Electronic Supplementary Material (ESI) for Environmental Science: Advances.

Supporting Information: Seasonal and spatial patterns differ between intracellular and extracellular antibiotic resistance genes in urban stormwater runoff

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Stormwater Sampling

Fig. S1 Map of the sampling locations for this study across Milwaukee County in Wisconsin, USA. The sites include a stormwater inlet, a stormwater outfall, the Menomonee River, and Lake Michigan. The map was created using ArcGIS software by Ersi.

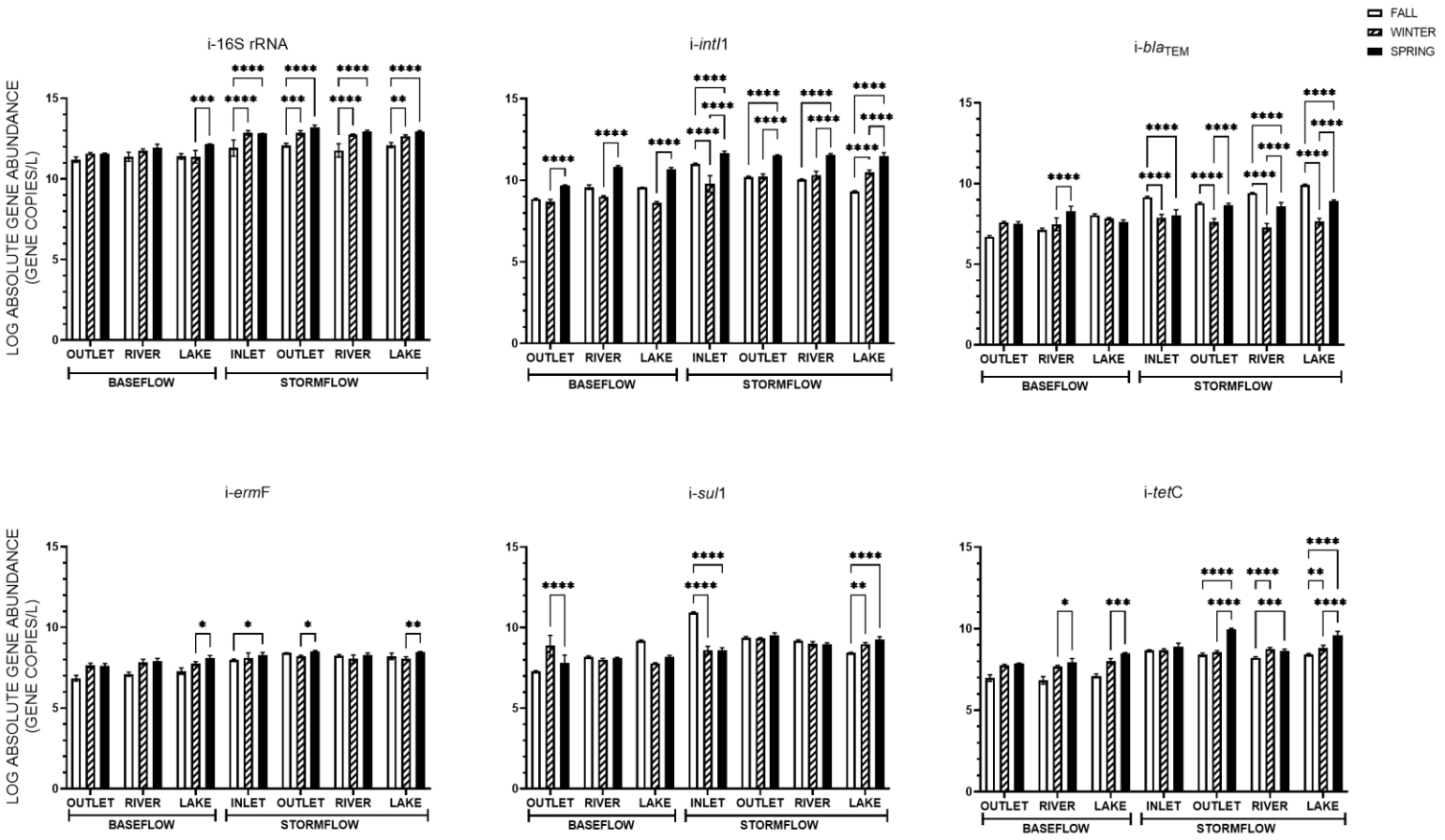


Fig. S2 Absolute gene abundance of intracellular genes by sampling location that occurred in the fall, winter, and spring under baseflow (n=9) and stormflow conditions (n=11). Error bars represent the standard deviation of the mean and statistically significant relationships between the average absolute abundance of the extracellular and intracellular genes were evaluated by Tukey's posthoc test and shown with * ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$), and ****($p<0.0001$).

