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ORIGINAL ARTICLE

Mycobial community assemblages in sink drains across a university campus

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Abstract

Multiple fungal species, including potential opportunistic pathogens have been previously identified in water systems. Here, we investigated over 250 restroom sink fungal communities across a university campus and evaluated their diversity and core taxa present. Remarkable similarity in mycobial community composition was observed across buildings with Ascomycota consistently dominating. We found a core mycobiome independent of the building sampled, that included Exophiala species, potential opportunistic pathogenic black yeasts. Other prevalent and dominant taxa included *Saccharomyces* and *Fusarium*, common built environment fungi. The frequent presence of *Malassezia*, a common skin commensal, showed the external influence of human activities as a source of fungi to sinks. The study represents a novel exploration of sink P-traps mycobial communities from a public area and highlights their importance as reservoirs of possible pathogenic fungi, as well as emphasizing the relevance of further research in this understudied ecosystem within the built environment.

KEYWORDS
built environment, fungi, mycobial community, mycobiome, next-generation sequencing, sink P-trap

1 | INTRODUCTION

Buildings have become our most intimate ecosystems, and our interactions with microorganisms that colonize the built environment (BE) can help shape our microbiome and can have effects on inhabitants' health. Fungi are a highly diverse domain, and their presence has long been established in the BE (Solomon, 1975). Previous studies have shown the BE mycobiome is composed mainly of saprotrophs; mold and yeasts such as *Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium*, and *Wallemia* (Martin-Sanchez et al., 2021; Ren et al., 2001; Samson et al., 2011; Taylor et al., 2014). Research has focused on buildings with excess moisture due to leaks caused by building damage, plumbing faults, or condensation (Adams et al., 2020; Jayaprakash et al., 2017; Pasanen et al., 2000; Sudakin, 1998; Torvinen et al., 2006; Trout et al., 2001). Under these conditions, fungi can flourish and function as sources of indoor pollutants by emitting spores, fungal fragments, mycotoxins, and volatile organic compounds which can exacerbate the onset of disease including asthma, trigger allergies, and have been associated with sick building syndrome and other respiratory diseases (Cooley et al., 1998; Fu et al., 2021; Karvala et al., 2010; Li & Yang, 2004; Simon-Nobbe et al., 2008; Soeria-Atmadja et al., 2010; Trout et al., 2001). Besides the health concerns, fungi can also cause structural damage to buildings resulting in considerable economic costs (Gámez-Espinosa et al., 2020; Haas et al., 2019; Schmidt, 2007).

Seasonal patterns, environmental gradients and other extrinsic factors primarily determine the indoor fungal diversity and composition, but more local features such as building function and construction can also contribute to shaping the mycobiome within individual buildings (Adams et al., 2013a, 2014, 2016; Amend et al., 2010; Barberán, Ladau, et al., 2015; Martin-Sanchez et al., 2021; Stephens, 2016; Wong et al., 2008). Outdoor air is an important source of indoor fungi. Culturable and non-culturable fungi concentrations and composition of species correlate in outdoor and indoor air and other BE surfaces (Adams et al., 2014, 2013a, 2013b). However, the most common indoor fungi are not necessarily identical to that of outdoors; for example, *Penicillium* is usually more common in indoor air (Hyvarinen et al., 1993; Li & Kendrick, 1995). Interestingly, while occupants are the primary source of bacteria to the BE (Hospodsky et al., 2012; Lax et al., 2014; Meadow et al., 2014), residents have been shown to either minimally (Adams et al., 2014) (Adams et al., 2014) influence or not determine fungal community structure (Dannemiller et al., 2016; Martin-Sanchez et al., 2021). A study comparing indoor air (private homes) and outdoor air, revealed a positive correlation between occupants and mycobiome composition (Martin-Sanchez et al., 2021). The study showed that increased number of occupants resulted in higher exchange and transport of air particles which drove indoor communities toward outdoor species composition. It is evident that humans can be a direct source of fungi especially dermatophytes such as *Malassezia* (Adams et al., 2013b; Pitkäranta et al., 2008). Restroom surfaces in particular were found to host highly diverse mycobiomes, and evidence suggests that they are sourced from human activities such as shoes (Fouquier et al., 2016).

The plumbing or water distribution systems (WDS) are one of the most favorable environments for microbial growth in healthy buildings (Adams et al., 2013b). Experiments with temporarily wetted surfaces have shown to encourage the growth of fungi within days or weeks (Pasanen et al., 1992). Endogenous growth has been shown on sink surfaces, in sink drains and the wider WDS (Adams et al., 2013b; Hamada & Abe, 2010; Short et al., 2011; Zupančič et al., 2016). Adams et al. (2013b) revealed differences in drains between kitchens and bathrooms in private homes and suggested a distant drain niche due to the high frequency of which thermotolerant fungi were observed, namely *Fusarium* and *Exophiala*. Aerosolization of fungal material rather than direct contact poses a greater risk for health (Górny et al., 2002; Kuhn & Ghannoum, 2003), and WDS including sinks have demonstrated aerosolization of fungi resulting in adverse effects on health (Anaissie, Kuchar, et al., 2001; Anaissie, Stratton, et al., 2001; Chang et al., 2006; Short et al., 2011). Moreover, drains have been suggested to be a reservoir of potentially serious fungal pathogens that could result in outbreaks through droplet-mediated dispersion (Hino et al., 2020). Despite the importance, there has been relatively little research into how fungal communities in WDS and drainage piping are structured, particularly in the public domain. In this study, we investigated mycobial community composition and structure of sink P-traps distributed across a university campus, specifically addressing the following objectives: (i) which fungi dominate P-trap mycobiome and do they correspond to taxa previously found in similar environments; (ii) whether the identified dominant taxa are found ubiquitously across all sinks; and (iii) how the mycobial communities are structured and whether or not they are influenced by the BE types.

