2.6 Tables

Table 1 | Differential abundance of significant taxa between treatment stages for combined ARB communities. Underlined locations indicate the locations containing the higher taxonomic abundance.

Phylum	Class	Family	*p* value	Locations
Actinobacteria	*Actinobacteria*	*Microbacteriaceae*	<0.001	UV - <u>DSA</u>
			0.017	ATE - <u>FCE</u>
		Propionibacteriaceae	0.047	ATE - <u>FCE</u>
Proteobacteria	*Alphaproteobacteria*	*Sphingomonadaceae*	<0.001	UV - <u>DSA</u>
	Betaproteobacteria	*Burkholderiaceae*	0.027	<u>PCE</u> - ATE
	Gammaproteobacteria	*Enterobacteriaceae*	0.012	<u>PCI</u> - ATE
			0.001	<u>PCE</u> - ATE
			0.006	<u>PCE</u> - UV
		Halomonadaceae	0.017	ATE - <u>FCE</u>
		Pasteurellaceae	<0.001	<u>UPA</u> - DSA
		Pseudomonadaceae	0.020	<u>PCE</u> - ATE

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.

2.7 Figures

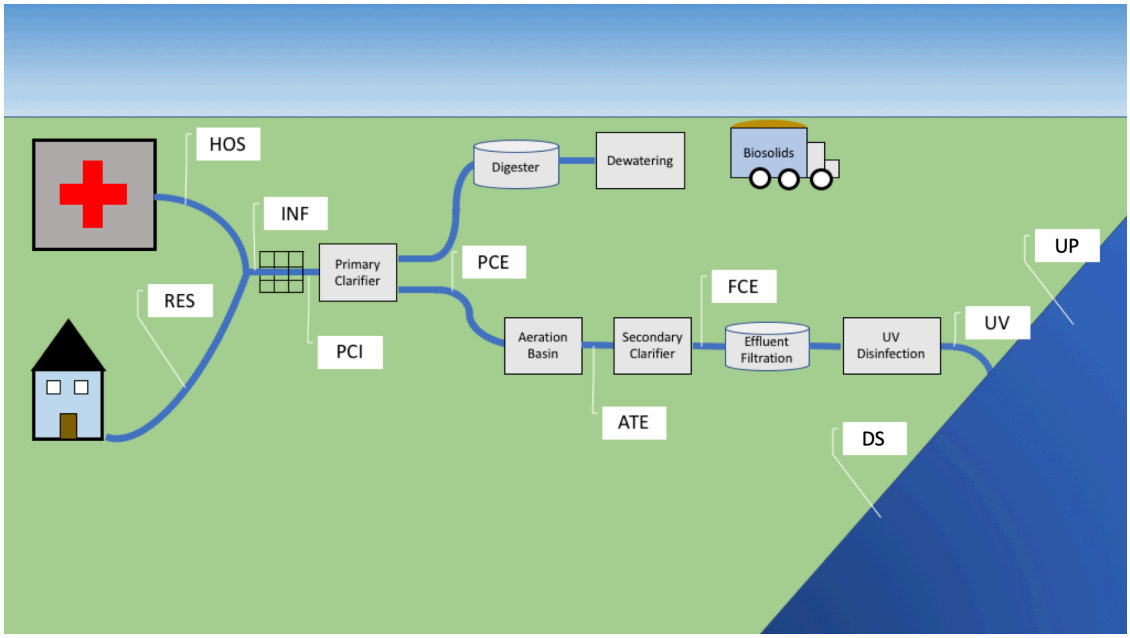

Figure 1 | Schematic of the treatment process and sampling sites of the wastewater treatment plants utilized for this study. Raw sewage from residential (RES) and hospital (HOS) sources are routed to a main sewer line. The combined raw influent is then passed through a physical screen to filter out large solids. The screen-filtered wastewater (PCI) undergoes primary clarification for an additional solid removal step. The primary clarifier effluent (PCE) is routed to an aeration basin for biological nutrient removal. The aeration tank effluent (ATE) then undergoes a secondary clarification process. The final clarification effluent (FCE) undergoes a final filtration step before UV treatment (UV) for microbial disinfection prior to stream release. The large solids removed during primary clarification are routed to a digester and dewatered for the production of biosolids. (Downstream, DS; Upstream, UP).

