

Longitudinal bacterial community dynamics and sodium hypochlorite intervention in a newly built university building

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Longitudinal bacterial community dynamics and sodium hypochlorite intervention in a newly built university building

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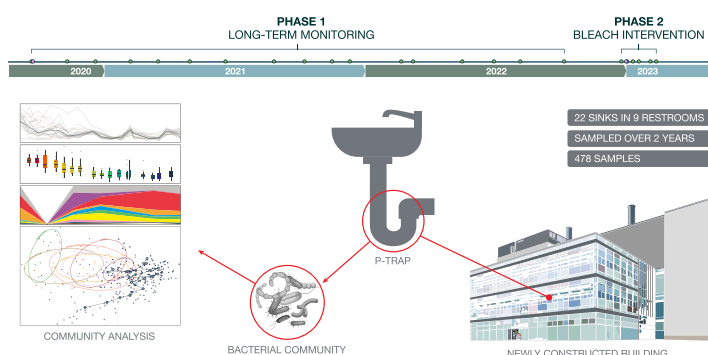
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HIGHLIGHTS

- Sink wastewater bacterial communities in new building sampled over 2.5 years
- Bacteria recover to pre-treatment levels in four weeks after bleach intervention
- Homogenisation of microbial communities observed
- Identified dynamics of key dominant bacterial families in P-trap wastewater

GRAPHICAL ABSTRACT



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ABSTRACT

Urbanisation and building advancements have increased microbial growth in indoor environments, altering human interactions with these microorganisms. Restrooms and their sinks harbour diverse bacterial communities, that differ from those found in natural environments, that could have negative implications for human health. Over two and a half years, this study examined the diversity, temporal dynamics, and resilience of bacterial communities in restroom sink P-traps in a newly built university building. Structured into two phases, the first phase consisted of continuous monitoring of bacterial community dynamics for two years ($n = 352$), while the second phase involved an intervention with sodium hypochlorite (bleach) and subsequent sampling ($n = 132$). In the first phase, we show that sink communities converge, becoming more compositionally similar to other sinks within the building. Bacterial families such as *Rhodocyclaceae* and *Flavobacteriaceae* dominated across the sinks, and others such as *Comamonadaceae*, *Moraxellaceae* and *Enterobacteriaceae* were highly prevalent. When comparing bacterial structure and composition to other sinks located on the university campus, the mean bacterial dissimilarity decreased over time, indicating compositional similarity, particularly with the newer buildings on campus. The second phase demonstrated resilience by the bacterial sink communities. Following bleach treatments, a distinct increase in *Acinetobacter* was observed. However, by the fourth week after bleach intervention, bacterial communities had re-established to levels observed prior to treatment. This study had the unique opportunity to sample a newly built building before occupancy and for the subsequent two and a half years. The

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findings provide crucial insights into the development and resilience of sink P-trap bacterial communities in restrooms, laying the groundwork for more targeted approaches to disinfection strategies.

1. Introduction

Urbanisation and improvement of our building utilities have created novel niches and opportunities for microbial colonisation and proliferation within our indoor environment, altering the exposure and interactions we have with microbial inhabitants. With an increasingly indoor bound human population, we are continuously exposed to indoor microorganisms which can differ substantially from those present in natural environments (Lee et al., 2021a, 2021b; Lehtimäki et al., 2017; Meadow et al., 2014; Rai et al., 2021). The indoor built environment provides a unique site for interactions between microorganisms arising from human and non-human origins that could favour negative health outcomes particularly regarding antibiotic resistance. Owing to adverse abiotic conditions, including water scarcity, extreme temperatures, and exposure to stressors like antimicrobial chemicals or sodium hypochlorite solutions in indoor environments, the selection of the most resilient microbial species may be favoured. This selection process may promote the exchange of genetic material and retention of antibiotic resistance genes. Moreover, studies have demonstrated that microorganisms within indoor settings can contribute to allergies and infectious disease, particularly in vulnerable populations such as immunocompromised individuals and infants (Borella et al., 2004; Kool et al., 1999; Richardson et al., 2019; Soeria-Atmadja et al., 2010; Zhang et al., 2016).

Microorganisms enter buildings from a variety of sources, from humans and their pets to outdoor air, soil, plants, and water (Fujimura et al., 2010; Hospodsky et al., 2012; Mahnert et al., 2015; Meadow et al., 2014). Before entering indoor systems, water sourced from either groundwater or surface water undergoes diverse treatment procedures aimed at removing microorganisms and other particulate matter. However, the microorganisms that can survive harsh treatment procedures may be further enriched in indoor habitats, and their potential impact on human inhabitants could be underestimated. While research on microbiomes within drinking water distribution systems have received more focus due to the direct implications for human health (Berry et al., 2006; Bitton, 2014; Lee et al., 2021a, 2021b; Meier and Bender, 2016), investigations into water pipes associated with wastewater are equally crucial, particularly in areas where occupants may be exposed. Sinks and their connected pipes, including the P-traps, harbour microbial communities and have been identified as significant reservoirs of pathogens in clinical settings, posing serious health risks to patients (Kotsanas et al., 2013; Snitkin, 2019; Williams et al., 2013). Water from taps not only serves as an important source of microorganisms to sink traps, but also contributes to the core composition of the sink microbiome, likely originating from humans (Withey et al., 2021). Previous studies have highlighted the high variability of sink drain biofilm microbial communities due to diverse environmental factors influencing sink conditions (Furuhata et al., 2010; Moen et al., 2015). Given their open nature, the continuous flow of waste containing various nutrients, and consistent hydration, sink traps present a challenging environment for monitoring and control (Ledwoch et al., 2020).

The proliferation of microorganisms in water distribution systems has long been recognised as a concern for public health due to biofilm formation, pathogen growth and water quality deterioration (Boe-Hansen et al., 2002; Lee, 2013). Biofilms are often regarded as chronic containments of drinking water distribution systems, providing several advantages to bacteria (Gomes et al., 2016). They facilitate the sharing of nutrients and metabolic products, provides protection against environmental stress and antimicrobial agents, and promotes the development and transfer of antibiotic resistance genes (Douterelo et al., 2018; Garrett et al., 2008; Wingender and Flemming, 2011).

There are multiple strategies to control microbial adhesion and

biofilm formation in water systems and sinks, the most common method being chemical disinfectant, in particular the use of sodium hypochlorite (bleach) (Caselli et al., 2016; Cole and Talmadge, 2019; Mi et al., 2015; Nocker et al., 2021). Household bleach contains 5 % - 9 % sodium hypochlorite and is used widely due to having a broad spectrum of antimicrobial activity (Centers for Disease Control and Prevention, 2022; Rutala and Weber, 2015). Sodium hypochlorite has been shown to have varying effects on microorganisms and biofilms. Studies focusing on the effects of sodium hypochlorite on specific and isolated microbial species have shown that variations in strain and species, bactericidal concentration and the presence of organic matter led to differing efficacies of bacterial reduction (Elmaksoud et al., 2014; Gomes et al., 2016; Köhler et al., 2018; Reynolds et al., 2012). Moreover, following biofilm formation in certain strains, there is a shift in their resistance levels to disinfectants (Lim et al., 2017). When compared to these individual species biofilms, multispecies biofilms exhibit greater resistance to chlorine inactivation (Simões et al., 2010). Research into disinfection of microbial communities from water distribution systems has found that chlorine treatment alters composition, lowers microbial richness and diversity (Mi et al., 2015; Paduano et al., 2020; Roeder et al., 2010; Vaz-Moreira et al., 2013). Despite treatment of water in these systems, certain bacterial phyla can dominate during chlorination or colonize after (Mi et al., 2015; Vaz-Moreira et al., 2013). Additionally, in environments where disinfectants are present at elevated concentrations, certain bacterial biofilms display resilience to chlorine and minimal cellular damage (Lin et al., 2017). In contrast, Mi et al. (2015) demonstrated that at low concentrations of chlorine disinfectants, there was an increase in diversity, underscoring their inefficacy and the importance of employing the appropriate dosage.