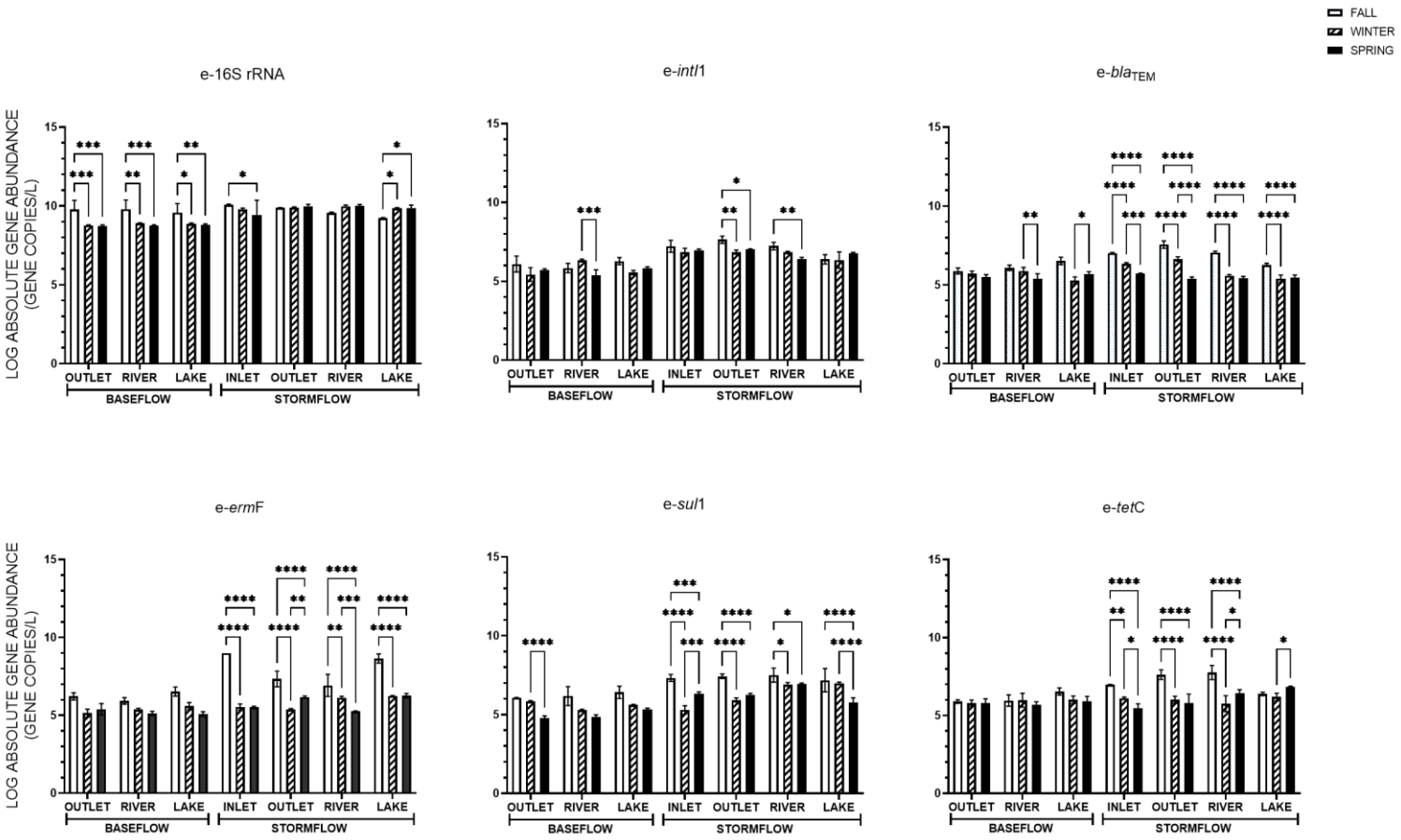
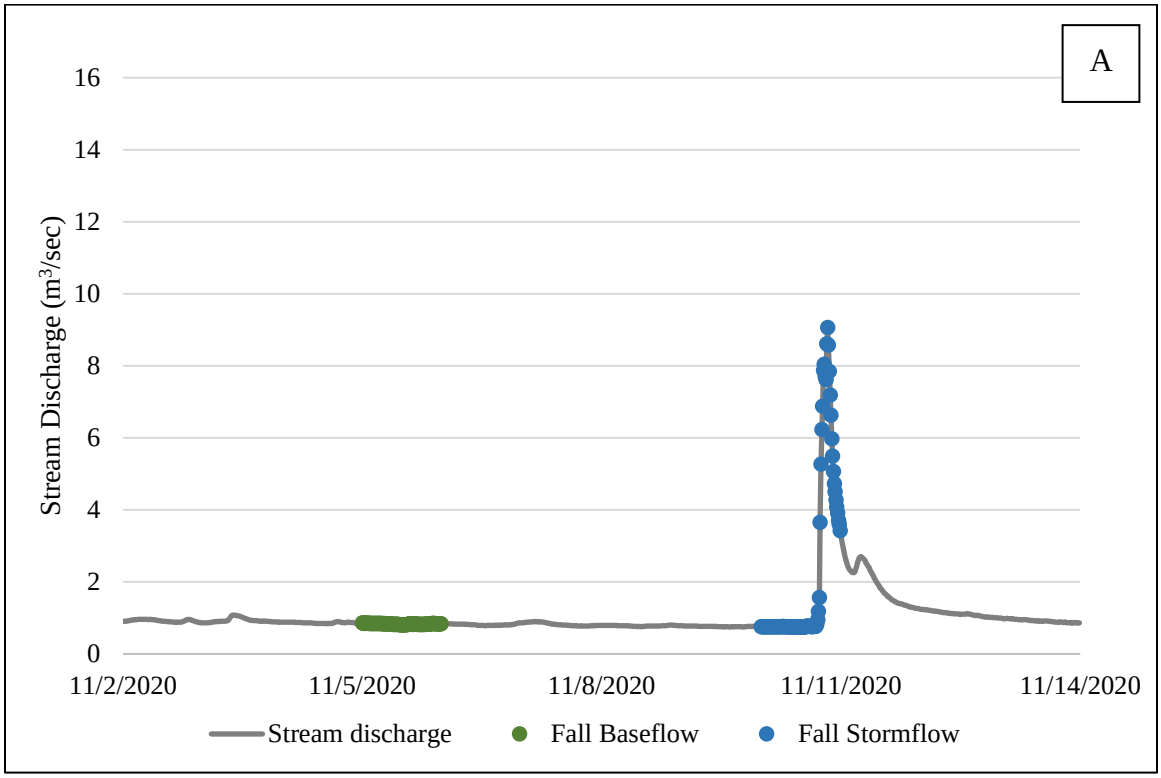
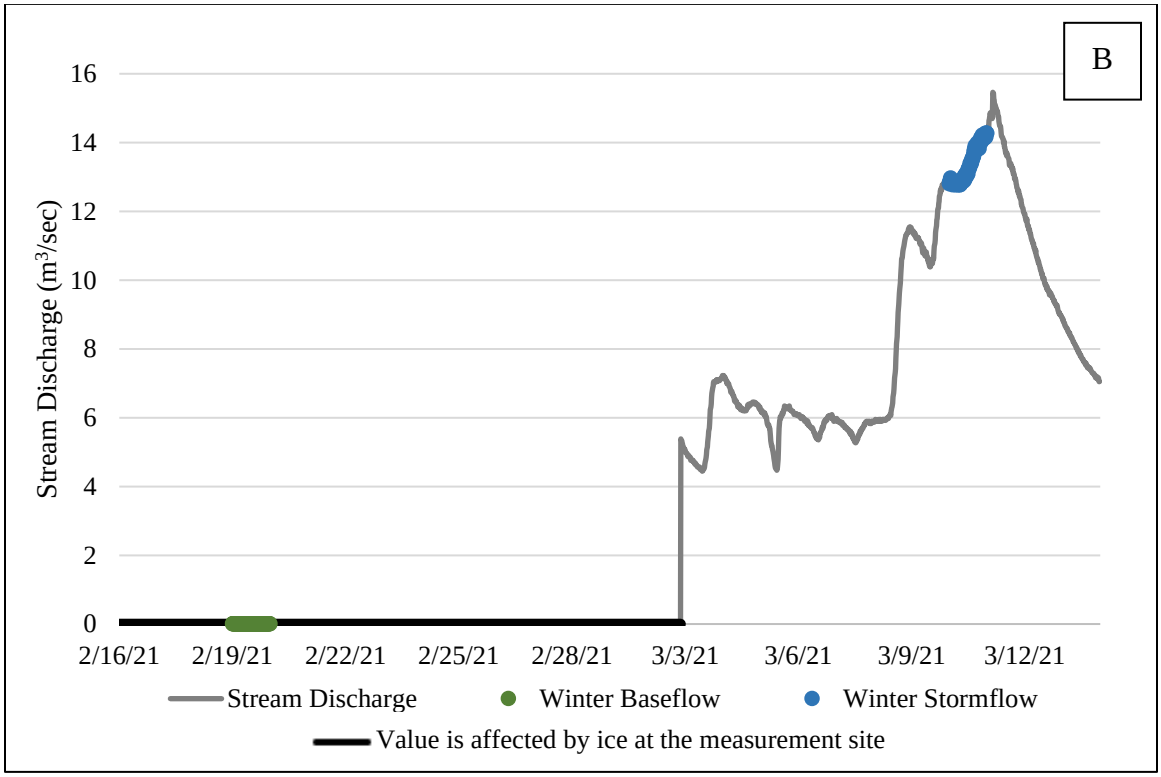


Fig. S3 Absolute gene abundance of extracellular genes by sampling location that occurred in the fall, winter, and spring under baseflow (n=3) and stormflow conditions (n=3). Error bars represent the standard deviation of the mean and statistically significant relationships between the average absolute abundance of the extracellular and intracellular genes were evaluated by Tukey's posthoc test and shown with * ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$), and ****($p<0.0001$).