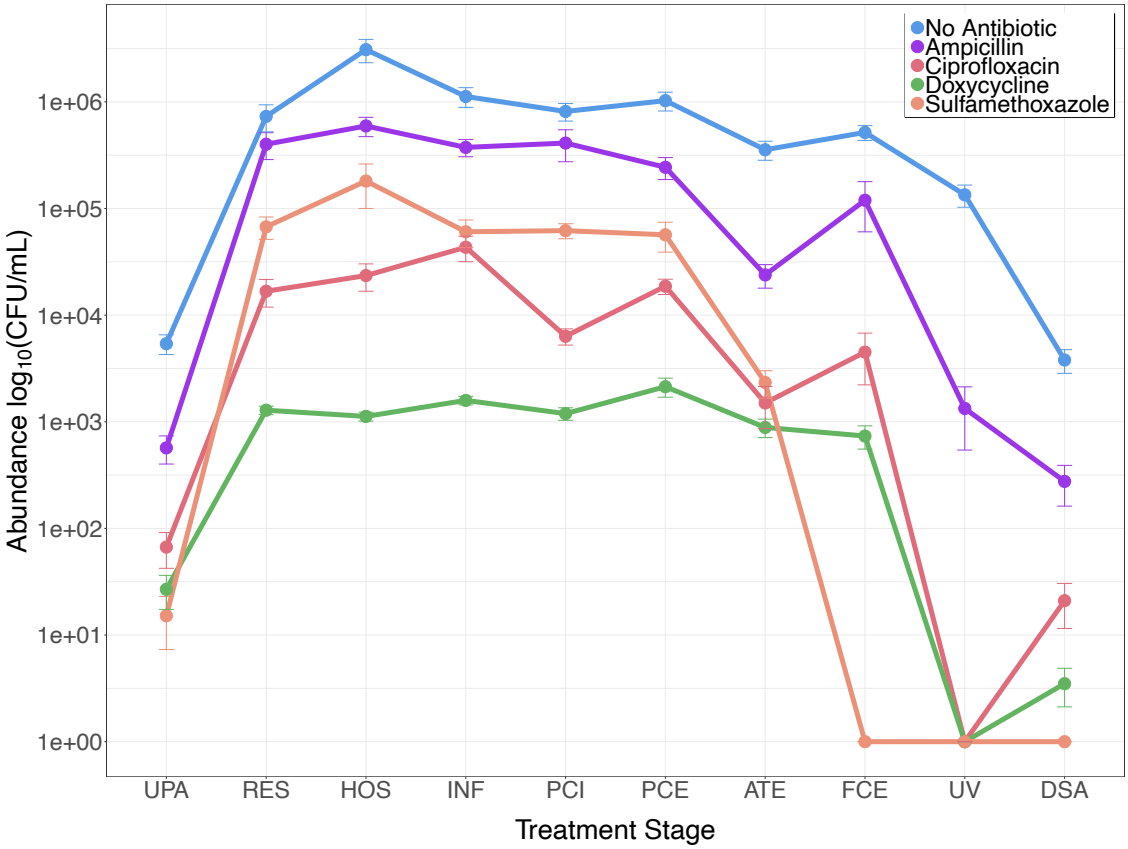

Figure 2 | Abundance of microbial colonies grown on the four antibiotics and a control at each stage of the treatment process. Total heterotrophic and resistant microbial concentrations saw significant reductions from the initial raw sewage to the final UV treated effluent. Error bars indicate the standard error of the mean for each antibiotic treatment at each site. 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.