Biofilms in microbial sink drains, particularly in hospital settings, pose a persistent challenge in terms of eradication and control. Recolonisation often occurs due to exposure to contaminated material deposited in the sink or upward growth from P-traps (Bourdin et al., 2023; Kotay et al., 2017). Numerous studies and reports highlight the intricacies of removing pathogens and controlling outbreaks from sink and drain environments. The predominant strategies to combat these outbreaks involve repeated exposure to sodium hypochlorite or complete removal and replacement of contaminated components such as the P-trap (Ahmad et al., 2004; Bert et al., 1998; Chapuis et al., 2016; Clarivet et al., 2016; Hota et al., 2009; Ling and How, 2013; Wendel et al., 2015). Alternative interventions include heating devices or other chemical treatments such as formalin, peracetic acid, Virox and foaming hydrogen peroxide (Döring et al., 1991; Jones et al., 2020; Lowe et al., 2012; Stjärne Aspelund et al., 2016; Wolf et al., 2014). In the most cases, intervention successfully reduced or prevented further cases. However, some instances required additional interventions before successful eradication, and certain studies lacked clarity on durability due to no long-term follow up. More recently, Ledwoch et al. (2020) investigated the efficacy of a variety of disinfectant chemicals in reducing viable cell counts in an in-vitro sink drain environment. They found bleach only partially effective against drain biofilms and that bacterial regrowth occurs within four days of the final treatment. Notably, none of these studies explored how the microbial communities changed upon exposure to the treatments.

Overall, disinfectants have a major impact on biofilm communities; however, it is of concern that intervention may favour the selection of persisters and more resilient microorganisms (Jin et al., 2020; Roeder et al., 2010). Many of these studies overlook the long-term consequences on biofilm communities and the success of the treatment (Buchan et al., 2019). A recent study by Zhang et al. (2021) demonstrated that chlorine disinfection can stimulate transformation of plasmid-encoded

antimicrobial resistance genes (Zhang et al., 2021). Although chlorine-based water disinfection processes are widely used and can inactivate antibiotic resistant bacteria, they may induce the release of antibiotic resistance genes that can naturally transform into other microorganisms. Another study corroborated these findings and highlighted the transfer of chlorine-injured opportunistic pathogens from non-antibiotic-resistant bacteria to antibiotic-resistant bacteria (Jin et al., 2020). Thus, effective treatment and a comprehensive understanding of the long-term effects of disinfectants on microbial communities is imperative to mitigate public health risks and manage antibiotic resistance in our sinks and water systems.

This present study aims to understand the temporal dynamics of sink bacterial communities in sinks within a newly built university building and further investigate their responses to an intervention consisting of applying sodium hypochlorite (bleach). To this end, we conducted initial sampling before the building's occupation, followed by a two-year sampling regimen focussing on all accessible restroom sinks. The objectives were to: (i) assess the long-term variations and stability of bacterial communities within restroom sink P-traps over a two-year period; (ii) identify the bacterial colonizers and ascertain their integration into the core microbiome; (iii) determine how diversity may change over time; (iv) determine the impact of bleach on bacterial structure and diversity, and assess whether communities could revert to their previous structure and composition. This long-term study, incorporating intervention, provides a unique perspective into the dynamics of sink bacterial communities and a basis for identifying cleaning regimes to ensure the safety of the occupants and the stability of a "healthy" sink microbiome.

2. Methodology

2.1. Location and sample collection

As part of the first phase of this study, sampling took place in the

newly built university building, Health and Life Sciences (HLS, University of Reading, Reading, UK, coordinates; 51.4384893513451, -0.9433138) (Fig. 1). A total of 22 sinks on the first three floor levels were selected for the study. On floor level one, sinks were open to the public and served the large teaching laboratory, while the remaining two floor levels were accessible only to authorized users and employees. The sampling initiative commenced on 23 August 2020, the day before the construction had completed. Subsequently, samples were collected approximately every six weeks over the span of two years concluding on 4 September 2022. This resulted in a total of 16 time points and 352 samples. The methods for collecting P-trap samples were consistent with previous studies (Withey et al., 2021, 2023). Briefly, a sterile 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 inner P-trap surface. All samples were stored in a -20°C freezer pending further processing. Occupancy data for the building was obtained by monitoring users' card access from 1 August 2022 to 30 September 2023 (data prior to August 2022 was unobtainable). Card access was required for entry to the stairwell granting access to floor level two and three. While this number provided an approximate occupancy, it may not capture all individuals entering without card access. Floor level one does not require access, and the card monitoring would not account for large practical classes occurring on this level.

2.2. Bleach intervention

The second phase of this study implemented an intervention using 10 % sodium hypochlorite (Honeywell Fluka™) (Fig. 1). On 24 December 2022, sinks were subjected to resampling, and subsequently, two-thirds of the sinks underwent bleach treatment the following week. Each restroom had at least one "control" sink left untreated (Table S1). The bleach treatment entailed pouring 500 ml of 10 % bleach into the selected sinks in the evening (8 pm) and allowing it to sit overnight. The following morning (6 am), 500 ml of sodium thiosulfate (70 mg/l) was