Streamflow Data





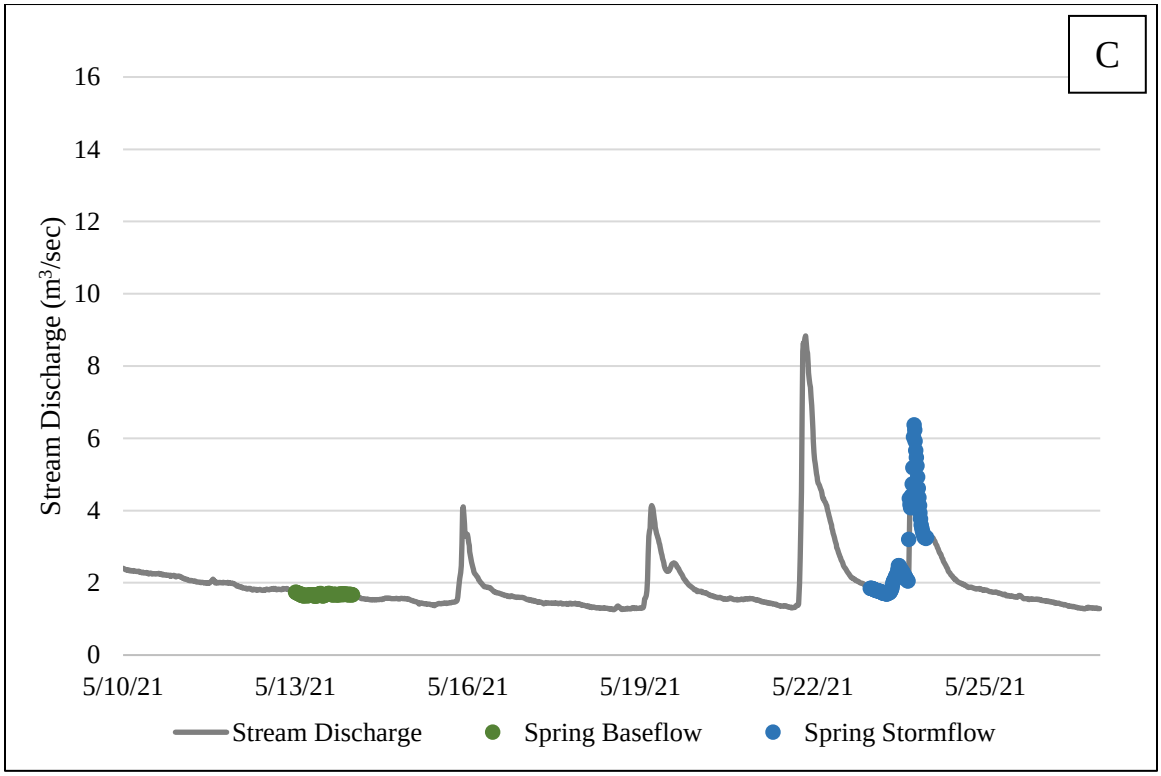
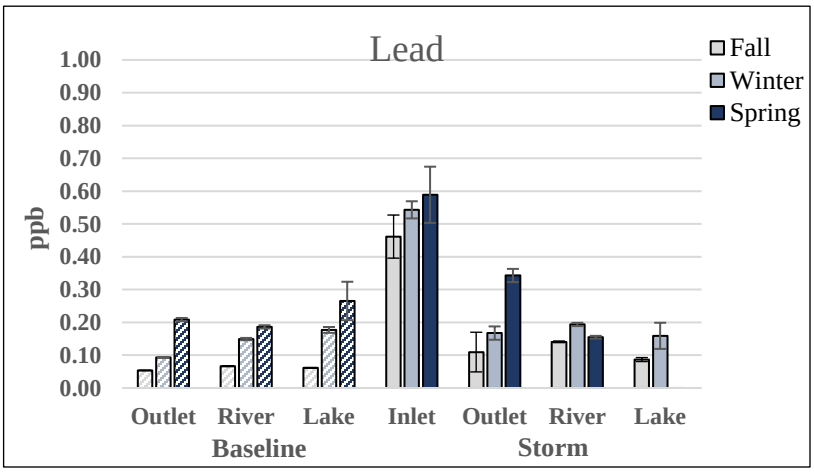
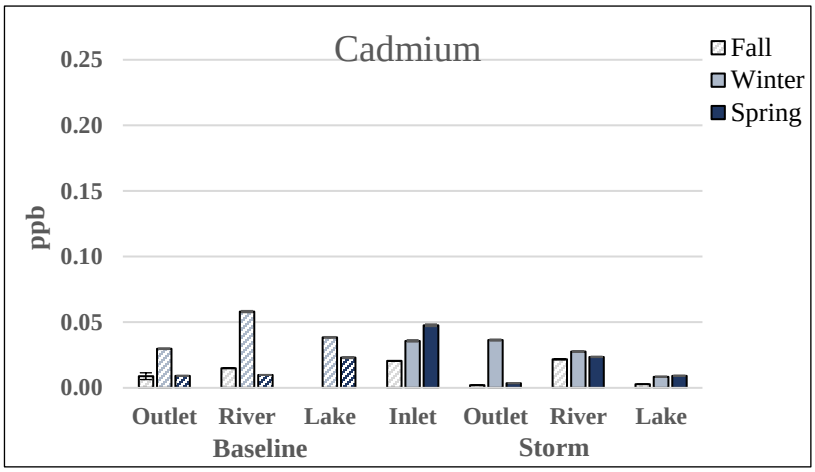
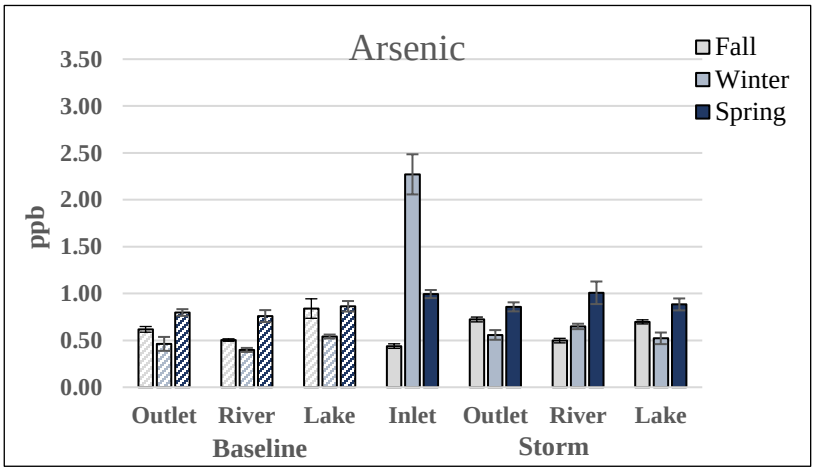


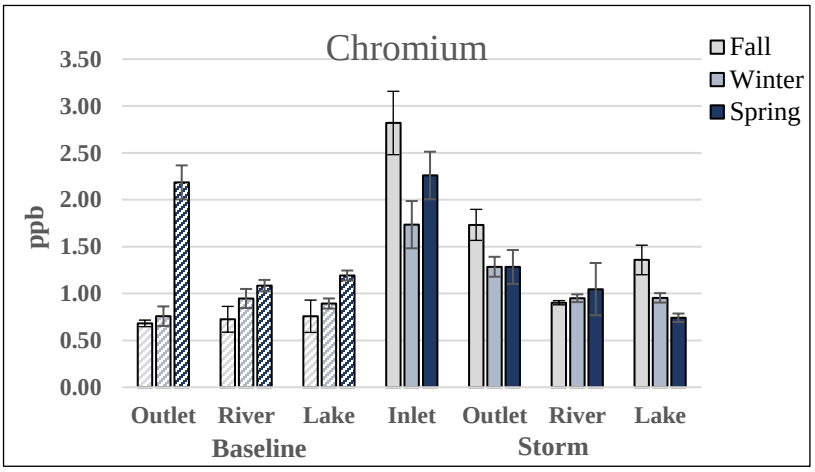
Fig. S4 Stream discharge recorded by USGS stream gage 04087120 during the baseflow and stormflow sampling events in the fall (A), winter (B), and spring (C).