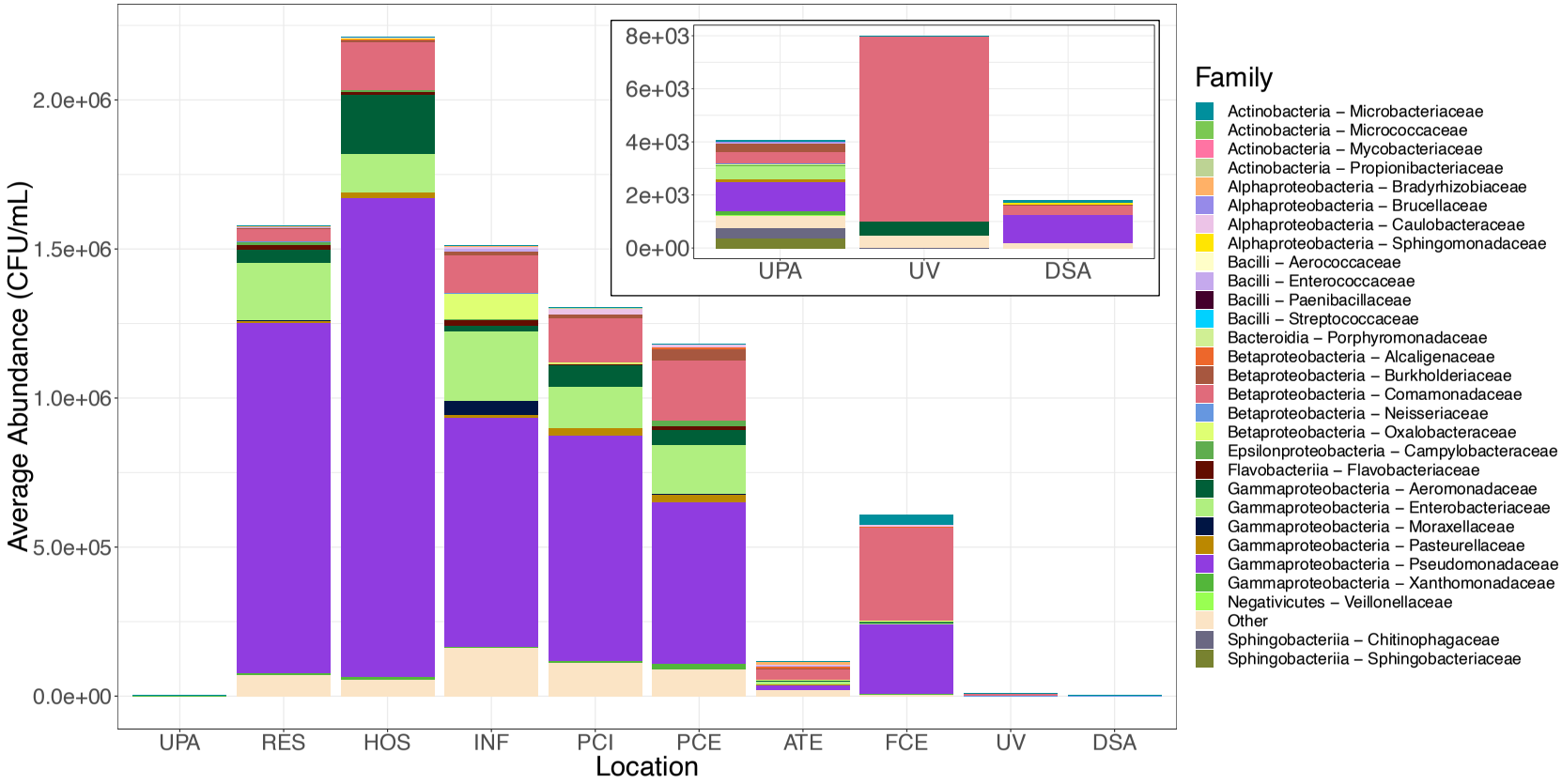

Figure 3 | CFU/mL normalized counts of 16S rRNA gene relative abundance data for antibiotic-resistant communities at each sampling location. Combined Mallard and Sugar Creek samples at each sampling location including combined antibiotic-amended cultured communities. Relative abundance determined through 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, UV treated, 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.

