University of Reading

# Understanding Microbial Community Dynamics and Resilience in Communal Sink Drains

A thesis submitted for the degree of

Doctor of Philosophy

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## Abstract

The built environment has often been referred to as a microbial wasteland, that is heavily shaped by the microbiota of human occupants, building design and environmental factors. While persistence of microorganisms in the built environment is typically attributed to frequent deposition of microorganisms from external sources, there is evidence to suggest that within the built environment there are areas of endogenous growth and microbial proliferation. Handwashing sinks and traps are environments that favour microbial colonisation and proliferation and are increasingly identified as reservoirs of antibiotic-resistant pathogens in clinical environments. Despite the importance of sinks and their traps in clinical outbreaks, there are still large gaps in our knowledge regarding the composition and diversity of sink microbial communities, particularly in non-clinical communal areas. This thesis focuses on characterising the microbial communities and their dynamics in P-traps present in communal restrooms to better understand the potential implications of interactions between human occupants and these environments, and to determine their importance as reservoirs. Firstly, the bacterial and fungal sink trap communities were characterised from a variety of university buildings, identifying the core microbial community, and demonstrating the influence of humans and their activities on sink community composition. Secondly, sink trap bacterial community diversity and composition was investigated temporally, followed by an intervention event with sodium hypochlorite to explore stability. Results showed communities becoming more stable over time, converging to similar compositions across all individual sinks and that the effects of sodium hypochlorite were short-lived. This suggests that the environment selects and those that colonise will persist. Finally, an alternative restroom P-trap microbial community was investigated, those of urinals. This study showed considerable variability in community composition and structure across individual urinals, however similar bacterial taxa were observed, notably the high prevalence and abundance of the genus *Dolosicoccus* was observed. Collectively this thesis provides insight into trap microbial communities emphasising the importance of traps as reservoirs of active microorganisms and provides evidence that sink trap communities are stable and resilient to perturbations in non-clinical environments. Furthermore, highlights the application of combining different sequencing techniques to identify novel species.

## Declaration

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

Zoe Withey

Reading, December 2023

Author contributions for each chapter are detailed below.

### Chapter 2

Withey, Z., Goodall, T., MacIntyre, S. and Gweon, H.S. (2021). **Characterization of communal sink drain communities of a university campus.** *Environmental DNA*, 3: 901-911. https://doi.org/10.1002/edn3.196

ZW performed the field work and the molecular work in the laboratory. TG performed next-generation sequencing. ZW performed sequence analysis, statistical analyses, interpreted statistical findings, and wrote the manuscript. ZW, SM, and HSG contributed to the final preparation of the manuscript. HSG was in charge of overall direction and planning.

### Chapter 3

Withey, Z., Awan, A., Duguma, N., Fell, E., Martinez, N. J., Neary, E., Goodall, T. and Gweon, H. S. (2023). **Mycobial community assemblages in sink drains across a university campus.** *Environmental DNA*, 5, 212–224. https://doi.org/10.1002/edn3.375

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 was in charge of overall direction and planning. All authors discussed the results and commented on the manuscript.

**Chapter 4**

Withey, Z., and Gweon, H. S. **Longitudinal bacterial community dynamics and sodium hypochlorite intervention in a newly opened university building.** *In preparation for submission.*

ZW conducted the sampling and molecular work. ZW and HSG performed the bioinformatics processing of the data, sequence analysis, statistical analysis, visualization, and the writing of the manuscript. ZW was responsible for overall direction and planning.

**Chapter 5**

Withey, Z. and Gweon, H. S. **Microbial Landscape of Public Urinals: a 16S rRNA Survey of the Bacterial Communities in Urinal P-traps and the Discovery of Their Most Abundant and Prevalent Species.**

ZW conducted the sampling and molecular work. ZW and HSG performed the bioinformatics processing of the data, sequence analysis, statistical analysis, visualization, and the writing of the manuscript. ZW and HSG were responsible for overall direction and planning.

**Additional Research**

French, P.[+], Withey, Z.[+], Wright, E., Kaur, A., Gweon, H. S. ***Chitinophaga spargani* sp. nov., isolated from rhizosphere of *Sparganium erectum.***

+ Authors contributed equally to this work. PF and ZW performed fieldwork, microbiology, and molecular work. Bioinformatics processing of the data was performed by HSG. PF analysed the genome and wrote the manuscript with assistance from ZW and HSG. AK performed Transmission Electron Microscopy imagining. HSG was responsible for overall direction and planning.

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## Chapter 1. Introduction

Industrialisation and urbanisation have resulted in humans spending nearly 90% of their time indoors (Kelley & Gilbert, 2013). This indoor or built environment (BE) is diverse and dynamic with variations due to age, purpose, occupancy, and locations of BEs. For the purpose of this research, the BE is defined as a humanmade environment providing surroundings for human occupancy, activities and settlement (Rai et al., 2021). The variety and number of BEs means the interactions between the BE microbiome and human microbiome is not fully understood and is subject to change as building design evolves. Therefore, by investigating microbial community composition and dynamics, the impact on human health can be further understood and future building design informed. For this thesis the term microbiome is defined as a characteristic microbial community in a well-defined habitat, encompassing not only the microorganisms involved but, also their theatre of activity (i.e., metabolites, mobile genetic elements). The microbiome includes of all microorganisms; Prokaryotes (Bacteria, Archaea) and Eukaryotes (e.g., Protozoa, Fungi and Algae) (Berg et al., 2020). Further, the term mycobiome is used to describe subset of the microbiome, specifically containing all fungi (mycobiota).

Despite the inhospitably of BE surfaces, many microorganisms are able to survive indoors (Gibbons, 2016; Gibbons et al., 2015). BEs can provide unique habitats with chemical and physical compositions different to natural environments, as well as harbouring many concealed environments such as sink P-traps which are difficult to monitor and reach. These unique and concealed habitats are colonised by numerous microorganisms and can have implications for health and diseases as they can increase exposure to certain microorganisms i.e., *Enterobacteriaceae* resistant to the antibiotic Carapenem (CRE) and serve as reservoirs for opportunistic pathogens such as *Serratia marcescens* (Bourdin et al., 2023; Kotsanas et al., 2013; Lowe et al., 2012; Parcell et al., 2018; Roberts et al., 2013; Sexton et al., 2006). Buildings can serve as microbial reservoirs specifically, indoor air, water, and surfaces and although the indoor environment lacks features of the outdoor environment, such as the decay of organic materials, the BE is still diverse and facilitates microbial colonisation and growth. The indoor environment shares some similarities with the outdoor and microorganisms can be distributed between environments but with a decrease in biomass observed in indoor environments (Adams et al., 2013, 2015). There are three major sources of microorganisms to the BE, the primary source is human occupants, followed by water and thirdly the outdoor environment (Brumfieldid et al., 2020; Góralska et al., 2020; Hospodsky et al., 2012; Meadow et al., 2014a; Prussin & Marr, 2015; Rai et al., 2021). Sources of microorganism from humans can be via shedding directly from their skin or

transportation on clothing and shoes (Coil et al., 2019; Hospodsky et al., 2012; McDonagh & Byrne, 2014). Water can disseminate microorganisms throughout buildings i.e., drinking water distribution systems (DWSD) and directly contact human occupants (Berry et al., 2006; Feazel et al., 2009; Hageskal et al., 2007; Yang et al., 2020). Other sources of colonising microorganism can be from pets, air, plants or further environmental sources (Fujimura et al., 2010; Hewitt et al., 2012; Mahnert et al., 2015). These microorganisms can form established communities or be transient dependent upon building conditions or routines such as cleaning or remediation (Kwan et al., 2018; Mcbain et al., 2003; Wingender & Flemming, 2011). Microbial communities and their viability in the BE are driven by the building's occupants and their activities, the surrounding external environment and building design (Leung & Lee, 2016). Indoor bacterial communities are driven more by building use than seasonal variation whereas, fungal communities are determined more by local environmental factors (Adams et al., 2014; Rintala et al., 2008; Tong et al., 2017).

This chapter will explore the key factors that influence microbial community composition and dynamics in the BE as well as highlighting the origins of microbes found in the BE. Specifically, the importance of sinks and associated pipes in clinical outbreaks will be discussed, along with any non-clinical studies. A brief insight into the methods used for microbiome studies will also be included.

## 1.1 Microorganisms of the Built Environment

### *1.1.1 The Impact of Human Occupancy*

Occupants and their behaviour drastically shape the microbiome of the BE. Humans directly shed between $10^6$ and $10^7$ skin-associated microbes per hour (Hospodsky et al., 2015) contributing a significant amount of biomass including bioaerosols to the BE (Yamamoto et al., 2015). Not only through shedding can humans be a source of bioaerosols but also through respiration. Qian and colleagues observed emission rates of $3.7 \times 10^7$ bacterial and $7.3 \times 10^6$ fungal genome copies per person per hour (Qian et al., 2012). Thus, it is unsurprising human occupancy might one of the most important factors affecting BE microbial communities particularly in areas heavily occupied or poorly ventilated (Adams et al., 2015).

Within hours or days, occupants can colonise new spaces with their own microbial fingerprint or cloud, and this can even be used to identify individuals or families (Klassert et al., 2021; Lax et al., 2014, 2017; Meadow et al., 2015). Humans harbour microbiomes which are unique to individuals that can fluence their environment for example, the bacteria in patient rooms in a hospital consistently resembles the

skin microbiota of the patient occupying the room, especially on surfaces such as bedrails (Lax et al., 2017). This study further suggested a constant transfer of microbes within the hospital environment, particularly with patients receiving more microbes from staff members than from patients to staff members. Moreover, a newly opened kindergarten in Norway showed that over 11 months there were significant changes in community composition due to changes in building occupants. *Propionibacterium* was initially one of most abundant skin commensals but with time, there was a decrease potentially due to the primary occupants of the building being pre-pubescent children whereas, before the construction workers were male adults (Nygaard & Charnock, 2018). For private residences, microbiota in homes were identifiable by family and upon a move to a new house there was rapid colonisation of the new home by the microbial signature of the occupants (Lax et al., 2014). Contrastingly, another study showed that although the indoor air microbiome of households was shown to be dominated by human-associated bacteria, the resemblance to the skin microbiome of the occupants was none when compared with occupants of other households (Wilkins et al., 2016). The conflicting results between these studies can be accounted for by the different samples collected, the first sampled surfaces, the latter air. Gender can also influence the bacterial communities of the BE (Barberán et al., 2015a; Hewitt et al., 2012; Luongo et al., 2017). For instance, offices inhabited by men were more contaminated when compared to women (Hewitt et al., 2012). Hewitt and colleagues speculated that this may be explained by differences in hygiene (Fierer et al., 2008). Conversely, sex had no effect on fungal communities (Luongo et al., 2017).

Not only can different buildings display unique microbiomes but so can different rooms and surfaces (Dunn et al., 2013). Kitchen surfaces showed high numbers of skin associated bacteria (Flores et al., 2013). Microbes associated with particular body parts can be found on distinct surfaces (Flores et al., 2011; Kembel et al., 2014). For example, a strong association between toilet surfaces and gut and vaginal communities, which is expected due to the direct contact between surface and the human body (Flores et al., 2011). Oral bacteria have observed throughout university laboratory surfaces i.e., desks, demonstrating their dispersion throughout the BE due to occupant activity (Yanagi et al., 2022). Surprisingly, indirect contact i.e., sitting fully clothed, can also result in transmission of human-associated microbes and surface proximity to other surrounding surfaces does not influence community composition (Meadow et al., 2014b). Additionally, microbial profiles from surfaces may be indicative of an individual's interaction with that environment and some have proposed its use for forensic application for personal identification or tracing due to the uniqueness of the microbiomes of individuals (Fierer et al., 2010; Lax et al., 2015; Park et al., 2017; Richardson et al., 2019). For example, the microbial community found on personal mobile phones and keyboards can be traced to

the owner (Fierer et al., 2010; Meadow et al.,2014c). However, a more recent study has determined that although microbial signatures of occupants can be detected within the BE, in comparison to current accepted forensic standards, microbial signatures cannot be used as a reliable trace (Hampton-Marcell et al., 2020).

Human occupancy affects the concentration and community structure of microorganisms in the BE, particularly bacterial communities (Bouillard et al., 2005; Goh et al., 2000; Meadow et al., 2014a). Humans are the primary source of bacterial transmission to many indoor environments such as offices, retail stores, gyms, public restrooms and hospitals (Flores et al., 2011; Hewitt et al., 2012; Hoisington et al., 2016; Hospodsky et al., 2015; Lax et al., 2017; Mukherjee et al., 2014; Sharma et al., 2019; Taylor et al., 2014; Wood et al., 2015; Yano et al., 2017). Notably, residences were demonstrated to have significantly higher bacterial concentrations than schools, offices and hospitals. The authors suggest the results were owed to differences in BEs and human activities; residences had a higher number of different human activities i.e., cooking, folding clothes/blankets, when compared to the other buildings (Wang et al., 2023). Studies of occupied indoor environments have shown an increase in bacterial concentrations and human-associated bacteria when compared to unoccupied indoor environments (Hospodsky et al., 2012; Meadow et al., 2014a; Park et al., 2013). Hospodsky and colleagues demonstrated elevated concentrations of bacteria (81 times) and fungi (15 times) in occupied school classrooms versus vacant conditions (Hospodsky et al., 2015). In high occupancy areas such as libraries high concentrations of airborne human pathogens, including bacteria and fungi, have been detected (Hayleeyesus & Manaye, 2014). Occupancy number and frequency otherwise known as human traffic further impacts the diversity and composition of communities throughout BEs (Cao et al., 2021). Many BEs such as universities, have high population densities and human traffic with buildings of varying usage. A study investigating door handles of a university showed that building sampled was most influential on door handle community due to human traffic. In buildings of low human throughput, temporally persistent communities were present (Ross & Neufeld, 2015). Hallways in particular experience high human traffic and have different microbial signatures to locations with lower traffic (Kembel et al., 2014). However, even within large retail stores with low occupant density, the presence of humans still affects microbial community structure (Hoisington et al., 2016). The effect of human occupancy can be observed when comparing indoor and outdoor bacterial communities. Bacterial taxa related to human pathogens have been found in indoor air whilst absent in outdoor air demonstrating the influence of human occupancy (Kembel et al., 2012). Moreover, Goh et al. collected samples in a library building and showed bacterial levels were approximately 10 times higher indoors than outdoors whereas, fungal levels in indoor air were

approximately 50 times lower than in outdoor air (Goh et al., 2000). There is more evidence for human occupancy impacting bacterial community composition, when compared to the weaker links to fungal composition.

The behaviour and activities of human occupants can also shape the indoor microbiome and increase microbial exposure. For example, pet ownership, or activities such as dry dusting, folding clothes and bed making (Ferro et al., 2004; Fujimura et al., 2010; Heo et al., 2017; Song et al., 2013). Simple activities such as moving and talking were demonstrated to have a positive correlation with concentration of bacterial bioaerosols (Heo et al., 2017). However, these activities had no influence on fungal bioaerosol concentrations. Furthermore, human and pet movements can affect the indoor microbiome by resuspending previously deposited materials from flooring and clothing, as well as introducing exogenous microbiota from outdoor environments (Adams et al., 2015; Barberán et al., 2015a; Fujimura et al., 2010; McDonagh & Byrne, 2014).

Overall occupants influence the microbiome of the BE through the release of their unique microbial signature, type of physical contact, frequency of movement and as a secondary source via passive transport and resuspension. Nevertheless, other factors such as the design and purpose of a building could influence human behaviour, their activities and traffic, as well as exerting its own influence, thus potentially play an integral role in what microbes are found within these environments.

### *1.1.2 The Impact of Building Design*

Buildings are complex ecosystems and their design drives indoor microbial community dynamics and composition (Kembel et al., 2012, 2014). With urbanisation and humanities transition to the BE, building design has undergone changes and exposure to the vast outdoor environmental microbes that humans coevolved with is reduced. These changes may potentially affect human health i.e., immune development and human microbiome diversity (Roslund et al., 2020). Similarly, to living organisms, buildings change over time which makes longitudinal studies of interest especially those investigating colonisation of newly opened buildings (Lax et al., 2017; Nygaard & Charnock, 2018). Buildings range from residential individual households to complex communal environments such as universities, shopping centres or airports. Key features of buildings or architectural design that shape the indoor microbial community include, room type, ventilation, connectedness to neighbouring spaces and materials used.

BEs appear to share microbial taxa and are dominated by bacteria frequently detected on humans i.e., *Acinetobacter*, and members of environmental origin i.e., soil (Kembel et al., 2014; Leung & Lee, 2016)

but variation between different rooms has been observed (Adams et al., 2014; Flores et al., 2011; Lax et al., 2014). A study investigating a gradient of urbanisation across South America proposed that the presence of walls dividing spaces i.e., to make rooms, explained differences in microbial composition rather than other building design features such as ventilation (Ruiz-Calderon et al., 2016). However, this variation could be primarily attributed to human occupancy density and use of rooms rather than solely building design.

A building's ventilation strategy shapes the BE microbial communities (Arundel et al., 1986; Dannemiller et al., 2017; Kembel et al., 2012). Type of ventilation system, number and location of ventilation points, window positioning, air speeds and flow can vary greatly between buildings and affect concentrations of airborne particles (Sattar et al., 2016). Historically, natural or passive ventilation was the preferred method for increasing air circulation and reducing pathogen concentrations indoors (Hobday & Dancer, 2013). However, a study showed bacterial aerosol concentration is higher in naturally ventilated offices than (mechanically) air-conditioned offices (Bragoszewska & Biedroń, 2018). This agrees with previous results, that buildings with passive ventilation have more complex microbial communities that resemble the outdoor environment than buildings with mechanically filtrated ventilated air (Kembel et al., 2014; Meadow et al., 2014a). Thus, in large buildings, rooms or spaces furthest from access to the outdoor environment are less diverse and less like outdoor microbial communities or rooms with outdoor access (Kembel et al., 2014; Meadow et al., 2014a; Weikl et al., 2016). Restrooms, which are very isolated rooms, had highly distinct communities compared to other rooms and spaces (Kembel et al., 2014). Moreover, higher ventilation can lower the bacterial concentration in buildings therefore, reducing the effect of occupants on indoor bacterial concentrations (Wang et al., 2023). The type of ventilation, mechanical versus natural, has been shown to influence indoor fungal diversity (Irga & Torpy, 2016) but the local environmental factors such as air currents is a stronger determinant of the indoor airborne mycobiome than ventilation type (Tong et al., 2017).

Indoor environmental parameters like room temperature and relative humidity can also affect indoor microbial concentrations (Guo et al., 2020). One of the most important factors of survival and proliferation of indoor microbes is moisture; a high relative humidity (>80%) supports microbial growth on surfaces (Qiu et al., 2022). Buildings with moisture problems had higher levels of airborne fungi observed (Haas et al., 2007; Pasanen et al., 2000). Furthermore, higher relative humidity indoors showed a significant positive correlation with microbial growth particularly in water associated spaces such as kitchens and restrooms including the sink P-trap (Frankel et al., 2012; Kotay et al., 2017). Concurrent with other studies Wang and colleagues found lower relative humidity resulted in lower

indoor fungi concentrations (Wang et al., 2023). Positive correlations between indoor fungi and indoor temperature and air exchange rate have been recorded. However, for bacteria, indoor temperature and air exchange rate correlated negatively (Frankel et al., 2012). Further studies have shown no significant correlation with temperature or relative humidity and indoor airborne bacteria concentration (Balasubramanian et al., 2012; Cho et al., 2019). Yet, Wang and colleagues demonstrated the lower the temperature indoors, the lower the bacterial concentrations (Wang et al., 2023). However, this study was performed in more extreme temperature zones whereas the previous studies were performed in more temperature-controlled facilities thus, a limited temperature distribution. Natural lighting has also been perceived to have some influences on the spread and emergence of pathogens in the BE (Hobday & Dancer, 2013; Koh et al., 2013).

All buildings are characterised by widespread use of different materials. Materials have different resistance to mould growth (Johansson et al., 2012) and moisture content (Torvinen et al., 2006). This needs to be considered when selecting suitable materials for the indoor environment. Flooring type influences microorganism dispersion, as resuspension of dust occurs less on hard flooring relative to carpet (Ferro et al., 2004; Qian & Ferro, 2008) and carpet is a known reservoir for microorganisms (Becher et al., 2018). However, Chase and colleagues demonstrated no bacterial community differences between surface material i.e., carpet or tile but, showed differences due to location within a room i.e., ceiling or floor (Chase et al., 2016). They suggested the previous observed differences between surface materials was a result of detecting differences based on usage patterns. This agrees with another study that stated human traffic levels affected the bacterial levels on different floor surfaces (Gupta et al., 2019). This does not suggest material has no impact on microbial communities but that within an indoor environment variation of conditions is limited so that occupants are comfortable, and this restricted range may not be enough to drive microbial change. Moreover, biophilic design (indoor planting) is becoming more popular and integrated into BE design as it plays an important role in human physical and mental well-being. Indoor planting dramatically changes the composition of the airborne microbiome; this has implications for controlling the indoor airborne microbiome, therefore will need to be considered alongside all the other parameters of building design going forward (Toyoda et al., 2023; Zhong et al., 2022).

Overall, the design of the indoor environment plays a vital role in mediating how air and microbes are circulated within a “closed” environment. Connectedness via ventilation of air had the biggest impact on bacterial community structure particularly in rooms with lower occupancy whereas rooms with higher occupancy were more influenced by human activities (Adams et al., 2014; Kembel et al., 2014; Meadow et al., 2014a). This demonstrates the direct and indirect impact humans have on microbial

diversity in the built environment. Directly through occupancy and their behaviours and indirectly by determining building design. “Bioinformed” and biophilic design is emerging to promote a healthy indoor environment by taking account of design, materials and occupant behaviour (Green, 2014).

### *1.1.3 Environmental Influences*

The outdoor environment is a complex ecosystem composed of extensive and diverse microbial communities arising from different sources such as soil, aquatic environments, and wildlife. The outdoor environment influences the composition of the indoor built environment due to fluctuations and transportation of microbes between the two environments via biotic i.e., humans, or abiotic vectors i.e., air (Flores et al., 2011; Stamper et al., 2016). In a study investigating an office, the main source of bacterial contamination was environmental in origin as many soil-associated bacteria were observed (Hewitt et al., 2012). For fungi, studies show fungi indoor are dominated by those from outdoor (Adams et al., 2013; Barberán et al., 2015a). Additionally, indoor air and outdoor air have strong microbial connections (Adams et al., 2015). This suggests that microbial communities in built environments are composed of “migrant” microbes rather than microbes originating from within built environments (Adams et al., 2015). The geographic location, climatic conditions and season impact environmental microbial communities and thus built environment communities.

Outdoor air influences the indoor air microbes of built environments. Air is an important transport mechanism of microbes as it connects room and surfaces therefore, aids in microbial distribution. Abundance of bacteria in outdoor air positively correlates with that in indoor air (Miletto & Lindow, 2015). Particularly in well-ventilated indoor spaces, the indoor air microbiome is comprised of a greater proportion of outdoor air-associated microorganisms (Kembel et al., 2012; Leung et al., 2014; Meadow et al., 2014a; Miletto & Lindow, 2015; Wilkins et al., 2016). Surface fungal communities are less influenced by occupants than bacterial communities but more so by environmental factors including air currents (Tong et al., 2017). Moreover, indoor pollen fungal concentrations parallel outdoor air concentration trends. However, outdoor air microbial communities can have a human commensal signal in heavily populated environments, demonstrating the diverse range of sources which contribute to microbial air communities (Qian et al., 2012). Although, humans can influence their surrounding environment, connectedness to external environments greatly influences air microbial composition. In a New York Subway, which is an open built environment with heavy human traffic, 20% of observed taxa in the air was associated with human skin (Robertson et al., 2013). Whereas a study in a university classroom showed 85% of the sequences were human associated (Meadow et al., 2014b). So, although the classroom is less populated than the subway there is still a

higher percent of human associated taxa observed, suggesting the subway is more under the influence of the environment due to connectedness to the outdoor air and environment. Furthermore, another subway system in Hong Kong had air compositionally indistinguishable from outdoor air (Leung et al., 2014).

Since the outdoor environment is an important microbial source for BE communities, changes in the outdoor microbial community will potentially influence nearby BEs. Geography contributes strongly to microbial community structure (Chase et al., 2016; Gibbons, 2016). Contrasting terrains i.e., agricultural, suburban and forests have distinctive air microbiomes (Bowers et al., 2010). Geographic location governs fungal diversity in settled-dust samples, the further from the equator the higher the diversity, despite differences in building designs, as well as driving microbial dynamics in house dust samples across the US (Amend et al., 2010; Barberán et al., 2015b). It is worth noting that the human microbiome varies across the world and since human occupancy and behaviour is a major force shaping the microbiome of the built environment this could result in variation (Yatsunenko et al., 2012). For example, skin microbial composition in specific ages is different between rural and urban children (Lehtimäki et al., 2017). Differences in microbial community composition due to geography could be due to differences in the climate of the regions studied.

Climatic and meteorological conditions contribute to differences found between microbiomes of geographical regions. Specifically, temperature and UV radiation have been found to be the most important meteorological factors in the viability of airborne bacteria (Brągoszewska et al., 2017). Weather conditions can also correspond to viable airborne microbial concentrations (Li et al., 2017). Certain microbial species can survive and grow in different climatic conditions i.e., cold-tolerant microorganism (Bej et al., 2000). Further, weather can determine dispersal and sources of outdoor microbial communities. Dry and warm conditions may result in desiccation of soil microbes, promoting spore dispersal or aerosolization (Brodie et al., 2007). This was demonstrated when comparing the office dust of buildings in Tucson, New York and San Francisco. Tucson was particularly abundant in desert soil bacteria suggesting a strong climatic signal (Hewitt et al., 2012).

Seasons have varied effects on microbial communities. Season can influence the levels of microorganisms and result in variation in bacterial and fungal community composition and structure (Du et al., 2018; Park et al., 2013). Outdoor air bacterial concentrations have been shown to vary diurnally but the effect on indoor communities may be reduced (Bowers et al., 2011). However, indoor dust bacterial communities do show seasonal variation, they peak in spring and are lowest in summer (Frankel et al., 2012). An early study investigating settled dust of two buildings found bacteria differed between most seasons but differences between the buildings was greater than the effect of seasons

(Rintala et al., 2008). In a more recent study of a newly opened kindergarten, it was found the sampling rounds were significant, suggesting observed microbial differences related to accumulation over time or seasonal variation. Yet, since the kindergarten had only been open 11 months, a stable community may not have developed, thus it is unclear the role of seasonal variation (Nygaard & Charnock, 2018). Fungal communities generally exhibit more obvious seasonal variation than bacterial communities (Pitkäranta, 2011; Reponen et al., 1992). Fungal concentrations particularly in indoor and outdoor dust and air are known to vary between seasons (Adams et al., 2013; Koch et al., 2000; Pitkäranta, 2011; Weikl et al., 2016). Contrasting to bacterial communities, fungal indoor dust communities peak in summer and are lowest in winter (Frankel et al., 2012). Additionally, during winter, fungi can disperse farther whereas bacteria are limited to local scale dispersion (Tong et al., 2017). Not many studies have specifically investigated how seasons affect the built environment virome. The airborne viral community in a daycare centre was strongly influenced by seasons compared to bacterial communities which were not (Prussin et al., 2019). During winter human-associated viruses were more diverse and dominant compared to summer which had a higher proportion of plant-associated viruses. Overall, the outdoor seasonal changes can affect the indoor microbiome and the extent of these affects vary for different microorganisms.

In conclusion, the built environment is shaped by the surrounding outdoor environment and many different microbial communities from diverse sources comprise this. Air and its movement are an important tool for the interactions between the built environment microbiome and the surrounding environmental microbiome. Geographic areas and their associated climatic conditions are strong determinates of environmental microbial community composition and their dispersion. Seasons have varied effects on microbial concentrations in outdoor air which reflects in indoor air communities. The extent of the affect appears to differ for different microorganisms. Importantly, the information about variation in microbiomes due to environmental influences can be applied to design predictive and computational tools to help identify environment-associated microbial community changes and how BEs could be affected. For example, urbanisation has resulted in less diverse outdoor environments and these urban areas exhibit less continental-scale geographic variability to rural areas (Barberán et al., 2015b). This decreased exposure to microbes associated with the shift from rural to urban environments rises concerns for human health and development. Furthermore, with climate change causing an increase in extreme weather events, buildings without adequate temperature control or resistance to these events could be subject to more intense variations in indoor temperature and changes in conditions. How this may affect our microbial inhabitants is unknown, but environmental influences will continue to play an important role in manipulating microorganisms of the BE.

### *1.1.4 Importance of Studying BE Microbiomes: AMR and Pathogens*

Antimicrobial resistance (AMR) bacteria are recognized as one of the biggest threats to public health, posing a global crisis that endangers the lifesaving role of antibiotics (Hutchings et al., 2019; O'Neil, 2014). It is estimated that AMR will cause up to 10 million deaths annually by 2050 (Li et al., 2018). AMR is not a modern phenomenon; genes encoding resistance to antibiotics are found naturally and have been identified in 30,000-year-old permafrost sediments (Bhullar et al., 2012; D'Costa et al., 2011). Human activities, including the overreliance and misuse of antibiotics in healthcare (Chang et al., 2019; Llor & Bjerrum, 2014) and agricultural settings (Chandler, 2019, Cole and Desphande, 2019), are driving factors leading to the current rapid increase and spread of AMR. AMR can arise through evolution and random mutations driven by selection due to antibiotic pressure or other environmental stress factors, as well as through horizontal gene transfer between species (Martinez & Baquero, 2000; Munita & Arias, 2016). These resistance genes can be transmitted via human movement or through the food chain to other environments (Cave et al., 2019, Conceição et al., 2013; Ruiz & Alvarez-Ordóñez, 2017).

To date most AMR studies have focused on surveying AMR in healthcare and agricultural settings. A study sampling airborne fine particulate matter showed that compared to outside ambient air, hospitals harboured nearly twice the abundance of antibiotic resistance genes (Wu et al., 2022). Research into human BEs is limited but, studies have generally shown high abundances of AMR genes, particularly in comparison with outdoor environments (Cave et al., 2019, Kang et al., 2018, Roberts et al., 2013; Zhao et al., 2021). As well as demonstrating resistant bacteria are transmissible in urban BEs, such as public transport systems and schools (Conceição et al., 2013; Kahsay et al., 2019; Zhou and Wang, 2013). In recent years the COVID-19 pandemic has highlighted the critical importance of clean and well-regulated indoor environments (Warmbrod et al., 2021) and has put more emphasis on understanding the microbes of the BE and the transmission of AMR genes. However, comprehensive AMR surveillance in public settings requires considerable time, cost, expertise and is often directed at observing clinically important pathogens (Conceição et al., 2013; Kahsay et al., 2019; Zhou and Wang, 2013). Although culture-independent metagenomic studies are overcoming some of these limitations and starting to reveal more about the abundance and prevalence of AMR genes in public BEs, including classrooms (Hartmann et al., 2016), athletic facilities (Fahimipour et al., 2018) and mass transit systems (Afshinnekoo et al., 2015; Kang et al., 2018)

The indoor microbiome is comprised of various bacteria including pathogens such as *Staphylococcus spp*. and *Enterobacteriaceae* with AMR genes present (Afshinnekoo et al., 2015; de Sousa, 2020; Fahimipour et al., 2018; Hartmann et al., 2016; Mkrtchyan et al., 2013; Wu et al., 2021). Increasingly studies are demonstrating that Methicillin-resistant *Staphylococcus aureus* (MRSA) can be found in public BEs, such as transport systems (Conceição et al., 2013; Lin et al., 2017; Peng et al., 2015), shopping centres (Domon et al., 2015), and university campuses (Roberts et al., 2013), with recovery of MRSA isolates varying from 1.5% to 36%. Furthermore, care homes have been shown to be reservoirs for antibiotic-resistant bacteria (ARB) including *Klebsiella* spp. and *E. coli* (Wiener et al., 1999). In contrast, a metagenomic study of mass transit systems across 60 cities around the world found a low abundance of antibiotic resistance genes, especially when compared to housekeeping genes (Danko et al, 2021). Yet, the authors suggest AMR genes could be higher than observed as samples may contain undetected and unidentified AMR genes. In this study the most common antibiotic resistance was detected for macrolides, lincosamides, streptogamines, and beta lactams, which agrees with AMR genes detected in other studies, as these are some off the most commonly used antibiotics to treat bacterial infections in humans (Hartmann et al., 2016). High usage of disinfectants and antimicrobial cleaning products indoors has been suggested to contribute to the selection pressure favouring resistant strains (Aeillo & Larson, 2003; Buffet-Bataillon et al., 2015; Jin et al., 2020; Kampf, 2018; Maki et al., 2023). Mahnert and colleagues suggested that a healthy indoor environment is characterized by a diverse and stable microbiome, which can help reduce the presence of antibiotic-resistant genes (Mahnert et al., 2019). The need to control the indoor airborne microbiome is increasingly evident, but it is a complex challenge with AMR genes and ARB abundances varying dependent on location, building type, and use.

Strategies to enhance microbial diversity indoors, such as increasing ventilation or incorporating green plants, can promote a healthier microbiome and potentially lower resistance exposure (Berg et al., 2014; Mahnert et al., 2015). Additionally, human density and activities may play a role in the transmission of airborne antimicrobial resistance genes (ARGs) in indoor environments; therefore, understanding users' behaviours may aid in reducing transmission. Addressing AMR requires comprehensive surveillance in BEs environments to better understand its prevalence and impact. Enhanced metagenomic technologies that link AMR genes to their bacterial hosts could significantly improve our ability to monitor and manage AMR in these settings (Arango-Argoty et al., 2019; Kalmar et al., 2022; Nurk et al., 2017; Stalder et al., 2019). Furthermore, long-term studies are needed to understand how AMR is changing. Given the serious public health implications, controlling the indoor

airborne microbiome and ensuring the maintenance of healthy indoor environments are critical steps in mitigating the spread of AMR and protecting public health.

## 1.2 Microorganisms in the Built Environment: Pipes, P-traps and Water as a Source

### *1.2.1 Microorganisms and Water Distribution Systems*

Water is essential to the growth and survival of bacteria and fungi. There are multiple sources of water throughout the BE from plumbing pipes, hot water storage tanks to small appliances and water features. These indoor sources of water contribute to the human microbiome via ingestion (drinking water), skin contact, inhalation of aerosolised water droplets (Johnson et al., 2013) and biofilm formation on water-associated surfaces (Adams et al., 2017). Premise plumbing systems, the water distribution system located within a building including distribution pipes, various devices (i.e., hot water heater), fixtures (i.e., showers), and drains (i.e., sinks), are known to harbour distinct microbiomes (Wang et al., 2013). Proteobacteria, Firmicutes, Nitrospirae and Actinobacteria are dominating phyla found within these environments (Bruno et al., 2022; Chao et al., 2013; Jing et al., 2023). Fungi such as *Fusarium* are widespread throughout plumbing drains (Short, 2011). Additionally, opportunistic pathogens are harboured by and colonise water distribution systems and the building plumbing environment itself (e.g., water heaters and shower heads), which makes their control particularly challenging (Ashbolt, 2015; Falkinham et al., 2015; Feazel et al., 2009; Marciano-Cabral et al., 2010; Wang et al., 2013). Opportunistic pathogens such as *Pseudomonas*, *Mycobacteria*, *Legionella,* and protozoans have been documented in these systems (Bédard et al., 2016; King, 2014; Nisar et al., 2023; van der Wielen & van der Kooij, 2013). Opportunistic pathogens are ideally adapted to premise plumbing conditions due to certain characteristics; biofilm formation, survival and propagation in free-living amoeba, disinfectant-resistance, and ability to grow at low oxygen or organic carbon levels (Falkinham et al., 2015). Furthermore, biofilms inevitably line premise plumbing pipes, and can contribute microbes to running water as it flows over microbial communities. Most biofilm communities consist of benign and even beneficial microorganisms but as mentioned they can provide a key environment for the proliferation of opportunistic pathogens. A biofilm starvation experiment revealed biofilms can survive under prolonged conditions of no water flow, and upon addition of nutrients, viable cells in biofilms can more than double within 24 hours (Hota et al., 2009). Two more recent studies using more comprehensive methods, further validated the ability of microbial diversity to rebound after stagnation demonstrating the important role of biofilms in recontamination (Dai et al., 2018; Ji et al., 2017).

### *1.2.2 Environmental Conditions of Water Distribution Systems and their effects on Microbial Communities*

Most studies focus on exploring the microbiome of drinking water due to its direct exposure to humans and the negative health implications if potable water pipes harbour pathogens. In these studies, the temperature (Inkinen et al., 2016), age of water pipes (Henne et al., 2012), water chemistry (Ji et al., 2015; Wang et al., 2014), stagnation within the pipes (Lautenschlager et al., 2010; Ye et al., 2022) and disinfection method (Baron et al., 2014; Paduano et al., 2020; Wang et al., 2012) influenced variation in microbial communities. Likewise, research into hot water premise plumbing determined that stagnation and especially temperature impacted taxonomic and functional gene composition (Dai et al., 2018). Moreover, higher water temperatures can increase the risk of opportunistic pathogens, as well as water use frequency (Ji et al., 2017). With lower usage, stagnation occurs, increasing the potential for pathogen growth (Ciesielski et al., 1984; Rhoads et al., 2015). Ciesielski and colleagues (1984) verified *L. pneumophila* numbers did not decrease when hot water heaters were not in use. Further, stagnation results in physiological changes in communities as genes involved in stress-associated cellular functions such as antibiotic resistance significantly increased whereas genes involved in metabolism and growth were reduced (Dai et al., 2018). Stagnation period in hot water taps had the least effect on total microbial numbers (Lipphaus et al., 2014), corresponding with Dai and colleagues (2018) more recent study. Although, the period of stagnation can be critical for specific pathogens (Rhoads et al., 2015). Conversely, in cold water taps, the period of stagnation can induce substantial changes in microbiome composition and microbial cell concentrations (Ji et al., 2015; Lautenschlager et al., 2010; Ling et al., 2018) and alongside warmer indoor temperatures, microbial metabolic activity level increases (Zhang et al., 2015). The stagnation studies above processed filtered water samples. It is recognised that microbial communities differ between water and biofilms. However, similar impact of stagnation was observed in biofilms as well, as microbial community members differed in their activity dependent upon temperature (Inkinen et al., 2016).

In another drinking water study pH was the strongest regulator of bacterial community (Pinto et al., 2012) and *Legionella* spp. have been shown to be associated with high pH drinking water (Ji et al., 2015). Other factors shaping both bacterial and eukaryotic community structures are disinfectant and water age, whereas pipe material only influences bacterial community structure (Wang et al., 2014; Yu et al., 2010). The variables tested also can interact with each other, resulting in different outcomes. For example, pipe material effects on microbial communities only became apparent at water ages corresponding to low disinfectant residuals (Wang et al., 2012). Water flow conditions can result in

differences in bacterial communities. Under low flow regimes in hospital water there was an increase in potentially pathogenic taxa, biofilm forming and environmental stress resistant bacterial taxa (Nisar et al., 2023). Additionally, in low flow rate water systems there are higher concentrations of antimicrobial resistant bacteria (ARB) and antimicrobial resistance genes (ARG) (Zhang et al., 2019).

The variation of indoor water microbial communities can be explained by the differences in environmental conditions, as discussed, how humans mediate them and raw water type. Microbial communities of eukaryotes and bacteria correlate (Wang et al., 2014), highlighting areas of future research opportunity, to explore interspecies relationships and the ecological roles of eukaryotes. For example, free-living amoeba graze on biofilms and help maintain its density, while also being host to amoeba-resistant opportunistic pathogens (Greub & Raoult, 2004). Microbial diversity is not necessarily negative and may even have health benefits in drinking water. Despite this, specific conditions can promote proliferation of opportunistic pathogen therefore, understanding and monitoring environmental conditions and their affects is essential.

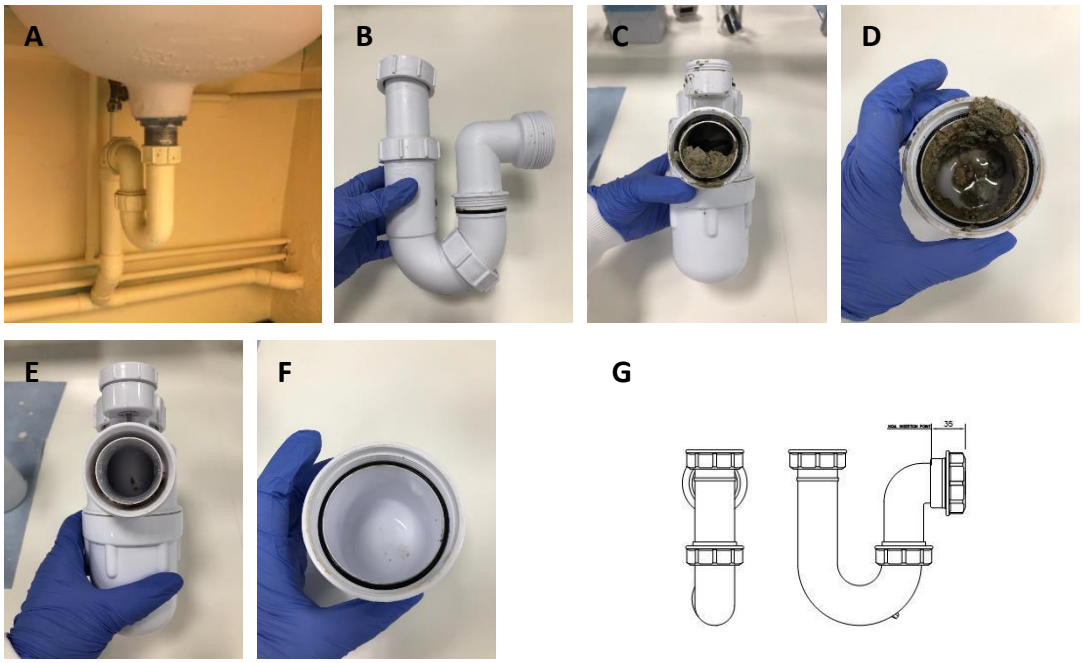


**Figure 1.1.** Images of P-trap. (A) In-situ sink P-trap, (B) Dismantled P-trap, (C, D) Dismantled bottle P-trap from a urinal (thick deposits), (E, F) Dismantled bottle P-trap from urinal (clean), (G) P-trap drawing.

### *1.2.3 Sinks and their Traps as a Reservoir*

Sinks and their traps, such as the P-trap (Figure 1.1) are rich microbial environments and known reservoirs of nosocomial pathogens and resistance genes (Apanga et al., 2022; Bourdin et al., 2023; Kizny Gordon et al., 2017; Lalancette et al., 2017). The purpose of sink traps, such as the P-trap, is to

form a barrier against noxious sewer gas escaping from the piping below. P-traps provide a relatively protected environment that is hydrated due to the retained water, therefore favours microbial growth, proliferation, and propagation of resilient biofilms (Kearney et al., 2021; Kotay et al., 2017; Valentin et al., 2021). Once biofilms are established in these environments, they can be difficult to eradicate and may require alternative or repeat interventions (Jones et al., 2020; Lemarié et al., 2021; Lowe et al., 2012; Otter et al., 2015; Regev-Yochay et al., 2018; Smolders et al., 2019). P-traps can be inoculated from two directions, firstly from above due to microorganisms from a variety of sources; shedding via handwashing, disposal of bodily fluids, tap water itself and any other waste (Figure 1.2). Interestingly, a study assessing the impact of handwashing soap on population dynamics of microorganisms found that certain soap could increase growth of isolates (Boyle et al., 2020). Therefore, as well as inoculating, nutrients can be provided from above. Secondly due to back-flow from connecting pipes downstream of the P-trap (Kotay et al., 2017). Contamination of sink drains can propagate to proximate rooms via plumbing (Hopman et al., 2019). Wastewater systems such as sinks are designed that all waste flows down. However, biofilms inside the plumbing can spread even against gravity (Aranega-Bou et al., 2021). Kotay and colleagues demonstrated motile *E. coli* can travel up from the sink trap at a rate of 2.5cm per day (Kotay et al., 2017). Thus, there is the potential through splash water and aerosols, that microorganisms from the sink trap can be transmitted to the surrounding environment.

Most research on sink traps has been associated with those found in hospital environments due to the direct implications to human health. Sinks have long been identified as sources of contamination in hospitals and implicated in outbreaks, pathogens identified include *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, *Serratia marcescens* and *Klebsiella oxytoca* (Ayliffe et al., 1974; Bourdin et al., 2023; Landelle et al., 2013; Lowe et al., 2012). Additionally, sink traps have been found to be reservoirs of genes coding antimicrobial resistance (AMR) (Decker & Palmore, 2014; Kizny Gordon et al., 2017; Kotsanas et al., 2013) and viable highly resistant microorganisms (van der Schoor et al., 2023). Compared to other hospital surfaces, sink drains have been shown to have the highest burden of antibiotic resistant bacteria (ARB) (Sukhum et al., 2022). Concerningly, bacteria with more antimicrobial resistance genes (ARGs) in their genomes have been isolated from ICU sinks more than other hospital spaces. Additionally, this study observed that ARGs were harboured on mobilised plasmids and shared in genomes of unrelated bacteria (Diorio-Toth et al., 2023). From their results, they suggested dependent on bacterial species, antimicrobial resistance could be maintained within such species by strain colonisation and/or horizontal gene transfer. Moreover, there is evidence of

shared strains with resistance across the hospital environment (sinks) and patients (Sukhum et al., 2022).

