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ORIGINAL ARTICLE

Characterization of communal sink drain communities of a university campus

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Abstract

Microorganisms are widely distributed throughout the built environment and even those found in concealed environments such as sink P-traps can have an impact on our health. To date, most studies on sink bacterial communities focused on those present in hospitals with no to little information regarding sinks in residential or communal settings. Here, we conducted a characterization using 16S rRNA sequencing of the bacterial communities of communal restroom sinks located on a university campus to investigate the diversity, prevalence, and abundances of the bacteria that reside in this understudied environment. The study found that community composition and structure were highly variable across individual sinks, and there were marginal differences between buildings and the two different parts of sink examined. Proteobacteria were the most abundant phylum in the sink communities, and the families *Burkholderiaceae*, *Moraxellaceae*, and *Sphingomonadaceae* were found to be ubiquitous across all sinks. Notably, human skin was identified as a primary contributor to the below-strainer sink bacterial community. These data provide novel insight into the sink bacterial communities' constituents and serve as the foundation for subsequent studies that might explore community stability and resilience of in situ sinks.

KEYWORDS
built environment, DNA barcoding, environmental microbiology, microbiome, sink

1 | INTRODUCTION

With humans in developed countries spending up to 90% of their lives indoors, there has been an increased effort to understand the mechanisms that influence microorganisms and their community dynamics (Klepeis et al., 2001). It is now necessary to recognize that buildings are complex ecosystems and microbial communities are present throughout the built environment (BE). The interactions microorganisms have with one another, their environment and specifically human occupants can have consequences that may beneficially or negatively affect human health and wellbeing (Hoisington et al., 2015; Stamper et al., 2016).

Indoor BEs are purposely designed to remain dry for human occupants and are therefore expected to be ecological sinks (Pulliam, 1988). Studies have shown this to be the case with BEs consisting of migrant, mainly human-associated microorganism rather than residential microorganisms (Lax et al., 2017). There is a greater influence of dispersal into the BE, for example, by occupants directly and indirectly depositing microorganisms, than by endogenous growth (Coil et al., 2019; Hospodsky et al., 2012; McDonagh et al., 2014). An exception to this may be areas which receive intentional and frequent water use such as bathrooms and their associated sinks and pipes. Periodic water use and flushing of waste fluid down sink alongside

warmer indoor temperatures and pipes being a relatively protected environment favors formation of biofilms (Bitton, 2014; Ji et al., 2017). The body of water in P-traps also allows for periodic stagnation, further promoting bacterial growth and biofilm formation (Bédard et al., 2018; Prest et al., 2013). Biofilms display higher tolerance to disinfectants, facilitate resistance to environmental stress, and allow embedded microorganisms to share nutrients and metabolic products (Chao et al., 2015; Douterelo et al., 2018; Poitelon et al., 2010; Revetta et al., 2010; Williams et al., 2004). This suggests the P-traps of sinks, invented to prevent sewer gases rising from the sink drain into the building, are an ideal environment for proliferation of microbial communities.

Built or indoor surfaces experience strong selective pressures (Martin et al., 2015). To a lesser extent, P-traps are also a selective environment due to the presence of antibacterial soap, low available carbon, repeat flushing, and competing microorganisms (Douterelo et al., 2016; Hibbing et al., 2010). In restrooms, previous work showed that both dispersal and selective pressures determine microbial composition as bathroom surfaces clustered based on their dominant source populations (Flores et al., 2011). Besides humans influencing community composition, environmental influences and building design can have an impact (Kembel et al., 2012; Meadow et al., ,2014, 2015). Environmental sources of colonizing microorganism can be from pets, air, water, or plants (Hewitt et al., 2012; Kelley & Gilbert, 2013). These microorganisms can form established communities or be transient dependent upon building conditions or routines such as cleaning or remediation (Adams et al., 2016; Wingender & Flemming, 2011). The P-Trap of sinks is often inaccessible, and thorough cleaning is limited suggesting stable communities could form.

Previous studies have highlighted the importance of sinks and their traps as a source in nosocomial outbreaks (Cholley et al., 2008; Gillespie et al., 2000; Lowe et al., 2012). Sink traps harbored opportunistic and antimicrobial-resistant bacteria, which were not easily controlled or removed (Hota et al., 2009; Stjärne Aspelund et al., 2016). An experimental study showed how biofilms can extend from the P-trap to basin, and upon addition of faucet water, microorganisms can be splashed to the surrounding area (Kotay et al., 2017). More recently, a study was released detailing the formation of biofilms in an in vitro drain biofilm model (Ledwoch et al., 2020). This further demonstrated the establishment of a rigid thick layer of embedded cells within eight days in a P-trap-simulated environment. Additionally, upon disinfection, the back sections of the trap were not controlled by Sodium Hypochlorite disinfection, and within days post treatment, the biofilm had recovered. This finding is similar to other studies where biofilms recovered within seven days after treatment with bleach or foaming products (Buchan et al., 2019; Jones et al., 2020). These studies were again hospital associated as they treated sinks found in patient rooms. Ledwoch's et al. model provides a reproducible and simple testing methodology for investigating trap formation and disinfection, but it does not represent complex biofilms formed over years of in situ sinks. While other studies have explored the surfaces of universities and restrooms (Dobbler et al., 2017; Flores et al., 2011; Ross & Neufeld, 2015), currently there is no literature describing the microbiome of P-traps of sinks in situ in non-clinical communal or public buildings. Universities offer an interesting study site, because they are subject to high population densities of healthy individuals from culturally diverse backgrounds. Individual behavior dependent upon building may influence the microbial diversity and composition of sink P-traps.