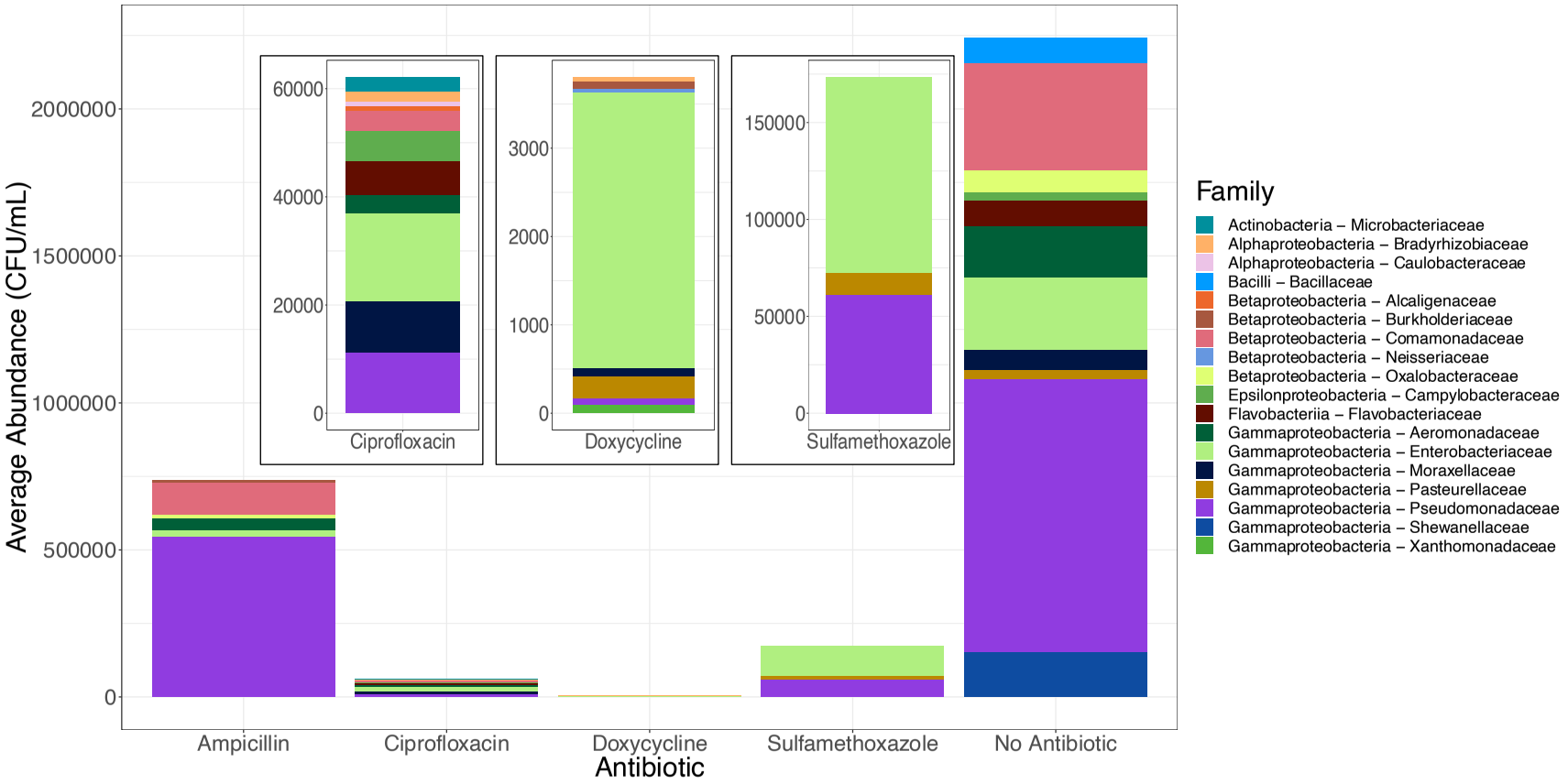

Figure 4 | CFU/mL normalized counts from relative abundance data of the taxonomic families from each cultured antibiotic treatment. The average colony counts for each location were normalized to CFUs per mL and adjusted to the calculated relative abundance determined through 16s rRNA sequences to obtain approximated absolute abundances. Families making up <1% of the total community at each site were excluded.

