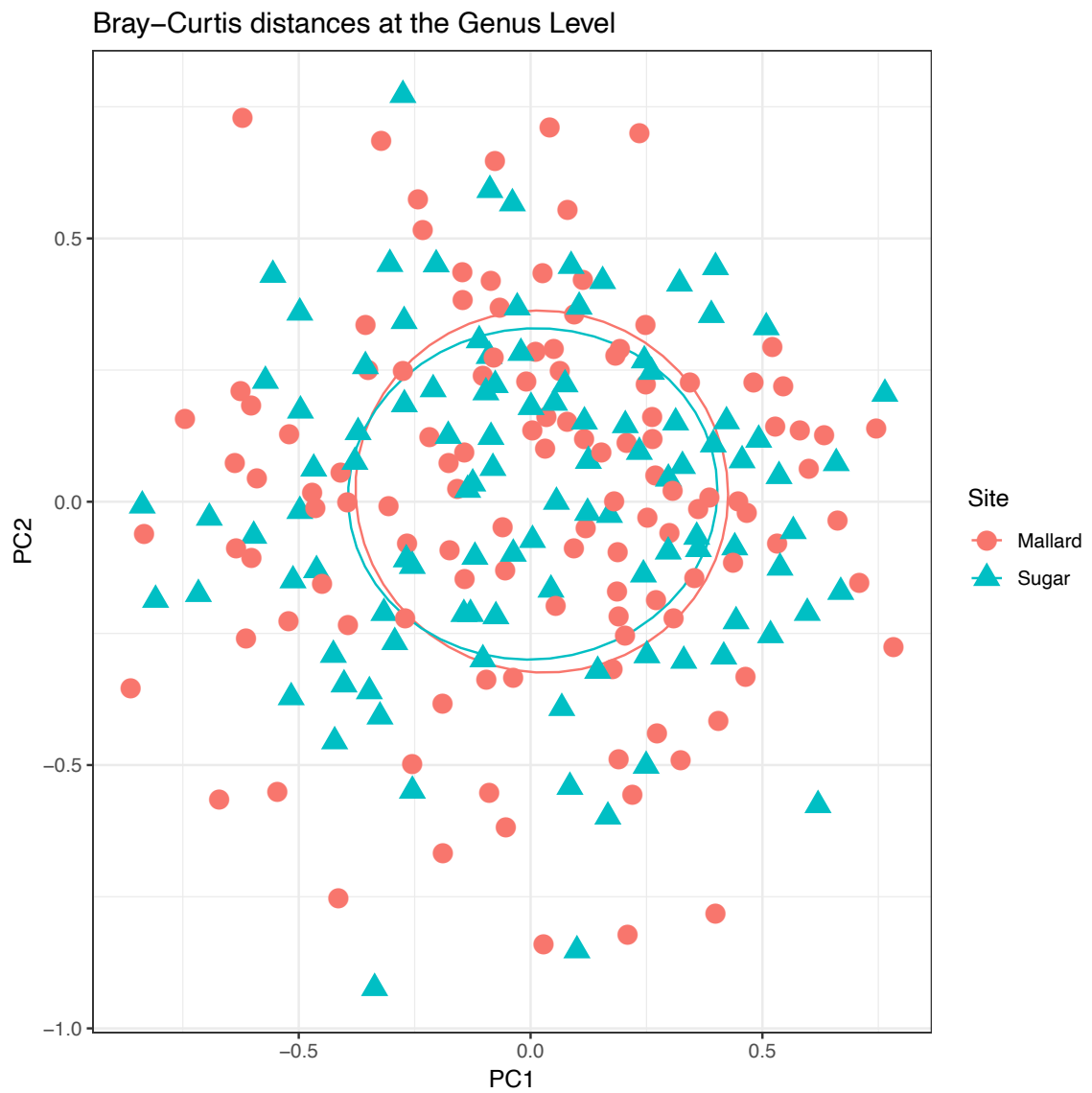
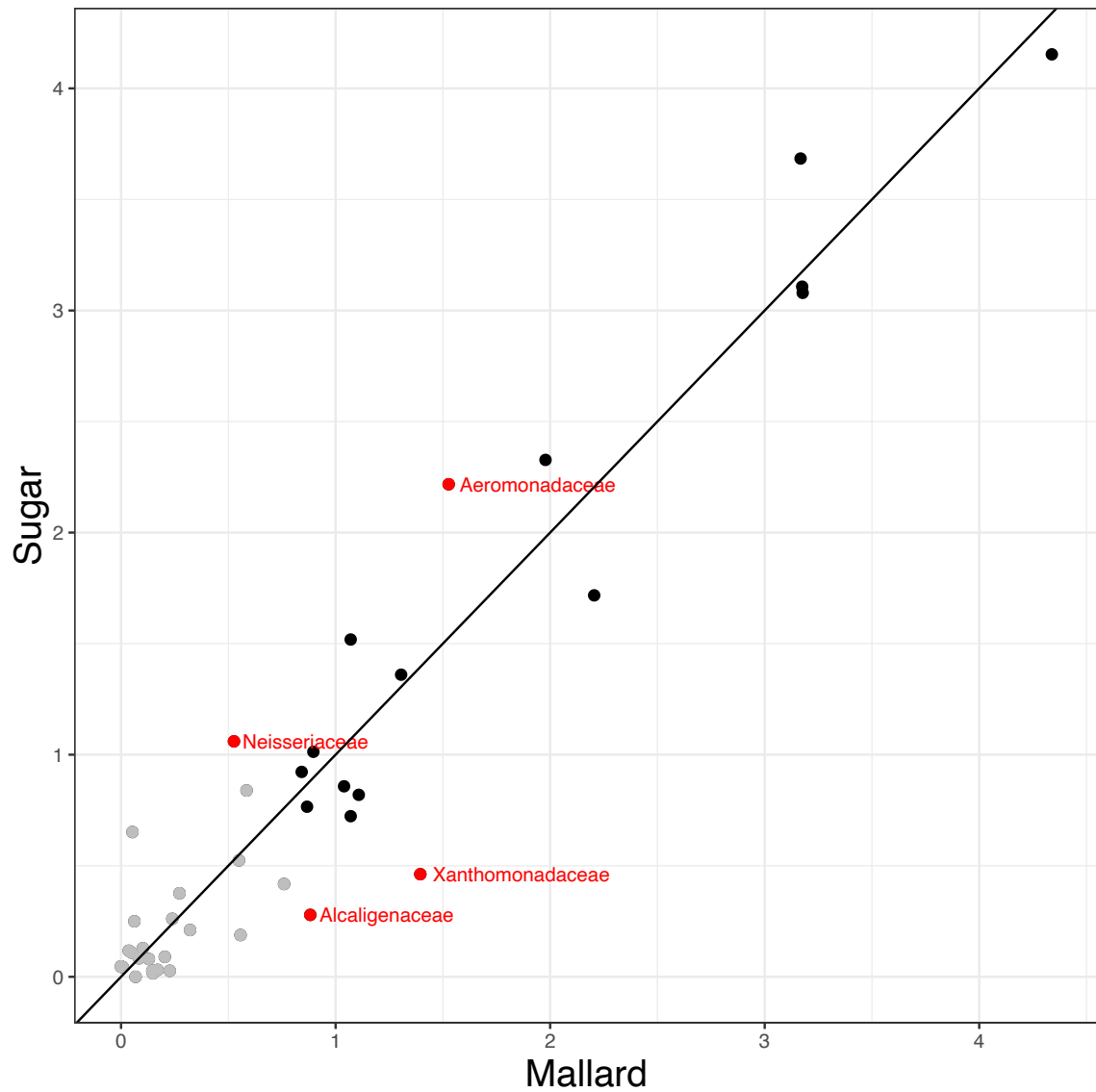


## APPENDIX A: Chapter 2 Supplemental Material



**Figure A1** | Beta diversities at the genus level from 16S rRNA gene sequencing. PCoA ordination for Mallard and Sugar Creek samples with PC1 and PC2 components. Data colored by the two sampling sites.

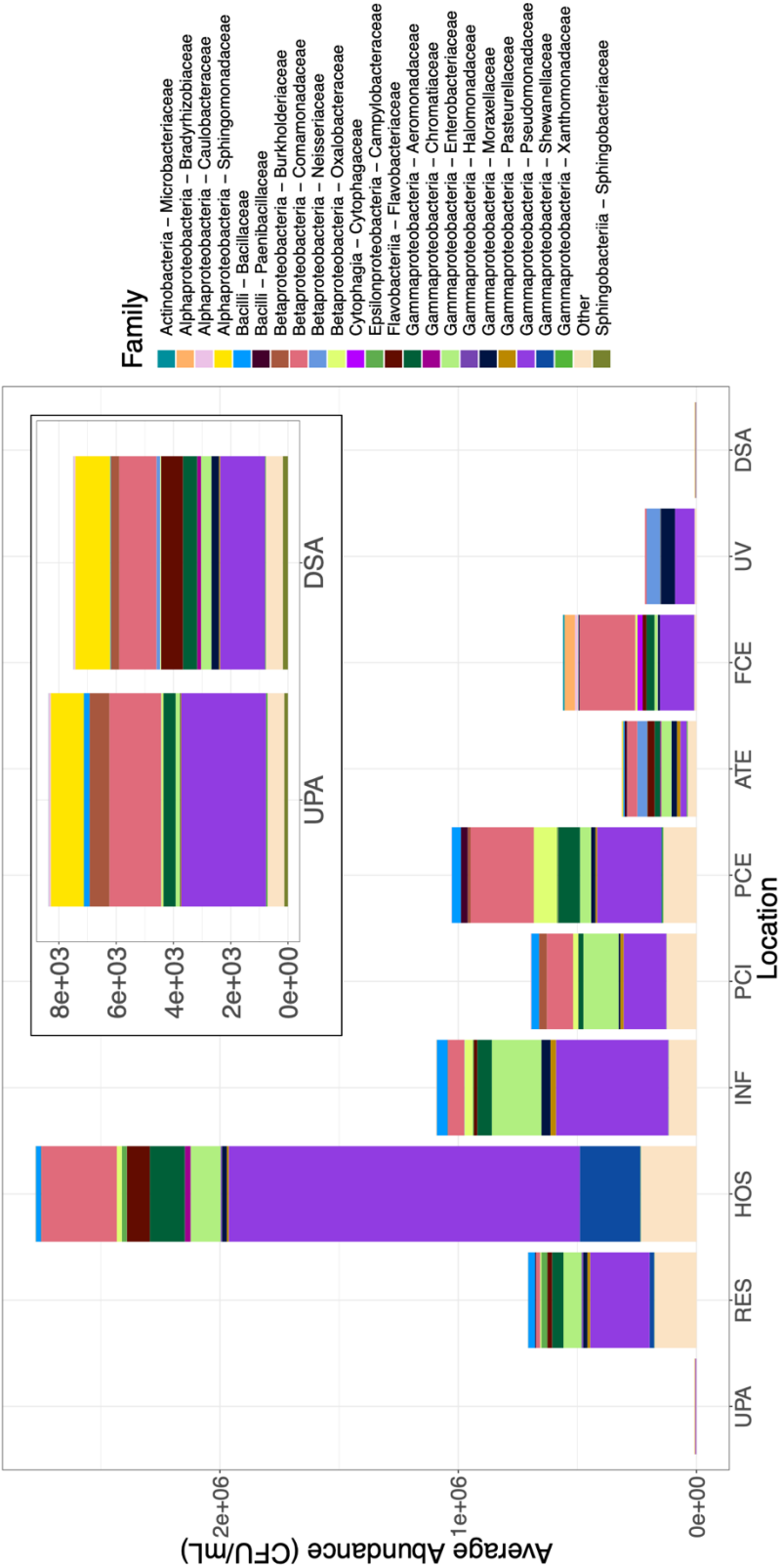


**Figure A2** | The mean relative abundance of each family from Mallard and Sugar Creek WWTPs. Non-rare taxa found to significantly differ using linear regression models at a 5% FDR threshold (Table A3) are indicated in red. Black symbols represent non-rare taxa found in 3% of all samples. Grey symbols represent rare taxa. The black line is the identity line (Mallard=Sugar).

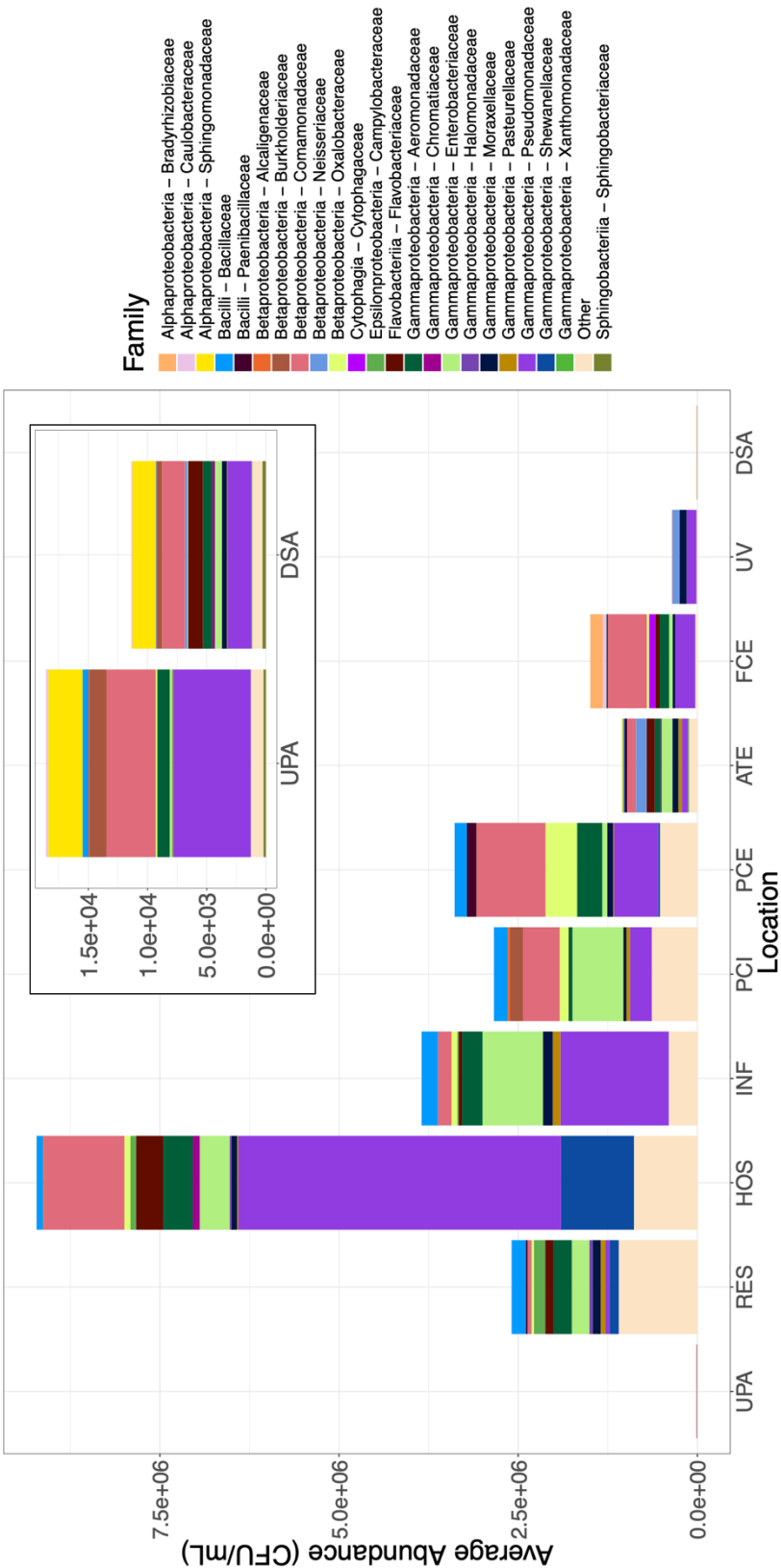
**Table A1** | Differential abundance  $p$  values of colony counts (normalized to CFU/mL) between each WWTP site, incubation temperature and media, stage of treatment, and antibiotic utilized for combined ARB, the total heterotrophic growth (HPC), and each individual antibiotic treatments.