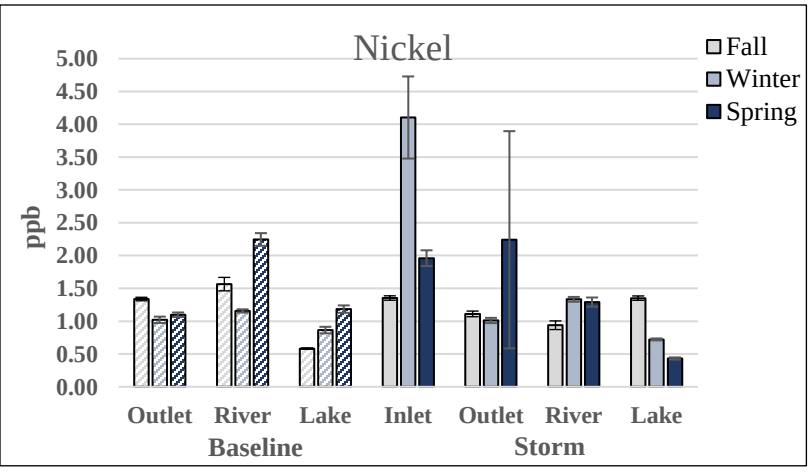
Water Quality Results

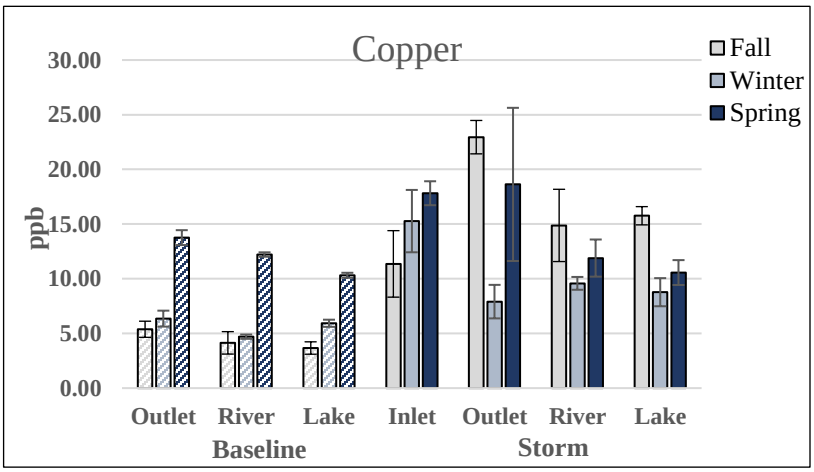


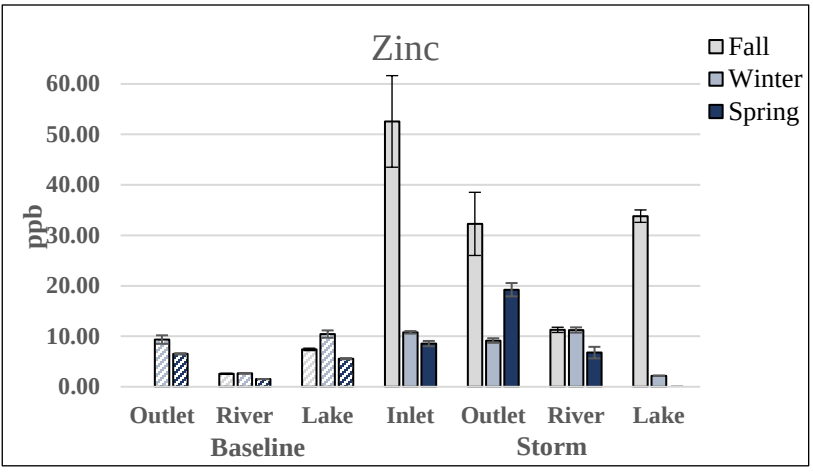












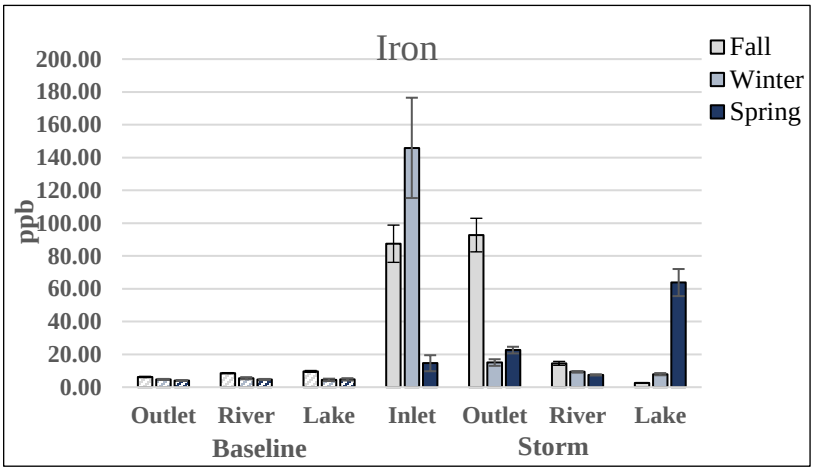
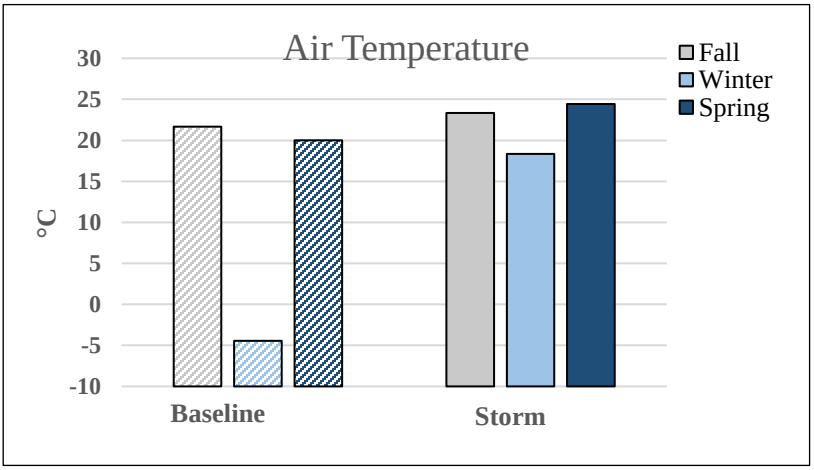


Figure S5. Concentration of various metals quantified under baseline and storm conditions. Error bars represent the standard deviation of the means (n=3).