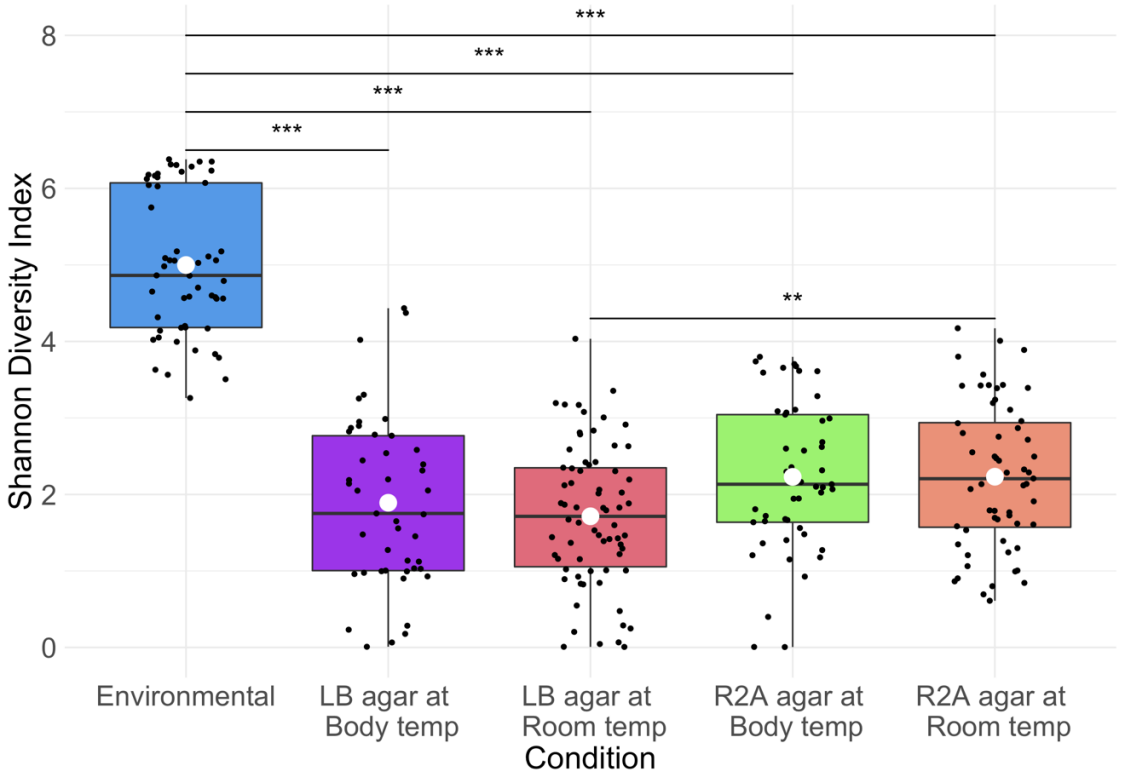

Figure 5 | Average OTU level Shannon diversity for the culture-independent and each culture-dependent community. Significant differences are indicated with bars between the locations with statistically differential diversity values. The statistical mean is represented by a white circle. "*" indicates a *p* value of 0.01 – 0.05; "**" indicates a *p* value of 0.001 – 0.01; "***" indicates a p value <0.001.