Terms	ALL	ARB	HPC	AMP	CIP	DOX	SUL
Mallard - Sugar	0.740	0.026	2.0E-16	0.004	0.553	3.3E-04	0.518
BT - RT	2.0E-16	2.0E-16	8.2E-07	2.0E-16	4.3E-14	1.9E-13	5.0E-10
LB - R2A	0.048	0.545	1.8E-12	9.7E-07	0.667	0.661	1.8E-11
UPA - DSA	0.168	0.138	0.759	0.378	0.759	0.017	0.443
RES - HOS	0.001	0.004	0.003	3.1E-05	0.106	0.677	0.819
RES - INF	0.008	0.004	0.629	2.7E-04	0.025	0.590	0.781
HOS - INF	0.416	0.973	0.012	0.583	0.530	0.339	0.613
INF - ATE	2.0E-16	2.0E-16	0.832	5.7E-08	7.0E-15	0.001	7.6E-12
PCI - PCE	0.104	0.469	0.003	0.163	0.612	0.177	4.6E-05
PCI - ATE	1.2E-13	9.4E-15	0.029	0.003	1.6E-06	0.195	5.2E-13
PCE - ATE	3.7E-13	2.0E-16	0.312	1.1E-07	1.0E-10	0.001	1.5E-05
ATE - FCE	0.001	9.1E-05	0.594	0.089	0.842	6.7E-06	7.1E-06
ATE - UV	1.0E-13	1.0E-14	0.023	2.8E-06	0.056	2.0E-16	1.8E-04
FCE - UV	7.6E-07	2.6E-06	0.007	0.001	0.039	1.5E-15	0.897
UV - DSA	0.424	0.548	1.1E-04	0.958	0.334	0.925	0.897
HPC - AMP	2.0E-16						
HPC - CIP	2.0E-16						
HPC - DOX	2.0E-16						
HPC - SULF	2.0E-16						
AMP - CIP	2.0E-16						
AMP - DOX	2.0E-16						
AMP - SULF	2.0E-16						
CIP - DOX	0.829						
CIP - SULF	0.652						
DOX - SULF	0.814						

ALL, total heterotrophic (no antibiotic amendment) and antibiotic resistant; HPC, total heterotrophic growth; AMP, ampicillin; CIP, ciprofloxacin; DOX, doxycycline; SUL, sulfamethoxazole  
 UPA, upstream; RES, residential sewage; HOS, hospital sewage; INF, sewage influent; PCI, primary clarification influent; PCE, primary clarification effluent; ATE, aeration tank effluent; FCE, final clarification effluent; UV, UV treated effluent; DSA, downstream



**Figure A3** | CFU/mL normalized counts of 16S rRNA gene relative abundance for all communities at each sampling location. Combined Mallard and Sugar Creek samples at each sampling location including control and antibiotic amended cultured communities. Relative abundance determined through 16S rRNA gene sequencing was multiplied by CFU/mL at each location to determine estimated taxonomic abundances. The inset shows the enlarged taxonomic abundances for the upstream and downstream locations. Families making up <1% of the total community at each site were excluded. UPA, upstream; RES, residential sewage; HOS, hospital sewage; INF, sewage influent; PCI, primary clarification influent; PCE, primary clarification effluent; ATE, aeration tank effluent; FCE, final clarification effluent; UV, UV treated effluent; DSA, downstream.



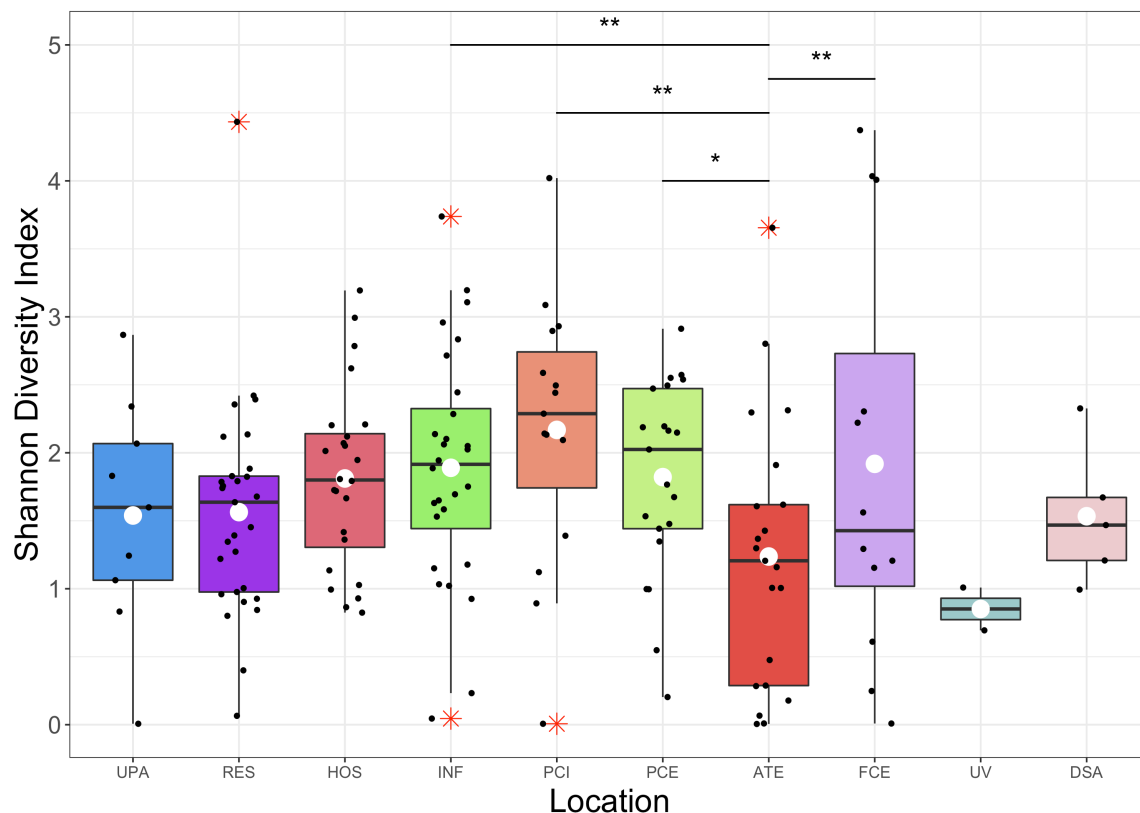
**Figure A4** | CFU/mL normalized counts of 16S rRNA gene relative abundance for total heterotrophic communities at each sampling location. Combined Mallard and Sugar Creek samples at each sampling location including total heterotrophic cultured communities. Relative abundance determined through 16S rRNA gene sequencing was multiplied by CFU/mL at each location to determine estimated taxonomic abundances. The inset shows the enlarged taxonomic abundances for the upstream and downstream locations. Families making up <1% of the total community at each site were excluded. UPA, upstream; RES, residential sewage; HOS, hospital sewage; INF, sewage influent; PCI, primary clarification influent; PCE, primary clarification effluent; ATE, aeration tank effluent; FCE, final clarification effluent; UV, UV treated effluent; DSA, downstream.

**Table A2** | Differential comparison  $p$  values for Shannon diversity between each WWTP site, incubation temperature and media, stage of treatment, and antibiotic utilized for combined ARB, the total heterotrophic growth (HPC), and each individual antibiotic treatments.

Terms	ALL	ARB	HPC	AMP	CIP	DOX	SULF
Mallard - Sugar	0.786	0.330	0.046	0.053	0.110	0.198	0.720
BT - RT	0.583	0.898	0.067	0.765	0.057	0.587	0.397
LB - R2A	2.2e-04	0.002	0.001	1.9e-04	0.205	0.347	0.360
UPA - DSA	0.707	0.885	0.936	0.962	0.896	-	-
RES - HOS	0.437	0.270	0.734	0.535	0.029	0.565	0.482
RES - INF	0.373	0.144	0.226	0.095	0.101	0.937	0.688
HOS - INF	0.926	0.764	0.314	0.295	0.508	0.516	0.321
INF - ATE	0.118	0.003	0.004	0.009	0.216	0.606	0.022
PCI - PCE	0.174	0.367	0.209	0.421	0.904	0.090	0.079
PCI - ATE	0.007	0.002	0.756	0.138	0.942	0.055	0.003
PCE - ATE	0.125	0.016	0.197	0.004	0.861	0.921	0.121
ATE - FCE	0.234	0.005	0.003	0.116	0.468	0.008	-
ATE - UV	0.183	0.648	0.005	0.935	-	-	-
FCE - UV	0.047	0.074	0.897	0.303	-	-	-
UV - DSA	0.019	0.302	0.005	0.414	-	-	-
NEG - AMP	6.1e-11						
NEG - CIP	1.4e-14						
NEG - DOX	7.0e-09						
NEG - SULF	0.001						
AMP - CIP	0.024						
AMP - DOX	0.873						
AMP - SULF	0.029						
CIP - DOX	0.022						
CIP - SULF	6.9e-05						
DOX - SULF	0.047						

ALL, total heterotrophic (no antibiotic amendment) and antibiotic resistant; HPC, total heterotrophic growth; AMP, ampicillin; CIP, ciprofloxacin; DOX, doxycycline; SUL, sulfamethoxazole

UPA, upstream; RES, residential sewage; HOS, hospital sewage; INF, sewage influent; PCI, primary clarification influent; PCE, primary clarification effluent; ATE, aeration tank effluent; FCE, final clarification effluent; UV, UV treated effluent; DSA, downstream



**Figure A5** | Average OTU level Shannon diversity of combined antibiotic resistant communities for all sampling locations. Mallard and Sugar Creek samples were combined by sampling location. Significant differences are indicated with bars between the locations with statistically differential diversity values. “\*” indicates a  $p$  value of 0.01 – 0.05; “\*\*” indicates a  $p$  value of 0.001 – 0.01; “\*\*\*” indicates a  $p$  value <0.001. Outliers are indicated with red asterisks and the statistical mean is represented by a white circle. UPA, upstream; RES, residential sewage; HOS, hospital sewage; INF, sewage influent; PCI, primary clarification influent; PCE, primary clarification effluent; ATE, aeration tank effluent; FCE, final clarification effluent; UV, UV treated effluent; DSA, downstream.

**Table A3** | Relative abundance and significant *p* values of the taxonomic families recovered from the Mallard and Sugar Creek WWTPs.

Phylum	Class	Family	Mallard	Sugar	<i>p</i> value
Actinobacteria	<i>Actinobacteria</i>	<i>Microbacteriaceae</i>	0.01%	0.07%	n.s.
		<i>Propionibacteriaceae</i>	0.02%	0.09%	n.s.
Bacteroidetes	<i>Bacteroidia</i>	<i>Porphyromonadaceae</i>	0.01%	<0.01%	n.s.
	<i>Cytophagia</i>	<i>Cytophagaceae</i>	<0.01%	0.27%	n.s.
	<i>Flavobacteriia</i>	<i>Flavobacteriaceae</i>	0.84%	3.74%	n.s.
	<i>Sphingobacteriia</i>	<i>Sphingobacteriaceae</i>	0.01%	<0.01%	n.s.
Firmicutes	<i>Bacilli</i>	<i>Bacillaceae</i>	5.87%	0.08%	n.s.
		<i>Paenibacillaceae</i>	1.56%	<0.01%	n.s.
		<i>Planococcaceae</i>	0.35%	<0.01%	n.s.
		<i>Enterococcaceae</i>	0.07%	0.01%	n.s.
Proteobacteria	<i>Alphaproteobacteria</i>	<i>Canlobacteraceae</i>	0.25%	0.16%	n.s.
		<i>Bradyrhizobiaceae</i>	0.89%	0.03%	n.s.
		<i>Brucellaceae</i>	0.02%	0.03%	n.s.
		<i>Rhodospirillaceae</i>	0.08%	<0.01%	n.s.
		<i>Sphingomonadaceae</i>	0.18%	0.06%	n.s.
	<i>Betaproteobacteria</i>	<i>Alcaligenaceae</i>	0.27%	<0.01%	0.0001
		<i>Burkholderiaceae</i>	1.26%	0.14%	n.s.
		<i>Comamonadaceae</i>	14.72%	11.54%	n.s.
		<i>Oxalobacteraceae</i>	4.66%	1.02%	n.s.
		<i>Neisseriaceae</i>	0.03%	1.20%	0.020
		<i>Rhodocyclaceae</i>	0.01%	<0.01%	n.s.
	<i>Epsilonproteobacteria</i>	<i>Campylobacteraceae</i>	0.25%	1.27%	n.s.
	<i>Gammaproteobacteria</i>	<i>Aeromonadaceae</i>	4.82%	6.63%	0.037
		<i>Shewanellaceae</i>	<0.01%	7.03%	n.s.
		<i>Chromatiaceae</i>	<0.01%	0.71%	n.s.
		<i>Enterobacteriaceae</i>	9.19%	9.06%	n.s.
		<i>Halomonadaceae</i>	0.04%	0.34%	n.s.
		<i>Pasteurellaceae</i>	1.21%	1.01%	n.s.
		<i>Moraxellaceae</i>	2.24%	1.89%	n.s.
		<i>Pseudomonadaceae</i>	40.46%	40.86%	n.s.
		<i>Xanthomonadaceae</i>	0.48%	0.03%	4.94E-05
Other	Other	Other	10.23%	12.73%	n.s.

n.s. – not significant



**Table A4** | Average colony counts (normalized to CFU/mL) for each sampling location and individual antibiotic amendment.

Location	HPC	AMP	CIP	DOX	SUL	Location Avg	Location Avg (ARB only)
Upstream	$5.4 \times 10^3$	569	67	27	15	$1.2 \times 10^3$	169
Residential sewage	$7.3 \times 10^5$	$4.0 \times 10^5$	$1.7 \times 10^4$	$1.3 \times 10^3$	$6.7 \times 10^4$	$2.4 \times 10^5$	$1.2 \times 10^5$
Hospital sewage	$3.1 \times 10^6$	$5.9 \times 10^5$	$2.4 \times 10^4$	$1.1 \times 10^3$	$1.8 \times 10^5$	$7.8 \times 10^5$	$2.0 \times 10^5$
Influent	$1.1 \times 10^6$	$3.7 \times 10^5$	$4.3 \times 10^4$	$1.6 \times 10^3$	$6.1 \times 10^4$	$3.2 \times 10^5$	$1.2 \times 10^5$
Primary Clarifier Influent	$8.1 \times 10^5$	$4.1 \times 10^5$	$6.3 \times 10^3$	$1.2 \times 10^3$	$6.2 \times 10^4$	$2.6 \times 10^5$	$1.2 \times 10^5$
Primary Clarifier Effluent	$1.0 \times 10^6$	$2.4 \times 10^5$	$1.9 \times 10^4$	$2.1 \times 10^3$	$5.7 \times 10^4$	$2.7 \times 10^5$	$8.0 \times 10^4$
Aeration Tank Effluent	$3.6 \times 10^5$	$2.4 \times 10^4$	$1.5 \times 10^3$	885	$2.3 \times 10^3$	$7.7 \times 10^4$	$7.1 \times 10^3$
Final Clarifier Effluent	$5.2 \times 10^5$	$1.2 \times 10^5$	$4.5 \times 10^3$	735	0	$1.3 \times 10^5$	$3.1 \times 10^4$
UV treated effluent	$1.3 \times 10^5$	$1.3 \times 10^3$	0	0	0	$2.7 \times 10^4$	333
Downstream	$3.8 \times 10^3$	276	21	4	0	819	74
Antibiotic Avg	$8.1 \times 10^5$	$2.2 \times 10^5$	$1.2 \times 10^4$	929	$4.4 \times 10^4$		

HPC, total heterotrophic growth; AMP, ampicillin; CIP, ciprofloxacin; DOX, doxycycline; SUL, sulfamethoxazole; ARB, combined antibiotic resistant bacteria

**Table A5** | Average Shannon diversity for all sampling locations within the wastewater treatment process and individual antibiotic treatments.

Location	HPC	AMP	CIP	DOX	SUL	Location Avg	Location Avg (ARB only)
UPA	3.29	1.73	1.42	0.01	2.34	2.30	1.54
RES	2.83	1.36	1.11	1.64	2.20	1.75	1.56
HOS	2.57	1.74	1.81	1.96	1.78	2.01	1.81
INF	2.34	2.13	1.59	1.60	2.38	1.98	1.89
PCI	3.22	2.23	1.20	2.39	2.88	2.34	2.17
PCE	2.84	2.38	1.35	1.29	1.99	2.03	1.82
ATE	3.22	1.14	1.38	1.36	1.09	1.78	1.24
FCE	2.17	1.79	0.72	2.64	-	1.94	1.92
UV	1.99	0.85	-	-	-	1.54	0.85
DSA	3.22	1.61	1.21	-	-	2.57	1.53
Antibiotic Avg	2.83	1.74	1.37	1.74	2.09		

HPC, total heterotrophic growth; AMP, ampicillin; CIP, ciprofloxacin; DOX, doxycycline; SUL, sulfamethoxazole; ARB, combined antibiotic resistant bacteria

UPA, upstream; RES, residential sewage; HOS, hospital sewage; INF, sewage influent; PCI, primary clarification influent; PCE, primary clarification effluent; ATE, aeration tank effluent; FCE, final clarification effluent; UV, UV treated effluent; DSA, downstream

**Table A6** | Relative abundance of the taxonomic families recovered from all sampling locations within the wastewater treatment process.