2 | METHODS

2.1 | Sample collection and DNA extraction

Samples from P-traps were collected from 20 different buildings across the University of Reading's Whiteknights campus during early November 2021. All buildings selected had accessible restrooms. Buildings selected were mainly those used for teaching; however, some buildings were used for dining or recreational activities. A total of 412 samples were collected. The methods for collecting P-trap samples were the same as described in Withey et al., 2021. Briefly, sterile cotton swabs were inserted using a sampling rod into the P-Traps and circumference of pipe swabbed for 5 s. Swabs were stored in 1.5 ml tubes in a freezer at −20°C until required for DNA extraction. Metadata was recorded on each of the swabs collected (Table S1). Genomic DNA was isolated from the swabs using HigherPurity Soil DNA Isolation kit (Canvax Biotech), according to the manufacturer's protocol. Negative controls were blank swabs extracted by the same method.

2.2 | PCR amplification and Illumina sequencing

The ITS2 region of the extracted DNA was amplified using forward primer fITS7 (GTGARTCATCGAATCTTTG) and reverse primer ITS4 (TCCTCCGCTTATTGATATGC) (Ihrmark et al., 2012). Each PCR reaction contained the following components; 22 µl of ReadyMix Taq PCR Reaction Mix (Sigma-Aldrich), 0.5 µl of each 10 µM forward and reverse primers, 5 µl of template DNA, and 22 µl of UltraPure DNase/RNase-free distilled water (Invitrogen). Thermocycling conditions were 30 s initial denaturation at 95°C, followed by 35 cycles of 30 s denaturation at 95°C, 30 s annealing at 50°C, 2 min extension at 72°C, and a final elongation at 72°C for 5 min. PCR reactions included negative template controls in which the template DNA was replaced with 5 µl of UltraPure DNase/RNase-free distilled water to ensure PCR reagents and equipment were not contaminated. After PCR amplification, PCR products were purified with Agencourt AMPure XP magnetic beads (Beckman Coulter).

Samples that did not amplify, and those post clean-up that had no band present on gel were excluded from barcoding and subsequent sequencing. Those samples that did not amplify were

mostly associated with particular buildings (Table S2). A total of 343 purified PCR products underwent a second PCR reaction to add Illumina-specific adapters and unique barcodes. In short, 25 µl reaction mixtures were prepared by adding 9.5 µl of ReadyMix Taq PCR Reaction Mix (Sigma-Aldrich), 2.5 µl of both forward index and reverse index primers (4 µM each), 9.5 µl Nuclease-free water and 1 µl of the purified PCR product. The thermocycle conditions for the second round of PCR were initial denaturation of 95°C for 2 min, and then 8 cycles of denaturation at 95°C for 15 s, annealing at 55°C for 30 s and extension at 72°C for 30 s, followed by a final extension of 72°C for 10 min. NGS normalization 96-Well Kit (Norgen) purified and normalized the samples before being pooled. An amplicon library spanning ITS2 region was sequenced at a concentration of 10 pM and merged with 5% PhiX on an Illumina Miseq platform using V3 chemistry (Illumina Inc.) at UK Centre for Ecology & Hydrology.

2.3 | Bioinformatics pipeline and statistical analyses

The obtained sequenced paired-end reads were processed using PIPITS (Gweon et al., 2015). All further data processing and statistical analysis were performed in R, version 3.6.3 (R Core Team, 2022) through RSTUDIO.

Phyloseq version 1.30.0, Tidyverse version 1.3.1, and vegan version 2.5.7, were used for data manipulation, plotting, and ecological analyses (Mcmurdie & Holmes, 2013; Oksanen et al., 2020; Wickham et al., 2019). Plots were further refined, and results visualized using ggplot2 version 3.3.5. Initially, low abundant OTUs (<10 reads) were removed from the ITS data, to reduce spurious taxa, and only OTUs identifiable to phylum were included for analysis. Three buildings were then removed from subsequent statistical analysis due to 5 or less samples remaining after rarefaction.

Beta diversity was evaluated and visualized with non-metric multidimensional scaling (NMDS) ordination of sink samples using Bray–Curtis dissimilarity distances and Jaccard indices constructed using the *vegdist* function. To assess the correlation between environmental variables (Building and Gender of restroom sampled) permutational multivariate analysis of variance (PERMANOVA; 999 permutations) was performed individually on the two variables using *adonis*. Additionally, Tukey's test was used for post-hoc analysis to further investigate the significant differences or similarities between pairs of buildings. *Betadisper* was used to test the homogeneity of variance among groups and analysis of variance (ANOVA) tested for the significant difference in these variances. Alpha diversity was also assessed by calculating species richness (number of OTUs), Shannon diversity and Pielous evenness. Significant differences in alpha diversity across building and restroom gender were calculated using the non-parametric Kruskal-Wallis test. Taxonomic analysis of the data was performed from Phylum to Genus and core mycobiome identified by their prevalence and relative abundance. *Plot_core* from the microbiome package version 1.8.0, was applied to visualize the core OTUs (Lahti & Shetty, 2017).

3 | RESULTS

3.1 | Data features

After bioinformatic processing through PIPITS, the fungal dataset contained 3862 OTUs (9,265,250 sequences), distributed across 343 samples from 20 buildings throughout the University of Reading's campus. The number of reads per sample varied between 2 and 81,693 (mean/median = 27,012/27,215). Rarefying to an even sequencing depth of 5000 reads per sample resulted in 42 samples being removed (301 samples remaining) (Figure S5). Furthermore, removal of buildings with not enough individual samples resulted in a total of 289 samples for downstream analysis. The remaining data comprised 2432 OTUs, with an average of 217 OTUS per sample (Min 36 OTUs, Max 417 OTUs) (Table S3). The highly abundant fungal OTUs (relative abundance below 1%) were also widely distributed (prevalence of 50% or more). Of the OTUs assigned to the domain fungi, there were seven identifiable phyla. Those identified to phylum, were further classified into 25 known classes, 88 orders, 220 families, 375 genera and 605 species (>85% identity).