The objectives of this study were to (a) determine the structure and diversity of bacterial communities in communal sinks across the University campus; (b) explore if sinks had a core microbiome or if community composition was specific to building and/or restroom gender; and (c) ascertain the dominant sources of the microorganisms to the university campus sinks.

2 | METHODS

2.1 | Sampling sites and procedure

Restroom sinks from nine buildings located on the main campus of the University of Reading were sampled. Five of the buildings belonged to the School of Biological Sciences, two were large humanity teaching buildings and the remaining two buildings were centrally located communal buildings: the library and student union. Between November to December 2019 during termtime, 123 sinks were sampled, resulting in a total of 215 samples to be sequenced. Routine cleaning of the sinks throughout all buildings was consistent and involved a daily surface wipe down of tap with Virucidal surface cleaner disinfectant. Drains and P-trap are not routinely treated. Each sample was classified by building (nine buildings), drain type (P-trap or below-strainer), and restroom gender (male, female, or unisex) (Figure S1). For each sink, two samples were taken where possible using sterile, cotton-tipped buds. For the P-trap drain type, the cotton bud was attached to a 40 cm metal rod ("sampling rod"), inserted and swirled in a circular motion for 5 s while touching the surface. For the below-strainer drain type, the circumference of the top of the pipe, just below the drain, was swabbed using the same swirling motion. Swabs were then cut using ethanol sterilized scissors directly into beaded microtubes. Prior to swabbing, the sink was flushed with cold water for 1 min to eliminate recent usage as a confounding factor. Samples were stored in the freezer at −20°C and thawed before DNA extraction.

2.2 | DNA extraction and sequencing

Genomic DNA was extracted from the swabs using the HigherPurity Soil DNA Isolation kit (Canvax Biotech), following the manufacturers protocol. The DNA was eluted in a final volume of 50 µl and stored at −20°C until needed. The first round of PCR targeted the V4 hypervariable region of the 16S ribosomal RNA gene with primers, 515F (Forward: GTGYCAGCMGCCGCGGTAA) and 806R (Reverse: GGACTACNVGGGTWTCTAAT) as used by the Earth

Microbiome Project (EMP, https://press.igsb.anl.gov/earthmicrobiome/protocols-and-standards/16s/). Each PCR amplification mix contained 8.5 µl of Nuclease-free water, 12.5 µl of 1X PCR Mastermix, 0.5 µl of each 10 µM forward and reverse primers, and 3.0 µl of gDNA, resulting in a total volume of 25 µl. Thermocycling conditions were followed as described by the EMP protocol. PCR products were purified with AMPure XP beads (Beckman Coulter) in accordance with manufacturers PCR purification workflow. The second PCR reaction adds Illumina-specific adapters and unique barcodes to either side of PCR product, allowing for samples to be pooled. The thermocycle conditions for the second round of PCR were 95°C for 2 min and 8 cycles of 95°C for 15 s, 55°C for 30 s, 72°C for 30 s, and a final extension of 72°C for 10 min. SequalPrep™ Normalization Plate Kit (ThermoFisher) cleaned and normalized the samples before being pooled. Samples were sequenced on the Illumina Miseq Platform (250PE) at UK Centre for Ecology & Hydrology.

2.3 | Data processing

The sequences were quality filtered and adapters removed using TrimGalore (https://github.com/FelixKrueger/TrimGalore). The resulting quality-filtered reads were processed with R using the DADA2 pipeline (v1.14.1, Callahan et al., 2016) generating an Amplicon sequence variant (ASV) abundance table. Each ASV was classified using the naive Bayesian classifier (Wang et al., 2007) against SILVA database (v.132, Quast et al., 2013) for kingdom to species assignments.

2.4 | Statistical analysis

All microbial community statistical analyses were conducted in R (v.3.6.3) using the packages vegan (v.2.5–6) and phyloseq (v.1.30.0). Visualization of results used the ggplot2 (v.3.3.2) package. Prior to statistical analysis, ASVs that were classified as Eukaryota, Archaea, or unclassified at domain were removed from the ASV abundance table. The ASV table was rarefied to an even sampling depth of 9000 resulting in 199 samples that met the threshold. A further two samples were removed from analyses as they appeared to be outliers. To assess beta diversity, the vegdist function was used to construct Bray–Curtis dissimilarity distances and visualized as a Non-metric multidimensional scaling (NMDS). Then dispersion within groups and between groups (groups being tested were building, drain type and gender) was tested for statistical significance. Betadisper was used to test homogeneity of dispersions among groups, coupled with ANOVA to test for their significance. The adonis function was used to perform permutational analysis of variance (PERMANOVA) to compare Bray–Curtis distances against drain type, building, and restroom gender (Oksanen et al., 2015). PERMANOVA tests whether composition among groups is similar or not. The number of permutations was set at the default 999 to calculate *p*-values. Alpha diversity was assessed with ASV richness and Shannon diversity indices. The Kruskal–Wallis test was applied to look for significant differences in alpha diversity across drain type, building, and restroom gender. LEfSe analysis (Segata et al., 2011) was calculated with Galaxy modules provided by the Huttenhower laboratory. LEfSe was used to compare below-strainer and P-trap samples and find the ASVs that contributed more to differences between the two groups. Statistical analysis of the data set was performed at ASV taxonomic level.

