	0.134	0.102	0.100
	<i>mexC</i>	Efflux: RND	0.392	0.063	0.220	0.035	0.044	0.076	0.037	0.034	0.084
	<i>mexD</i>	Efflux: RND	1.094	0.096	0.415	0.077	0.073	0.052	0.063	0.051	0.057
	<i>mexE</i>	Efflux: RND	0.052	0.037	0.048	0.088	0.107	0.022	0.098	0.042	0.084
	<i>mexF</i>	Efflux: RND	0.411	0.396	0.515	0.667	0.410	1.128	1.132	0.810	0.630
	<i>mexI</i>	Efflux: RND	0.055	0.025	0.088	0.192	0.096	0.200	0.186	0.161	0.160
	<i>mexT</i>	Efflux: RND	1.062	0.092	0.258	0.012	0.024	0.089	0.083	0.012	0
	<i>mexW</i>	Efflux: RND	0.204	0.239	0.243	0.204	0.160	0.324	0.278	0.171	0.138
	<i>mexY</i>	Efflux: RND	0.036	0.032	0.018	0.021	0.008	0.010	0.020	0.024	0.035
	<i>mtrE</i>	Efflux: RND	0.007	0	0	0.007	0	0.028	0.008	0	0.013
	<i>opcM</i>	Efflux: RND	0.004	0	0	0	0	0	0	0	0
	<i>oprA</i>	Efflux: RND	0.006	0.033	0.014	0	0.011	0	0.012	0	0.005
	<i>oprC</i>	Efflux: RND	0.013	0.028	0.021	0.019	0.022	0.028	0.028	0.024	0.011

Antibiotic resistance			Proportion of genes (%)								
Resistance type	Resistance subtype (ARG)	Resistance mechanism	Water			Stream sediment					
			E	SW _U	SW _{0.3}	SS _U	SS _{0.3}	SS _{2.7}	SS _{3.2}	RS _U	RS _{3.7}
	<i>emrE</i>	Efflux: SMR	0	0.079	0.068	0.027	0	0	0.064	0.076	0.021
	<i>qacEΔ1</i>	Efflux: SMR	0.912	0	0.498	0	0.325	0	0	0	0
Polymyxin	<i>arnA</i>	Target alteration	0.106	0.104	0.094	0.147	0.115	0.104	0.069	0.164	0.116
	<i>Icr-Mo</i>	Target alteration	0	0.008	0	0	0	0.006	0.005	0	0
	<i>mcr-1.5</i>	Target alteration	0.003	0	0	0	0	0	0	0	0
	<i>mcr-3</i>	Target alteration	0	0.010	0	0	0	0	0	0	0
	<i>mcr-4</i>	Target alteration	0	0.008	0	0	0.010	0	0	0	0
	<i>mcr-5</i>	Target alteration	0.011	0.006	0	0	0	0	0.008	0	0.004
P	<i>Puromycin resistance protein</i>	Efflux: MFS	0	0	0.009	0	0	0	0	0	0
Q	<i>abaQ</i>	Efflux: MFS	0	0	0	0	0	0.017	0	0	0
	<i>norB</i>	Efflux: MFS	0	0	0	0	0	0	0.014	0	0
	<i>qepA</i>	Efflux: MFS	0.003	0.049	0.047	0.075	0.020	0.138	0.105	0.074	0.042
	<i>qnrB</i>	Target protection	0.014	0	0	0	0	0	0	0	0
R	<i>arr</i>	Inactivation	0.648	0.025	0.513	0.079	0.496	0.523	0.333	0.293	0.140
	<i>Rifampin monoxygenase</i>	Inactivation	0	0.009	0.008	0	0.005	0.055	0.021	0.025	0.008
SUL	<i>sul1</i>	Target replacement	5.383	0	1.803	0	0.817	0.018	0.035	0	0.047
	<i>sul2</i>	Target replacement	0.230	0	0.436	0	0.398	0	0	0	0
	<i>sul4</i>	Target replacement	0.079	0	0.046	0.135	0.048	0.075	0.084	0.071	0.260
Tetracycline	<i>otrA</i>	Target protection	0.005	0.002	0	0.009	0.010	0.052	0.030	0.042	0.012
	<i>tet32</i>	Target protection	0	0.008	0.012	0	0.004	0	0	0	0
	<i>tet35</i>	Efflux: ABC	0	0	0.011	0	0.012	0	0	0	0
	<i>tet39</i>	Efflux: MFS	0.020	0	0	0	0	0	0.005	0	0
	<i>tet41</i>	Efflux: MFS	0.008	0	0.010	0	0	0	0	0	0
	<i>tet43</i>	Efflux: MFS	0.034	0	0.012	0	0.024	0.026	0.021	0	0
	<i>tet44</i>	Target protection	0.009	0	0.006	0	0	0	0	0	0
	<i>tetA</i>	Efflux: MFS	0.037	0.015	0.038	0	0.010	0.022	0.021	0	0.008
	<i>tetC</i>	Efflux: MFS	0	0.012	0.052	0	0.017	0.006	0	0	0