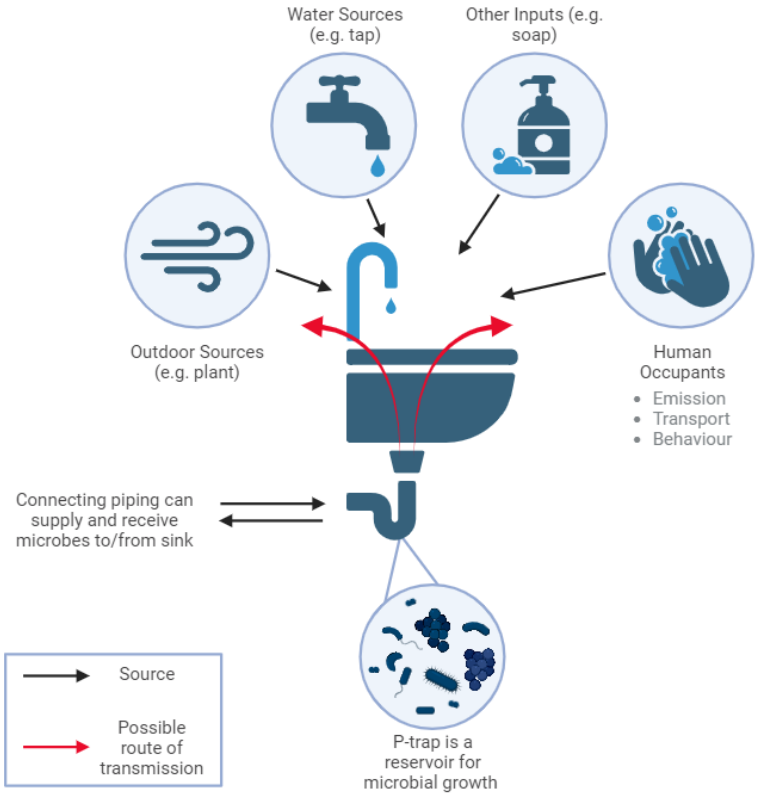


**Figure 1.2.** Diagram demonstrating potential sources of microorganisms to the sink environment. Microorganisms from the outdoor environment could enter the sink through passive movement of air depending on how enclosed the surrounding environment. Further tap water could directly contribute microorganism to the P-trap. Other inputs such as soap or improper disposal of biological fluids, are potential sources, as well as providing additional nutrients to the microbial communities established in the P-trap. Human-associated microorganisms could be introduced into the P-trap through washing of hands, additionally humans can transport microorganisms from environments previously in contact with. The behaviours of human occupants could influence the composition of microorganisms, i.e. inappropriate use of sinks, temperature used for hand washing. All these sources could contribute to the P-trap serving as a microbial reservoir which can spread horizontally and vertically along connecting pipes. The red arrows show a possible route of transmission of microorganism from the P-trap; upon supply of tap water, microorganisms could spread to the surrounding surfaces or onto the hands of users.

Methods to eradicate or control microorganisms associated with clinical outbreaks include disinfectant with sodium hypochlorite and replacement of contaminated sinks. In ICUs this has been

shown to reduce infection rates, but bacteria can reside further down the system and reappear despite installation of new sinks (Hota et al., 2009; Stjärne Aspelund et al., 2016). Removal of all horizontal drainage systems can help to fully eradicate an outbreak (Vergara-López et al., 2013). Other cases of elimination using replacement and disinfection have had success on Carbapenem-resistant *Acinetobacter baumannii* (CRAB) (La Forgia et al., 2010; Landelle et al., 2013). In a neonatal ICU, after implementation of intensified environmental cleaning, sink management protocol, sink dismantling and sodium hypochlorite soaking, CRAB was no longer isolated (Woon et al., 2023). Several studies have suggested pouring liquid disinfectants alone, may have a transient effect due to inadequate contact time in a fluidic system, therefore disinfectants might not penetrate the biofilms adequately (Carling, 2018; Kizny Gordon et al., 2017; Parkes & Hota, 2018). Instilling devices such as a stop valve or urinary catheter balloons alongside disinfectant, to allow for increased contact time with pipes, reduced colonisation for several days whereas, without these devices reduction was transient (Cadnum et al., 2019; Jones et al., 2019). These studies do not investigate how the communities changed, and which bacterial taxa could be more tolerant to disinfectant. Moreover, other chemical treatments, i.e., formalin, acetic acid, Virox and foaming hydrogen peroxide have been implemented and been effective in controlling outbreaks or reducing microbial load (Buchan et al., 2019; Jones et al., 2020; Lowe et al., 2012; Smolders et al., 2019; Stjärne Aspelund et al., 2016; Wolf et al., 2014). In a study using a drain biofilm model, sodium hypochlorite was partially effective, as bacterial growth reoccurred within 4 days, agreeing with the previous studies suggesting liquid disinfectants have a temporary affect. Other interventions installed sink drain plugs as a physical barrier against splashing. Reduction in the target microorganism was observed after installation and particularly in the sink basin the microorganism was rarely cultured. Self-disinfecting sinks are also efficient at reducing bacterial load (de Jonge et al., 2019; Fusch et al., 2015). By cyclically heating up to high temperatures they can reduce transmission. However, purchase and installation of these have additional costs; a cheaper alternative of pouring boiling water down the sink reduced bacterial load but self-disinfecting sinks were significantly more efficient (Gideskog et al., 2023). Further engineered sinks that decontaminate the P-trap using UV and regularly rinsing with ozonated water showed to decrease levels of bacterial and fungal contamination in the P-trap compared to initial levels (Cole & Talmadge, 2019). Often improper disposal of clinical waste down handwashing sinks has been associated with the outbreaks, so alongside other eradication measures, education and guidelines for sink management and practices need to be established for long-term control (Lowe et al., 2012; Woon et al., 2023).

Sink design facilitates the spread of pathogens in two main ways, firstly by promoting biofilm formation and secondly by encouraging disruption of established biofilms. Studies have suggested

aerosols are the primary dispersion route for microorganisms. However, these claims are based on rudimentary findings (Fusch et al., 2015; Hota et al., 2009). More extensive research has shown that bacteria cannot aerosolise from sink P-traps and disperse but, can upon tap use be spread to surrounding area via large droplet size particles (Kotay et al., 2019). Dispersal from beneath strainer to sink bowl then onto users can occur by splashing of flowing water (Hajar et al., 2019). Equipment within 2m from a sink can be at risk of potential contamination and faster water velocity of water exiting the tap results in a larger "splash zone" (Garvey et al., 2023). Taps that directly flow into the drain may increase the risk of microorganism's dispersal (Aranega-Bou et al., 2019; Hota et al., 2009) as well as shallow bowls are more likely to disperse microorganisms just below the strainer (Gestrich et al., 2018). Material of pipes can influence bacterial counts, for example copper water pipes have been shown to reduce bacterial counts compared to PVC (Learbuch et al., 2021). These findings suggest that rethinking or modifying sink design could reduce the risk for dissemination of potentially harmful microorganisms from sinks and their traps.

Studies of sinks outside a hospital are very limited, particularly those that directly investigate the sink P-trap microbial communities. Surfaces of kitchen sinks have been studied using predominately culture-based approaches and some include 16S rRNA sequencing (Borrusso & Quinlan, 2017; Flores et al., 2013; Mcbain et al., 2003; Moen et al., 2015, 2023). *Morexellaceae*, *Micrococcaceae*, *Streptococcaceae*, and *Enterobacteriaceae* were bacterial taxa identified as dominate in household kitchen sinks (Moen et al., 2015, 2023). Other studies have specifically investigated fungi isolated from domestic bathroom sink drains (Hamada & Abe, 2010; Short, 2011). Mostafa and colleagues cultured bacteria from public female restrooms including sink surfaces (Mostafa & Sabra, 2013). However, by only using culture-based techniques, the study is limited to only identifying culturable microorganisms. Additionally, the study only focused on female restrooms from buildings identical in design.

Overall, there is a clear lack of research regarding public restrooms sink microbial communities. The studies of hospital sinks have shown the importance of sinks as a reservoir of pathogens and AMR genes and the challenges with eradiating pathogenic outbreaks. As well as the long-term persistence of resistance-conferring plasmids in unrelated hosts in the sink environment. In buildings such as hospitals, there is more risk for immunocompromised and vulnerable people and the behaviours relating to sink usage i.e., improper use, may shape the microbial community to be more resilient and resistant. In public buildings where, sinks may not experience the same practices as in hospitals but are exposed to variety of occupants and their activities, identifying the prevalence and abundance of associated microbial taxa is yet to be established. Considering the health concerns associated with

hospital sinks, the microbial communities of sinks within public non-clinical buildings should be investigated. Therefore, the microbial community of a "healthy" sink can be established and the potential implications on the human occupants of the public buildings be considered.

## 1.3 Review of Methodologies

The ability to investigate and understand the composition, dynamics and resilience of complex microbial communities inhabiting diverse environments has improved significantly in recent years. Microbiology as a distinct science dates to 1857, when Louis Pasteur demonstrated that formation of fluids was due to microorganism (Opal, 2010). Since then, the field of modern microbiology launched and over a century later the first bacterium was sequenced (Fleischmann et al., 1995). The traditional method of culturing provides a semiquantitative enumeration of viable cells but, is often time-consuming and further biochemical, serological or molecular genetics methods are required to confirm the species. Advances in modern molecular techniques such as DNA sequencing and data analysis now offer unparalleled insights into the composition and dynamics of microbial communities as well as overcoming the limitations of culture-based analysis. Culture-based limitations include only viable cells being represented, preferential selection of fast-growing microorganisms (Posten & Cooney, 1993) and only confirming microorganisms capable of growing on the selected medium (Amann et al., 1995). Additionally, cultivation cannot quantify microorganisms in the viable but non-culturable state, which is of concern as these organisms can be pathogens (Ramamurthy et al., 2014). Due to these limitations most bacterial and archaeal taxa remain uncultured (Steen et al., 2019) as culturing may be ineffective at identifying novel or unculturable microorganism. Furthermore, the diversity and scale of environmental microorganisms are massively underrepresented in culture-based studies and extraordinarily abundant microorganisms found in diverse habitats may only be very remotely related to any strains that have been previously isolated and characterized (Staley and Konopka, 1985; Rappe and Giovannoni, 2003). To overcome the limitations and problems associated with culturing, culture-independent molecular methods can be used.

Next-generation sequencing (NGS) technology enables high-throughput parallel sequencing of DNA and has resulted in a flood of sequencing data (Thompson et al., 2017). NGS can include metabarcoding, whole metagenome and metatranscriptome sequencing. These methods can provide information on the presence, abundance, or function of genes of microorganisms from environmental or clinical samples. Metabarcoding also known as short gene marker or amplicon sequencing is often

the method of choice for studies investigating microbiomes due to usability, affordability and providing a high-level but low-resolution overview. Metabarcoding involves targeting a specific region of interest, known as the marker genes with primers. Marker genes are conserved genes containing a highly variable region flanked by highly conserved regions (Knight et al., 2018). The most frequently targeted genes for molecular analysis are those which encode for the small subunit ribosomal RNA molecule (herein, 16S rRNA for prokaryotes and 18S rRNA for eukaryotes) and the internal transcribed spacer regions (ITS) for fungi. The variable regions allow for taxonomic identification (Janda & Abbott, 2007) and the flanking conserved regions allow for design of universal primers e.g. that can be used to amplify the 16S rRNA across all prokaryotes. For example, the 16S rRNA gene is approximately 1500 base pairs long and comprised of nine hypervariable regions (V1-V9), commonly only one or two regions are selected for sequencing due to constraints of number of base pairs able to be sequenced on an Illumina platform. Dependent upon the region selected, certain bacterial genera can be underrepresented or missing therefore selection of appropriate V-region for sample of interest is essential (Abellan-Schneyder et al., 2021). This is true in the case of other regions of interest, for example dependent upon ITS region (ITS1 or ITS2) selected there can be differences in fungal community profiles (Blaalid et al., 2013; Mbareche et al., 2020). For prokaryotes, the V4, V5, V6 regions of the 16S rRNA gene are recognised as highly conserved and can provide accurate classifications of organisms at higher levels which is useful for unknown microbial communities (Bukin et al., 2019). Metabarcoding is well tested, quick, and cost-effective, making it applicable for many different studies and sample types (Caporaso et al., 2010; Thompson et al., 2017). Due to its usability, there are large existing public data sets (i.e., Human Microbiome Project, Earth Microbiome Project) that can be used in conjunction with other investigations. Additionally, metabarcoding works well for low-biomass samples and samples contaminated with host DNA. However, targeting isolated genomic DNA (gDNA) in this way does not differentiate between dead, inactive or active cells. It has been demonstrated recently that the majority of gDNA present in the BE is composed of dead cells or those with a compromised cell membrane (Vaishampayan et al., 2013). Additionally, the method is subject to biases. Firstly, amplification biases, as the number of amplification PCR cycles increases the diversity may be affected due to reannealing of major PCR products becoming more probable than primer binding as PCR proceeds (Bonnet et al., 2002; Suzuki & Giovannoni, 1996). Secondly, bias arises from choice of primers as primers are specific to a region in the sequence and do not have equal affinity for all possible DNA sequences (Albertsen et al., 2015; Hong et al., 2009; Walker et al., 2015). Bias can be mitigated by optimising primer selection but a priori knowledge of the microbial community is required. Metabarcoding resolution can be limited to genus level or higher taxonomic levels, dependent upon choice of taxa, gene, primers, choice of identification database and quality of

sequencing run. In some experiments resolution is better than genus but, often distinguishing between closely related prokaryotic strains is limited (Jovel et al., 2016; Lan et al., 2016). Functional information is limited and much better characterised in whole metagenome studies (Aßhauer et al., 2015).

Whole metagenomic sequencing or shotgun sequencing obtains a more detailed resolution such as strain-level (Heilbronner et al., 2011; Roetzer et al., 2013) and as stated can provide information on molecular functions. Specific functional pathways in samples can be determined at gene level providing insight into the functional capacity of an entire community (Aßhauer et al., 2015). In contrast to metabarcoding a greater depth of information is achieved as all microbial genomes within a sample can be captured, including viral and eukaryotic DNA without the need for targeting different marker genes. Additionally, there are no PCR related biases, and the method can provide an accurate estimate of growth dynamics of the microbiota (Korem et al., 2015). Novel genomes can be mined from metagenomic datasets which increases the availability of taxonomic data (Sangwan et al., 2016). However, in comparison to metabarcoding, whole metagenomic sequencing is relatively expensive, more complex, requires demanding bioinformatic processes and is limited by sequencing depth. Moreover, contamination from host-derived DNA can occur and shotgun sequencing is rendered inefficient with low biomass samples (Fuks et al., 2018). Similarly, to metabarcoding, there is no discrimination between live, dead or active microorganisms, and computational analysis and assigning taxonomic classification is largely dependent upon reference microbial genomes. Regarding bacterial genomes, the database is expanding, but contains around an order of magnitude less sequences than 16S rRNA gene databases (Fuks et al., 2018). Consequently, species may be missed when analysing shotgun/whole metagenomic sequences since its genome is currently not in a database.

High-throughput short-read sequencing such as those mentioned above have been the methods of choice for many years, but they are limited to the size of the library so, can fail to resolve larger repeat regions (Sereika et al., 2022). In metagenomic samples this can be particularly problematic as they can often contain related species of strains with near-identical long sequences of DNA. Long-read sequencing can overcome this, providing species level of identification of complex bacterial communities i.e., full length 16S rRNA (Matsuo et al., 2021). When constructing metagenome-assembled genomes (MAGs), from short-read metagenomics they can be highly fragmented even with high-quality MAGs (Liu et al., 2020). Long-reads generated on Nanopore or PacBio platforms can bridge genome gaps as well as help with detecting complex structural variants like large inversions or translocations (Mantere et al., 2019). A hybrid assembly of both short- and long-read can enable reference-quality genome reconstruction from diverse microbiomes (Bertrand et al., 2019; Jin et al.,

2022; Singleton et al., 2021). Although PacBio HiFi reads can generate near-finished microbial genomes from pure cultures or metagenomes due to its high accuracy (> 99%), cost per base is high making it economically unfeasible (Bickhart et al., 2022; Feng et al., 2022). Whereas Oxford Nanopore Technologies (ONT) developed Nanopore sequencing, which is more accessible, rapid, and affordable for many research labs (Gorzynski et al., 2022; Yang et al., 2022). Nanopore sequencing has previously experienced difficulties with fully characterising long homopolymer regions and having higher error rates (Delahaye & Nicolas, 2021) but with the release of new technologies (R10.4), ONT have enabled generation of complete bacterial and plasmid genomes without the need for short-read sequencing (Sanderson et al., 2023; Zhao et al., 2023). Reference-quality genomes from complex metagenomes using only nanopore long reads can now be obtained (Liu et al., 2022). This massively opens up the black box of uncultured microorganisms as begins to provide genome-level insights of organisms that cannot be isolated, therefore expanding the tree of life (Hug et al., 2016).

For determining gene expression and functional output of microbial communities' meta-transcriptomics can be applied by the sequencing of total RNA (Mann et al., 2018). When this method is paired with amplicon analysis the microorganisms actively transcribing can be identified. This means that only live organisms are identified overcoming a concern of the previous sequencing methods, however bias can arise towards organisms with higher rates of transcription. Transcriptomes can reveal microbial responses to changes in the environment e.g., xenobiotic exposure (Maurice et al., 2013). However, it is the most expensive and complex as samples require careful storage to prevent degradation and contaminating host mRNA and rRNA must be removed.

Overall, NGS has revolutionised microbial ecology as a reliance on culture-based surveys limits our understanding of microbial populations. Bioinformatic approaches have improved simultaneously with the growth of larger databases containing reference genomes. The advantages and disadvantages of culture-independent techniques have been discussed and depending on the study and what is being investigated determines the sequencing methods selected. Short-read sequencing such as metabarcoding is an affordable and a powerful tool, useful for larger studies with low biomass samples. Although functional capabilities of communities can get lost when restricted to metabarcoding the efficiency and effectiveness can outweigh the disadvantages. Long-read sequencing is emerging as an alternative or complementary choice for metagenomic studies and is advantageous for resolving taxonomic discrepancies. A combination of short- and long-read sequencing where applicable will greatly enhance many microbiome studies. Further additional high throughput "omics" approaches to study microbial proteins and metabolic products provide valuable information on species present and their activities. When possible, efforts should be made to combine

culture-independent and culture-dependent techniques, alongside use of computer modelling and collection of environmental metadata. Together this will increase our knowledge about the microbial communities and their activities within the BE. As well as the implications of microbial interactions with occupants on both, their microbiome and health, and other the microorganisms within the BE.

## 1.4 Thesis Outline

The general objectives of this thesis were to investigate the microbial communities of P-traps found in public restrooms, to evaluate how they develop and react to perturbations, and how these microbial communities could potentially affect us. We hypothesize that sink microbial communities will reflect microbial sources input into P-traps and that microbes associated with humans will be present. Research into sink traps and other P-trap communities was conducted in-situ to provide results that reflect P-trap communities, under real-world conditions, as wholly as possible with the methodologies available. In this thesis, each chapter advances the knowledge and builds upon the previous, to expand our understanding of P-trap microbial communities. Described below are the specific features of each chapter:

- **Chapter 2: Characterization of Communal Sink Drain Communities of a University Campus**
  This study addressed three specific aims in order to gain a fundamental understanding of the bacterial communities of sink P-traps found in different public restroom sinks across buildings on a university campus. Firstly, to determine the structure and diversity of bacterial communities in communal sinks across campus and then secondly, to explore if sinks had a core (>70% prevalence) bacterial community. If no core bacteria were present, are bacterial communities influenced by specific buildings or locations and/or restroom gender? The final aim was to ascertain the dominant sources of microorganisms to the university campus sinks. The data collected in this study led to the identification of bacterial taxa, primarily to the family level and the data was used to assess the differences between buildings with varying functions on a university campus. In the context of this thesis this study established a core bacterial community for sinks and demonstrated they are diverse ecosystems, with high variance among some bacterial taxa across individual sinks however, there is commonality in the highly abundant taxa observed across buildings. Further, the results emphasised the importance of humans as a primary contributor of bacteria to the sink P-trap and as such demonstrated the complexity of interactions between humans and sinks.

- **Chapter 3: Mycobial Community Assemblages in Sink Drains Across a University Campus**
  Similarly, to the first manuscript, this chapters focuses on characterising microbial communities, specifically identifying the fungal communities in communal sink P-traps. The research questions of this chapter were: Which fungi dominate P-traps, and are they found ubiquitously across all sinks? Additionally, how are mycobial communities structured, and are they influenced by different types of BE? The results elucidated the fungal taxa that dominated in sinks and concurred with previous research into water-associated BEs. Remarkable similarity in the mycobial community was observed across the different university buildings. This could suggest some stability of mycobial communities to perturbations from differing sink usage. Additionally, the external influence of human activities was demonstrated as a common skin commensal was present in many sinks. Overall, the study highlighted the importance of sink P-traps as reservoirs of possible opportunistic pathogenic fungi although the risks may be negligible in non-clinical environments.
- **Chapter 4: Longitudinal bacterial community dynamics and sodium hypochlorite intervention in a newly opened university building**
  Over two years samples were collected from the P-traps of sinks in a newly opened university building to reveal bacterial community dynamics (Phase One). This longitudinal sampling regime was then followed by an intervention study (Phase Two), where sinks were treated with sodium hypochlorite (10% bleach). Specifically in the first phase, we aimed to assess the long-term variations and stability of bacterial communities within restroom sink P-traps and identify the bacterial colonizers. In the second phase of this chapter, we aimed to determine the impact of sodium hypochlorite on bacterial community structure and diversity, and assess the reliance and resistance of these communities. In this chapter the temporal dynamics and development of sink P-trap bacterial communities are explored to reveal formation of a stable microbial community. Following intervention with bleach there is an acute affect to the bacterial community structure but within four weeks communities resemble those of sinks that were left untreated during the study. Understanding sink p-trap community dynamics and resilience to stressors provides meaningful insights to developing disinfection strategies and what constitutes a “healthy” or “normal” sink microbiome.
- **Chapter 5: Microbial Landscape of Public Urinals: a 16S rRNA Survey of the Bacterial Communities in Urinal P-traps and the Discovery of Their Most Abundant and Prevalent Species**
  Alternative P-traps in restrooms could provide a viable environment for microbial proliferation as has been observed in sink P-traps. This study investigates the bacterial

communities found in P-traps of urinals from restrooms across a university campus as well as a central train station. Similarly to Chapter 2 and 3, the research aims were to provide insight into bacterial community composition of urinal P-traps and to analyse the effect of building on bacterial populations. Further, to identify the core bacterial family and genera and whether human-associated bacterial signatures, especially those related to the urogenital tract or urine, are reflected in P-traps? Despite the increased levels of ammonia and low nutrients in urinals, the sequencing data showed that some bacterial taxa are able to dominate in this environment and are found across a variety of buildings. In contrast to sinks P-traps, urinals P-traps were much more variable in their community structure. However, like sinks, the bacterial taxa observed had could be associated with humans, particularly urine. A comprehensive examination of urinal P-trap communities generates insights to built environment niches that are potentially unnoticed but still could pose a health risk.

Together, this research aims to provide a comprehensive view of the composition, dynamics, and resilience of microbial communities found in university restroom P-traps.

### *1.4.1 Additional Research*

Provided at the end of the thesis is research that is outside the scope of the main thesis aims but offers extra insight into technologies available. The work was performed alongside the main body of research.

- ***Chitinophaga spargani* sp. nov., isolated from rhizosphere of *Sparganium erectum***

  With the array of sequencing technologies accessible today, identifying and isolating novel bacterial species is possible. In this section, a novel species was isolated from the rhizosphere of *Sparganium erectum* and the whole genome sequenced and assembled using a hybrid approach; short- and long-read sequencing. Methodologies used in the chapter could be applied to other environments such as the sink or urinal P-trap. Mining novel species of bacteria can lead to identification of potentially novel metabolic products and antibiotics, as well as understanding the functionality of bacteria around us and how we could mitigate their proliferation and dissemination if required.

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# Chapter 2. Characterization of Communal Sink Drain Communities of a University Campus

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## 2.1 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 characterisation 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**

DNA Barcoding, Sink, Microbiome, Built Environment, Environmental Microbiology.

## 2.2 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 (Klepesis et al., 2001). It is now necessary to recognise 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., 2020; 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 sinks, alongside warmer indoor temperatures, and pipes being a relatively protected environment favours 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 allows 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 prevents 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 colonising microorganism can be from pets, air, water, or plants (Hewitt et al., 2012; Kelly & 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 harboured 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 and colleagues 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 behaviour dependent upon building may influence the microbial diversity and composition of sink P-traps.

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

## 2.3 Methods

### *2.3.1 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) (Supplementary File: Figure A.1). 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 five seconds 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 sterilised scissors directly into beaded microtubes. Prior to swabbing, the sink was flushed with cold water for one minute to eliminate recent usage as a confounding factor. Samples were stored in the freezer at -20°C and thawed before DNA extraction.

### *2.3.2 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 minutes and 8 cycles of 95°C for 15 seconds, 55°C for 30 seconds, 72°C for 30 seconds and a final extension of 72°C for 10 minutes. SequalPrep™ Normalization Plate Kit (ThermoFisher) cleaned and normalised the samples before being pooled. Samples were sequenced on the Illumina Miseq Platform (250PE) at UK Centre for Ecology & Hydrology.

### *2.3.3 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 (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 (Quast et al., 2013) for kingdom to species assignments.

### *2.3.4 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). Visualisation 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 visualised 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., 2020). PERMANOVA tests whether composition among groups are 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 lab. 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 (i) sequenced V4 region; (ii) processed sequences through Deblur pipeline; (iii) sequence length of 90bp; and (iv) 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). 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 90bp limits taxonomic resolution but some studies accessible through Qiita only met that length such as soil sources, therefore 90bp was chosen for comparability. Default parameters were used unless otherwise stated.

## 2.4 Results

### *2.4.1 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 2,741 ASVs where they were distributed and classified into 31 phyla, 51 classes, 118 orders and 186 families. An average of 64 ASVs were 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.

### *2.4.2 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 2.1A) and R2 the differences were marginal with only 19% of the variation explained (PERMANOVA, DF = 8, F.model = 5.5998, R2 = 0.19243, P = 0.001). Moreover, pairwise comparisons showed that the average R2 of all comparisons was below 0.1 (Supplementary Table A.1). HBS was significantly different from all other buildings (R2 values ranging from 0.06 to 0.15) (Supplementary Table A.1). 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 2.1A, Supplementary Table A.2). ASV richness (Figure 2.1C) and diversity (Figure 2.1D) varied significantly between buildings (Kruskal-Wallis test, Richness: DF = 8, $p$ <0.05; Shannon: DF = 8, $p$ <0.001; Supplementary Table A.3). 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 R2 (Figure 2.1B; PERMANOVA, DF = 1, F = 24.096, R2 = 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 analysed in their separate drain types

(Supplementary Table A.4). An average number of 66 ASVs (min 20, max 167) and 61 ASVs (min 18, max 160) were 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 (Supplementary Figure A.2). Notably there were no significant difference among sink ASV richness and diversity when categorised by restroom gender (Supplementary Table A.3). Regarding gender beta diversity metrics, the bacterial communities were statistically different, however gender had the lowest variance explained, i.e. only 2% of the variation in bacterial communities was explained by the Gender of restrooms (PERMANOVA, DF = 2, F = 2.1941, R2 = 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, Supplementary Figure A.3 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) (Supplementary Figure A.4). 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 2.2). At the family level, compositional differences were more pronounced as *Moraxellaceae* was the most 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 (Supplementary Figure A.5). Analysis of taxonomic composition of individual sinks at the family level showed highly variable taxonomic profiles between sinks (Supplementary Figure A.6).

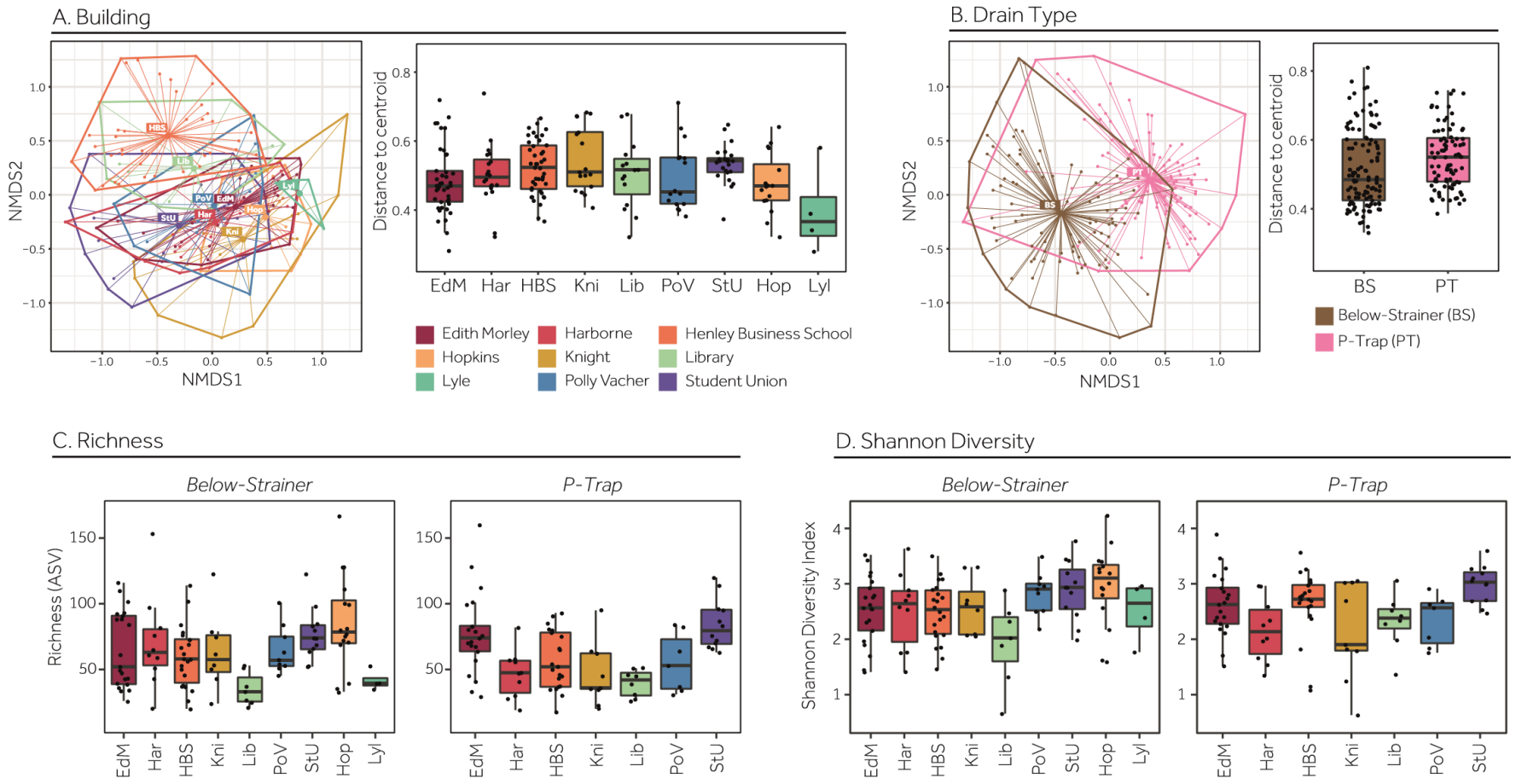


**Figure 2.1.** (A) Non-metric multidimensional scaling (NMDS) resulting from Bray-Curtis dissimilarity matrices of community composition between nine different buildings sampled; Distances to centroid in multivariate homogeneity of group variance analysis for sink bacterial communities for each building. (B) Aforementioned NMDS and distances to centroid for drain types. (C) 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.

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 (Supplementary Figure A.5B). 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).

### *2.4.3 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 2.3). 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 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 was 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, Supplementary Figure A.7). When looking at core families, three families, namely *Burkholderiaceae*, *Moraxellaceae* and *Sphingomonadaceae*, were identified in 100% of all sinks sampled.

### *2.4.4 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 2.4). 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.

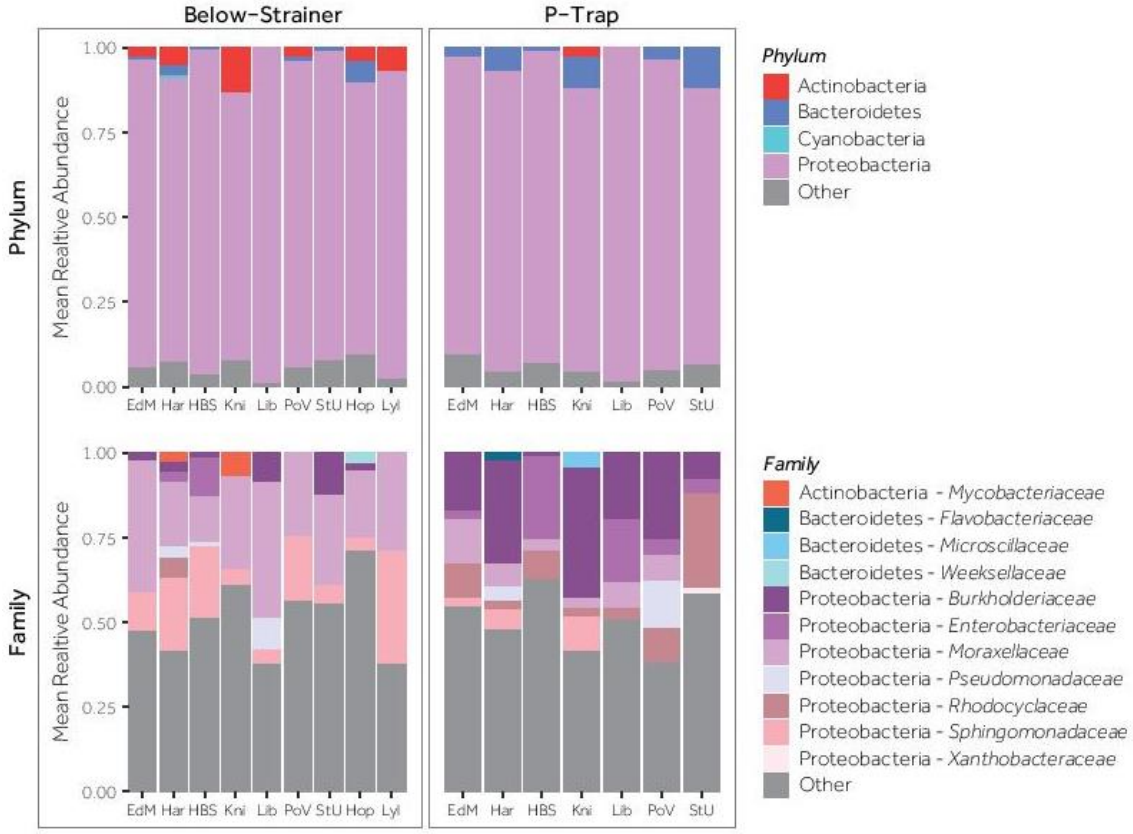


**Figure 2.2.** Average relative abundance of the top 5 phyla and top 12 families found in the university restroom sinks. The average data represent pooled sequences from the 9 buildings spilt by drain type. Proteobacteria is the dominant bacterial phylum across all sinks regardless of building and drain 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.

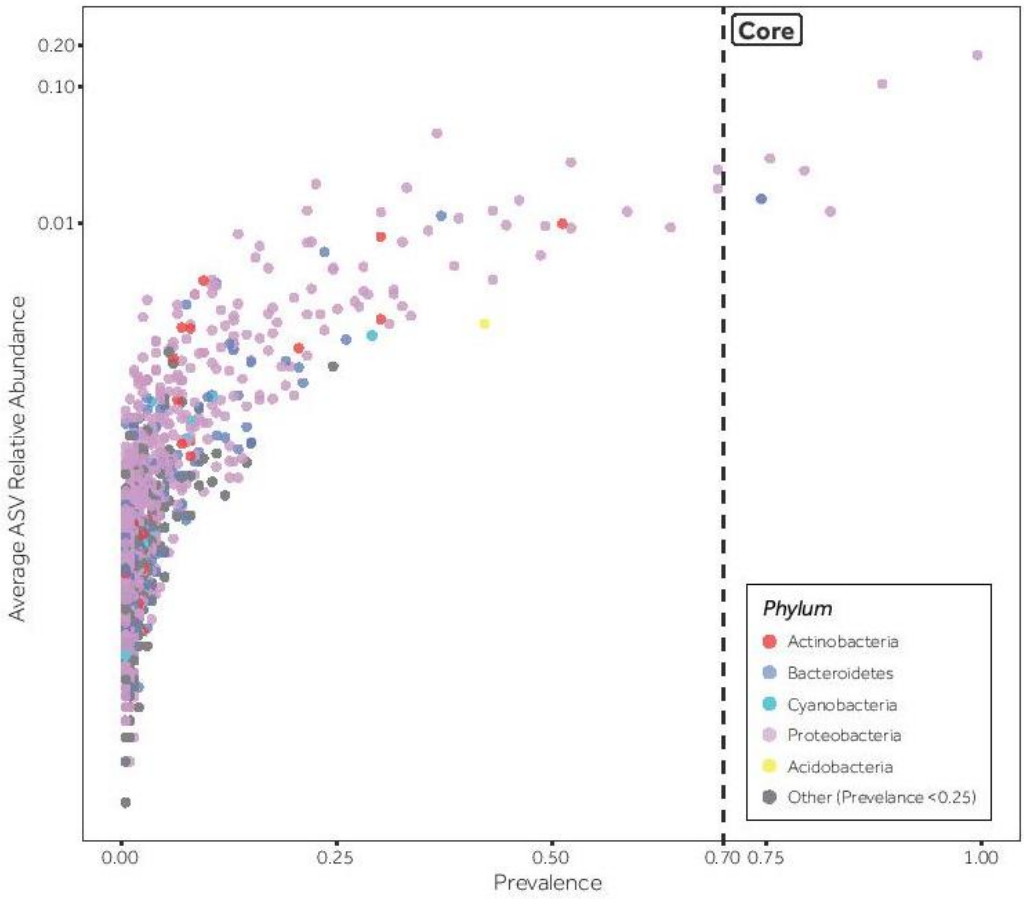


**Figure 2.3.** Prevalence of total 2,741 ASVs across 199 sink samples and their average relative abundance. ASVs are coloured by phylum. The dotted line shows the cut-off for taxa defined as core sink ASVs, prevalence ≥0.7. Seven ASVs were present in the core region belonging to the families; *Moraxellaceae*, *Beijerinckiaceae*, *Burkholderiaceae*, *Sphingomonadaceae* and *Weeksellaceae*.

## 2.5 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 2.1A), the small effect sizes as well as marginal significant pairwise differences (Supplementary Table A.1) 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 2.1A) (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 two to three 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 & Kelley, 2005; 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 was the most dominant phylum in both Drain types, and the phylum Actinobacteria was 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 i.e., the body of water in P-Trap versus the “drier” drain. Differences between the two environments was further supported by LEfSe reporting a large number of bacterial taxa between the two drain

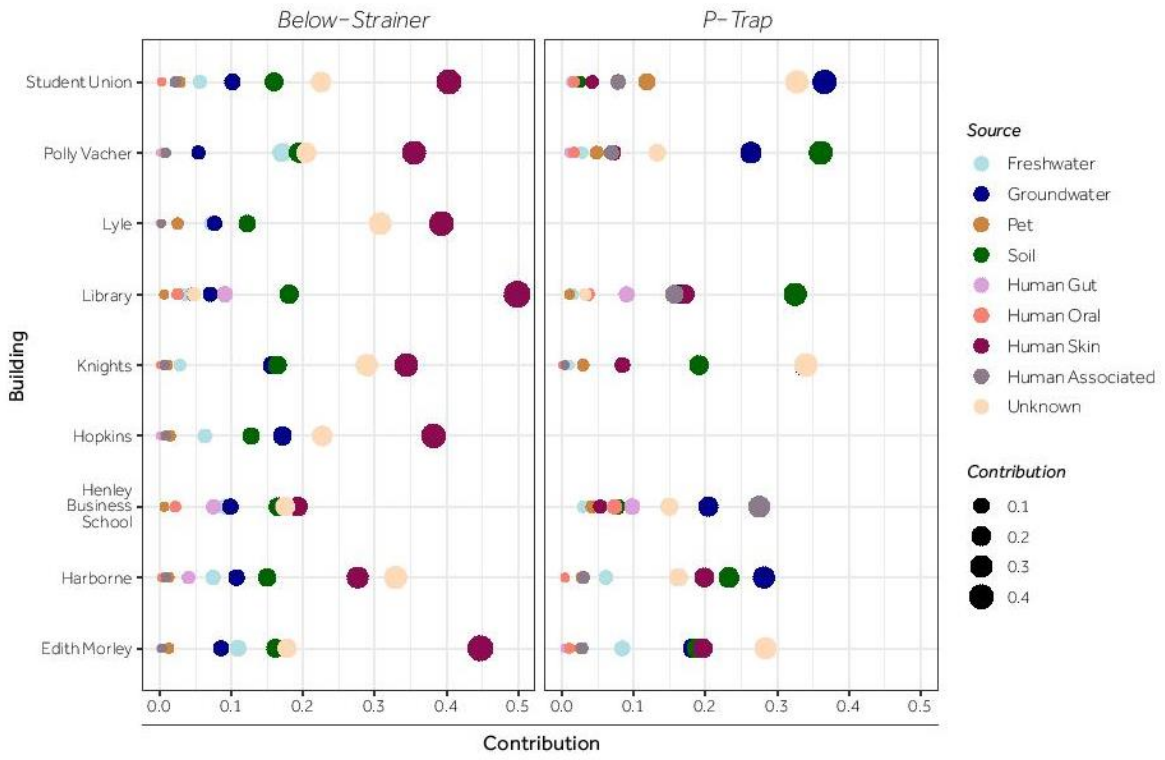


**Figure 2.4.** Predicted source contribution to each building generated from SourceTracker output. Source environments were taken from studies deposited in Qiita. Point size represents predicted source contribution to each building. Human skin is a dominant source across below-strainer communities. P-trap samples do not have a dominating source and there is more variation in contributing sources across buildings.

types. There was also a strong presence of *Enterobacteriaceae* in P-traps particularly in HBS building and the Library building.

ASV 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; Merino et al., 2019; 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 colonising both dry and moist areas of human skin (Powell et al., 2012). *Acinetobacter's* ability to survive harsh treatments and to colonise human skin may explain why it was the most abundant genus found in sinks. *Acinetobacter* spp. have been implicated in various nosocomial outbreaks (Hong et al., 2012; 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 coloniser 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. Previously 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 2.4). Of the 211 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 faeces 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 if it represents the water sources (Freshwater and Groundwater), our results suggests 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 harbours 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 2.3). 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 was 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

emphasises 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.

## 2.6 Declaration

### *2.6.1 Ethics approval and consent to participate*

Not applicable

### *2.6.2 Consent for publication*

Not applicable

### *2.6.3 Availability of data and materials*

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 Supplementary Table A.5.

### *2.6.4 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.

### *2.6.5 Supplementary material link*

The supplementary material for this article can be found at: https://doi.org/10.1002/edn3.196

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## Appendix A

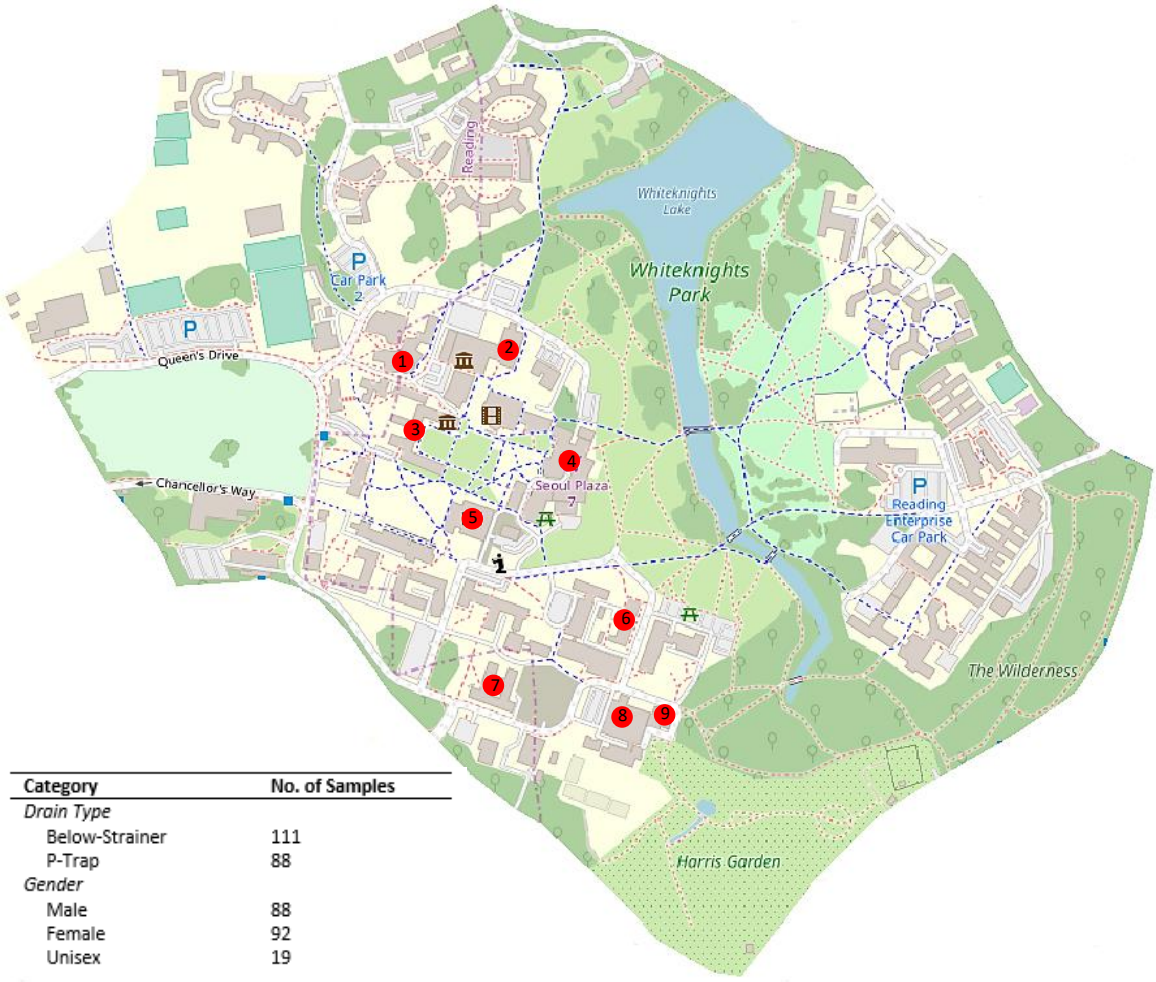
## Supplementary material for Chapter 2

### Characterization of communal sink drain communities of a university campus

This appendix includes:

- **Figure A.1** - Image showing the buildings sampled. 1. Henley Business School (HBS) n= 43, 2. Knights (Kni) n= 17, 3. Edith Morley (EdM) n= 42, 4. Student Union (StU) n= 24, 5. Library (Lib) n= 15, 6. Hopkins (Hop) n= 16, 7. Polly Vacher (PoV) n= 16, 8. Harborne (Har) n= 22, 9. Lyle (Lyl) n= 4 (taken from www.openstreetmap.org). n = number of samples taken from that building.
- **Figure A.2** - Rarefaction curves comparing the number of reads with the number of ASVs.
- **Figure A.3** - LEfSe at ASV level. Linear discriminant analysis (LDA) combined with effect size measurements revealed a list of features that enable discrimination between below-strainer and P-Trap sink samples. A P-value of <0.05 and an LDA score of ≥ 3 were used to identify bacterial groups with statistical significance.
- **Figure A.4** - LEfSe at ASV level. Three ASVs identified as discriminatory based on gender. A P-value of <0.05 and an LDA score of ≥ 3 were used to identify bacterial groups with statistical significance.
- **Figure A.5** - The top 20 (A) Families and (B) ASVs that accounted for the highest percentage of reads.
- **Figure A.6** - Bacterial composition at family across all samples, ordered by building. 23 families are shown, “Other” groups families that had <0.1% mean relative abundance.
- **Figure A.7** - Diagram comparing the core ASVs when the data is split into the two drain types and cores ASVs identified. Below-strainer has 10 core AVSs (identified in 70% of all below-strainer samples). P-trap has 6 core ASVs (identified in 70% of all P-trap samples). 5 ASVs were common between the two sets of core ASVs.
- **Table A.1** - Pairwise comparisons for all pairs of levels of the factor “Building” by using PERMANOVA. P-Bonferroni corrected p-values shown, stars indicated the p-value significance $p < 0.05$; *. The R2 values indicate the amount of variation explained by the comparisons in the model.