To ascertain the potential sources of bacteria in university restroom sinks, the SourceTracker software package was used (Knights et al., 2013). SourceTracker was supplied with source environments from selected studies accessed from Qiita (Gonzalez et al., 2018) that met the following criteria (a) sequenced V4 region; (b) processed sequences through Deblur pipeline; (c) sequence length of 90 bp; and (d) logical source environment for restroom sink. These studies contained samples from humans and outdoor environments (Chase et al., 2016; Flores et al., 2013, 2014; Lax et al., 2014; https://qiita.ucsd.edu/study/description/1521). Biom files for each of these studies were accessible for download from Qiita. The biom tables from Qiita had been processed through the Deblur pipeline, so for compatibility and to merge tables, the sink quality-filtered reads were processed again using Deblur QIIME 2 (trimmed to 90 bp) (https://github.com/biocore/deblur). Using sequences with a length of 90 bp limits taxonomic resolution but some studies accessible through Qiita only met that length such as soil sources; therefore, 90 bp was chosen for comparability. Default parameters were used unless otherwise stated.

3 | RESULTS

3.1 | Sequences and ASVs

The 215 samples from the nine sites across the university campus generated a total of 3 358 721 paired-end raw sequences, with a median/average of 14 821/15 622 sequences per sample. After rarefaction, 1 791 000 sequences remained which were grouped into 2741 ASVs where they were distributed and classified into 31 phyla, 51 classes, 118 orders, and 186 families. An average of 64 ASVs was observed in all the samples (min 18 ASV, max 165 ASVs). In the samples of all university sinks, 95.8% of sequences were assigned to the phylum level, 91.2% to the class level, 82.2% to the order level, 74.1% to the family level, 48.5% to genus level, and 6% to species level.

3.2 | Sink bacterial community structure and composition

While there were significant differences in bacterial community structure and composition between buildings, as indicated by the NMDS plot (Figure 1a) and R^2, the differences were marginal with only 19% of the variation explained (PERMANOVA, DF = 8, F model = 5.5998, R^2 = 0.19243, p = 0.001). Moreover, pairwise

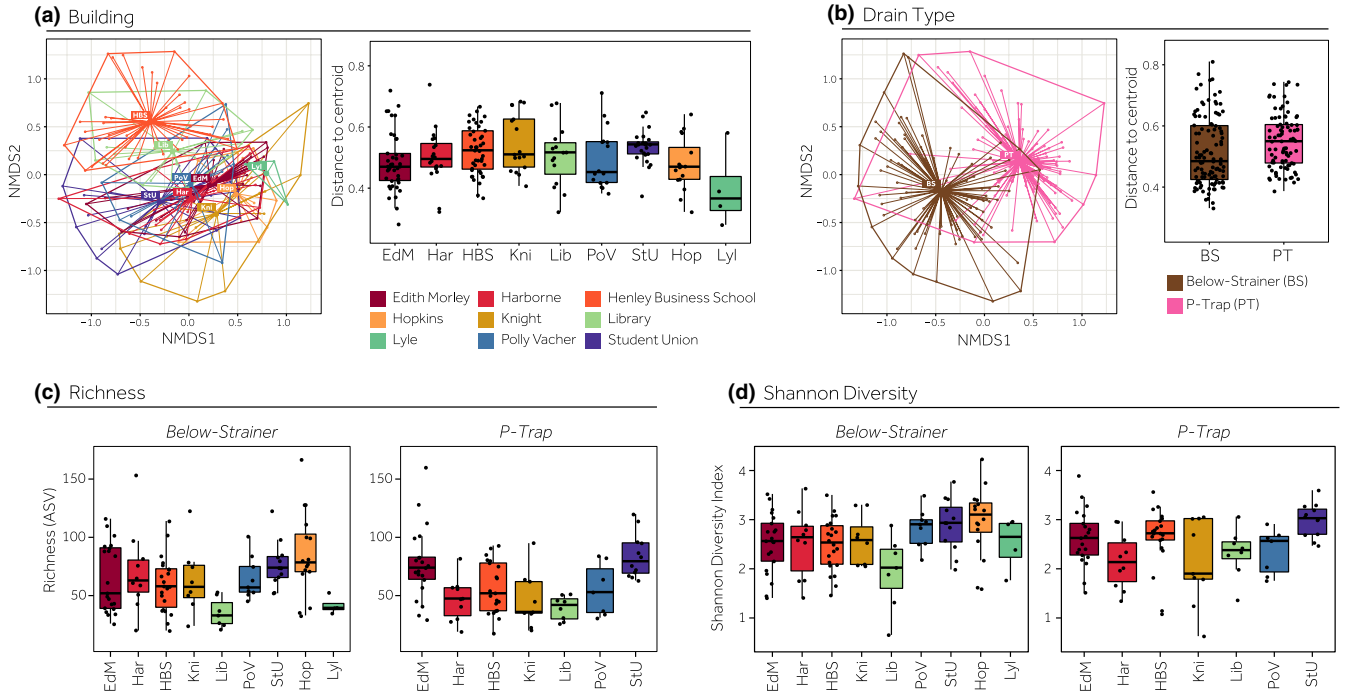

FIGURE 1 (a) Non-metric multidimensional scaling (NMDS) resulting from Bray–Curtis dissimilarity matrices of community composition between nine different buildings sampled; distances to centroid in multivariate homogeneity of group variance analysis for sink bacterial communities for each building. (b) Aforementioned NMDS and distances to centroid for drain types. (c) Amplicon sequence variant (ASV) richness in sink communities across buildings for each drain type. (d) Shannon diversity index in sink communities across buildings sampled for each drain type. P-Traps in Hopkins building and Lyle building were inaccessible due to the design of the sinks