3.2 | Taxonomic distribution

The fungi identified to Phylum were represented by seven phyla, of which two accounted for the majority of taxa (<99%); Ascomycota (91.89%) and Basidiomycota (7.99%). Ascomycota dominated across all buildings sampled (Figure 1a; Figure S1a). The top three classes were Sordariomycetes (39%), Eurotiomycetes (24.37%) and Saccharomycetes (12.46%). The main orders were Hypocreales (37.26%), Chaetothyriales (23.9%), Saccharomycetales (12.46%). The dominant identifiable families were *Nectriaceae* (21.87%), *Herpotrichiellaceae* (20.06%) and *Saccharomycetaceae* (10.94%). Of the 375 genera classified, *Exophiala* (19.33%), *Saccharomyces* (10.92%), *Fusarium* (5.36%), *Cyphellophora* (3.42%), *Malassezia* (2.87%), *BisiFusarium* (1.51%), and *Ramularia* (1.35%) had a relative abundance >1%. The majority of the genus *Exophiala* was identified as the species *Exophiala lecanii-corni* (61.2% of the reads classified as the genus *Exophiala*). *Exophiala lecanii-corni* was the top identifiable species and accounted for 11.84% of reads across all species. The OTUs that had >1% RA accounted for 60.82% of all reads (Table 1). Moreover, the phyla Ascomycota was highly prevalent (100% of samples) and, across buildings a notable similarity was observed in phyla and family taxonomic compositions as well as at the genus level when looking at the average relative abundance (Figures 1b and 2a; Figure S1b). However, taxonomic analysis of individual samples showed variation in relative abundances of the top genera between some sinks within a building (Figures S2 and S3, Table S4).

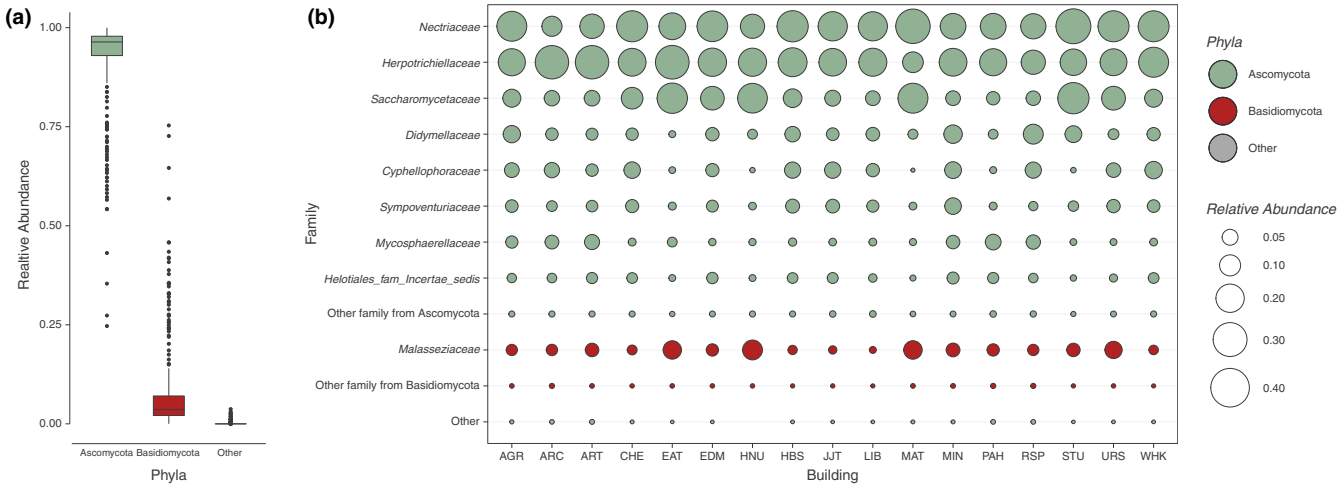

FIGURE 1 Taxonomic analysis. (a) Boxplot showing the distribution of the dominant phyla. "Other" represents remaining 5 phyla. (b) Bubble plot of mean relative abundance of the most abundant fungal families (>1%) by building. Across all buildings, the mean distribution of families is generally uneven as a few taxa tend to dominate. No strong compositional difference is observed between buildings based on families when comparing mean relative abundances. Circle size indicates relative abundance and color of bubble represents the phylum from which the family is found. Abbreviations on x-axis correspond to the following buildings; AGR, Agriculture; ARC, Archaeology; ART, Art; CHE, Chemistry; EAT, Eat at the Square; EDM, Edith Morely; HNU, Harry Nunsten; HBS, Henley Business School; JJT, JJ Thompson; LIB, Library; MAT, Maths; MINL, Mingella; PAH, Park House; RSP, Sports Park; STU, Student Union; URS, URS; WHK, Whiteknights.