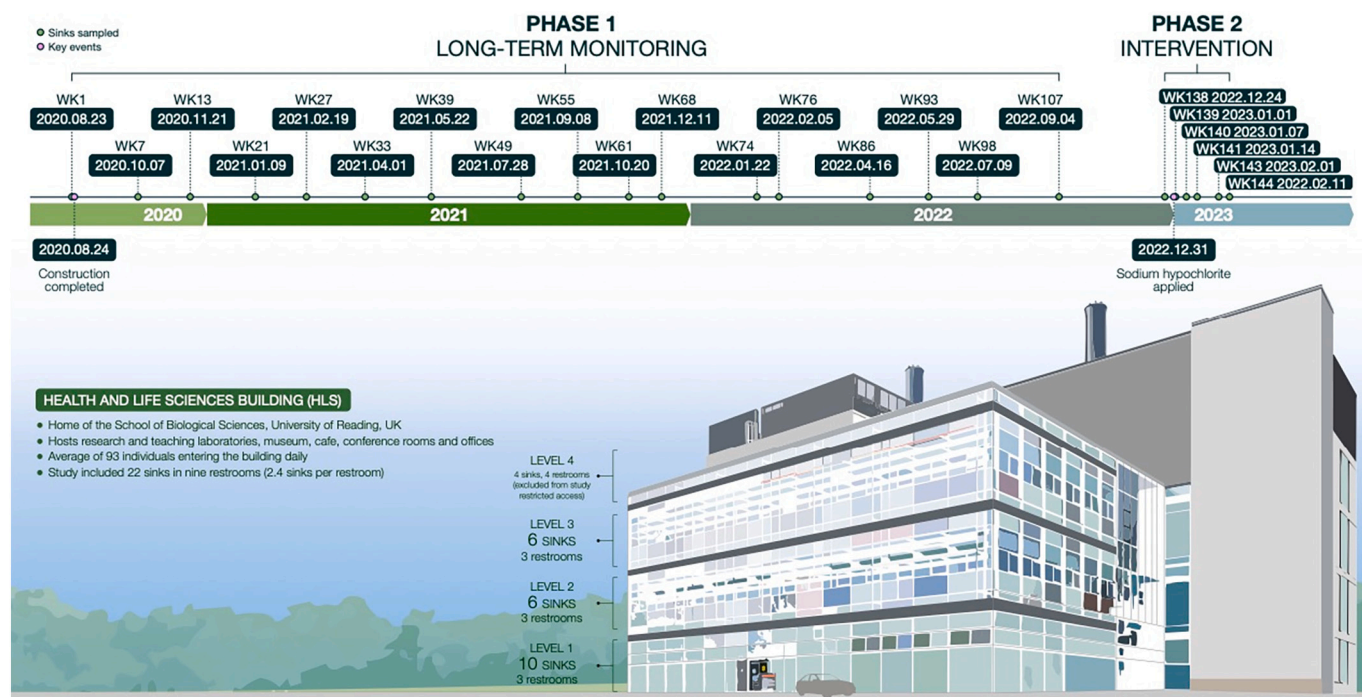


Fig. 1. Summary diagram outlining the two phases of the study and providing details of the study site (Health and Life Sciences, HLS). Phase 1 sampling began on the 23 August 2020 one day before the construction of the building completed. Sampling occurred approximately every six weeks across the first three floor levels of the HLS building, comprising a total of 22 sinks. Phase 1 sampling finished 4 September 2022. Phase 2 sampling commenced on the 24 December 2022. Sinks were treated with sodium hypochlorite on the evening of 31 December 2022 and left overnight. The following morning, 1 January 2023, samples were collected, subsequently collection occurred two, four and five weeks from treatment.

added to quench any residual reactions and the sinks were flushed with tap water for five minutes. Samples were collected in the morning after treatment, as well as one week, two weeks, four weeks, and five weeks following the initial treatment. The sampling methodology differed slightly from the previous approach. Briefly, similar to the previous method, sterile cotton swabs were inserted using a sampling rod into the P-traps. However, instead of swabbing the circumference of the pipe, only one ordinal point of the circular P-trap was swabbed per sampling time point. The swab was carefully rotated and moved up and down for 10 s in the designated P-trap area to ensure sufficient biomass collection. This adjustment was necessitated by the more frequent collection of samples, as destructive sampling was considered potentially problematic.

2.3. Sample processing and data processing

Following the manufacturer's instructions, the HigherPurity Soil DNA Isolation kit from Canvax Biotech was used to extract genomic DNA from the swabs. Samples collected for the bleach intervention study and negative controls were quantified using Qubit fluorometer 3.0 (High Sensitivity assay). Samples that had no detectable DNA were excluded from subsequent downstream processing, encompassing all bleach-treated samples from the morning after intervention (WK139), three from the bleach-treated samples after one week (WK140) and all negative controls (Table S1). The amplification of the V4 region of the bacterial 16S rRNA gene and metabarcoding was performed using 515F (Forward: GTGYCAGCMGCCGCGGTAA) and 806R (Reverse: GGACTACNCGGTGTCTAAT) primers (Thompson et al., 2017). The reaction quantities and thermocycling conditions for PCR remained consistent with those previously described in Withey et al. (2021). Ampure XP beads (Beckman Coulter) were used to purify the PCR products, and their concentration was assessed using the Qubit fluorometer 3.0. Subsequently, the purified PCR products were sent to Novogene (UK) for sequencing on the Illumina MiSeq platform (2 × 250 bp paired-end).

The raw pair-end sequences were demultiplexed, then quality filtered and trimmed using TrimGalore (v.0.6.10, <https://github.com/FelixKrueger/TrimGalore>). The quality filtered reads were then dereplicated, denoised and merged using DADA2 (v.1.26.0, Callahan et al., 2016) and produced an amplicon sequence variant (ASV) abundance table (Table S7). ASVs were classified using the naïve Bayesian classifier (Wang et al., 2007) against the SILVA database (v.138, Quast et al., 2013). ASVs were subjected to filtering, excluding those not assigned to the bacterial domain and also implementing a length filter to exclude those exceeding 300 bp. ASVs with low abundance below 10 counts across the feature table were systematically removed to reduce the likelihood of spurious taxa.

2.4. Statistical analysis

Statistical analyses were performed in R (v.4.3.1, R Core Team, 2022) using the packages phyloseq (v.1.44.0, McMurdie and Holmes, 2013) and vegan (v.2.6–4, Oksanen et al., 2020). To account for uneven sampling depth, the samples were rarefied to 5000 reads per sample (Weiss et al., 2017), resulting in the loss of 28 samples. The data analysis was divided into three parts. Initially, the focus was on the development of bacterial communities and temporal dynamics during the first two years of the recently built university building. Subsequently, analysis of the bleach intervention study was conducted, and finally, a comparison was made between all untreated sinks in the new HLS building across all sampling time points, along with sinks from other campus buildings sampled in 2019 (Withey et al., 2021).

The alpha diversity indices were computed using the phyloseq (v.1.44.0, McMurdie and Holmes, 2013) R package and microbiome R package (v1.23.1, <http://microbiome.github.com/microbiome>) from the ASV relative abundance table. Linear mixed effects models from lme4 R package (1.1–35.1, Bates et al., 2015), featuring both a random

intercept and random slope, were employed to investigate trends in alpha diversity including Shannon diversity, ASV richness and Pielou's evenness, and the interaction between treatment with sampling time point.

To estimate beta diversity, Bray-Curtis dissimilarity was determined from the ASV relative abundance tables. The beta diversity was visualised using the NMDS through the vegan R package. The among-group and sampling time point differences in sink microbial composition were tested through the PERMANOVA with function adonis from the vegan R package. Adonis.pair() from the R package EcolUtils (v.0.1, Salazar, 2023) was used for pairwise beta diversity comparisons. The *p*-values for multiple comparisons were adjusted using the Benjamini-Hochberg method.

Additionally, the CODYN package (v.2.0.5, Hallett et al., 2016) was used to elucidate trends in temporal dynamics for the first two phases including mean rank shift using their rank_shift() function and turnover calculated using turnover() (Hallett et al., 2016).

Assessing the potential convergence in composition between HLS building and other campus buildings involved plotting Bray-Curtis distance against sampling time points. This comparison was made with a subset of sinks from HLS that were untreated during the bleach intervention, providing an extended timeseries (two and a half years) for comparison. A linear model was used as the smoothing method in these plots.

To identify bacterial genera that significantly differ between untreated and treated sinks at each sampling time point during bleach intervention, wilcox.test() was used to compare their relative abundances.

3. Results

Processing and filtering of reads resulted in a feature table containing 11,212,944 merged reads from 478 samples (378 from the time series and 100 from the bleach intervention). After rarefaction, 456 samples (365 from the time series samples and 91 from the bleach intervention), comprising a total of 1731 ASVs remained. On average, each sample contained 38 ASVs, with a minimum of 5 and a maximum of 145. The ASVs were taxonomically classified into 27 identified phyla, 47 classes, 107 orders, 181 families, 296 genera, and 124 species.

Regarding the building's occupancy, an average of 93 people registered into the building daily from 1 August 2022 to 30 September 2023, with a minimum of eight and a maximum 176 individuals. Occupancy remained relatively consistent throughout the year, with an average of 130 individuals checking into the building on weekdays and 20 individuals on weekends (Fig. S1). The last week of December and first week of January had the lowest number of occupants, followed by a slight decrease in entries in months of April, August and September.