comparisons showed that the average R^2 of all comparisons was below 0.1 (Table S1). HBS was significantly different from all other buildings (R^2 values ranging from 0.06 to 0.15) (Table S1). There was a significant difference in beta diversity between the buildings (ANOVA, DF = 8, F = 2.3291, $p < 0.05$), where Student Union building had the most homogenous community while Lyle building had the least (Figure 1a, Table S2). ASV richness (Figure 1c) and diversity (Figure 1d) varied significantly between buildings (Kruskal–Wallis test, Richness: DF = 8, $p < 0.05$; Shannon: DF = 8, $p < 0.001$; Table S3). There was a significant difference in community structure and composition between the upper part of the drain (below-strainer) and the P-Trap albeit with a low R^2 (Figure 1b; PERMANOVA, DF = 1, F = 24.096, R^2 = 0.10998, p = 0.001). The beta diversity between below-strainer and P-trap samples was also shown to be significantly different (ANOVA, DF = 1, F = 4.935, p = 0.027). The difference between buildings was still significant when buildings were analyzed in their separate drain types (Table S4). An average number of 66 ASVs (min 20, max 167) and 61 ASVs (min 18, max 160) was observed in below-strainer samples and P-trap samples, respectively. ASV richness and diversity were not significantly different between the two drain types (Wilcoxon test, Richness: W = 4400, p = 0.32; Shannon: W = 4444, p = 0.38). Rarefaction curves of the two drain types indicated that additional sequencing efforts will not result in changes in abundance (Figure S2). Notably, there was no significant difference among sink ASV richness and diversity when categorized by restroom gender (Table S3). Regarding gender beta diversity metrics, the bacterial communities were statistically different; however, gender had the lowest variance explained, that is, only 2% of the variation in bacterial communities was explained by the Gender of restrooms (PERMANOVA, DF = 2, F = 2.1941, R^2 = 0.02212, p = 0.002) while the dispersion among gender groups was homogeneous (ANOVA, DF = 2, F = 0.4784, p = 0.62).

LEfSe analysis identified 53 taxa that were more relatively abundant in either of the drain types (below-strainer and P-trap had 29 taxa and 24 taxa, respectively, Figure S3 both with Linear Discriminant Analysis (LDA) score >3.0). ASV2 belonging to the family *Burkholderiaceae* and ASV1 belonging to *Moraxellaceae* were the most differentially abundant ASVs in below-strainer and P-trap samples, respectively (LDA >4.5). For restroom gender, three ASVs were identified as discriminatory, one for each category (Female, Male, Unisex) (Figure S4). No discriminatory taxa were found for sink samples between buildings.

At the phylum level, the dominant bacterial phylum across all sink samples was Proteobacteria (88.75% of sequences), followed by Bacteroidetes (5.93%), then Actinobacteria (3.20%). The remaining phyla had mean relative abundances of less than 1%. The relative abundance of Proteobacteria was consistent across samples but the relative abundance of Actinobacteria was higher overall in below-strainer samples whereas, Bacteroidetes was more prevalent in P-trap samples (Figure 2). At the family level, compositional differences were more pronounced as *Moraxellaceae* was the most