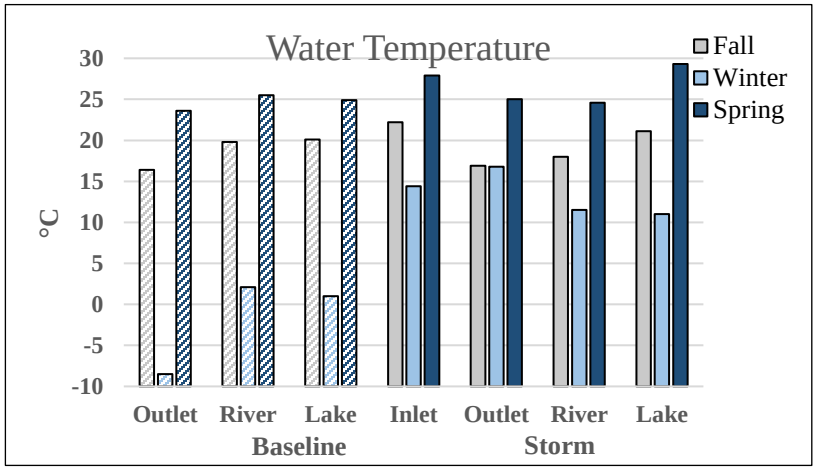


Fig. S6 Air and water temperatures recorded at the time of baseline and storm sampling.

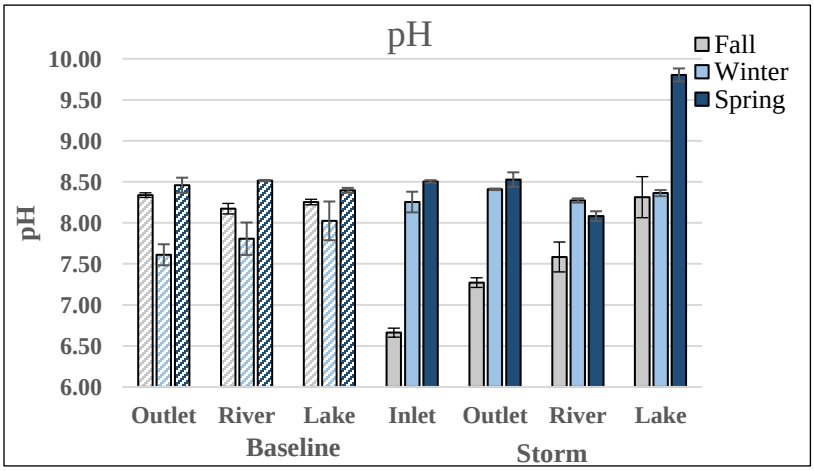


Fig. S7 pH of baseline and stormflow samples. Error bars represent the standard deviation of the means (n=3).

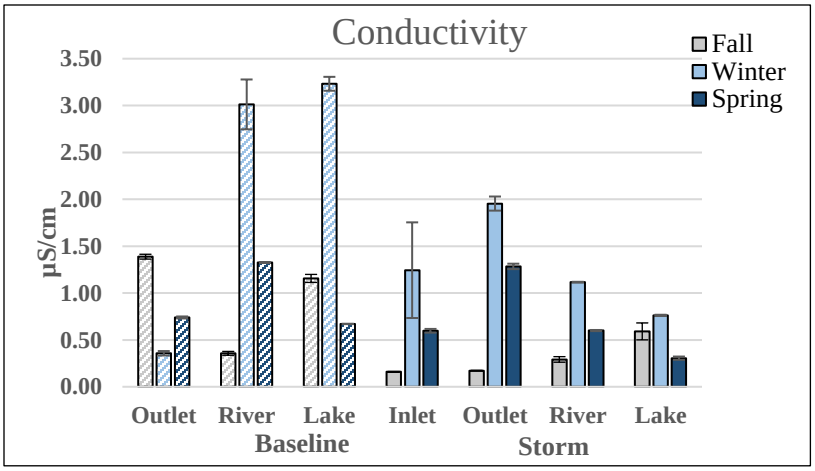


Fig. S8 Conductivity of baseline and stormflow samples. Error bars represent the standard deviation of the means (n=3).

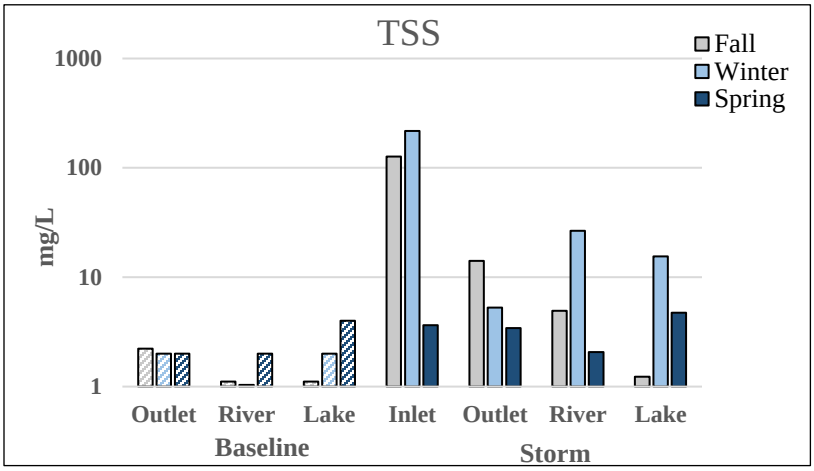
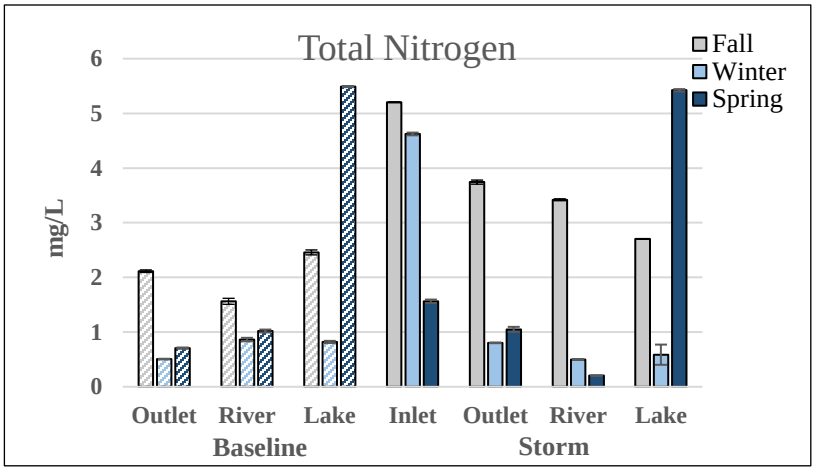


Fig. S9 Concentration of total suspended solids (TSS) quantified in baseline and storm samples.