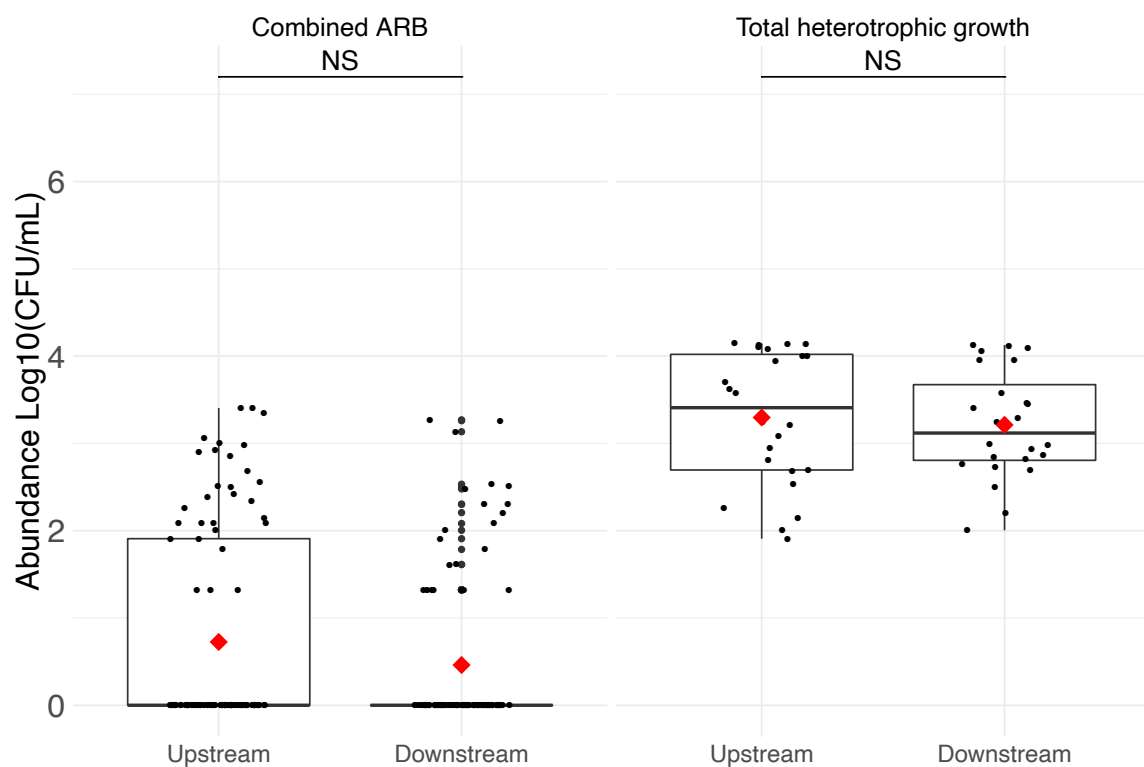
Phylum	Class	Family	UPA	RES	HOS	INF	PCI	PCE	ATE	FCE	UV	DSA
Actinobacteria	<i>Actinobacteria</i>	<i>Microbacteriaceae</i>	0.05%	0.01%	<0.01%	0%	<0.01%	<0.01%	<0.01%	1.09%	<0.01%	0.06%
Bacteroidetes	<i>Cytophagia</i>	<i>Cytophagaceae</i>	0%	0%	0%	0%	0%	<0.01%	0%	3.84%	0%	<0.01%
	<i>Flavobacteriia</i>	<i>Flavobacteriaceae</i>	<0.01%	2.82%	3.45%	1.32%	0.09%	0.17%	9.54%	2.82%	<0.01%	10.17%
	<i>Sphingobacteriia</i>	<i>Sphingobacteriaceae</i>	1.57%	<0.01%	<0.01%	0%	0%	<0.01%	<0.01%	<0.01%	0%	2.41%
Firmicutes	<i>Bacilli</i>	<i>Bacillaceae</i>	2.26%	4.07%	0.78%	4.14%	4.58%	3.66%	1.72%	0.22%	<0.01%	0.30%
		<i>Paenibacillaceae</i>	0.14%	0.54%	0.04%	<0.01%	<0.01%	2.83%	3.23%	0.74%	0%	0.01%
Proteobacteria	<i>Alphaproteobacteria</i>	<i>Caenobacteraceae</i>	0.88%	0.03%	0.02%	0.09%	0.42%	0.17%	0.51%	2.24%	<0.01%	0.75%
		<i>Bradyrhizobiaceae</i>	0.01%	0.09%	0.01%	0.06%	0.05%	<0.01%	0.18%	7.80%	0%	0.07%
		<i>Sphingomonadaceae</i>	13.67%	<0.01%	0.03%	0.04%	<0.01%	<0.01%	1.41%	0.25%	0%	16.20%
	<i>Betaproteobacteria</i>	<i>Alcaligenaceae</i>	0.27%	<0.01%	0.11%	<0.01%	0.76%	0.08%	0.13%	0.03%	0%	0.29%
		<i>Burkholderiaceae</i>	8.08%	0.18%	0.07%	0.29%	4.70%	1.13%	0.87%	0.21%	<0.01%	3.91%
		<i>Comamonadaceae</i>	21.26%	2.23%	11.31%	6.11%	15.69%	25.72%	12.85%	41.51%	3.20%	17.29%
		<i>Oxalobacteraceae</i>	0.81%	0.63%	0.76%	3.18%	3.06%	9.42%	0.11%	1.70%	0.11%	0.41%
		<i>Neisseriaceae</i>	0.13%	0.15%	<0.01%	0.10%	<0.01%	0.01%	13.03%	0.02%	26.12%	1.72%
	<i>Epsilonproteobacteria</i>	<i>Campylobacteraceae</i>	<0.01%	3.59%	0.75%	0.41%	0.05%	0.42%	0.15%	<0.01%	<0.01%	<0.01%
	<i>Gammaproteobacteria</i>	<i>Aeromonadaceae</i>	5.05%	6.68%	5.27%	5.58%	2.98%	8.78%	7.43%	6.01%	0.68%	6.58%
		<i>Shewanellaceae</i>	0%	2.70%	9.11%	0.13%	0%	0.45%	<0.01%	<0.01%	0.01%	0%
		<i>Chromatiaceae</i>	0%	0%	0.87%	0%	0%	0%	1.27%	0%	0.56%	1.82%
		<i>Enterobacteriaceae</i>	1.80%	10.73%	4.52%	19.02%	20.98%	4.53%	13.50%	2.38%	0.04%	4.78%
		<i>Halomonadaceae</i>	0%	1.03%	0.20%	<0.01%	0.02%	0.01%	0.01%	0.23%	0.16%	0.01%
		<i>Pasteurellaceae</i>	0.29%	1.64%	0.27%	2.11%	1.97%	0.70%	4.45%	0.04%	0.06%	0.54%
		<i>Moraxellaceae</i>	0.18%	2.41%	0.73%	3.48%	1.04%	1.76%	7.30%	1.50%	27.31%	3.52%
		<i>Pseudomonadaceae</i>	34.95%	35.22%	52.81%	43.06%	25.55%	25.99%	8.36%	25.52%	38.02%	20.88%
		<i>Xanthomonadaceae</i>	0.60%	0.21%	0.07%	0.06%	0.19%	0.51%	1.34%	0.15%	0%	0.36%
Other	Other	Other	7.99%	25.01%	8.83%	10.79%	17.89%	13.64%	12.61%	1.69%	3.74%	7.91%

UPA, upstream; RES, residential sewage; HOS, hospital sewage; INF, sewage influent; PCI, primary clarification influent; PCE, primary clarification effluent; ATE, aeration tank effluent; FCE, final clarification effluent; UV, UV treated effluent; DSA, downstream

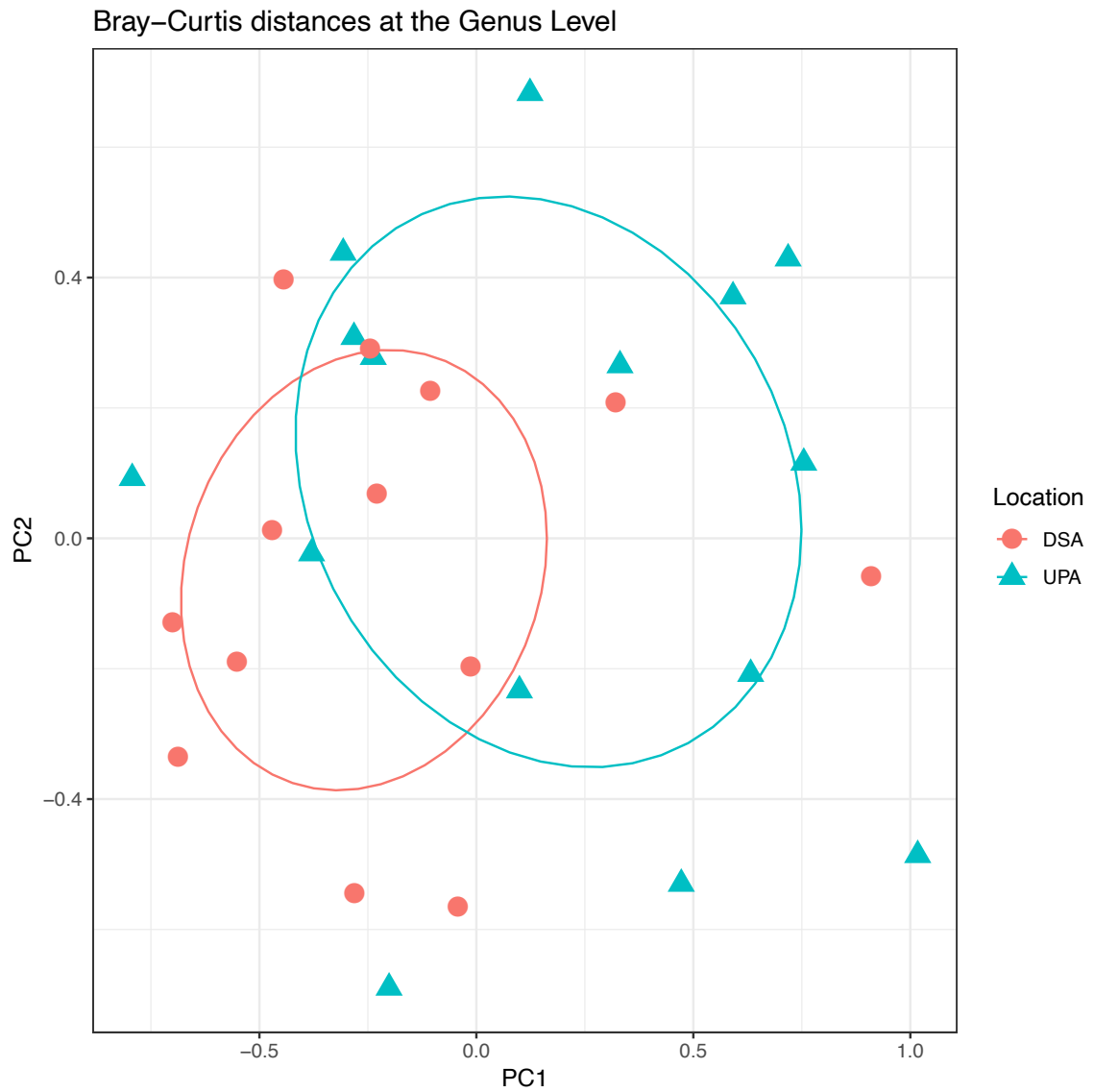
**Table A7** | Relative abundance of the antibiotic resistant taxonomic families recovered from all sampling locations within the wastewater treatment process.

Phylum	Class	Family	UPA	RES	HOS	INF	PCI	PCE	ATE	FCE	UV	DSA	
Actinobacteria	Actinobacteria	Mycobacteriaceae	0%	0%	0%	<0.01%	<0.01%	0%	0.07%	<0.01%	0%	0%	
		Microbacteriaceae	0.43%	0.03%	<0.01%	0%	0.01%	0%	<0.01%	3.27%	0%	0.84%	
		Micrococcaceae	0%	<0.01%	0%	0%	0%	0%	0%	0.01%	0%	0%	
		Propionibacteriaceae	<0.01%	0.01%	0.01%	<0.01%	0.16%	0.07%	0.01%	0.08%	<0.01%	0.01%	
Bacteroidetes	Bacteroidia	Bacteroidaceae	0%	0%	0.01%	0%	0%	0%	0%	0%	0%	0%	
		Porphyromonadaceae	0%	0.01%	0.02%	0%	0%	0%	0.44%	0%	0%	0%	
		Cytophagia	Cytophagaceae	0%	0%	0%	0%	0%	<0.01%	0%	<0.01%	0%	0%
	Flavobacteriia	Flavobacteriaceae	<0.01%	1.18%	0.42%	1.45%	0.17%	0.62%	0.01%	<0.01%	0%	<0.01%	
		Sphingobacteriia	Chitinophagaceae	5.05%	<0.01%	<0.01%	0%	0%	0%	<0.01%	<0.01%	0%	0%
		Sphingobacteriaceae	4.80%	<0.01%	<0.01%	0%	0%	0%	<0.01%	<0.01%	0%	0%	
	Firmicutes	Bacilli	Bacillaceae	0%	<0.01%	<0.01%	0.02%	0.01%	<0.01%	<0.01%	<0.01%	0%	0%
Family XII			0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	
Paenibacillaceae			0%	0%	0%	<0.01%	<0.01%	<0.01%	<0.01%	<0.01%	0%	<0.01%	
Planococcaceae			0%	<0.01%	<0.01%	<0.01%	<0.01%	<0.01%	<0.01%	<0.01%	0%	0%	
Aerococcaceae			0%	0%	<0.01%	0%	<0.01%	0%	0%	0.02%	0%	0%	
Enterococcaceae			0.07%	0.10%	0.06%	0.40%	0.04%	0.01%	0.42%	<0.01%	0%	0%	
Streptococcaceae			0.14%	<0.01%	<0.01%	0%	<0.01%	<0.01%	<0.01%	0.05%	0%	0%	
Negativicutes			Veillonellaceae	0%	<0.01%	<0.01%	0%	0%	<0.01%	<0.01%	0.01%	0%	0%
Proteobacteria		Alphaproteobacteria	Caulobacteraceae	0.07%	0.07%	0.10%	0.30%	1.30%	0.64%	4.46%	0.95%	0%	0.61%
			Bradyrhizobiaceae	0.01%	0.20%	0.06%	0.22%	0.14%	0.01%	2.45%	0.04%	0%	<0.01%
			Brucellaceae	1.21%	0.05%	0.01%	<0.01%	0.01%	0.09%	1.46%	<0.01%	0%	0%
			Acetobacteraceae	0%	0%	0.01%	0%	<0.01%	<0.01%	0%	0%	0%	0%
			Rhodospirillaceae	0%	0%	<0.01%	0%	<0.01%	<0.01%	<0.01%	0%	0%	0%
			Sphingomonadaceae	0%	<0.01%	0.10%	0.15%	<0.01%	<0.01%	<0.01%	0.02%	0%	1.23%
			Betaproteobacteria	Alcaligenaceae	0%	<0.01%	<0.01%	<0.01%	<0.01%	0.28%	1.50%	0.08%	0%
		Burkholderiaceae	10.03%	0.17%	0.25%	0.97%	1.11%	3.54%	5.41%	0.38%	<0.01%	1.14%	
		Comamonadaceae	14.20%	2.69%	7.50%	8.81%	10.89%	18.76%	34.87%	52.09%	87.48%	21.62%	
		Oxalobacteraceae	1.79%	0.01%	0.02%	5.83%	0.42%	0.14%	1.32%	0.78%	0%	0.13%	
		Neisseriaceae	0.74%	0.07%	0.01%	0.33%	0.01%	0.02%	<0.01%	0.07%	0%	0.14%	
		Rhodocyclaceae	0%	<0.01%	0.03%	0%	<0.01%	0.03%	<0.01%	<0.01%	0%	0.23%	
	Epsilonproteobacteria	Campylobacteraceae	<0.01%	0.64%	0.28%	0.29%	0.12%	1.03%	0.06%	<0.01%	0%	<0.01%	
	Gammaproteobacteria	Aeromonadaceae	0.58%	2.75%	9.28%	1.17%	5.31%	4.74%	3.55%	0.75%	6.34%	<0.01%	
		Shewanellaceae	0%	0.01%	0.01%	0.22%	0%	<0.01%	0%	<0.01%	0%	0%	
		Chromatiaceae	0%	0%	0%	0%	0%	0%	<0.01%	0%	0%	0%	
		Enterobacteriaceae	5.89%	12.30%	4.53%	12.18%	12.71%	11.12%	6.93%	0.35%	<0.01%	<0.01%	
Halomonadaceae		0.01%	0.11%	0.05%	0.01%	0.04%	0.01%	0.01%	0.60%	<0.01%	0.02%		
Pasteurellaceae		0.85%	0.54%	0.69%	0.60%	2.56%	1.68%	1.62%	0.10%	0.01%	<0.01%		
Moraxellaceae		<0.01%	0.18%	0.07%	3.27%	0.01%	<0.01%	0.01%	0.06%	0%	<0.01%		
Pseudomonadaceae		34.74%	73.91%	73.47%	52.20%	56.10%	47.22%	15.12%	39.13%	0.01%	63.13%		
	Xanthomonadaceae	4.06%	0.46%	0.42%	0.18%	0.52%	1.66%	0.46%	0.08%	0%	<0.01%		
Other	Other	Other	15.35%	4.51%	2.58%	11.38%	8.35%	8.33%	19.81%	1.09%	6.15%	10.89%	