3.1. Diversity and composition of university sinks over two years

Alpha diversity, measured by ASV richness (Fig. 2a), Shannon diversity (Fig. 2b) and Pielou's evenness (Fig. 2c) exhibited a decreasing trend over time, with the variation in diversity among individual sinks converging to the median. Linear mixed effects models were used to test the association between alpha diversity indices and sampling time points. Sampling time point was a significant predictor of Shannon diversity and ASV richness (Shannon, $p < 0.001$; ASV richness, $p < 0.001$, Table S2). Gender and floor level were shown not to significantly predict Shannon diversity or ASV richness. For Pielou's evenness, restroom gender was the only significant predictor, although not highly statistically significant ($p > 0.01$, Table S2). While showing an overall decrease over time, the ASV richness exhibited fluctuation throughout the sampling time points. Periodic spikes in ASV richness occurred at WK49 (28 July 2021), WK74 (22 January 2022) and WK98 (9 July 2022). Shannon diversity remained relatively unaffected as these influxes of ASVs during these periods had low relative abundances. Peaks at these six-month

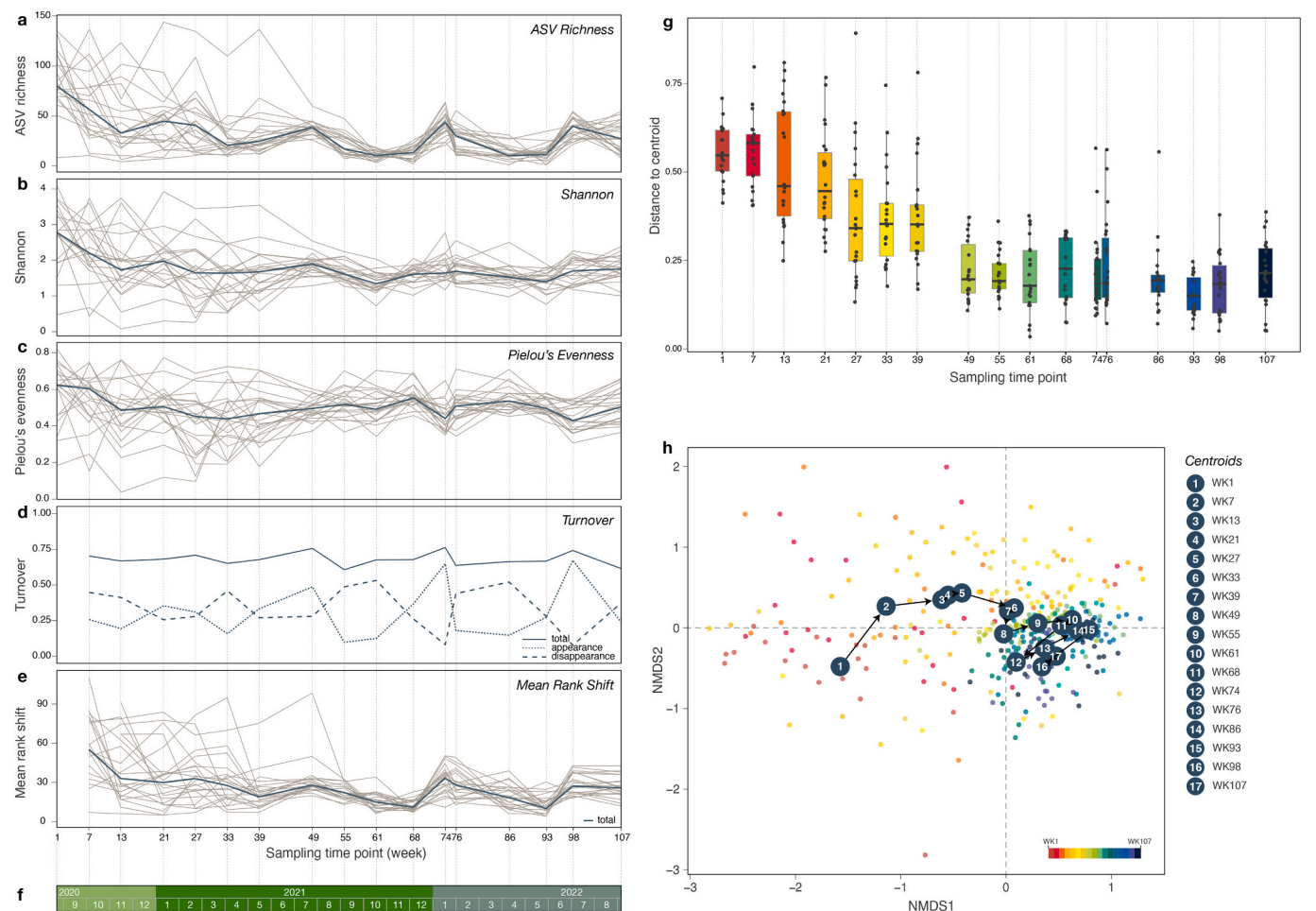


Fig. 2. Alpha and beta diversity. (a - c) Alpha diversity indices over sampling round. Dark blue line is the median of the diversity metric, lighter grey lines represent individual sinks. Alpha diversity indices exhibit a decreasing trend over time, with the variation in diversity among individual sinks converging to the median. (c) Turnover of ASVs. (d) Mean rank shifts. Note that in (c) and (d) x axis starts at sampling time point WK1, but this refers to a difference between sampling timepoints thus sampling WK1 refers to the turnover (or mean rank shift) from WK1 to WK7, sapling time point WK13 in x axis refers to difference between WK7 and WK13 and so on. (f) Time scale in years for the sampling time points (weeks). (g) Distances to centroid in multivariate homogeneity of group variance analysis for sink bacterial communities over sampling time points. (h) Non-metric multidimensional scaling (NMDS) resulting from Bray-Curtis dissimilarity matrices of community composition between sampling time points (weeks). Blue circles indicate centroid of sampling time points and black arrows indicates direction in time. Communities are becoming more similar and homogenous over time.

intervals were also evident in total turnover (Fig. 2d) aligning with an increase in ASV appearance and mean rank shift (Fig. 2e). A reduction in evenness was observed at these sampling time points (Fig. 2c) suggesting that the increase in diversity did not result from a more even distribution within the community. Subsequent weeks exhibited a recovery to the levels of richness or evenness observed before, remaining relatively stable until the sampling point six months later. During weeks, WK49, WK74 and WK98, characterised by elevated ASV richness, 7, 17 and 22 ASVs, respectively, were identified with significant differences in their relative abundances compared to the preceding week (Table S3). Although these ASVs significantly increased in relative abundance, their overall contribution to the bacterial community remained small (relative abundances < 1 %).

There were overall significant differences among the bacterial communities across different sampling time points (PERMANOVA, DF = 16, F model = 8.2682, R2 = 0.25570, $p = 0.001$, Table 1, S4). The variation in bacterial communities was most strongly associated with sampling time point, explaining 25 % of the variation, whereas gender and floor level explained only 4.5 % and 3.6 %, respectively. The association between sampling time point and beta diversity (distances to centroid) was shown to be significant using linear mixed effects models and became more homogenous over time (Linear mixed effects model:

Table 1
Results of PERMANOVA analysis of similarity based on ASVs tables of Bray-Curtis distance matrices. Abbreviations: DF degrees of freedom; SS sum of squares; F, F value by permutation. p -values are based on 999 permutations. Stars indicate the p -value significance $p < 0.05$; *, $p < 0.01$; **, $p < 0.001$; ***, $p < 0.0001$.

Factor	DF	SS	F	R2	P-value
Sampling Round	16	15.888	8.2682	0.25570	0.001 ***
Gender	2	2.792	11.6241	0.04494	0.001 ***
Floor Level	2	2.261	9.4150	0.03640	0.001 ***

Sum sq. = 5.5116, Mean sq. = 5.5116, Num DF = 1, Den DF = 341.35, F value = 382.52, $p < 2.2e-16$). The non-metric multidimensional scaling (NDMS) based on Bray-Curtis distance matrix (Fig. 2h) and distances to centroid in multivariate homogeneity of group variance analysis for sink bacterial communities over sampling time points, showed separation among the initial sampling time points, followed by a gradual clustering of later sampling time points. Overall beta diversity showed communities becoming more compositionally similar over time (Fig. 2g).