TABLE 1 Identity of top OTUs (>1% relative abundance). Overall abundance (total percentage of reads) and prevalence shown

		Total reads (%)	Prevalence (%)
OTU2835	f_Nectriaceae	11.67	88.59
OTU1942	g_Saccharomyces	9.89	96.31
OTU2067	o_Hypocreales	9.07	89.60
OTU956	s_Exophiala_lecanii-corni_SH1508706.08FU	6.59	88.59
OTU1988	o_Hypocreales	3.38	71.48
OTU1844	s_Cyphellophora_europaea_SH1636081.08FU	2.90	60.07
OTU2526	f_Didymellaceae	2.69	71.81
OTU712	s_Exophiala_aquamarina_SH1240520.08FU	2.13	65.77
OTU1710	g_Fusarium	2.01	85.23
OTU196	s_Malasseziaceae_sp_SH1547563.08FU	1.90	91.28
OTU1289	f_Sympoventuriaceae	1.80	65.77
OTU1713	g_Bisifusarium	1.51	70.13
OTU1607	g_Fusarium	1.49	79.53
OTU3500	f_Helotiales_fam_Incertae_sedis	1.35	57.72
OTU1264	s_Exophiala_equina_SH1635779.08FU	1.35	56.04
OTU919	s_Exophiala_phaeomuriformis_SH1529587.08FU	1.09	52.01

3.3 | Core mycobiome

Thousand eight ninty one OTUs were found in <10% of samples. No OTU was identified in all samples, however the three OTUS with RA > 1% were present in 90% or more of sinks samples. The most prevalent OTU (OTU1942, 96% of samples) was also the second most abundant and was classified to the genus *Saccharomyces* (Table 1). A core microbiome analysis was performed to check the prevalence of OTUs across sinks sampled. An OTU was considered part of the core mycobiome if it was present in at least 80% of samples. Eight OTUs were considered part of the core mycobiome (Figure 3). Following OTU1942 (classified as g_*Saccharomyces*), maximum prevalence was shown by OTU196 (91% of samples, classified as g_*Malassezia*), OTU2067 (90%, o_Hypocreales), OTU2835 (89%, f_*Nectriaceae*), OTU956 (89%, s_*Exophiala_lecanii-corni_SH1508706.08FU*), OTU1710 (85%, g_*Fusarium*), OTU204 (84%, g_*Saccharomyces*), OTU1607 (80%, g_*Fusarium*). The second most prevalent OTU was classified to the genus *Malassezia*. The remaining six core OTUs corresponded to

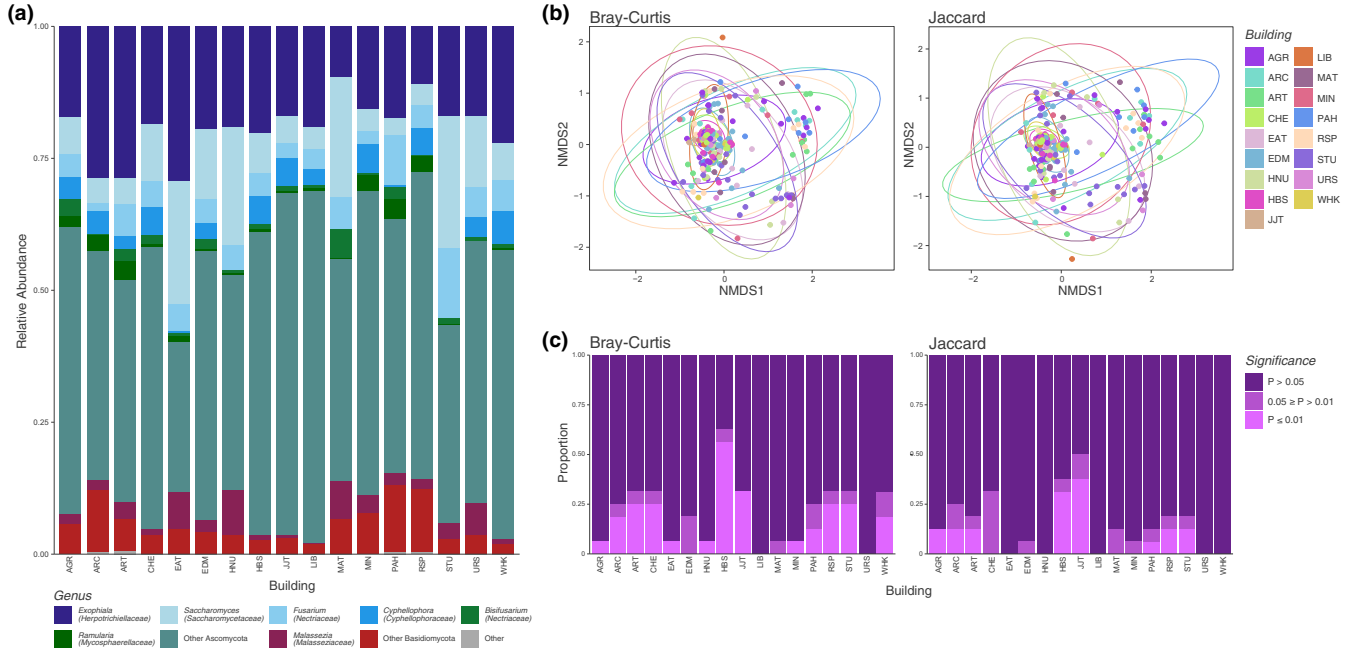

FIGURE 2 Composition of mycobial communities by building. (a) Fungal composition: Relative abundances of top genera (>1%) by building shown. Family of genera is italicized and in brackets below genus in the legend. (b) Beta diversity. Non-metric multidimensional scaling (NMDS) plots of dissimilarity metrics. Each point represents a sample; color indicates building. (left) Bray-Curtis (abundance) and (right) Jaccard (presence-absence). (c) Post-hoc Tukey analysis: Percentage on *y*-axis of non-significant ($p > 0.05$), significant ($0.05 \geq p > 0.01$), and highly significant ($p \leq 0.01$), as indicated by color, building interactions. Henley business school (HBS) had the highest percentage of significant values (50% or more) therefore, its composition significantly differed from half or more of the buildings. Building abbreviations as follows; AGR, Agriculture; ARC, Archaeology; ART, Art; CHE, Chemistry; EAT, Eat at the Square; EDM, Edith Morely; HNU, Harry Nunsten; HBS, Henley Business School; JJT, JJ Thompson; LIB, Library; MAT, Maths; MINL, Mingella; PAH, Park House; RSP, Sports Park; STU, Student Union; URS, URS; WHK, Whiteknights.