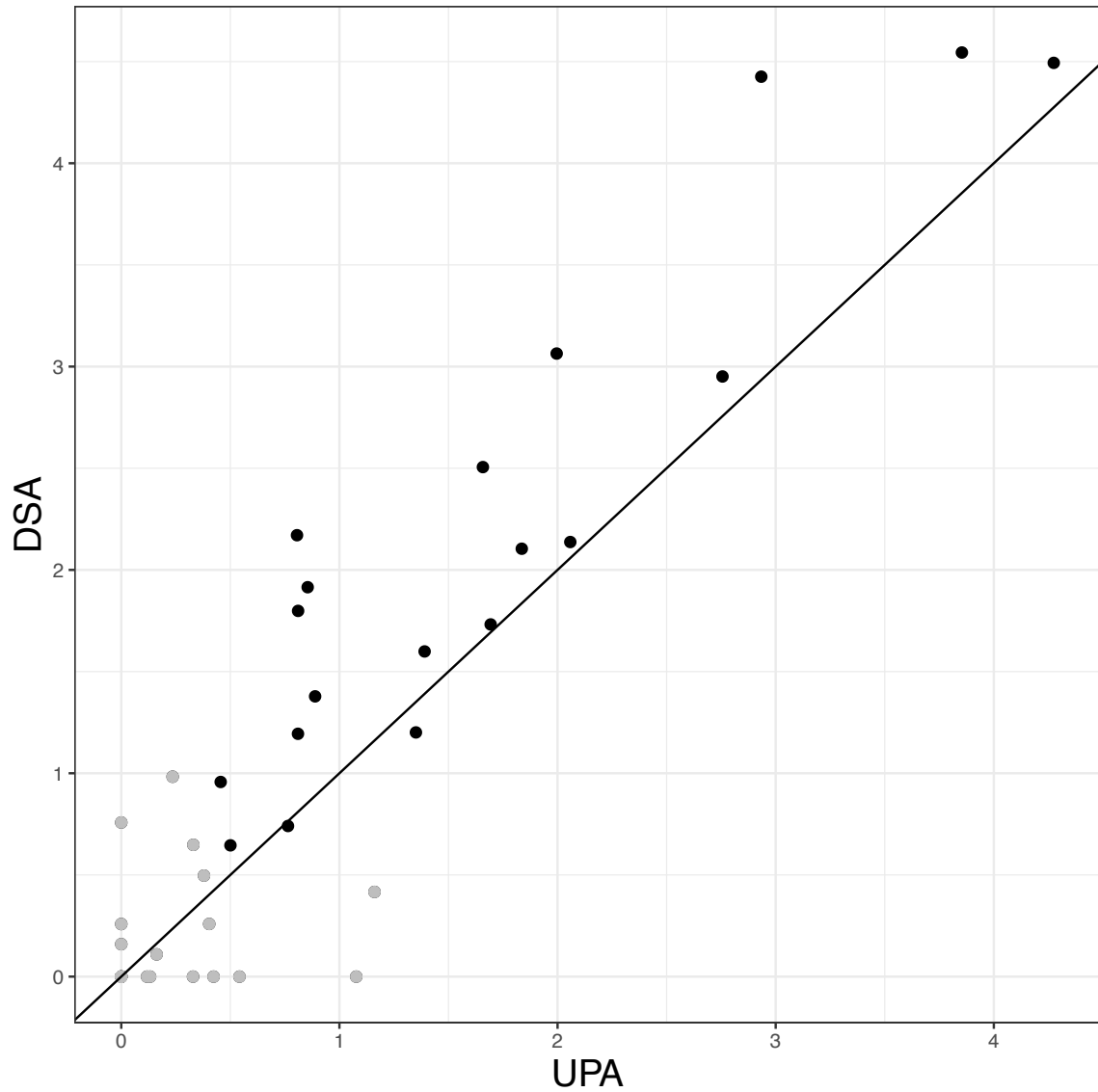
UPA, upstream; RES, residential sewage; HOS, hospital sewage; INF, sewage influent; PCI, primary clarification influent; PCE, primary clarification effluent; ATE, aeration tank effluent; FCE, final clarification effluent; UV, UV treated effluent; DSA, downstream



**Figure A6** | Significant differences in bacterial enumeration for combined ARB and total heterotrophic growth between upstream and downstream waters. ♦ represents the statistical mean. “NS” indicates a  $p$  value  $>0.05$ .



**Figure A7** | No significant differences in beta diversities of the stream communities at the genus level from 16S rRNA gene sequencing ( $p=0.079$ ). PCoA ordination for upstream (UPA) and downstream (DSA) communities with PC1 and PC2 components. Data colored by the two sampling locations.



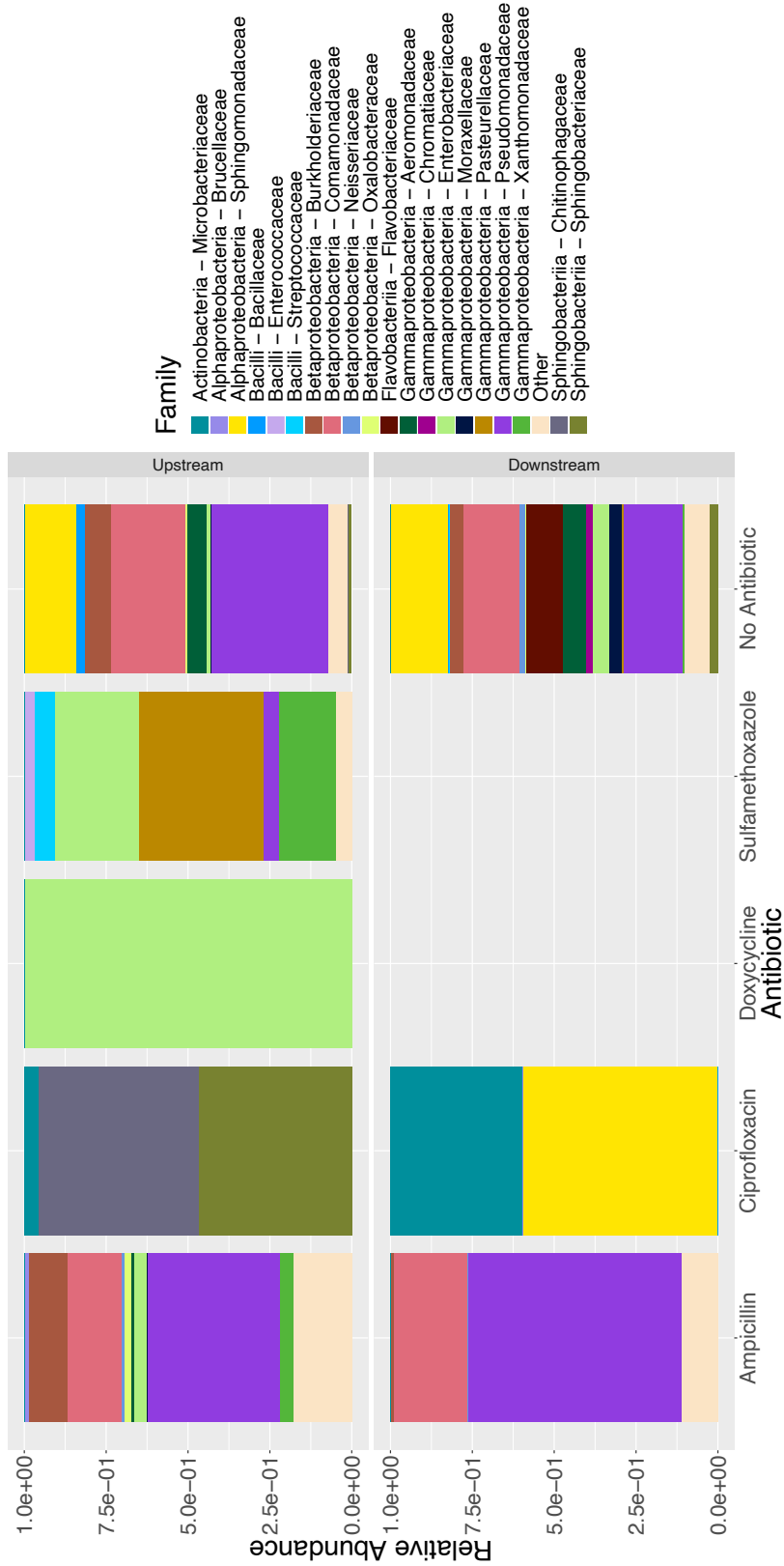
**Figure A8** | The mean relative abundance of each family from upstream and downstream catchment waters. No non-rare taxa were found to significantly differ between upstream (UPA) and downstream (DSA) waters using linear regression models at a 5% FDR threshold. Black symbols represent non-rare taxa found in 3% of all samples. Grey symbols represent rare taxa. The black line is the identity line (Upstream=Downstream).

**Table A8** | Average colony counts (normalized to CFU/mL) and relative abundance of taxonomic families in individual antibiotic treatments among upstream and downstream sampling locations.

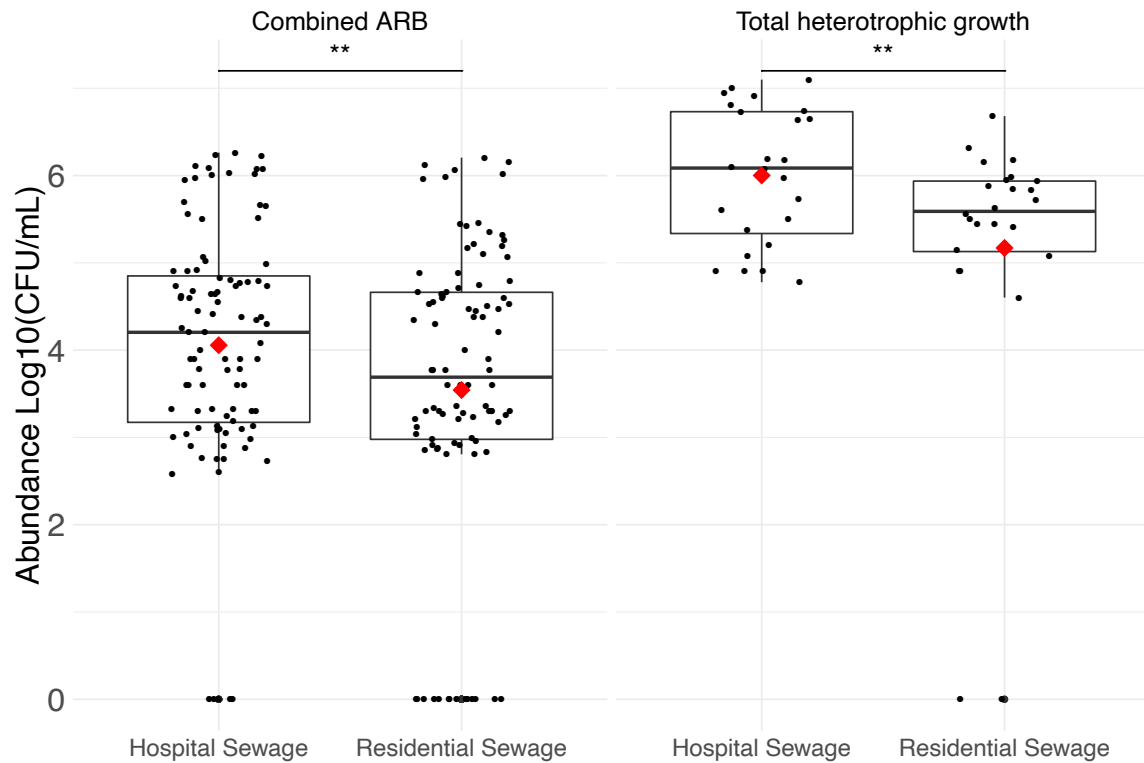
Phylum	Class	Family	Upstream					Downstream				
			HPC	AMP	CIP	DOX	SULF	HPC	AMP	CIP	DOX	SULF
Actinobacteria	Actinobacteria	Microbacteriaceae	0 (0%)	1 (<0.01%)	33 (4.16%)	0 (0%)	0 (0%)	0 (0%)	1 (<0.01%)	57 (40.36%)	-	-
		Flavobacteriaceae	1 (<0.01%)	1 (<0.01%)	0 (0%)	0 (0%)	0 (0%)	1243 (10.92%)	1 (<0.01%)	0 (0%)	-	-
		Chitinophagaceae	40 (0.22%)	1 (<0.01%)	388 (49.08%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	-	-
Bacteroidetes	Sphingobacteriia	Sphingobacteriaceae	209 (1.12%)	1 (<0.01%)	369 (46.66%)	1 (<0.01%)	0 (0%)	295 (2.59%)	0 (0%)	0 (0%)	-	-
		Bacillaceae	480 (2.57%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	37 (0.33%)	0 (0%)	0 (0%)	-	-
		Enterovacciae	1 (<0.01%)	0 (0%)	0 (0%)	0 (0%)	10 (2.97%)	3 (0.02%)	0 (0%)	0 (0%)	-	-
Firmicutes	Bacilli	Streptococcaceae	0 (0%)	1 (<0.01%)	0 (0%)	0 (0%)	21 (6.28%)	0 (0%)	0 (0%)	0 (0%)	-	-
		Caulobacteraceae	185 (0.99%)	2 (0.08%)	0 (0%)	0 (0%)	0 (0%)	87 (0.76%)	10 (0.62%)	1 (<0.01%)	-	-
		Brucellaceae	14 (0.07%)	37 (1.41%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	-	-
Proteobacteria	Alphaproteobacteria	Sphingomonadaceae	2901 (15.54%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1970 (17.31%)	1 (<0.01%)	83 (59.44%)	-	-
		Burkholderiaceae	1458 (7.81%)	308 (11.70%)	1 (<0.01%)	1 (0.01%)	0 (0%)	468 (4.11%)	19 (1.17%)	1 (0.01%)	-	-
		Comamonadaceae	4149 (22.23%)	436 (16.57%)	1 (0.04%)	1 (<0.01%)	1 (<0.01%)	1931 (16.96%)	364 (22.08%)	1 (0.05%)	-	-
Betaproteobacteria	Betaproteobacteria	Oxalobacteriaceae	125 (0.67%)	55 (2.09%)	0 (0%)	0 (0%)	0 (0%)	49 (0.43%)	2 (0.14%)	0 (0%)	-	-
		Neisseriaceae	8 (0.04%)	23 (0.86%)	0 (0%)	1 (<0.01%)	0 (0%)	209 (1.84%)	2 (0.14%)	0 (0%)	-	-
		Aeromonadaceae	1056 (5.66%)	18 (0.68%)	0 (0%)	0 (0%)	0 (0%)	805 (7.07%)	1 (<0.01%)	1 (<0.01%)	-	-
Gammaproteobacteria	Gammaproteobacteria	Chromatiaceae	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	223 (1.96%)	0 (0%)	0 (0%)	-	-
		Enterobacteriaceae	232 (1.24%)	107 (4.08%)	1 (0.01%)	280 (99.97%)	87 (25.66%)	585 (5.14%)	1 (<0.01%)	1 (0.03%)	-	-
		Pasteurellaceae	40 (0.22%)	1 (<0.01%)	1 (<0.01%)	1 (0.01%)	130 (38.23%)	66 (0.58%)	1 (<0.01%)	1 (0.01%)	-	-
Other	Other	Moraxellaceae	39 (0.21%)	1 (<0.01%)	0 (0%)	0 (0%)	0 (0%)	431 (3.78%)	1 (<0.01%)	1 (0.01%)	-	-
		Pseudomonadaceae	6528 (34.98%)	1064 (40.42%)	1 (<0.01%)	0 (0%)	16 (4.72%)	2021 (17.76%)	1064 (64.46%)	1 (0.03%)	-	-
		Xanthomonadaceae	23 (0.12%)	113 (4.29%)	1 (<0.01%)	0 (0%)	59 (17.28%)	44 (0.39%)	0 (0%)	1 (0.01%)	-	-
Other	Other	Other	1056 (6.30%)	468 (17.80%)	1 (0.04%)	1 (0.01%)	17 (4.87%)	868 (8.05%)	183 (11.38%)	1 (0.06%)	-	-