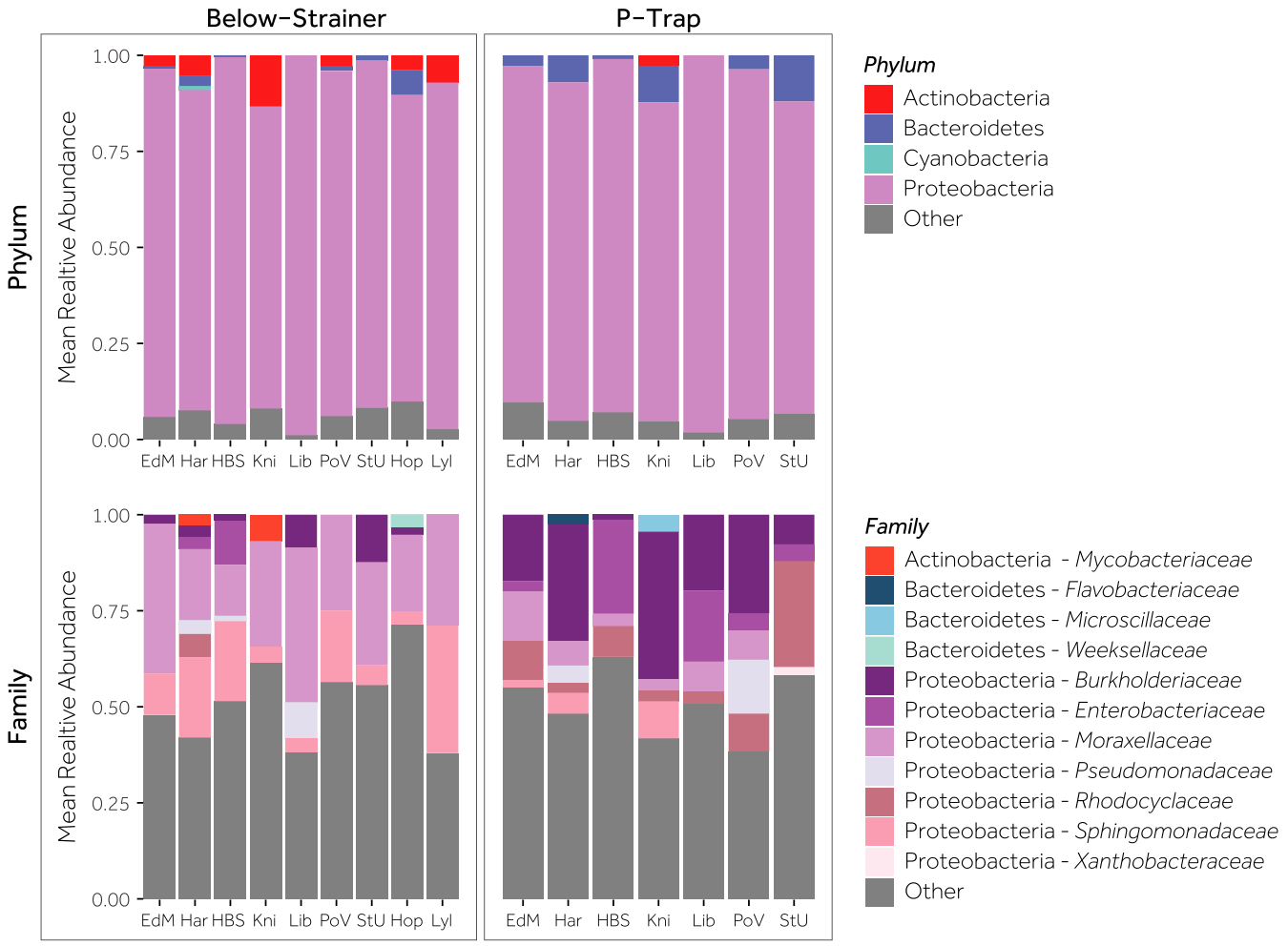

FIGURE 2 Average relative abundance of the top 5 phyla and top 12 families found in the university restroom sinks. The average data represent pooled sequences from the 9 buildings spilt by drain type. Proteobacteria are the dominant bacterial phylum across all sinks regardless of building and drain type. Taxonomic differences were observed between drain types at family level. *Moraxellaceae* is more prevalent in below-strainer samples while *Burkholderiaceae* is more dominant in P-trap samples

prevalent family in below-strainer samples while *Burkholderiaceae* was more dominant in P-trap samples. Markedly, *Acinetobacter* of the Family *Moraxellaceae* was the dominant genera across all sinks (19.7% of reads) with ASV1 accounting for the majority of those (16.8% of reads), followed by *Acidovorax* (ASV2) of the family *Burkholderiaceae*, (10.4% of reads). Overall, the five most abundant families (70.86% of sequence) were *Moraxellaceae*, *Burkholderiaceae*, *Sphingomonadaceae*, *Rhodocyclaceae*, and *Enterobacteriaceae*, all belonging to the phylum Proteobacteria (Figure S5A). Analysis of taxonomic composition of individual sinks at the family level showed highly variable taxonomic profiles between sinks (Figure S6). Additionally, there were no observable patterns in relative abundances of taxa when grouped by restroom gender or building, except for Henley Business School building which appeared to have higher abundances of *Enterobacteriaceae* in both drain types when compared to other buildings. The 20 most common ASVs represented 60.44% of all reads and all except for 6 ASVs belonged to the 5 most abundant families (Figure S5B). Notably, of all the ASVs classified to genus level, except for two (*Xenophilus and Cloacibacterium*), have been identified in biofilms of drinking water faucet microbiome (Liu et al., 2012).

3.3 | Core sink microbiome

To detect the core microbiome of sinks, shared ASVs were identified by prevalence and their average relative abundance for each of the 2,741 identified ASVs. No ASV was observed in all sink samples; however, if split into drain type, one ASV from the genus *Acinetobacter* was identified in all P-trap samples. In this study, an ASV was considered to be part of the core microbiome if it was present in at least 70% of samples (Figure 3). Seven ASVs were considered to belong to the "core" sink microbiome. Their average relative abundances ranged from 1.21% to 16.81% per ASV. Of the seven ASVs, six were Proteobacteria belonging to the four families, *Moraxellaceae*, *Beijerinckiaceae*, *Burkholderiaceae*, and *Sphingomonadaceae*. The