Antibiotic resistance			Proportion of genes (%)								
Resistance type		Resistance mechanism	Water			Stream sediment					
Resistance subtype (ARG)			E	SW _U	SW _{0.3}	SS _U	SS _{0.3}	SS _{2.7}	SS _{3.2}	RS _U	RS _{3.7}
	<i>tetE</i>	Efflux: MFS	0.015	0	0	0	0	0	0	0	0
	<i>tetG</i>	Efflux: MFS	0.041	0	0.107	0	0.050	0	0	0	0
	<i>tetM</i>	Target protection	0	0	0.031	0	0.020	0	0.023	0	0
	<i>tetO</i>	Target protection	0	0	0	0	0	0	0.003	0	0
	<i>tetP</i>	Efflux: MFS	0.040	0.015	0.034	0.003	0.047	0.004	0.039	0.010	0.029
	<i>tetQ</i>	Target protection	0.042	0	0.018	0	0	0	0	0	0
	<i>tetR</i>	Regulation	0.016	0	0	0	0	0	0	0	0
	<i>tetT</i>	Target protection	0.017	0.002	0.024	0	0.010	0	0	0	0.004
	<i>tetV</i>	Efflux: MFS	0.015	0.012	0	0	0	0.091	0.016	0.029	0
	<i>tetW</i>	Target protection	0.037	0	0.006	0	0.021	0	0	0	0
	<i>tetX</i>	Inactivation	0.039	0	0	0	0.009	0	0	0	0
	<i>tetX1</i>	Inactivation	0.009	0	0	0	0	0	0	0	0
	<i>tetX2</i>	Inactivation	0.128	0	0.034	0	0.023	0	0	0	0
	<i>tetX3</i>	Inactivation	0	0	0	0	0.017	0	0	0	0
	<i>tetX4</i>	Inactivation	0.008	0	0.010	0	0.009	0	0	0	0
	<i>Tetracycline resistance protein</i>	Efflux: MFS	0.005	0.004	0.019	0	0	0.011	0	0	0.005
TMP	<i>dfrA1</i>	Target replacement	0	0	0	0	0	0	0.012	0	0.019
	<i>dfrB1</i>	Target replacement	0.032	0	0	0	0.034	0	0	0	0
	<i>dfrB2</i>	Target replacement	0.021	0.095	0.044	0	0.085	0.193	0.159	0.228	0.200
	<i>dfrB3</i>	Target replacement	0.038	0	0.101	0.049	0	0.262	0	0.237	0.026
	<i>dfrB6</i>	Target replacement	0.112	0	0	0.070	0.066	0.055	0.291	0.048	0.088
Vancomycin	<i>vanA</i>	Target alteration	0	0	0.007	0	0	0	0	0	0.022
	<i>vanC</i>	Target alteration	0	0	0.006	0	0.008	0	0.039	0.006	0.007
	<i>vanD</i>	Target alteration	0.006	0.026	0	0	0	0	0	0	0.011
	<i>vanG</i>	Target alteration	0	0	0.012	0	0.006	0	0	0	0.006
	<i>vanH</i>	Target alteration	0	0.016	0	0	0	0.013	0.030	0.023	0

Antibiotic resistance			Proportion of genes (%)								
Resistance type		Resistance mechanism	Water			Stream sediment					
Resistance subtype (ARG)			E	SW _U	SW _{0.3}	SS _U	SS _{0.3}	SS _{2.7}	SS _{3.2}	RS _U	RS _{3.7}
	<i>vanR</i>	Regulation	0.085	0.127	0.079	0.254	0.090	0.961	0.646	0.262	0.130
	<i>vanS</i>	Regulation	0.021	0	0	0.065	0.028	0.179	0.042	0.046	0.034
	<i>vanX</i>	Target alteration	0	0	0	0	0	0	0.019	0	0
	<i>vanY</i>	Target alteration	0	0	0	0	0	0	0.007	0	0
Other/regulation	<i>gadX</i>	Regulation	0	0.019	0	0	0	0	0	0	0
	<i>H-NS</i>	Regulation	0	0.024	0	0	0	0	0.045	0	0
	<i>LuxR</i>	Regulation	0	0.021	0	0	0	0	0	0	0
	<i>cAMP-regulatory protein</i>	Regulation	0.194	0.312	0.109	0.115	0.085	0.164	0.099	0.043	0.047
	<i>cob(I)</i>	Regulation	0.030	0.039	0	0.031	0	0	0.015	0	0
	<i>rpsD</i>	Regulation	0	0	0	0.014	0	0	0	0	0
	<i>tsnR</i>	Regulation	0	0	0	0	0	0.016	0	0	0
	<i>cpxR</i>	Regulation	1.178	0.562	0.975	0.853	0.726	1.217	1.170	0.983	0.838
	<i>arlR</i>	Regulation	0.068	0	0.011	0	0.008	0.046	0.053	0	0.018
ARG proportion (%)			31.886	24.332	23.904	14.968	15.600	20.836	23.416	17.262	14.715
ARG subtypes detected			160	130	147	66	129	91	109	73	82

Table S10. Statistically significant Pearson correlations (R) between physicochemical parameters in water as well as sediment and (A) target gene abundances, and (B) target gene relative abundances in prokaryotic community. Statistically significant partial correlations between physicochemical parameters and ARGs (in case of ARGs abundances *16Stot* is used as covariable and in case of ARGs relative abundances *B16S* relative abundance in prokaryotic community is used as covariable) are given in bold italic. Temp – temperature; N_{tot} – total nitrogen; P_{tot} – total phosphorous; TOC – total organic carbon; *16Stot* – total prokaryotic community abundance; *B16S* – bacteria specific 16S rRNA gene; *A16S* – archaea specific 16S rRNA gene.