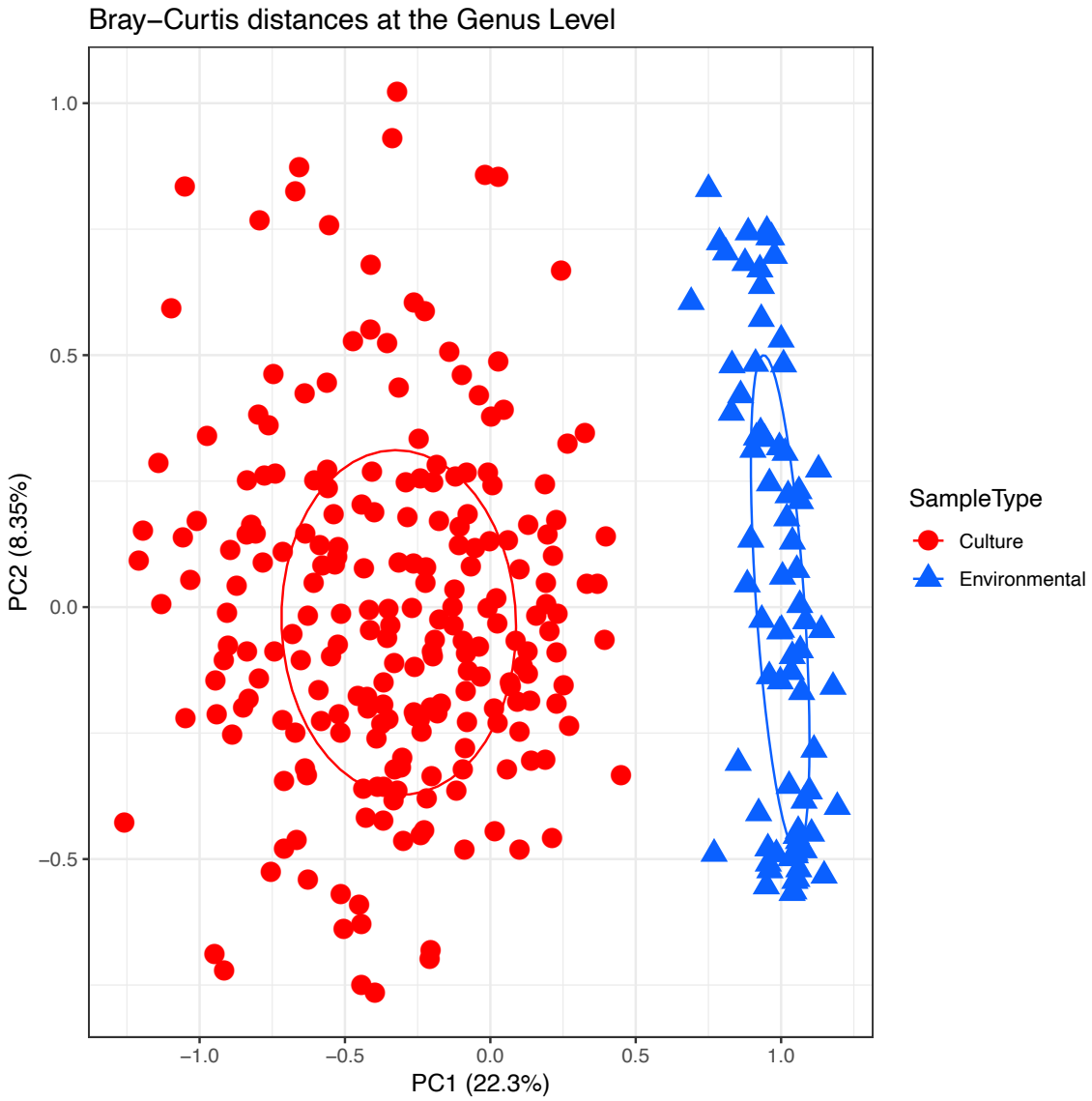

Figure 6 | Bray Curtis PCoA ordination for all culture-independent and culture-dependent samples. Beta diversities at the genus level from 16S rRNA gene sequencing are shown with PC1 and PC2 components. Data are clustered and colored by the two sequencing approaches utilized.