- **Table A.2** - Mean distance to centroid from multivariate homogeneity of group variance analysis for bacterial communities in each building sampled. SD represents Standard Deviation.
- **Table A.3A & B** - Results of Kruskal-Wallis test. Chi-Squared, degrees of freedom (DF) and P-values are given.
- **Table A.4** - Results of PERMANOVA analysis of Bray-Curtis dissimilarity distances for bacterial ASVs community structure in relation to sample variables. Abbreviations: Df, degrees of freedom; SS sum of squares; MS, mean sum of squares, F, F value by permutation. p-values are based on 999 permutations. Stars indicate the p-value significance $p < 0.05$; *, $p < 0.01$; **, $P < 0.001$; ***.
- **Table A.5** - Information for each sample including what part of the sink drain was sampled, where the sink was, and the restroom gender associated with the sink.




| Category | No. of Samples |
|---|---|
| *Drain Type* | |
| Below-Strainer | 111 |
| P-Trap | 88 |
| *Gender* | |
| Male | 88 |
| Female | 92 |
| Unisex | 19 |

**Figure A.1.** Image showing the buildings sampled. 1. Henley Business School (HBS) n= 43, 2. Knights (Kni) n= 17, 3. Edith Morley (EdM) n= 42, 4. Student Union (StU) n= 24, 5. Library (Lib) n= 15, 6. Hopkins (Hop) n= 16, 7. Polly Vacher (PoV) n= 16, 8. Harborne (Har) n= 22, 9. Lyle (Lyl) n= 4 (taken from www.openstreetmap.org). n = number of samples taken from that building.

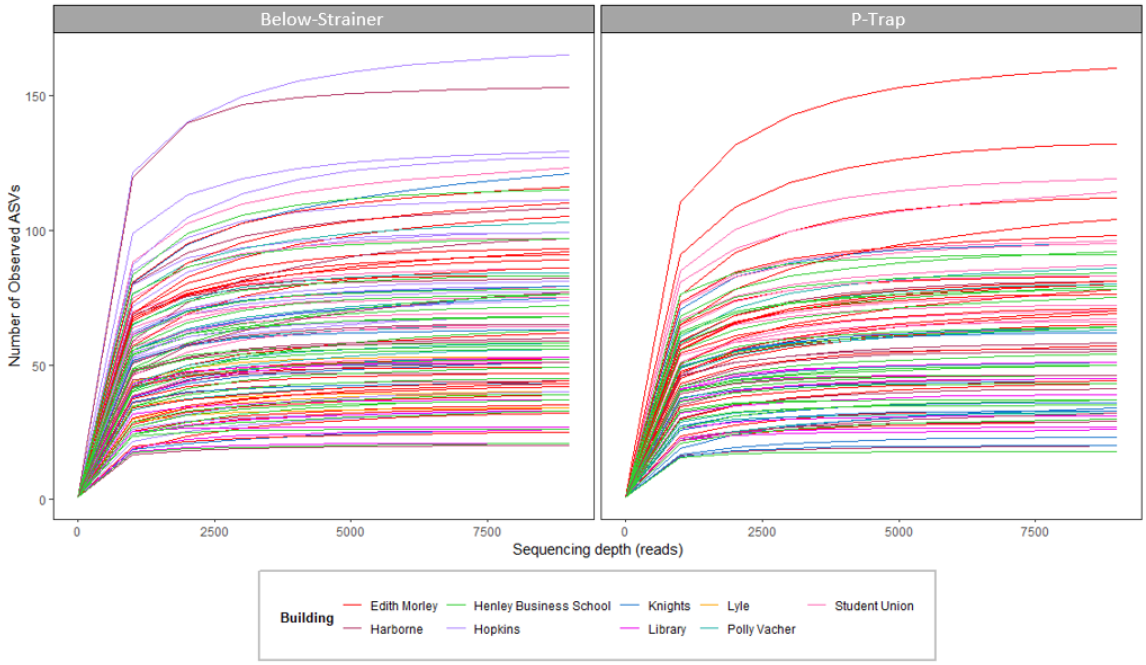



**Figure A.2.** Rarefaction curves comparing the number of reads with the number of ASVs.

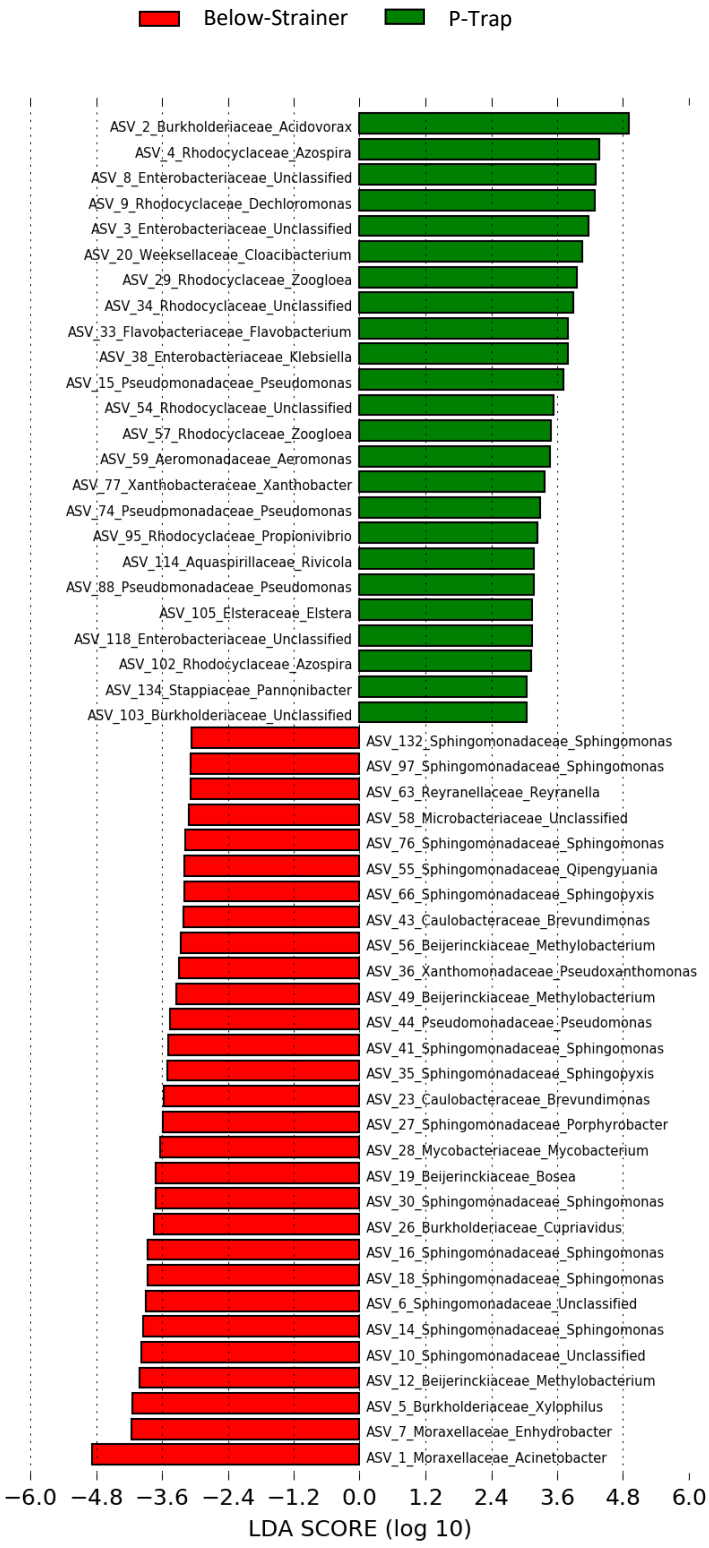



**Figure A.3.** LEfSe at ASV level. Linear discriminant analysis (LDA) combined with effect size measurements revealed a list of features that enable discrimination between Below-Strainer and P-Trap sink samples. A P-value of <0.05 and an LDA score of ≥ 3 were used to identify bacterial groups with statistical significance.

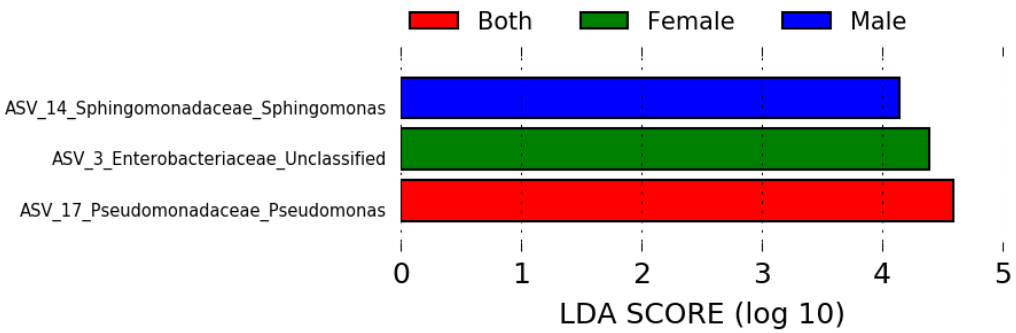



**Figure A.4 -** LEfSe at ASV level. Three ASVs identified as discriminatory based on gender. A P-value of <0.05 and an LDA score of ≥ 3 were used to identify bacterial groups with statistical significance.

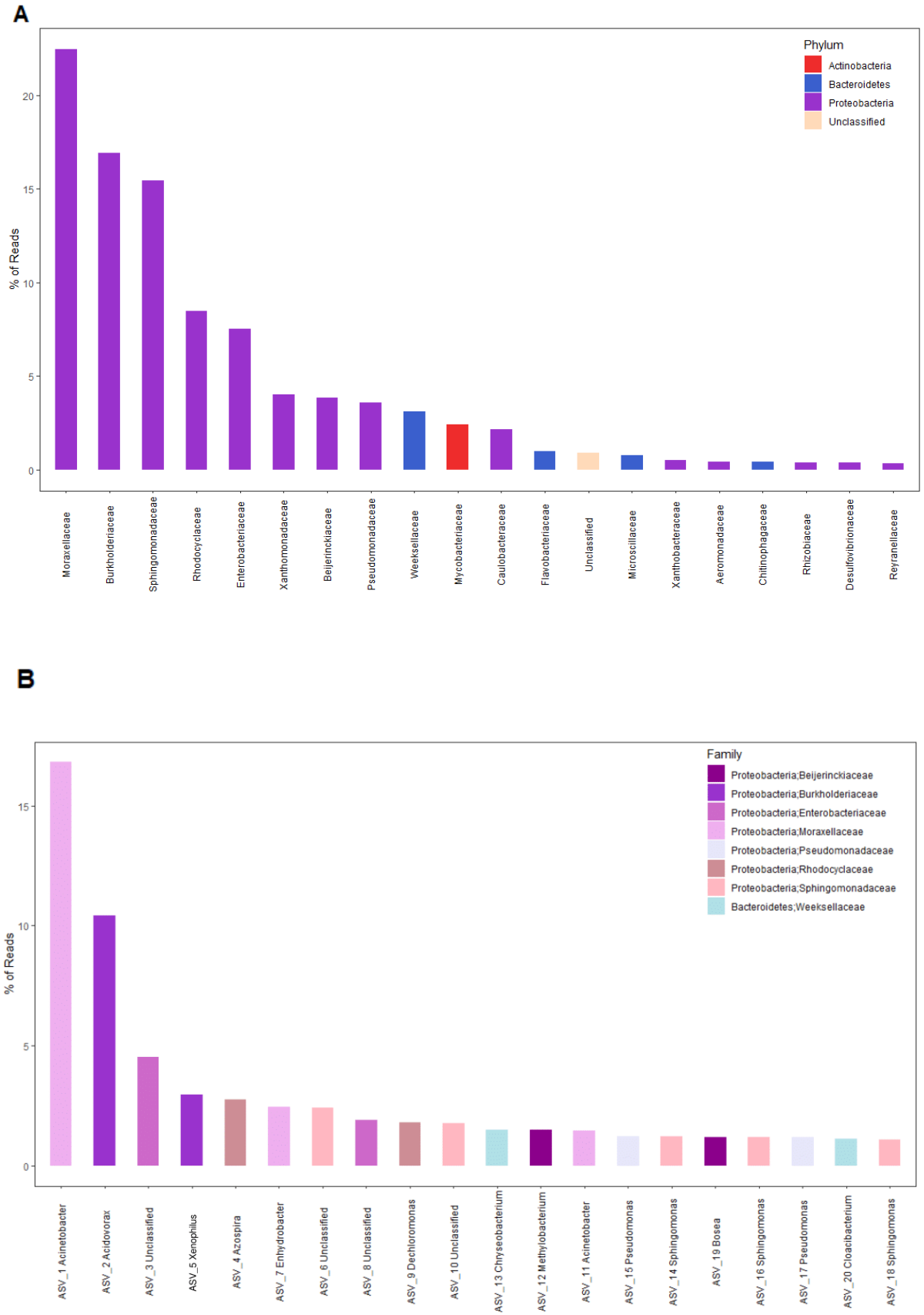



**Figure A.5.** The top 20 (A) Families and (B) ASVs that accounted for the highest percentage of reads.

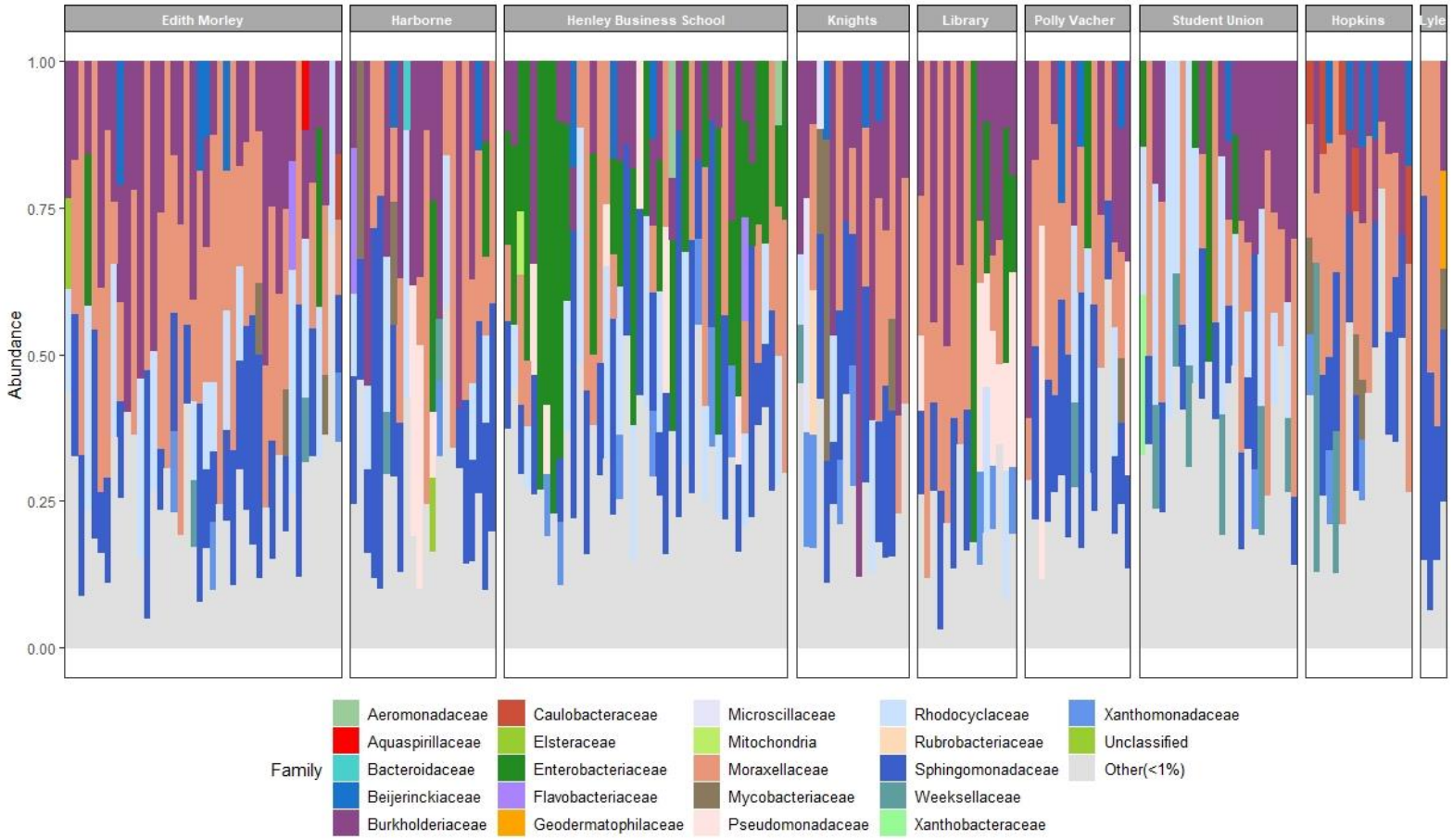



**Figure A.6.** Bacterial composition at family across all samples, ordered by building. 23 families are shown, "Other" groups families that had <0.1% mean relative abundance.

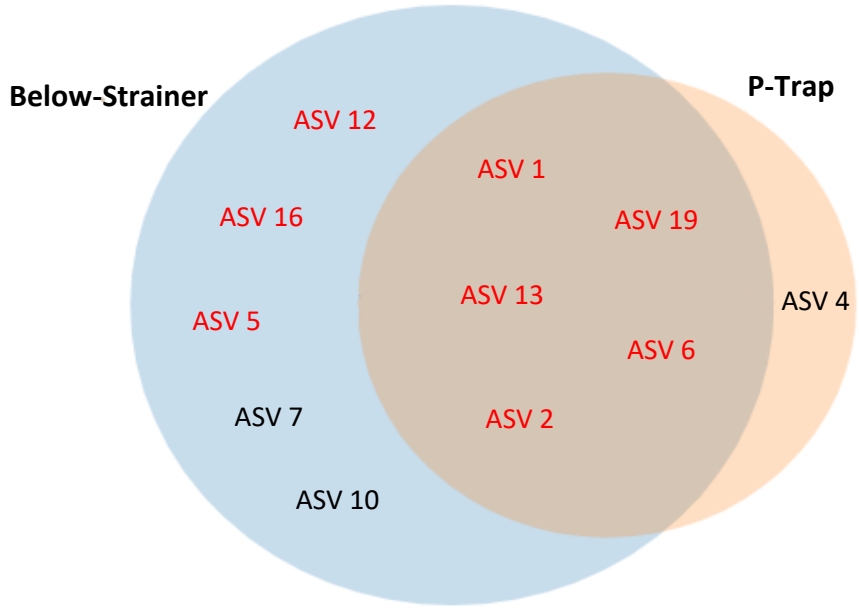



**Figure A.7.** Diagram comparing the core ASVs when the data is split into the two drain types and cores ASVs identified. Below-strainer has 10 core AVSs (identified in 70% of all below-strainer samples). P-trap has 6 core ASVs (identified in 70% of all P-trap samples). 5 ASVs were common between the two sets of core ASVs.

| *Pairwise Comparison* | *F* | *R2* | *p-value (Bonferroni corrected)* | *Significance* |
|---|---|---|---|---|
| **HBS x StU** | **9.335706** | **0.125588** | **0.036** | * |
| **HBS x Har** | **10.79826** | **0.150397** | **0.036** | * |
| **HBS x EdM** | **16.67572** | **0.1673** | **0.036** | * |
| **HBS x Lyl** | **4.258423** | **0.086451** | **0.036** | * |
| **HBS x Hop** | **10.21354** | **0.151957** | **0.036** | * |
| **HBS x PoV** | **7.925529** | **0.122071** | **0.036** | * |
| **HBS x Kni** | **9.243968** | **0.137469** | **0.036** | * |
| **HBS x Lib** | **3.680807** | **0.061675** | **0.036** | * |
| **StU x Har** | **3.720334** | **0.081372** | **0.036** | * |
| **StU x EdM** | **6.444907** | **0.091489** | **0.036** | * |
| StU x Lyl | 3.076216 | 0.105798 | 0.144 | |
| **StU x Hop** | **5.602858** | **0.128497** | **0.036** | * |
| StU x PoV | 3.503905 | 0.084423 | 0.108 | |
| **StU x Kni** | **3.668394** | **0.085975** | **0.036** | * |
| **StU x Lib** | **3.713513** | **0.091211** | **0.036** | * |
| Har x EdM | 4.245332 | 0.06608 | 0.072 | |
| Har x Lyl | 2.479082 | 0.101273 | 0.504 | |
| **Har x Hop** | **5.145021** | **0.131435** | **0.036** | * |
| Har x PoV | 2.070745 | 0.057408 | 0.936 | |
| Har x Kni | 1.795356 | 0.048793 | 1 | |
| Har x Lib | 3.172374 | 0.087702 | 0.252 | |
| EdM x Lyl | 2.233738 | 0.048314 | 0.936 | |
| **EdM x Hop** | **4.351099** | **0.072096** | **0.036** | * |
| EdM x PoV | 3.772481 | 0.063114 | 0.108 | |
| EdM x Kni | 4.166259 | 0.068114 | 0.072 | |
| **EdM x Lib** | **5.162415** | **0.085808** | **0.036** | * |
| Lyl x Hop | 2.142518 | 0.106368 | 0.54 | |
| Lyl x PoV | 2.59143 | 0.12585 | 0.216 | |
| Lyl x Kni | 1.616193 | 0.078394 | 1 | |
| Lyl x Lib | 3.071188 | 0.153015 | 0.216 | |
| **Hop x PoV** | **3.855428** | **0.113879** | **0.036** | * |

| | | | | |
|---|---|---|---|---|
| **Hop x Kni** | **3.237939** | **0.094572** | **0.036** | * |
| **Hop x Lib** | **5.044158** | **0.148165** | **0.036** | * |
| **PoV x Kni** | **2.640585** | **0.078494** | **0.036** | * |
| PoV x Lib | 2.423092 | 0.077112 | 0.468 | |
| **Kni x Lib** | **3.698353** | **0.109749** | **0.036** | * |

**Table A.1.** Pairwise comparisons for all pairs of levels of the factor "Building" by using PERMANOVA. P-Bonferroni corrected p-values shown, stars indicated the p-value significance $p < 0.05$; *. The R2 values indicate the amount of variation explained by the comparisons in the model.

| ***Building*** | ***Mean Distance to the Centroid*** | ***SD*** |
|---|---|---|
| Edith Morley | 0.4859015 | 0.09478499 |
| Harborne | 0.5024314 | 0.08940724 |
| Henley Business School | 0.5238127 | 0.07757995 |
| Hopkins | 0.4791152 | 0.08730570 |
| Knights | 0.5475142 | 0.09447974 |
| Library | 0.5050253 | 0.09959272 |
| Lyle | 0.3984704 | 0.12768986 |
| Polly Vacher | 0.4980293 | 0.09825886 |
| Student Union | 0.5341402 | 0.05538182 |

**Table A.2.** Mean distance to centroid from multivariate homogeneity of group variance analysis for bacterial communities in each building sampled. SD represents Standard Deviation.

*A. Observed ASVs/Richness*

| ***Drain Type*** | ***Group*** | ***DF*** | ***Chi-squared*** | ***P-value*** |
|---|---|---|---|---|
| Below- Strain | **Building** | **8** | **22.05** | **0.004824** |
| | Gender | 2 | 1.1074 | 0.5748 |
| P-Trap | **Building** | **6** | **27.977** | **0.000095** |
| | Gender | 2 | 1.4213 | 0.4913 |

*B. Shannon Diversity*

| ***Drain Type*** | ***Group*** | ***DF*** | ***Chi-squared*** | ***P-value*** |
|---|---|---|---|---|
| Below- Strain | **Building** | **8** | **17.196** | **0.02814** |
| | Gender | 2 | 0.31114 | 0.8559 |
| P-Trap | **Building** | **6** | **17.863** | **0.006583** |
| | Gender | 2 | 2.2147 | 0.3304 |

**Table A.3A & B.** Results of Kruskal-Wallis test. Chi-Squared, degrees of freedom (DF) and P-values are given.

| | *DF* | *SS* | *MS* | *F* | $2^2$ | *P values* |
|---|---|---|---|---|---|---|
| *Below-Strainer* | | | | | | |
| Building | 8 | 7.4702 | 0.93377 | 4.0901 | 0.24654 | 0.001 *** |
| Gender | 2 | 0.879 | 0.43951 | 1.5835 | 0.02901 | 0.021* |
| *P-Trap* | | | | | | |
| Building | 6 | 7.5584 | 1.25974 | 5.2115 | 0.27852 | 0.001 *** |
| Gender | 2 | 1.2524 | 0.62618 | 2.0562 | 0.04615 | 0.004 ** |

**Table A.4.** Results of PERMANOVA analysis of Bray-Curtis dissimilarity distances for bacterial ASVs community structure in relation to sample variables. Abbreviations: Df, degrees of freedom; SS sum of squares; MS, mean sum of squares, F, F value by permutation. p-values are based on 999 permutations. Stars indicate the p-value significance $p < 0.05$; *, $p < 0.01$; **, $P < 0.001$; ***.

| ID | Sample Number | Drain Type | Sample Location | Floor | Gender |
|---|---|---|---|---|---|
| **ZW.E01.S100B** | 100B | P-Trap | Henley Business School | Ground | Male |
| **ZW.E01.S100T** | 100T | Below-Strain | Henley Business School | Ground | Male |
| **ZW.E01.S101T** | 101T | Below-Strain | Student Union | Ground | Female |
| **ZW.E01.S102B** | 102B | P-Trap | Student Union | Ground | Female |
| **ZW.E01.S102T** | 102T | Below-Strain | Student Union | Ground | Female |
| **ZW.E01.S103B** | 103B | P-Trap | Student Union | Ground | Female |
| **ZW.E01.S103T** | 103T | Below-Strain | Student Union | Ground | Female |
| **ZW.E01.S104B** | 104B | P-Trap | Student Union | Ground | Female |
| **ZW.E01.S104T** | 104T | Below-Strain | Student Union | Ground | Female |
| **ZW.E01.S105B** | 105B | P-Trap | Student Union | Ground | Female |
| **ZW.E01.S105T** | 105T | Below-Strain | Student Union | Ground | Female |
| **ZW.E01.S106B** | 106B | P-Trap | Student Union | Ground | Female |
| **ZW.E01.S106T** | 106T | Below-Strain | Student Union | Ground | Female |
| **ZW.E01.S107B** | 107B | P-Trap | Student Union | Ground | Female |
| **ZW.E01.S107T** | 107T | Below-Strain | Student Union | Ground | Female |
| **ZW.E01.S108B** | 108B | P-Trap | Student Union | Ground | Female |
| **ZW.E01.S108T** | 108T | Below-Strain | Student Union | Ground | Female |
| **ZW.E01.S109B** | 109B | P-Trap | Student Union | Ground | Female |
| **ZW.E01.S109T** | 109T | Below-Strain | Student Union | Ground | Female |
| **ZW.E01.S10B** | 10B | P-Trap | Harborne | Ground | Female |
| **ZW.E01.S10T** | 10T | Below-Strain | Harborne | Ground | Female |
| **ZW.E01.S110B** | 110B | P-Trap | Edith Morley | Ground | Female |
| **ZW.E01.S110T** | 110T | Below-Strain | Edith Morley | Ground | Female |
| **ZW.E01.S111T** | 111T | Below-Strain | Edith Morley | Ground | Female |
| **ZW.E01.S112B** | 112B | P-Trap | Edith Morley | Ground | Female |
| **ZW.E01.S112T** | 112T | Below-Strain | Edith Morley | Ground | Female |
| **ZW.E01.S113B** | 113B | P-Trap | Edith Morley | First | Female |
| **ZW.E01.S113T** | 113T | Below-Strain | Edith Morley | First | Female |
| **ZW.E01.S114B** | 114B | P-Trap | Edith Morley | First | Unisex |
| **ZW.E01.S114T** | 114T | Below-Strain | Edith Morley | First | Unisex |

| | | | | | |
|---|---|---|---|---|---|
| **ZW.E01.S115B** | 115B | P-Trap | Edith Morley | First | Female |
| **ZW.E01.S115T** | 115T | Below-Strain | Edith Morley | First | Female |
| **ZW.E01.S116B** | 116B | P-Trap | Edith Morley | First | Female |
| **ZW.E01.S116T** | 116T | Below-Strain | Edith Morley | First | Female |
| **ZW.E01.S117B** | 117B | P-Trap | Edith Morley | First | Female |
| **ZW.E01.S117T** | 117T | Below-Strain | Edith Morley | First | Female |
| **ZW.E01.S118B** | 118B | P-Trap | Edith Morley | Second | Female |
| **ZW.E01.S118T** | 118T | Below-Strain | Edith Morley | Second | Female |
| **ZW.E01.S119B** | 119B | P-Trap | Edith Morley | Second | Female |
| **ZW.E01.S119T** | 119T | Below-Strain | Edith Morley | Second | Female |
| **ZW.E01.S11B** | 11B | P-Trap | Harborne | Ground | Female |
| **ZW.E01.S11T** | 11T | Below-Strain | Harborne | Ground | Female |
| **ZW.E01.S120B** | 120B | P-Trap | Edith Morley | Second | Female |
| **ZW.E01.S120T** | 120T | Below-Strain | Edith Morley | Second | Female |
| **ZW.E01.S121B** | 121B | P-Trap | Edith Morley | Second | Female |
| **ZW.E01.S121T** | 121T | Below-Strain | Edith Morley | Second | Female |
| **ZW.E01.S122B** | 122B | P-Trap | Henley Business School | Ground | Male |
| **ZW.E01.S122T** | 122T | Below-Strain | Henley Business School | Ground | Male |
| **ZW.E01.S123B** | 123B | P-Trap | Henley Business School | Ground | Male |
| **ZW.E01.S123T** | 123T | Below-Strain | Henley Business School | Ground | Male |
| **ZW.E01.S12T** | 12T | Below-Strain | Harborne | Ground | Female |
| **ZW.E01.S13B** | 13B | P-Trap | Harborne | Ground | Female |
| **ZW.E01.S13T** | 13T | Below-Strain | Harborne | Ground | Female |
| **ZW.E01.S14T** | 14T | Below-Strain | Lyle | Ground | Female |
| **ZW.E01.S15T** | 15T | Below-Strain | Lyle | Ground | Female |
| **ZW.E01.S16T** | 16T | Below-Strain | Lyle | Ground | Female |
| **ZW.E01.S17T** | 17T | Below-Strain | Lyle | Ground | Male |
| **ZW.E01.S18T** | 18T | Below-Strain | Lyle | Ground | Male |
| **ZW.E01.S19T** | 19T | Below-Strain | Hopkins | Ground | Male |
| **ZW.E01.S1B** | 1B | P-Trap | Harborne | First | Male |
| **ZW.E01.S1T** | 1T | Below-Strain | Harborne | First | Male |
| **ZW.E01.A0T** | 20T | Below-Strain | Hopkins | Ground | Male |
| **ZW.E01.A1T** | 21T | Below-Strain | Hopkins | Ground | Male |

| | | | | | |
|---|---|---|---|---|---|
| **ZW.E01.A2T** | 22T | Below-Strain | Hopkins | Ground | Female |
| **ZW.E01.A3T** | 23T | Below-Strain | Hopkins | Ground | Female |
| **ZW.E01.A4T** | 24T | Below-Strain | Hopkins | Ground | Female |
| **ZW.E01.A5T** | 25T | Below-Strain | Hopkins | First | Female |
| **ZW.E01.A6T** | 26T | Below-Strain | Hopkins | First | Female |
| **ZW.E01.A7T** | 27T | Below-Strain | Hopkins | First | Female |
| **ZW.E01.A8T** | 28T | Below-Strain | Hopkins | First | Male |
| **ZW.E01.A9T** | 29T | Below-Strain | Hopkins | First | Male |
| **ZW.E01.AB** | 2B | P-Trap | Harborne | First | Male |
| **ZW.E01.AT** | 2T | Below-Strain | Harborne | First | Male |
| **ZW.E01.B0T** | 30T | Below-Strain | Hopkins | First | Male |
| **ZW.E01.B1T** | 31T | Below-Strain | Hopkins | Second | Female |
| **ZW.E01.B2T** | 32T | Below-Strain | Hopkins | Second | Female |
| **ZW.E01.B3T** | 33T | Below-Strain | Hopkins | Second | Female |
| **ZW.E01.B4T** | 34T | Below-Strain | Hopkins | Second | Male |
| **ZW.E01.B5T** | 35T | Below-Strain | Hopkins | Second | Male |
| **ZW.E01.B6T** | 36T | Below-Strain | Hopkins | Second | Male |
| **ZW.E01.B7B** | 37B | P-Trap | Polly Vacher | Ground | Female |
| **ZW.E01.B7T** | 37T | Below-Strain | Polly Vacher | Ground | Female |
| **ZW.E01.B8B** | 38B | P-Trap | Polly Vacher | Ground | Male |
| **ZW.E01.B8T** | 38T | Below-Strain | Polly Vacher | Ground | Male |
| **ZW.E01.B9T** | 39T | Below-Strain | Polly Vacher | Ground | Male |
| **ZW.E01.BB** | 3B | P-Trap | Harborne | First | Male |
| **ZW.E01.BT** | 3T | Below-Strain | Harborne | First | Male |
| **ZW.E01.C0T** | 40T | Below-Strain | Polly Vacher | Ground | Male |
| **ZW.E01.C1T** | 41T | Below-Strain | Polly Vacher | Ground | Male |
| **ZW.E01.C2B** | 42B | P-Trap | Polly Vacher | Ground | Male |
| **ZW.E01.C2T** | 42T | Below-Strain | Polly Vacher | Ground | Male |
| **ZW.E01.C3B** | 43B | P-Trap | Polly Vacher | Ground | Male |
| **ZW.E01.C3T** | 43T | Below-Strain | Polly Vacher | Ground | Male |
| **ZW.E01.C4B** | 44B | P-Trap | Polly Vacher | Ground | Male |
| **ZW.E01.C4T** | 44T | Below-Strain | Polly Vacher | Ground | Male |
| **ZW.E01.C5B** | 45B | P-Trap | Polly Vacher | Ground | Female |

| | | | | | |
|---|---|---|---|---|---|
| **ZW.E01.C5T** | 45T | Below-Strain | Polly Vacher | Ground | Female |
| **ZW.E01.C6B** | 46B | P-Trap | Polly Vacher | Ground | Female |
| **ZW.E01.C6T** | 46T | Below-Strain | Polly Vacher | Ground | Female |
| **ZW.E01.C7B** | 47B | P-Trap | Knight | Ground | Male |
| **ZW.E01.C7T** | 47T | Below-Strain | Knight | Ground | Male |
| **ZW.E01.C8B** | 48B | P-Trap | Knight | Ground | Male |
| **ZW.E01.C8T** | 48T | Below-Strain | Knight | Ground | Male |
| **ZW.E01.C9B** | 49B | P-Trap | Knight | Ground | Male |
| **ZW.E01.C9T** | 49T | Below-Strain | Knight | Ground | Male |
| **ZW.E01.CB** | 4B | P-Trap | Harborne | First | Male |
| **ZW.E01.CT** | 4T | Below-Strain | Harborne | First | Male |
| **ZW.E01.S50B** | 50B | P-Trap | Knight | Ground | Male |
| **ZW.E01.S50T** | 50T | Below-Strain | Knight | Ground | Male |
| **ZW.E01.S51B** | 51B | P-Trap | Knight | Ground | Male |
| **ZW.E01.S51T** | 51T | Below-Strain | Knight | Ground | Male |
| **ZW.E01.S52B** | 52B | P-Trap | Knight | Ground | Female |
| **ZW.E01.S52T** | 52T | Below-Strain | Knight | Ground | Female |
| **ZW.E01.S53B** | 53B | P-Trap | Knight | Ground | Female |
| **ZW.E01.S53T** | 53T | Below-Strain | Knight | Ground | Female |
| **ZW.E01.S54B** | 54B | P-Trap | Knight | Ground | Female |
| **ZW.E01.S54T** | 54T | Below-Strain | Knight | Ground | Female |
| **ZW.E01.S55B** | 55B | P-Trap | Knight | Ground | Female |
| **ZW.E01.S55T** | 55T | Below-Strain | Knight | Ground | Female |
| **ZW.E01.S56B** | 56B | P-Trap | Student Union | Ground | Male |
| **ZW.E01.S56T** | 56T | Below-Strain | Student Union | Ground | Male |
| **ZW.E01.S57B** | 57B | P-Trap | Student Union | Ground | Male |
| **ZW.E01.S57T** | 57T | Below-Strain | Student Union | Ground | Male |
| **ZW.E01.S58B** | 58B | P-Trap | Student Union | Ground | Male |
| **ZW.E01.S58T** | 58T | Below-Strain | Student Union | Ground | Male |
| **ZW.E01.S59B** | 59B | P-Trap | Student Union | Ground | Male |
| **ZW.E01.S59T** | 59T | Below-Strain | Student Union | Ground | Male |
| **ZW.E01.S5T** | 5T | Below-Strain | Harborne | First | Female |
| **ZW.E01.S60B** | 60B | P-Trap | Student Union | Ground | Male |

| | | | | | |
|---|---|---|---|---|---|
| **ZW.E01.S60T** | 60T | Below-Strain | Student Union | Ground | Male |
| **ZW.E01.S61B** | 61B | P-Trap | Student Union | Ground | Male |
| **ZW.E01.S61T** | 61T | Below-Strain | Student Union | Ground | Male |
| **ZW.E01.S62B** | 62B | P-Trap | Edith Morley | Ground | Male |
| **ZW.E01.S62T** | 62T | Below-Strain | Edith Morley | Ground | Male |
| **ZW.E01.S63B** | 63B | P-Trap | Edith Morley | Ground | Male |
| **ZW.E01.S63T** | 63T | Below-Strain | Edith Morley | Ground | Male |
| **ZW.E01.S64B** | 64B | P-Trap | Edith Morley | Ground | Male |
| **ZW.E01.S64T** | 64T | Below-Strain | Edith Morley | Ground | Male |
| **ZW.E01.S65B** | 65B | P-Trap | Edith Morley | Ground | Male |
| **ZW.E01.S65T** | 65T | Below-Strain | Edith Morley | Ground | Male |
| **ZW.E01.S66B** | 66B | P-Trap | Edith Morley | First | Male |
| **ZW.E01.S66T** | 66T | Below-Strain | Edith Morley | First | Male |
| **ZW.E01.S67B** | 67B | P-Trap | Edith Morley | First | Unisex |
| **ZW.E01.S67T** | 67T | Below-Strain | Edith Morley | First | Unisex |
| **ZW.E01.S68B** | 68B | P-Trap | Edith Morley | First | Male |
| **ZW.E01.S68T** | 68T | Below-Strain | Edith Morley | First | Male |
| **ZW.E01.S69B** | 69B | P-Trap | Edith Morley | Second | Male |
| **ZW.E01.S69T** | 69T | Below-Strain | Edith Morley | Second | Male |
| **ZW.E01.S6T** | 6T | Below-Strain | Harborne | First | Female |
| **ZW.E01.S70B** | 70B | P-Trap | Edith Morley | Second | Unisex |
| **ZW.E01.S70T** | 70T | Below-Strain | Edith Morley | Second | Unisex |
| **ZW.E01.S71B** | 71B | P-Trap | Edith Morley | Second | Male |
| **ZW.E01.S71T** | 71T | Below-Strain | Edith Morley | Second | Male |
| **ZW.E01.S72B** | 72B | P-Trap | Library | Fifth | Female |
| **ZW.E01.S72T** | 72T | Below-Strain | Library | Fifth | Female |
| **ZW.E01.S73B** | 73B | P-Trap | Library | Fifth | Female |
| **ZW.E01.S73T** | 73T | Below-Strain | Library | Fifth | Female |
| **ZW.E01.S74B** | 74B | P-Trap | Library | Fifth | Male |
| **ZW.E01.S74T** | 74T | Below-Strain | Library | Fifth | Male |
| **ZW.E01.S75B** | 75B | P-Trap | Library | Fifth | Male |
| **ZW.E01.S75T** | 75T | Below-Strain | Library | Fifth | Male |
| **ZW.E01.S76B** | 76B | P-Trap | Library | Fourth | Unisex |

| | | | | | |
|---|---|---|---|---|---|
| **ZW.E01.S76T** | 76T | Below-Strain | Library | Fourth | Unisex |
| **ZW.E01.S77B** | 77B | P-Trap | Library | Third | Unisex |
| **ZW.E01.S77T** | 77T | Below-Strain | Library | Third | Unisex |
| **ZW.E01.S78B** | 78B | P-Trap | Library | Second | Unisex |
| **ZW.E01.S78T** | 78T | Below-Strain | Library | Second | Unisex |
| **ZW.E01.S79B** | 79B | P-Trap | Library | First | Unisex |
| **ZW.E01.S79T** | 79T | Below-Strain | Library | First | Unisex |
| **ZW.E01.S7B** | 7B | P-Trap | Harborne | Ground | Male |
| **ZW.E01.S7T** | 7T | Below-Strain | Harborne | Ground | Male |
| **ZW.E01.S80B** | 80B | P-Trap | Henley Business School | Second | Female |
| **ZW.E01.S80T** | 80T | Below-Strain | Henley Business School | Second | Female |
| **ZW.E01.S81B** | 81B | P-Trap | Henley Business School | Second | Female |
| **ZW.E01.S81T** | 81T | Below-Strain | Henley Business School | Second | Female |
| **ZW.E01.S82B** | 82B | P-Trap | Henley Business School | Second | Male |
| **ZW.E01.S82T** | 82T | Below-Strain | Henley Business School | Second | Male |
| **ZW.E01.S83B** | 83B | P-Trap | Henley Business School | Second | Male |
| **ZW.E01.S83T** | 83T | Below-Strain | Henley Business School | Second | Male |
| **ZW.E01.S84B** | 84B | P-Trap | Henley Business School | Second | Male |
| **ZW.E01.S84T** | 84T | Below-Strain | Henley Business School | Second | Male |
| **ZW.E01.S85B** | 85B | P-Trap | Henley Business School | First | Female |
| **ZW.E01.S85T** | 85T | Below-Strain | Henley Business School | First | Female |
| **ZW.E01.S86B** | 86B | P-Trap | Henley Business School | First | Female |
| **ZW.E01.S86T** | 86T | Below-Strain | Henley Business School | First | Female |
| **ZW.E01.S87B** | 87B | P-Trap | Henley Business School | First | Female |
| **ZW.E01.S87T** | 87T | Below-Strain | Henley Business School | First | Female |
| **ZW.E01.S88B** | 88B | P-Trap | Henley Business School | Ground | Unisex |
| **ZW.E01.S88T** | 88T | Below-Strain | Henley Business School | Ground | Unisex |
| **ZW.E01.S89B** | 89B | P-Trap | Henley Business School | Ground | Unisex |
| **ZW.E01.S89T** | 89T | Below-Strain | Henley Business School | Ground | Unisex |
| **ZW.E01.S8B** | 8B | P-Trap | Harborne | Ground | Male |
| **ZW.E01.S8T** | 8T | Below-Strain | Harborne | Ground | Male |
| **ZW.E01.S90B** | 90B | P-Trap | Henley Business School | Ground | Unisex |
| **ZW.E01.S90T** | 90T | Below-Strain | Henley Business School | Ground | Unisex |

| | | | | | |
|---|---|---|---|---|---|
| **ZW.E01.S91B** | 91B | P-Trap | Henley Business School | Ground | Female |
| **ZW.E01.S91T** | 91T | Below-Strain | Henley Business School | Ground | Female |
| **ZW.E01.S92B** | 92B | P-Trap | Henley Business School | Ground | Female |
| **ZW.E01.S92T** | 92T | Below-Strain | Henley Business School | Ground | Female |
| **ZW.E01.S93B** | 93B | P-Trap | Henley Business School | Ground | Female |
| **ZW.E01.S93T** | 93T | Below-Strain | Henley Business School | Ground | Female |
| **ZW.E01.S94B** | 94B | P-Trap | Henley Business School | Ground | Female |
| **ZW.E01.S94T** | 94T | Below-Strain | Henley Business School | Ground | Female |
| **ZW.E01.S95B** | 95B | P-Trap | Henley Business School | Ground | Female |
| **ZW.E01.S95T** | 95T | Below-Strain | Henley Business School | Ground | Female |
| **ZW.E01.S96B** | 96B | P-Trap | Henley Business School | Ground | Female |
| **ZW.E01.S96T** | 96T | Below-Strain | Henley Business School | Ground | Female |
| **ZW.E01.S97B** | 97B | P-Trap | Henley Business School | Ground | Female |
| **ZW.E01.S97T** | 97T | Below-Strain | Henley Business School | Ground | Female |
| **ZW.E01.S98B** | 98B | P-Trap | Henley Business School | Ground | Male |
| **ZW.E01.S98T** | 98T | Below-Strain | Henley Business School | Ground | Male |
| **ZW.E01.S99B** | 99B | P-Trap | Henley Business School | Ground | Male |
| **ZW.E01.S99T** | 99T | Below-Strain | Henley Business School | Ground | Male |
| **ZW.E01.S9B** | 9B | P-Trap | Harborne | Ground | Male |
| **ZW.E01.S9T** | 9T | Below-Strain | Harborne | Ground | Male |

**Table A.5.** Information for each sample including what part of the sink drain was sampled, where the sink was, and the restroom gender associated with the sink.