Target gene	Physicochemical parameters												
	Water									Sediment			
	Temp	O ₂ conc	pH	N _{tot}	NH ₄ -N	NO ₃ -N	P _{tot}	PO ₄ ³⁻ -P	SO ₄ ²⁻	pH	TOC %	N _{tot}	P _{tot}
A													
<i>16Stot</i>					0.87*		0.95**	0.88*		-0.84*			
<i>B16S</i>					0.86*		0.95**	0.87*		-0.83*			
<i>A16S</i>					0.95**		0.99***	0.93**		-0.83*	0.87*		
<i>aadA</i>										-0.86*	0.87*	0.89*	0.91*/ 0.88*
<i>acrB</i>					0.92**		0.96**	0.90*		-0.81*			
<i>blaCTX-M</i>					0.96**		0.99***/ 0.90*	0.98***/ 0.97**					
<i>blaOXA2</i>										-0.93**	0.89*	0.85*	0.81*
<i>mexF</i>					0.94**		0.99***	0.95**					
<i>sul1</i>					0.87*		0.94**	0.85*			0.82*	0.84*	0.85*
<i>sul2</i>					0.98***/ 0.92*		0.99***/ 0.95*	0.98***/ 0.90*		-0.90*		0.82*	
<i>tetW</i>							0.88*			-0.85*			
B													
<i>B16S</i>					-0.96**		-0.93**	-0.91*			-0.84*	-0.86*	-0.83*
<i>A16S</i>					0.96**		0.93**	0.91*			0.84*	0.86*	0.83*
<i>aadA</i>												0.87*	0.95**/ 0.93*
<i>acrB</i>										0.83*	-0.87*		
<i>blaCTX-M</i>					0.95**/ 0.96*		0.92**/ 0.98**		0.91*	0.94**/ 0.90*		-0.81*	
<i>blaOXA2</i>													0.83*
<i>blaTEM1</i>					-0.83*		-0.90*	-0.86*		0.82*			
<i>mexF</i>					0.86*			0.86*					

<i>sul1</i>					0.84*		0.93**	0.82*			0.85*	0.92**	0.98***/ 0.96**
<i>sul2</i>					0.97**		0.91*	0.95**					
<i>tetA</i>							-0.85*			0.90*			
<i>tetB</i>			0.85*		-0.85*		-0.95**	-0.86*		0.87*			
<i>tetW</i>	0.92**/ 0.93*	-0.89* / - 0.89*											

* p<0.05; ** p<0.01; *** p<0.001

Table S11. Proportions of pathogens in the bacterial communities of studied samples. E is the effluent of the wastewater treatment plant (WWTP), SW and RW are water samples, and SS and RS are sediment samples of the stream and river, respectively. SW_U and SS_U are the samples taken upstream of the WWTP discharge point and RW_U and RS_U are the samples taken from the river upstream of the stream inflow point. The number in the subscript of the sample code shows the distance (km) of the sampling point downstream from the WWTP discharge point.