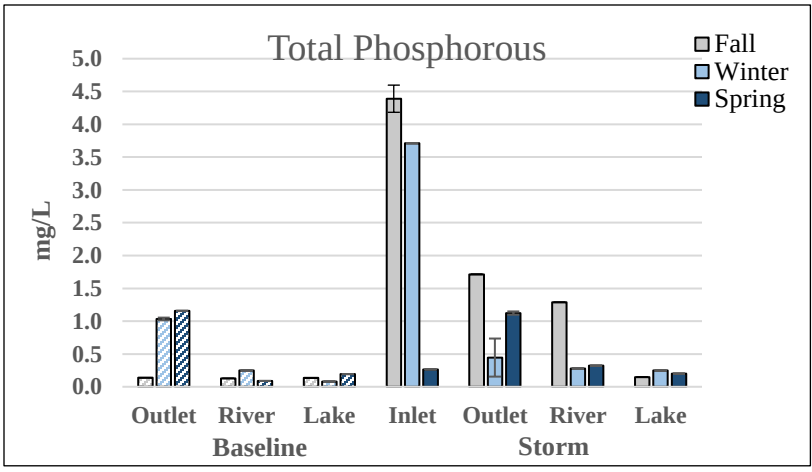


Fig. S10 Concentration of nutrients (total nitrogen and phosphorous) quantified in baseline and storm samples. Error bars represent the standard deviation of the means (n=3).

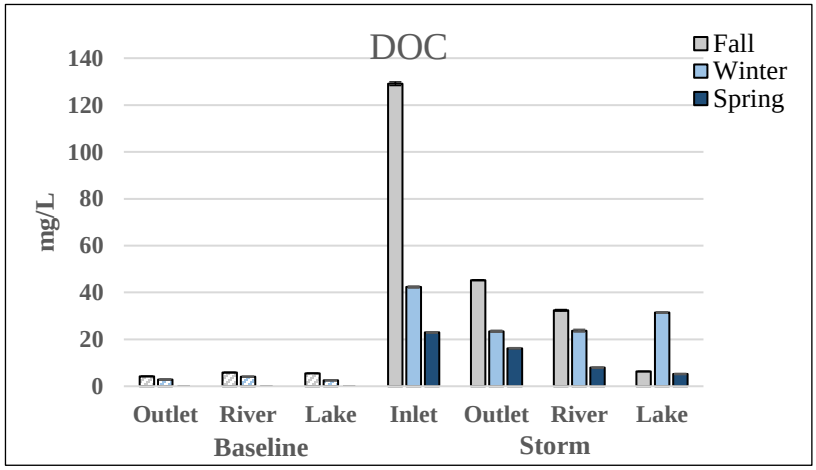


Fig. S11 Concentration of dissolved organic carbon (DOC) quantified in baseline and storm samples. Error bars represent the standard deviation of the means (n=3).

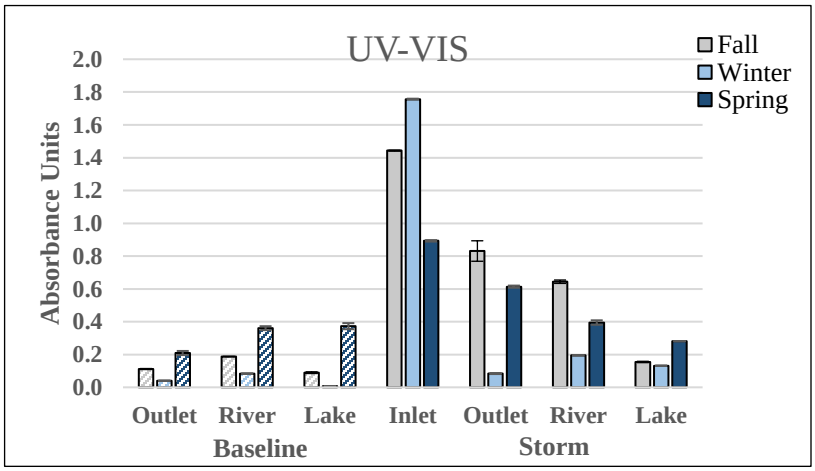


Fig. S12 Ultraviolet–visible spectrophotometry (UV-Vis) measured in baseline and storm samples.

Correlation Analysis

Table S1 Correlation analysis (Pearson's r) of ARGs and *intI*1 by sampling event

	fall baseflow	fall stormwater	winter baseflow	winter stormflow	spring baseflow	spring stormflow
i BLATEM	**0.73**	**-0.68**	-0.49	-0.26	0.62	-0.26
i ERMF	**0.67**	-0.46	**0.67**	-0.06	**0.73**	-0.06
i SUL1	**0.83**	**0.98**	-0.33	0.29	0.54	0.29
i TETC	-0.02	0.54	-0.59	0.34	0.49	0.34
e BLATEM	-0.09	**0.85**	0.45	0.32	-0.08	0.32
e ERMF	0.64	-0.46	0.23	-0.44	0.13	-0.44
e SUL1	0.56	0.04	**-0.80**	-0.39	0.30	-0.39
e TETC	0.50	**0.70**	0.31	-0.25	0.27	-0.25

Bolded indicates a p-values<0.05

Table S2 Correlation analysis (Pearson's r) of ARGs and *intI*1 by sampling location

	outlet baseflow	river baseflow	lake baseflow	inlet storm	outlet storm	river storm	lake storm
i BLATEM	0.24	**0.73**	-0.49	0.19	0.35	-0.05	-0.56
i ERMF	0.34	0.26	0.44	0.22	0.66	0.35	0.51
i SUL1	-0.36	0.46	0.23	0.13	0.66	-0.49	**0.97**
i TETC	0.45	0.43	0.38	0.62	**0.99**	0.45	**0.92**
e BLATEM	-0.09	0.32	**0.82**	0.39	0.63	**0.83**	-0.35
e ERMF	**0.70**	0.32	0.66	0.53	**0.87**	**0.84**	-0.25
e SUL1	0.06	0.42	**0.70**	0.39	**0.89**	0.56	-0.58
e TETC	0.11	0.53	**0.77**	0.37	**0.87**	0.49	0.57