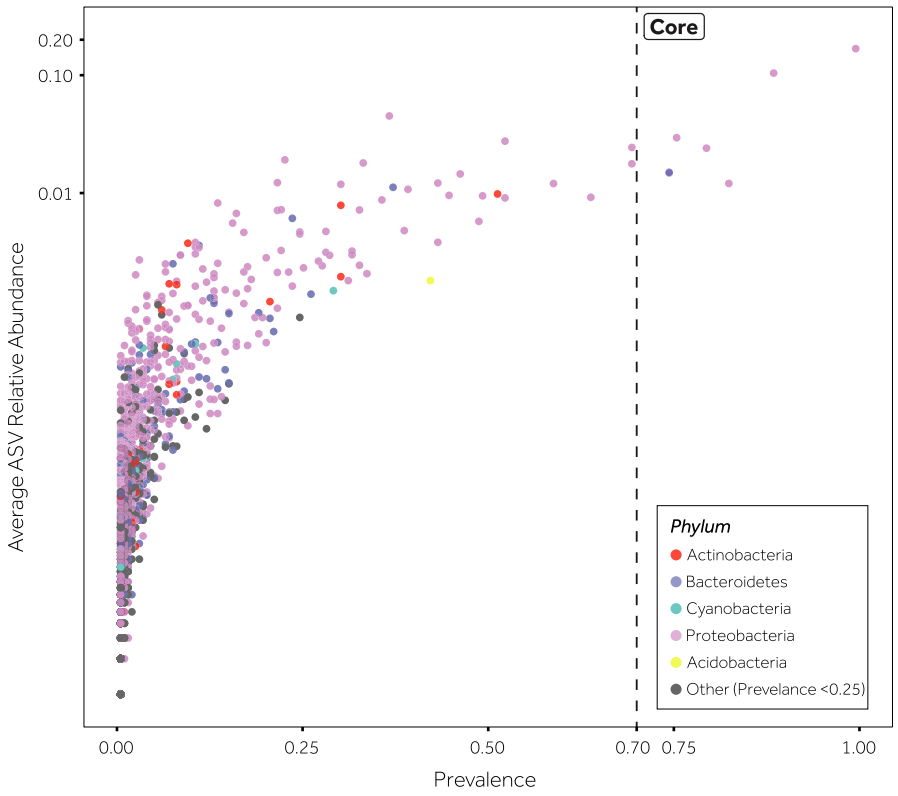

FIGURE 3 Prevalence of total 2741 Amplicon sequence variant (ASVs) across 199 sink samples and their average relative abundance. ASVs are colored by phylum. The dotted line shows the cutoff for taxa defined as core sink ASVs, prevalence ≥0.7. Seven ASVs were present in the core region belonging to the families: *Moraxellaceae*, *Beijerinckiaceae*, *Burkholderiaceae*, *Sphingomonadaceae*, and *Weeksellaceae*

remaining ASV belonged to the *Weeksellaceae* family of the phylum Bacteroidetes. Differences were seen in the number of ASVs classified as core when the data were split into below-strainer and P-trap where below-strainer and P-trap had 10 core ASVs and six core ASVs, respectively (five ASVs were shared in both, Figure S7). When looking at core families, three families, namely *Burkholderiaceae*, *Moraxellaceae*, and *Sphingomonadaceae*, were identified in 100% of all sink sampled.

3.4 | SourceTracker

Human skin was identified as a primary source of the bacterial taxa found across all sinks and was particularly associated with below-strainer biofilm samples (Figure 4). P-trap samples had a less distinct pattern with changes in leading sources dependent upon building. However, "unknown" source was the second largest overall of the source categories. This is not uncommon in microbial samples as the source samples selected for SourceTracker may not be a complete representation of microorganism found in/on the Reading area and associated occupants.

4 | DISCUSSION

Through this study, we have investigated the structure of the bacterial community and diversity of communal restroom sinks collected from a university campus. The results indicate that while building sampled as well as drain type had some effect on bacterial community structure (Figure 1A), the small effect sizes as well as marginal significant pairwise differences (Table S1) meant that the buildings were not too dissimilar in their restroom sink bacterial communities. It is also worth noting that the significant differences derived from PERMANOVA may have been influenced by the asymmetrical design and heterogeneous dispersions (Figure 1A) (Anderson, 2017). Differences in microbial communities between buildings have been previously reported (Rintala et al., 2008; Ross & Neufeld, 2015). Ross and Neufeld (2015) identified temporally stable bacterial communities on university door handles and demonstrated human frequency impacted door handle communities. Similarly, sinks in the Student Union building which is used by primarily students from across campus due to its central locality had one of the highest alpha diversity. However, the library despite being widely used as well as centrally located did not have a high alpha diversity. This potentially is because the sinks in the library were relatively new as the building had been recently refurbished and subsequently opened only 2–3 months prior to sampling (opened September 2019).

The bacterial communities of university sinks examined in this study were dominated by Proteobacteria. Previous studies indicate that BE surface bacterial communities are often dominated by Proteobacteria due to the strong influence of humans in an indoor environment (Lax et al., 2014). Within drinking water, Proteobacteria frequently dominate 50%–80% of bacterial communities (El-Chakhtoura et al., 2015; Ji et al., 2015; Pinto et al., 2012, 2014). As well as Proteobacteria being associated with the BE, the next top two phyla; Bacteroidetes and Actinobacteria have also been associated with a variety of built environments including restroom surfaces (Flores et al., 2011; Kelley et al., 2004; Lee et al., 2007; McManus &