Pathogen	Proportion of pathogens (%)								
	E	SW _U	SW _{0.3}	SS _U	SS _{0.3}	SS _{2.7}	SS _{3.2}	RS _U	RS _{3.7}
<i>Achromobacter xylosoxidans</i>	0.01663	0.01584	0.01324	0.00788	0.00843	0.01001	0.01046	0.00891	0.00829
<i>Acinetobacter baumannii</i>	0.02481	0.02799	0.02086	0.00769	0.01301	0.00843	0.01256	0.00761	0.00858
<i>Acinetobacter haemolyticus</i>	0.00100	0.00059	0.00036	0.00038	0.00028	0.00019	0.00037	0.00032	0.00034
<i>Acinetobacter junii</i>	0.00380	0.00261	0.00123	0.00074	0.00067	0.00062	0.00064	0.00053	0.00051
<i>Acinetobacter lwoffii</i>	0.00072	0.00069	0.00036	0.00020	0.00024	0.00021	0.00023	0.00024	0.00029
<i>Acinetobacter nosocomialis</i>	0.00105	0.00168	0.00087	0.00034	0.00032	0.00033	0.00039	0.00018	0.00030
<i>Bacillus anthracis</i>	0.00061	0.00089	0.00074	0.00077	0.00086	0.00057	0.00061	0.00071	0.00114
<i>Bordetella pertussis</i>	0.01306	0.00946	0.01225	0.01190	0.01030	0.01242	0.01347	0.01194	0.01238
<i>Brucella abortus</i>	0.00036	0.00027	0.00024	0.00016	0.00039	0.00033	0.00038	0.00024	0.00025
<i>Brucella canis</i>	0.00013	0.00002	0.00018	0.00003	0.00018	0.00008	0.00010	0.00007	0.00002
<i>Brucella melitensis</i>	0.00021	0.00041	0.00018	0.00021	0.00020	0.00046	0.00046	0.00030	0.00029
<i>Brucella ovis</i>	0.00007	0.00002	0.00006	0.00006	0.00008	0.00008	0.00005	0.00006	0.00010
<i>Brucella suis</i>	0.00056	0.00041	0.00047	0.00040	0.00044	0.00043	0.00052	0.00033	0.00025
<i>Burkholderia cenocepacia</i>	0.01184	0.01003	0.01028	0.00783	0.00773	0.00897	0.00972	0.00795	0.00772
<i>Burkholderia cepacia</i>	0.01096	0.00883	0.00948	0.00697	0.00652	0.00829	0.00839	0.00730	0.00664
<i>Burkholderia pseudomallei</i>	0.03463	0.03884	0.03168	0.01960	0.01979	0.02180	0.02084	0.02078	0.02009
<i>Campylobacter coli</i>	0.00099	0.00183	0.00089	0.00082	0.00104	0.00046	0.00057	0.00046	0.00067
<i>Campylobacter jejuni</i>	0.00190	0.00262	0.00198	0.00193	0.00218	0.00134	0.00127	0.00177	0.00184
<i>Chlamydia pneumoniae</i>	0.00075	0.00095	0.00085	0.00066	0.00087	0.00055	0.00053	0.00071	0.00073
<i>Chlamydia trachomatis</i>	0.00670	0.00451	0.00484	0.00223	0.00425	0.00149	0.00181	0.00159	0.00212
<i>Citrobacter amalonaticus</i>	0.00075	0.00047	0.00049	0.00046	0.00043	0.00047	0.00056	0.00042	0.00051
<i>Citrobacter freundii</i>	0.03459	0.00944	0.05084	0.00771	0.07140	0.02725	0.02520	0.01002	0.01242
<i>Citrobacter koseri</i>	0.00046	0.00045	0.00037	0.00047	0.00040	0.00057	0.00040	0.00034	0.00044
<i>Citrobacter youngae</i>	0.00021	0.00019	0.00012	0.00016	0.00008	0.00013	0.00017	0.00019	0.00008
<i>Clostridioides difficile</i>	0.00413	0.00545	0.00529	0.00324	0.00525	0.00259	0.00240	0.00285	0.00361
<i>Clostridium botulinum</i>	0.00460	0.00413	0.00431	0.00420	0.00591	0.00280	0.00293	0.00362	0.00448
<i>Clostridium perfringens</i>	0.00204	0.00249	0.00228	0.00225	0.00313	0.00113	0.00114	0.00136	0.00221
<i>Clostridium tetani</i>	0.00038	0.00058	0.00088	0.00080	0.00089	0.00039	0.00040	0.00056	0.00075