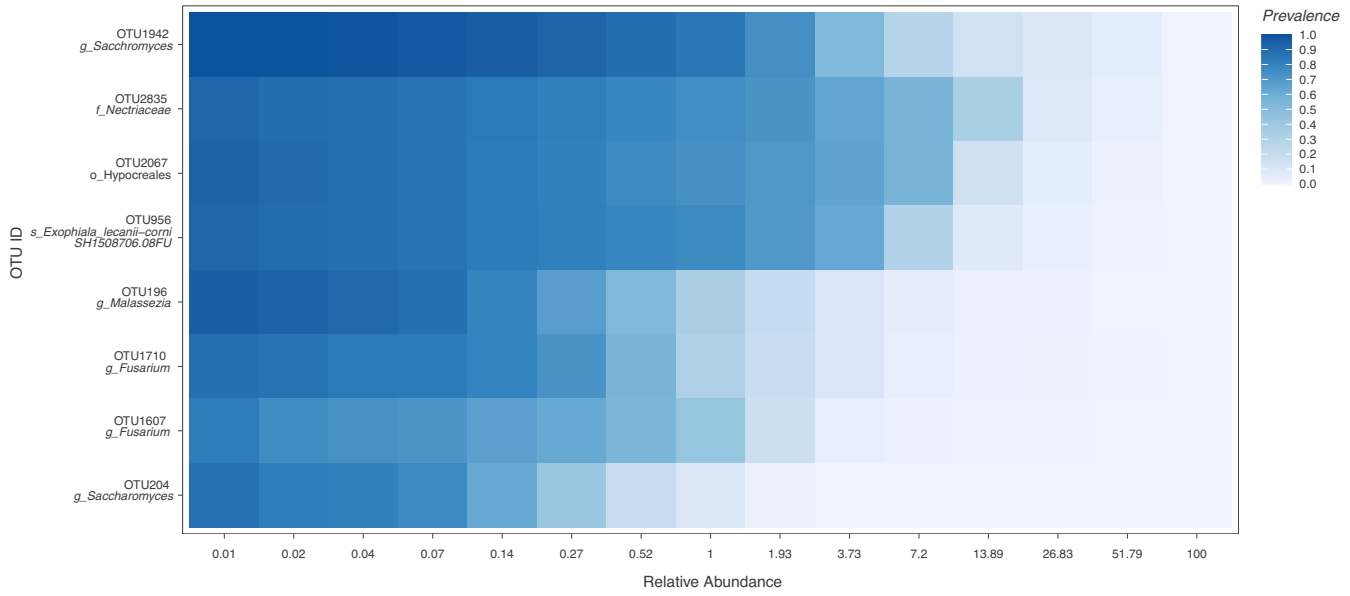

FIGURE 3 Heatmap of the core microbiome analysis. Shows the eight OTUs that were considered part of the core mycobiome (>80% prevalence of 289 samples). The *y*-axis shows the eight core OTUs. The relative abundance derived from count data is plotted on the *x*-axis. The gradient of color indicates the variation of prevalence of each OTU.

three orders Saccharomycetales (one OTU), Hypocreales (four OTUs), Chaetothyriales (one OTUs). Although these eight OTUs represent a small fraction of the total number of OTUs they were among some of the most abundant OTUs (together accounting for 42.97% of all reads). If the threshold for what was considered a core OTU was lowered to more than 70%, 30 OTUs would be deemed core.

3.4 | Mycobiome composition and diversity

Associations of microbiome compositions with factors were assessed qualitatively and quantitatively using PERMANOVA and two beta-diversity metrics (Bray-Curtis distance and Jaccard index), respectively. For both metrics, there was no clear separation observed in the NMDS plot of samples by their building (Figure 2b). PERMANOVA showed groups to be significantly different when samples were grouped by building (F.model = 2.379, $R^2 = 0.12643$, $p = 0.001$ (Bray-Curtis); F.model = 1.6981, $R^2 = 0.09364$, $p = 0.001$ (Jaccard)); however, only a low proportion of the variance in mycobial community composition was explained. A post-hoc Tukey test showed that a few specific buildings were significantly different from others and could be partly accountable for the significant PERMANOVA result (Figure 2c, Table S5). But overall, post-hoc analysis showed that the majority of buildings were not significantly different from one another. One building in particular, Henley Business School (HBS) differed significantly from 50% or more of buildings. However, removing this building from PERMANOVA did not change the overall result F.model = 2.0739, $R^2 = 0.11267$, $p = 0.001$ (Bray-Curtis); F.model = 1.5774, $R^2 = 0.08807$, $p = 0.001$ (Jaccard). There were also significant differences in beta diversity (homogeneity of group dispersions) between the buildings (ANOVA, $df = 16$, $F = 6.9652$, $p < 0.001$ (Bray-Curtis); $df = 16$, $F = 5.7269$, $p < 0.001$ (Jaccard)) (Figures S4 and S6). It is important to note that PERMANOVA is sensitive to heterogeneous group dispersions within an unbalanced design (Anderson, 2017), and the unequal number of samples across buildings could be partially responsible for the significant differences between the buildings. PERMANOVA is conservative when high dispersions occur in larger groups and liberal when high dispersions occur in smaller groups (Anderson & Walsh, 2013). High dispersion was observed in many of the smaller groups (i.e., Art and Math), potentially causing increased rejection rates of the null hypothesis, thus more likely to find a significant result. Gender had no significant effect on community composition (PERMANOVA, F.model = 0.98694, $R^2 = 0.01064$, $p = 0.469$ (Bray-Curtis); F.model = 0.97977, $R^2 = 0.01054$, $p = 0.49$ (Jaccard)), and their dispersions were homogenous when using both indices (ANOVA, $df = 3$, $F = 2.5618$, $p = 0.05519$ (Bray-Curtis); $df = 3$, $F = 1.4294$, $p = 0.2344$ (Jaccard)).