HPC, total heterotrophic growth; AMP, ampicillin; CIP, ciprofloxacin; DOX, doxycycline; SUL, sulfamethoxazole

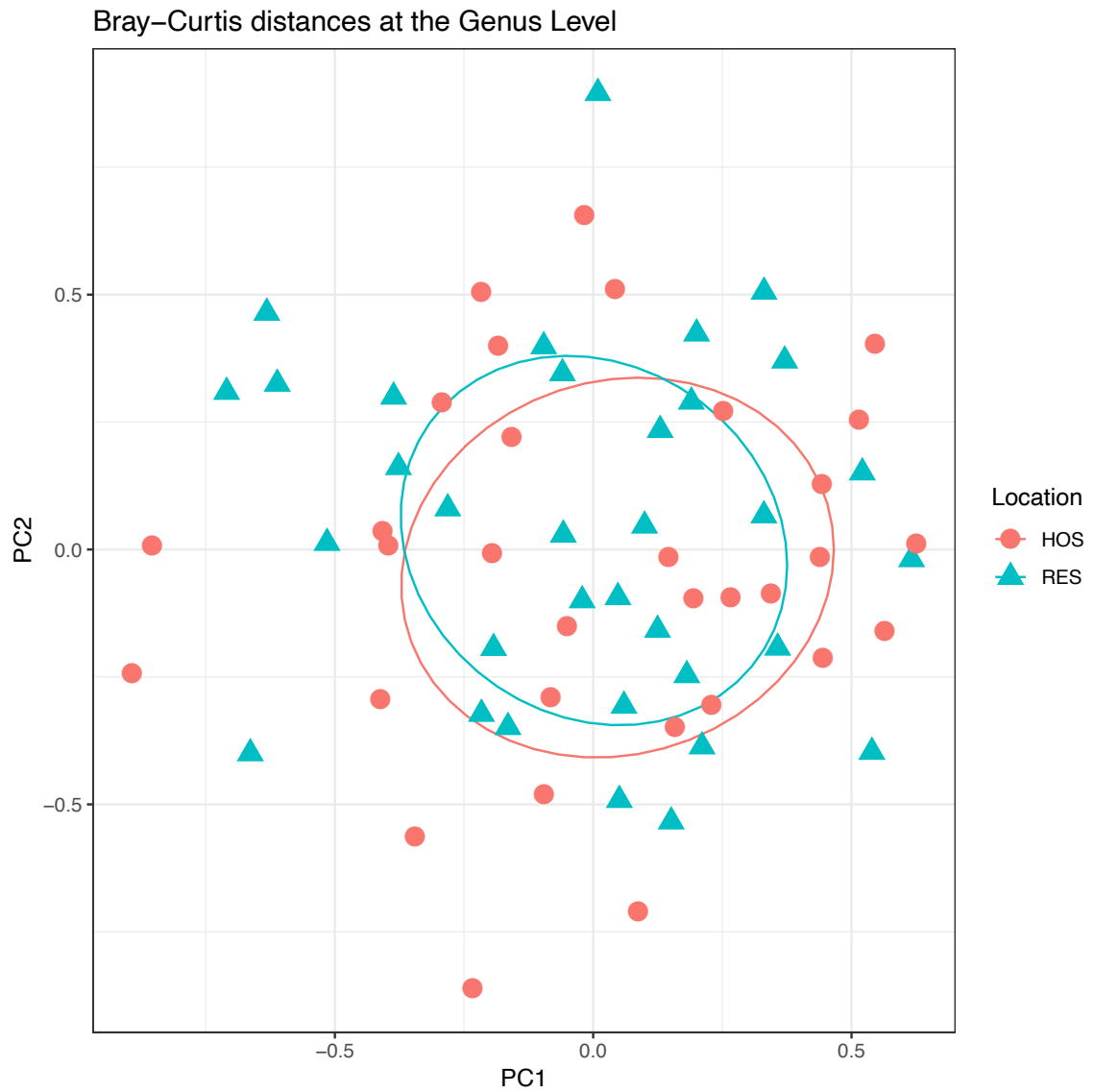




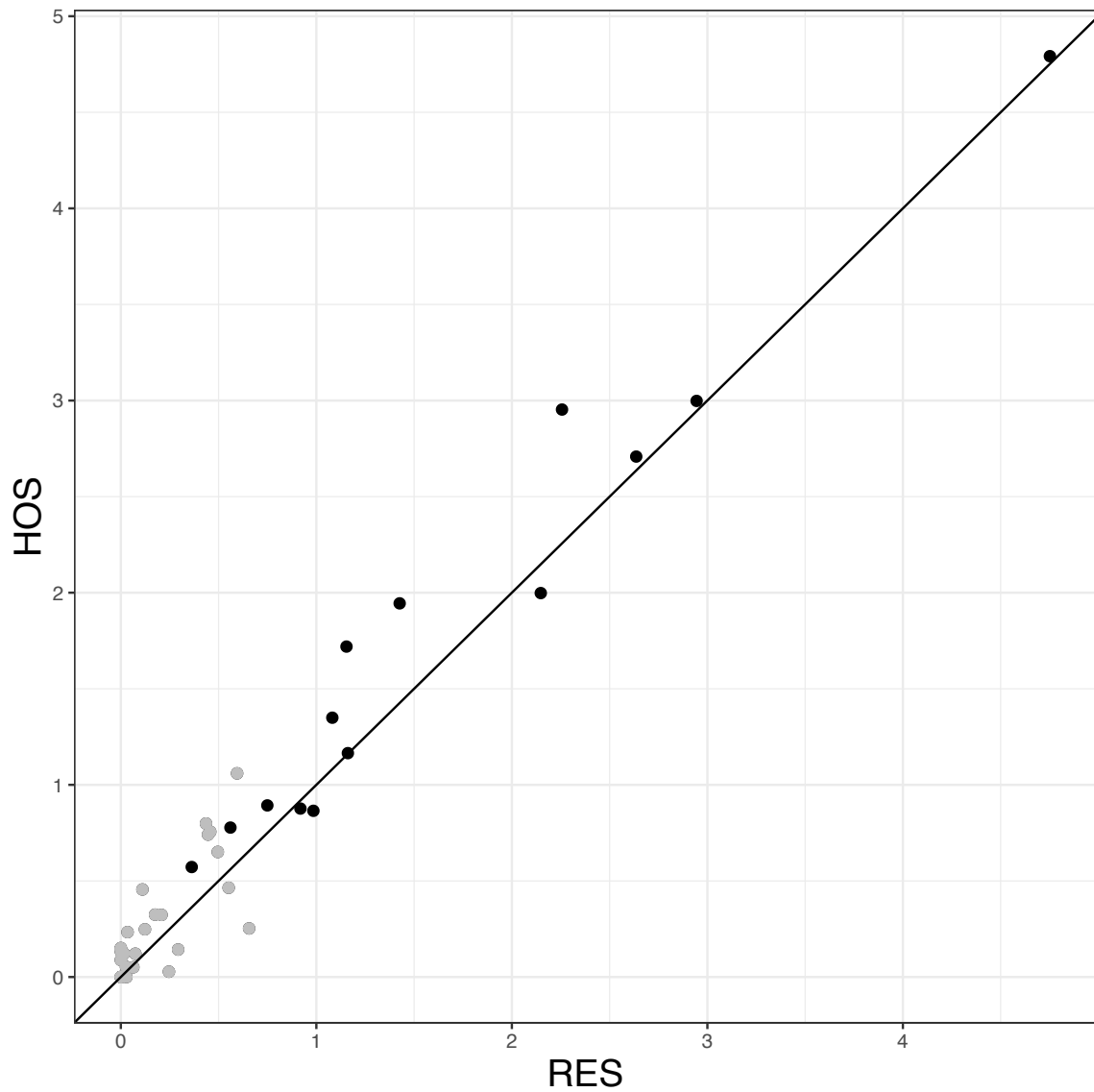
**Figure A9** | Relative abundance for total heterotrophic and antibiotic resistant communities from upstream and downstream sampling locations.



**Figure A10** | Significant differences in bacterial enumeration for combined ARB and total heterotrophic growth between hospital and residential sewage. ♦ represents the statistical mean. “\*” indicates a *p* value of 0.01 – 0.05; “\*\*” indicates a *p* value of 0.001 – 0.01; “\*\*\*” indicates a *p* value < 0.001.



**Figure A11** | No significant differences in beta diversities of the sewage communities at the genus level from 16S rRNA gene sequencing ( $p=0.731$ ). PCoA ordination for residential (RES) and hospital (HOS) sewage communities with PC1 and PC2 components. Data colored by the two sampling locations.

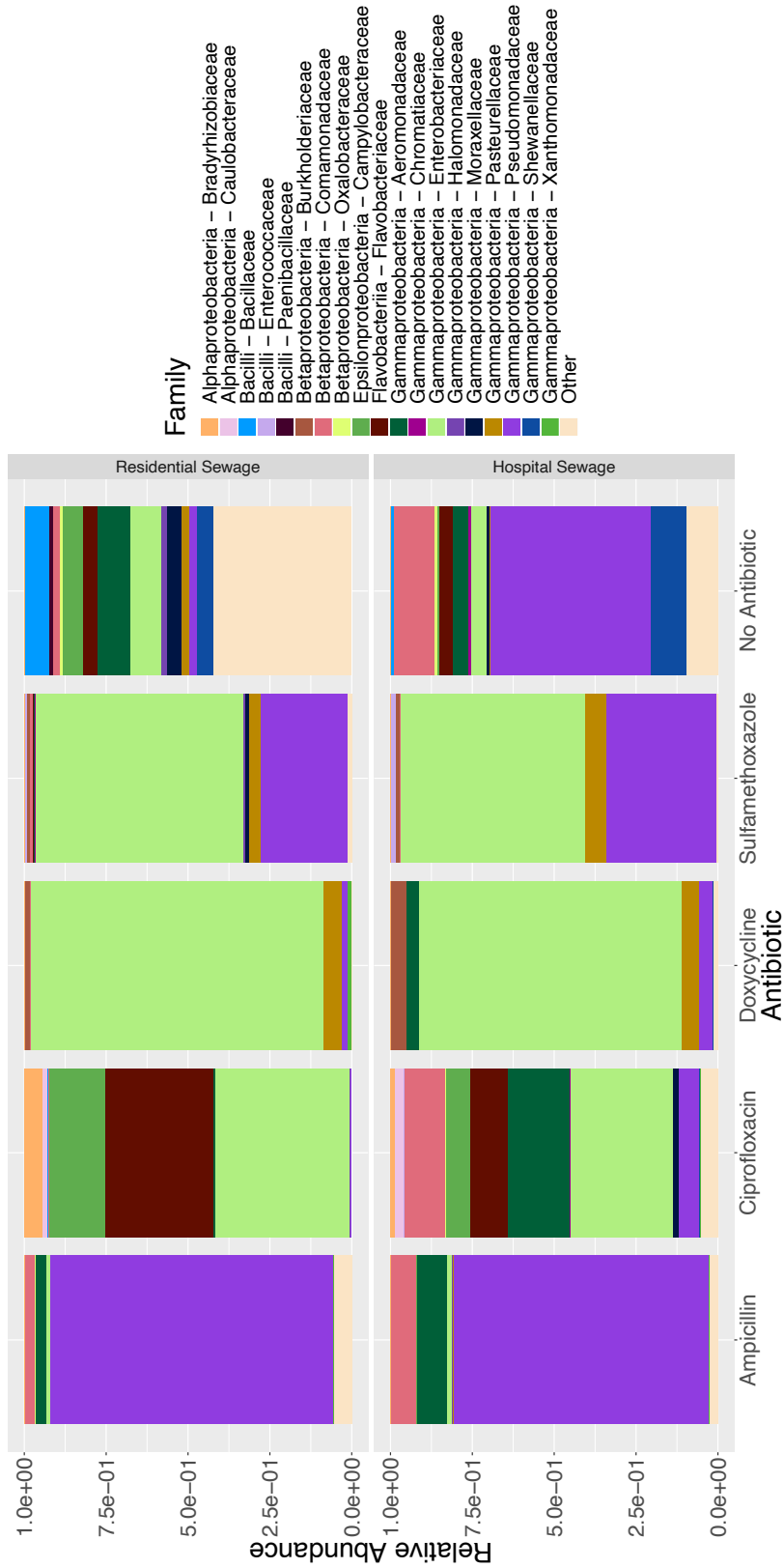


**Figure A12** | The mean relative abundance of each family from raw residential and hospital sewage lines. No non-rare taxa were found to significantly differ between residential (RES) and hospital (HOS) sewage using linear regression models at a 5% FDR threshold. Black symbols represent non-rare taxa found in 3% of all samples. Grey symbols represent rare taxa. The black line is the identity line (Residential=Hospital).

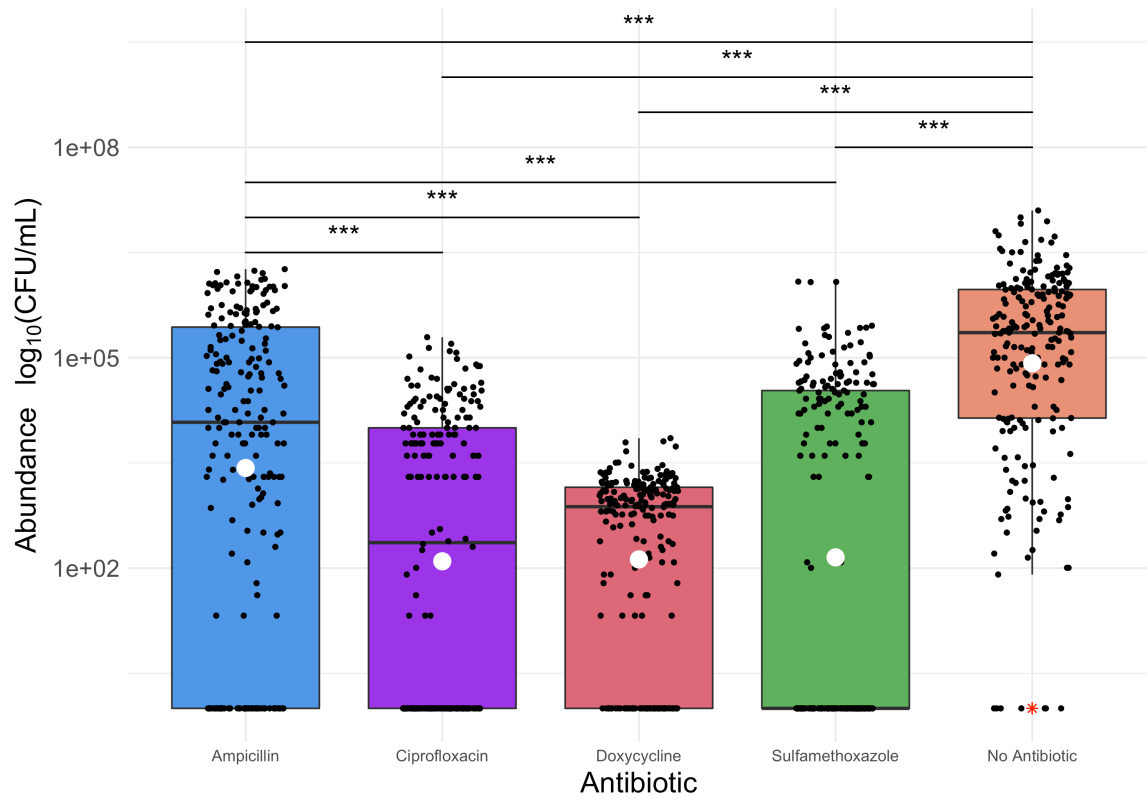
**Table A9** | Average colony counts (normalized to CFU/mL) and relative abundance of taxonomic families in individual antibiotic treatments among residential and hospital sewage.