Bolded indicates a p-values<0.05

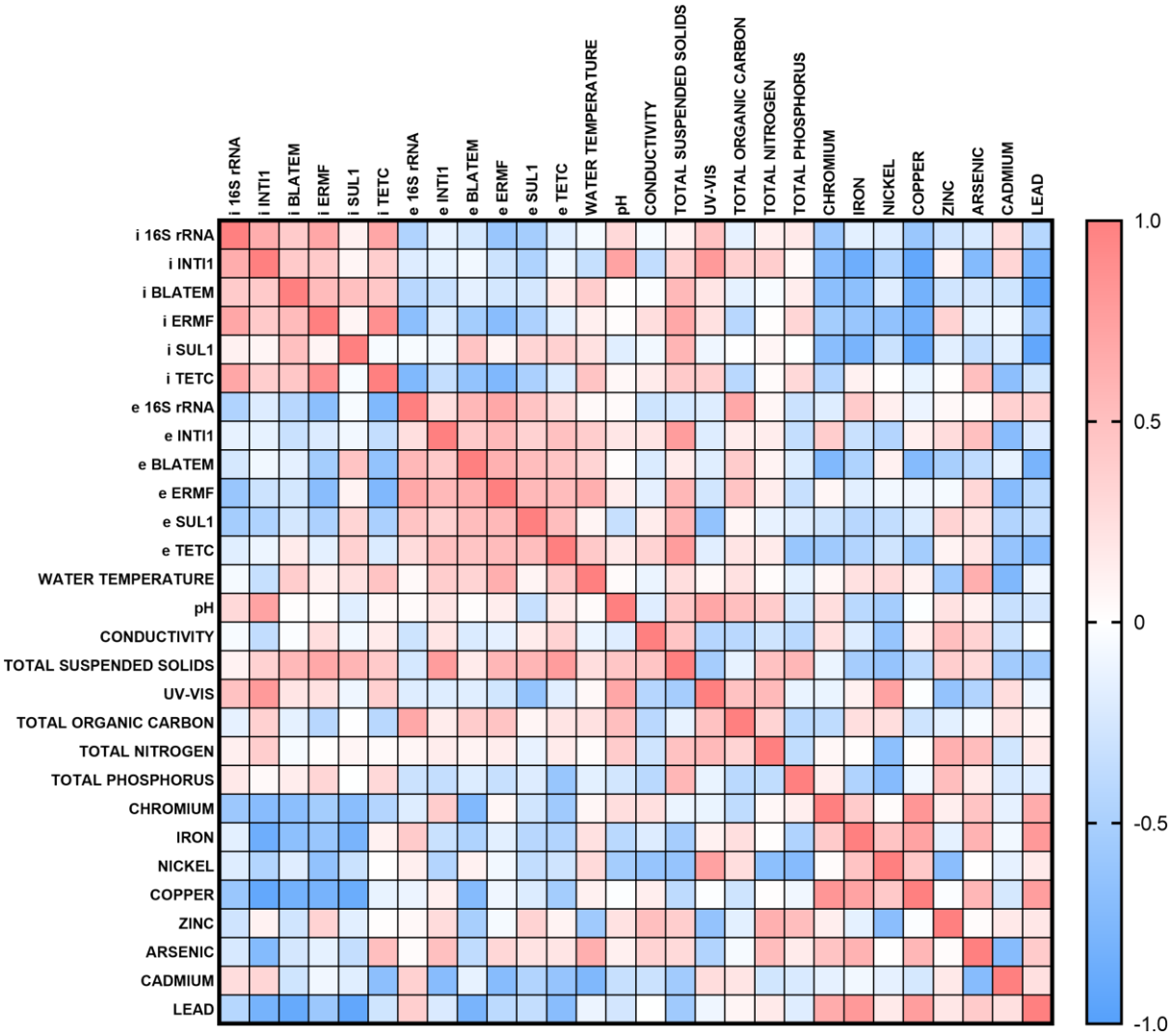


Fig. S13 Pearson correlation matrix of ARGs and water quality parameters during baseflow conditions

Table S3 Pearson's r from correlation analysis of ARGs and water quality parameters during baseflow conditions

	INTRACELLULAR						EXTRACELLULAR					
	16S rRNA	INTI1	BLATEM	ERMF	SUL1	TETC	16S rRNA	INTI1	BLATEM	ERMF	SUL1	TETC
WATER TEMPERATURE	-0.06	-0.34	0.39	0.12	0.23	0.46	0.05	0.39	0.33	0.63	0.08	0.42
pH	0.30	**0.72**	0.01	0.02	-0.18	0.06	0.03	0.19	0.02	0.14	-0.33	0.17
CONDUCTIVITY	-0.05	-0.36	-0.02	0.25	-0.08	0.16	-0.29	0.20	-0.22	-0.16	0.15	0.34
TOTAL SUSPENDED SOLIDS	0.09	0.35	0.55	**0.68**	0.58	0.42	-0.25	**0.77**	0.15	0.56	0.57	**0.77**
UV-VIS	**0.48**	**0.79**	0.20	0.23	-0.10	0.37	-0.20	-0.19	-0.19	-0.27	**-0.64**	**-0.18**
TOTAL ORGANIC CARBON	-0.15	0.36	-0.16	**-0.42**	-0.01	**-0.42**	**0.68**	0.14	**0.40**	**0.46**	0.07	0.21
TOTAL NITROGEN	0.13	**0.38**	-0.05	0.01	0.07	0.03	0.07	0.14	0.09	0.14	-0.14	0.15
TOTAL PHOSPHORUS	0.17	0.04	0.14	0.32	-0.01	0.30	-0.31	-0.35	-0.21	-0.34	-0.20	**-0.61**
CHROMIUM	-0.58	**-0.71**	**-0.68**	-0.54	**-0.69**	-0.44	-0.20	0.39	**-0.75**	0.07	-0.28	-0.56
IRON	-0.17	**-0.86**	**-0.67**	-0.60	**-0.79**	0.11	0.41	-0.32	-0.47	-0.17	-0.42	-0.45
NICKEL	-0.20	-0.45	-0.19	-0.65	-0.31	0.00	0.12	-0.44	0.10	-0.08	-0.36	-0.29
COPPER	-0.60	**-0.92**	**-0.82**	**-0.80**	**-0.87**	-0.14	-0.12	0.12	**-0.72**	-0.10	-0.21	-0.54
ZINC	-0.29	0.10	-0.28	0.34	-0.17	0.01	0.05	0.27	-0.52	-0.06	0.34	0.09
ARSENIC	-0.24	**-0.72**	-0.25	-0.15	-0.35	0.50	0.03	0.49	-0.38	0.31	0.21	0.20
CADMIUM	0.26	0.32	-0.28	-0.08	-0.18	**-0.68**	0.36	**-0.71**	-0.14	**-0.71**	-0.45	-0.62
LEAD	-0.43	**-0.81**	**-0.90**	-0.58	**-0.93**	-0.28	0.38	-0.22	**-0.79**	-0.40	-0.35	**-0.69**