Variation in alpha diversities across the buildings sampled were analyzed (Figure 4). Among buildings, Henley Business School (HBS) was observed to have the highest mean richness (mean 295 OTUs). This finding was replicated with the two other alpha-diversity metrics. Whereas, Student Union (STU) was found to have the lowest means for all alpha diversity metrics. Kruskal-Wallis tests were used to determine the influence of building on community alpha-diversity (Figure 4). OTU richness, diversity (Shannon) and Pielou's evenness differed significantly by building. Pairwise comparisons for buildings were calculated using Wilcoxon tests for each of the alpha diversity metrics (Table S6). Multiple pairs of buildings were highly significant from one another which may contribute to the overall significant difference across all buildings. No significant associations of alpha diversity were detected with restroom gender ($df = 3$, Observed $p = 0.09388$, Shannon diversity index $p = 0.09433$, Pielou's evenness $p = 0.1852$).

4 | DISCUSSION

Sinks, drains and their associated pipes offer a unique niche in the BE due to their continuous moisture, temporary fluctuations in temperature, high pH due to regular use of detergents and potentially increased concentrations of organic matter. In this study, we observed that the sink P-traps of various university buildings harbored diverse mycobial communities which were markedly similar between most buildings. There was a distinct core mycobiome with the most dominant taxa present across the majority of samples (>70%). Drains in residential settings were previously established to have shown clear evidence of both, harboring fungi due to deposition patterns and endogenous growth (Adams et al., 2013b). This was reflected in the public P-traps of this study with similar taxa identified, namely *Exophiala*, *Fusarium* and *Malassezia*. Also, overlapping with taxa in the above-mentioned study, taxa found also matched those found in other cultures and culture-independent studies of fungi identified in the BE, specifically restroom and plumbing environments.

In our study, of the identifiable genera, *Exophiala* was found to be the most abundant and ubiquitous. *Exophiala* is a saprotrophic "black yeast" and includes both terrestrial and waterborne species. It has also been shown to be oligotrophic, thermotolerant, survive high pH, and able to utilize surfactants as a source of carbon, namely detergents (Hamada & Abe, 2009; Isola et al., 2013; Nishimura et al., 1987; Zalar et al., 2011). *Exophiala* species can be considered opportunistic pathogens causing cutaneous and superficial infections (Chromomycosis) however, fatal systemic infections have been documented (Fothergill, 1996; Gold et al., 1994; Greig et al., 2003; Hiruma et al., 1993; Hopf et al., 2020; Martínez-González et al., 2008; Nachman et al., 1996; Woo et al., 2013; Zeng et al., 2007). This genus has previously been isolated from other water sources in the BEs such as, dishwashers, steam bath facilities, swimming pools, bathrooms, and associated drainpipes (Babič et al., 2015; Hamada & Abe, 2009; Lian & de Hoog, 2010; Matos et al., 2002; Nishimura et al., 1987; Porteous et al., 2003; Ruoff, 2002; Zalar et al., 2011). As well as from tap water and public drinking reservoirs (Biedunkiewicz & Schulz, 2012; Göttlich et al., 2002; Heinrichs, Hübner, et al., 2013; Heinrichs, Hü, et al., 2013). The most common identifiable species present in sinks P-traps was *Exophiala* lecanii-corni which was formerly proven to be a dominant component of water tap biofilms (Heinrichs, Hü, et al., 2013). Moreover, it is known to efficiently remove volatile organic compounds (VOC) from the air, therefore potentially explaining its dominance in biofilms growing at the water-air interface (Pirnie-Fisker & Woertz, 2007; Woertz et al., 2001). *Exophiala lecanii-cornii* has been reported to mainly result in superficial mycoses affecting skin and nails but, in a rare occurrence caused keratitis (Lee et al., 2016; Miyakubo et al., 2020; Zeng et al., 2007). *Exophiala*'s widespread distribution across a variety of indoor water