# Chapter 3. Mycobial Community Assemblages in Sink Drains across a University Campus

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## 3.1 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, Mycobiome, Mycobial community, Fungi, Sink, P-trap, Next-generation sequencing

## 3.2 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, et al., 2015b; 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) 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, et al., 2001a, 2001b; 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 research questions: (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.

## 3.3 Methods

### *3.3.1 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 B.1). Genomic DNA was isolated from the swabs using HigherPurity Soil

DNA Isolation kit (Canvax Biotech), according to the manufacturers protocol. Negative controls were blank swabs extracted by the same method.

### *3.3.2 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 5µl 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 building (Table B.2). 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.

### *3.3.3 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 was 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).

## 3.4 Results

### *3.4.1 Data Features*

After bioinformatic processing through PIPITS, the fungal data set contained 3862 OTUs (9,265,250 sequences), distributed across 343 samples from 20 buildings throughout the University of Readings campus. The number of reads per sample varied between two to 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 B.5). Further, 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 B.3). 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% percentage identity).

### *3.4.2 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 3.1A, Figure B.1A). 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 greater than 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 3.1). 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 (Figure 3.1B, 2A Figure B.1B). However, taxonomic analysis of individual samples showed variation in relative abundances of the top genera between some sinks within a building (Figure B.2, B.3, Table B.4).

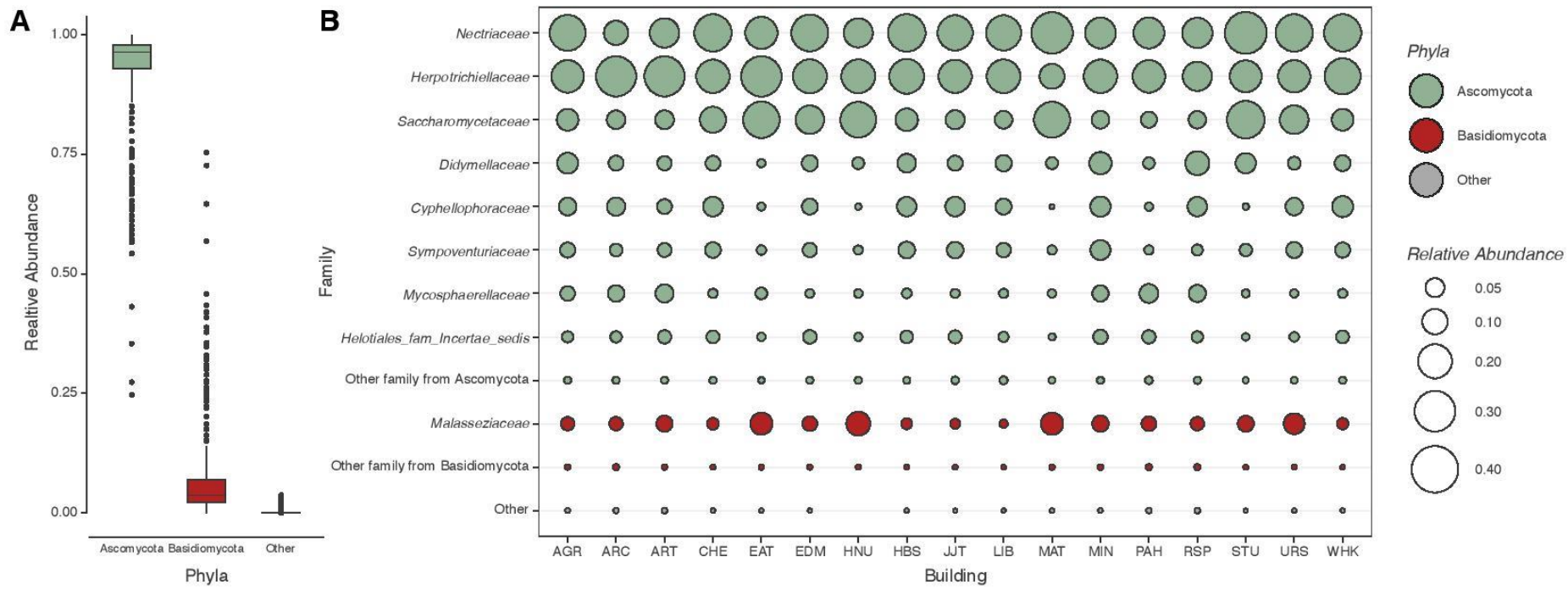


**Figure 3.1.** Taxonomic analysis. A) Boxplot showing the distribution of the dominant phyla. "Other" represents remaining 5 phyla. B) Bubble plot of mean relative abundance of the most abundant fungal families (>1%) by building. Across all buildings, the mean distribution of families is generally uneven 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 colour 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.

| | | 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 |

**Table 3.1.** Identity of top OTUs (>1% Relative abundance). Overall abundance (total percentage of reads) and prevalence shown.

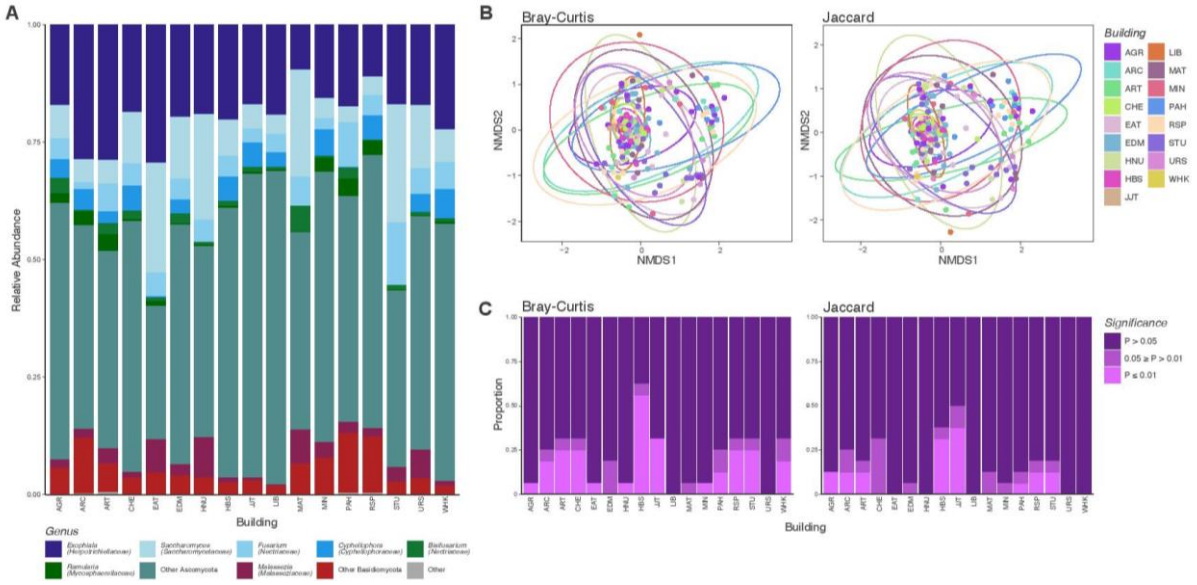


**Figure 3.2.** Composition of mycobial communities by building. A) Fungal composition: Relative abundances of top genera (>1%) by building shown. Family of genera is italicised and in brackets below genus in the legend. B) Beta Diversity. Non-metric multidimensional scaling (NMDS) plots of dissimilarity metrics. Each point represents a sample, colour indicated 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 colour, 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.

### *3.4.3 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 B.3). 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 3.3). 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 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.

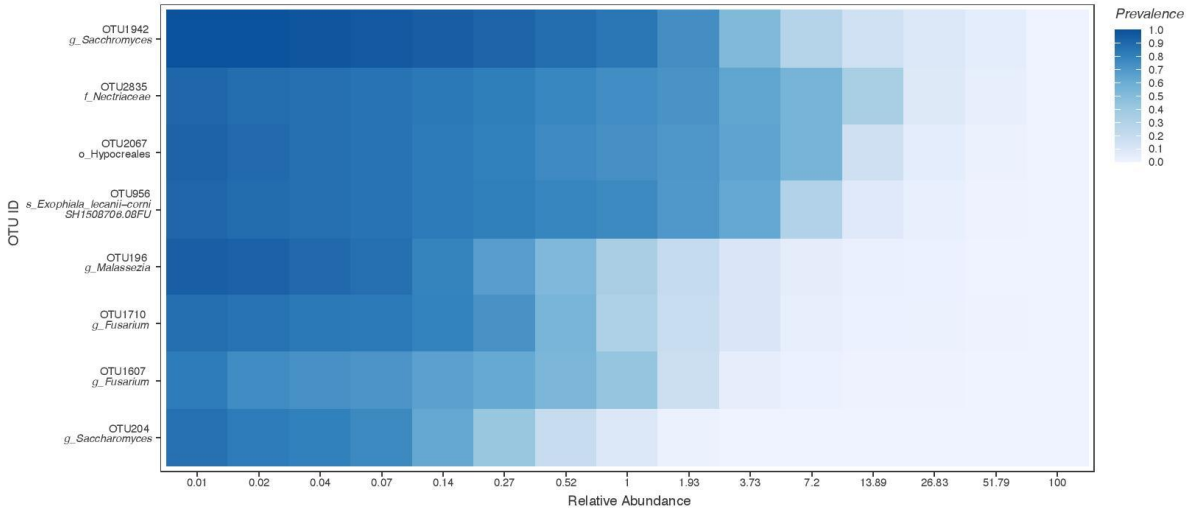


**Figure 3.3.** Heatmap of the core microbiome analysis. Shows the eight OTUs that were considered part of the core mycobiome (>80% prevalence of 289 samples). The y-axis shows the eight core OTUs. The relative abundance derived from count data is plotted on the x-axis. The gradient of colour indicates the variation of prevalence of each OTU.

### *3.4.4 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 3.2B). 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 3.2C, Table B.5). But overall, post-hoc analysis showed that the majority of building 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)) (Figure B.4). 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. PERMNAOVA is conservative when high dispersions occur in larger groups and liberal when high dispersion 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 3.4). 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 3.4). 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 B.6). Multiple pairs of buildings were highly significant from one another which may contribute to the

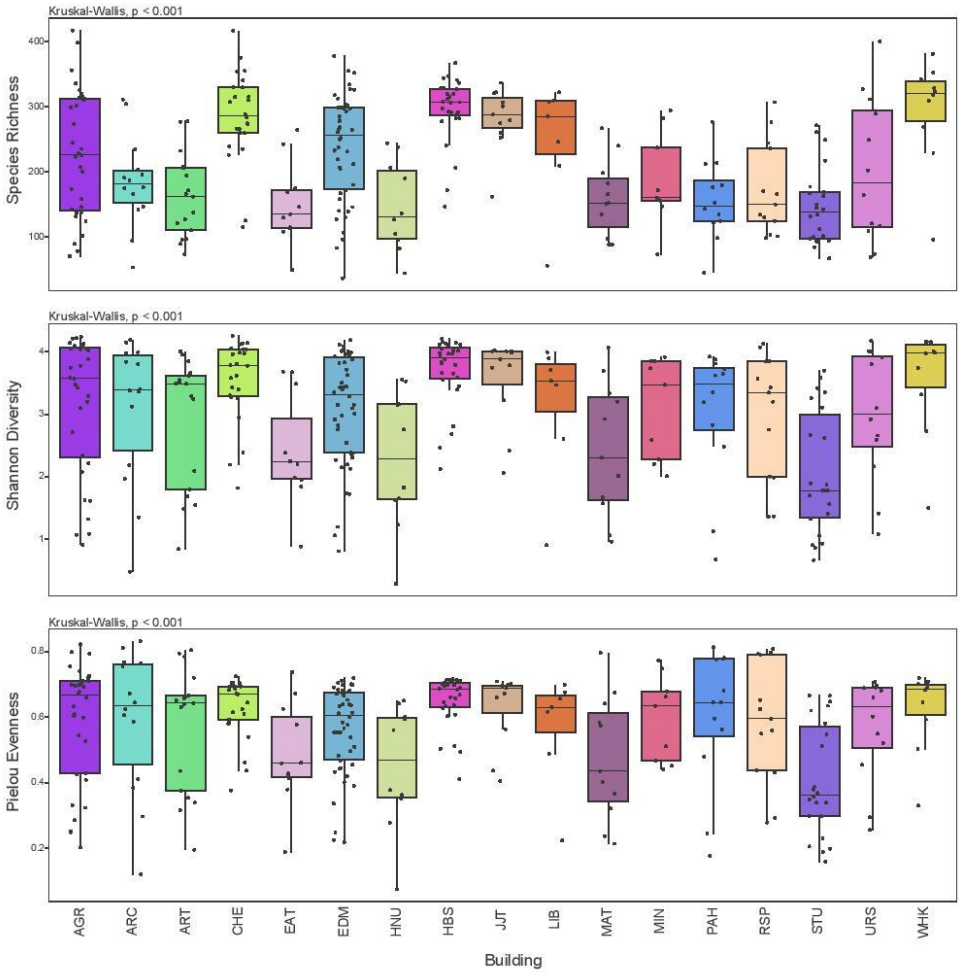


**Figure 3.4.** Fungal alpha diversity. Boxplot of alpha diversity of fungal communities by building sampled. Species richness (number of OTUs), Shannon and Pielou's evenness shown. Each point represents a sample. P-value obtained from Kruskal-Wallis test shown above each plot.

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).

## 3.5 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 agreed with findings of this study, with the high abundance and prevalence of *Exophiala* and *Fusarium* suggesting their presence due to endogenous growth and the occurrence of *Malassezia* likely present due to deposition from handwashing. Bacterial taxa found in our study not only overlapped with those from Adams et al., 2013b but also have been found in other culture dependent 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 isolated from potable water sources i.e., tap water and public drinking reservoirs (Biedunkiewicz & Schulz, 2012; Göttlich et al., 2002; Heinrichs, et al., 2013a,2013b). 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 et al.,

2013a). 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 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, et al., 2015a, 2015b; 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 restrooms 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 et al., 2001a; 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 on 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., 2013a; 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 et al., 2013a; 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 analysed 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 et al., 2001a; 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.

## 3.6 Declaration

### *3.6.1 Data Availability*

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 Supplementary Table B.1.

### *3.6.2 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.

### *3.6.3 Supplementary material link*

The supplementary material for this article can be found at: https://doi.org/10.1002/edn3.375

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## Appendix B

## Supplementary material for Chapter 3

### Mycobial community assemblages in sink drains across a university campus

This appendix includes:

- **Figure B.1** - Mycobiome composition by building. Colours indicate the average fungal phylum/family distribution in different buildings. A) At the Phylum level. B) At the genus level. Ascomycota dominate across buildings at the phylum level.
- **Figure B.2** - Genus level composition of the sink mycobiome from public restrooms. Each line represents a single sample. Coloured bar underneath bar plot shows from which building the sample was taken. Sink samples grouped by building along x-axis.
- **Figure B.3** - The top three genera and their contribution across all samples plotted as a rank abundance curve. Red dotted line represents the mean relative abundance for the genus and the dark blue represents the median. Figure shows some variation in relative abundances of the top genera across and within buildings. Coloured bar underneath plot corresponds to building from which that sample was taken.
- **Figure B.4** - Distances (Bray-Curtis and Jaccard matrices used) to centroid in multivariate homogeneity of group variance analysis for sink fungal communities for each building. The spread of some buildings is more variable in comparison to others.
- **Figure B.5** - Rarefaction analysis: Most of the samples, showed rarefaction curves that did not reach a plateau suggesting further sequencing may be required for a full taxonomic representation of the fungal community.
- **Figure B.6** - Document of beta diversity analysis including all samples. NMDS of Bray-Curtis and Jaccard, betadisper results and statistical analysis shown. Overall results were no different from when the outliers were removed (data presented in manuscript).
- **Table B.1** - Table of sample metadata.
- **Table B.2** - Number of samples successfully amplified ITS2 region and purified using Agencourt AMPure XP magnetic beads (Beckman Coulter).
- **Table B.3** - OTU table with associated taxonomy (not included, link provided as table too large to include)

- **Table B.4** - Average relative abundance (RA) of top classified genera by A) Building and B) Gender of restroom from which sample was taken. Some samples were collected from kitchens, so this was included as an additional group under gender.
- **Table B.5** - Post hoc Tukey test results. A) Using Bray-Curtis dissimilarity matrix, B) Jaccard. Pairs of buildings shown in tables are only those with significant differences observed. Stars indicate the p-value significance * $p < 0.05$ , ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.
- **Table B.6** - Results of Paired Wilcoxon comparisons between buildings based on alpha diversity measures A) Observed, B) Shannon, C) Evenness. Pairs of buildings shown in tables are only those with significant differences observed. P.adj shows the P-Bonferroni corrected p-values, stars indicate the p-value significance * $p < 0.05$ , ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

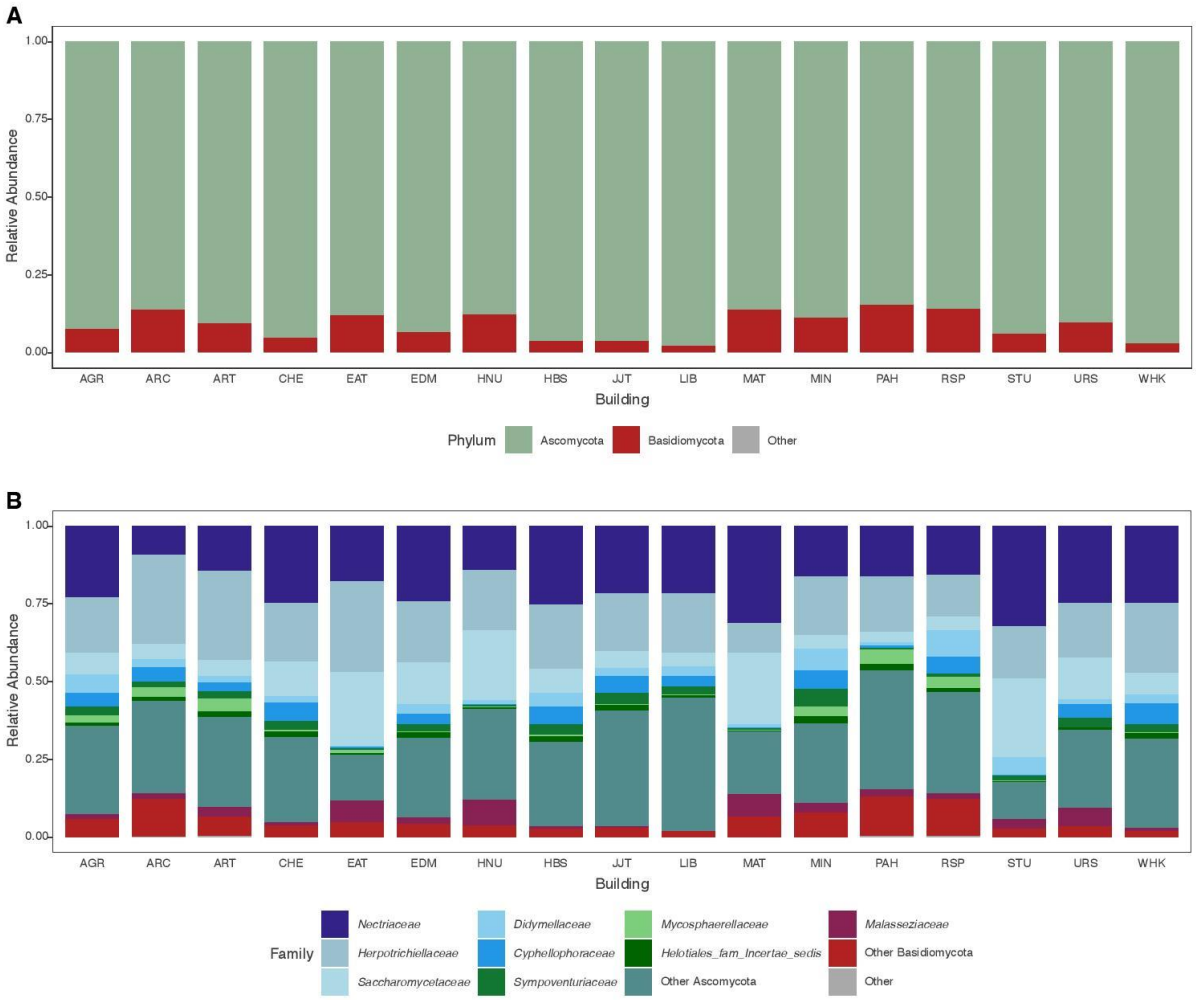



**Figure B.1.** Mycobiome composition by building. Colours indicate the average fungal phylum/family distribution in different buildings. A) At the Phylum level. B) At the genus level. Ascomycota dominate across buildings at the phylum level

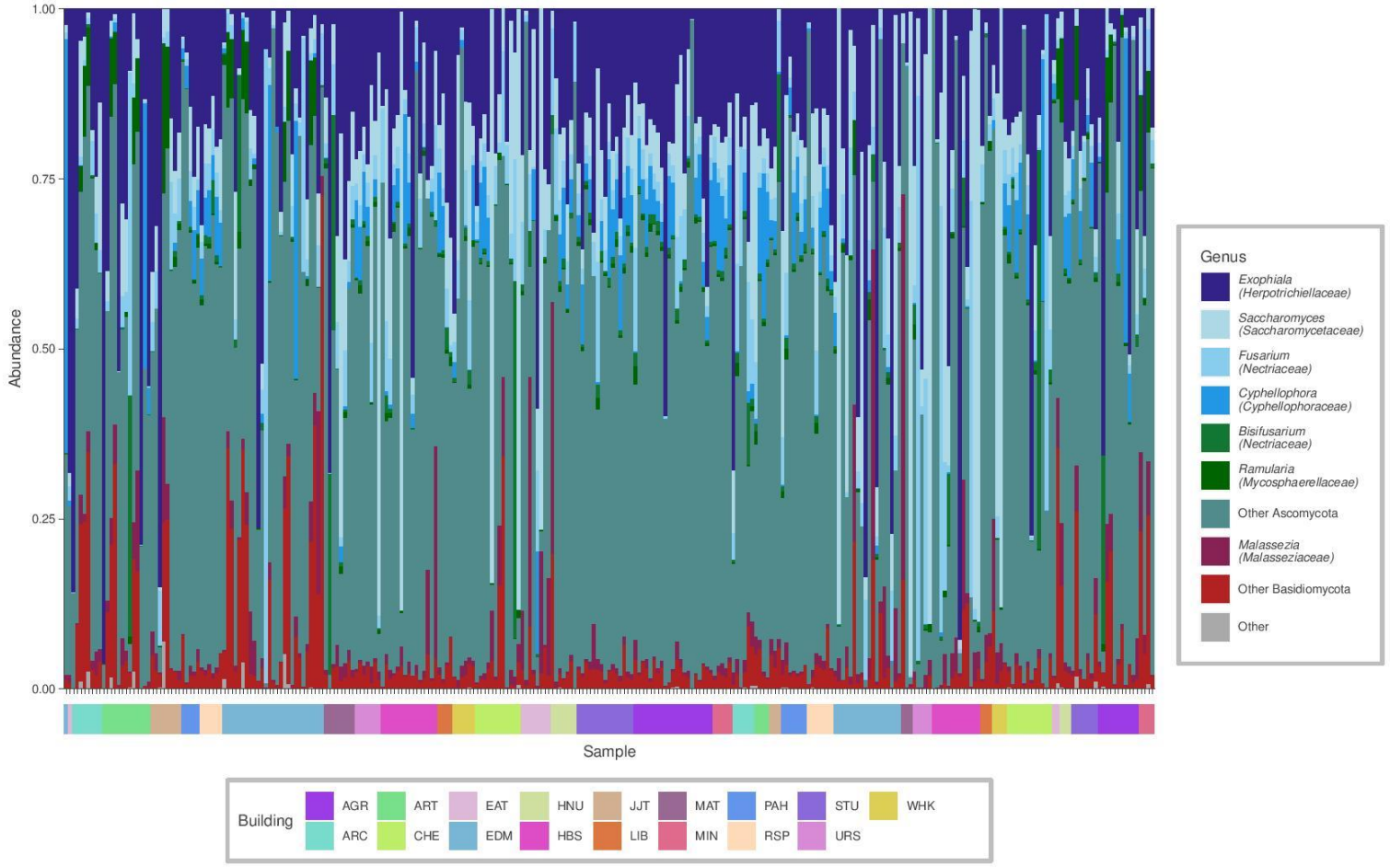



**Figure B.2.** Genus level composition of the sink mycobiome from public restrooms. Each line represents a single sample. Coloured bar underneath bar plot shows from which building the sample was taken. Sink samples grouped by building along x-axis.

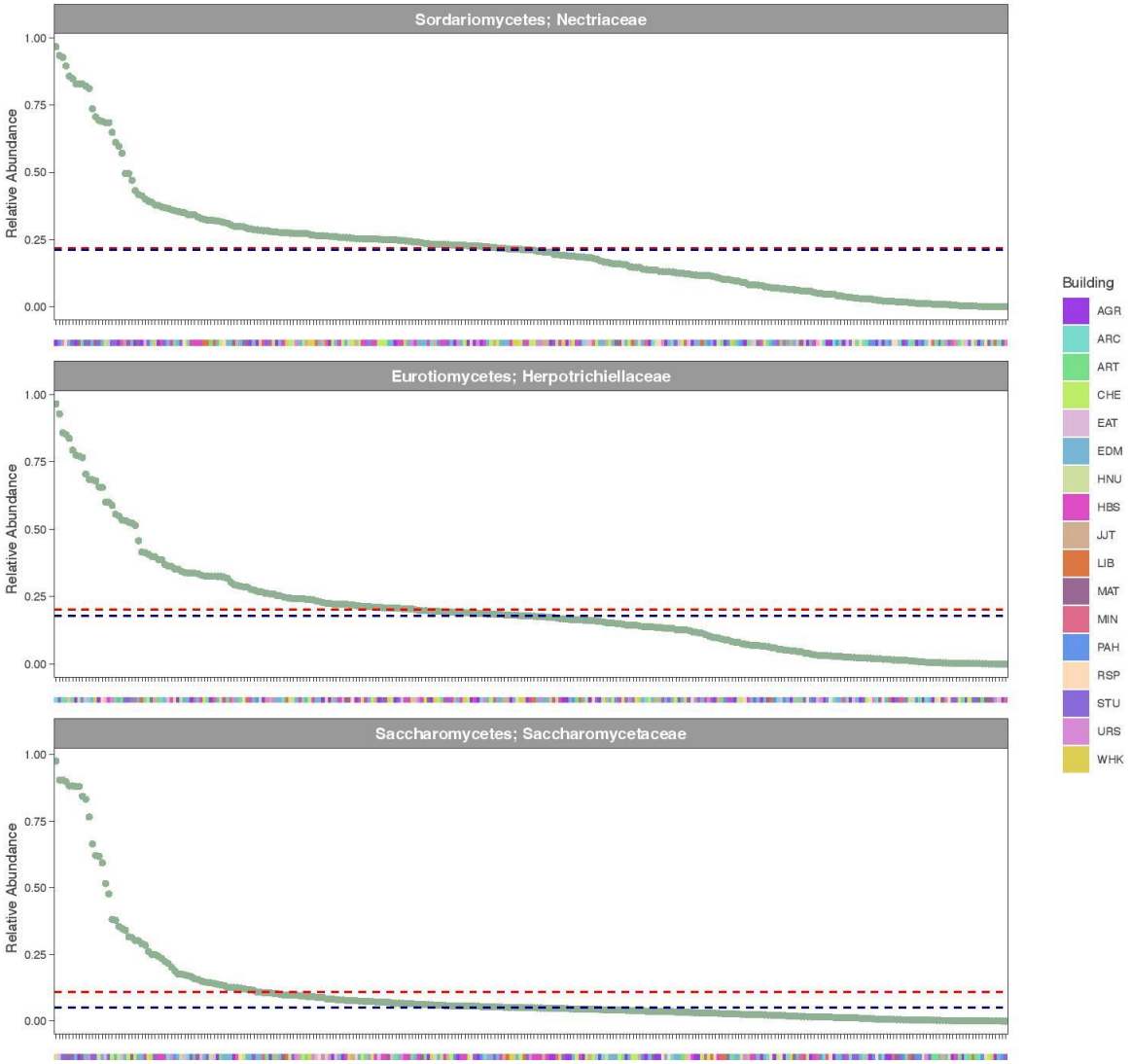



**Figure B.3.** The top three genera and their contribution across all samples plotted as a rank abundance curve. Red dotted line represents the mean relative abundance for the genus and the dark blue represents the median. Figure shows some variation in relative abundances of the top genera across and within buildings. Coloured bar underneath plot corresponds to building from which that sample was taken.

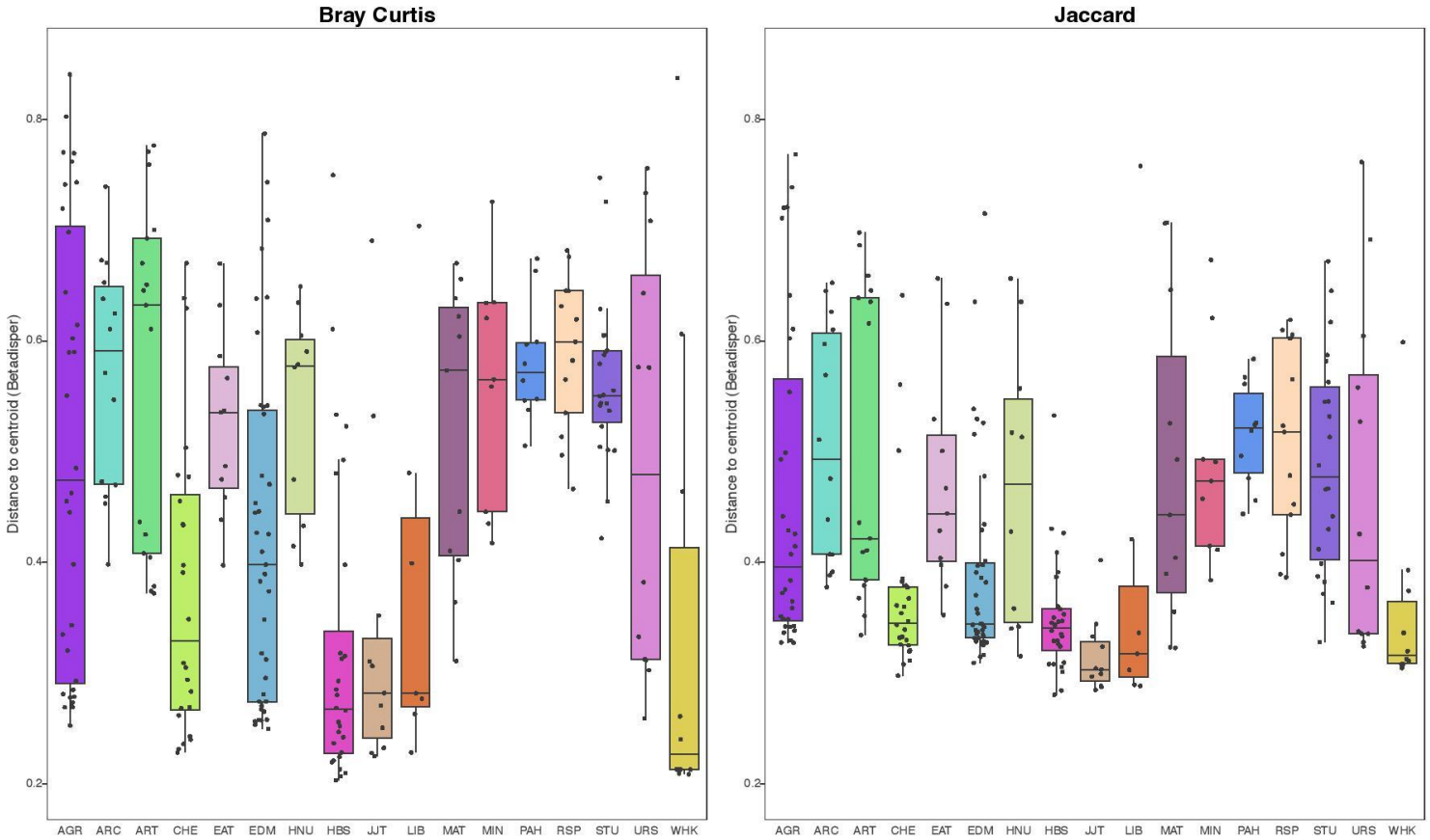



**Figure B.4.** Distances (Bray-Curtis and Jaccard matrices used) to centroid in multivariate homogeneity of group variance analysis for sink fungal communities for each building. The spread of some buildings is more variable in comparison to others.

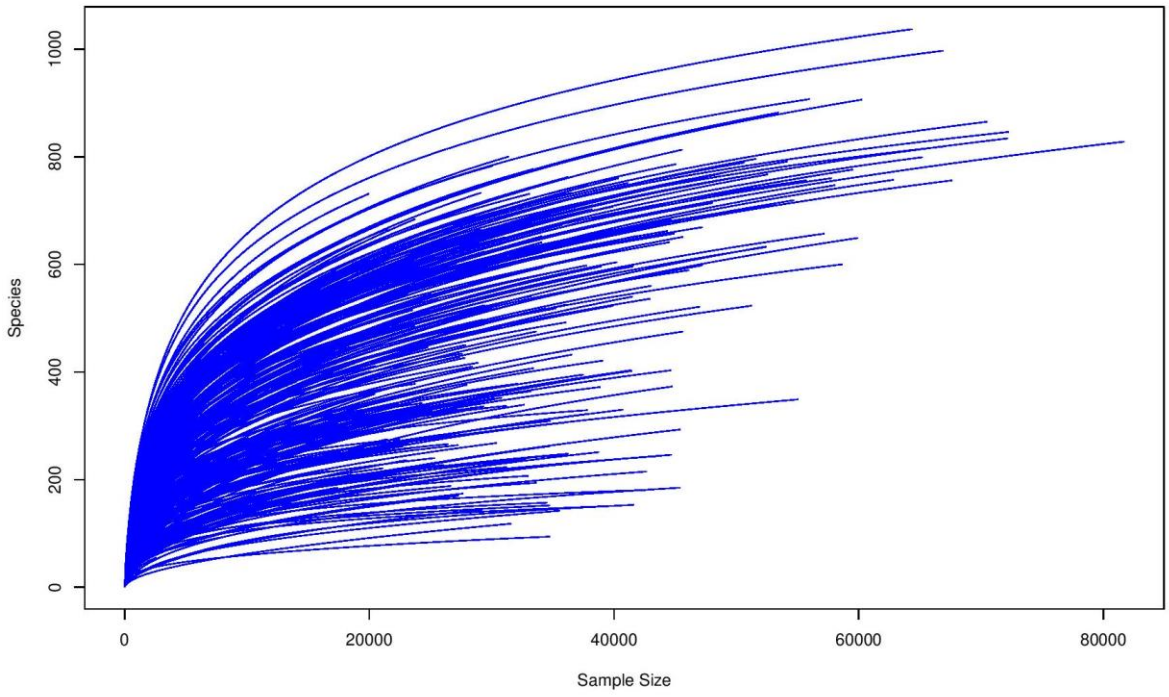
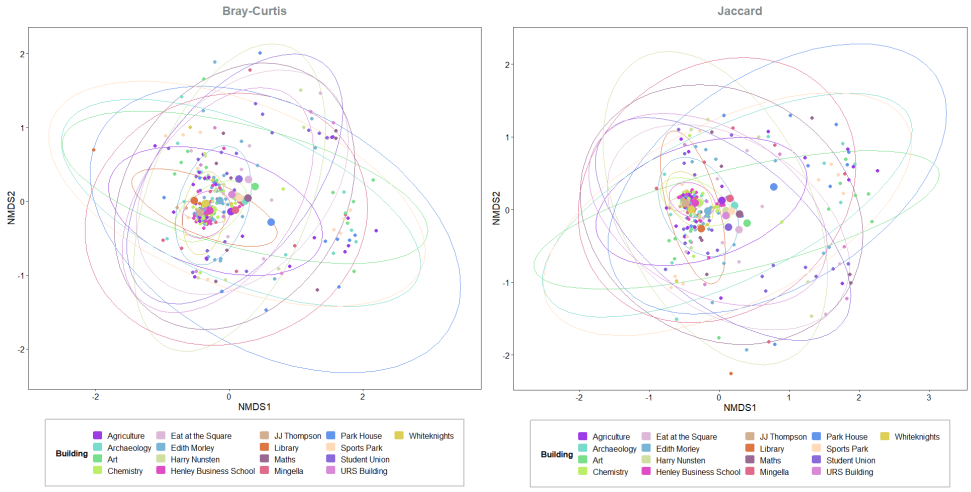


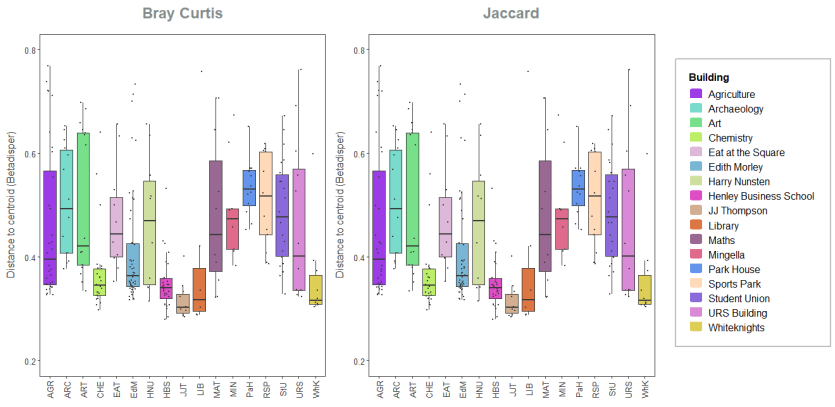

**Figure B.5.** Rarefaction analysis: Most of the samples, showed rarefaction curves that did not reach a plateau suggesting further sequencing may be required for a full taxonomic representation of the fungal community.

**Figure B.6. (Below)** Document of beta diversity analysis including all samples. NMDS of Bray-Curtis and Jaccard, betadisper results and statistical analysis shown. Overall results were no different from when the outliers were removed (data presented in manuscript).

Three samples were removed (two from Park House, one from Edith Morley) for analysis in the main text of the paper due to them being outliers. Analysis as performed in main text, was also carried out on the full dataset of 289 samples for comparison and completeness. As concluded in the main text no clear separation of buildings was observed in NMDS (below). Centroids of building groups are also shown in NMDS plot.




PERMANOVA was performed and the null hypothesis rejected. There are significant differences among different buildings (F.model = 2.3225, R2 = 0.12019 , P = 0.001 (Bray-Curtis); F.model = 1.6907, R2 = 0.09046, P = 0.001 (Jaccard)). This further agrees with results stated in main text. Results from betadisper are shown below:




ANOVA, DF = 16, F = 6.7917 p < 0.001 (Bray-Curtis); DF = 16, F = 5.4683, P < 0.001 (Jaccard)).

Overall results were no different from when the outliers were removed (data presented in manuscript).

| Seq ID | Building | Gender | Floor | Building Purpose | Location | P-Trap material | Sink Trap design | Building Temperature (°C) |
|---|---|---|---|---|---|---|---|---|
| **ND.E01.SU01** | Student Union | Male | Ground | Recreational | Central | Unknown | Uknown | 14.5 |
| **ND.E01.SU02** | Student Union | Male | Ground | Recreational | Central | Unknown | Uknown | 14.5 |
| **ND.E01.SU03** | Student Union | Male | Ground | Recreational | Central | Unknown | Uknown | 14.5 |
| **ND.E01.SU04** | Student Union | Male | Ground | Recreational | Central | Unknown | Uknown | 14.5 |
| **ND.E01.SU05** | Student Union | Male | Ground | Recreational | Central | Unknown | Uknown | 14.5 |
| **ND.E01.SU06** | Student Union | Male | Ground | Recreational | Central | Unknown | Uknown | 14.5 |
| **ND.E01.SU07** | Student Union | Male | Ground | Recreational | Central | Unknown | Uknown | 14.5 |
| **ND.E01.SU08** | Student Union | Male | Ground | Recreational | Central | Unknown | Uknown | 16.8 |
| **ND.E01.SU10** | Student Union | Male | Ground | Recreational | Central | Unknown | Uknown | 16.8 |
| **ND.E01.SU11** | Student Union | Male | Ground | Recreational | Central | Unknown | Uknown | 16.8 |
| **ND.E01.SU12** | Student Union | Female | Ground | Recreational | Central | Unknown | Uknown | 15.9 |
| **ND.E01.SU13** | Student Union | Female | Ground | Recreational | Central | Unknown | Uknown | 15.9 |
| **ND.E01.SU14** | Student Union | Female | Ground | Recreational | Central | Unknown | Uknown | 15.9 |
| **ND.E01.SU15** | Student Union | Female | Ground | Recreational | Central | Unknown | Uknown | 15.9 |
| **ND.E01.SU16** | Student Union | Female | Ground | Recreational | Central | Unknown | Uknown | 15.9 |
| **ND.E01.SU17** | Student Union | Female | Ground | Recreational | Central | Unknown | Uknown | 15.9 |
| **ND.E01.SU18** | Student Union | Female | Ground | Recreational | Central | Unknown | Uknown | 15.9 |
| **ND.E01.SU19** | Student Union | Female | Ground | Recreational | Central | Unknown | Uknown | 16.9 |
| **ND.E01.SU20** | Student Union | Female | Ground | Recreational | Central | Unknown | Uknown | 16.9 |
| **ND.E01.SU21** | Student Union | Female | Ground | Recreational | Central | Unknown | Uknown | 16.9 |
| **ND.E01.SU23** | Student Union | Female | Ground | Recreational | Central | Unknown | Uknown | 16.9 |
| **ND.E01.SU24** | Student Union | Neutral | First | Recreational | Central | Unknown | Uknown | 15.9 |
| **ND.E01.SU26** | Student Union | Neutral | First | Recreational | Central | Unknown | Uknown | 15.9 |
| **ND.E01.EATS12** | Eat at the Square | Neutral | Ground | Recreational | Central | Plastic | Bottle Trap | 20.1 |
| **ND.E01.SU27** | Student Union | Neutral | First | Recreational | Central | Unknown | Uknown | 15.9 |
| **ND.E01.SU28** | Student Union | Neutral | First | Recreational | Central | Unknown | Uknown | 15.9 |
| **ND.E01.EATS01** | Eat at the Square | Male | Ground | Recreational | Central | Unknown | Bottle Trap | 21.7 |
| **ND.E01.EATS02** | Eat at the Square | Male | Ground | Recreational | Central | Unknown | Bottle Trap | 21.7 |
| **ND.E01.EATS03** | Eat at the Square | Male | Ground | Recreational | Central | Unknown | Bottle Trap | 21.7 |

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|---|---|---|---|---|---|---|---|---|
| **ND.E01.EATS04** | Eat at the Square | Male | Ground | Recreational | Central | Unknown | Bottle Trap | 21.7 |
| **ND.E01.EATS05** | Eat at the Square | Female | Ground | Recreational | Central | Unknown | Bottle Trap | 24 |
| **ND.E01.EATS06** | Eat at the Square | Female | Ground | Recreational | Central | Unknown | Bottle Trap | 24 |
| **ND.E01.EATS07** | Eat at the Square | Female | Ground | Recreational | Central | Unknown | Bottle Trap | 24 |
| **ND.E01.EATS08** | Eat at the Square | Female | Ground | Recreational | Central | Unknown | Bottle Trap | 24 |
| **ND.E01.EATS09** | Eat at the Square | Female | Ground | Recreational | Central | Unknown | Bottle Trap | 24 |
| **ND.E01.EATS10** | Eat at the Square | Female | Ground | Recreational | Central | Unknown | Bottle Trap | 24 |
| **ND.E01.EATS11** | Eat at the Square | Female | Ground | Recreational | Central | Unknown | Bottle Trap | 24 |
| **ND.E01.HN01** | Harry Nunsten | Male | Ground | Teaching | Central | Unknown | Bottle Trap | 26.4 |
| **ND.E01.HN02** | Harry Nunsten | Male | Ground | Teaching | Central | Unknown | Bottle Trap | 26.4 |
| **ND.E01.HN03** | Harry Nunsten | Female | Ground | Teaching | Central | Unknown | Bottle Trap | 25.1 |
| **ND.E01.HN04** | Harry Nunsten | Female | Ground | Teaching | Central | Unknown | Bottle Trap | 25.1 |
| **ND.E01.HN05** | Harry Nunsten | Neutral | Ground | Teaching | Central | Unknown | Bottle Trap | 28.3 |
| **ND.E01.HN06** | Harry Nunsten | Kitchen | Ground | Teaching | Central | Unknown | Bottle Trap | 25.4 |
| **ND.E01.HN07** | Harry Nunsten | Male | First | Teaching | Central | Plastic | Bottle Trap | 21.6 |
| **ND.E01.HN08** | Harry Nunsten | Male | First | Teaching | Central | Unknown | Bottle Trap | 21.6 |
| **ND.E01.HN09** | Harry Nunsten | Male | First | Teaching | Central | Unknown | Bottle Trap | 21.6 |
| **ND.E01.HN10** | Harry Nunsten | Female | First | Teaching | Central | Unknown | Bottle Trap | 30.6 |
| **ND.E01.HN11** | Harry Nunsten | Female | First | Teaching | Central | Unknown | Bottle Trap | 30.6 |
| **EF.E01.URS03** | URS Building | Female | Ground | Teaching | Central | Plastic | Bottle Trap | 11 |
| **EF.E01.URS04** | URS Building | Female | Ground | Teaching | Central | Plastic | Bottle Trap | 11 |
| **EF.E01.URS05** | URS Building | Neutral | Ground | Teaching | Central | Plastic | Bottle Trap | 8.1 |
| **EF.E01.URS06** | URS Building | Kitchen | Ground | Teaching | Central | Plastic | S-Trap | 11.2 |
| **EF.E01.URS07** | URS Building | Male | Second | Teaching | Central | Plastic | Bottle Trap | 15.6 |
| **EF.E01.URS08** | URS Building | Male | Second | Teaching | Central | Plastic | Bottle Trap | 18.8 |
| **EF.E01.MILL01** | Miller | Male | Ground | Teaching | Central | Plastic | Bottle Trap | 8 |
| **EF.E01.MILL02** | Miller | Male | Ground | Teaching | Central | Plastic | Bottle Trap | 8 |
| **EF.E01.MILL03** | Miller | Female | Ground | Teaching | Central | Plastic | Bottle Trap | 8.9 |
| **EF.E01.MILL05** | Miller | Female | Ground | Teaching | Central | Plastic | Bottle Trap | 8.9 |
| **EF.E01.MILL07** | Miller | Male | First | Teaching | Central | Unknown | Bottle Trap | 12.5 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **EF.E01.EDM03** | Edith Morley | Male | Ground | Teaching | Central | Metal | Bottle Trap | 8.6 |
| **EF.E01.EDM04** | Edith Morley | Male | Ground | Teaching | Central | Metal | Bottle Trap | 8.6 |
| **EF.E01.EDM05** | Edith Morley | Male | Ground | Teaching | Central | Metal | Bottle Trap | 14.8 |
| **EF.E01.EDM06** | Edith Morley | Male | Ground | Teaching | Central | Metal | Bottle Trap | 14.8 |
| **EF.E01.EDM07** | Edith Morley | Female | Ground | Teaching | Central | Metal | Bottle Trap | 10.1 |
| **EF.E01.EDM08** | Edith Morley | Female | Ground | Teaching | Central | Metal | Bottle Trap | 10.1 |
| **EF.E01.EDM10** | Edith Morley | Female | Ground | Teaching | Central | Metal | Bottle Trap | 12.6 |
| **EF.E01.MAT01** | Maths | Female | Ground | Teaching | Central | Unknown | Bottle Trap | 21.1 |
| **EF.E01.MAT02** | Maths | Female | Ground | Teaching | Central | Unknown | Bottle Trap | 21.1 |
| **EF.E01.MAT03** | Maths | Female | Ground | Teaching | Central | Unknown | Bottle Trap | 21.1 |
| **EF.E01.MAT04** | Maths | Male | First | Teaching | Central | Unknown | Bottle Trap | 19.1 |
| **EF.E01.MAT05** | Maths | Male | First | Teaching | Central | Unknown | Bottle Trap | 19.1 |
| **EF.E01.EDM09** | Edith Morley | Female | Ground | Teaching | Central | Metal | Bottle Trap | 12.6 |
| **EF.E01.MAT06** | Maths | Male | First | Teaching | Central | Unknown | Bottle Trap | 19.1 |
| **EF.E01.MAT07** | Maths | Female | Second | Teaching | Central | Unknown | Bottle Trap | 22.8 |
| **EF.E01.MAT08** | Maths | Female | Second | Teaching | Central | Unknown | Bottle Trap | 22.8 |
| **EF.E01.MAT09** | Maths | Female | Second | Teaching | Central | Unknown | Bottle Trap | 22.8 |
| **EF.E01.MAT10** | Maths | Male | Third | Teaching | Central | Unknown | Bottle Trap | 19 |
| **EF.E01.MAT12** | Maths | Male | Third | Teaching | Central | Unknown | Bottle Trap | 19 |
| **EF.E01.URS11** | URS Building | Neutral | Second | Teaching | Central | Plastic | S-Trap | 13.4 |
| **EF.E01.EDM12** | Edith Morley | Female | Ground | Teaching | Central | Metal | Bottle Trap | 14.2 |
| **EF.E01.EDM18** | Edith Morley | Neutral | Ground | Teaching | Central | Metal | Bottle Trap | 16.9 |
| **EF.E01.EDM20** | Edith Morley | Male | First | Teaching | Central | Metal | Bottle Trap | 14.7 |
| **EF.E01.EDM26** | Edith Morley | Female | First | Teaching | Central | Metal | Bottle Trap | 14.8 |
| **EF.E01.EDM34** | Edith Morley | Male | Second | Teaching | Central | Metal | Bottle Trap | 19.4 |
| **EF.E01.EDM36** | Edith Morley | Male | Second | Teaching | Central | Metal | Bottle Trap | 15.7 |
| **EF.E01.EDM38** | Edith Morley | Male | Second | Teaching | Central | Metal | Bottle Trap | 7.8 |
| **EF.E01.EDM41** | Edith Morley | Female | Second | Teaching | Central | Metal | Bottle Trap | 18.1 |
| **EF.E01.EDM42** | Edith Morley | Female | Second | Teaching | Central | Metal | Bottle Trap | 15 |
| **EF.E01.EDM44** | Edith Morley | Female | Second | Teaching | Central | Metal | Bottle Trap | 11.7 |