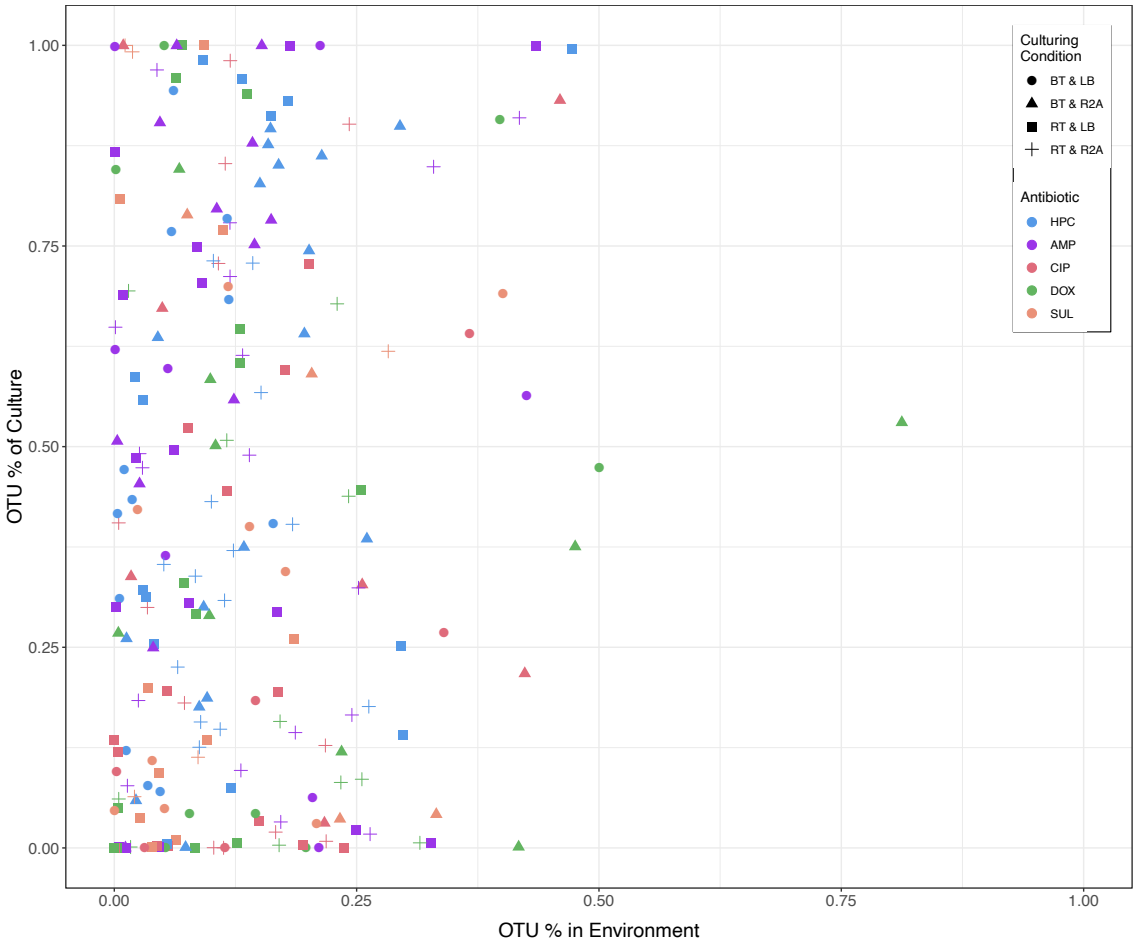

Figure 7 | Relative abundances of the shared composition within culture and environmental communities at the OTU level. BT, body temperature (37°C); RT, room temperature (22°C); LB, lysogeny broth agar; R2A, Reasoner's 2 agar.