Throughout all time points, sink communities were predominantly composed of sequences classified to the phyla Proteobacteria (71.23 %) and Bacteroidota (27.34 %). The top families with an overall relative

abundance >1 % included *Rhodocyclaceae* (36.93 %), *Flavobacteriaceae* (25.86 %), *Sphingomonadaceae* (8.56 %), *Comamonadaceae* (6 %), *Xanthomonadaceae* (3.98 %), *Pseudomonadaceae* (3.60 %), *Caulobacteraceae* (3.30 %), *Enterobacteriaceae* (3.05 %) and *Moraxellaceae* (2.41 %). The remaining 175 identifiable families collectively accounted for 5.78 % of all reads, while 0.59 % of reads were unidentifiable to family. All families belonged to Proteobacteria, except for *Flavobacteriaceae* which is part of Bacteroidota. Fig. 3 shows *Rhodocyclaceae* increased in relative abundance over the first four sampling time points (WK1 – WK27) then remained between 25 and 50 % in relative abundance for the remaining duration. By the following sampling time points (WK33), *Rhodocyclaceae* occurred in all sampled sinks. *Flavobacteriaceae* took longer to reach its maximum relative abundance, starting to plateau by WK55, although it was already present in all sinks by this week. While there was more variation in relative abundances between sinks in earlier sampling time points for all families, overall, there appeared to be less variation by WK49. The top two genera, comprising over 50 % of the total reads, were *Azospira* (34.58 %) and *Flavobacterium* (25.86 %). Of the aforementioned nine core ASVs, eight were identified to the genus level (Table S5). The most prevalent and abundant ASV was classified as *Azospira oryzae* establishing itself in all sinks after WK27.

3.2. Bleach intervention

Following the bleach treatment (the day before WK139), the intervened sinks showed an absence of quantifiable DNA the morning after (WK139), indicating a significant impact of the bleach on bacterial community and composition (Fig. S2). From WK140, there was no difference among the treatment groups across alpha diversity indices when analysing trends post intervention (WK140–WK144, Shannon, $p = 0.4515$; ASV richness, $p = 0.3039$; Pielou's evenness, $p = 0.4732$, Table S6). In terms of beta-diversity, significant differences were observed between two treatments during the immediate three weeks post-intervention, namely WK139, WK140, WK141 (PERMANOVA: WK140, $DF = 1$, $F_{\text{model}} = 7.2776$, $R^2 = 0.34203$, $P < 0.001$; WK141, $DF = 1$, $F_{\text{model}} = 3.2289$, $R^2 = 0.26404$, $P = 0.004$, Fig. 4b). It was only from WK143 onward that no significant differences were observed between the treatments (PERMANOVA: WK143, $DF = 1$, $F_{\text{model}} = 1.861$, $R^2 = 0.09867$, $P = 0.102$; WK144, $DF = 1$, $F_{\text{model}} = 1.0731$, R^2

$= 0.07625$, $P = 0.405$). From WK140 there were no differences among treatment groups in terms of their distances to centroids (Linear mixed effects model: Treatment, Sum sq. = 0.034417, Mean sq. = 0.034417, Num DF = 1, Den DF = 13.682, F value = 2.5625, $p = 0.1322550$; Week, Sum sq. = 0.195514, Mean sq. = 0.195514, Num DF = 1, Den DF = 46.393, F value = 14.5568, $p = 0.0004009$, Fig. S3). Overall, it required four weeks for the bacterial community and structure to homogenise with the treated sinks.

The week immediately following the bleach treatment (WK140), a distinct increase in the mean relative abundance of *Acinetobacter* was observed in bleached sinks at (Fig. 4a). This distinctive peak in *Acinetobacter* appears in most bleached sinks at the individual level (Figs. S4, S5). By WK141 the mean relative abundance of *Acinetobacter* had greatly diminished. Six bacterial genera, including *Acinetobacter*, were identified as significantly different in their relative abundances between untreated and bleached sinks at WK140 (Fig. S6). In bleached sinks compared to untreated sinks, there was an elevation in the relative abundances of *Acinetobacter* and a decrease in *Azospira*, *Flavobacterium*, and *Acidovorax*. Although there was more variation in *Acinetobacter* relative abundances (3.16 % - 74.26 %) among bleached sinks at WK140, the median/mean (median 24.76 %, mean 38.76 %) was higher than those untreated sinks at WK140 (median 4.06 %, mean 3.97 %) and WK138 (median 0.3 %, mean 0.53 %, before intervention). No significantly different genera were identified between untreated and treated sinks in the subsequent sampling time points (WK141, WK143, WK144). Moreover, Fig. 4a shows that the bacterial community of bleach-treated sinks had, by WK141, returned to taxonomic compositions that were more similar to WK138 (before treatment) and the untreated sinks.

3.3. Comparison to other buildings on campus

The bacterial communities within the newly built university building (HLS) gradually became more similar in structure and composition to the sinks sampled from other buildings on the same university campus in 2019. Despite significant differences in community structure and composition between HLS and other university buildings across sampling time points (PERMANOVA: $DF = 31$, $F = 7.5285$, $R^2 = 0.44422$, $p < 0.001$ ***), the NMDS analysis indicated that the later sampling time points of HLS were closer to the campus sinks (Fig. 5). Notably, the mean

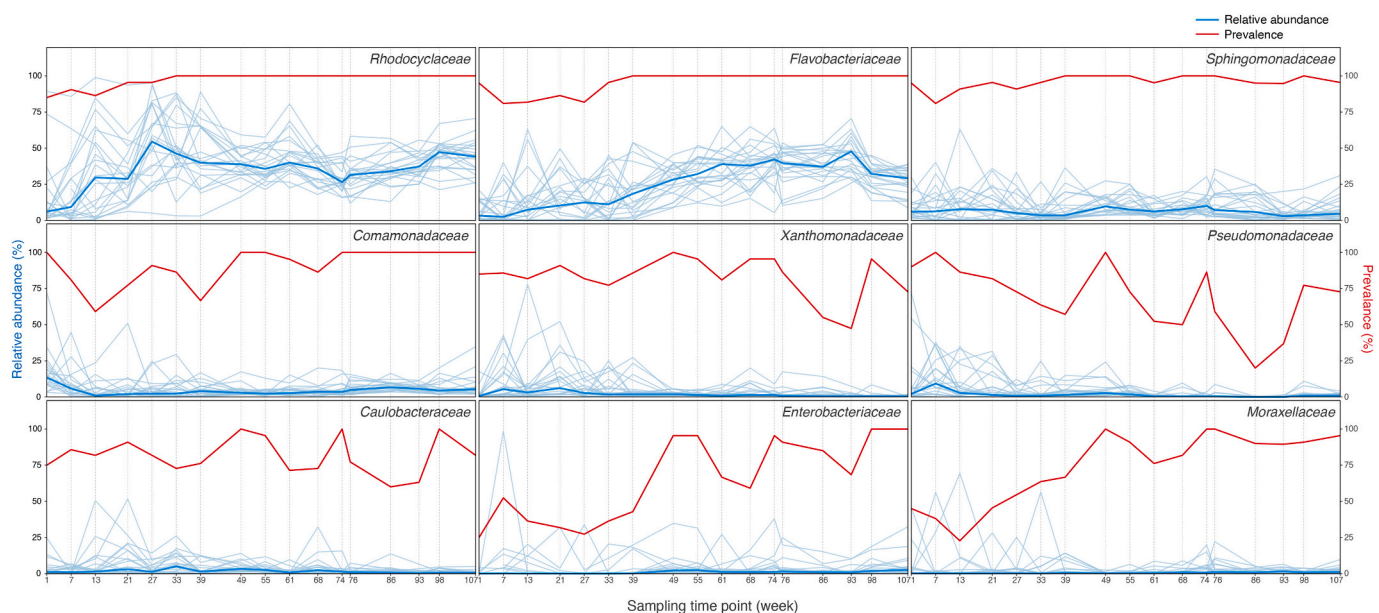


Fig. 3. Relative abundances (%) of the top genera over the sampling period. The darker blue line is the median relative abundance across all sink samples, the lighter blue lines represent individual sinks. Prevalence at each time point of top genera is indicated by the red line. *Rhodocyclaceae* was the abundant bacterial family across all samples and by WK33 was present in all sink samples. All families in the plot, by the final sampling time point (WK107) were prevalent (> 70 % of sink samples).