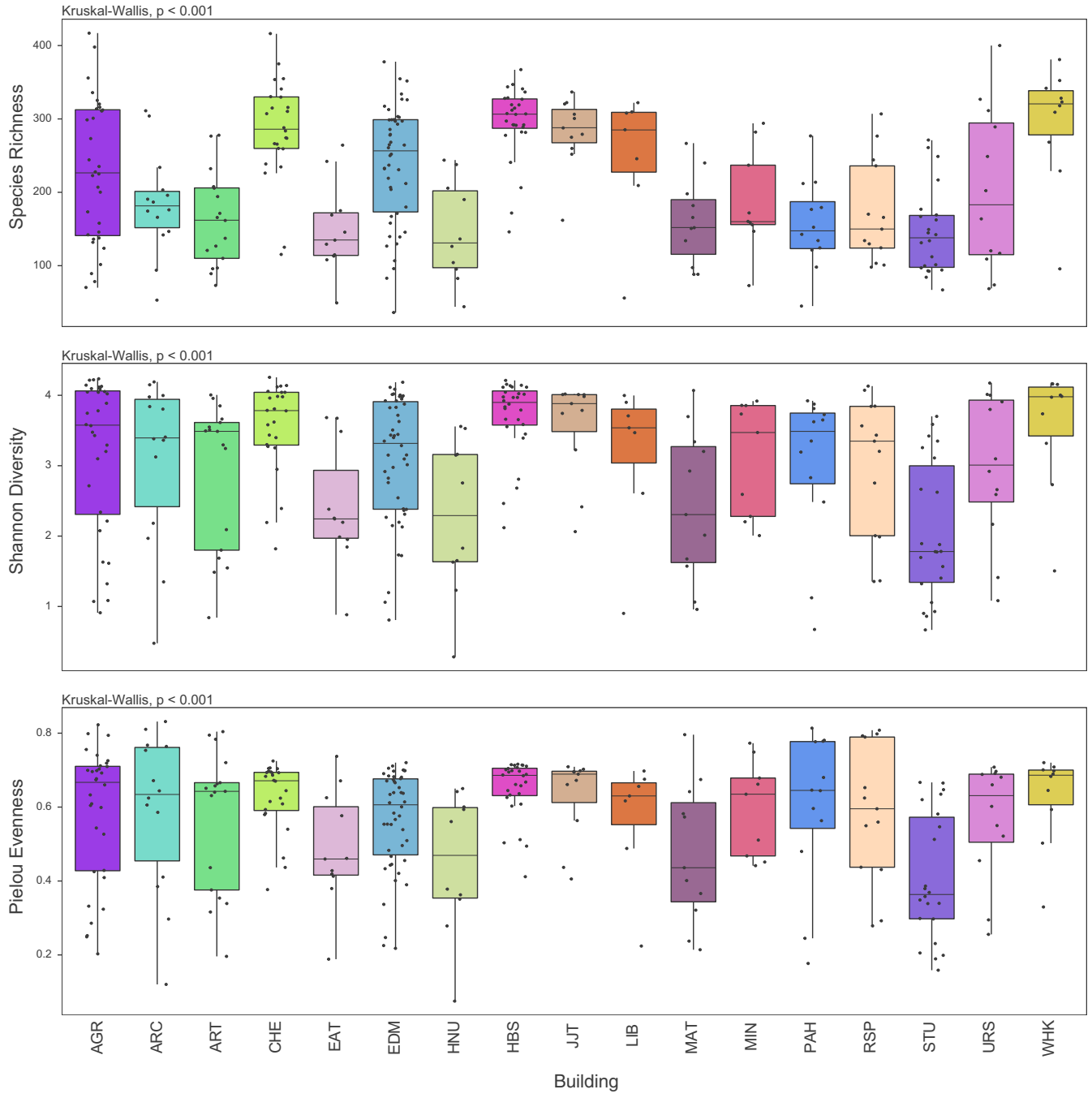

FIGURE 4 Fungal alpha diversity. Boxplot of alpha diversity of fungal communities by building sampled. Species richness (number of OTUs), Shannon and Pielou's evenness shown. Each point represents a sample. *p*-value obtained from Kruskal-Wallis test shown above each plot.

source environments, and its ability to survive more challenging ecological pressures results in its unsurprising presence and dominance across sinks samples.

The second most dominant classifiable genus was *Saccharomyces*, and like *Exophiala* was highly prevalent (96% of samples). *Saccharomyces* is a common genus in indoor environments (i.e., dust) and is usually associated with humans (Barberán, Dunn, et al., 2015; Barberán, Ladau, et al., 2015; Dannemiller et al., 2016; Estensmo et al., 2021; Fouquier et al., 2016; Gupta et al., 2020; Martin-Sanchez et al., 2021; Viel et al., 2017). Fouquier and colleagues identified it as the most abundant and ubiquitous fungi in restroom floors. Furthermore, the most prevalent OTU (OTU1942) belonged to this genus, and was also the second most abundant OTU. OTU1942 was blasted against the NCBI database and classified as *Saccharomyces cerevisiae* at 97.05% percentage identity giving some clarity on what this OTU might be or its closest relative. *S. cerevisiae* is found in many natural niches in the environment and is also known for being a common fruit-associated fungus, gastronomically relevant, and is used in

research laboratories (Moon & Lo, 2014). Similar to *Exophiala* spp., *S. cerevisiae* can utilize VOCs and is also tolerant to metals (Krauter & Krauter, 2002; Pirnie-Fisker & Woertz, 2007).

Fusarium of the family *Nectriaceae* (most abundant family in present study) was another highly prevalent and abundant genus. Members of the family *Nectriaceae* are important plant and human pathogens, specifically, some *Fusarium* spp. are emerging fungal pathogens of increasing importance (Batista et al., 2020; Garber, 2001; O'Donnell et al., 2010; Pfaller & Diekema, 2004). It is thought that there are approximately 10 *Fusarium* species complexes that are related to human pathogens, of these, the notable two complexes are members of the *Fusarium solani* species complex (FSSC), and the *Fusarium oxysporum* species complex (FOSC) which together comprise ~80% of infections (Batista et al., 2020). Moreover, certain FSSC and FOSC appear to be common in water systems, including those of hospitals, posing a significant risk for nosocomial infections (Anaissie, Kuchar, et al., 2001; Babič et al., 2015; Hageskal et al., 2006; O'Donnell et al., 2004, 2007; Oliveira et al., 2016; Short et al., 2011). Infections caused by *Fusarium* spp. range from superficial and locally invasive to disseminated (van Diepeningen et al., 2015). For example, infections can vary from melanonychia to sinusitis to neutropenia (Anaissie & Nucci, 2002; Lee et al., 2002; Nucci & Anaissie, 2007). Additionally, the most abundant OTU (OTU2835) was classified to the family *Nectriaceae*. Upon blasting against NCBI database this OTU was further identified as a *Fusarium* (closest relative was *Fusarium foetens*, 96.71% percentage identity). Thus, the overall relative abundance of the genus *Fusarium* may be underrepresented, as only OTUs classified to genus were included. Therefore, the overall relative abundance of *Fusarium* may be similar to that of *Exophiala* (~19%). Alongside *Exophiala*, *Fusarium* was more frequently detected in drains of bathrooms and kitchens when compared to other residential surfaces and, in another bathroom study, *Fusarium* was identified as one of the most common fungi (Adams et al., 2013b; Hamada & Abe, 2009). It is worth noting, however, that the ITS region has been shown to work poorly in differentiating between species of *Fusarium* as well as other highly speciose genera including *Aspergillus*, *Fusarium*, *Penicillium* and *Trichoderma* (Al-Hatmi et al., 2016; Stielow et al., 2015).