Bolded indicates a p-values<0.05

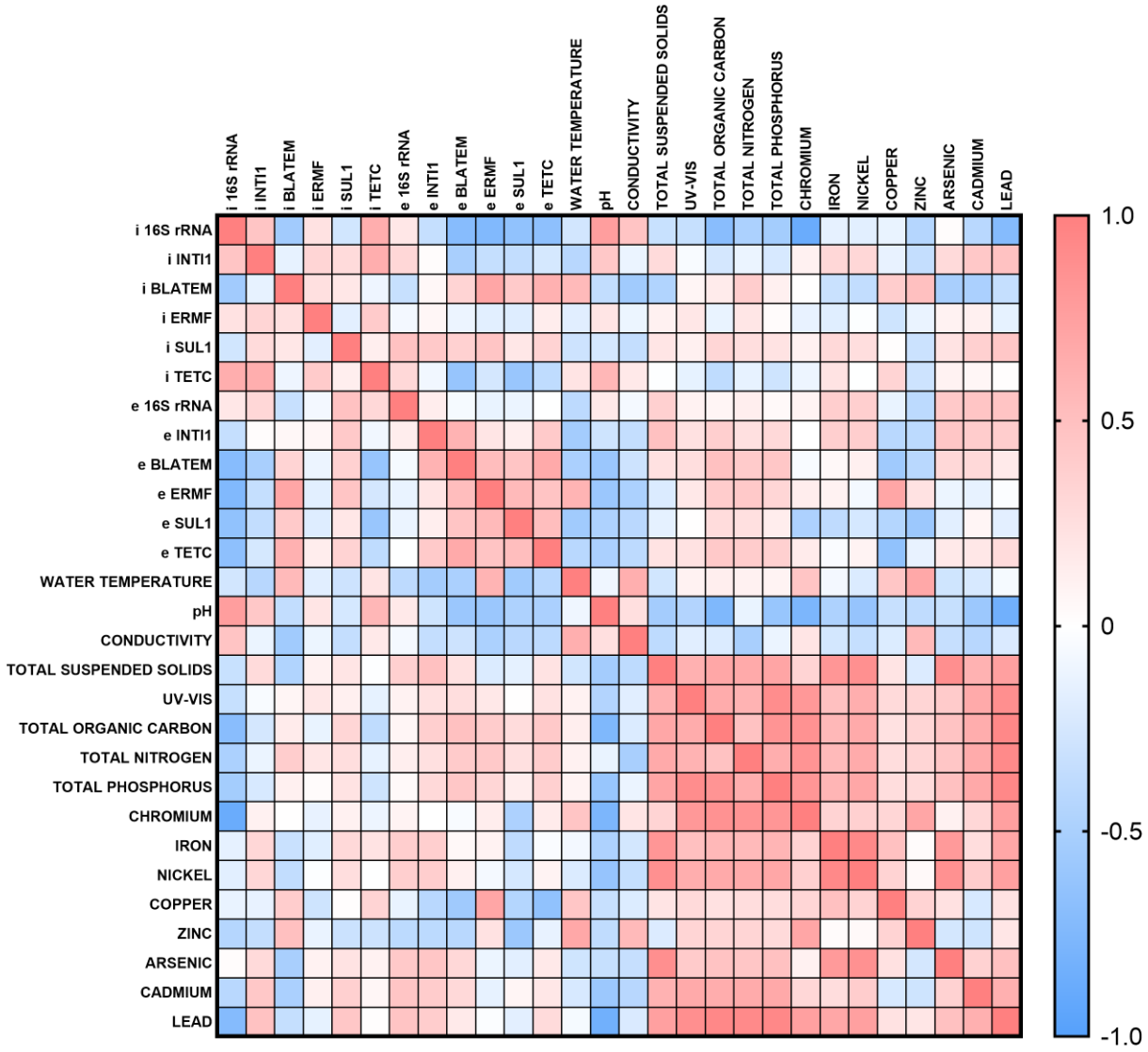


Fig. S14 Pearson correlation matrix of ARGs and water quality parameters during stormflow conditions

Table S4 Pearson's r from correlation analysis of ARGs and water quality parameters during stormflow conditions

	INTRACELLULAR						EXTRACELLULAR					
	16S rRNA	INTI1	BLATEM	ERMF	SUL1	TETC	16S rRNA	INTI1	BLATEM	ERMF	SUL1	TETC
WATER TEMPERATURE	-0.27	-0.42	0.54	-0.18	-0.30	0.21	-0.39	-0.54	-0.50	**0.59**	-0.56	-0.42
pH	**0.76**	**0.43**	**-0.36**	0.20	-0.25	**0.56**	0.17	-0.29	**-0.59**	**-0.59**	**-0.48**	**-0.50**
CONDUCTIVITY	**0.46**	-0.12	**-0.55**	-0.11	**-0.35**	0.17	-0.07	**-0.35**	**-0.30**	**-0.49**	**-0.41**	**-0.39**
TOTAL SUSPENDED SOLIDS	-0.32	0.28	-0.46	0.10	0.20	-0.01	0.37	0.48	0.23	-0.22	-0.15	0.22
UV-VIS	**-0.34**	-0.05	0.08	0.19	0.11	-0.15	0.10	0.23	0.25	0.18	0.00	0.22
TOTAL ORGANIC CARBON	**-0.70**	-0.26	0.15	-0.13	0.32	**-0.38**	0.07	**0.37**	**0.48**	**0.40**	0.27	**0.42**
TOTAL NITROGEN	**-0.49**	-0.12	**0.39**	0.19	0.26	-0.14	0.13	0.24	**0.40**	**0.42**	0.24	**0.40**
TOTAL PHOSPHORUS	**-0.54**	-0.24	0.11	0.03	0.22	-0.29	0.05	0.30	**0.43**	0.32	0.13	**0.37**
CHROMIUM	**-0.88**	0.10	0.00	-0.14	0.10	-0.11	0.09	0.00	-0.05	0.14	-0.49	0.15
IRON	-0.15	0.31	-0.32	-0.19	0.30	0.22	0.39	0.38	0.05	0.10	-0.38	-0.03
NICKEL	-0.17	0.31	-0.36	-0.02	0.26	-0.01	0.37	0.38	0.11	-0.07	-0.25	0.09
COPPER	-0.13	-0.14	0.39	-0.29	0.01	0.34	-0.14	-0.41	-0.56	**0.69**	-0.44	**-0.65**
ZINC	-0.44	-0.35	0.49	-0.13	-0.31	-0.30	-0.40	-0.40	-0.41	0.23	**-0.58**	-0.14
ARSENIC	0.02	0.29	-0.51	0.10	0.22	0.10	0.42	0.45	0.31	-0.11	-0.17	0.17
CADMIUM	-0.41	0.43	-0.50	0.12	0.36	0.06	0.45	0.40	0.30	-0.14	0.08	0.19
LEAD	**-0.72**	0.48	-0.34	-0.15	0.44	0.01	0.46	0.39	0.16	-0.03	-0.17	0.28

Bolded indicates a p-values<0.05

DNA Extraction and ddPCR

All DNA extractions were completed within 24 hours of sampling. A one-liter water sample was vacuum filtered through a 0.22 µm Merck Millipore Express Plus® membrane filter. iDNA was extracted from the filters via FastDNA Spin Kit manufacturer's protocol (MP Biomedicals, Santa Ana, CA). eDNA was concentrated from the filtrate by pumping the filtrate through a column containing nucleic acid adsorption particles. Following, 100 mL of an organic eluent (15 g/L NaCl, 30 g/L tryptone, 15 g/L beef extract, 3.75 g/L glycine, 0.28 g/L Na(OH), pH = 9.3 ± 0.2; autoclaved at 120°C for 20 min) was pumped through the column and collected. eDNA was extracted from the eluent via isopropanol-ethanol precipitation.

The ddPCR assays (22 µL) consisted of a reaction mixture of QX200 ddPCR EvaGreen Supermix (Bio-Rad) (11 µL), forward and reverse primers (250 nM each) (Table S5), diluted DNA extracts (4 µL), and Sigma® Life Science Molecular Biology Reagent (5.9 µL). The quantitative digital PCR experiments (dMIQE) checklist was completed for quality assurance and control [1]. The samples from this study averaged 14,966 positive droplets per reaction. If the number of droplets generated was <10,000 per 20 µL PCR, the reaction was rejected [2].

Table S5 ddPCR conditions and primers

Gene	Annealing Temperature	Forward Primer and Reverse Primer	Reference
16S rRNA	60°C	For. (5'-CCTACGGGAGGCAGCAG-3') Rev. (5'-ATTACCGCGGCTGCTGG-3')	3
intI1	60°C	For. (5'-CCTCCCGCACGATGATC-3') Rev. (5'-TCCACGCATCGTCAGGC-3')	4
bla$_{TEM}$	60°C	For. (5'-GCKGCCAACTTACTTCTGACAACG-3') Rev. (5'- CTTTATCCGCCTCCATCCAGTCTA-3')	5
*erm*F	60°C	For. (5'-TCGTTTTACGGGTCAGCACTT-3') Rev. (5'- CAACCAAAGCTGTGTCGTTT-3')	6
*sul*1	60°C	For. (5'-CCGTTGGCCTTCCTGTAAAG -3') Rev. (5'- TTGCCGATCGCGTGAAGT-3')	7
*tet*C	60°C	For. (5'-GCGGGATATCGTCCATTCCG -3') Rev. (5'-GCGTAGAGGATCCACAGGACG-3')	8

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