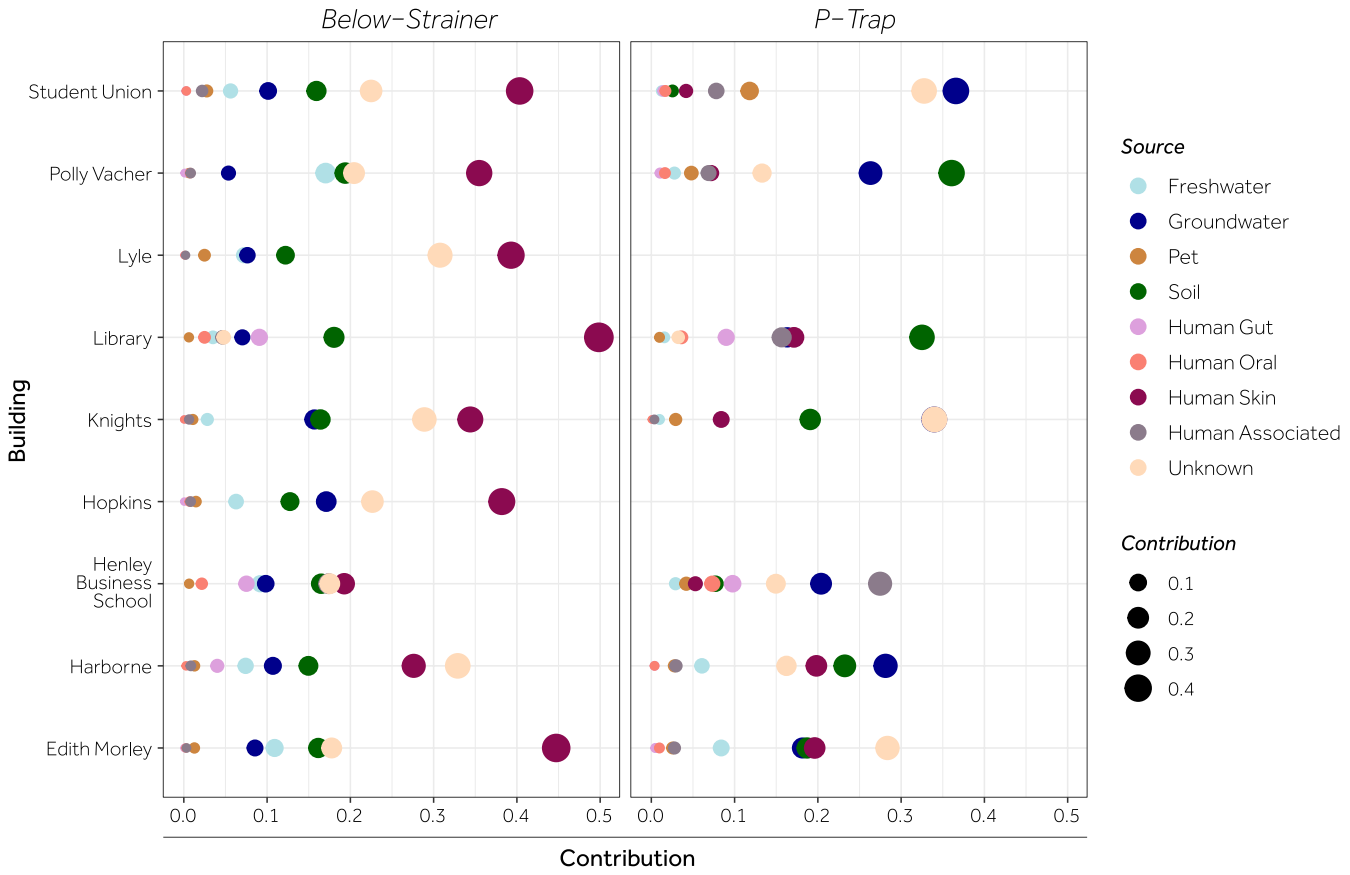

FIGURE 4 Predicted source contribution to each building generated from SourceTracker output. Source environments were taken from studies deposited in Qiita. Point size represents predicted source contribution to each building. Human skin is a dominant source across below-strainer communities. P-trap samples do not have a dominating source, and there is more variation in contributing sources across buildings

Kelley, 2005a; Rintala et al., 2008; Ross & Neufeld, 2015). Similarly, both bulk water and biofilms of drinking water pipes share these top phyla (Inkinen et al., 2014; Lin et al., 2014; Liu et al., 2014).

Overall, Proteobacteria were the most dominant phylum in both Drain types, and the phylum Actinobacteria were relatively more abundant in below-strainer samples while Bacteroidetes was more abundant in P-trap samples. Additionally, compositional differences were more pronounced at family level between below-strainer and P-trap samples. *Moraxellaceae* was the most prevalent family in below-strainer samples while *Burkholderiaceae* was more dominant in P-trap samples. Differences may be attributed to the fundamental difference in environmental conditions of the two drain types, that is, the body of water in P-Trap versus the "drier" drain. Differences between the two environments were further supported by LEfSe reporting a large number of bacterial taxa between the two drain types. There was also a strong presence of *Enterobacteriaceae* in P-traps particularly in HBS building and the Library building.