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|---|---|---|---|---|---|---|---|---|
| **EF.E01.EDM52** | Edith Morley | Kitchen | Second | Teaching | Central | Plastic | S-Trap | 18.5 |
| **NJ.E01.AGR05** | Agriculture | Male | Ground | Teaching | East Side | Plastic | P-Trap | |
| **NJ.E01.AGR10** | Agriculture | Female | Ground | Teaching | East Side | Plastic | P-Trap | |
| **NJ.E01.AGR13** | Agriculture | Female | Ground | Teaching | East Side | Plastic | P-Trap | 26.4 |
| **NJ.E01.AGR15** | Agriculture | Neutral | Ground | Teaching | East Side | Plastic | Bottle Trap | 24.6 |
| **NJ.E01.AGR16** | Agriculture | Neutral | Ground | Teaching | East Side | Plastic | Bottle Trap | 20 |
| **NJ.E01.AGR17** | Agriculture | Male | First | Teaching | East Side | Plastic | P-Trap | 32 |
| **NJ.E01.RC06** | Russell & Chancellors | Female | Ground | Teaching | West Side | Plastic | Uknown | 18.9 |
| **NJ.E01.RC07** | Russell & Chancellors | Female | Ground | Teaching | West Side | Plastic | Uknown | 18.9 |
| **NJ.E01.RC08** | Russell & Chancellors | Female | Ground | Teaching | West Side | Plastic | Uknown | 18.9 |
| **NJ.E01.RC10** | Russell & Chancellors | Female | Ground | Teaching | West Side | Plastic | Uknown | 18.9 |
| **NJ.E01.RC11** | Russell & Chancellors | Neutral | Ground | Teaching | West Side | Plastic | Bottle Trap | 16 |
| **NJ.E01.MIN01** | Mingella | Male | Ground | Theather | Central | Unknown | Bottle Trap | 17.1 |
| **NJ.E01.MIN03** | Mingella | Neutral | First | Theather | Central | Plastic | Bottle Trap | 17.8 |
| **NJ.E01.MIN05** | Mingella | Female | Ground | Theather | Central | Unknown | Uknown | 17.2 |
| **NJ.E01.MIN06** | Mingella | Female | Ground | Theather | Central | Unknown | Uknown | 17.2 |
| **NJ.E01.MIN07** | Mingella | Female | Ground | Theather | Central | Unknown | Uknown | 17.2 |
| **NJ.E01.MIN09** | Mingella | Neutral | First | Theather | Central | Unknown | Uknown | 15.5 |
| **NJ.E01.MIN11** | Mingella | Neutral | First | Theather | Central | Unknown | Uknown | 18.2 |
| **NJ.E01.MIN12** | Mingella | Neutral | First | Theather | Central | Unknown | Uknown | 18.2 |
| **NJ.E01.MIN13** | Mingella | Neutral | Second | Theather | Central | Plastic | Bottle Trap | 19.8 |
| **NJ.E01.MIN14** | Mingella | Neutral | Ground | Theather | Central | Plastic | Bottle Trap | 14.6 |
| **NJ.E01.AGR18** | Agriculture | Male | First | Teaching | East Side | Plastic | P-Trap | 32 |
| **NJ.E01.AGR20** | Agriculture | Male | First | Teaching | East Side | Plastic | P-Trap | 32 |
| **NJ.E01.AGR22** | Agriculture | Female | First | Teaching | East Side | Plastic | P-Trap | 29.9 |
| **NJ.E01.AGR24** | Agriculture | Female | First | Teaching | East Side | Plastic | P-Trap | |
| **NJ.E01.AGR26** | Agriculture | Female | First | Teaching | East Side | Plastic | P-Trap | |
| **NJ.E01.AGR27** | Agriculture | Neutral | First | Teaching | East Side | Plastic | Bottle Trap | 27.3 |
| **NJ.E01.AGR32** | Agriculture | Neutral | Second | Teaching | East Side | Plastic | Bottle Trap | 24.6 |
| **NJ.E01.AGR35** | Agriculture | Female | Third | Teaching | East Side | Plastic | P-Trap | 20 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **NJ.E01.AGR38** | Agriculture | Kitchen | Third | Teaching | East Side | Plastic | S-Trap | 19.4 |
| **NJ.E01.AGR39** | Agriculture | Male | Fourth | Teaching | East Side | Plastic | P-Trap | 19.6 |
| **NJ.E01.AGR42** | Agriculture | Male | Fourth | Teaching | East Side | Plastic | P-Trap | 16.7 |
| **NJ.E01.AGR43** | Agriculture | Female | Fourth | Teaching | East Side | Plastic | P-Trap | 16.4 |
| **NJ.E01.AGR44** | Agriculture | Female | Fourth | Teaching | East Side | Plastic | P-Trap | 16.4 |
| **NJ.E01.AGR45** | Agriculture | Female | Fourth | Teaching | East Side | Plastic | P-Trap | 12.7 |
| **NJ.E01.AGR46** | Agriculture | Female | Fourth | Teaching | East Side | Plastic | P-Trap | 12.7 |
| **NJ.E01.AGR47** | Agriculture | Neutral | Fourth | Teaching | East Side | Plastic | Bottle Trap | 14.5 |
| **EN.E01.LIB01** | Library | Neutral | First | Study | Central | Metal | Bottle Trap | 16.9 |
| **AA.E01.ART01** | Art | Male | Ground | Teaching | East Side | Plastic | S-Trap | 15.8 |
| **AA.E01.ART02** | Art | Male | Ground | Teaching | East Side | Plastic | S-Trap | 10.2 |
| **AA.E01.ART03** | Art | Male | Ground | Teaching | East Side | Plastic | S-Trap | 10.2 |
| **AA.E01.ART04** | Art | Male | Ground | Teaching | East Side | Plastic | S-Trap | 6.5 |
| **AA.E01.ART05** | Art | Male | Ground | Teaching | East Side | Plastic | S-Trap | 6.5 |
| **AA.E01.ART06** | Art | Male | Ground | Teaching | East Side | Plastic | Bottle Trap | 6 |
| **AA.E01.ART07** | Art | Male | Ground | Teaching | East Side | Metal | S-Trap | 6 |
| **AA.E01.ART08** | Art | Female | Ground | Teaching | East Side | Plastic | S-Trap | 5 |
| **AA.E01.ART09** | Art | Female | Ground | Teaching | East Side | Plastic | Bottle Trap | 16.4 |
| **AA.E01.ART10** | Art | Female | Ground | Teaching | East Side | Plastic | Bottle Trap | 16.4 |
| **AA.E01.ART13** | Art | Female | Ground | Teaching | East Side | Plastic | Bottle Trap | 5 |
| **AA.E01.ART14** | Art | Female | Ground | Teaching | East Side | Plastic | Bottle Trap | 5 |
| **AA.E01.ART15** | Art | Female | Ground | Teaching | East Side | Plastic | Bottle Trap | 5 |
| **AA.E01.ART16** | Art | Female | Ground | Teaching | East Side | Plastic | Bottle Trap | 5 |
| **AA.E01.ART17** | Art | Female | Ground | Teaching | East Side | Plastic | Bottle Trap | 5 |
| **AA.E01.ART18** | Art | Neutral | Ground | Teaching | East Side | Plastic | Bottle Trap | 11.4 |
| **AA.E01.PAH13** | Park House | Female | First | Recreational | Central | Plastic | S-Trap | 14.7 |
| **AA.E01.PAH14** | Park House | Kitchen | First | Recreational | Central | Plastic | S-Trap | 14.1 |
| **AA.E01.PAH1** | Park House | Male | Ground | Recreational | Central | Plastic | Bottle Trap | 12 |
| **AA.E01.PAH2** | Park House | Male | Ground | Recreational | Central | Plastic | Bottle Trap | 12 |
| **AA.E01.PAH3** | Park House | Male | Ground | Recreational | Central | Plastic | Bottle Trap | 12 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **AA.E01.PAH4** | Park House | Male | Ground | Recreational | Central | Plastic | Bottle Trap | 12 |
| **AA.E01.PAH5** | Park House | Female | Ground | Recreational | Central | Plastic | Bottle Trap | 11.5 |
| **AA.E01.PAH6** | Park House | Female | Ground | Recreational | Central | Plastic | Bottle Trap | 11.5 |
| **AA.E01.PAH7** | Park House | Female | Ground | Recreational | Central | Plastic | Bottle Trap | 11.5 |
| **AA.E01.PAH8** | Park House | Female | Ground | Recreational | Central | Plastic | Bottle Trap | 11.5 |
| **AA.E01.PAH9** | Park House | Female | Ground | Recreational | Central | Plastic | Bottle Trap | 11.5 |
| **AA.E01.PAH10** | Park House | Neutral | Ground | Recreational | Central | Plastic | Bottle Trap | 16.8 |
| **AA.E01.PAH11** | Park House | Male | First | Recreational | Central | Plastic | S-Trap | 13.8 |
| **AA.E01.PAH12** | Park House | Female | First | Recreational | Central | Plastic | S-Trap | 14.7 |
| **AA.E01.ARC01** | Archaeology | Male | Ground | Teaching | South Central | Plastic | Bottle Trap | 17.6 |
| **AA.E01.ARC02** | Archaeology | Male | Ground | Teaching | South Central | Plastic | Bottle Trap | 17.6 |
| **AA.E01.ARC03** | Archaeology | Male | Ground | Teaching | South Central | Plastic | Bottle Trap | 17.6 |
| **AA.E01.ARC04** | Archaeology | Female | Ground | Teaching | South Central | Plastic | Bottle Trap | 15.4 |
| **AA.E01.ARC05** | Archaeology | Female | Ground | Teaching | South Central | Plastic | Bottle Trap | 15.4 |
| **AA.E01.ARC06** | Archaeology | Female | Ground | Teaching | South Central | Plastic | Bottle Trap | 15.4 |
| **AA.E01.ARC07** | Archaeology | Male | First | Teaching | South Central | Plastic | Bottle Trap | 14.2 |
| **AA.E01.ARC08** | Archaeology | Male | First | Teaching | South Central | Plastic | Bottle Trap | 14.2 |
| **AA.E01.ARC09** | Archaeology | Female | First | Teaching | South Central | Plastic | Bottle Trap | 19.2 |
| **AA.E01.ARC10** | Archaeology | Female | First | Teaching | South Central | Plastic | Bottle Trap | 19.2 |
| **AA.E01.ARC11** | Archaeology | Female | First | Teaching | South Central | Plastic | Uknown | 7.8 |
| **AA.E01.ARC13** | Archaeology | Kitchen | First | Teaching | South Central | Plastic | S-Trap | 11.3 |
| **AA.E01.ARC14** | Archaeology | Kitchen | First | Teaching | South Central | Plastic | S-Trap | 13 |
| **AA.E01.ARC15** | Archaeology | Female | First | Teaching | South Central | Unknown | Uknown | 7.8 |
| **AA.E01.ARC16** | Archaeology | Female | First | Teaching | South Central | Unknown | Uknown | 7.8 |
| **AA.E01.ART19** | Art | Female | Ground | Teaching | East Side | Plastic | S-Trap | 5 |
| **AA.E01.SPK01** | Sports Park | Male | Ground | Recreational | West Side | Unknown | Bottle Trap | 16.5 |
| **AA.E01.SPK02** | Sports Park | Male | Ground | Recreational | West Side | Unknown | Bottle Trap | 16.5 |
| **AA.E01.SPK03** | Sports Park | Male | Ground | Recreational | West Side | Unknown | Bottle Trap | 16.5 |
| **AA.E01.SPK04** | Sports Park | Male | Ground | Recreational | West Side | Unknown | Bottle Trap | 16.5 |
| **AA.E01.SPK05** | Sports Park | Male | Ground | Recreational | West Side | Unknown | Bottle Trap | 16.5 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **AA.E01.SPK06** | Sports Park | Male | Ground | Recreational | West Side | Unknown | Bottle Trap | 16.5 |
| **AA.E01.SPK07** | Sports Park | Male | Ground | Recreational | West Side | Unknown | Bottle Trap | 16.5 |
| **AA.E01.SPK08** | Sports Park | Male | Ground | Recreational | West Side | Unknown | Bottle Trap | 16.5 |
| **AA.E01.SPK09** | Sports Park | Female | Ground | Recreational | West Side | Unknown | Bottle Trap | 18.7 |
| **AA.E01.SPK10** | Sports Park | Female | Ground | Recreational | West Side | Unknown | Bottle Trap | 18.7 |
| **AA.E01.SPK11** | Sports Park | Female | Ground | Recreational | West Side | Plastic | Bottle Trap | 16.5 |
| **AA.E01.SPK12** | Sports Park | Female | Ground | Recreational | West Side | Plastic | Bottle Trap | 16.5 |
| **AA.E01.SPK13** | Sports Park | Neutral | Ground | Recreational | West Side | Plastic | Bottle Trap | 17 |
| **AA.E01.SPK14** | Sports Park | Neutral | Ground | Recreational | West Side | Plastic | Bottle Trap | 17 |
| **AA.E01.SPK15** | Sports Park | Neutral | Ground | Recreational | West Side | Plastic | Bottle Trap | 16.3 |
| **EN.E01.POV03** | Polly Vacher | Male | Ground | Teaching | South Central | Plastic | P-Trap | 14.5 |
| **EN.E01.POV05** | Polly Vacher | Male | Ground | Teaching | South Central | Plastic | P-Trap | 13.3 |
| **EN.E01.POV07** | Polly Vacher | Male | Ground | Teaching | South Central | Plastic | P-Trap | 13.3 |
| **EN.E01.LIB02** | Library | Neutral | First | Study | Central | Metal | Bottle Trap | 16.9 |
| **EN.E01.LIB04** | Library | Neutral | Second | Study | Central | Metal | Bottle Trap | 21.2 |
| **EN.E01.LIB05** | Library | Neutral | Third | Study | Central | Metal | Bottle Trap | 22.5 |
| **EN.E01.LIB06** | Library | Neutral | Third | Study | Central | Metal | Bottle Trap | 22.5 |
| **EN.E01.LIB07** | Library | Neutral | Fourth | Study | Central | Metal | Bottle Trap | 22.2 |
| **EN.E01.LIB09** | Library | Neutral | Fifth | Study | Central | Plastic | Bottle Trap | 24.8 |
| **EN.E01.LIB10** | Library | Male | Fifth | Study | Central | Metal | Bottle Trap | 25 |
| **EN.E01.WHI24** | Whiteknights | Female | Third | Office | Central | Plastic | Bottle Trap | 17.5 |
| **EN.E01.WHI20** | Whiteknights | Neutral | Ground | Office | Central | Plastic | Bottle Trap | 18.4 |
| **EN.E01.WHI19** | Whiteknights | Female | Second | Office | Central | Metal | Bottle Trap | 17.3 |
| **EN.E01.WHI17** | Whiteknights | Female | Second | Office | Central | Metal | Bottle Trap | 17.3 |
| **EN.E01.WHI16** | Whiteknights | Female | Second | Office | Central | Metal | Bottle Trap | 14.4 |
| **EN.E01.HBS01** | Henley Business School | Male | Ground | Teaching | Central | Plastic | Bottle Trap | 16.4 |
| **EN.E01.HBS02** | Henley Business School | Male | Ground | Teaching | Central | Plastic | Bottle Trap | |
| **EN.E01.HBS03** | Henley Business School | Male | Ground | Teaching | Central | Plastic | Bottle Trap | |
| **EN.E01.HBS04** | Henley Business School | Male | Ground | Teaching | Central | Plastic | Bottle Trap | |
| **EN.E01.HBS05** | Henley Business School | Male | Ground | Teaching | Central | Plastic | Bottle Trap | 16.4 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **EN.E01.HBS06** | Henley Business School | Male | Ground | Teaching | Central | Plastic | P-Trap | 24.9 |
| **EN.E01.HBS08** | Henley Business School | Female | Ground | Teaching | Central | Plastic | Bottle Trap | 18 |
| **EN.E01.HBS09** | Henley Business School | Female | Ground | Teaching | Central | Plastic | Bottle Trap | 18 |
| **EN.E01.HBS10** | Henley Business School | Female | Ground | Teaching | Central | Plastic | Bottle Trap | 18 |
| **EN.E01.HBS12** | Henley Business School | Female | Ground | Teaching | Central | Plastic | Bottle Trap | 18 |
| **EN.E01.HBS13** | Henley Business School | Female | Ground | Teaching | Central | Plastic | Bottle Trap | 18 |
| **EN.E01.HBS14** | Henley Business School | Female | Ground | Teaching | Central | Plastic | Bottle Trap | 18 |
| **EN.E01.HBS15** | Henley Business School | Female | Ground | Teaching | Central | Plastic | P-Trap | 24.4 |
| **EN.E01.HBS18** | Henley Business School | Neutral | Ground | Teaching | Central | Plastic | Bottle Trap | 21.4 |
| **EN.E01.HBS19** | Henley Business School | Neutral | Ground | Teaching | Central | Plastic | Bottle Trap | 21.4 |
| **EN.E01.HBS17** | Henley Business School | Neutral | Ground | Teaching | Central | Plastic | Bottle Trap | 21.4 |
| **EN.E01.HBS26** | Henley Business School | Female | First | Teaching | Central | Plastic | Bottle Trap | 18.1 |
| **EN.E01.HBS27** | Henley Business School | Female | First | Teaching | Central | Plastic | Bottle Trap | 18.1 |
| **EN.E01.HBS29** | Henley Business School | Male | Second | Teaching | Central | Plastic | Bottle Trap | 20 |
| **EN.E01.HBB0** | Henley Business School | Male | Second | Teaching | Central | Plastic | Bottle Trap | 20 |
| **EN.E01.HBB1** | Henley Business School | Male | Second | Teaching | Central | Plastic | Bottle Trap | 20 |
| **EN.E01.HBB6** | Henley Business School | Neutral | Second | Teaching | Central | Metal | Bottle Trap | 17.9 |
| **EN.E01.HBB7** | Henley Business School | Kitchen | Ground | Teaching | Central | Plastic | Bottle Trap | 24.3 |
| **EN.E01.HBB8** | Henley Business School | Kitchen | First | Teaching | Central | Plastic | S-Trap | 25 |
| **EF.E01.URS12** | URS Building | Female | Ground | Teaching | Central | Plastic | Bottle Trap | 7.7 |
| **EF.E01.URS13** | URS Building | Female | Second | Teaching | Central | Plastic | Bottle Trap | 8.8 |
| **EF.E01.URS14** | URS Building | Female | Second | Teaching | Central | Plastic | Bottle Trap | 8.8 |
| **EF.E01.URS15** | URS Building | Female | Second | Teaching | Central | Plastic | Bottle Trap | 8.8 |
| **EF.E01.URS16** | URS Building | Female | Second | Teaching | Central | Plastic | Bottle Trap | 8.8 |
| **EF.E01.EDM11** | Edith Morley | Female | Ground | Teaching | Central | Metal | Bottle Trap | 14.2 |
| **EF.E01.EDM13** | Edith Morley | Neutral | Ground | Teaching | Central | Metal | Bottle Trap | 16.9 |
| **EF.E01.EDM14** | Edith Morley | Neutral | Ground | Teaching | Central | Metal | Bottle Trap | 16.9 |
| **EF.E01.EDM15** | Edith Morley | Neutral | Ground | Teaching | Central | Metal | Bottle Trap | 16.9 |
| **EF.E01.EDM16** | Edith Morley | Neutral | Ground | Teaching | Central | Metal | Bottle Trap | 16.9 |
| **EF.E01.EDM17** | Edith Morley | Neutral | Ground | Teaching | Central | Metal | Bottle Trap | 16.9 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **EF.E01.EDM19** | Edith Morley | Male | First | Teaching | Central | Metal | Bottle Trap | 14.7 |
| **EF.E01.EDM21** | Edith Morley | Male | First | Teaching | Central | Metal | Bottle Trap | 17.9 |
| **EF.E01.EDM22** | Edith Morley | Male | First | Teaching | Central | Metal | Bottle Trap | 17.9 |
| **EF.E01.EDM23** | Edith Morley | Male | First | Teaching | Central | Metal | Bottle Trap | 17.2 |
| **EF.E01.EDM24** | Edith Morley | Male | First | Teaching | Central | Metal | Bottle Trap | 17.2 |
| **EF.E01.EDM25** | Edith Morley | Female | First | Teaching | Central | Metal | Bottle Trap | 14.8 |
| **EF.E01.EDM28** | Edith Morley | Female | First | Teaching | Central | Metal | Bottle Trap | 15.5 |
| **EF.E01.EDM29** | Edith Morley | Female | First | Teaching | Central | Metal | Bottle Trap | 16.9 |
| **EF.E01.EDM30** | Edith Morley | Female | First | Teaching | Central | Metal | Bottle Trap | 16.9 |
| **EF.E01.EDM32** | Edith Morley | Neutral | First | Teaching | Central | Metal | Bottle Trap | 14.2 |
| **EF.E01.EDM33** | Edith Morley | Kitchen | First | Teaching | Central | Metal | Bottle Trap | 18.9 |
| **EF.E01.EDM35** | Edith Morley | Male | Second | Teaching | Central | Metal | Bottle Trap | 19.4 |
| **EF.E01.EDM37** | Edith Morley | Male | Second | Teaching | Central | Metal | Bottle Trap | 15.7 |
| **EF.E01.EDM39** | Edith Morley | Male | Second | Teaching | Central | Metal | Bottle Trap | 7.8 |
| **EF.E01.EDM40** | Edith Morley | Female | Second | Teaching | Central | Metal | Bottle Trap | 18.1 |
| **EF.E01.EDM43** | Edith Morley | Female | Second | Teaching | Central | Metal | Bottle Trap | 15 |
| **EF.E01.EDM46** | Edith Morley | Neutral | Second | Teaching | Central | Metal | Bottle Trap | 16.3 |
| **EF.E01.EDM47** | Edith Morley | Male | Third | Teaching | Central | Metal | Bottle Trap | 13.9 |
| **EF.E01.EDM49** | Edith Morley | Female | Third | Teaching | Central | Metal | Bottle Trap | 15.7 |
| **EF.E01.EDM50** | Edith Morley | Female | Third | Teaching | Central | Metal | Bottle Trap | 15.7 |
| **EF.E01.EDM51** | Edith Morley | Male | Fourth | Teaching | Central | Metal | Bottle Trap | 11.8 |
| **EF.E01.EDM54** | Edith Morley | Female | Fourth | Teaching | Central | Metal | Bottle Trap | 13.6 |
| **EF.E01.EDM55** | Edith Morley | Neutral | Ground | Teaching | Central | Metal | Bottle Trap | 16.1 |
| **EF.E01.EDM56** | Edith Morley | Neutral | Ground | Teaching | Central | Metal | Bottle Trap | 16.1 |
| **ND.E01.CHEM01** | Chemistry | Male | Second | Teaching | South Central | Plastic | Uknown | 8.4 |
| **ND.E01.CHEM02** | Chemistry | Male | Second | Teaching | South Central | Plastic | Uknown | 8.4 |
| **ND.E01.CHEM03** | Chemistry | Male | Second | Teaching | South Central | Plastic | Uknown | 8.4 |
| **ND.E01.CHEM04** | Chemistry | Male | Second | Teaching | South Central | Plastic | Uknown | 8.4 |
| **ND.E01.CHEM05** | Chemistry | Male | Second | Teaching | South Central | Plastic | Uknown | 8.4 |
| **ND.E01.CHEM06** | Chemistry | Female | Ground | Teaching | South Central | Plastic | S-Trap | 4.2 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **ND.E01.CHEM07** | Chemistry | Female | Ground | Teaching | South Central | Plastic | S-Trap | 4.2 |
| **ND.E01.CHEM08** | Chemistry | Female | Ground | Teaching | South Central | Plastic | S-Trap | 4.2 |
| **ND.E01.CHEM09** | Chemistry | Neutral | Second | Teaching | South Central | Plastic | Uknown | 12.5 |
| **ND.E01.CHEM10** | Chemistry | Neutral | Second | Teaching | South Central | Plastic | Uknown | 12.5 |
| **ND.E01.CHEM11** | Chemistry | Neutral | Ground | Teaching | South Central | Plastic | Bottle Trap | 19 |
| **ND.E01.CHEM13** | Chemistry | Female | First | Teaching | South Central | Plastic | Uknown | 18.7 |
| **ND.E01.CHEM14** | Chemistry | Female | First | Teaching | South Central | Plastic | Uknown | 18.7 |
| **ND.E01.CHEM15** | Chemistry | Female | First | Teaching | South Central | Plastic | Uknown | 18.7 |
| **ND.E01.CHEM16** | Chemistry | Female | First | Teaching | South Central | Plastic | Uknown | 18.7 |
| **ND.E01.CHEM17** | Chemistry | Female | First | Teaching | South Central | Plastic | Uknown | 18.7 |
| **ND.E01.CHEM18** | Chemistry | Male | Third | Teaching | South Central | Plastic | Uknown | 13.5 |
| **ND.E01.CHEM19** | Chemistry | Male | Third | Teaching | South Central | Plastic | Uknown | 13.5 |
| **ND.E01.CHEM20** | Chemistry | Male | Third | Teaching | South Central | Plastic | Uknown | 13.5 |
| **ND.E01.CHEM21** | Chemistry | Male | Third | Teaching | South Central | Plastic | Uknown | 13.5 |
| **ND.E01.CHEM22** | Chemistry | Male | Third | Teaching | South Central | Plastic | Uknown | 13.5 |
| **ND.E01.CHEM23** | Chemistry | Neutral | Second | Teaching | South Central | Plastic | Uknown | 12.5 |
| **ND.E01.CHEM24** | Chemistry | Neutral | Second | Teaching | South Central | Plastic | Uknown | 12.5 |
| **ND.E01.CHEM25** | Chemistry | Neutral | Second | Teaching | South Central | Plastic | Uknown | 12.5 |
| **AA.E01.JJT01** | JJ Thompson | Male | Ground | Teaching | Central | Unknown | Bottle Trap | 18.5 |
| **AA.E01.JJT02** | JJ Thompson | Male | Ground | Teaching | Central | Unknown | Bottle Trap | 18.5 |
| **AA.E01.JJT03** | JJ Thompson | Female | Ground | Teaching | Central | Unknown | Bottle Trap | 18.2 |
| **AA.E01.JJT04** | JJ Thompson | Female | Ground | Teaching | Central | Unknown | Bottle Trap | 18.2 |
| **AA.E01.JJT05** | JJ Thompson | Female | Ground | Teaching | Central | Unknown | Bottle Trap | 18.2 |
| **AA.E01.JJT06** | JJ Thompson | Neutral | Ground | Teaching | Central | Plastic | Bottle Trap | 18.9 |
| **AA.E01.JJT08** | JJ Thompson | Neutral | First | Teaching | Central | Unknown | Bottle Trap | 17.7 |
| **AA.E01.JJT09** | JJ Thompson | Male | Second | Teaching | Central | Unknown | Bottle Trap | 21.2 |
| **AA.E01.JJT10** | JJ Thompson | Male | Second | Teaching | Central | Unknown | Bottle Trap | 21.2 |
| **AA.E01.JJT11** | JJ Thompson | Female | Second | Teaching | Central | Unknown | Bottle Trap | 19.8 |
| **AA.E01.JJT12** | JJ Thompson | Kitchen | Second | Teaching | Central | Plastic | Bottle Trap | 22.1 |
| **AA.E01.JJT13** | JJ Thompson | Neutral | Third | Teaching | Central | Plastic | Bottle Trap | 22.7 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **AA.E01.JJT14** | JJ Thompson | Neutral | Third | Teaching | Central | Plastic | Bottle Trap | 22.7 |
| **AA.E01.JJT15** | JJ Thompson | Neutral | Third | Teaching | Central | Plastic | Bottle Trap | 22.7 |
| **NJ.E01.AGR06** | Agriculture | Male | Ground | Teaching | East Side | Plastic | P-Trap | 22.9 |
| **NJ.E01.AGR07** | Agriculture | Male | Ground | Teaching | East Side | Plastic | P-Trap | 22.9 |
| **NJ.E01.AGR08** | Agriculture | Female | Ground | Teaching | East Side | Plastic | P-Trap | 27.4 |
| **NJ.E01.AGR09** | Agriculture | Female | Ground | Teaching | East Side | Plastic | P-Trap | |
| **NJ.E01.AGR11** | Agriculture | Female | Ground | Teaching | East Side | Plastic | P-Trap | |
| **NJ.E01.AGR12** | Agriculture | Female | Ground | Teaching | East Side | Plastic | P-Trap | 26.4 |
| **NJ.E01.AGR14** | Agriculture | Female | Ground | Teaching | East Side | Plastic | P-Trap | 24.6 |
| **NJ.E01.AGR19** | Agriculture | Male | First | Teaching | East Side | Plastic | P-Trap | 32 |
| **NJ.E01.AGR21** | Agriculture | Male | First | Teaching | East Side | Plastic | P-Trap | 32 |
| **NJ.E01.AGR23** | Agriculture | Female | First | Teaching | East Side | Plastic | P-Trap | |
| **NJ.E01.AGR25** | Agriculture | Female | First | Teaching | East Side | Plastic | P-Trap | |
| **NJ.E01.AGR28** | Agriculture | Male | Second | Teaching | East Side | Plastic | P-Trap | 23 |
| **NJ.E01.AGR30** | Agriculture | Female | Second | Teaching | East Side | Unknown | Uknown | 22.7 |
| **NJ.E01.AGR31** | Agriculture | Female | Second | Teaching | East Side | Unknown | Uknown | 22.7 |
| **NJ.E01.AGR33** | Agriculture | Male | Third | Teaching | East Side | Plastic | P-Trap | 19 |
| **NJ.E01.AGR34** | Agriculture | Male | Third | Teaching | East Side | Plastic | P-Trap | 19 |
| **NJ.E01.AGR36** | Agriculture | Female | Third | Teaching | East Side | Plastic | P-Trap | 20 |
| **NJ.E01.AGR37** | Agriculture | Neutral | Third | Teaching | East Side | Plastic | Bottle Trap | |
| **NJ.E01.AGR41** | Agriculture | Male | Fourth | Teaching | East Side | Plastic | P-Trap | 16.7 |
| **NJ.E01.AGR48** | Agriculture | Neutral | Fourth | Teaching | East Side | Plastic | Bottle Trap | 13.4 |
| **EN.E01.WHI05** | Whiteknights | Female | Ground | Office | Central | Metal | Bottle Trap | 14.8 |
| **EN.E01.WHI06** | Whiteknights | Kitchen | Ground | Office | Central | Plastic | S-Trap | 5.7 |
| **EN.E01.WHI07** | Whiteknights | Male | First | Office | Central | Metal | Bottle Trap | 19 |
| **EN.E01.WHI08** | Whiteknights | Male | First | Office | Central | Plastic | S-Trap | 19 |
| **EN.E01.WHI09** | Whiteknights | Female | First | Office | Central | Plastic | Bottle Trap | 21.2 |
| **EN.E01.WHI10** | Whiteknights | Female | First | Office | Central | Plastic | Bottle Trap | 19.2 |
| **EN.E01.WHI11** | Whiteknights | Female | First | Office | Central | Plastic | Bottle Trap | 19.2 |
| **EN.E01.WHI12** | Whiteknights | Male | Second | Office | Central | Metal | Bottle Trap | 14.5 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **EN.E01.HBS07** | Henley Business School | Male | Ground | Teaching | Central | Plastic | P-Trap | 24.9 |
| **EN.E01.HBS11** | Henley Business School | Female | Ground | Teaching | Central | Plastic | Bottle Trap | 18 |
| **EN.E01.HBS16** | Henley Business School | Female | Ground | Teaching | Central | Plastic | P-Trap | 24.5 |
| **EN.E01.HBS20** | Henley Business School | Neutral | Ground | Teaching | Central | Metal | Bottle Trap | 20.8 |
| **EN.E01.HBS21** | Henley Business School | Male | First | Teaching | Central | Plastic | P-Trap | 24.3 |
| **EN.E01.HBS22** | Henley Business School | Male | First | Teaching | Central | Plastic | P-Trap | 24.3 |
| **EN.E01.HBS23** | Henley Business School | Female | First | Teaching | Central | Plastic | P-Trap | 26.2 |
| **EN.E01.HBS24** | Henley Business School | Female | First | Teaching | Central | Plastic | P-Trap | 26.2 |
| **EN.E01.HBS28** | Henley Business School | Neutral | First | Teaching | Central | Metal | Bottle Trap | 20.2 |
| **EN.E01.HBB2** | Henley Business School | Male | Second | Teaching | Central | Plastic | P-Trap | 13.9 |
| **EN.E01.HBB3** | Henley Business School | Male | Second | Teaching | Central | Plastic | P-Trap | 13.9 |
| **EN.E01.HBB4** | Henley Business School | Female | Second | Teaching | Central | Plastic | P-Trap | 17.9 |
| **EN.E01.HBB5** | Henley Business School | Female | Second | Teaching | Central | Plastic | P-Trap | 17.9 |
| **EN.E01.HBB9** | Henley Business School | Kitchen | First | Teaching | Central | Plastic | Bottle Trap | 19 |

**Table B.1.** Table of sample metadata.

| Building name | Total number of samples collected | Number of samples that did not amplify | Amplification success percentage |
|---|---|---|---|
| **Whiteknights** | 24 | 11 | 54.17 |
| **Library** | 13 | 5 | 61.54 |
| **Henley Business School** | 38 | 0 | 100.00 |
| **Polly Vacher** | 13 | 10 | 23.08 |
| **Sports Park** | 15 | 0 | 100.00 |
| **Park House** | 14 | 0 | 100.00 |
| **JJ Thompson** | 15 | 1 | 93.33 |
| **Archeology** | 16 | 1 | 93.75 |
| **Art** | 19 | 2 | 89.47 |
| **Math** | 12 | 1 | 91.67 |
| **Edith Morley** | 51 | 2 | 96.08 |
| **URS** | 14 | 2 | 85.71 |
| **Miller** | 8 | 3 | 62.50 |
| **Mingella** | 14 | 4 | 71.43 |
| **Agriculture** | 46 | 4 | 91.30 |
| **Russell & Chancellors** | 24 | 19 | 20.83 |
| **Student Union** | 28 | 3 | 89.29 |
| **Eat at the Square** | 12 | 0 | 100.00 |
| **Chemistry** | 25 | 1 | 96.00 |
| **Harry Nursten** | 11 | 0 | 100.00 |
| **Total** | **412** | **69** | **83.25** |

**Table B.2.** Number of samples successfully amplified ITS2 region and purified using Agencourt AMPure XP magnetic beads (Beckman Coulter).

Please use the link for Supplementary Table B: https://doi.org/10.1002/edn3.375. The csv file contains over 2000 rows of OTUs therefore is too large to include in the appendix.

**Table B.3**. OTU table with associated taxonomy.

*A. Building*

| | AGR | ARC | ART | CHE | EAT | EDM | HNU | HBS | JJT | LIB | MAT | MIN | PAH | RSP | STU | URS | WHK |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Exophiala** | 17.17 | 28.69 | 28.82 | 18.63 | 29.40 | 19.54 | 19.04 | 20.19 | 16.93 | 19.10 | 9.53 | 15.69 | 17.43 | 10.98 | 17.01 | 17.11 | 22.21 |
| **Saccharomyces** | 6.88 | 4.63 | 4.83 | 10.68 | 23.21 | 13.15 | 22.25 | 7.51 | 5.06 | 4.11 | 22.67 | 4.12 | 3.20 | 3.95 | 25.01 | 13.32 | 6.85 |
| **Fusarium** | 4.49 | 1.59 | 6.01 | 4.77 | 5.09 | 4.45 | 4.79 | 4.51 | 2.92 | 3.82 | 6.20 | 2.31 | 9.46 | 4.23 | 13.22 | 5.53 | 5.81 |
| **Cyphellophora** | 4.15 | 4.54 | 2.60 | 5.53 | 0.35 | 3.11 | 0.12 | 5.28 | 5.37 | 3.17 | 0.01 | 5.76 | 0.40 | 5.27 | 0.13 | 4.01 | 6.33 |
| **Malassezia** | 1.95 | 2.04 | 3.30 | 1.33 | 7.21 | 2.53 | 8.70 | 1.09 | 0.76 | 0.39 | 7.33 | 3.45 | 2.54 | 1.99 | 3.23 | 6.20 | 1.21 |
| **Bisifusarium** | 3.07 | 0.10 | 2.22 | 1.66 | 0.57 | 1.87 | 0.49 | 0.89 | 0.93 | 0.44 | 5.43 | 0.28 | 2.16 | 0.06 | 0.95 | 0.58 | 0.69 |
| **Ramularia** | 2.25 | 3.08 | 3.65 | 0.57 | 1.29 | 0.43 | 0.37 | 0.66 | 0.49 | 0.52 | 0.34 | 3.14 | 3.84 | 3.24 | 0.31 | 0.29 | 0.50 |

*B. Gender*

| | Female | Kitchen | Male | Neutral |
|---|---|---|---|---|
| **Exophiala** | 22.29 | 18.95 | 16.92 | 16.93 |
| **Saccharomyces** | 11.37 | 7.97 | 10.78 | 10.60 |
| **Fusarium** | 4.99 | 3.41 | 6.64 | 4.12 |
| **Cyphellophora** | 3.60 | 2.40 | 3.29 | 3.44 |
| **Malassezia** | 2.37 | 1.20 | 3.75 | 2.65 |
| **Bisifusarium** | 1.72 | 0.45 | 1.67 | 0.88 |
| **Ramularia** | 1.29 | 1.65 | 1.18 | 1.78 |

**Table B.4.** Average relative abundance (RA) of top classified genera by A) Building and B) Gender of restroom from which sample was taken. Some samples were collected from kitchens, so this was included as an additional group under gender.

*A. Bray Curtis*

| Building Comparison | | | P value | Significance |
|---|---|---|---|---|
| Student Union | vs | Henley Business School | 2.77E-06 | **** |
| Henley Business School | vs | Art | 9.86E-06 | **** |
| Sports Park | vs | Henley Business School | 1.59E-05 | **** |
| Henley Business School | vs | Archaeology | 5.74E-05 | **** |
| Henley Business School | vs | Agriculture | 0.000309 | *** |
| Park House | vs | Henley Business School | 0.000313 | *** |
| Student Union | vs | Chemistry | 0.002079 | ** |
| Sports Park | vs | JJ Thompson | 0.002929 | ** |
| Student Union | vs | JJ Thompson | 0.003112 | ** |
| Sports Park | vs | Chemistry | 0.003182 | ** |
| Chemistry | vs | Art | 0.003489 | ** |
| Mingella | vs | Henley Business School | 0.003587 | ** |
| JJ Thompson | vs | Art | 0.003851 | ** |
| JJ Thompson | vs | Archaeology | 0.007806 | ** |
| Chemistry | vs | Archaeology | 0.009563 | ** |
| Whiteknights | vs | Sports Park | 0.009598 | ** |
| Henley Business School | vs | Harry Nunsten | 0.010558 | ** |
| Whiteknights | vs | Student Union | 0.011923 | ** |
| Henley Business School | vs | Eat at the Square | 0.012574 | ** |
| Park House | vs | JJ Thompson | 0.012791 | ** |
| Whiteknights | vs | Art | 0.01334 | ** |
| Park House | vs | Chemistry | 0.020586 | * |
| Maths | vs | Henley Business School | 0.021488 | * |
| Whiteknights | vs | Archaeology | 0.023459 | * |
| Whiteknights | vs | Park House | 0.03282 | * |
| Student Union | vs | Edith Morley | 0.033004 | * |
| Sports Park | vs | Edith Morley | 0.038545 | * |
| Edith Morley | vs | Art | 0.046949 | * |

*A. Jaccard*

| Building Comparisons | | | P values | Significance |
|---|---|---|---|---|
| Student Union | vs | Henley Business School | 0.001212 | *** |
| Henley Business School | vs | Art | 0.001461 | *** |
| Henley Business School | vs | Archaeology | 0.001476 | *** |
| JJ Thompson | vs | Archaeology | 0.001747 | ** |
| JJ Thompson | vs | Art | 0.002099 | ** |
| Sports Park | vs | Henley Business School | 0.002141 | ** |
| Sports Park | vs | JJ Thompson | 0.002204 | ** |
| Student Union | vs | JJ Thompson | 0.002361 | ** |
| Park House | vs | JJ Thompson | 0.003432 | ** |
| Park House | vs | Henley Business School | 0.004684 | ** |
| Henley Business School | vs | Agriculture | 0.008715 | ** |
| JJ Thompson | vs | Agriculture | 0.013638 | ** |
| Chemistry | vs | Archaeology | 0.021354 | * |
| Chemistry | vs | Art | 0.025149 | * |
| Sports Park | vs | Chemistry | 0.026921 | * |

| | | | | |
|---|---|---|---|---|
| Student Union | vs | Chemistry | 0.027229 | * |
| Maths | vs | JJ Thompson | 0.029625 | * |
| Mingella | vs | JJ Thompson | 0.032579 | * |
| Park House | vs | Chemistry | 0.040516 | * |
| Edith Morley | vs | Archaeology | 0.050522 | * |
| Maths | vs | Henley Business School | 0.051766 | * |

**Table B.5.** Post hoc Tukey test results. A) Using Bray-Curtis dissimilarity matrix, B) Jaccard. Pairs of buildings shown in tables are only those with significant differences observed. Stars indicate the p-value significance * $p < 0.05$ , ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

*A. Observed*

| Building Comparison | | | p-value | p.adj | Significance |
|---|---|---|---|---|---|
| Archaeology | vs | Chemistry | 0.000335 | 0.046 | * |
| Archaeology | vs | Henley Business School | 6.61E-05 | 0.009 | ** |
| Art | vs | Chemistry | 1.92E-05 | 0.003 | ** |
| Art | vs | Henley Business School | 4.50E-07 | 6.12E-05 | **** |
| Art | vs | JJ Thompson | 0.000221 | 0.03 | * |
| Chemistry | vs | Eat at the Square | 0.000107 | 0.015 | * |
| Chemistry | vs | Harry Nunsten | 0.000107 | 0.015 | * |
| Chemistry | vs | Maths | 0.000177 | 0.024 | * |
| Chemistry | vs | Park House | 9.22E-05 | 0.013 | * |
| Chemistry | vs | Student Union | 9.92E-07 | 0.000135 | *** |
| Eat at the Square | vs | Henley Business School | 9.27E-06 | 0.001 | ** |
| Eat at the Square | vs | JJ Thompson | 8.51E-05 | 0.012 | * |
| Edith Morley | vs | Student Union | 3.53E-05 | 0.005 | ** |
| Harry Nunsten | vs | Henley Business School | 2.19E-05 | 0.003 | ** |
| Harry Nunsten | vs | JJ Thompson | 6.80E-05 | 0.009 | ** |
| Henley Business School | vs | Maths | 1.33E-05 | 0.002 | ** |
| Henley Business School | vs | Park House | 6.72E-06 | 0.000914 | *** |
| Henley Business School | vs | Sports Park | 1.92E-05 | 0.003 | ** |
| Henley Business School | vs | Student Union | 2.14E-08 | 2.91E-06 | **** |
| JJ Thompson | vs | Park House | 9.91E-05 | 0.013 | * |
| JJ Thompson | vs | Student Union | 3.14E-05 | 0.004 | ** |
| Student Union | vs | Whiteknights | 9.56E-05 | 0.013 | * |

*B. Shannon Diversity*

| Building Comparison | | | p-value | p.adj | Significance |
|---|---|---|---|---|---|
| Agriculture | vs | Student Union | 0.000268 | 0.036 | * |
| Chemistry | vs | Harry Nunsten | 0.000313 | 0.043 | * |
| Chemistry | vs | Student Union | 4.88E-07 | 6.64E-05 | **** |
| Eat at the Square | vs | Henley Business School | 2.69E-05 | 0.004 | ** |
| Edith Morley | vs | Student Union | 0.000102 | 0.014 | * |
| Harry Nunsten | vs | Henley Business School | 2.57E-05 | 0.003 | ** |
| Henley Business School | vs | Maths | 0.000298 | 0.041 | * |
| Henley Business School | vs | Student Union | 3.93E-09 | 5.34E-07 | **** |
| JJ Thompson | vs | Student Union | 3.33E-05 | 0.005 | ** |
| Student Union | vs | Whiteknights | 0.000233 | 0.032 | * |

*C. Pielou's Evenness*

| Building Comparison | | | p-value | p.adj | Significance |
|---|---|---|---|---|---|
| Chemistry | vs | Student Union | 3.33E-06 | 0.000453 | *** |
| Edith Morley | vs | Student Union | 0.000186 | 0.025 | * |
| Harry Nunsten | vs | Henley Business School | 0.000105 | 0.014 | * |
| Henley Business School | vs | Student Union | 1.31E-07 | 1.78E-05 | **** |
| JJ Thompson | vs | Student Union | 6.66E-05 | 0.009 | ** |

**Table B.6.** Results of Paired Wilcoxon comparisons between buildings based on alpha diversity measures A) Observed, B) Shannon, C) Evenness. Pairs of buildings shown in tables are only those with significant differences observed. P.adj shows the P-Bonferroni corrected p-values, stars indicate the p-value significance * $p < 0.05$ , ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

# Chapter 4. Longitudinal Bacterial Community Dynamics and Sodium Hypochlorite Intervention in a Newly Built University Building

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## 4.1 Abstract

Sink P-traps harbour diverse bacterial communities that are increasingly acknowledged as potential reservoirs for pathogens and antimicrobial resistance in clinical settings. Yet, they remain understudied in environments outside of hospital settings. 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 *Enterbacteriaceae* were highly prevalent. When comparing bacterial structure and composition to other sinks located on the university campus, the mean bacterial dissimilarity (Bray-Curtis) 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 invention, bacterial communities had reestablished 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 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.