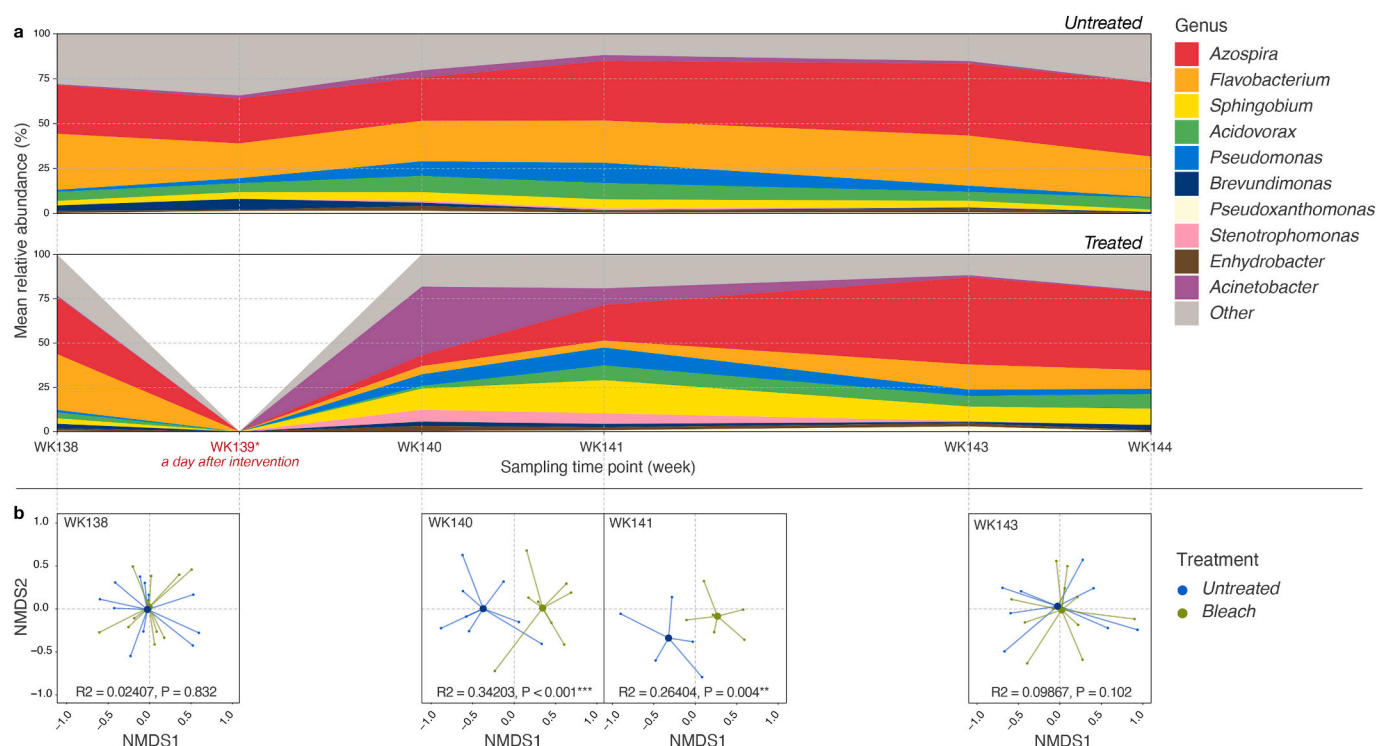


Fig. 4. (a) Average relative abundance of the top bacterial genera found in restroom sinks. The average data represent pooled sequences of sinks, split by treatment. “Other” represents all other genera and sequences unclassified to the genus level. WK139*: No data was present for bleach treated samples at this time point due to no quantifiable DNA. WK139 was plotted at zero for relative abundance visualisations. Taxonomic differences were observed after treatment. *Acinetobacter* was more prevalent in bleached samples at WK140 than untreated. For untreated samples, taxonomic composition appeared relatively stable. (b) Non-metric multidimensional scaling (NMDS) resulting from Bray-Curtis dissimilarity matrices of community composition at each sampling time point. Centroid of group is represented by darker coloured point. Bottom of each plot displays the result from PERMANOVA. Prior to bleach treatment (W138) sink samples were similar in composition, following bleach intervention, treated sinks diverged from untreated sinks in their bacterial community composition and structure. By WK143 (four weeks after intervention) communities overlapped and there were no significant differences between the treatment groups.

bacterial dissimilarity (Bray-Curtis) between HLS sinks and campus sinks decreased over time, indicating compositional similarity (Fig. S7). By the final sampling time points (WK144), the bacterial communities of HLS were most similar to Polly Vacher (Mean Bray-Curtis Distance = 0.63), followed by Library (Mean Bray-Curtis Distance = 0.73) and Henley Business School (Mean Bray-Curtis Distance = 0.72) (Fig. S8). Concerning common taxa between HLS sinks across all sampling time points, WK1 to WK144, and campus sinks, there were 82 families and 134 genera in common. When comparing the final timepoint of the HLS sinks to all campus sinks, out of 23 identified families in the HLS sinks (WK144), 21 families were shared with campus sinks. For the 32 identified genera in the final sampling time point of HLS, 24 were present in campus sinks. Core sink families including *Comamonadaceae* (100 % prevalence), *Sphingomonadaceae* (99 %), *Rhodocyclaceae* (98 %), *Xanthomonadaceae* (94 %) and *Moraxellaceae* (91 %) were present in at least 90 % of sinks (289 out of 321 sinks; 91 campus sinks, 230 HLS sinks). These core sink families were also some of the most abundant. At the genus level *Sphingobium* (96 % prevalence), *Azospira* (95 %) and *Acidovorax* (90 %) were identified as core sink taxa. Overall, the bacterial communities of the sinks located in HLS became more similar to the bacterial communities in sinks from the surrounding campus over the two years of sampling, sharing numerous bacterial taxa.

4. Discussion

In this study, we observed the stabilisation and increased similarity among bacterial communities over an extended observation period spanning over two years. Alpha diversity showed a reduction in variability among individual sinks over time, and beta diversity indicated a trend towards sinks becoming more homogenous and compositionally

similar. After 28 May 2021 (WK49) similar bacterial community compositions were consistently observed across individual sinks, with occasional variations in proportions of each top genera in specific sinks (Figs. S4, S5).

One possible explanation for this convergence and relative stability is that sinks within a building, primarily designated for handwashing in restrooms as sampled in this study, should generally be exposed to similar sources of microbial taxa and nutrients. Previous work has identified human skin as a primary contributor to the sink microbiome (Withey et al., 2021), reinforcing the expectation of compositional similarity among sinks within the same building. However, variations in the relative abundances of genera in specific sinks could be attributed to non-hand hygiene activities in those sinks. For example, in hospitals improper sink usage has been observed i.e., washing of medical items, disposal of antimicrobials and patient nutrition items (Grabowski et al., 2018; Woon et al., 2023). Similarly, university handwashing sinks may be used to dispose of nutritional products, washing of eating utensils and other miscellaneous equipment, or even washing of other body parts. But without surveying occupants’ behaviour in the building, it remains inconclusive whether this is the case.