Phylum	Class	Family	Residential Sewage				Hospital Sewage					
			HPC	AMP	CIP	DOX	SUL	HPC	AMP	CIP	DOX	SUL
Bacteroidetes	Flavobacteriia	Flavobacteriaceae	1.1E+05 (4.23%)	0 (0%)	1.6E+04 (32.69%)	0 (0%)	2 (<0.01%)	3.8E+05 (4.07%)	0 (0%)	9.0E+03 (11.39%)	0 (0%)	3 (<0.01%)
Firmicutes	Bacilli	Bacillaceae	2.0E+05 (7.55%)	9 (<0.01%)	0 (0%)	0 (0%)	2 (<0.01%)	8.7E+04 (0.93%)	6 (<0.01%)	0 (0%)	0 (0%)	0 (0%)
		Proteobacteria	2.6E+04 (1.01%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4.3E+03 (0.05%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
		Enterococcaceae	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1.6E+03 (0.68%)	62 (<0.01%)	0 (0%)	290 (0.37%)	0 (0%)	1.8E+03 (1.50%)
		Caulobacteraceae	24 (<0.01%)	273 (0.02%)	763 (1.52%)	0 (0%)	3 (<0.01%)	646 (0.01%)	109 (0.01%)	2.0E+03 (2.56%)	0 (0%)	0 (0%)
		Bradyrhizobiaceae	24 (<0.01%)	0 (0%)	2.7E+03 (5.43%)	8 (0.21%)	9 (<0.01%)	368 (<0.01%)	243 (0.01%)	1.0E+03 (1.27%)	0 (0%)	1 (<0.01%)
		Beaproteobacteria	5.1E+03 (0.19%)	41 (<0.01%)	1 (<0.01%)	62 (1.52%)	2.5E+03 (1.07%)	2.6E+03 (0.03%)	4.4E+03 (0.22%)	2 (<0.01%)	152 (4.76%)	1.6E+03 (1.39%)
		Comamonadaceae	4.8E+04 (1.84%)	4.1E+04 (3.18%)	219 (0.44%)	0 (0%)	1.6E+03 (0.66%)	1.1E+06 (12.08%)	1.5E+05 (7.57%)	9.7E+03 (12.28%)	0 (0%)	9 (0.01%)
		Oxalobacteraceae	3.0E+04 (1.16%)	10 (<0.01%)	0 (0%)	0 (0%)	162 (0.07%)	8.4E+04 (0.90%)	468 (0.02%)	0 (0%)	0 (0%)	0 (0%)
		Epsilonproteobacteria	1.6E+05 (6.11%)	0 (0%)	8.5E+03 (16.93%)	0 (0%)	454 (0.19%)	7.9E+04 (0.85%)	106 (0.01%)	5.9E+03 (7.48%)	0 (0%)	4 (<0.01%)
		Gammaproteobacteria	2.6E+05 (10.05%)	4.2E+04 (3.26%)	272 (0.54%)	0 (0%)	1.4E+03 (0.59%)	4.1E+05 (4.45%)	1.9E+05 (9.22%)	1.5E+04 (18.74%)	125 (3.90%)	0 (0%)
		Shewanellaceae	1.3E+05 (5.01%)	0 (0%)	0 (0%)	0 (0%)	91 (0.04%)	1.0E+06 (10.97%)	0 (0%)	302 (0.38%)	0 (0%)	0 (0%)
		Chromatiaceae	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	9.7E+04 (1.05%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
		Enterobacteriaceae	2.4E+05 (9.40%)	1.7E+04 (1.35%)	2.0E+04 (40.69%)	3.6E+03 (89.48%)	1.5E+05 (63.38%)	4.2E+05 (4.52%)	3.4E+04 (1.67%)	2.5E+04 (30.96%)	2.6E+03 (80.06%)	6.6E+04 (56.41%)
		Halomonadaceae	4.7E+04 (1.82%)	85 (0.01%)	1 (<0.01%)	0 (0%)	1.6E+03 (0.68%)	2.2E+04 (0.23%)	1.1E+03 (0.05%)	5 (0.01%)	0 (0%)	5 (<0.01%)
		Pasteurellaceae	6.7E+04 (2.59%)	9 (<0.01%)	0 (0%)	238 (5.85%)	8.3E+03 (3.49%)	1.7E+04 (0.18%)	1.0E+04 (0.52%)	0 (0%)	171 (5.34%)	7.4E+03 (6.36%)
		Monasellaceae	1.1E+05 (4.32%)	16 (<0.01%)	0 (0%)	0 (0%)	2.8E+03 (1.18%)	8.0E+04 (0.86%)	43 (<0.01%)	1.5E+03 (1.86%)	0 (0%)	0 (0%)
		Pseudomonadaceae	5.5E+04 (2.11%)	1.1E+06 (86.15%)	174 (0.35%)	60 (1.48%)	6.3E+04 (26.62%)	4.5E+06 (48.59%)	1.6E+06 (77.53%)	4.9E+03 (6.14%)	138 (4.31%)	3.9E+04 (33.71%)
		Xanthomonadaceae	0 (0%)	7.3E+03 (0.56%)	0 (0%)	55 (1.35%)	14 (0.01%)	349 (<0.01%)	9.1E+03 (0.45%)	0 (0%)	3 (0.09%)	2 (<0.01%)
Other	Other	Other	1.1E+06 (42.62%)	7.0E+04 (5.47%)	707 (1.41%)	4 (0.09%)	3.2E+03 (1.36%)	9.5E+05 (10.21%)	5.5E+04 (2.71%)	5.2E+03 (6.56%)	49 (1.52%)	703 (6.06%)

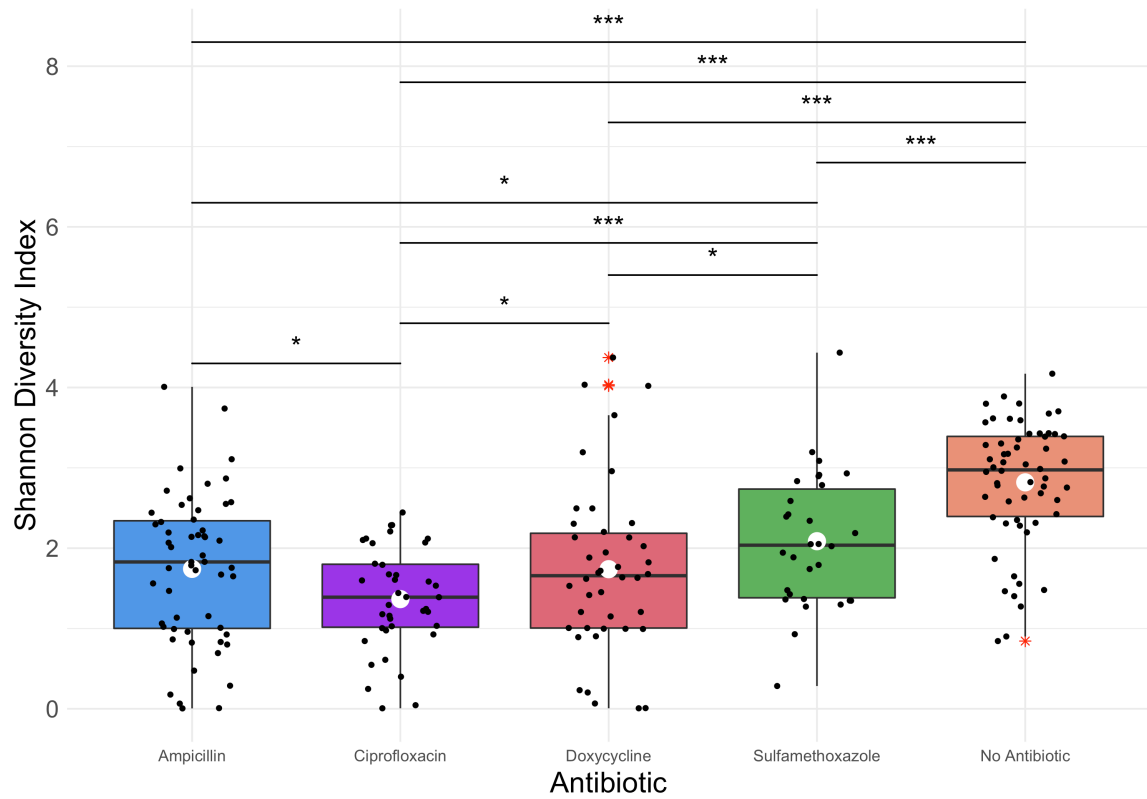
HPC, total heterotrophic growth; AMP, ampicillin; CIP, ciprofloxacin; DOX, doxycycline; SUL, sulfamethoxazole



**Figure A13** | Relative abundance for total heterotrophic and antibiotic resistant communities from residential and hospital sewage sampling locations.



**Figure A14** | Average colony counts for each cultured antibiotic treatment. Significant differences are indicated with bars between the locations with statistically differential abundances. The statistical means are represented with white circles. “\*” indicates a  $p$  value of 0.01 – 0.05; “\*\*” indicates a  $p$  value of 0.001 – 0.01; “\*\*\*” indicates a  $p$  value < 0.001.



**Figure A15** | Average OTU level Shannon diversity for each cultured antibiotic treatment. Significant differences are indicated with bars between the locations with statistically differential diversity values. “\*” indicates a  $p$  value of 0.01 – 0.05; “\*\*” indicates a  $p$  value of 0.001 – 0.01; “\*\*\*” indicates a  $p$  value <0.001. Outliers are indicated with red asterisks and statistical means are represented with white circles.



**Table A10** | Average colony counts (normalized to CFU/mL) and relative abundance of taxonomic families in individual antibiotic treatments.

Phylum	Class	Family	HPC	AMP	CIP	DOX	SUL	p value
Actinobacteria	<i>Actinobacteria</i>	<i>Microbacteriaceae</i>	4 (<0.01%)	1 (<0.01%)	2.6E+03 (3.92%)	0 (0%)	0 (0%)	n.s.
		<i>Propionibacteriaceae</i>	2.0E+03 (0.07%)	73 (0.01%)	131 (0.20%)	14 (0.35%)	243 (0.13%)	0.003
Bacteroidetes	<i>Flavobacteriia</i>	<i>Flavobacteriaceae</i>	8.7E+04 (3.19%)	3.0E+03 (0.37%)	6.4E+03 (9.58%)	25 (0.61%)	1 (<0.01%)	n.s.
Firmicutes	<i>Bacilli</i>	<i>Bacillaceae</i>	8.7E+04 (3.17%)	40 (<0.01%)	2 (<0.01%)	0 (0%)	9 (<0.01%)	3.03E-21
Proteobacteria	<i>Alphaproteobacteria</i>	<i>Caulobacteraceae</i>	3.4E+03 (0.12%)	3.3E+03 (0.41%)	878 (1.32%)	0 (0%)	8 (<0.01%)	0.003
		<i>Bradyrhizobiaceae</i>	1.2E+04 (0.44%)	35 (<0.01%)	1.9E+03 (2.90%)	49 (1.18%)	26 (0.01%)	1.05E-05
	<i>Betaproteobacteria</i>	<i>Alcaligenaceae</i>	3.4E+03 (0.12%)	50 (0.01%)	807 (1.22%)	0 (0%)	2 (<0.01%)	4.03E-05
		<i>Burkholderiaceae</i>	1.2E+04 (0.45%)	8.4E+03 (1.03%)	334 (0.50%)	76 (1.84%)	1.6E+03 (0.89%)	4.03E-05
		<i>Comamonadaceae</i>	3.6E+05 (13.23%)	1.1E+05 (13.28%)	3.8E+03 (5.66%)	33 (0.80%)	651 (0.36%)	7.62E-31
		<i>Oxalobacteraceae</i>	7.7E+04 (2.83%)	1.3E+04 (1.62%)	6 (0.01%)	16 (0.39%)	41 (0.02%)	8.94E-13
		<i>Neisseriaceae</i>	2.6E+04 (0.95%)	863 (0.11%)	13 (0.02%)	43 (1.03%)	10 (0.01%)	0.006
	<i>Epsilonproteobacteria</i>	<i>Campylobacteraceae</i>	2.8E+04 (1.01%)	147 (0.02%)	5.5E+03 (8.33%)	40 (0.98%)	111 (0.06%)	7.87E-12
	<i>Gammaproteobacteria</i>	<i>Aeromonadaceae</i>	1.7E+05 (6.33%)	4.2E+04 (5.19%)	3.3E+03 (5.04%)	31 (0.75%)	476 (0.26%)	6.13E-15
		<i>Shewanellaceae</i>	1.5E+05 (5.63%)	452 (0.06%)	54 (0.08%)	0 (0%)	21 (0.01%)	n.s.
		<i>Enterobacteriaceae</i>	2.5E+05 (9.09%)	1.9E+04 (2.37%)	1.6E+04 (24.24%)	3.1E+03 (75.71%)	1.0E+05 (55.39%)	1.94E-10
		<i>Pasteurellaceae</i>	3.1E+04 (1.15%)	2.2E+03 (0.27%)	218 (0.33%)	245 (5.94%)	1.1E+04 (6.19%)	1.53E-14
		<i>Moraxellaceae</i>	6.7E+04 (2.45%)	77 (0.01%)	9.6E+03 (14.49%)	100 (2.42%)	656 (0.36%)	2.87E-18
		<i>Pseudomonadaceae</i>	9.3E+05 (33.86%)	5.5E+05 (67.41%)	1.1E+04 (16.94%)	69 (1.66%)	6.1E+04 (33.48%)	7.31E-19
		<i>Xanthomonadaceae</i>	2.7E+03 (0.10%)	5.1E+03 (0.63%)	43 (0.07%)	100 (2.42%)	66 (0.04%)	3.00E-03
		<i>Halomonadaceae</i>	7.2E+03 (0.26%)	502 (0.06%)	8 (0.01%)	5 (0.13%)	406 (0.22%)	1.70E-07
Other	Other	Other	4.3E+05 (15.53%)	5.8E+04 (7.16%)	3.4E+03 (5.13%)	1.5E+02 (3.75%)	4.7E+03 (2.55%)	n.s.

HPC, total heterotrophic growth; AMP, ampicillin; CIP, ciprofloxacin; DOX, doxycycline; SUL, sulfamethoxazole

n.s. – not significant

**Table A11** | Average colony counts (normalized to CFU/mL) for incubation temperatures and culturing media for  
a) total heterotrophic growth

Temperature	LB	R2A	Temp Avg
BT	$2.1 \times 10^5$	$7.5 \times 10^5$	$4.8 \times 10^5$
RT	$8.2 \times 10^5$	$1.5 \times 10^6$	$1.2 \times 10^5$
Media Avg	$5.2 \times 10^5$	$1.1 \times 10^6$	

b) combined antibiotic resistant communities.

Temperature	LB	R2A	Temp Avg
BT	$1.1 \times 10^4$	$2.3 \times 10^4$	$1.7 \times 10^4$
RT	$1.0 \times 10^5$	$1.4 \times 10^5$	$1.2 \times 10^5$
Media Avg	$5.6 \times 10^4$	$8.2 \times 10^4$	

BT, body temperature (37°C); RT, room temperature (22°C); LB, lysogeny broth agar; R2A, Reasoner's 2 agar

**Table A12** | Average Shannon diversity for incubation temperatures and culturing media for

a) total heterotrophic growth

Temperature	LB	R2A	Media Avg
BT	2.48	2.87	2.70
RT	2.62	3.28	2.95
Temperature Avg	2.55	3.05	

b) combined antibiotic resistant communities.