## 4.2 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; 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., 2021b; Meier & Bendinger, 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, provide protection against environmental stress and antimicrobial agents, and promote the development and transfer of antibiotic resistance genes (Douterelo et al., 2018; Garrett et al., 2008; Wingender & 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 & 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 & 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 & 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, Lechwoch 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.

## 4.3 Methodology

### *4.3.1 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) (Figure 4.1). 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 seconds 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. While this number provided an approximate occupancy, it may not capture all individuals entering without card access, and it does not account for large practicals occurring on floor level one (data prior to August 2022 was unobtainable).

### *4.3.2 Bleach Intervention*

The second phase of this study implemented an intervention using 10% sodium hypochlorite (Honeywell Fluka) (Figure 4.1). 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 C.1). The bleach treatment entailed pouring 500 ml of 10% bleach into the selected sinks in the evening and allowing it to sit overnight. The following morning, 500 ml of sodium thiosulfate (70mg/l) was 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 seconds 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.

### *4.3.3 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 C.1). The amplification of the V4 region of the bacterial 16S rRNA gene and metabarcoding was performed using 515F (Forward: GTGYCAGCMGCCGCGGTAA) and 806R (Reverse: GGACTACNVGGGTWTCTAAT) 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 Illuminia MiSeq platform (2x250 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. 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 300bp. ASVs with low abundance below 10 counts across the feature table were systematically removed to reduce the likelihood of spurious taxa.

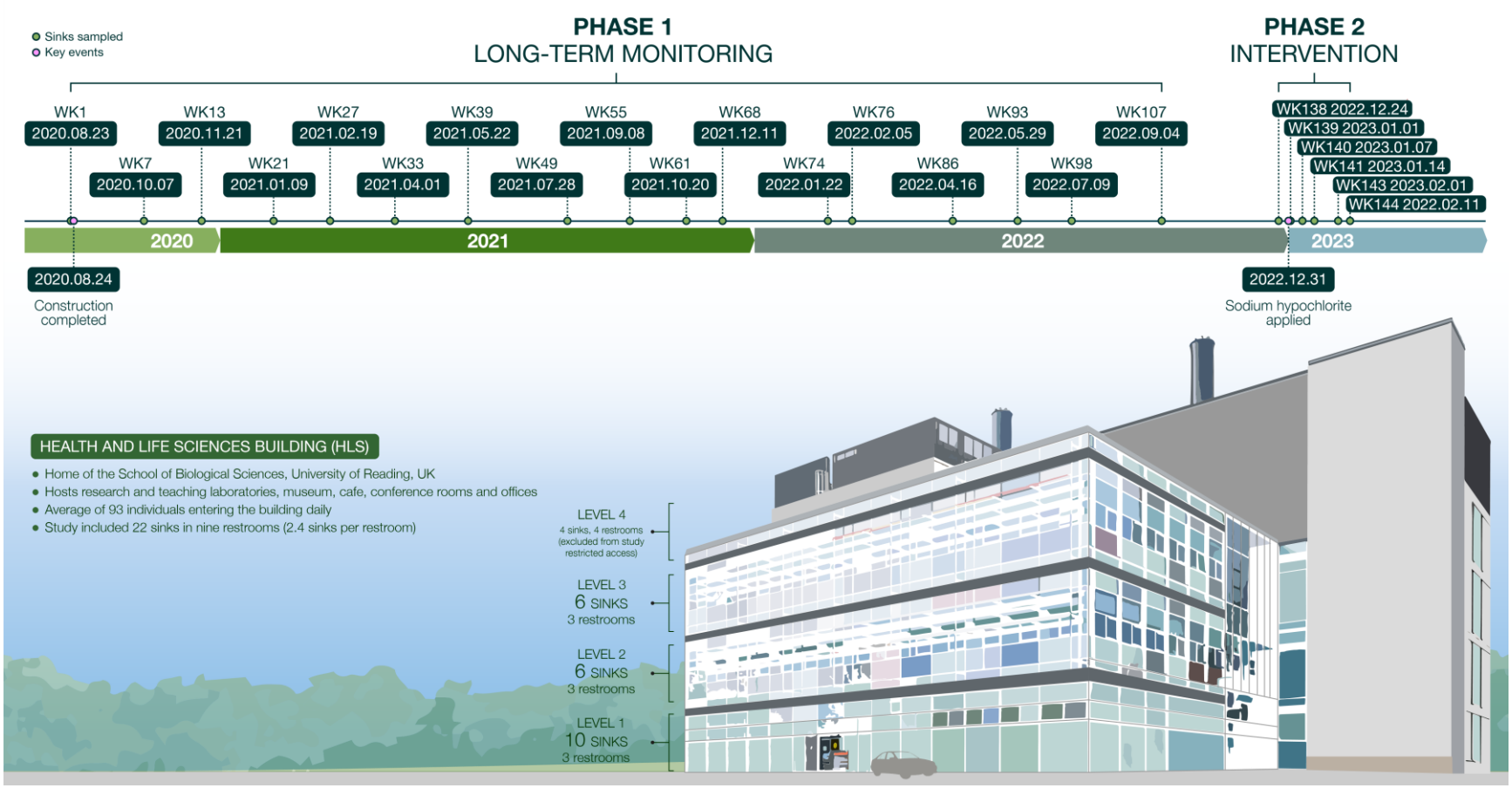


**Figure 4.1.** Summary diagram outlining the two phases of the study and providing details of the study site (Health and Life Sciences, HLS). Phase 1 sampling began on the 23 August 2020 one day before the construction of the building completed. Sampling occurred approximately every six weeks across 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.

### *4.3.4 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 & 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 & 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.

## 4.4 Results

Processing and filtering of reads resulted in a feature table containing 11,212,944 merged reads from 484 samples (384 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 (Figure C.1). 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.

### *4.4.1 Diversity and composition of university sinks over two years*

Alpha diversity, measured by ASV richness (Figure 4.2a), Shannon diversity (Figure 4.2b) and Pielou's evenness (Figure 4.2c) 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 C.2). 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 C.2). 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 intervals were also evident in total turnover (Figure 4.2d) aligning with an increase in ASV appearance and mean rank shift (Figure 4.2e). A reduction in evenness

was observed at these sampling time points (Figure 4.2c) 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 C.3). 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 4.1, C.4). 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: 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 (Figure 4.2h) 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 (Figure 4.2g).

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 greater than 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 *Flavobacteriaecae* which is part of Bacteroidota. Figure 4.3 shows *Rhodocyclaceae* increased in relative abundance over the first four sampling time points (WK1 – WK27) then remained between 25-50% 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 C.5). The most prevalent and abundant ASV was classified as *Azospira oryzae* establishing itself in all sinks after WK27.

| 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 *** |

**Table 4.1.** 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 $p < 0.05$; *, $p < 0.01$; **, $P < 0.001$; ***.

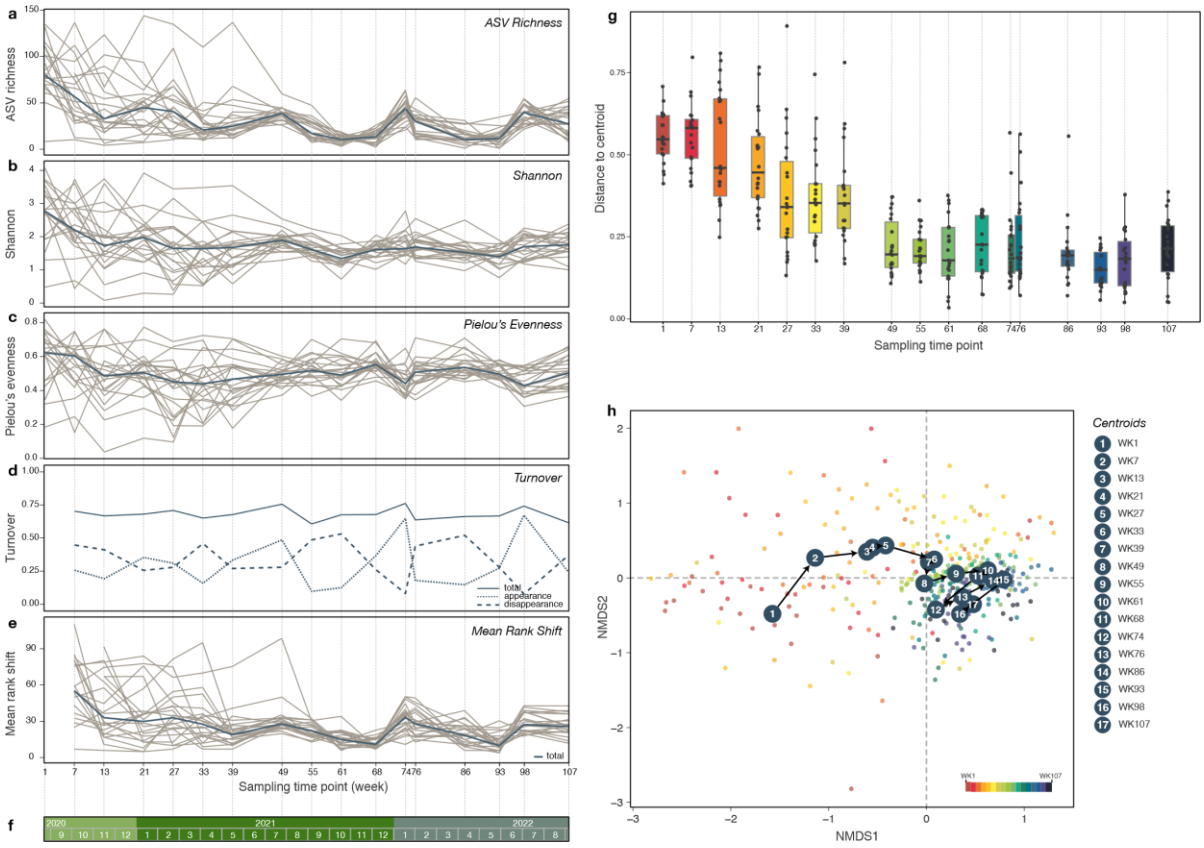


**Figure 4.2.** Alpha and beta diversity. (a - c) Alpha diversity indices over sampling round. Dark blue line is the median of the diversity metric, lighter grey lines represent individual sinks. Alpha diversity indices exhibit a decreasing trend over time, with the variation in diversity among individual 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 WK7, 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.

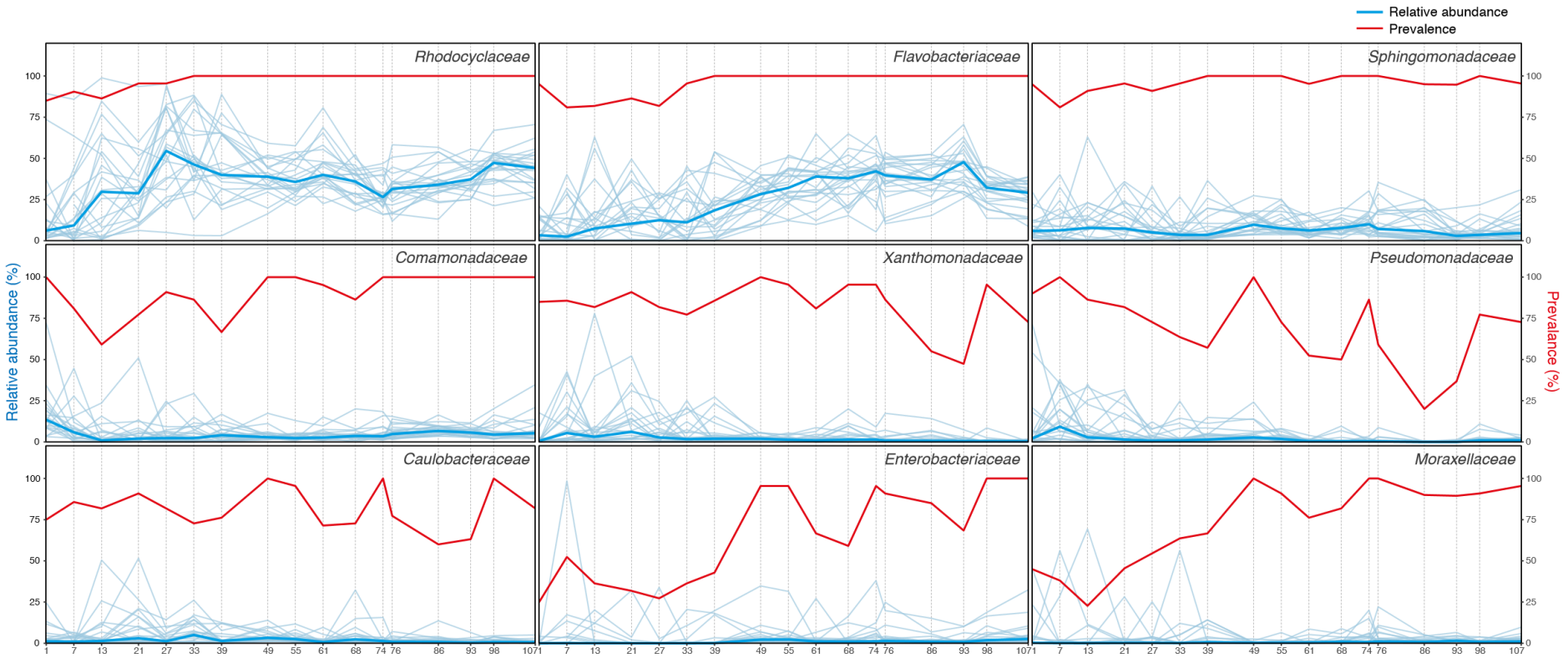


**Figure 4.3.** Relative abundances (%) of the top genera over the sampling period. The darker blue line is the median relative abundance across all sink samples, the lighter blue lines represent individual sinks. Prevalence at each time point of top genera is indicated by the red line. *Rhodocyclaceae* 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).

### *4.4.2 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 (Figure C.2). 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 C.6). 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.model = 7.2776, R2 = 0.34203, P < 0.001; WK141 , DF = 1, F.model = 3.2289 , R2 = 0.26404, P = 0.004, Figure 4.4b). It was only from WK143 onward that no significant differences were observed between the treatments (PERMANOVA: WK143, DF = 1, F.model = 1.861, R2 = 0.09867, P = 0.102 ; WK144, DF = 1, F.model = 1.0731, R2 = 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, Figure C.3). Overall, it required four weeks for the bacterial community and structure to homogenize 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 (Figure 4.4a). This distinctive peak in *Acinetobacter* appears in most bleached sinks at the individual level (Figure C.4, C.5). 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 (Figure C.6). 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, Figure 4.4a 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.

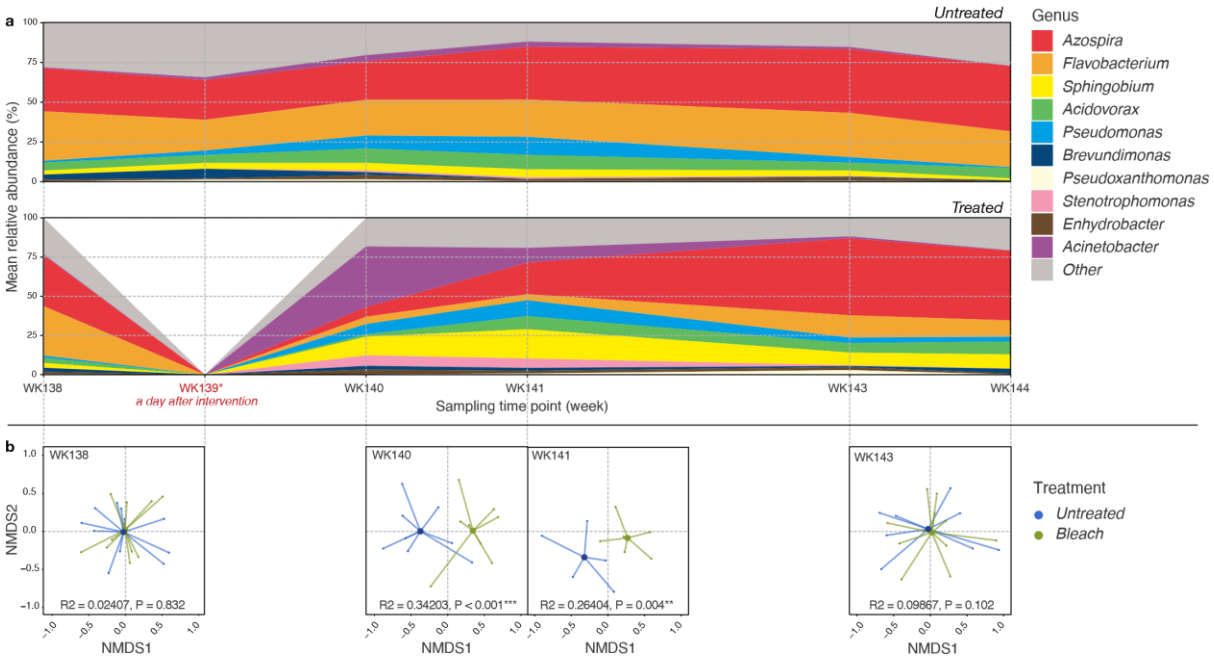


**Figure 4.4.** (a) Average relative abundance of the top bacterial genera found in restroom sinks. The average data represent pooled sequences of sinks, split by treatment. "Other" represents all other genera and sequences unclassified to the genus level. Abbreviations: B.I Before Intervention; A.I After Intervention. WK139 (A.I)* 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.

### *4.4.3 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, R2 = 0.44422, $p < 0.001$ ***), the NMDS analysis indicated that the later sampling time points of HLS were closer to the campus sinks (Figure 4.5). Notably, the mean bacterial dissimilarity (Bray-Curtis) between HLS sinks and campus sinks decreased over time, indicating compositional similarity (Figure C.7). 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) (Figure C.8). 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.

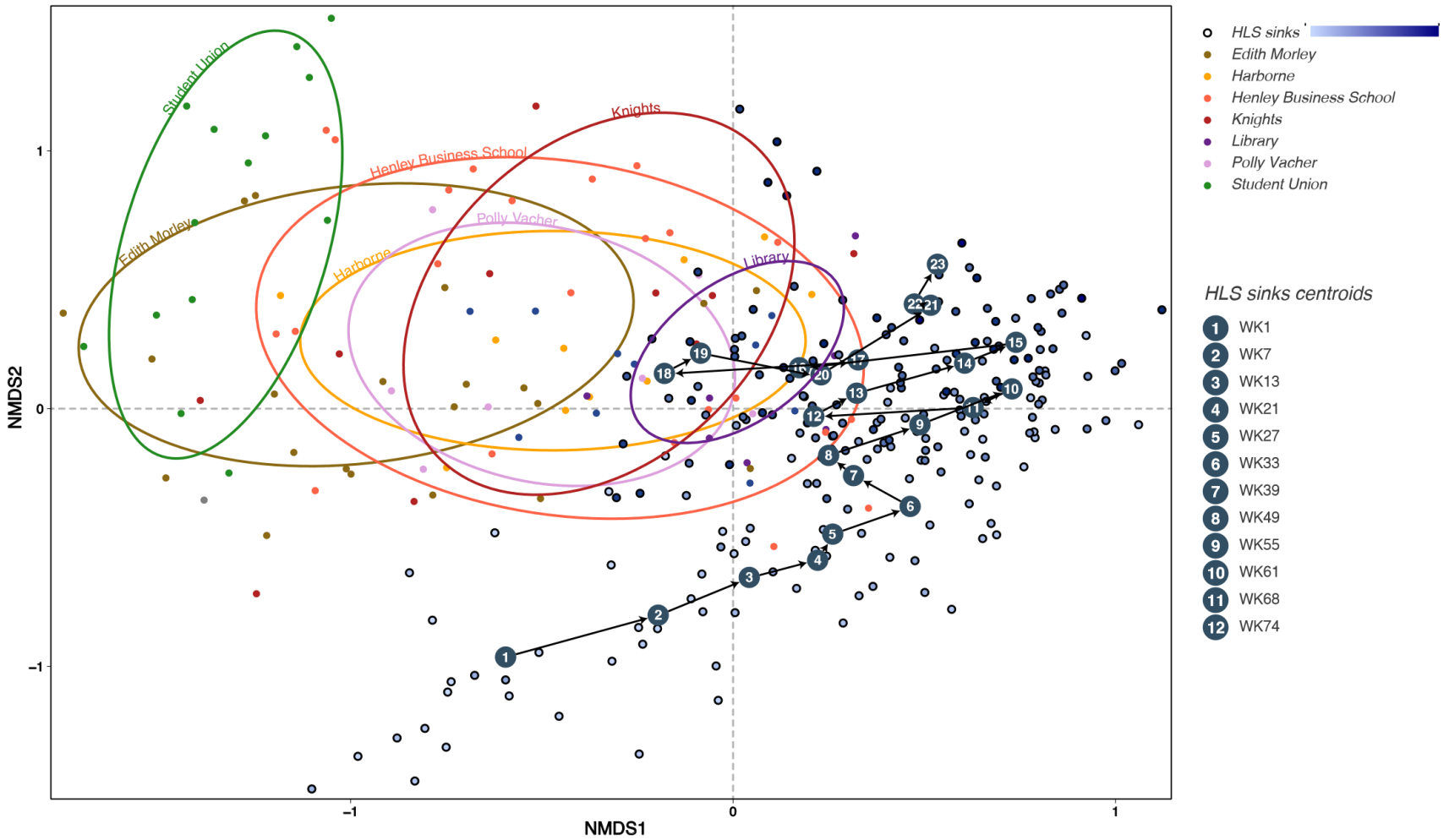


**Figure 4.5.** Non-metric multidimensional scaling (NMDS) resulting from Bray-Curtis dissimilarity matrices of community composition between buildings from the university campus, including the new HLS building by sampling rounds. Centroids for each sampling round for HLS and ellipses for all other buildings are shown on the plot. Arrows indicates HLS communities becoming more similar over time in composition to the other sinks present on campus.

## 4.5 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 (Figure C.4, C.5).

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 additional waste or products being poured down the sinks, or other unconventional use of sinks. 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 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 colonise 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 colonisers 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; Martins 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.

Limitations of this study include the insufficient metadata collected on the occupancy and behaviour of occupants. However, we did acquire approximate occupancy data for the latter phase of the study, providing 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 & Camper, 2008).

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 the anthropogenic influences on these microbial communities and their potential implications for human health.

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## Appendix C

## Supplementary material for Chapter 4

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

This appendix includes:

- **Figure C.1 -** Plot of the approximate occupancy of HLS from August 2022 to September 2023. Data obtained from the recorded number of entries into the building via the use of the card reader. Entries recorded into the building prior to August 2022 was not obtainable.
- **Figure C.2** - Alpha diversity indices between bleach treated and untreated samples. (A) Shannon Diversity, (B) ASV Richness, (C) Pielou's Evenness, (D) Total Turnover. Thicker line indicates mean by treatment. For the bleached sinks with no DNA (all sinks at WK139 and three sinks at WK140), these samples were included with the values as zero for the plots. Note that in (D) for untreated sinks the x axis starts at WK139, but this refers to the difference between sampling time points (weeks), WK138 to WK139. WK140 refers to difference between WK139 and WK140 and so on. For WK139 total turnover was plotted as 1 as there was a complete removal of the community between WK138 and WK139. WK140 (WK139 to WK140) was also plotted as 1 as a new community had established. Abbreviations: B.I Before Intervention; A.I After Intervention.
- **Figure C.3 -** Distances (Bray-Curtis matrices) to centroid in multivariate homogeneity of group variance analysis for sink bacterial communities for untreated and treated over sampling rounds.
- **Figure C.4 -** Bacterial composition at genus level across sampling rounds of all sinks treated with bleach. Includes the data from the timeseries. "Other" groups genera that had less than 1% mean relative abundance and those reads unclassified to the genus level. Blank spaces indicate where there was no data present due to no quantifiable DNA.
- **Figure C.5 -** Bacterial composition at genus level across sampling rounds of all untreated sinks. Includes the data from the timeseries. "Other" groups genera that had less than 1% mean relative abundance and those reads unclassified to the genus level. Sink IDs starting with D were only included in the timeseries study.

- **Figure C.6 -** Relative abundance of significant genera between untreated and bleach treated samples at WK140. Median and interquartile range displayed for each genus by treatment. WK138 included as reference to relative abundances of these genera prior to treatment.
- **Figure C.7 -** Mean beta dissimilarity between sinks in HLS by sampling time point (week) and other buildings on campus. Based on Bray-Curtis distances. Over time sinks from HLS are becoming more similar in community composition to sinks from the surrounding campus.
- **Figure C.8 -** Comparisons of the mean Bray-Curtis distance of the last sampling time point (WK107) of HLS and the other individual buildings. Lower values indicate higher similarity in composition.

- **Table C.1** - Data collected for each sink P-trap sample for both studies. Bleach study also includes the gDNA concentrations recorded for each sample.
- **Table C.2** - Results table from ANOVA of linear mixed effects model for the alpha diversity indices for phase 1. Sum Sq, sum of squares; Mean Sq, mean square; Num DF, degrees of freedom, DEN DF, denominator degrees of freedom. Stars indicate the p-value significance $p < 0.05$; *, $p < 0.01$; **, $P < 0.001$; ***.
- **Table C.3** - Significant ASVs between WKS with increased peak in ASV richness. (A) WK39 vs WK49, (B) WK68 vs WK74, (C) WK93 vs WK98. Mean relative abundances of ASVs at WKs being compared and p-adjusted values from Wilcox test. P values adjusted with Benjamini-Hochberg (BH). Highlighted cells indicate ASVs shared between the peaks. Taxonomy of ASVs included.
- **Table C.4** - Pairwise comparisons for all significant pairs of levels of sampling time point (week) by using PERMANOVA. P values corrected with Benjamini-Hochberg (BH) are shown. The R2 values indicated the amount of variation explained.
- **Table C.5** - ASVs classified as core (>70% prevalence). Overall abundance (counts), prevalence and classification are shown.
- **Table C.6** - Results table from ANOVA of linear mixed effects model for the alpha diversity indices for phase 2. Sum Sq, sum of squares; Mean Sq, mean square; Num DF, degrees of freedom, DEN DF, denominator degrees of freedom. Stars indicate the p-value significance $p < 0.05$; *, $p < 0.01$; **, $P < 0.001$; ***.

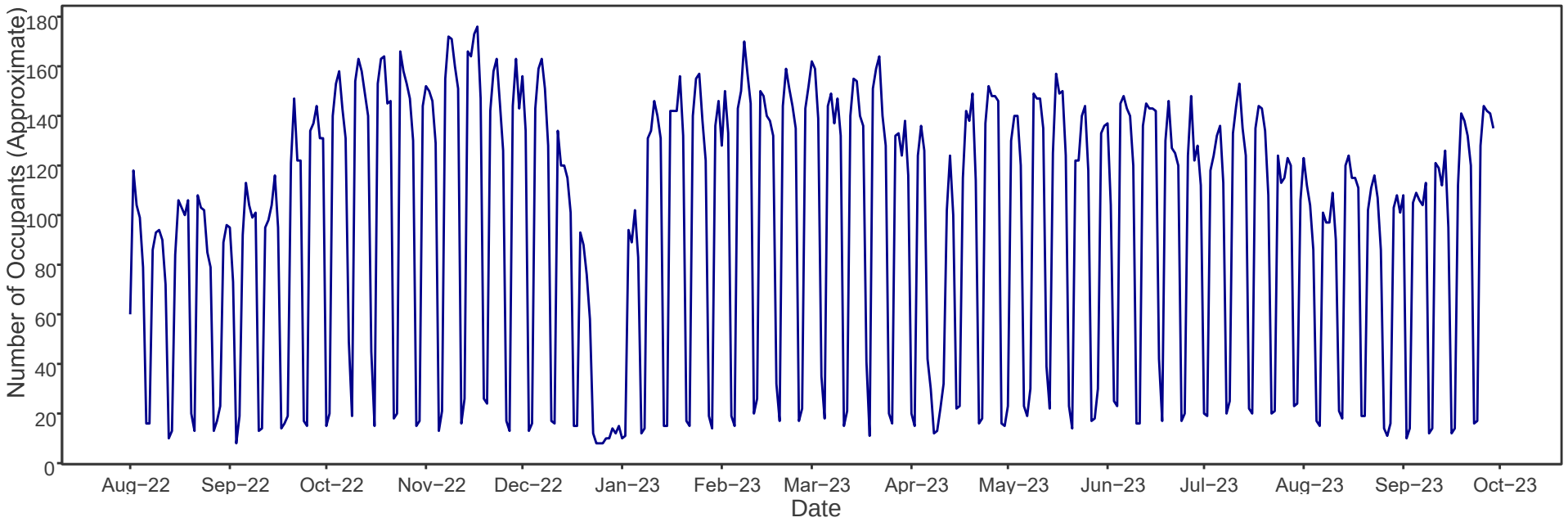


**Figure C.1.** Plot of the approximate occupancy of HLS from August 2022 to September 2023. Data obtained from the recorded number of entries into the building via the use of the card reader. Entries recorded into the building prior to August 2022 was not obtainable.

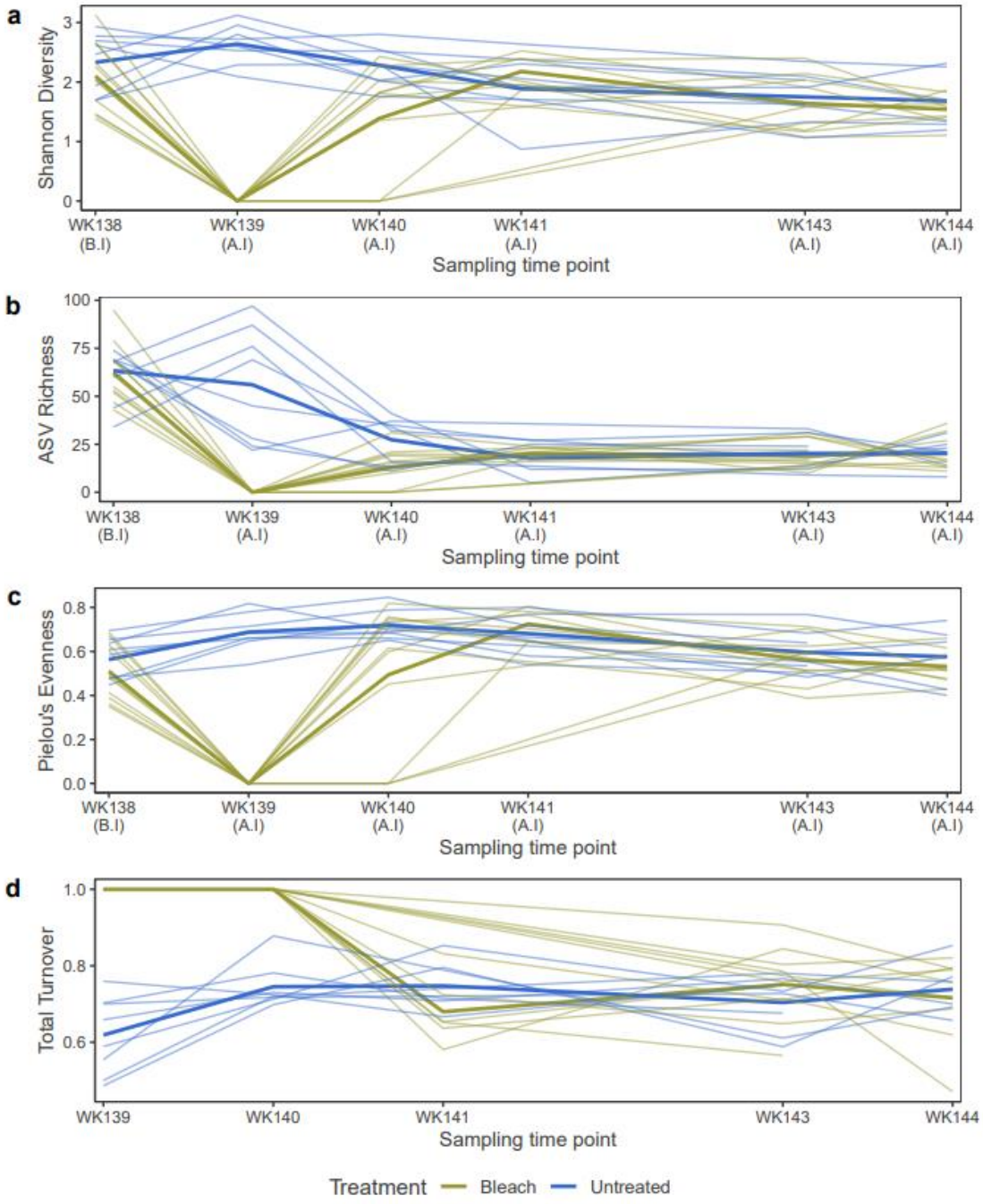


**Figure C.2.** Alpha diversity indices between bleach treated and untreated samples. (A) Shannon Diversity, (B) ASV Richness, (C) Pielou's Evenness, (D) Total Turnover. Thicker line indicates mean by treatment. For the bleached sinks with no DNA (all sinks at WK139 and three sinks at WK140), these samples were included with the values as zero for the plots. Note that in (D) for untreated sinks the x axis starts at WK139, but this refers to the difference between sampling time points (weeks), WK138 to WK139. WK140 refers to difference between WK139 and WK140 and so on. For WK139 total turnover was plotted as 1 as there was a complete removal of the community between WK138 and WK139. WK140 (WK139 to WK140) was also plotted as 1 as a new community had established. Abbreviations: B.I Before Intervention; A.I After Intervention.

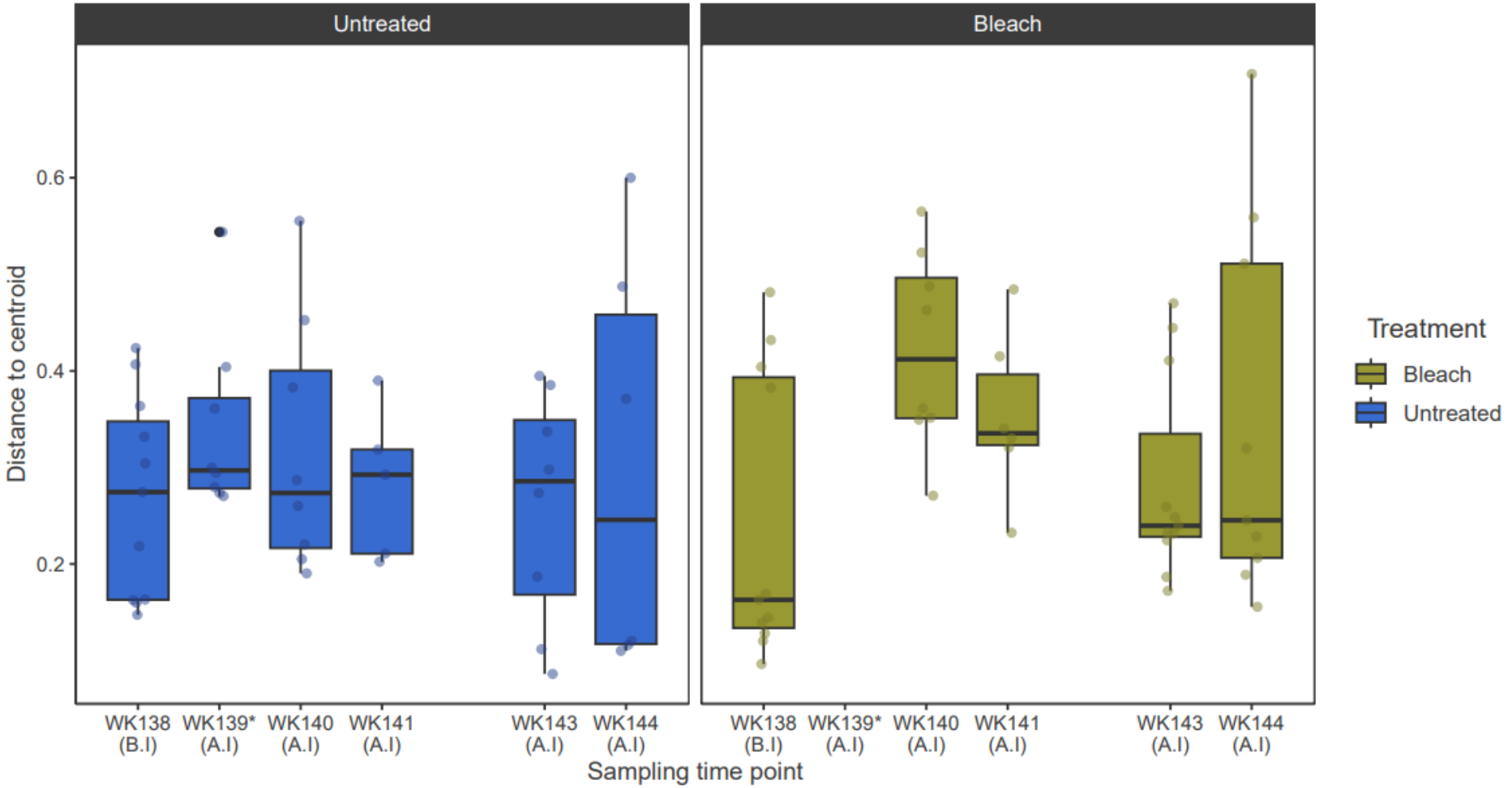


**Figure C.3.** Distances (Bray-Curtis matrices) to centroid in multivariate homogeneity of group variance analysis for sink bacterial communities for untreated and treated over sampling rounds.

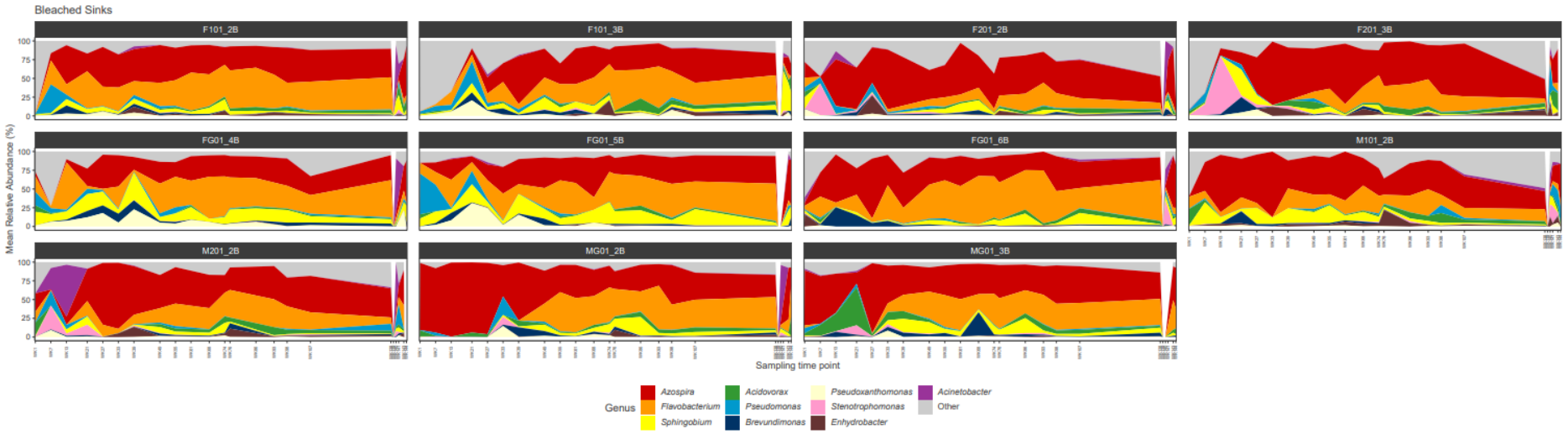


**Figure C.4.** Bacterial composition at genus level across sampling rounds of all sinks treated with bleach. Includes the data from the timeseries. "Other" groups genera that had less than 1% mean relative abundance and those reads unclassified to the genus level. Blank spaces indicate where there was no data present due to no quantifiable DNA.

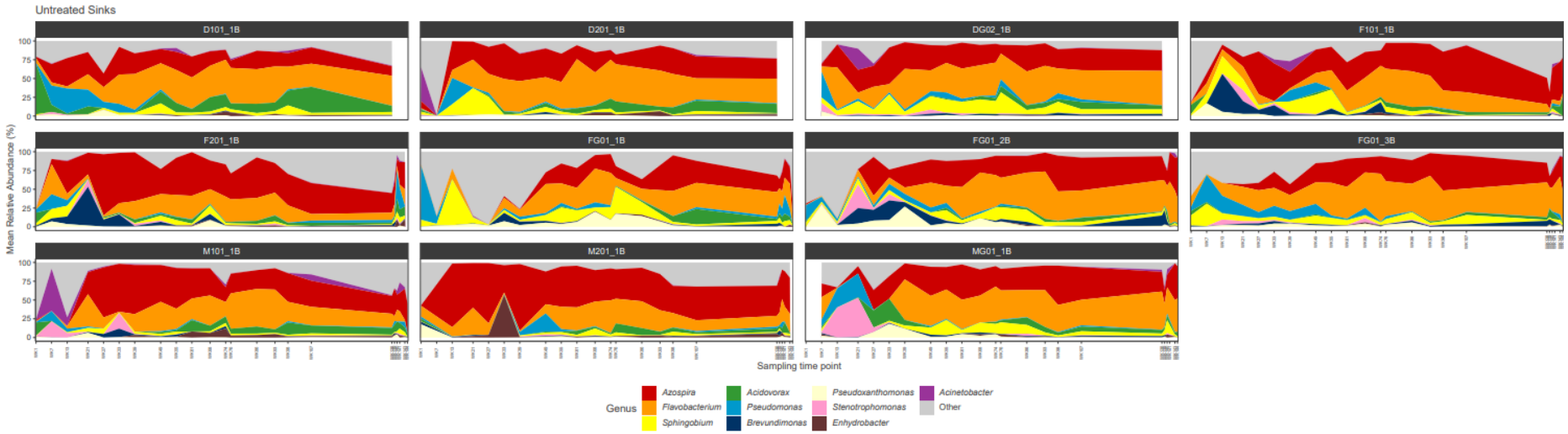


**Figure C.5.** Bacterial composition at genus level across sampling rounds of all untreated sinks. Includes the data from the timeseries. "Other" groups genera that had less than 1% mean relative abundance and those reads unclassified to the genus level. Sink IDs starting with D were only included in the timeseries study.

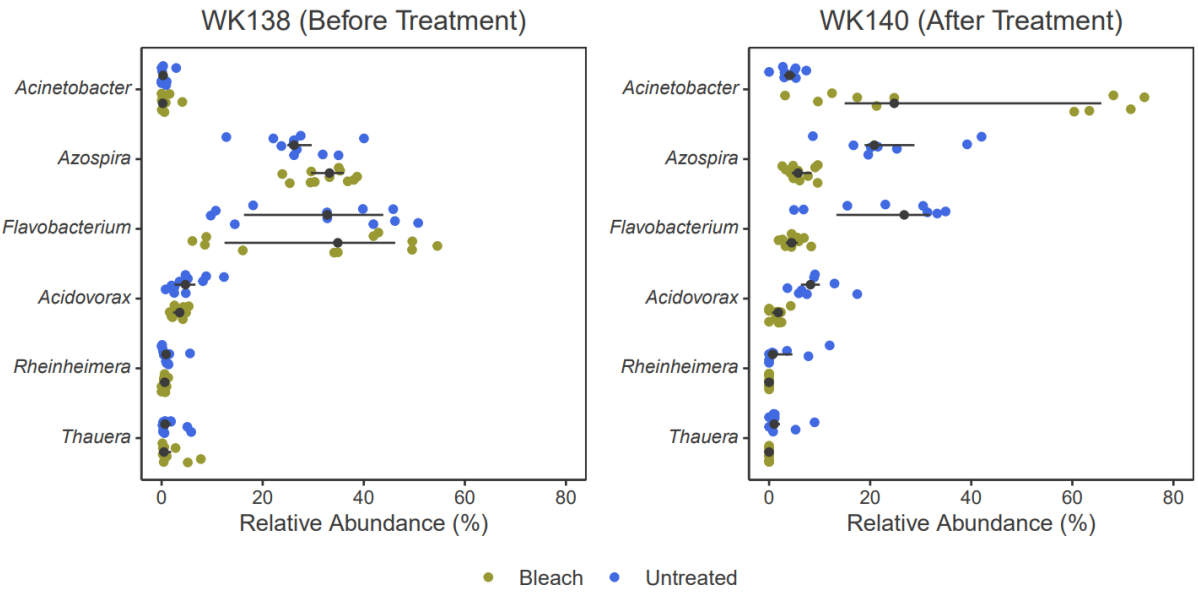


**Figure C.6.** Relative abundance of significant genera between untreated and bleach treated samples at WK140. Median and interquartile range displayed for each genus by treatment. WK138 included as reference to relative abundances of these genera prior to treatment.