Another justification for sink stability lies in the environmental conditions sinks impose on microbial communities. Although sink P-traps may be conducive to microbial colonisation, the bacteria persisting in sinks must withstand temperature fluctuations due to hot tap water usage, physical disturbance from water pressure, the use of chemicals (i.e., soap and disinfectants), and survive in a low-nutrient environment. Thus, the sink environment selects for bacteria that can endure these conditions.

Additionally, we observed periodic spikes (WK49, WK74, WK98) in ASV richness during the study, coinciding with a decrease in Pielou’s

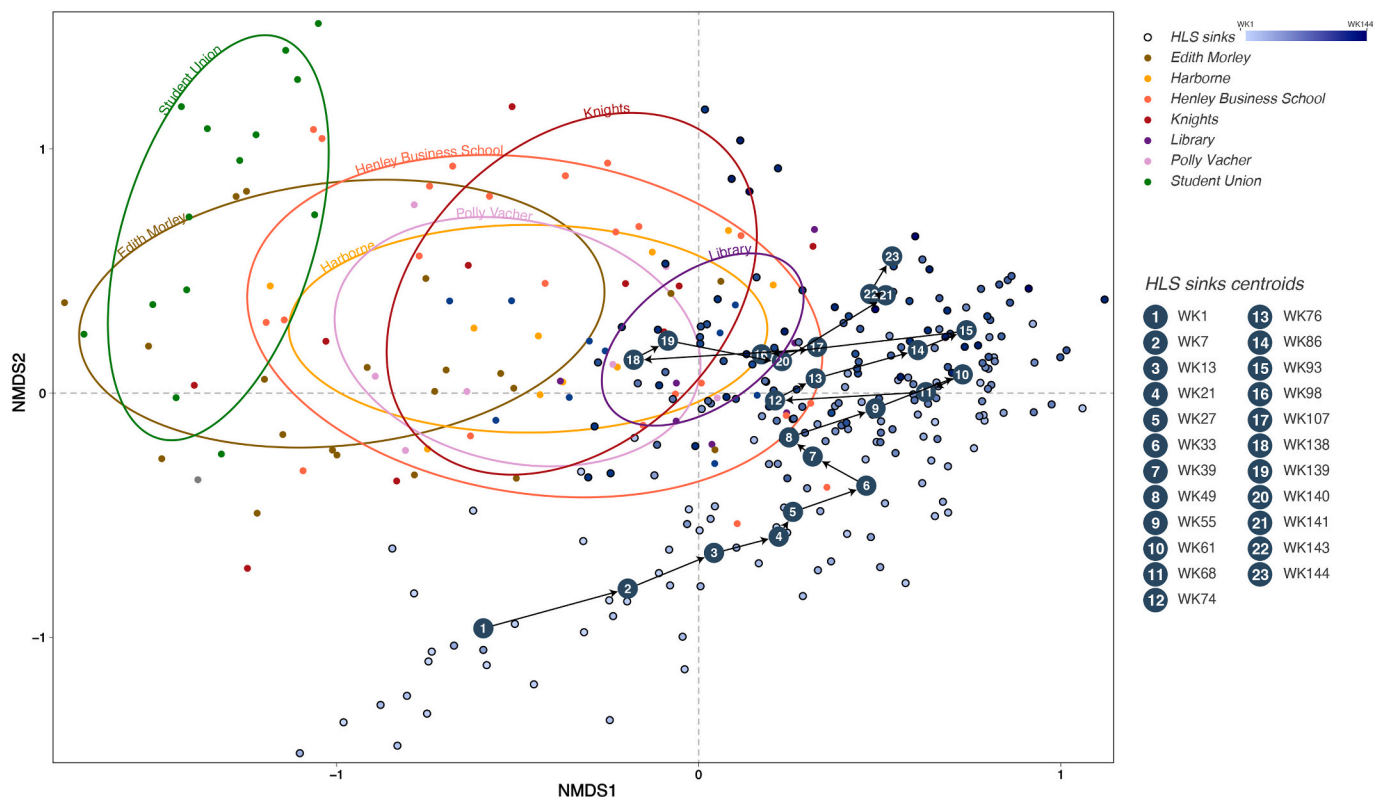


Fig. 5. Non-metric multidimensional scaling (NMDS) resulting from Bray-Curtis dissimilarity matrices of community composition between buildings from the university campus, including the new HLS building by sampling rounds. Centroids for each sampling round for HLS and ellipses for all other buildings are shown on the plot. Arrows indicates HLS communities becoming more similar over time in composition to the other sinks present on campus.

evenness. A similar phenomenon was observed at WK21, though the peak was less evident, possibly due to larger variations in alpha diversity indices earlier in the sampling regimen. These spikes occurred in January and July, corresponding to months with closure periods. Stagnation in the water pipes during closure periods may contribute to an increase in ASVs (Ji et al., 2015; Lautenschlager et al., 2010; Ling et al., 2018; Lipphaus et al., 2014; Ye et al., 2022). However, occupancy data from 2023 indicated low occupancy only in the first week of January, and overall, the month had the average number of daily occupants. For July there was no apparent reduction in the number of daily occupants. Alternatively, changes in tap water treatment by the supplying company every six months might influence tap water community and, consequently, bacterial sink diversity. However, this theory remains unconfirmed. The following six months after these spikes, richness and evenness recovered to levels observed before, further demonstrating the stability of sink bacterial communities.

In alignment with previous studies of sinks and water distribution systems, the dominant phylum observed was Proteobacteria (Dai et al., 2020; El-Chakhtoura et al., 2015; Liu et al., 2018; Withey et al., 2021). The second most abundant phylum, Bacteroidota, has been identified in various stages of drinking water treatment, from river water to drinking water (Pinar-Méndez et al., 2022). The prominent families identified in this study have also been documented as dominant in tap water, wastewater and sink drains (Douterelo et al., 2014; Eichler et al., 2006; Numberger et al., 2019; Pinto et al., 2012; Pirzadian et al., 2020; Saunders et al., 2016; Vaz-Moreira et al., 2013).

Clear shifts in bacterial community composition were evident at the onset of the sampling regime. The most abundant family *Rhodocyclaceae*, increased in relative abundance until February 2021 (WK27), after which it plateaued (median relative abundance above 25 %). Present from the initial sampling time point, *Rhodocyclaceae* may have been among the first bacteria to colonize and establish itself. This family,

known for degrading various carbon sources, has been isolated from diverse environments, including sewage, polluted and unpolluted pond waters, and aquifers (Oren, 2014). The most abundant ASV identified, *Azospira oryzae*, accounted for the majority of reads classified as *Rhodocyclaceae*. *Azospira* sp. are perchlorate reducers found in biological reactors, wastewater, aquifers, heavily polluted river water and rivers (Adedire et al., 2022; Bellini et al., 2013; Guarino et al., 2020; Hunter, 2007; Jiao et al., 2023; Li et al., 2010; Zhang et al., 2020).

Following *Azospira oryzae*, the second most abundant ASVs were classified to the genus *Flavobacterium*. Similarly, to *Azospira*, *Flavobacterium* has been isolated from wastewater, drinking water systems and sinks (LaMartina et al., 2021; Pirzadian et al., 2020; Schmeisser et al., 2003; Simões et al., 2010). Moreover, *Flavobacterium* readily adhere to surfaces, forming multispecies biofilms, and can withstand intermediate hydrodynamic pressures, making these taxa ideal colonizers of sink environments. Overall, *Flavobacterium* (*Flavobacteriaceae*) took longer to plateau but became established and remained at a relative abundance of ~30 % in most sinks for the remainder of the timeseries. Notably, *Flavobacterium* sp. are known opportunistic pathogens in humans and have been associated with sinks and their taps (Hoque et al., 2001).