Temperature	LB	R2A	Temperature Avg
BT	1.65	1.89	1.77
RT	1.49	1.91	1.68
Media Avg	1.55	1.90	

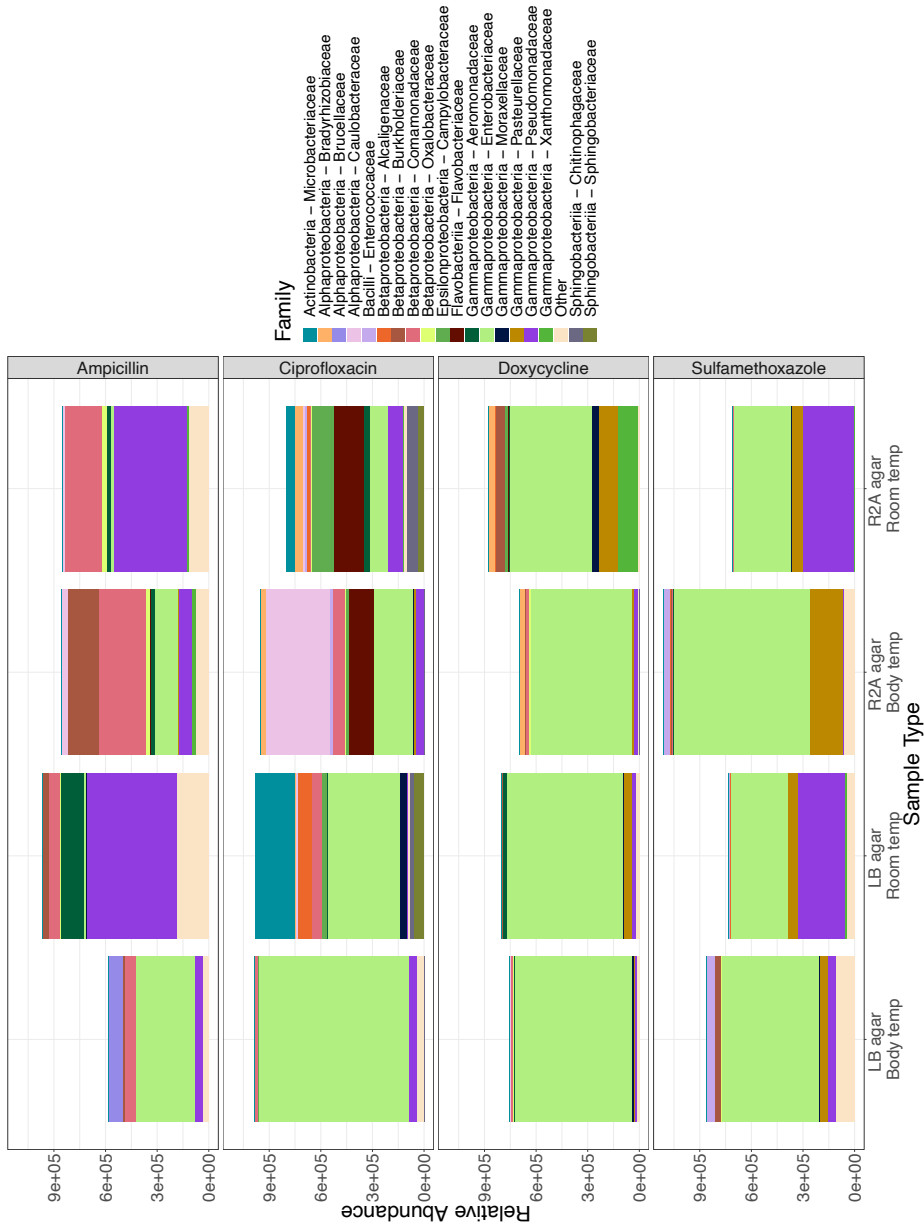
BT, body temperature (37°C); RT, room temperature (22°C); LB, lysogeny broth agar; R2A, Reasoner's 2 agar

**Table A13** | Differential abundance of taxa between incubation temperatures for total and combined antibiotic resistant communities.

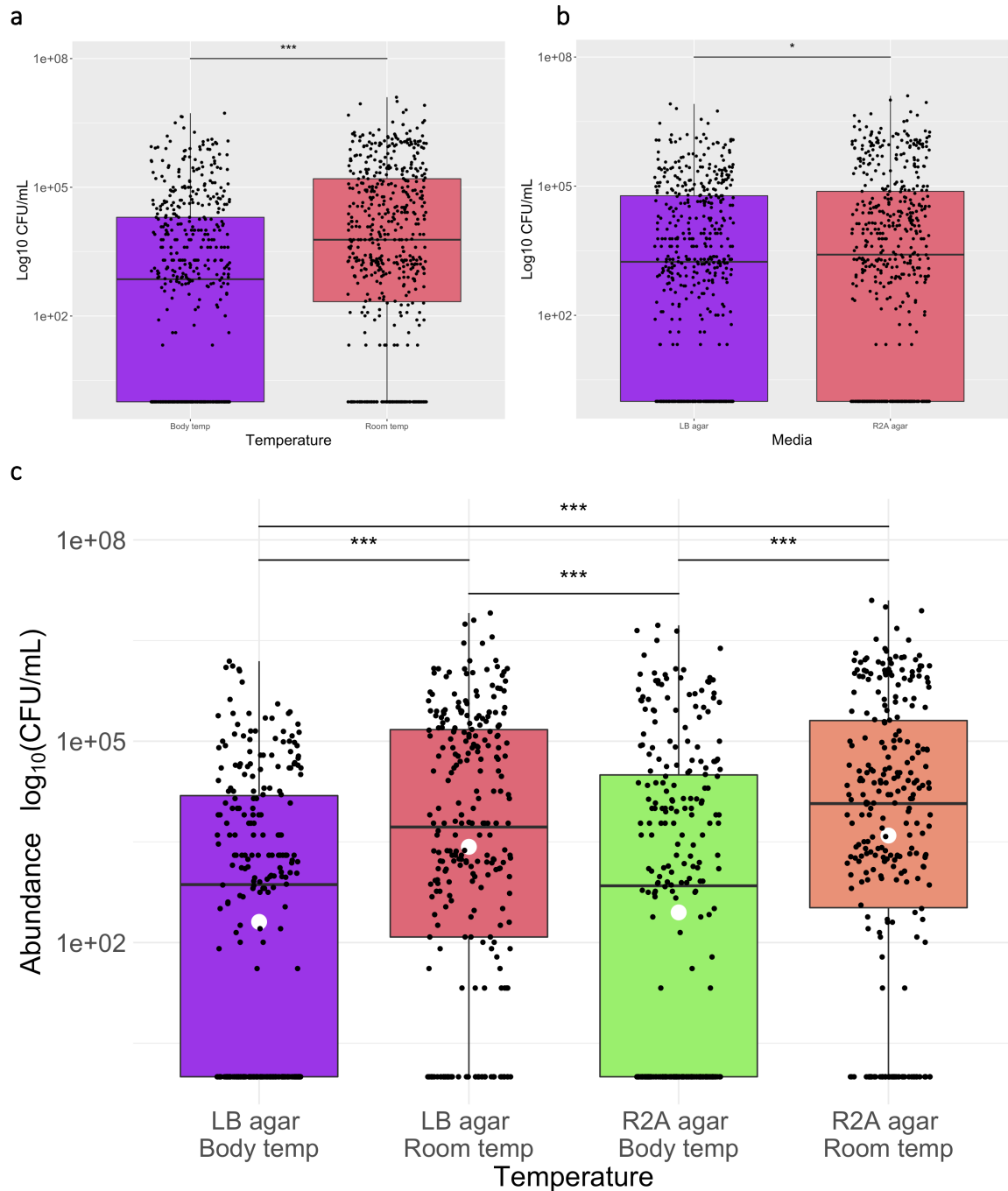
Phylum	Class	Family	Higher abundance	<i>p</i> value
Firmicutes	<i>Bacilli</i>	<i>Bacillaceae</i>	BT	0.014
Proteobacteria	<i>Epsilonproteobacteria</i>	<i>Campylobacteraceae</i>	RT	0.006
	<i>Gammaproteobacteria</i>	<i>Pseudomonadaceae</i>	RT	0.014

**Table A14** | Differential abundance of taxa between culture media for total and combined antibiotic resistant communities.

Phylum	Class	Family	Higher abundance	<i>p</i> value
Proteobacteria	<i>Alphaproteobacteria</i>	<i>Caulobacteraceae</i>	R2A	2.05E-07
		<i>Bradyrhizobiaceae</i>	R2A	4.04E-07
	<i>Betaproteobacteria</i>	<i>Burkholderiaceae</i>	R2A	0.010
		<i>Comamonadaceae</i>	R2A	3.83E-06
		<i>Oxalobacteraceae</i>	R2A	5.50E-07
	<i>Gammaproteobacteria</i>	<i>Enterobacteriaceae</i>	LB	0.010
		<i>Xanthomonadaceae</i>	R2A	0.003



**Figure A16** | CFU/mL normalized counts of 16S rRNA gene relative abundance for antibiotic-resistant communities at in each culturing condition. Relative abundance determined through 16S rRNA gene sequencing was multiplied by CFU/mL at each location to determine estimated taxonomic abundances. Families making up <1% of the total community at each site were excluded.

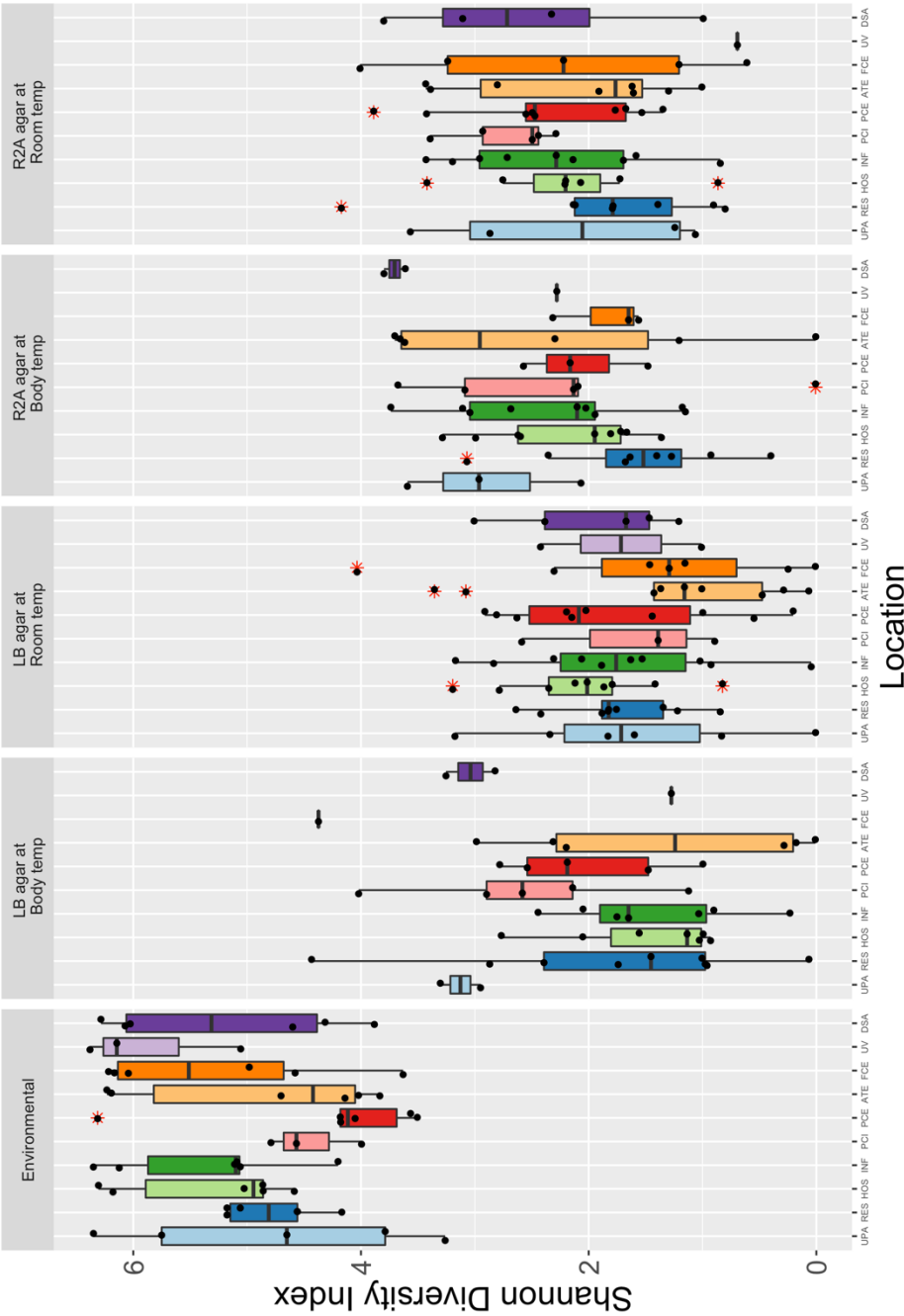


**Figure A17** | Abundance of microbial colonies grown under different culturing conditions. The average combined total heterotrophic and ARB microbial abundance when cultured using (a) two incubation temperatures and (b) two media types. Differences in individual culturing conditions can be seen in panel c. Significant differences are indicated with bars between the locations with statistically differential abundances. Statistical means are represented with white circles. “\*” indicates a  $p$  value of 0.01 – 0.05; “\*\*” indicates a  $p$  value of 0.001 – 0.01; “\*\*\*” indicates a  $p$  value < 0.001.

**Table A15** | Differential comparisons of Shannon diversity between the environmental source material and individual culturing conditions.

<b>Terms</b>	<b><i>p</i> value</b>
Environmental - BT & LB	2.00E-16
Environmental - BT & R2A	2.00E-16
Environmental - RT & LB	2.00E-16
Environmental - RT & R2A	2.00E-16
RT & LB - BT & LB	0.916
RT & R2A - RT & LB	9.85E-04
RT & LB - BT & R2A	0.030
BT & R2A - BT & LB	0.058
RT & R2A - BT & LB	0.005
RT & R2A - BT & R2A	0.373
Environmental – Culture (all)	2.0E-16
BT, body temperature (37°C); RT, room temperature (22°C); LB, lysogeny broth agar; R2A, Reasoner's 2 agar	





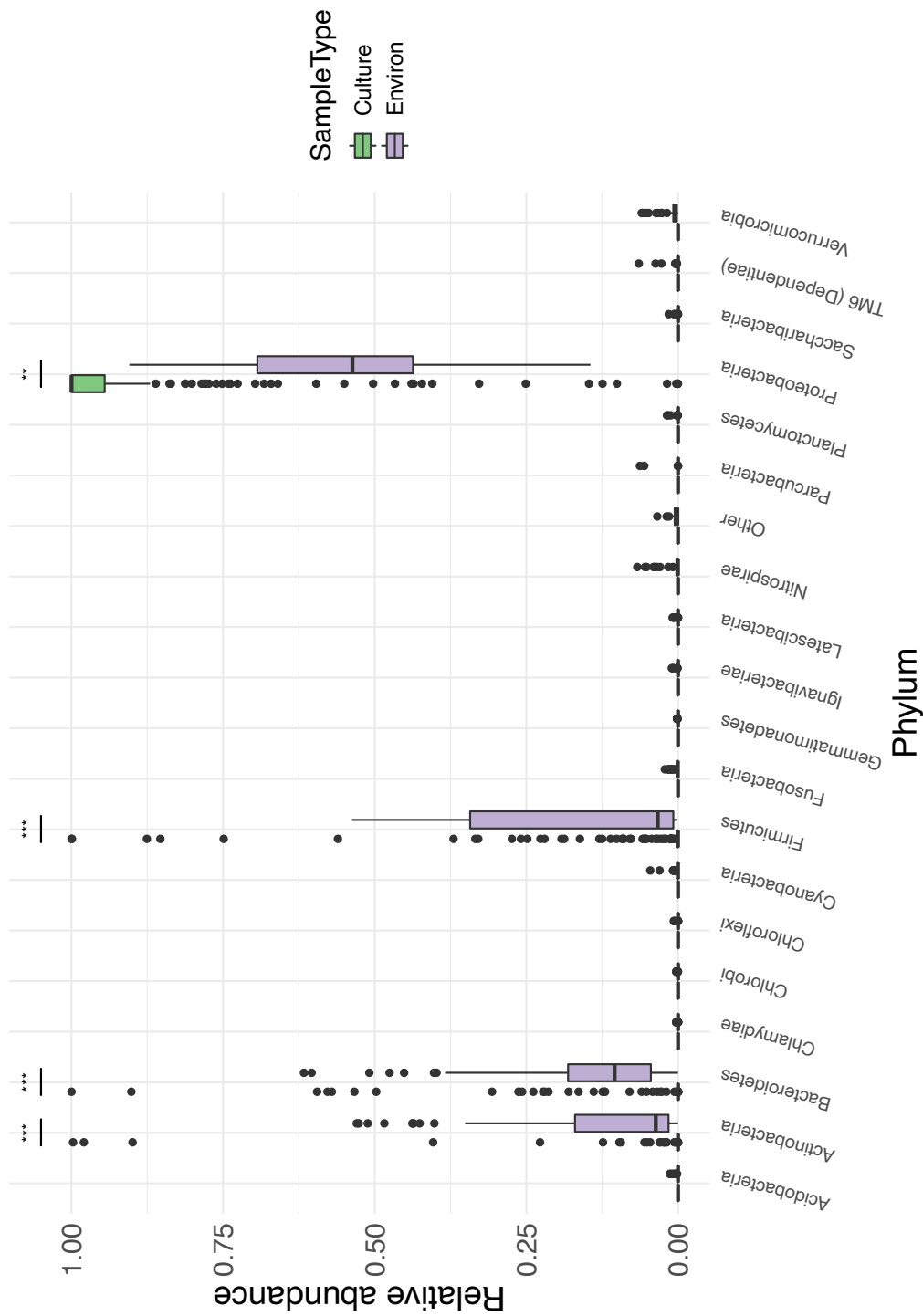
**Figure A18** | Average OTU level Shannon diversity for each culturing condition and environmental source material at each location in the treatment process. Outliers are indicated with red asterisks. UPA, upstream; RES, residential sewage; HOS, hospital sewage; INF, sewage influent; PCI, primary clarification influent; PCE, primary clarification effluent; ATE, aeration tank effluent; FCE, final clarification effluent; UV, UV treated effluent; DSA, downstream.