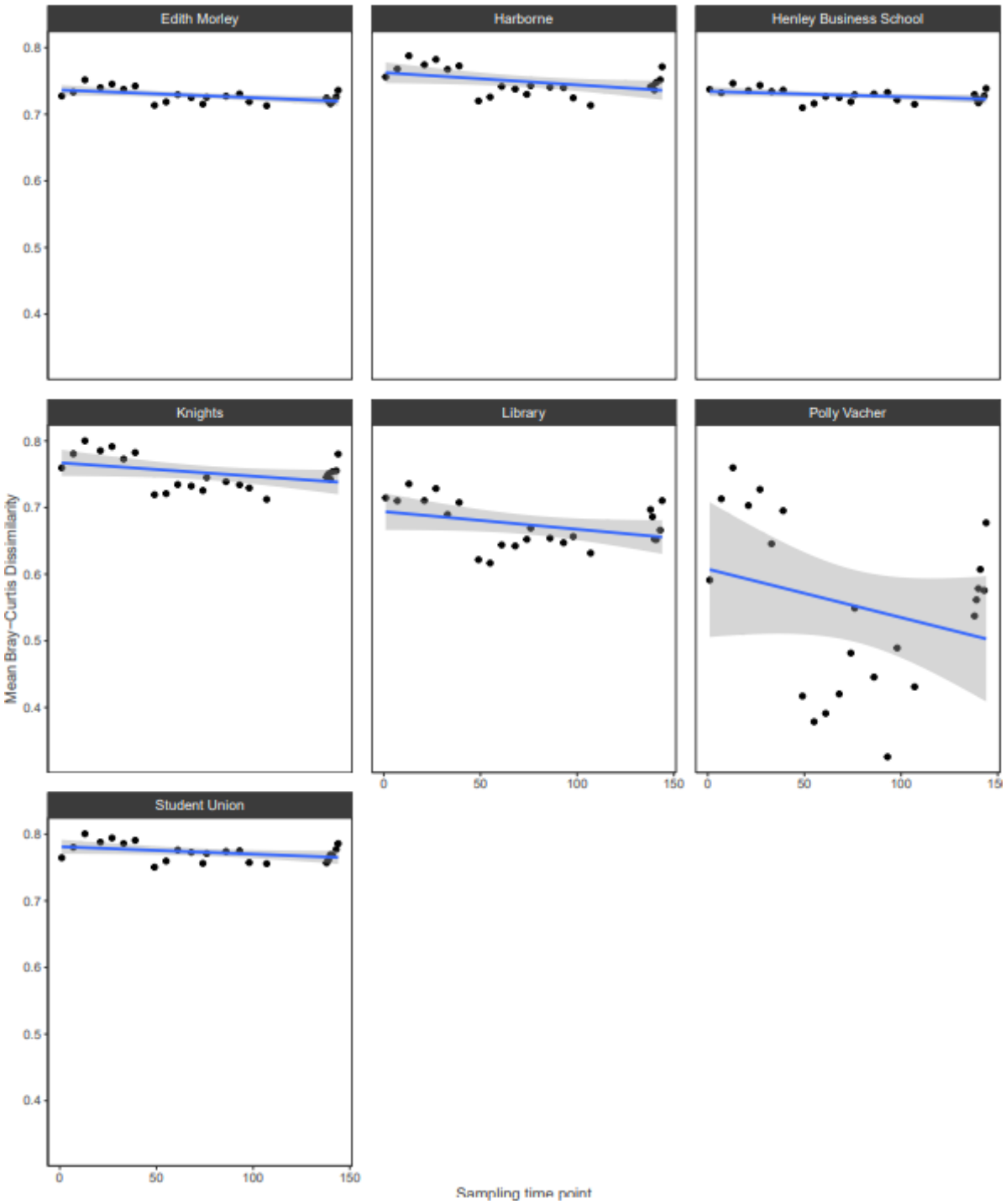


**Figure C.7.** Mean beta dissimilarity between sinks in HLS by sampling time point (week) and other buildings on campus. Based on Bray-Curtis distances. Over time sinks from HLS are becoming more similar in community composition to sinks from the surrounding campus.

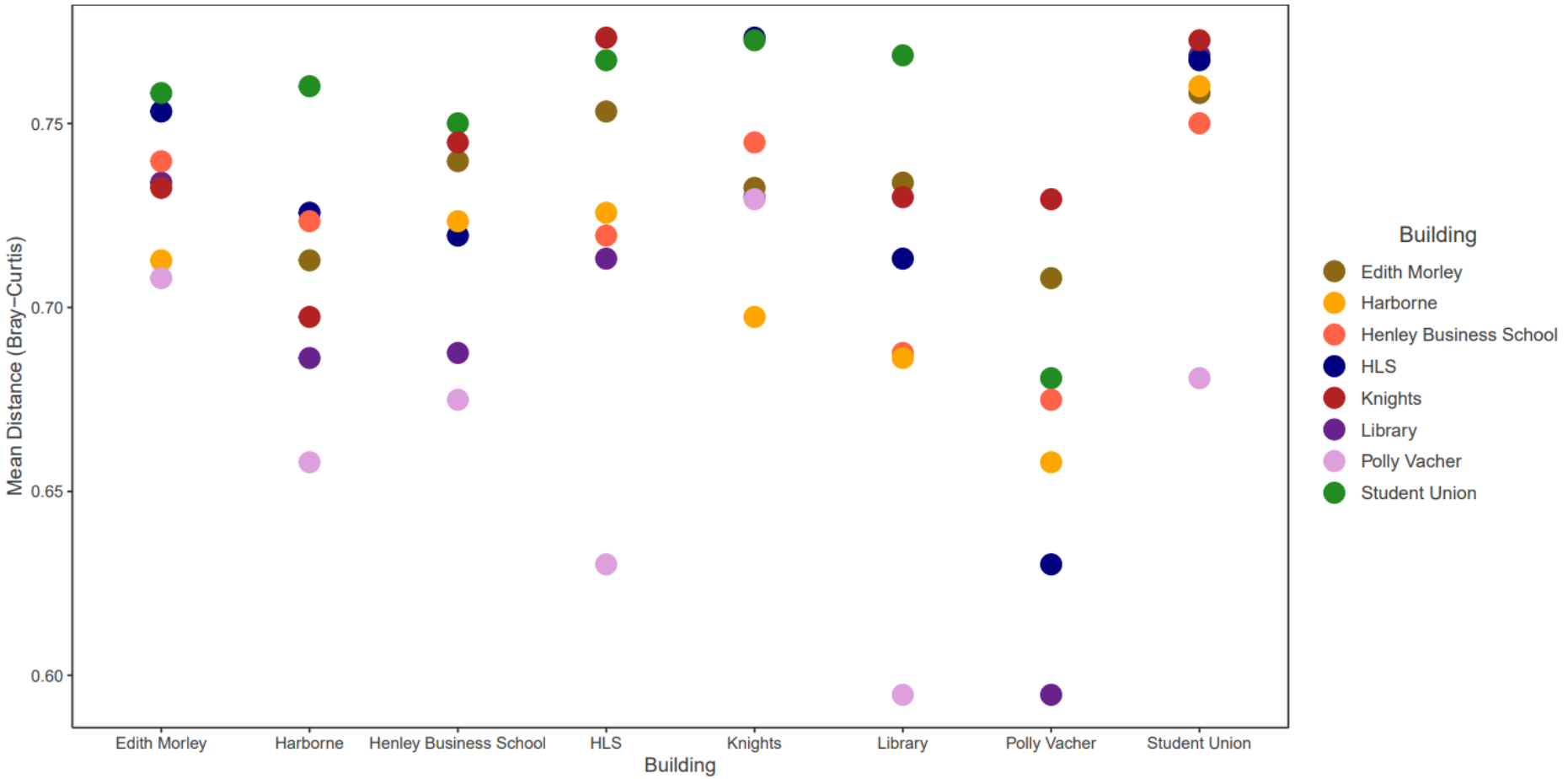


**Figure C.8.** Comparisons of the mean Bray-Curtis distance of the last sampling time point (WK107) of HLS and the other individual buildings. Lower values indicate higher similarity in composition.

| seq_id | sample_id | s_id | sample_date | sample_week | round_id | dataset | treatment | gender | floor | gDNA ng/ul |
|---|---|---|---|---|---|---|---|---|---|---|
| ZW.E03.HLS391 | FG01_1B_T0 | FG01_1B | 24/12/2022 | 138 | T0 | Bleach | Untreated | Female | Ground | 27.7 |
| ZW.E03.HLS392 | FG01_2B_T0 | FG01_2B | 24/12/2022 | 138 | T0 | Bleach | Untreated | Female | Ground | 23.9 |
| ZW.E03.HLS393 | FG01_3B_T0 | FG01_3B | 24/12/2022 | 138 | T0 | Bleach | Untreated | Female | Ground | 6.32 |
| ZW.E03.HLS394 | FG01_4B_T0 | FG01_4B | 24/12/2022 | 138 | T0 | Bleach | Untreated | Female | Ground | 17.2 |
| ZW.E03.HLS395 | FG01_5B_T0 | FG01_5B | 24/12/2022 | 138 | T0 | Bleach | Untreated | Female | Ground | 19.2 |
| ZW.E03.HLS396 | FG01_6B_T0 | FG01_6B | 24/12/2022 | 138 | T0 | Bleach | Untreated | Female | Ground | 9.57 |
| ZW.E03.HLS397 | MG01_1B_T0 | MG01_1B | 24/12/2022 | 138 | T0 | Bleach | Untreated | Male | Ground | 4.94 |
| ZW.E03.HLS398 | MG01_2B_T0 | MG01_2B | 24/12/2022 | 138 | T0 | Bleach | Untreated | Male | Ground | 3.26 |
| ZW.E03.HLS399 | MG01_3B_T0 | MG01_3B | 24/12/2022 | 138 | T0 | Bleach | Untreated | Male | Ground | 3.27 |
| ZW.E03.HLS400 | DG02_1B_T0 | DG02_1B | 24/12/2022 | 138 | T0 | Bleach | Untreated | Unisex | Ground | 2.75 |
| ZW.E03.HLS401 | F101_1B_T0 | F101_1B | 24/12/2022 | 138 | T0 | Bleach | Untreated | Female | First | 6.24 |
| ZW.E03.HLS402 | F101_2B_T0 | F101_2B | 24/12/2022 | 138 | T0 | Bleach | Untreated | Female | First | 0.963 |
| ZW.E03.HLS403 | F101_3B_T0 | F101_3B | 24/12/2022 | 138 | T0 | Bleach | Untreated | Female | First | 2.61 |
| ZW.E03.HLS404 | M101_1B_T0 | M101_1B | 24/12/2022 | 138 | T0 | Bleach | Untreated | Male | First | 3.61 |
| ZW.E03.HLS405 | M101_2B_T0 | M101_2B | 24/12/2022 | 138 | T0 | Bleach | Untreated | Male | First | 3.69 |
| ZW.E03.HLS406 | D101_1B_T0 | D101_1B | 24/12/2022 | 138 | T0 | Bleach | Untreated | Unisex | First | 7.6 |
| ZW.E03.HLS407 | F201_1B_T0 | F201_1B | 24/12/2022 | 138 | T0 | Bleach | Untreated | Female | Second | 6.32 |
| ZW.E03.HLS408 | F201_2B_T0 | F201_2B | 24/12/2022 | 138 | T0 | Bleach | Untreated | Female | Second | 17.7 |
| ZW.E03.HLS409 | F201_3B_T0 | F201_3B | 24/12/2022 | 138 | T0 | Bleach | Untreated | Female | Second | 15.1 |
| ZW.E03.HLS410 | M201_1B_T0 | M201_1B | 24/12/2022 | 138 | T0 | Bleach | Untreated | Male | Second | 7.53 |
| ZW.E03.HLS411 | M201_2B_T0 | M201_2B | 24/12/2022 | 138 | T0 | Bleach | Untreated | Male | Second | 3.13 |
| ZW.E03.HLS412 | D201_1B_T0 | D201_1B | 24/12/2022 | 138 | T0 | Bleach | Untreated | Unisex | Second | 1.7 |
| ZW.E03.HLS415 | FG01_1B_T1 | FG01_1B | 01/01/2023 | 139 | T1 | Bleach | Untreated | Female | Ground | 24.5 |
| ZW.E03.HLS416 | FG01_2B_T1 | FG01_2B | 01/01/2023 | 139 | T1 | Bleach | Untreated | Female | Ground | 7.12 |
| ZW.E03.HLS417 | FG01_3B_T1 | FG01_3B | 01/01/2023 | 139 | T1 | Bleach | Untreated | Female | Ground | 2.34 |
| ZW.E03.HLS418 | FG01_4B_T1 | FG01_4B | 01/01/2023 | 139 | T1 | Bleach | Bleach | Female | Ground | Too low |
| ZW.E03.HLS419 | FG01_5B_T1 | FG01_5B | 01/01/2023 | 139 | T1 | Bleach | Bleach | Female | Ground | Too low |
| ZW.E03.HLS420 | FG01_6B_T1 | FG01_6B | 01/01/2023 | 139 | T1 | Bleach | Bleach | Female | Ground | Too low |
| ZW.E03.HLS421 | MG01_1B_T1 | MG01_1B | 01/01/2023 | 139 | T1 | Bleach | Untreated | Male | Ground | 1.33 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ZW.E03.HLS422 | MG01_2B_T1 | MG01_2B | 01/01/2023 | 139 | T1 | Bleach | Bleach | Male | Ground | Too low |
| ZW.E03.HLS423 | MG01_3B_T1 | MG01_3B | 01/01/2023 | 139 | T1 | Bleach | Bleach | Male | Ground | Too low |
| ZW.E03.HLS424 | F101_1B_T1 | F101_1B | 01/01/2023 | 139 | T1 | Bleach | Untreated | Female | First | 0.72 |
| ZW.E03.HLS425 | F101_2B_T1 | F101_2B | 01/01/2023 | 139 | T1 | Bleach | Bleach | Female | First | Too low |
| ZW.E03.HLS426 | F101_3B_T1 | F101_3B | 01/01/2023 | 139 | T1 | Bleach | Bleach | Female | First | Too low |
| ZW.E03.HLS427 | M101_1B_T1 | M101_1B | 01/01/2023 | 139 | T1 | Bleach | Untreated | Male | First | 4.45 |
| ZW.E03.HLS428 | M101_2B_T1 | M101_2B | 01/01/2023 | 139 | T1 | Bleach | Bleach | Male | First | Too low |
| ZW.E03.HLS429 | F201_1B_T1 | F201_1B | 01/01/2023 | 139 | T1 | Bleach | Untreated | Female | Second | 5.4 |
| ZW.E03.HLS430 | F201_2B_T1 | F201_2B | 01/01/2023 | 139 | T1 | Bleach | Bleach | Female | Second | Too low |
| ZW.E03.HLS431 | F201_3B_T1 | F201_3B | 01/01/2023 | 139 | T1 | Bleach | Bleach | Female | Second | Too low |
| ZW.E03.HLS432 | M201_1B_T1 | M201_1B | 01/01/2023 | 139 | T1 | Bleach | Untreated | Male | Second | 7.43 |
| ZW.E03.HLS433 | M201_2B_T1 | M201_2B | 01/01/2023 | 139 | T1 | Bleach | Bleach | Male | Second | Too low |
| ZW.E03.HLS434 | FG01_1B_T2 | FG01_1B | 07/01/2023 | 140 | T2 | Bleach | Untreated | Female | Ground | 8.47 |
| ZW.E03.HLS435 | FG01_2B_T2 | FG01_2B | 07/01/2023 | 140 | T2 | Bleach | Untreated | Female | Ground | 4.17 |
| ZW.E03.HLS436 | FG01_3B_T2 | FG01_3B | 07/01/2023 | 140 | T2 | Bleach | Untreated | Female | Ground | 1.8 |
| ZW.E03.HLS437 | FG01_4B_T2 | FG01_4B | 07/01/2023 | 140 | T2 | Bleach | Bleach | Female | Ground | 0.2 |
| ZW.E03.HLS438 | FG01_5B_T2 | FG01_5B | 07/01/2023 | 140 | T2 | Bleach | Bleach | Female | Ground | Too low |
| ZW.E03.HLS439 | FG01_6B_T2 | FG01_6B | 07/01/2023 | 140 | T2 | Bleach | Bleach | Female | Ground | 0.229 |
| ZW.E03.HLS440 | MG01_1B_T2 | MG01_1B | 07/01/2023 | 140 | T2 | Bleach | Untreated | Male | Ground | 8.68 |
| ZW.E03.HLS441 | MG01_2B_T2 | MG01_2B | 07/01/2023 | 140 | T2 | Bleach | Bleach | Male | Ground | 1.16 |
| ZW.E03.HLS442 | MG01_3B_T2 | MG01_3B | 07/01/2023 | 140 | T2 | Bleach | Bleach | Male | Ground | Too low |
| ZW.E03.HLS443 | F101_1B_T2 | F101_1B | 07/01/2023 | 140 | T2 | Bleach | Untreated | Female | First | 11.9 |
| ZW.E03.HLS444 | F101_2B_T2 | F101_2B | 07/01/2023 | 140 | T2 | Bleach | Bleach | Female | First | 0.743 |
| ZW.E03.HLS445 | F101_3B_T2 | F101_3B | 07/01/2023 | 140 | T2 | Bleach | Bleach | Female | First | Too low |
| ZW.E03.HLS446 | M101_1B_T2 | M101_1B | 07/01/2023 | 140 | T2 | Bleach | Untreated | Male | First | 43.9 |
| ZW.E03.HLS447 | M101_2B_T2 | M101_2B | 07/01/2023 | 140 | T2 | Bleach | Bleach | Male | First | 1.64 |
| ZW.E03.HLS448 | F201_1B_T2 | F201_1B | 07/01/2023 | 140 | T2 | Bleach | Untreated | Female | Second | 1.56 |
| ZW.E03.HLS449 | F201_2B_T2 | F201_2B | 07/01/2023 | 140 | T2 | Bleach | Bleach | Female | Second | 0.422 |
| ZW.E03.HLS450 | F201_3B_T2 | F201_3B | 07/01/2023 | 140 | T2 | Bleach | Bleach | Female | Second | 1.14 |
| ZW.E03.HLS451 | M201_1B_T2 | M201_1B | 07/01/2023 | 140 | T2 | Bleach | Untreated | Male | Second | 11.2 |
| ZW.E03.HLS452 | M201_2B_T2 | M201_2B | 07/01/2023 | 140 | T2 | Bleach | Bleach | Male | Second | 0.675 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ZW.E03.HLS453 | FG01_1B_T3 | FG01_1B | 14/01/2023 | 141 | T3 | Bleach | Untreated | Female | Ground | 10.3 |
| ZW.E03.HLS454 | FG01_2B_T3 | FG01_2B | 14/01/2023 | 141 | T3 | Bleach | Untreated | Female | Ground | 12.1 |
| ZW.E03.HLS455 | FG01_3B_T3 | FG01_3B | 14/01/2023 | 141 | T3 | Bleach | Untreated | Female | Ground | 7.88 |
| ZW.E03.HLS456 | FG01_4B_T3 | FG01_4B | 14/01/2023 | 141 | T3 | Bleach | Bleach | Female | Ground | 7.36 |
| ZW.E03.HLS457 | FG01_5B_T3 | FG01_5B | 14/01/2023 | 141 | T3 | Bleach | Bleach | Female | Ground | 4.4 |
| ZW.E03.HLS458 | FG01_6B_T3 | FG01_6B | 14/01/2023 | 141 | T3 | Bleach | Bleach | Female | Ground | 2.44 |
| ZW.E03.HLS459 | MG01_1B_T3 | MG01_1B | 14/01/2023 | 141 | T3 | Bleach | Untreated | Male | Ground | 32.8 |
| ZW.E03.HLS460 | MG01_2B_T3 | MG01_2B | 14/01/2023 | 141 | T3 | Bleach | Bleach | Male | Ground | 14.2 |
| ZW.E03.HLS461 | MG01_3B_T3 | MG01_3B | 14/01/2023 | 141 | T3 | Bleach | Bleach | Male | Ground | 3.14 |
| ZW.E03.HLS462 | F101_1B_T3 | F101_1B | 14/01/2023 | 141 | T3 | Bleach | Untreated | Female | First | 20.3 |
| ZW.E03.HLS463 | F101_2B_T3 | F101_2B | 14/01/2023 | 141 | T3 | Bleach | Bleach | Female | First | 42.7 |
| ZW.E03.HLS464 | F101_3B_T3 | F101_3B | 14/01/2023 | 141 | T3 | Bleach | Bleach | Female | First | 5.17 |
| ZW.E03.HLS465 | M101_1B_T3 | M101_1B | 14/01/2023 | 141 | T3 | Bleach | Untreated | Male | First | 34.7 |
| ZW.E03.HLS466 | M101_2B_T3 | M101_2B | 14/01/2023 | 141 | T3 | Bleach | Bleach | Male | First | 6.29 |
| ZW.E03.HLS467 | F201_1B_T3 | F201_1B | 14/01/2023 | 141 | T3 | Bleach | Untreated | Female | Second | 22.4 |
| ZW.E03.HLS468 | F201_2B_T3 | F201_2B | 14/01/2023 | 141 | T3 | Bleach | Bleach | Female | Second | 10.6 |
| ZW.E03.HLS469 | F201_3B_T3 | F201_3B | 14/01/2023 | 141 | T3 | Bleach | Bleach | Female | Second | 15 |
| ZW.E03.HLS470 | M201_1B_T3 | M201_1B | 14/01/2023 | 141 | T3 | Bleach | Untreated | Male | Second | 54 |
| ZW.E03.HLS471 | M201_2B_T3 | M201_2B | 14/01/2023 | 141 | T3 | Bleach | Bleach | Male | Second | 0.998 |
| ZW.E03.HLS472 | FG01_1B_T4 | FG01_1B | 01/02/2023 | 143 | T4 | Bleach | Untreated | Female | Ground | 4.39 |
| ZW.E03.HLS473 | FG01_2B_T4 | FG01_2B | 01/02/2023 | 143 | T4 | Bleach | Untreated | Female | Ground | 4.99 |
| ZW.E03.HLS474 | FG01_3B_T4 | FG01_3B | 01/02/2023 | 143 | T4 | Bleach | Untreated | Female | Ground | 3.99 |
| ZW.E03.HLS475 | FG01_4B_T4 | FG01_4B | 01/02/2023 | 143 | T4 | Bleach | Bleach | Female | Ground | 5.99 |
| ZW.E03.HLS476 | FG01_5B_T4 | FG01_5B | 01/02/2023 | 143 | T4 | Bleach | Bleach | Female | Ground | 1.17 |
| ZW.E03.HLS477 | FG01_6B_T4 | FG01_6B | 01/02/2023 | 143 | T4 | Bleach | Bleach | Female | Ground | 9.47 |
| ZW.E03.HLS478 | MG01_1B_T4 | MG01_1B | 01/02/2023 | 143 | T4 | Bleach | Untreated | Male | Ground | 21.2 |
| ZW.E03.HLS479 | MG01_2B_T4 | MG01_2B | 01/02/2023 | 143 | T4 | Bleach | Bleach | Male | Ground | 14.1 |
| ZW.E03.HLS480 | MG01_3B_T4 | MG01_3B | 01/02/2023 | 143 | T4 | Bleach | Bleach | Male | Ground | 3.17 |
| ZW.E03.HLS481 | F101_1B_T4 | F101_1B | 01/02/2023 | 143 | T4 | Bleach | Untreated | Female | First | 8.53 |
| ZW.E03.HLS482 | F101_2B_T4 | F101_2B | 01/02/2023 | 143 | T4 | Bleach | Bleach | Female | First | 46 |
| ZW.E03.HLS483 | F101_3B_T4 | F101_3B | 01/02/2023 | 143 | T4 | Bleach | Bleach | Female | First | 2.42 |

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|---|---|---|---|---|---|---|---|---|---|---|
| ZW.E03.HLS484 | M101_1B_T4 | M101_1B | 01/02/2023 | 143 | T4 | Bleach | Untreated | Male | First | 54 |
| ZW.E03.HLS485 | M101_2B_T4 | M101_2B | 01/02/2023 | 143 | T4 | Bleach | Bleach | Male | First | 5.27 |
| ZW.E03.HLS486 | F201_1B_T4 | F201_1B | 01/02/2023 | 143 | T4 | Bleach | Untreated | Female | Second | 14.8 |
| ZW.E03.HLS487 | F201_2B_T4 | F201_2B | 01/02/2023 | 143 | T4 | Bleach | Bleach | Female | Second | 1.69 |
| ZW.E03.HLS488 | F201_3B_T4 | F201_3B | 01/02/2023 | 143 | T4 | Bleach | Bleach | Female | Second | 5.53 |
| ZW.E03.HLS489 | M201_1B_T4 | M201_1B | 01/02/2023 | 143 | T4 | Bleach | Untreated | Male | Second | 29.6 |
| ZW.E03.HLS490 | M201_2B_T4 | M201_2B | 01/02/2023 | 143 | T4 | Bleach | Bleach | Male | Second | 8.55 |
| ZW.E03.HLS491 | FG01_1B_T5 | FG01_1B | 11/02/2023 | 144 | T5 | Bleach | Untreated | Female | Ground | 3.47 |
| ZW.E03.HLS492 | FG01_2B_T5 | FG01_2B | 11/02/2023 | 144 | T5 | Bleach | Untreated | Female | Ground | 12 |
| ZW.E03.HLS493 | FG01_3B_T5 | FG01_3B | 11/02/2023 | 144 | T5 | Bleach | Untreated | Female | Ground | 10.8 |
| ZW.E03.HLS494 | FG01_4B_T5 | FG01_4B | 11/02/2023 | 144 | T5 | Bleach | Bleach | Female | Ground | 3.56 |
| ZW.E03.HLS495 | FG01_5B_T5 | FG01_5B | 11/02/2023 | 144 | T5 | Bleach | Bleach | Female | Ground | 12.6 |
| ZW.E03.HLS496 | FG01_6B_T5 | FG01_6B | 11/02/2023 | 144 | T5 | Bleach | Bleach | Female | Ground | 9.82 |
| ZW.E03.HLS497 | MG01_1B_T5 | MG01_1B | 11/02/2023 | 144 | T5 | Bleach | Untreated | Male | Ground | 6.11 |
| ZW.E03.HLS498 | MG01_2B_T5 | MG01_2B | 11/02/2023 | 144 | T5 | Bleach | Bleach | Male | Ground | 0.582 |
| ZW.E03.HLS499 | MG01_3B_T5 | MG01_3B | 11/02/2023 | 144 | T5 | Bleach | Bleach | Male | Ground | 9.45 |
| ZW.E03.HLS500 | F101_1B_T5 | F101_1B | 11/02/2023 | 144 | T5 | Bleach | Untreated | Female | First | Too High |
| ZW.E03.HLS501 | F101_2B_T5 | F101_2B | 11/02/2023 | 144 | T5 | Bleach | Bleach | Female | First | 10.4 |
| ZW.E03.HLS502 | F101_3B_T5 | F101_3B | 11/02/2023 | 144 | T5 | Bleach | Bleach | Female | First | 6.1 |
| ZW.E03.HLS503 | M101_1B_T5 | M101_1B | 11/02/2023 | 144 | T5 | Bleach | Untreated | Male | First | 44.2 |
| ZW.E03.HLS504 | M101_2B_T5 | M101_2B | 11/02/2023 | 144 | T5 | Bleach | Bleach | Male | First | 15.2 |
| ZW.E03.HLS505 | F201_1B_T5 | F201_1B | 11/02/2023 | 144 | T5 | Bleach | Untreated | Female | Second | 17.4 |
| ZW.E03.HLS506 | F201_2B_T5 | F201_2B | 11/02/2023 | 144 | T5 | Bleach | Bleach | Female | Second | 3.93 |
| ZW.E03.HLS507 | F201_3B_T5 | F201_3B | 11/02/2023 | 144 | T5 | Bleach | Bleach | Female | Second | 9.96 |
| ZW.E03.HLS508 | M201_1B_T5 | M201_1B | 11/02/2023 | 144 | T5 | Bleach | Untreated | Male | Second | 15.9 |
| ZW.E03.HLS509 | M201_2B_T5 | M201_2B | 11/02/2023 | 144 | T5 | Bleach | Bleach | Male | Second | 5.54 |
| ZW.E02.HLS003R | FG01_3B_00 | FG01_3B | 23/08/2020 | 1 | 0 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS006R | FG01_6B_00 | FG01_6B | 23/08/2020 | 1 | 0 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS029R | MG01_1B_01 | MG01_1B | 07/10/2020 | 7 | 1 | Timeseries | NA | Male | Ground | NA |
| ZW.E02.HLS037R | M101_2B_01 | M101_2B | 07/10/2020 | 7 | 1 | Timeseries | NA | Male | First | NA |
| ZW.E02.HLS058R | M101_1B_02 | M101_1B | 21/11/2020 | 13 | 2 | Timeseries | NA | Male | First | NA |

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|---|---|---|---|---|---|---|---|---|---|---|
| ZW.E02.HLS064R | M201_1B_02 | M201_1B | 21/11/2020 | 13 | 2 | Timeseries | NA | Male | Second | NA |
| ZW.E02.HLS133R | F101_3B_05 | F101_3B | 01/04/2021 | 33 | 5 | Timeseries | NA | Female | First | NA |
| ZW.E02.HLS159R | M101_2B_06 | M101_2B | 22/05/2021 | 39 | 6 | Timeseries | NA | Male | First | NA |
| ZW.E02.HLS166R | D201_1B_06 | D201_1B | 22/05/2021 | 39 | 6 | Timeseries | NA | Unisex | Second | NA |
| ZW.E03.HLS169 | FG01_1B_07 | FG01_1B | 28/07/2021 | 49 | 7 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS170 | FG01_2B_07 | FG01_2B | 28/07/2021 | 49 | 7 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS171 | FG01_3B_07 | FG01_3B | 28/07/2021 | 49 | 7 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS172 | FG01_4B_07 | FG01_4B | 28/07/2021 | 49 | 7 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS173 | FG01_5B_07 | FG01_5B | 28/07/2021 | 49 | 7 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS174 | FG01_6B_07 | FG01_6B | 28/07/2021 | 49 | 7 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS175 | MG01_1B_07 | MG01_1B | 28/07/2021 | 49 | 7 | Timeseries | NA | Male | Ground | NA |
| ZW.E03.HLS176 | MG01_2B_07 | MG01_2B | 28/07/2021 | 49 | 7 | Timeseries | NA | Male | Ground | NA |
| ZW.E03.HLS177 | MG01_3B_07 | MG01_3B | 28/07/2021 | 49 | 7 | Timeseries | NA | Male | Ground | NA |
| ZW.E03.HLS178 | DG02_1B_07 | DG02_1B | 28/07/2021 | 49 | 7 | Timeseries | NA | Unisex | Ground | NA |
| ZW.E03.HLS179 | F101_1B_07 | F101_1B | 28/07/2021 | 49 | 7 | Timeseries | NA | Female | First | NA |
| ZW.E03.HLS180 | F101_2B_07 | F101_2B | 28/07/2021 | 49 | 7 | Timeseries | NA | Female | First | NA |
| ZW.E03.HLS181 | F101_3B_07 | F101_3B | 28/07/2021 | 49 | 7 | Timeseries | NA | Female | First | NA |
| ZW.E03.HLS182 | M101_1B_07 | M101_1B | 28/07/2021 | 49 | 7 | Timeseries | NA | Male | First | NA |
| ZW.E03.HLS183 | M101_2B_07 | M101_2B | 28/07/2021 | 49 | 7 | Timeseries | NA | Male | First | NA |
| ZW.E03.HLS184 | D101_1B_07 | D101_1B | 28/07/2021 | 49 | 7 | Timeseries | NA | Unisex | First | NA |
| ZW.E03.HLS185 | F201_1B_07 | F201_1B | 28/07/2021 | 49 | 7 | Timeseries | NA | Female | Second | NA |
| ZW.E03.HLS186 | F201_2B_07 | F201_2B | 28/07/2021 | 49 | 7 | Timeseries | NA | Female | Second | NA |
| ZW.E03.HLS187 | F201_3B_07 | F201_3B | 28/07/2021 | 49 | 7 | Timeseries | NA | Female | Second | NA |
| ZW.E03.HLS188 | M201_1B_07 | M201_1B | 28/07/2021 | 49 | 7 | Timeseries | NA | Male | Second | NA |
| ZW.E03.HLS189 | M201_2B_07 | M201_2B | 28/07/2021 | 49 | 7 | Timeseries | NA | Male | Second | NA |
| ZW.E03.HLS190 | D201_1B_07 | D201_1B | 28/07/2021 | 49 | 7 | Timeseries | NA | Unisex | Second | NA |
| ZW.E03.HLS191 | FG01_1B_08 | FG01_1B | 08/09/2021 | 55 | 8 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS192 | FG01_2B_08 | FG01_2B | 08/09/2021 | 55 | 8 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS193 | FG01_3B_08 | FG01_3B | 08/09/2021 | 55 | 8 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS194 | FG01_4B_08 | FG01_4B | 08/09/2021 | 55 | 8 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS195 | FG01_5B_08 | FG01_5B | 08/09/2021 | 55 | 8 | Timeseries | NA | Female | Ground | NA |

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|---|---|---|---|---|---|---|---|---|---|---|
| ZW.E03.HLS196 | FG01_6B_08 | FG01_6B | 08/09/2021 | 55 | 8 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS197 | MG01_1B_08 | MG01_1B | 08/09/2021 | 55 | 8 | Timeseries | NA | Male | Ground | NA |
| ZW.E03.HLS198 | MG01_2B_08 | MG01_2B | 08/09/2021 | 55 | 8 | Timeseries | NA | Male | Ground | NA |
| ZW.E03.HLS199 | MG01_3B_08 | MG01_3B | 08/09/2021 | 55 | 8 | Timeseries | NA | Male | Ground | NA |
| ZW.E03.HLS200 | DG02_1B_08 | DG02_1B | 08/09/2021 | 55 | 8 | Timeseries | NA | Unisex | Ground | NA |
| ZW.E03.HLS201 | F101_1B_08 | F101_1B | 08/09/2021 | 55 | 8 | Timeseries | NA | Female | First | NA |
| ZW.E03.HLS202 | F101_2B_08 | F101_2B | 08/09/2021 | 55 | 8 | Timeseries | NA | Female | First | NA |
| ZW.E03.HLS203 | F101_3B_08 | F101_3B | 08/09/2021 | 55 | 8 | Timeseries | NA | Female | First | NA |
| ZW.E03.HLS204 | M101_1B_08 | M101_1B | 08/09/2021 | 55 | 8 | Timeseries | NA | Male | First | NA |
| ZW.E03.HLS205 | M101_2B_08 | M101_2B | 08/09/2021 | 55 | 8 | Timeseries | NA | Male | First | NA |
| ZW.E03.HLS206 | D101_1B_08 | D101_1B | 08/09/2021 | 55 | 8 | Timeseries | NA | Unisex | First | NA |
| ZW.E03.HLS207 | F201_1B_08 | F201_1B | 08/09/2021 | 55 | 8 | Timeseries | NA | Female | Second | NA |
| ZW.E03.HLS208 | F201_2B_08 | F201_2B | 08/09/2021 | 55 | 8 | Timeseries | NA | Female | Second | NA |
| ZW.E03.HLS209 | F201_3B_08 | F201_3B | 08/09/2021 | 55 | 8 | Timeseries | NA | Female | Second | NA |
| ZW.E03.HLS210 | M201_1B_08 | M201_1B | 08/09/2021 | 55 | 8 | Timeseries | NA | Male | Second | NA |
| ZW.E03.HLS211 | M201_2B_08 | M201_2B | 08/09/2021 | 55 | 8 | Timeseries | NA | Male | Second | NA |
| ZW.E03.HLS212 | D201_1B_08 | D201_1B | 08/09/2021 | 55 | 8 | Timeseries | NA | Unisex | Second | NA |
| ZW.E03.HLS213 | FG01_1B_09 | FG01_1B | 20/10/2021 | 61 | 9 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS214 | FG01_2B_09 | FG01_2B | 20/10/2021 | 61 | 9 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS215 | FG01_3B_09 | FG01_3B | 20/10/2021 | 61 | 9 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS216 | FG01_4B_09 | FG01_4B | 20/10/2021 | 61 | 9 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS217 | FG01_5B_09 | FG01_5B | 20/10/2021 | 61 | 9 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS218 | FG01_6B_09 | FG01_6B | 20/10/2021 | 61 | 9 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS219 | MG01_1B_09 | MG01_1B | 20/10/2021 | 61 | 9 | Timeseries | NA | Male | Ground | NA |
| ZW.E03.HLS220 | MG01_2B_09 | MG01_2B | 20/10/2021 | 61 | 9 | Timeseries | NA | Male | Ground | NA |
| ZW.E03.HLS221 | MG01_3B_09 | MG01_3B | 20/10/2021 | 61 | 9 | Timeseries | NA | Male | Ground | NA |
| ZW.E03.HLS222 | DG02_1B_09 | DG02_1B | 20/10/2021 | 61 | 9 | Timeseries | NA | Unisex | Ground | NA |
| ZW.E03.HLS223 | F101_1B_09 | F101_1B | 20/10/2021 | 61 | 9 | Timeseries | NA | Female | First | NA |
| ZW.E03.HLS224 | F101_2B_09 | F101_2B | 20/10/2021 | 61 | 9 | Timeseries | NA | Female | First | NA |
| ZW.E03.HLS225 | F101_3B_09 | F101_3B | 20/10/2021 | 61 | 9 | Timeseries | NA | Female | First | NA |
| ZW.E03.HLS226 | M101_1B_09 | M101_1B | 20/10/2021 | 61 | 9 | Timeseries | NA | Male | First | NA |

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|---|---|---|---|---|---|---|---|---|---|---|
| ZW.E03.HLS227 | M101_2B_09 | M101_2B | 20/10/2021 | 61 | 9 | Timeseries | NA | Male | First | NA |
| ZW.E03.HLS228 | D101_1B_09 | D101_1B | 20/10/2021 | 61 | 9 | Timeseries | NA | Unisex | First | NA |
| ZW.E03.HLS229 | F201_1B_09 | F201_1B | 20/10/2021 | 61 | 9 | Timeseries | NA | Female | Second | NA |
| ZW.E03.HLS230 | F201_2B_09 | F201_2B | 20/10/2021 | 61 | 9 | Timeseries | NA | Female | Second | NA |
| ZW.E03.HLS231 | F201_3B_09 | F201_3B | 20/10/2021 | 61 | 9 | Timeseries | NA | Female | Second | NA |
| ZW.E03.HLS232 | M201_1B_09 | M201_1B | 20/10/2021 | 61 | 9 | Timeseries | NA | Male | Second | NA |
| ZW.E03.HLS233 | M201_2B_09 | M201_2B | 20/10/2021 | 61 | 9 | Timeseries | NA | Male | Second | NA |
| ZW.E03.HLS234 | D201_1B_09 | D201_1B | 20/10/2021 | 61 | 9 | Timeseries | NA | Unisex | Second | NA |
| ZW.E03.HLS235 | FG01_1B_10 | FG01_1B | 11/12/2021 | 68 | 10 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS236 | FG01_2B_10 | FG01_2B | 11/12/2021 | 68 | 10 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS237 | FG01_3B_10 | FG01_3B | 11/12/2021 | 68 | 10 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS238 | FG01_4B_10 | FG01_4B | 11/12/2021 | 68 | 10 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS239 | FG01_5B_10 | FG01_5B | 11/12/2021 | 68 | 10 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS240 | FG01_6B_10 | FG01_6B | 11/12/2021 | 68 | 10 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS241 | MG01_1B_10 | MG01_1B | 11/12/2021 | 68 | 10 | Timeseries | NA | Male | Ground | NA |
| ZW.E03.HLS242 | MG01_2B_10 | MG01_2B | 11/12/2021 | 68 | 10 | Timeseries | NA | Male | Ground | NA |
| ZW.E03.HLS243 | MG01_3B_10 | MG01_3B | 11/12/2021 | 68 | 10 | Timeseries | NA | Male | Ground | NA |
| ZW.E03.HLS244 | DG02_1B_10 | DG02_1B | 11/12/2021 | 68 | 10 | Timeseries | NA | Unisex | Ground | NA |
| ZW.E03.HLS245 | F101_1B_10 | F101_1B | 11/12/2021 | 68 | 10 | Timeseries | NA | Female | First | NA |
| ZW.E03.HLS246 | F101_2B_10 | F101_2B | 11/12/2021 | 68 | 10 | Timeseries | NA | Female | First | NA |
| ZW.E03.HLS247 | F101_3B_10 | F101_3B | 11/12/2021 | 68 | 10 | Timeseries | NA | Female | First | NA |
| ZW.E03.HLS248 | M101_1B_10 | M101_1B | 11/12/2021 | 68 | 10 | Timeseries | NA | Male | First | NA |
| ZW.E03.HLS249 | M101_2B_10 | M101_2B | 11/12/2021 | 68 | 10 | Timeseries | NA | Male | First | NA |
| ZW.E03.HLS250 | D101_1B_10 | D101_1B | 11/12/2021 | 68 | 10 | Timeseries | NA | Unisex | First | NA |
| ZW.E03.HLS251 | F201_1B_10 | F201_1B | 11/12/2021 | 68 | 10 | Timeseries | NA | Female | Second | NA |
| ZW.E03.HLS252 | F201_2B_10 | F201_2B | 11/12/2021 | 68 | 10 | Timeseries | NA | Female | Second | NA |
| ZW.E03.HLS253 | F201_3B_10 | F201_3B | 11/12/2021 | 68 | 10 | Timeseries | NA | Female | Second | NA |
| ZW.E03.HLS254 | M201_1B_10 | M201_1B | 11/12/2021 | 68 | 10 | Timeseries | NA | Male | Second | NA |
| ZW.E03.HLS255 | M201_2B_10 | M201_2B | 11/12/2021 | 68 | 10 | Timeseries | NA | Male | Second | NA |
| ZW.E03.HLS256 | D201_1B_10 | D201_1B | 11/12/2021 | 68 | 10 | Timeseries | NA | Unisex | Second | NA |
| ZW.E03.HLS259 | FG01_1B_11 | FG01_1B | 22/01/2022 | 74 | 11 | Timeseries | NA | Female | Ground | NA |

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|---|---|---|---|---|---|---|---|---|---|---|
| ZW.E03.HLS260 | FG01_2B_11 | FG01_2B | 22/01/2022 | 74 | 11 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS261 | FG01_3B_11 | FG01_3B | 22/01/2022 | 74 | 11 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS262 | FG01_4B_11 | FG01_4B | 22/01/2022 | 74 | 11 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS263 | FG01_5B_11 | FG01_5B | 22/01/2022 | 74 | 11 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS264 | FG01_6B_11 | FG01_6B | 22/01/2022 | 74 | 11 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS265 | MG01_1B_11 | MG01_1B | 22/01/2022 | 74 | 11 | Timeseries | NA | Male | Ground | NA |
| ZW.E03.HLS266 | MG01_2B_11 | MG01_2B | 22/01/2022 | 74 | 11 | Timeseries | NA | Male | Ground | NA |
| ZW.E03.HLS267 | MG01_3B_11 | MG01_3B | 22/01/2022 | 74 | 11 | Timeseries | NA | Male | Ground | NA |
| ZW.E03.HLS268 | DG02_1B_11 | DG02_1B | 22/01/2022 | 74 | 11 | Timeseries | NA | Unisex | Ground | NA |
| ZW.E03.HLS269 | F101_1B_11 | F101_1B | 22/01/2022 | 74 | 11 | Timeseries | NA | Female | First | NA |
| ZW.E03.HLS270 | F101_2B_11 | F101_2B | 22/01/2022 | 74 | 11 | Timeseries | NA | Female | First | NA |
| ZW.E03.HLS271 | F101_3B_11 | F101_3B | 22/01/2022 | 74 | 11 | Timeseries | NA | Female | First | NA |
| ZW.E03.HLS272 | M101_1B_11 | M101_1B | 22/01/2022 | 74 | 11 | Timeseries | NA | Male | First | NA |
| ZW.E03.HLS273 | M101_2B_11 | M101_2B | 22/01/2022 | 74 | 11 | Timeseries | NA | Male | First | NA |
| ZW.E03.HLS274 | D101_1B_11 | D101_1B | 22/01/2022 | 74 | 11 | Timeseries | NA | Unisex | First | NA |
| ZW.E03.HLS275 | F201_1B_11 | F201_1B | 22/01/2022 | 74 | 11 | Timeseries | NA | Female | Second | NA |
| ZW.E03.HLS276 | F201_2B_11 | F201_2B | 22/01/2022 | 74 | 11 | Timeseries | NA | Female | Second | NA |
| ZW.E03.HLS277 | F201_3B_11 | F201_3B | 22/01/2022 | 74 | 11 | Timeseries | NA | Female | Second | NA |
| ZW.E03.HLS278 | M201_1B_11 | M201_1B | 22/01/2022 | 74 | 11 | Timeseries | NA | Male | Second | NA |
| ZW.E03.HLS279 | M201_2B_11 | M201_2B | 22/01/2022 | 74 | 11 | Timeseries | NA | Male | Second | NA |
| ZW.E03.HLS280 | D201_1B_11 | D201_1B | 22/01/2022 | 74 | 11 | Timeseries | NA | Unisex | Second | NA |
| ZW.E03.HLS281 | FG01_1B_12 | FG01_1B | 05/02/2022 | 76 | 12 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS282 | FG01_2B_12 | FG01_2B | 05/02/2022 | 76 | 12 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS283 | FG01_3B_12 | FG01_3B | 05/02/2022 | 76 | 12 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS284 | FG01_4B_12 | FG01_4B | 05/02/2022 | 76 | 12 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS285 | FG01_5B_12 | FG01_5B | 05/02/2022 | 76 | 12 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS286 | FG01_6B_12 | FG01_6B | 05/02/2022 | 76 | 12 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS287 | MG01_1B_12 | MG01_1B | 05/02/2022 | 76 | 12 | Timeseries | NA | Male | Ground | NA |
| ZW.E03.HLS288 | MG01_2B_12 | MG01_2B | 05/02/2022 | 76 | 12 | Timeseries | NA | Male | Ground | NA |
| ZW.E03.HLS289 | MG01_3B_12 | MG01_3B | 05/02/2022 | 76 | 12 | Timeseries | NA | Male | Ground | NA |
| ZW.E03.HLS290 | DG02_1B_12 | DG02_1B | 05/02/2022 | 76 | 12 | Timeseries | NA | Unisex | Ground | NA |