Amplicon sequence variant level analysis showed many sequences associated with *Acinetobacter*, which was a genus found in all sink samples. Previous BE studies have identified *Acinetobacter* as a common BE genus due to its wide distribution from hospitals to subways and even in the international space station (Afshinnekoo et al., 2015; Baron et al., 2014; Castro et al., 2004; Chase et al., 2016; Hsu et al., 2020; Ross & Neufeld, 2015). *Acinetobacter* has also been identified on specific water-associated environments such as shower tiles and isolated from drinking water (Allen et al., 2004; Norton & Lechevallier, 2000). Furthermore, it was the most common genus of bacteria found in treated water and was present throughout the water treatment process suggesting they can withstand the harsh treatments (Lin et al., 2014). As well as being a common treated water-associated genus, *Acinetobacter* is also capable of colonizing both dry and moist areas of human skin (Powell & Marcon, 2012). *Acinetobacter's* ability to survive harsh treatments and to colonize human skin may explain why it was the most abundant genus found in sinks. *Acinetobacter* spp. have been implicated in various nosocomial outbreaks (Debast et al., 1996; Kappstein et al., 2000) and can be resistant to multiple antibiotics (Badave & Dhananjay, 2015; Kumari et al., 2019). *Acidovorax*, which has been previously identified in hospital sink pipes and drinking water distribution systems (Gilbert et al., 2010; Pinto et al., 2012), was also associated with the core ASV with the second highest prevalence belonged to this genus. Properties of *Acidovorax* species such as strong autoaggregating abilities and high whole-cell hydrophobicity are important in biofilm development in flowing environments (Rickard et al., 2004). Sink drains experience frequent disruption due to tap usage, and the autoaggregating properties of *Acidovorax* may explain why

it is a successful colonizer of sinks. The third most abundant ASV belonged to the Family *Enterobacteriaceae* which contains opportunistic and principal pathogens alongside human gut commensals and environmental species. Previous studies in hospitals identified handwashing sinks and drains as a possible reservoir of potentially harmful *Klebsiella pneumoniae* and *Klebsiella oxytoca* (Buchan et al., 2019; Leitner et al., 2015). This demonstrates that the sink environment is a suitable environment for clinically significant strains. Further investigation of what genera and species of the family *Enterobacteriaceae* are found in "healthy" sinks is required to confirm whether they could be a future risk.

One of the notable findings from this study is that human skin was identified as a primary contributor to the sink microbiome (Figure 4). Of the 186 Families identified, 32 have been found on human hands including the dominating Family, *Moraxellaceae*. We had expected a higher contribution from the human gut as it had been previously identified as a contributing source for surfaces near toilets (Flores et al., 2011). The low contributions of human gut could be due to either that not all bacteria of the bulk water are able to attach to the pipe wall biofilms (Inkinen et al., 2016), or more likely that the plumbing is not a suitable environment for proliferation of bacteria found in the gut. Arguably, prevalence of skin and gut bacteria in the sink basin and P-trap is expected as the process of washing hands would remove bacteria present on the skin. Moreover, skin-associated bacteria are generally resilient and can survive on surfaces for extended periods of time (Grice & Segre, 2011), and the dead skin, oils from hands and other organic matter such as feces may supply additional nutrients for microorganisms to form stable communities in sinks. While we would need to investigate the tap water itself in order to determine whether it represents the water sources (Freshwater and Groundwater), our results suggest that tap water may be another potential contributor to the sink microbiome, and this may also explain why the larger contribution from groundwater was seen as a source in P-trap samples. Faucet water generally harbors relatively low concentrations of bacteria (Flores et al., 2011), but a study of office drink water pipe biofilms suggested that the supply of fresh water, especially in stagnated areas, promotes new growth of active bacteria (Inkinen et al., 2016). Therefore, we can speculate that the body of water in a P-trap may provide a supply of faucet water microorganisms to the pipe wall biofilms, which is replenished upon sink usage. This study has shown that there was a general lack of ASVs that are ubiquitous in sinks (Figure 3). Previous studies have shown that between and within humans, there is great variation in taxonomic composition, and no core temporal microbiome exists at high abundances within a single body site (Caporaso et al., 2011; Turnbaugh et al., 2007). As such, one would expect a similar trend in sink microbiome if humans are driving sink bacterial community. Human palms particularly have a smaller core microbiome when compared to mouth and gut (Caporaso et al., 2011).

One of the limitations of this study is that sampling was restricted to a single time point, and no human occupancy or restroom use data were collected at the time. Also collecting physico-chemical data would have allowed investigating other potential drivers of the community. Furthermore, as previously mentioned faucet water may be sampled to determine its contribution to bacterial communities. While it is beyond the scope of this study, additional high throughput "omics" approaches such as metatranscriptomics may prove to be useful in identifying overall community activities in the sinks.

Overall, the results of this study showed diverse as well as highly variable taxonomic profiles among individual sinks while the differences between buildings were marginal indicating not too dissimilar bacterial community composition and structure. Below-strainer and P-trap were shown to differ in their bacterial communities and specific taxa were found to be more relatively abundant in either of the drain types. Variation in community structures particularly within a given building could be attributed to differences in human occupants since human skin was a primary contributor. This emphasizes the importance of external sources to the sink especially, those arising from human origin. These findings provide the foundation for subsequent studies that might explore community stability and resilience of in situ sinks, as well as defining what constitutes a viable population of this understudied ecosystem.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

AUTHOR CONTRIBUTIONS

ZW performed the field work and the molecular work in the laboratory. TG performed DNA quantification and sequencing. ZW performed the sequence analysis, statistical analyses, interpreted the statistical findings, and wrote the manuscript. ZW, SM, and HSG contributed to the final preparation of the manuscript. HSG devised the project and the main conceptual ideas.

DATA AVAILABILITY STATEMENT

The raw sequence data reported in this study have been deposited in the European Nucleotide Archive under the accession number PRJEB42256. The relevant information for each sample is shown in Table S5.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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