**Table A16** | Relative abundance of the taxonomic families recovered from environmental culture-independent and individual culture-dependent sampling conditions.

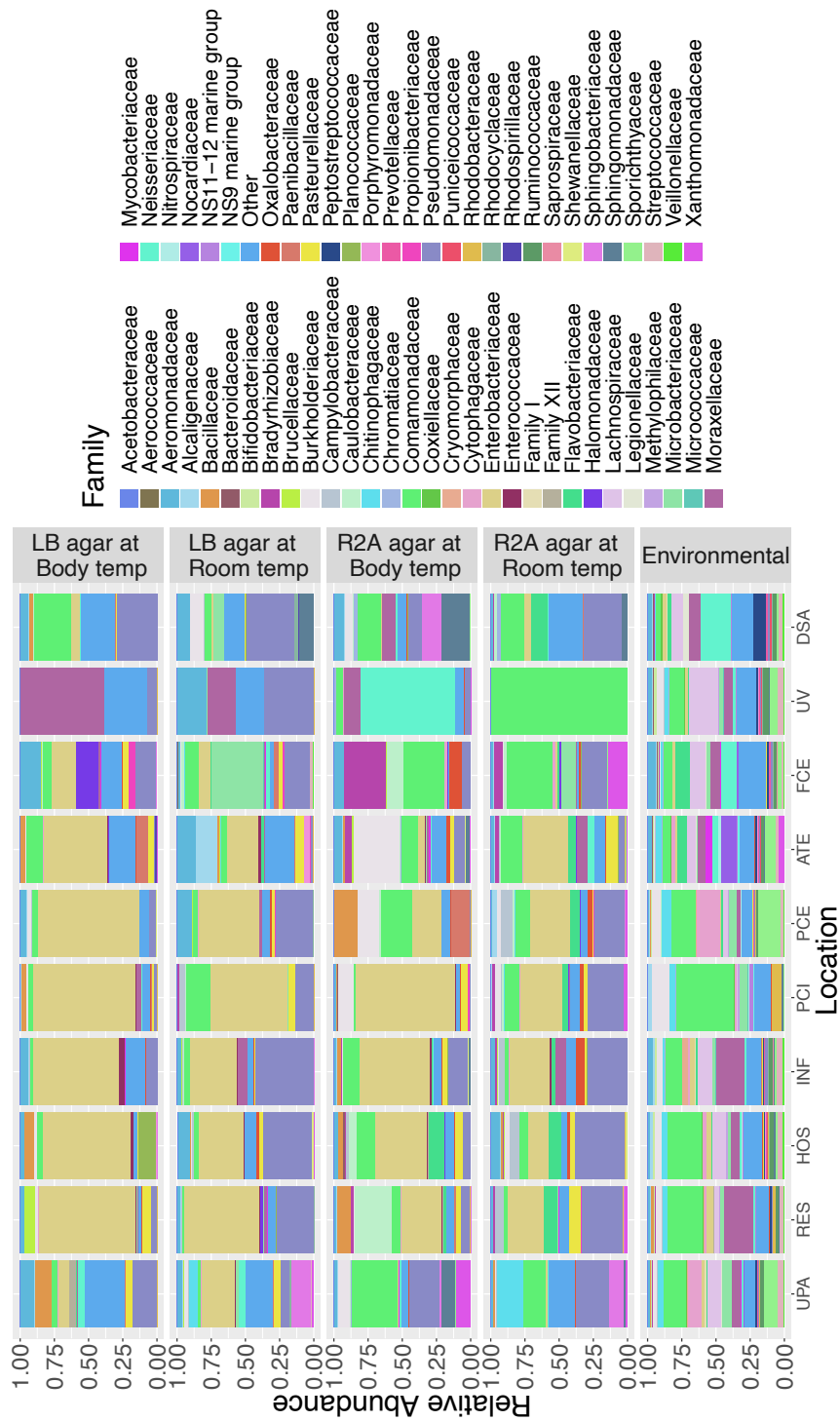
Phylum	Class	Family	Enviro	BT LB	BT R2A	RT LB	RT R2A
Actinobacteria	<i>Actinobacteria</i>	<i>Bifidobacteriaceae</i>	0.91%	0%	0%	0%	0%
		<i>Microbacteriaceae</i>	3.01%	<0.01%	<0.01%	5.02%	1.23%
		<i>Micrococcaceae</i>	0.04%	0%	0%	0.06%	0%
		<i>Mycobacteriaceae</i>	0.67%	0.10%	0.19%	<0.01%	<0.01%
		<i>Nocardiaceae</i>	1.54%	0%	0%	0%	0%
		<i>Propionibacteriaceae</i>	0%	0.09%	0.16%	0.23%	0.05%
		<i>Sporichthyaceae</i>	5.04%	0%	0%	0%	0.06%
		<i>Bacteroidaceae</i>	0.45%	0%	0.23%	0%	0%
		<i>Porphyromonadaceae</i>	0.35%	0%	0.02%	0.49%	0.08%
		<i>Prevotellaceae</i>	0.67%	0%	0%	0%	0%
Bacteroidetes	<i>Bacteroidia</i>	<i>Cytophagaceae</i>	3.99%	<0.01%	0.14%	<0.01%	0.16%
		<i>Cryomorphaceae</i>	0.84%	0%	0%	0%	0%
		<i>Flavobacteriaceae</i>	3.33%	0.01%	3.15%	0.28%	6.06%
		NS9 marine group	0.20%	0%	0%	0%	0%
		<i>Chitinophagaceae</i>	3.52%	<0.01%	0.01%	0.62%	1.55%
		NS11-12 marine group	0.24%	0%	0%	0%	0%
		<i>Saprospiraceae</i>	0.06%	0%	0%	0%	0%
		<i>Sphingobacteriaceae</i>	0%	0%	0.67%	1.26%	0.88%
		Family I	0.15%	0%	0%	0%	0%
		<i>Cyanobacteria</i>	0%	<0.01%	<0.01%	0.09%	<0.01%
Firmicutes	<i>Bacilli</i>	<i>Bacillaceae</i>	0.31%	2.72%	4.31%	0.31%	0.28%
		<i>Enterococcaceae</i>	0%	1.35%	0.66%	0.25%	0.20%
		Family XII	0.02%	0.26%	0%	0%	0%
		<i>Paenibacillaceae</i>	0.03%	1.29%	1.03%	0.48%	<0.01%
		<i>Planococcaceae</i>	0%	2.36%	0.01%	<0.01%	<0.01%
		<i>Streptococcaceae</i>	2.92%	0.01%	<0.01%	0.33%	<0.01%
		<i>Lachnospiraceae</i>	8.68%	0%	0%	0%	0%
		<i>Peptostreptococcaceae</i>	1.84%	0%	0%	0%	0%
		<i>Ruminococcaceae</i>	2.51%	0%	0%	0%	0%
		<i>Veillonellaceae</i>	0.78%	<0.01%	<0.01%	0.05%	<0.01%
Nitrospirae	<i>Negativicutes</i>	<i>Nitrospiraceae</i>	0.29%	0%	0%	0%	0%
	<i>Nitrospira</i>	<i>Nitrospiraceae</i>	0.29%	0%	0%	0%	0%
Proteobacteria	<i>Alphaproteobacteria</i>	<i>Acetobacteraceae</i>	0.02%	0%	0%	0.10%	0%
		<i>Bradyrhizobiaceae</i>	0.04%	0.01%	3.52%	0.18%	1.80%
		<i>Brucellaceae</i>	0%	1.94%	0.11%	0.09%	<0.01%
		<i>Caulobacteraceae</i>	0.06%	0.03%	7.13%	0.27%	0.83%
		<i>Rhodobacteraceae</i>	0.78%	<0.01%	0.01%	<0.01%	0.01%
		<i>Rhodospirillaceae</i>	0%	0.23%	0.06%	<0.01%	<0.01%
		<i>Sphingomonadaceae</i>	0.69%	0.01%	2.05%	0.76%	0.39%
		<i>Alcaligenaceae</i>	0.57%	0.05%	0.06%	1.68%	0.57%
		<i>Burkholderiaceae</i>	3.76%	1.39%	6.16%	1.26%	1.58%
		<i>Comamonadaceae</i>	14.51%	4.75%	13.09%	4.79%	12.35%
		<i>Methylophilaceae</i>	0.47%	0%	0%	0%	0%
		<i>Neisseriaceae</i>	5.49%	0.36%	2.32%	0.91%	0.63%
		<i>Oxalobacteraceae</i>	0.14%	0.03%	1.12%	0.58%	1.94%
		<i>Rhodocyclaceae</i>	0.30%	<0.01%	<0.01%	0.41%	<0.01%
		<i>Campylobacteraceae</i>	0.17%	0.15%	0.38%	0.71%	3.90%
		<i>Aeromonadaceae</i>	2.82%	3.47%	2.90%	6.59%	2.33%
		<i>Chromatiaceae</i>	0.07%	0%	0.37%	0%	0.17%
		<i>Coxiellaceae</i>	0.44%	0%	0%	0%	0%
		<i>Enterobacteriaceae</i>	2.50%	58.95%	30.95%	31.27%	20.53%
		<i>Halomonadaceae</i>	0%	0.46%	0.04%	0.45%	0.16%
		<i>Legionellaceae</i>	0.76%	0%	0%	0%	0%
		<i>Moraxellaceae</i>	8.99%	1.60%	1.05%	2.45%	2.06%
		<i>Pasteurellaceae</i>	0.41%	2.67%	3.26%	2.73%	3.37%
		<i>Pseudomonadaceae</i>	1.09%	5.44%	7.62%	25.31%	25.44%
		<i>Shewanellaceae</i>	0%	0.15%	0.26%	0.32%	0.38%
		<i>Xanthomonadaceae</i>	0.61%	0.06%	0.86%	0.32%	2.87%
		<i>Puniceococcaceae</i>	0.23%	0%	0%	0%	0%
Verrucomicrobia	<i>Opitutae</i>	<i>Opitutaceae</i>	12.66%	10.10%	6.10%	9.35%	8.12%
Other	Other	Other					

**Table A17** | Differential abundance of taxa between cultured and environmental source samples.

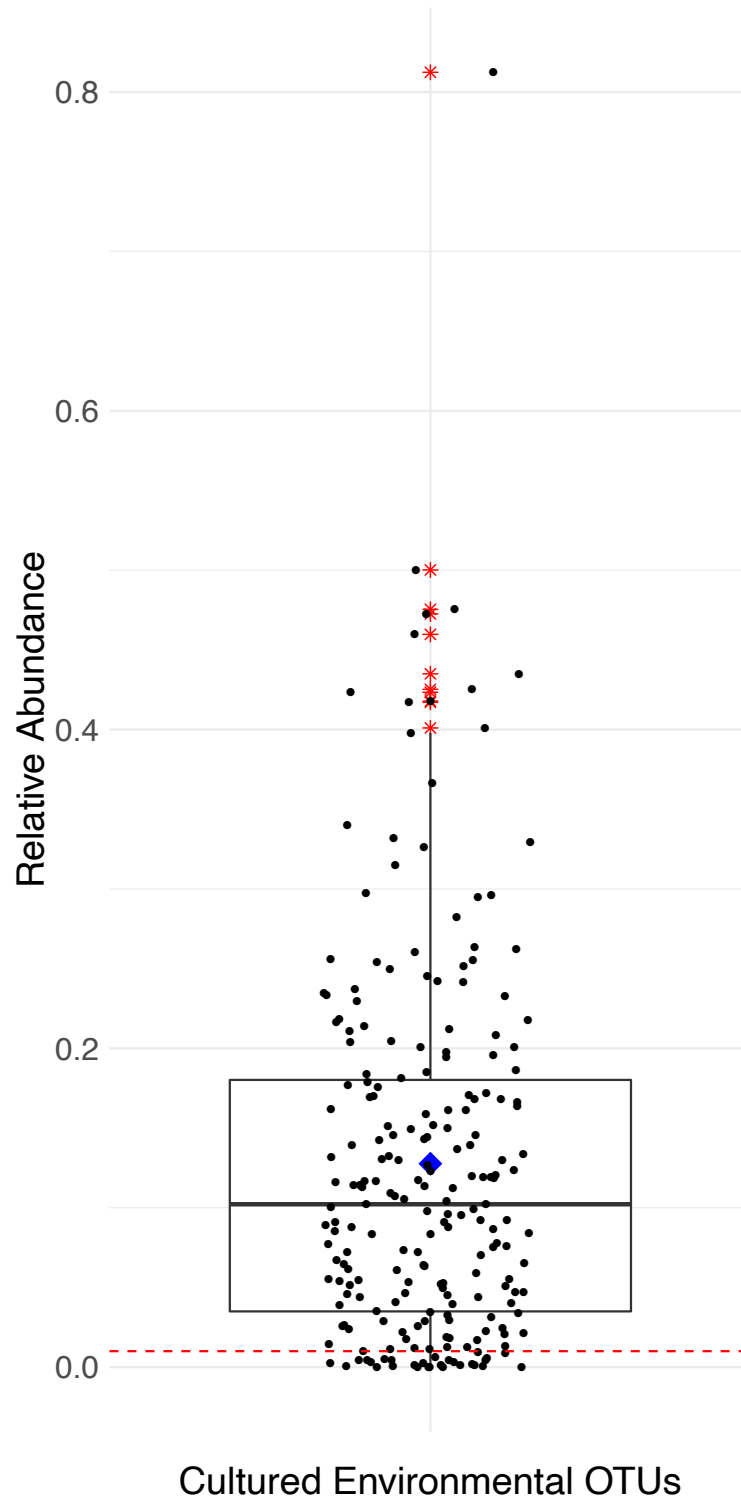
Phylum	Class	Family	Higher abundance	p value
Bacteroidetes	<i>Flavobacteriia</i>	<i>Flavobacteriaceae</i>	Environmental	2.04E-13
Firmicutes	<i>Bacilli</i>	<i>Bacillaceae</i>	Environmental	4.21E-06
Proteobacteria	<i>Alphaproteobacteria</i>	<i>Rhodobacteriaceae</i>	Environmental	5.28E-90
		<i>Sphingomonadaceae</i>	Environmental	2.05E-20
	<i>Betaproteobacteria</i>	<i>Alcaligenaceae</i>	Environmental	1.55E-23
		<i>Burkholderiaceae</i>	Environmental	9.21E-06
		<i>Comamonadaceae</i>	Environmental	1.21E-10
		<i>Oxalobacteraceae</i>	Environmental	1.10E-12
		<i>Neisseriaceae</i>	Environmental	1.40E-38
	<i>Epsilonproteobacteria</i>	<i>Campylobacteraceae</i>	Environmental	1.30E-07
	<i>Gamma proteobacteria</i>	<i>Aeromonadaceae</i>	Environmental	4.02E-08
		<i>Enterobacteriaceae</i>	Culture	0.00089717
		<i>Moraxellaceae</i>	Environmental	2.51E-21
		<i>Xanthomonadaceae</i>	Environmental	2.72E-13



**Figure A19** | Relative abundance of OTUs assigned to each phylum from environmental and cultured samples. “\*” indicates a  $p$  value of 0.01 – 0.05; “\*\*” indicates a  $p$  value of 0.001 – 0.01; “\*\*\*” indicates a  $p$  value < 0.001.



**Figure A20** | Relative abundance for total heterotrophic and antibiotic resistant communities from all sampling locations in the wastewater treatment process under individual culturing conditions. UPA, upstream; RES, residential sewage; HOS, hospital sewage; INF, sewage influent; PCI, primary clarification influent; PCE, primary clarification effluent; ATE, aeration tank effluent; FCE, final clarification effluent; UV, UV treated effluent; DSA, downstream.



**Figure A21** | Relative abundance of culturable OTUs found within the corresponding environmental source samples. On average 12.8% of the environmental OTUs were also recovered within cultivable growth. Red dashed line indicates the widely accepted 1% culturability cut off; ♦ indicates the mean; and \* identifies outliers.