Pathogen	Proportion of pathogens (%)								
	E	SW _U	SW _{0.3}	SS _U	SS _{0.3}	SS _{2.7}	SS _{3.2}	RS _U	RS _{3.7}
<i>Enterobacter asburiae</i>	0.00069	0.00066	0.00079	0.00043	0.00041	0.00056	0.00068	0.00058	0.00069
<i>Enterobacter cloacae</i>	0.00336	0.00409	0.00373	0.00231	0.00233	0.00222	0.00275	0.00229	0.00210
<i>Enterobacter hormaechei</i>	0.00393	0.00285	0.00266	0.00151	0.00150	0.00113	0.00130	0.00131	0.00110
<i>Enterobacter kobei</i>	0.00054	0.00037	0.00065	0.00024	0.00049	0.00033	0.00036	0.00015	0.00036
<i>Enterococcus faecalis</i>	0.00701	0.01864	0.00830	0.00618	0.01334	0.00735	0.00603	0.00724	0.01177
<i>Enterococcus faecium</i>	0.02379	0.06015	0.02404	0.01353	0.02889	0.01514	0.05648	0.03206	0.02221
<i>Escherichia coli</i>	0.03942	0.04339	0.02904	0.02168	0.02220	0.02148	0.02349	0.01870	0.02132
<i>Francisella tularensis</i>	0.00026	0.00045	0.00041	0.00040	0.00039	0.00008	0.00024	0.00020	0.00025
<i>Haemophilus influenzae</i>	0.00213	0.00424	0.00182	0.00132	0.00149	0.00107	0.00130	0.00091	0.00121
<i>Haemophilus parainfluenzae</i>	0.00272	0.00128	0.00111	0.00086	0.00080	0.00076	0.00086	0.00084	0.00092
<i>Helicobacter pylori</i>	0.00263	0.00366	0.00297	0.00226	0.00279	0.00181	0.00191	0.00205	0.00240
<i>Klebsiella aerogenes</i>	0.00168	0.00174	0.00167	0.00135	0.00144	0.00139	0.00134	0.00148	0.00140
<i>Klebsiella oxytoca</i>	0.00311	0.00206	0.00292	0.00221	0.00232	0.00201	0.00206	0.00189	0.00230
<i>Klebsiella pneumoniae</i>	0.02724	0.02839	0.02757	0.02024	0.01917	0.01940	0.02116	0.02380	0.01783
<i>Legionella pneumophila</i>	0.00610	0.00527	0.00628	0.00473	0.00544	0.00445	0.00487	0.00444	0.00507
<i>Listeria monocytogenes</i>	0.00213	0.00304	0.00283	0.00213	0.00252	0.00134	0.00177	0.00164	0.00191
<i>Micrococcus luteus</i>	0.00227	0.00292	0.00273	0.00219	0.00204	0.00215	0.00224	0.00207	0.00223
<i>Moraxella catarrhalis</i>	0.00218	0.00182	0.00119	0.00056	0.00065	0.00067	0.00070	0.00060	0.00052
<i>Morganella morganii</i>	0.00162	0.00162	0.00145	0.00104	0.00119	0.00105	0.00099	0.00105	0.00114
<i>Mycobacterium tuberculosis</i>	0.02436	0.02238	0.02197	0.01989	0.01837	0.02190	0.02029	0.02057	0.02022
<i>Mycobacteroides abscessus</i>	0.05611	0.03808	0.03870	0.01834	0.02480	0.02153	0.01895	0.01982	0.01832
<i>Mycoplasma genitalium</i>	0.00004	0.00006	0.00001	0.00006	0.00003	0.00002	0.00002	0.00004	0.00008
<i>Mycoplasma pneumoniae</i>	0.00008	0.00018	0.00004	0.00010	0.00010	0.00006	0.00005	0.00005	0.00008
<i>Neisseria gonorrhoeae</i>	0.00163	0.00101	0.00128	0.00093	0.00087	0.00117	0.00116	0.00118	0.00115
<i>Neisseria meningitidis</i>	0.00416	0.00375	0.00363	0.00309	0.00260	0.00258	0.00279	0.00286	0.00290
<i>Proteus mirabilis</i>	0.00275	0.00222	0.00119	0.00048	0.00071	0.00038	0.00066	0.00051	0.00052
<i>Proteus penneri</i>	0.00007	0.00010	0.00006	0.00007	0.00005	0.00003	0.00005	0.00005	0.00004
<i>Proteus vulgaris</i>	0.00013	0.00031	0.00028	0.00018	0.00032	0.00013	0.00015	0.00017	0.00018
<i>Providencia rettgeri</i>	0.00089	0.00112	0.00073	0.00072	0.00058	0.00071	0.00074	0.00053	0.00063
<i>Providencia stuartii</i>	0.00148	0.00070	0.00104	0.00061	0.00091	0.00053	0.00060	0.00060	0.00059
<i>Pseudomonas aeruginosa</i>	0.07725	0.03252	0.05980	0.03308	0.03852	0.03736	0.03948	0.03262	0.03488
<i>Pseudomonas fluorescens</i>	0.03167	0.02812	0.02593	0.02268	0.02268	0.02733	0.02907	0.02146	0.02180