The remaining top genera from the phylum Ascomycota; *Cyphellophora*, *BisiFusarium* and *Ramularia* have been found in the BE. *Cyphellophora* and *BisiFusarium* have been identified in drinking and environmental water supplies, indoor water fittings, and drain outlets (Babič et al., 2017; Góralska et al., 2020; Heinrichs, Hü, et al., 2013; Hino et al., 2020; Lian & de Hoog, 2010). Moreover, *Cyphellophora* is another black yeast-like fungi, with several species previously isolated from clinical samples, mostly nails and skin (Feng et al., 2014; Lian & de Hoog, 2010). The genus *Ramularia* includes numerous plant pathogens, and its presence has been detected in indoor dust (Adams et al., 2020; Martin-Sanchez et al., 2021; Videira et al., 2016).

Notably, *Malassezia* was frequently detected. *Malassezia* are dominant members of the human skin mycobiome; therefore, their presence in P-traps is expected due to the shedding of fungi from skin during handwashing (Findley et al., 2013; Hospodsky et al., 2012; Theelen et al., 2018; Xu, 2015). This is further supported by Adams et al., who detected *Malassezia* in bathroom drains but not kitchen drains (Adams et al., 2013b). These commensal yeasts can be associated with common skin disorders such as dandruff and eczema (Thayikkannu et al., 2015; Theelen et al., 2018). Additionally, *Malassezia* has been shown to be far more abundant in indoor dust than outdoors and particularly abundant in bathrooms (Martin-Sanchez et al., 2021). Surprisingly, the study of restroom surfaces found only trace evidence of *Malassezia*, however, the samples analyzed were limited to one surface, floors, as the other two surfaces tested did not yield many fungi (Fouquier et al., 2016). The other two surfaces were those in contact with skin more frequently, toilet seats and soap dispensers. However, these exposed dry surfaces may not provide ideal conditions for sustaining microbial life. Furthermore, multiple species of *Malassezia* have demonstrated adherence to and formation of biofilms on abiotic surfaces, namely polyurethane (Angiolella et al., 2018; Cannizzo et al., 2007; Zareei et al., 2018), suggesting that they are capable of colonizing P-traps.

Overall, taxa that dominated, consistently had high prevalence and have been previously identified in other similar wet indoor environments. The black yeasts from *Exophiala*, the filamentous fungi of *Fusarium*, and the white yeast from *Saccharomyces* were common inhabitants of P-traps and have all been retrieved from tap water (Anaissie, Kuchar, et al., 2001; Gonçalves et al., 2006; Göttlich et al., 2002; Hageskal et al., 2007, 2009). Their large contribution to the total composition of P-traps was expected and agrees with published research, specifically, studies that sampled the external drain of domestic sinks (Adams et al., 2013b).

The most striking findings from our results was that there was little difference in mycobial communities between buildings. While we cannot suggest what variables are specifically responsible for the differences between buildings due to lack of metadata collected, we speculate that the sinks sampled across a campus will largely experience similar usage as they are primarily for handwashing and under a strict as well as consistent cleaning regime. Gender of restroom had no effect on mycobial community composition. Previous studies have shown that there was no difference in bacterial and fungal communities between male and female restroom floor surfaces (Fouquier et al., 2016; Gibbons et al., 2015). It is also worth mentioning that this was the case for bacterial communities in P-traps (Withey et al., 2021).

Here, we provide a first insight into the mycobial communities of sink P-traps across publicly accessible and frequently used restrooms. The large sample size, in comparison to previous studies of domestic drains, has permitted a more extensive and generalizable observation of the communities present. Future studies may determine the community formation, stability over time, and responses to perturbations or stressors such as increased vigor and frequency of cleaning regimes. Furthermore, understanding mechanisms and routes of dispersion for fungi from sinks into the surrounding environment particularly in public areas is essential. This knowledge will inform future architectural and sink design, mitigation and

prevention of any prospective outbreaks. Little is known about the microbiology of sinks and their associate pipes, which we encounter in everyday life. Our findings present a glimpse of the mycobial community present in these understudied environments. Overall, we found that a diverse community of fungi are present in many sink P-traps, and P-traps appear to share similarities in their compositions, suggesting some stability to perturbations from differing sink usage. We also found that potentially pathogenic black fungi were prevalent in P-traps. Occurrence of black fungi in healthcare facilities with a large number of immunocompromised patients is of concern, but in areas such as universities the risk may be negligible. That said, maintaining good hygiene practices and regular cleaning should not be ignored.

AUTHOR CONTRIBUTIONS

AA, ND, EF, NJM, EN performed the fieldwork and majority of the molecular work in the laboratory. ZW assisted with remaining molecular work and performed library preparation. TG performed DNA quantification and sequencing. Bioinformatics processing of the data was performed by HSG. Sequence analysis, statistical analyses and interpretations were performed by ZW. ZW wrote the manuscript in consultation with HSG. HSG conceived the study and was in charge of overall direction and planning. All authors discussed the results and commented on the manuscript.

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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.

DATA AVAILABILITY STATEMENT

The sequencing data have been deposited with links to BioProject accession number PRJNA860571 in the NCBI BioProject database (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA860571). The relevant information for each sample is shown in Table S1.

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