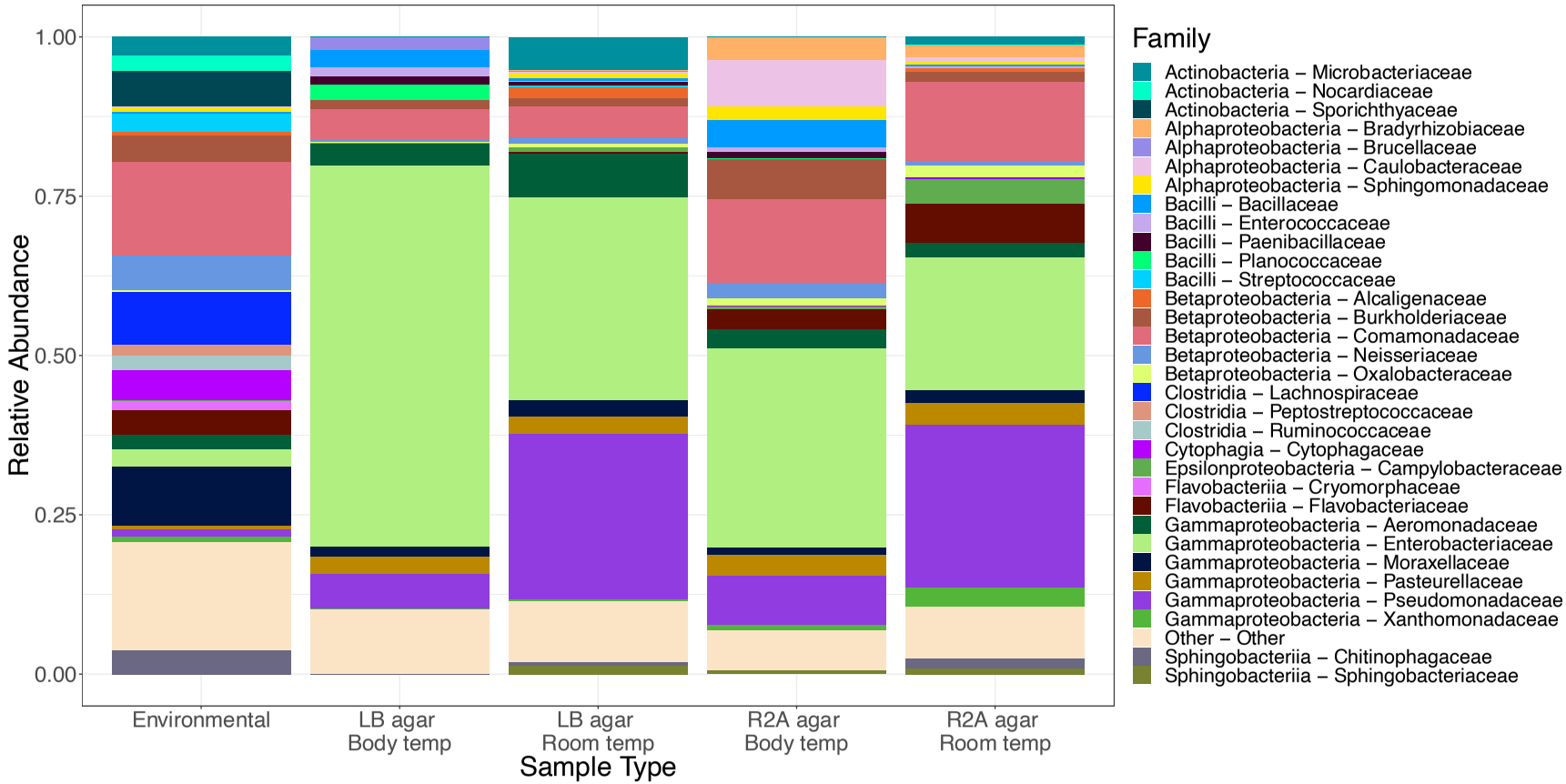

Figure 8 | Relative abundance of the taxonomic families for the culture-independent samples and each culture-dependent condition. Families making up <1% of the total community at each site were excluded.