<i>Pseudomonas putida</i>	0.01265	0.01138	0.01184	0.01026	0.01026	0.01168	0.01304	0.00994	0.00972

Pathogen	Proportion of pathogens (%)								
	E	SW _U	SW _{0.3}	SS _U	SS _{0.3}	SS _{2.7}	SS _{3.2}	RS _U	RS _{3.7}
<i>Pseudomonas sp.</i>	0.01832	0.01151	0.01020	0.00431	0.00625	0.00688	0.00943	0.00416	0.00488
<i>Pseudomonas stutzeri</i>	0.02123	0.01634	0.01860	0.01497	0.01401	0.01657	0.01835	0.01647	0.01535
<i>Ralstonia mannitolilytica</i>	0.00400	0.00380	0.00313	0.00148	0.00171	0.00187	0.00171	0.00170	0.00169
<i>Ralstonia pickettii</i>	0.00726	0.00480	0.00585	0.00518	0.00394	0.00636	0.00643	0.00593	0.00494
<i>Raoultella planticola</i>	0.00074	0.00083	0.00091	0.00072	0.00072	0.00058	0.00121	0.00129	0.00094
<i>Rickettsia prowazekii</i>	0.00020	0.00053	0.00017	0.00030	0.00032	0.00031	0.00038	0.00026	0.00033
<i>Salmonella enterica</i>	0.02172	0.01320	0.01731	0.01797	0.01221	0.01134	0.01024	0.00999	0.01044
<i>Serratia liquefaciens</i>	0.00064	0.00139	0.00066	0.00043	0.00038	0.00043	0.00048	0.00036	0.00051
<i>Serratia marcescens</i>	0.00687	0.00434	0.00476	0.00320	0.00337	0.00369	0.00370	0.00309	0.00345
<i>Serratia odorifera</i>	0.00066	0.00051	0.00057	0.00039	0.00042	0.00072	0.00069	0.00046	0.00051
<i>Shigella dysenteriae</i>	0.00019	0.00018	0.00010	0.00013	0.00015	0.00011	0.00012	0.00007	0.00015
<i>Shigella flexneri</i>	0.00351	0.00071	0.00084	0.00031	0.00056	0.00022	0.00034	0.00028	0.00037
<i>Shigella sonnei</i>	0.00055	0.00302	0.00057	0.00036	0.00053	0.00041	0.00042	0.00029	0.00034
<i>Staphylococcus aureus</i>	0.00358	0.00498	0.00445	0.00417	0.00388	0.00308	0.00286	0.00325	0.00379
<i>Staphylococcus epidermidis</i>	0.00078	0.00117	0.00067	0.00056	0.00064	0.00061	0.00067	0.00054	0.00060
<i>Staphylococcus intermedius</i>	0.00004	0.00005	0.00009	0.00005	0.00010	0.00002	0.00005	0.00003	0.00005
<i>Staphylococcus lugdunensis</i>	0.00008	0.00001	0.00004	0.00001	0.00001	NA	NA	0.00005	0.00001
<i>Staphylococcus pseudintermedius</i>	0.00279	0.00250	0.00140	0.00040	0.00066	0.00048	0.00034	0.00026	0.00029
<i>Stenotrophomonas maltophilia</i>	0.02502	0.01658	0.02350	0.01309	0.01800	0.01638	0.01806	0.01304	0.01343
<i>Streptococcus agalactiae</i>	0.00134	0.00239	0.00133	0.00112	0.00149	0.00069	0.00101	0.00091	0.00130
<i>Streptococcus anginosus</i>	0.00035	0.00110	0.00042	0.00024	0.00039	0.00022	0.00016	0.00010	0.00015
<i>Streptococcus constellatus</i>	0.00010	0.00007	0.00003	0.00009	0.00007	0.00008	0.00004	0.00002	0.00003
<i>Streptococcus pneumoniae</i>	0.02112	0.01588	0.01210	0.00361	0.00590	0.00385	0.00380	0.00330	0.00357
<i>Streptococcus pyogenes</i>	0.00058	0.00141	0.00101	0.00077	0.00099	0.00096	0.00061	0.00082	0.00117
<i>Vibrio cholerae</i>	0.00745	0.00575	0.00549	0.00419	0.00512	0.00425	0.00455	0.00431	0.00429
<i>Vibrio parahaemolyticus</i>	0.00966	0.00837	0.00805	0.00599	0.00677	0.00555	0.00623	0.00572	0.00633
<i>Vibrio vulnificus</i>	0.00260	0.00208	0.00202	0.00169	0.00182	0.00159	0.00177	0.00143	0.00173
<i>Yersinia enterocolitica</i>	0.00212	0.00229	0.00198	0.00093	0.00117	0.00128	0.00146	0.00089	0.00105
<i>Yersinia pestis</i>	0.00058	0.00033	0.00054	0.00034	0.00039	0.00027	0.00054	0.00027	0.00037
Total proportion of detected pathogens	0.688	0.606	0.591	0.373	0.487	0.411	0.470	0.384	0.387