The third most abundant ASV belonged to *Sphingobium yanoikuyae*. *Sphingobium* are metabolically versatile and well-studied due to their capabilities to degrade environmentally important pollutants (Balkwill et al., 2006; Mitra et al., 2020; Nielsen et al., 2017). These bacteria are able to degrade ibuprofen and are important microorganisms in wastewater settings (Balciunas et al., 2020; Nielsen et al., 2017). High abundances of *Sphingobium* have been identified in hospital sink drain outlets and specifically *Sphingobium yanoikuyae* has been isolated (Pirzadian et al., 2020). Other notable taxa, *Enterobacteriaceae* and *Moraxellaceae* had a lower prevalence at the beginning of the study but became prevalent in almost all sink samples. These families contain many taxa

associated with humans, suggesting that their increased prevalence may coincide with an increase in use by occupants (Conti et al., 2009; Pandey et al., 1999; Teixeira and Merquior, 2014).

Microbial communities tend to shift towards a stable state in the absence of external influences. Change in community state can be initiated by changes in the external conditions or perturbations that push the system into a new state (Faust et al., 2015). Following bleach intervention bacterial sink communities shifted away from untreated bacterial sink communities, with significant differences in relative abundances of certain taxa between the two groups. *Acinetobacter* became more abundant in sinks treated with bleach. However, by WK143, *Acinetobacter* had greatly reduced in relative abundance, and the bacterial communities of treated sinks had returned to a similar state as before intervention and the untreated sinks. *Acinetobacter* has been found in chemically treated waters (i.e., hydrogen peroxide, chlorine dioxide and monochloramine), and the family it belongs to, *Moraxellaceae*, has been described as chlorine-resistant (Paduano et al., 2020; Peters et al., 2018). Disinfection with bleach exerts selective pressures on the sink microbiome and may promote persisters, selecting for microorganisms able to utilise decayed microbial products (Dai et al., 2020). As well as being having resistant properties, *Acinetobacter* form a part of the human skin flora and utilise a wide variety of substrates. Consequently, it could be deposited in the sink drain environment after handwashing by occupants and exploiting the sink niche post bleach treatment (Carvalho et al., 2023; Seifert et al., 1997).

Intervention with bleach had a transient influence on the sink community, inducing a temporary selection pressure that led to a population shift. Disturbances such as bleach intervention, acted as a selection pressure by increasing mortality and decreasing biomass (Zhou et al., 2014). This was confirmed experimentally when no genomic DNA was recovered the morning following treatment. Due to the drastic shift in population, the growth of bacterial species reliant on interactions within the biofilm may have been constrained, resulting in an extended duration for their reestablishment. Moreover, niche selection will be stronger after a disturbance, providing an opportunity for some species to proliferate. In a fluidic system such as the sink system, any residual bleach (the disturbance) can be removed, and higher population dispersal rates could lead communities to converge towards the original ones after the disturbance effect is gone, as observed in this study. The sink communities had a high degree of resilience, returning to their original state.

Previous studies have found eradicating microorganisms from sinks challenging, with biofilms forming days after treatment (Ledwoch et al., 2020; Nocker et al., 2021; Stjärne Aspelund et al., 2016; Wendel et al., 2015). The disinfection strategy and age of biofilm in water distribution pipes can influence how disinfectants affect bacterial community structure (Liu et al., 2013; Zhang et al., 2019). In a model system, bleach was only partially effective against the drain biofilm (Ledwoch et al., 2020). Alternatives to bleach may provide more effective long-term solutions. Peracetic acid was highly successful at eradicating and preventing biofilm regrowth in every part of the drain model (Ledwoch et al., 2020). Other disinfectant alternatives include foam-based disinfectants (Jones et al., 2020), probiotic cleaning solutions (Caselli, 2017; Saito et al., 2016), and steam (Umemura et al., 2023). This study did not explore these or other methods of disinfectants on in-situ sinks, but further work could be conducted to observe how communities change in response and if they follow similar patterns of recovery, exhibiting a high degree of resilience.

This study also compared the communities of the newly built building to data previously collected on other sinks of the same campus. The results demonstrated that the newer HLS sinks were becoming more similar in composition to other campus sinks. The sinks from HLS were more similar to the newer buildings; Library (constructed in 2019) and Henley Business School (constructed in 2009), but also to Polly Vacher building which was most similar in composition to all buildings.

Management of disinfection protocols, particularly those in health-care settings, can be informed through our study regarding the actions

these strategies could have on sink bacterial communities. Our results demonstrated the potential eradication of a stable sink community following disinfection and the temporary dominance of specific bacterial taxa post treatment. This suggest under improper management, following disinfection, there could be an establishment of a reservoir of potentially pathogenic bacteria, unhindered by competition for nutrients with the previously established sink bacteria. Furthermore, this study provides the insights for designing intervention and management strategies to maintain a healthy microbial balance in sinks. Follow up research could implement the use of targeted cleaning or removal of specific microbial taxa. Additionally, the potential for the development of probiotic cleaners based on the microbial communities identified in public sinks. The implementation of probiotic cleaners designed to incorporate microbial communities found in sink P-traps might serve as a proactive measure to mitigate the possibility of repeated interventions with bleach and reduce the selection of persisters or antibiotic resistance genes (Dai et al., 2020; Jin et al., 2020; Zhang et al., 2021). Further, future research could consider expanding the research to include hospitals and other public settings where whole bacterial communities have not been analysed over prolonged periods of time. Moreover, identification of the bacterial communities to species level and identification of genes (including antimicrobial resistance genes) would enable clearer conclusions to be drawn regarding mechanisms of survival in this environment, pathogenicity and potentially transmissibility of genes between bacterial species. Once identified, we could investigate if bacterial species of notable interest can be isolated from surfaces surrounding the sink environment and start to investigate possible routes of transmission.

Limitations of this study include the insufficient metadata collected on the occupancy and behaviour of occupants. Whilst we were unable to obtain occupancy data for the whole duration of the study, we did acquire approximate occupancy data for the latter phase of the study (August 2022 to September 2023). This provided insights into the occupancy levels during full operational capacity of the building. For the bleach intervention, the inability to lock restroom meant that sinks treated with bleach may have been interrupted by the occupants. However, by implementing treatments after the working hours and overnight with access of the building restricted to most occupants, potential interruptions were minimised. While not included in this study, the inclusion of a method to differentiate between live and dead bacterial cells, such as propidium monoazide, would provide insights into the persistence of viable cells in sink drains (Nocker and Camper, 2008). Additionally, by selecting 16S sequencing as the method of choice, the study is limited to identifying bacterial communities to the genus level at best. A higher resolution, i.e. to species level, would provide additional insights on community composition and particularly if they are a pathogenic species. However, by selecting 16S sequencing, this enabled the analysis of many samples and still enabled the identification of some of the top ASVs to the species level.

In conclusion we have demonstrated that the temporal variation between samples reduced over time, leading to the formation of established bacterial communities in sink P-traps. Moreover, following an intervention with bleach, bacterial communities deviated from the structure of untreated sinks, and notably, this effect persisted for a four-week period. This study highlights the critical role of temporal studies across sinks, enhancing our understanding of bacterial community dynamics and their stability within the built environment.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2024.175349>.

CRediT authorship contribution statement

Zoe Withey: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Hyun S. Gweon:** Writing – review & editing, Supervision, Software, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The sequencing data have been deposited with links to NCBI BioProject accession number PRJNA1081805 in the NCBI BioProject database (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1081805>). The relevant information for each sample is shown in Table S1.

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