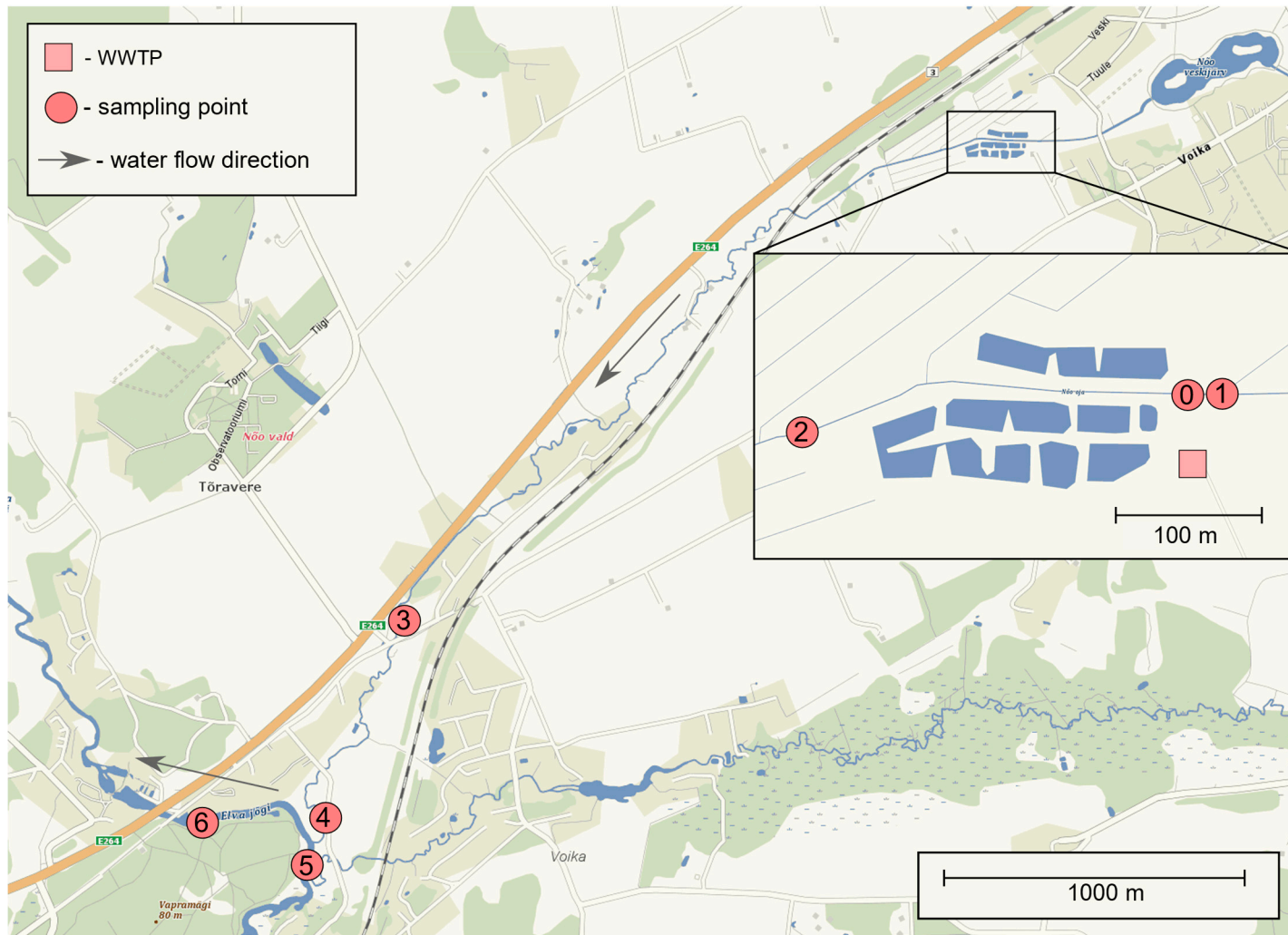


Figure S1. A map showing the locations of wastewater treatment plant (WWTP) of Nõo and sampling points on the Nõo stream and Elva river. 0 is the WWTP discharge point, 1 is the stream sampling point locating upstream of the WWTP discharge point; 2, 3 and 4 are the stream sampling points downstream at the distance of 0.3, 2.7 and 3.2 km of the WWTP discharge point; and 5 and 6 are the river sampling points locating upstream of the Nõo stream inflow point and downstream of the stream inflow at the 3.7 km distance from the WWTP discharge point, respectively.

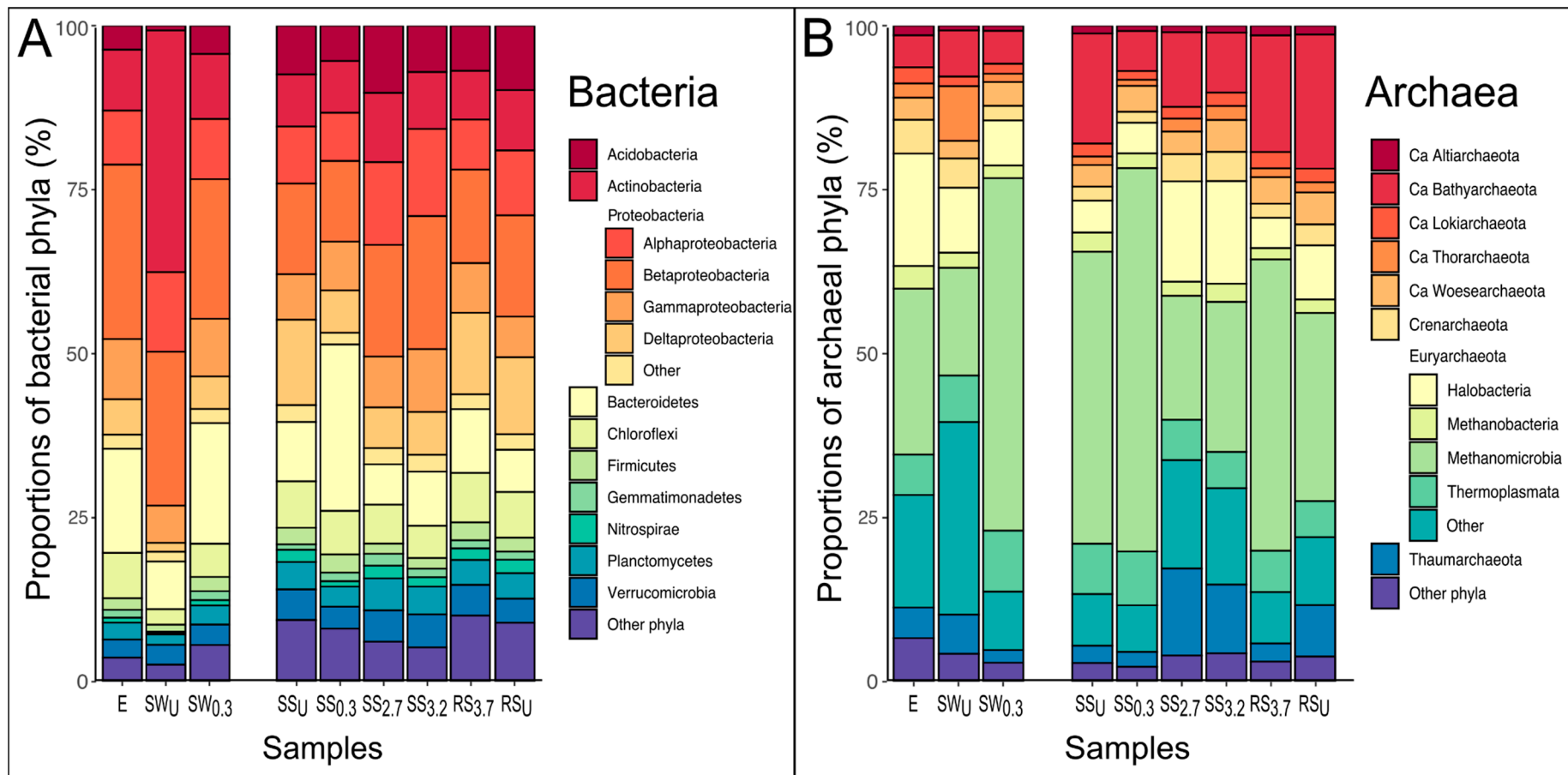


Figure S2. The bacterial (A) and archaeal (B) community taxonomy of wastewater treatment plant (WWTP) effluent (E), stream water upstream (SW_U) and downstream ($SW_{0.3}$) of WWTP discharge, as well as stream sediment upstream (SS_U) and downstream ($SS_{0.3}$ - $SS_{3.2}$) of WWTP discharge and river sediment upstream (RS_U) and downstream ($RS_{3.7}$) of stream inflow at phylum level. *Proteobacteria* and *Euryarchaeota* are presented at class level. The subscript number in the sample code indicates distance (in km) from the WWTP discharge.

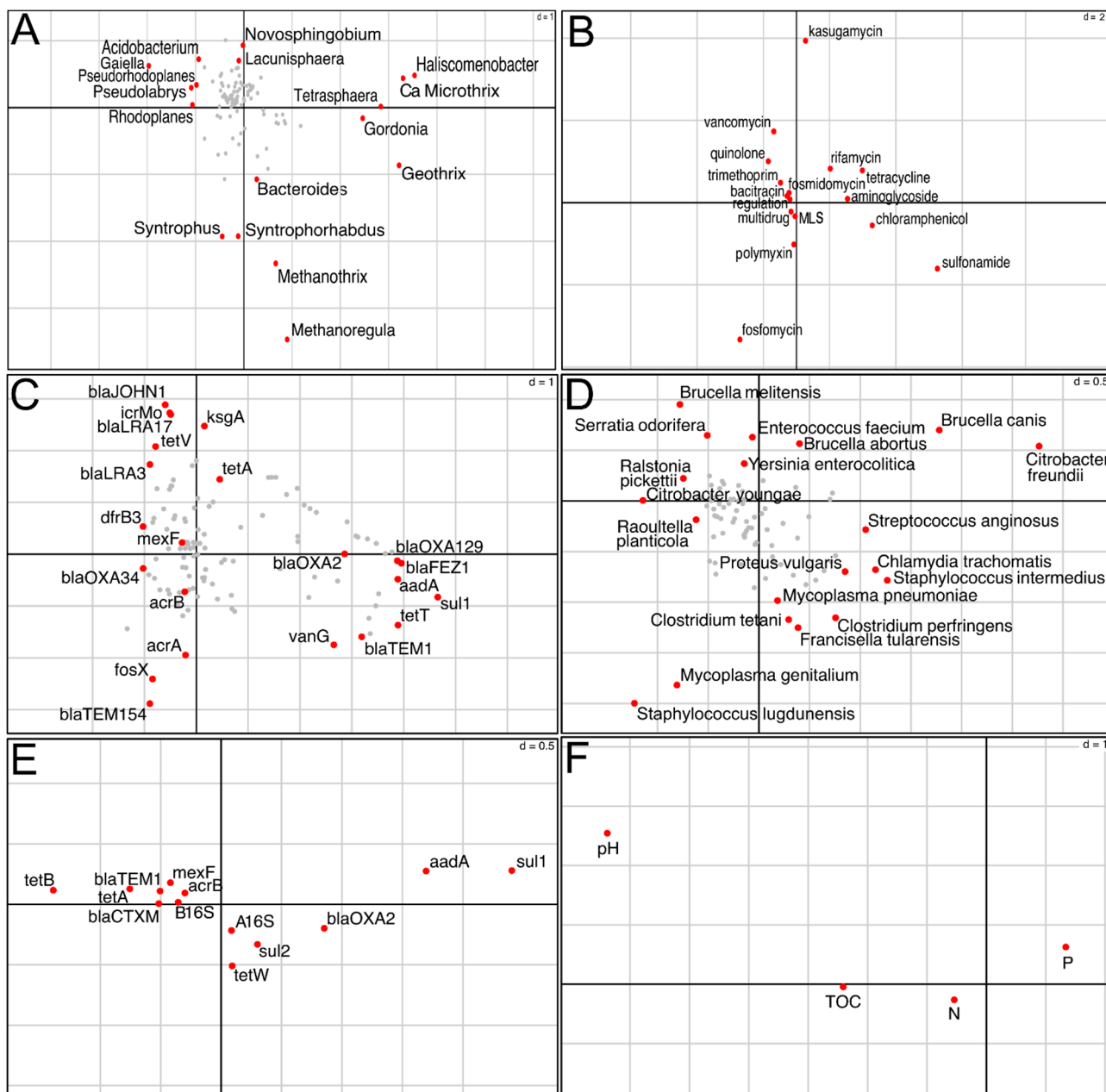


Figure S3. Multiple co-inertia analysis results based on six sediment-specific data sets. Shown are variable spaces for each data set (microbial genera proportions in prokaryotic community (A), antibiotic resistance type (B) and ARG subtype (C) proportions, pathogen proportions (D), target gene abundances (E) and sediment physicochemical parameters (F). TOC – total organic carbon, N – total nitrogen, P – total phosphorous content in sediment.

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