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|---|---|---|---|---|---|---|---|---|---|---|
| ZW.E03.HLS291 | F101_1B_12 | F101_1B | 05/02/2022 | 76 | 12 | Timeseries | NA | Female | First | NA |
| ZW.E03.HLS292 | F101_2B_12 | F101_2B | 05/02/2022 | 76 | 12 | Timeseries | NA | Female | First | NA |
| ZW.E03.HLS293 | F101_3B_12 | F101_3B | 05/02/2022 | 76 | 12 | Timeseries | NA | Female | First | NA |
| ZW.E03.HLS294 | M101_1B_12 | M101_1B | 05/02/2022 | 76 | 12 | Timeseries | NA | Male | First | NA |
| ZW.E03.HLS295 | M101_2B_12 | M101_2B | 05/02/2022 | 76 | 12 | Timeseries | NA | Male | First | NA |
| ZW.E03.HLS296 | D101_1B_12 | D101_1B | 05/02/2022 | 76 | 12 | Timeseries | NA | Unisex | First | NA |
| ZW.E03.HLS297 | F201_1B_12 | F201_1B | 05/02/2022 | 76 | 12 | Timeseries | NA | Female | Second | NA |
| ZW.E03.HLS298 | F201_2B_12 | F201_2B | 05/02/2022 | 76 | 12 | Timeseries | NA | Female | Second | NA |
| ZW.E03.HLS299 | F201_3B_12 | F201_3B | 05/02/2022 | 76 | 12 | Timeseries | NA | Female | Second | NA |
| ZW.E03.HLS300 | M201_1B_12 | M201_1B | 05/02/2022 | 76 | 12 | Timeseries | NA | Male | Second | NA |
| ZW.E03.HLS301 | M201_2B_12 | M201_2B | 05/02/2022 | 76 | 12 | Timeseries | NA | Male | Second | NA |
| ZW.E03.HLS302 | D201_1B_12 | D201_1B | 05/02/2022 | 76 | 12 | Timeseries | NA | Unisex | Second | NA |
| ZW.E03.HLS303 | FG01_1B_13 | FG01_1B | 16/04/2022 | 86 | 13 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS304 | FG01_2B_13 | FG01_2B | 16/04/2022 | 86 | 13 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS305 | FG01_3B_13 | FG01_3B | 16/04/2022 | 86 | 13 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS306 | FG01_4B_13 | FG01_4B | 16/04/2022 | 86 | 13 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS307 | FG01_5B_13 | FG01_5B | 16/04/2022 | 86 | 13 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS308 | FG01_6B_13 | FG01_6B | 16/04/2022 | 86 | 13 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS309 | MG01_1B_13 | MG01_1B | 16/04/2022 | 86 | 13 | Timeseries | NA | Male | Ground | NA |
| ZW.E03.HLS310 | MG01_2B_13 | MG01_2B | 16/04/2022 | 86 | 13 | Timeseries | NA | Male | Ground | NA |
| ZW.E03.HLS311 | MG01_3B_13 | MG01_3B | 16/04/2022 | 86 | 13 | Timeseries | NA | Male | Ground | NA |
| ZW.E03.HLS312 | DG02_1B_13 | DG02_1B | 16/04/2022 | 86 | 13 | Timeseries | NA | Unisex | Ground | NA |
| ZW.E03.HLS313 | F101_1B_13 | F101_1B | 16/04/2022 | 86 | 13 | Timeseries | NA | Female | First | NA |
| ZW.E03.HLS314 | F101_2B_13 | F101_2B | 16/04/2022 | 86 | 13 | Timeseries | NA | Female | First | NA |
| ZW.E03.HLS315 | F101_3B_13 | F101_3B | 16/04/2022 | 86 | 13 | Timeseries | NA | Female | First | NA |
| ZW.E03.HLS316 | M101_1B_13 | M101_1B | 16/04/2022 | 86 | 13 | Timeseries | NA | Male | First | NA |
| ZW.E03.HLS317 | M101_2B_13 | M101_2B | 16/04/2022 | 86 | 13 | Timeseries | NA | Male | First | NA |
| ZW.E03.HLS318 | D101_1B_13 | D101_1B | 16/04/2022 | 86 | 13 | Timeseries | NA | Unisex | First | NA |
| ZW.E03.HLS319 | F201_1B_13 | F201_1B | 16/04/2022 | 86 | 13 | Timeseries | NA | Female | Second | NA |
| ZW.E03.HLS320 | F201_2B_13 | F201_2B | 16/04/2022 | 86 | 13 | Timeseries | NA | Female | Second | NA |
| ZW.E03.HLS321 | F201_3B_13 | F201_3B | 16/04/2022 | 86 | 13 | Timeseries | NA | Female | Second | NA |

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|---|---|---|---|---|---|---|---|---|---|---|
| ZW.E03.HLS322 | M201_1B_13 | M201_1B | 16/04/2022 | 86 | 13 | Timeseries | NA | Male | Second | NA |
| ZW.E03.HLS323 | M201_2B_13 | M201_2B | 16/04/2022 | 86 | 13 | Timeseries | NA | Male | Second | NA |
| ZW.E03.HLS324 | D201_1B_13 | D201_1B | 16/04/2022 | 86 | 13 | Timeseries | NA | Unisex | Second | NA |
| ZW.E03.HLS325 | FG01_1B_14 | FG01_1B | 29/05/2022 | 93 | 14 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS326 | FG01_2B_14 | FG01_2B | 29/05/2022 | 93 | 14 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS327 | FG01_3B_14 | FG01_3B | 29/05/2022 | 93 | 14 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS328 | FG01_4B_14 | FG01_4B | 29/05/2022 | 93 | 14 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS329 | FG01_5B_14 | FG01_5B | 29/05/2022 | 93 | 14 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS330 | FG01_6B_14 | FG01_6B | 29/05/2022 | 93 | 14 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS331 | MG01_1B_14 | MG01_1B | 29/05/2022 | 93 | 14 | Timeseries | NA | Male | Ground | NA |
| ZW.E03.HLS332 | MG01_2B_14 | MG01_2B | 29/05/2022 | 93 | 14 | Timeseries | NA | Male | Ground | NA |
| ZW.E03.HLS333 | MG01_3B_14 | MG01_3B | 29/05/2022 | 93 | 14 | Timeseries | NA | Male | Ground | NA |
| ZW.E03.HLS334 | DG02_1B_14 | DG02_1B | 29/05/2022 | 93 | 14 | Timeseries | NA | Unisex | Ground | NA |
| ZW.E03.HLS335 | F101_1B_14 | F101_1B | 29/05/2022 | 93 | 14 | Timeseries | NA | Female | First | NA |
| ZW.E03.HLS336 | F101_2B_14 | F101_2B | 29/05/2022 | 93 | 14 | Timeseries | NA | Female | First | NA |
| ZW.E03.HLS337 | F101_3B_14 | F101_3B | 29/05/2022 | 93 | 14 | Timeseries | NA | Female | First | NA |
| ZW.E03.HLS338 | M101_1B_14 | M101_1B | 29/05/2022 | 93 | 14 | Timeseries | NA | Male | First | NA |
| ZW.E03.HLS339 | M101_2B_14 | M101_2B | 29/05/2022 | 93 | 14 | Timeseries | NA | Male | First | NA |
| ZW.E03.HLS340 | D101_1B_14 | D101_1B | 29/05/2022 | 93 | 14 | Timeseries | NA | Unisex | First | NA |
| ZW.E03.HLS341 | F201_1B_14 | F201_1B | 29/05/2022 | 93 | 14 | Timeseries | NA | Female | Second | NA |
| ZW.E03.HLS342 | F201_2B_14 | F201_2B | 29/05/2022 | 93 | 14 | Timeseries | NA | Female | Second | NA |
| ZW.E03.HLS343 | F201_3B_14 | F201_3B | 29/05/2022 | 93 | 14 | Timeseries | NA | Female | Second | NA |
| ZW.E03.HLS344 | M201_1B_14 | M201_1B | 29/05/2022 | 93 | 14 | Timeseries | NA | Male | Second | NA |
| ZW.E03.HLS345 | M201_2B_14 | M201_2B | 29/05/2022 | 93 | 14 | Timeseries | NA | Male | Second | NA |
| ZW.E03.HLS346 | D201_1B_14 | D201_1B | 29/05/2022 | 93 | 14 | Timeseries | NA | Unisex | Second | NA |
| ZW.E03.HLS347 | FG01_1B_15 | FG01_1B | 09/07/2022 | 98 | 15 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS348 | FG01_2B_15 | FG01_2B | 09/07/2022 | 98 | 15 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS349 | FG01_3B_15 | FG01_3B | 09/07/2022 | 98 | 15 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS350 | FG01_4B_15 | FG01_4B | 09/07/2022 | 98 | 15 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS351 | FG01_5B_15 | FG01_5B | 09/07/2022 | 98 | 15 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS352 | FG01_6B_15 | FG01_6B | 09/07/2022 | 98 | 15 | Timeseries | NA | Female | Ground | NA |

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|---|---|---|---|---|---|---|---|---|---|---|
| ZW.E03.HLS353 | MG01_1B_15 | MG01_1B | 09/07/2022 | 98 | 15 | Timeseries | NA | Male | Ground | NA |
| ZW.E03.HLS354 | MG01_2B_15 | MG01_2B | 09/07/2022 | 98 | 15 | Timeseries | NA | Male | Ground | NA |
| ZW.E03.HLS355 | MG01_3B_15 | MG01_3B | 09/07/2022 | 98 | 15 | Timeseries | NA | Male | Ground | NA |
| ZW.E03.HLS356 | DG02_1B_15 | DG02_1B | 09/07/2022 | 98 | 15 | Timeseries | NA | Unisex | Ground | NA |
| ZW.E03.HLS357 | F101_1B_15 | F101_1B | 09/07/2022 | 98 | 15 | Timeseries | NA | Female | First | NA |
| ZW.E03.HLS358 | F101_2B_15 | F101_2B | 09/07/2022 | 98 | 15 | Timeseries | NA | Female | First | NA |
| ZW.E03.HLS359 | F101_3B_15 | F101_3B | 09/07/2022 | 98 | 15 | Timeseries | NA | Female | First | NA |
| ZW.E03.HLS360 | M101_1B_15 | M101_1B | 09/07/2022 | 98 | 15 | Timeseries | NA | Male | First | NA |
| ZW.E03.HLS361 | M101_2B_15 | M101_2B | 09/07/2022 | 98 | 15 | Timeseries | NA | Male | First | NA |
| ZW.E03.HLS362 | D101_1B_15 | D101_1B | 09/07/2022 | 98 | 15 | Timeseries | NA | Unisex | First | NA |
| ZW.E03.HLS363 | F201_1B_15 | F201_1B | 09/07/2022 | 98 | 15 | Timeseries | NA | Female | Second | NA |
| ZW.E03.HLS364 | F201_2B_15 | F201_2B | 09/07/2022 | 98 | 15 | Timeseries | NA | Female | Second | NA |
| ZW.E03.HLS365 | F201_3B_15 | F201_3B | 09/07/2022 | 98 | 15 | Timeseries | NA | Female | Second | NA |
| ZW.E03.HLS366 | M201_1B_15 | M201_1B | 09/07/2022 | 98 | 15 | Timeseries | NA | Male | Second | NA |
| ZW.E03.HLS367 | M201_2B_15 | M201_2B | 09/07/2022 | 98 | 15 | Timeseries | NA | Male | Second | NA |
| ZW.E03.HLS368 | D201_1B_15 | D201_1B | 09/07/2022 | 98 | 15 | Timeseries | NA | Unisex | Second | NA |
| ZW.E03.HLS369 | FG01_1B_16 | FG01_1B | 04/09/2022 | 107 | 16 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS370 | FG01_2B_16 | FG01_2B | 04/09/2022 | 107 | 16 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS371 | FG01_3B_16 | FG01_3B | 04/09/2022 | 107 | 16 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS372 | FG01_4B_16 | FG01_4B | 04/09/2022 | 107 | 16 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS373 | FG01_5B_16 | FG01_5B | 04/09/2022 | 107 | 16 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS374 | FG01_6B_16 | FG01_6B | 04/09/2022 | 107 | 16 | Timeseries | NA | Female | Ground | NA |
| ZW.E03.HLS375 | MG01_1B_16 | MG01_1B | 04/09/2022 | 107 | 16 | Timeseries | NA | Male | Ground | NA |
| ZW.E03.HLS376 | MG01_2B_16 | MG01_2B | 04/09/2022 | 107 | 16 | Timeseries | NA | Male | Ground | NA |
| ZW.E03.HLS377 | MG01_3B_16 | MG01_3B | 04/09/2022 | 107 | 16 | Timeseries | NA | Male | Ground | NA |
| ZW.E03.HLS378 | DG02_1B_16 | DG02_1B | 04/09/2022 | 107 | 16 | Timeseries | NA | Unisex | Ground | NA |
| ZW.E03.HLS379 | F101_1B_16 | F101_1B | 04/09/2022 | 107 | 16 | Timeseries | NA | Female | First | NA |
| ZW.E03.HLS380 | F101_2B_16 | F101_2B | 04/09/2022 | 107 | 16 | Timeseries | NA | Female | First | NA |
| ZW.E03.HLS381 | F101_3B_16 | F101_3B | 04/09/2022 | 107 | 16 | Timeseries | NA | Female | First | NA |
| ZW.E03.HLS382 | M101_1B_16 | M101_1B | 04/09/2022 | 107 | 16 | Timeseries | NA | Male | First | NA |
| ZW.E03.HLS383 | M101_2B_16 | M101_2B | 04/09/2022 | 107 | 16 | Timeseries | NA | Male | First | NA |

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|---|---|---|---|---|---|---|---|---|---|---|
| ZW.E03.HLS384 | D101_1B_16 | D101_1B | 04/09/2022 | 107 | 16 | Timeseries | NA | Unisex | First | NA |
| ZW.E03.HLS385 | F201_1B_16 | F201_1B | 04/09/2022 | 107 | 16 | Timeseries | NA | Female | Second | NA |
| ZW.E03.HLS386 | F201_2B_16 | F201_2B | 04/09/2022 | 107 | 16 | Timeseries | NA | Female | Second | NA |
| ZW.E03.HLS387 | F201_3B_16 | F201_3B | 04/09/2022 | 107 | 16 | Timeseries | NA | Female | Second | NA |
| ZW.E03.HLS388 | M201_1B_16 | M201_1B | 04/09/2022 | 107 | 16 | Timeseries | NA | Male | Second | NA |
| ZW.E03.HLS389 | M201_2B_16 | M201_2B | 04/09/2022 | 107 | 16 | Timeseries | NA | Male | Second | NA |
| ZW.E03.HLS390 | D201_1B_16 | D201_1B | 04/09/2022 | 107 | 16 | Timeseries | NA | Unisex | Second | NA |
| ZW.E02.HLS1 | FG01_1B_00 | FG01_1B | 23/08/2020 | 1 | 0 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS10 | DG02_1B_00 | DG02_1B | 23/08/2020 | 1 | 0 | Timeseries | NA | Unisex | Ground | NA |
| ZW.E02.HLS100 | F101_2B_04 | F101_2B | 19/02/2021 | 27 | 4 | Timeseries | NA | Female | First | NA |
| ZW.E02.HLS101 | F101_3B_04 | F101_3B | 19/02/2021 | 27 | 4 | Timeseries | NA | Female | First | NA |
| ZW.E02.HLS102 | M101_1B_04 | M101_1B | 19/02/2021 | 27 | 4 | Timeseries | NA | Male | First | NA |
| ZW.E02.HLS103 | M101_2B_04 | M101_2B | 19/02/2021 | 27 | 4 | Timeseries | NA | Male | First | NA |
| ZW.E02.HLS104 | D101_1B_04 | D101_1B | 19/02/2021 | 27 | 4 | Timeseries | NA | Unisex | First | NA |
| ZW.E02.HLS105 | F201_1B_04 | F201_1B | 19/02/2021 | 27 | 4 | Timeseries | NA | Female | Second | NA |
| ZW.E02.HLS106 | F201_2B_04 | F201_2B | 19/02/2021 | 27 | 4 | Timeseries | NA | Female | Second | NA |
| ZW.E02.HLS107 | F201_3B_04 | F201_3B | 19/02/2021 | 27 | 4 | Timeseries | NA | Female | Second | NA |
| ZW.E02.HLS108 | M201_1B_04 | M201_1B | 19/02/2021 | 27 | 4 | Timeseries | NA | Male | Second | NA |
| ZW.E02.HLS109 | M201_2B_04 | M201_2B | 19/02/2021 | 27 | 4 | Timeseries | NA | Male | Second | NA |
| ZW.E02.HLS11 | F101_1B_00 | F101_1B | 23/08/2020 | 1 | 0 | Timeseries | NA | Female | First | NA |
| ZW.E02.HLS110 | D201_1B_04 | D201_1B | 19/02/2021 | 27 | 4 | Timeseries | NA | Unisex | Second | NA |
| ZW.E02.HLS12 | F101_2B_00 | F101_2B | 23/08/2020 | 1 | 0 | Timeseries | NA | Female | First | NA |
| ZW.E02.HLS121 | FG01_1B_05 | FG01_1B | 01/04/2021 | 33 | 5 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS122 | FG01_2B_05 | FG01_2B | 01/04/2021 | 33 | 5 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS123 | FG01_3B_05 | FG01_3B | 01/04/2021 | 33 | 5 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS124 | FG01_4B_05 | FG01_4B | 01/04/2021 | 33 | 5 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS125 | FG01_5B_05 | FG01_5B | 01/04/2021 | 33 | 5 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS126 | FG01_6B_05 | FG01_6B | 01/04/2021 | 33 | 5 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS127 | MG01_1B_05 | MG01_1B | 01/04/2021 | 33 | 5 | Timeseries | NA | Male | Ground | NA |
| ZW.E02.HLS128 | MG01_2B_05 | MG01_2B | 01/04/2021 | 33 | 5 | Timeseries | NA | Male | Ground | NA |
| ZW.E02.HLS129 | MG01_3B_05 | MG01_3B | 01/04/2021 | 33 | 5 | Timeseries | NA | Male | Ground | NA |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| ZW.E02.HLS13 | F101_3B_00 | F101_3B | 23/08/2020 | | 1 | 0 | Timeseries | NA | Female | First | NA |
| ZW.E02.HLS130 | DG02_1B_05 | DG02_1B | 01/04/2021 | | 33 | 5 | Timeseries | NA | Unisex | Ground | NA |
| ZW.E02.HLS131 | F101_1B_05 | F101_1B | 01/04/2021 | | 33 | 5 | Timeseries | NA | Female | First | NA |
| ZW.E02.HLS132 | F101_2B_05 | F101_2B | 01/04/2021 | | 33 | 5 | Timeseries | NA | Female | First | NA |
| ZW.E02.HLS133 | F101_3B_05 | F101_3B | 01/04/2021 | | 33 | 5 | Timeseries | NA | Female | First | NA |
| ZW.E02.HLS134 | M101_1B_05 | M101_1B | 01/04/2021 | | 33 | 5 | Timeseries | NA | Male | First | NA |
| ZW.E02.HLS135 | M101_2B_05 | M101_2B | 01/04/2021 | | 33 | 5 | Timeseries | NA | Male | First | NA |
| ZW.E02.HLS136 | D101_1B_05 | D101_1B | 01/04/2021 | | 33 | 5 | Timeseries | NA | Unisex | First | NA |
| ZW.E02.HLS137 | F201_1B_05 | F201_1B | 01/04/2021 | | 33 | 5 | Timeseries | NA | Female | Second | NA |
| ZW.E02.HLS138 | F201_2B_05 | F201_2B | 01/04/2021 | | 33 | 5 | Timeseries | NA | Female | Second | NA |
| ZW.E02.HLS139 | F201_3B_05 | F201_3B | 01/04/2021 | | 33 | 5 | Timeseries | NA | Female | Second | NA |
| ZW.E02.HLS14 | M101_1B_00 | M101_1B | 23/08/2020 | | 1 | 0 | Timeseries | NA | Male | First | NA |
| ZW.E02.HLS140 | M201_1B_05 | M201_1B | 01/04/2021 | | 33 | 5 | Timeseries | NA | Male | Second | NA |
| ZW.E02.HLS141 | M201_2B_05 | M201_2B | 01/04/2021 | | 33 | 5 | Timeseries | NA | Male | Second | NA |
| ZW.E02.HLS142 | D201_1B_05 | D201_1B | 01/04/2021 | | 33 | 5 | Timeseries | NA | Unisex | Second | NA |
| ZW.E02.HLS144 | HLS_Water | HLS_Water | 01/04/2021 | NA | | Tap_water | Timeseries | NA | Source | Source | NA |
| ZW.E02.HLS145 | FG01_1B_06 | FG01_1B | 22/05/2021 | | 39 | 6 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS146 | FG01_2B_06 | FG01_2B | 22/05/2021 | | 39 | 6 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS147 | FG01_3B_06 | FG01_3B | 22/05/2021 | | 39 | 6 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS148 | FG01_4B_06 | FG01_4B | 22/05/2021 | | 39 | 6 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS149 | FG01_5B_06 | FG01_5B | 22/05/2021 | | 39 | 6 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS15 | M101_2B_00 | M101_2B | 23/08/2020 | | 1 | 0 | Timeseries | NA | Male | First | NA |
| ZW.E02.HLS150 | FG01_6B_06 | FG01_6B | 22/05/2021 | | 39 | 6 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS151 | MG01_1B_06 | MG01_1B | 22/05/2021 | | 39 | 6 | Timeseries | NA | Male | Ground | NA |
| ZW.E02.HLS152 | MG01_2B_06 | MG01_2B | 22/05/2021 | | 39 | 6 | Timeseries | NA | Male | Ground | NA |
| ZW.E02.HLS153 | MG01_3B_06 | MG01_3B | 22/05/2021 | | 39 | 6 | Timeseries | NA | Male | Ground | NA |
| ZW.E02.HLS154 | DG02_1B_06 | DG02_1B | 22/05/2021 | | 39 | 6 | Timeseries | NA | Unisex | Ground | NA |
| ZW.E02.HLS155 | F101_1B_06 | F101_1B | 22/05/2021 | | 39 | 6 | Timeseries | NA | Female | First | NA |
| ZW.E02.HLS156 | F101_2B_06 | F101_2B | 22/05/2021 | | 39 | 6 | Timeseries | NA | Female | First | NA |
| ZW.E02.HLS157 | F101_3B_06 | F101_3B | 22/05/2021 | | 39 | 6 | Timeseries | NA | Female | First | NA |
| ZW.E02.HLS158 | M101_1B_06 | M101_1B | 22/05/2021 | | 39 | 6 | Timeseries | NA | Male | First | NA |

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|---|---|---|---|---|---|---|---|---|---|---|
| ZW.E02.HLS159 | M101_2B_06 | M101_2B | 22/05/2021 | 39 | 6 | Timeseries | NA | Male | First | NA |
| ZW.E02.HLS16 | D101_1B_00 | D101_1B | 23/08/2020 | 1 | 0 | Timeseries | NA | Unisex | First | NA |
| ZW.E02.HLS160 | D101_1B_06 | D101_1B | 22/05/2021 | 39 | 6 | Timeseries | NA | Unisex | First | NA |
| ZW.E02.HLS161 | F201_1B_06 | F201_1B | 22/05/2021 | 39 | 6 | Timeseries | NA | Female | Second | NA |
| ZW.E02.HLS162 | F201_2B_06 | F201_2B | 22/05/2021 | 39 | 6 | Timeseries | NA | Female | Second | NA |
| ZW.E02.HLS163 | F201_3B_06 | F201_3B | 22/05/2021 | 39 | 6 | Timeseries | NA | Female | Second | NA |
| ZW.E02.HLS164 | M201_1B_06 | M201_1B | 22/05/2021 | 39 | 6 | Timeseries | NA | Male | Second | NA |
| ZW.E02.HLS165 | M201_2B_06 | M201_2B | 22/05/2021 | 39 | 6 | Timeseries | NA | Male | Second | NA |
| ZW.E02.HLS166 | D201_1B_06 | D201_1B | 22/05/2021 | 39 | 6 | Timeseries | NA | Unisex | Second | NA |
| ZW.E02.HLS17 | F201_1B_00 | F201_1B | 23/08/2020 | 1 | 0 | Timeseries | NA | Female | Second | NA |
| ZW.E02.HLS18 | F201_2B_00 | F201_2B | 23/08/2020 | 1 | 0 | Timeseries | NA | Female | Second | NA |
| ZW.E02.HLS19 | F201_3B_00 | F201_3B | 23/08/2020 | 1 | 0 | Timeseries | NA | Female | Second | NA |
| ZW.E02.HLS2 | FG01_2B_00 | FG01_2B | 23/08/2020 | 1 | 0 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS20 | M201_1B_00 | M201_1B | 23/08/2020 | 1 | 0 | Timeseries | NA | Male | Second | NA |
| ZW.E02.HLS21 | M201_2B_00 | M201_2B | 23/08/2020 | 1 | 0 | Timeseries | NA | Male | Second | NA |
| ZW.E02.HLS22 | D201_1B_00 | D201_1B | 23/08/2020 | 1 | 0 | Timeseries | NA | Unisex | Second | NA |
| ZW.E02.HLS23 | FG01_1B_01 | FG01_1B | 07/10/2020 | 7 | 1 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS24 | FG01_2B_01 | FG01_2B | 07/10/2020 | 7 | 1 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS25 | FG01_3B_01 | FG01_3B | 07/10/2020 | 7 | 1 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS26 | FG01_4B_01 | FG01_4B | 07/10/2020 | 7 | 1 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS27 | FG01_5B_01 | FG01_5B | 07/10/2020 | 7 | 1 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS28 | FG01_6B_01 | FG01_6B | 07/10/2020 | 7 | 1 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS29 | MG01_1B_01 | MG01_1B | 07/10/2020 | 7 | 1 | Timeseries | NA | Male | Ground | NA |
| ZW.E02.HLS3 | FG01_3B_00 | FG01_3B | 23/08/2020 | 1 | 0 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS30 | MG01_2B_01 | MG01_2B | 07/10/2020 | 7 | 1 | Timeseries | NA | Male | Ground | NA |
| ZW.E02.HLS31 | MG01_3B_01 | MG01_3B | 07/10/2020 | 7 | 1 | Timeseries | NA | Male | Ground | NA |
| ZW.E02.HLS32 | DG02_1B_01 | DG02_1B | 07/10/2020 | 7 | 1 | Timeseries | NA | Unisex | Ground | NA |
| ZW.E02.HLS33 | F101_1B_01 | F101_1B | 07/10/2020 | 7 | 1 | Timeseries | NA | Female | First | NA |
| ZW.E02.HLS34 | F101_2B_01 | F101_2B | 07/10/2020 | 7 | 1 | Timeseries | NA | Female | First | NA |
| ZW.E02.HLS35 | F101_3B_01 | F101_3B | 07/10/2020 | 7 | 1 | Timeseries | NA | Female | First | NA |
| ZW.E02.HLS36 | M101_1B_01 | M101_1B | 07/10/2020 | 7 | 1 | Timeseries | NA | Male | First | NA |

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|---|---|---|---|---|---|---|---|---|---|---|
| ZW.E02.HLS37 | M101_2B_01 | M101_2B | 07/10/2020 | 7 | 1 | Timeseries | NA | Male | First | NA |
| ZW.E02.HLS38 | D101_1B_01 | D101_1B | 07/10/2020 | 7 | 1 | Timeseries | NA | Unisex | First | NA |
| ZW.E02.HLS39 | F201_1B_01 | F201_1B | 07/10/2020 | 7 | 1 | Timeseries | NA | Female | Second | NA |
| ZW.E02.HLS4 | FG01_4B_00 | FG01_4B | 23/08/2020 | 1 | 0 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS40 | F201_2B_01 | F201_2B | 07/10/2020 | 7 | 1 | Timeseries | NA | Female | Second | NA |
| ZW.E02.HLS41 | F201_3B_01 | F201_3B | 07/10/2020 | 7 | 1 | Timeseries | NA | Female | Second | NA |
| ZW.E02.HLS42 | M201_1B_01 | M201_1B | 07/10/2020 | 7 | 1 | Timeseries | NA | Male | Second | NA |
| ZW.E02.HLS43 | M201_2B_01 | M201_2B | 07/10/2020 | 7 | 1 | Timeseries | NA | Male | Second | NA |
| ZW.E02.HLS44 | D201_1B_01 | D201_1B | 07/10/2020 | 7 | 1 | Timeseries | NA | Unisex | Second | NA |
| ZW.E02.HLS45 | FG01_1B_02 | FG01_1B | 21/11/2020 | 13 | 2 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS46 | FG01_2B_02 | FG01_2B | 21/11/2020 | 13 | 2 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS47 | FG01_3B_02 | FG01_3B | 21/11/2020 | 13 | 2 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS48 | FG01_4B_02 | FG01_4B | 21/11/2020 | 13 | 2 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS49 | FG01_5B_02 | FG01_5B | 21/11/2020 | 13 | 2 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS5 | FG01_5B_00 | FG01_5B | 23/08/2020 | 1 | 0 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS50 | FG01_6B_02 | FG01_6B | 21/11/2020 | 13 | 2 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS51 | MG01_1B_02 | MG01_1B | 21/11/2020 | 13 | 2 | Timeseries | NA | Male | Ground | NA |
| ZW.E02.HLS52 | MG01_2B_02 | MG01_2B | 21/11/2020 | 13 | 2 | Timeseries | NA | Male | Ground | NA |
| ZW.E02.HLS53 | MG01_3B_02 | MG01_3B | 21/11/2020 | 13 | 2 | Timeseries | NA | Male | Ground | NA |
| ZW.E02.HLS54 | DG02_1B_02 | DG02_1B | 21/11/2020 | 13 | 2 | Timeseries | NA | Unisex | Ground | NA |
| ZW.E02.HLS55 | F101_1B_02 | F101_1B | 21/11/2020 | 13 | 2 | Timeseries | NA | Female | First | NA |
| ZW.E02.HLS56 | F101_2B_02 | F101_2B | 21/11/2020 | 13 | 2 | Timeseries | NA | Female | First | NA |
| ZW.E02.HLS57 | F101_3B_02 | F101_3B | 21/11/2020 | 13 | 2 | Timeseries | NA | Female | First | NA |
| ZW.E02.HLS58 | M101_1B_02 | M101_1B | 21/11/2020 | 13 | 2 | Timeseries | NA | Male | First | NA |
| ZW.E02.HLS59 | M101_2B_02 | M101_2B | 21/11/2020 | 13 | 2 | Timeseries | NA | Male | First | NA |
| ZW.E02.HLS6 | FG01_6B_00 | FG01_6B | 23/08/2020 | 1 | 0 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS60 | D101_1B_02 | D101_1B | 21/11/2020 | 13 | 2 | Timeseries | NA | Unisex | First | NA |
| ZW.E02.HLS61 | F201_1B_02 | F201_1B | 21/11/2020 | 13 | 2 | Timeseries | NA | Female | Second | NA |
| ZW.E02.HLS62 | F201_2B_02 | F201_2B | 21/11/2020 | 13 | 2 | Timeseries | NA | Female | Second | NA |
| ZW.E02.HLS63 | F201_3B_02 | F201_3B | 21/11/2020 | 13 | 2 | Timeseries | NA | Female | Second | NA |
| ZW.E02.HLS64 | M201_1B_02 | M201_1B | 21/11/2020 | 13 | 2 | Timeseries | NA | Male | Second | NA |

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|---|---|---|---|---|---|---|---|---|---|---|
| ZW.E02.HLS65 | M201_2B_02 | M201_2B | 21/11/2020 | 13 | 2 | Timeseries | NA | Male | Second | NA |
| ZW.E02.HLS66 | D201_1B_02 | D201_1B | 21/11/2020 | 13 | 2 | Timeseries | NA | Unisex | Second | NA |
| ZW.E02.HLS67 | FG01_1B_03 | FG01_1B | 09/01/2021 | 21 | 3 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS68 | FG01_2B_03 | FG01_2B | 09/01/2021 | 21 | 3 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS69 | FG01_3B_03 | FG01_3B | 09/01/2021 | 21 | 3 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS7 | MG01_1B_00 | MG01_1B | 23/08/2020 | 1 | 0 | Timeseries | NA | Male | Ground | NA |
| ZW.E02.HLS70 | FG01_4B_03 | FG01_4B | 09/01/2021 | 21 | 3 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS71 | FG01_5B_03 | FG01_5B | 09/01/2021 | 21 | 3 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS72 | FG01_6B_03 | FG01_6B | 09/01/2021 | 21 | 3 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS73 | MG01_1B_03 | MG01_1B | 09/01/2021 | 21 | 3 | Timeseries | NA | Male | Ground | NA |
| ZW.E02.HLS74 | MG01_2B_03 | MG01_2B | 09/01/2021 | 21 | 3 | Timeseries | NA | Male | Ground | NA |
| ZW.E02.HLS75 | MG01_3B_03 | MG01_3B | 09/01/2021 | 21 | 3 | Timeseries | NA | Male | Ground | NA |
| ZW.E02.HLS76 | DG02_1B_03 | DG02_1B | 09/01/2021 | 21 | 3 | Timeseries | NA | Unisex | Ground | NA |
| ZW.E02.HLS77 | F101_1B_03 | F101_1B | 09/01/2021 | 21 | 3 | Timeseries | NA | Female | First | NA |
| ZW.E02.HLS78 | F101_2B_03 | F101_2B | 09/01/2021 | 21 | 3 | Timeseries | NA | Female | First | NA |
| ZW.E02.HLS79 | F101_3B_03 | F101_3B | 09/01/2021 | 21 | 3 | Timeseries | NA | Female | First | NA |
| ZW.E02.HLS8 | MG01_2B_00 | MG01_2B | 23/08/2020 | 1 | 0 | Timeseries | NA | Male | Ground | NA |
| ZW.E02.HLS80 | M101_1B_03 | M101_1B | 09/01/2021 | 21 | 3 | Timeseries | NA | Male | First | NA |
| ZW.E02.HLS81 | M101_2B_03 | M101_2B | 09/01/2021 | 21 | 3 | Timeseries | NA | Male | First | NA |
| ZW.E02.HLS82 | D101_1B_03 | D101_1B | 09/01/2021 | 21 | 3 | Timeseries | NA | Unisex | First | NA |
| ZW.E02.HLS83 | F201_1B_03 | F201_1B | 09/01/2021 | 21 | 3 | Timeseries | NA | Female | Second | NA |
| ZW.E02.HLS84 | F201_2B_03 | F201_2B | 09/01/2021 | 21 | 3 | Timeseries | NA | Female | Second | NA |
| ZW.E02.HLS85 | F201_3B_03 | F201_3B | 09/01/2021 | 21 | 3 | Timeseries | NA | Female | Second | NA |
| ZW.E02.HLS86 | M201_1B_03 | M201_1B | 09/01/2021 | 21 | 3 | Timeseries | NA | Male | Second | NA |
| ZW.E02.HLS87 | M201_2B_03 | M201_2B | 09/01/2021 | 21 | 3 | Timeseries | NA | Male | Second | NA |
| ZW.E02.HLS88 | D201_1B_03 | D201_1B | 09/01/2021 | 21 | 3 | Timeseries | NA | Unisex | Second | NA |
| ZW.E02.HLS89 | FG01_1B_04 | FG01_1B | 19/02/2021 | 27 | 4 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS9 | MG01_3B_00 | MG01_3B | 23/08/2020 | 1 | 0 | Timeseries | NA | Male | Ground | NA |
| ZW.E02.HLS90 | FG01_2B_04 | FG01_2B | 19/02/2021 | 27 | 4 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS91 | FG01_3B_04 | FG01_3B | 19/02/2021 | 27 | 4 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS92 | FG01_4B_04 | FG01_4B | 19/02/2021 | 27 | 4 | Timeseries | NA | Female | Ground | NA |

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|---|---|---|---|---|---|---|---|---|---|---|
| ZW.E02.HLS93 | FG01_5B_04 | FG01_5B | 19/02/2021 | 27 | 4 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS94 | FG01_6B_04 | FG01_6B | 19/02/2021 | 27 | 4 | Timeseries | NA | Female | Ground | NA |
| ZW.E02.HLS95 | MG01_1B_04 | MG01_1B | 19/02/2021 | 27 | 4 | Timeseries | NA | Male | Ground | NA |
| ZW.E02.HLS96 | MG01_2B_04 | MG01_2B | 19/02/2021 | 27 | 4 | Timeseries | NA | Male | Ground | NA |
| ZW.E02.HLS97 | MG01_3B_04 | MG01_3B | 19/02/2021 | 27 | 4 | Timeseries | NA | Male | Ground | NA |
| ZW.E02.HLS98 | DG02_1B_04 | DG02_1B | 19/02/2021 | 27 | 4 | Timeseries | NA | Unisex | Ground | NA |
| ZW.E02.HLS99 | F101_1B_04 | F101_1B | 19/02/2021 | 27 | 4 | Timeseries | NA | Female | First | NA |

**Table C.1.** Data collected for each sink P-trap sample for both studies. Bleach study also includes the gDNA concentrations recorded for each sample.

| Term | Sum Sq | Mean Sq | Num DF | Den DF | F Value | P Value | Significance |
|---|---|---|---|---|---|---|---|
| ***Shannon*** | | | | | | | |
| Week | 11.311 | 11.311 | 1 | 341.51 | 36.1193 | 4.76E-09 | *** |
| Floor Level | 0.9184 | 0.4592 | 2 | 16.94 | 1.4663 | 0.25863 | NS |
| Gender | 1.9719 | 0.9859 | 2 | 17.02 | 3.1484 | 0.06864 | NS |
| ***ASV Richness*** | | | | | | | |
| Week | 50604 | 50604 | 1 | 341.47 | 98.8665 | <2E-16 | *** |
| Floor Level | 1710 | 855 | 2 | 16.74 | 1.6702 | 0.2181 | NS |
| Gender | 1769 | 884 | 2 | 16.85 | 1.7278 | 0.2077 | NS |
| ***Pielou Evenness*** | | | | | | | |
| Week | 0.000756 | 0.000756 | 1 | 341.72 | 0.058 | 0.8098 | NS |
| Floor Level | 0.045749 | 0.022875 | 2 | 17.07 | 1.7542 | 0.2028 | NS |
| Gender | 0.113894 | 0.056947 | 2 | 17.17 | 4.367 | 0.0293 | * |

**Table C.2.** Results table from ANOVA of linear mixed effects model for the alpha diversity indices for phase 1. Sum Sq, sum of squares; Mean Sq, mean square; Num DF, degrees of freedom, DEN DF, denominator degrees of freedom. Stars indicate the p-value significance $p < 0.05$; *, $p < 0.01$; **, $P < 0.001$; ***.

**A. WK39 vs WK49**

| ASV | WK39 Mean RA (%) | WK49 Mean RA (%) | p-adjust | Change in RA | Phylum | Class | Order | Family | Genus | Species |
|---|---|---|---|---|---|---|---|---|---|---|
| ASV_0000006 | 0.8400 | 3.7236 | 0.0016884 | Increase | Proteobacteria | Gammaproteobacteria | Enterobacterales | Enterobacteriaceae | Unclassified | Unclassified |
| ASV_0000011 | 1.2819 | 0.0000 | 0.0026395 | Decrease | Proteobacteria | Gammaproteobacteria | Burkholderiales | Comamonadaceae | Unclassified | Unclassified |
| ASV_0000021 | 1.2857 | 2.9527 | 0.0194184 | Increase | Proteobacteria | Gammaproteobacteria | Pseudomonadales | Pseudomonadaceae | Pseudomonas | Unclassified |
| ASV_0000034 | 0.9029 | 0.4773 | 0.0035328 | Decrease | Proteobacteria | Gammaproteobacteria | Enterobacterales | Alteromonadaceae | Rheinheimera | Unclassified |
| ASV_0000044 | 0.0305 | 0.4955 | 0.0035328 | Increase | Proteobacteria | Alphaproteobacteria | Caulobacterales | Caulobacteraceae | Caulobacter | Unclassified |
| ASV_0000055 | 0.1467 | 0.0000 | 0.0194184 | Decrease | Proteobacteria | Gammaproteobacteria | Pseudomonadales | Pseudomonadaceae | Pseudomonas | Unclassified |
| ASV_0000108 | 0.0819 | 0.2955 | 0.0016884 | Increase | Proteobacteria | Alphaproteobacteria | Caulobacterales | Caulobacteraceae | Phenylobacterium | Unclassified |

**B. WK68 vs WK74**

| ASV | WK68 Mean RA (%) | WK74 Mean RA (%) | p-adjust | Change in RA | Phylum | Class | Order | Family | Genus | Species |
|---|---|---|---|---|---|---|---|---|---|---|
| ASV_0000005 | 0.0355 | 0.3073 | 0.0210 | Increase | Proteobacteria | Gammaproteobacteria | Pseudomonadales | Moraxellaceae | Acinetobacter | Unclassified |
| ASV_0000022 | 0.3064 | 0.3955 | 0.0343 | Increase | Proteobacteria | Alphaproteobacteria | Caulobacterales | Caulobacteraceae | Brevundimonas | Unclassified |
| ASV_0000027 | 0.0000 | 0.2045 | 0.0130 | Increase | Proteobacteria | Alphaproteobacteria | Sphingomonadales | Sphingomonadaceae | Unclassified | Unclassified |
| ASV_0000030 | 0.0127 | 0.4618 | 0.0036 | Increase | Proteobacteria | Alphaproteobacteria | Rhizobiales | Beijerinckiaceae | Methylobacterium-Methylorubrum | Unclassified |
| ASV_0000037 | 0.1909 | 0.1164 | 0.0425 | Decrease | Proteobacteria | Alphaproteobacteria | Sphingomonadales | Sphingomonadaceae | Sphingomonas | Unclassified |
| ASV_0000067 | 0.0000 | 0.5300 | 0.0058 | Increase | Bacteroidota | Bacteroidia | Flavobacteriales | Weeksellaceae | Cloacibacterium | haliotis |
| ASV_0000069 | 0.0027 | 0.0573 | 0.0058 | Increase | Proteobacteria | Gammaproteobacteria | Burkholderiales | Methylophilaceae | Methylotenera | Unclassified |
| ASV_0000073 | 0.0564 | 0.1382 | 0.0337 | Increase | Proteobacteria | Gammaproteobacteria | Burkholderiales | Rhodocyclaceae | Unclassified | Unclassified |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ASV_0000000074 | 0.1218 | 0.1100 | 0.0210 | Decrease | Proteobacteria | Alphaproteobacteria | Sphingomonadales | Sphingomonadaceae | Unclassified | Unclassified |
| ASV_0000000077 | 0.0209 | 0.0927 | 0.0130 | Increase | Proteobacteria | Alphaproteobacteria | Sphingomonadales | Sphingomonadaceae | Novosphingobium | Unclassified |
| ASV_0000000096 | 0.0100 | 0.3291 | 0.0092 | Increase | Proteobacteria | Gammaproteobacteria | Burkholderiales | Comamonadaceae | Unclassified | Unclassified |
| ASV_0000000102 | 0.0000 | 0.0482 | 0.0058 | Increase | Firmicutes | Bacilli | Lactobacillales | Streptococcaceae | Streptococcus | Unclassified |
| ASV_0000000110 | 0.0000 | 0.0255 | 0.0092 | Increase | Proteobacteria | Gammaproteobacteria | Salinisphaerales | Solimonadaceae | Nevskia | ramosa |
| ASV_0000000174 | 0.0000 | 0.0809 | 0.0130 | Increase | Proteobacteria | Gammaproteobacteria | Enterobacterales | Unclassified | Unclassified | Unclassified |
| ASV_0000000253 | 0.0000 | 0.0645 | 0.0130 | Increase | Proteobacteria | Gammaproteobacteria | Burkholderiales | Oxalobacteraceae | Herminiimonas | Unclassified |
| ASV_0000000305 | 0.0000 | 0.0764 | 0.0224 | Increase | Proteobacteria | Gammaproteobacteria | Burkholderiales | Rhodocyclaceae | Azospira | Unclassified |
| ASV_0000000915 | 0.0000 | 0.0464 | 0.0397 | Increase | Proteobacteria | Alphaproteobacteria | Rhodobacterales | Rhodobacteraceae | Unclassified | Unclassified |

**C. WK93 vs WK98**

| ASV | WK93 Mean RA (%) | WK98 Mean RA (%) | p-adjust | Change in RA | Phylum | Class | Order | Family | Genus | Species |
|---|---|---|---|---|---|---|---|---|---|---|
| ASV_0000000002 | 46.08526316 | 31.00727273 | 0.0030642 | Decrease | Bacteroidota | Bacteroidia | Flavobacteriales | Flavobacteriaceae | Flavobacterium | Unclassified |
| ASV_0000000005 | 0.041052632 | 0.381818182 | 0.0061057 | Increase | Proteobacteria | Gammaproteobacteria | Pseudomonadales | Moraxellaceae | Acinetobacter | Unclassified |
| ASV_0000000008 | 0.173684211 | 0.707272727 | 0.0387379 | Increase | Proteobacteria | Gammaproteobacteria | Xanthomonadales | Xanthomonadaceae | Pseudoxanthomonas | mexicana |
| ASV_0000000010 | 2.032631579 | 0.623636364 | 0.0042283 | Decrease | Proteobacteria | Gammaproteobacteria | Pseudomonadales | Moraxellaceae | Enhydrobacter | aerosaccus |
| ASV_0000000015 | 0.069473684 | 0.389090909 | 0.0042283 | Increase | Proteobacteria | Alphaproteobacteria | Rhizobiales | Beijerinckiaceae | Bosea | Unclassified |
| ASV_0000000022 | 0.041052632 | 0.117272727 | 0.0188053 | Increase | Proteobacteria | Alphaproteobacteria | Caulobacterales | Caulobacteraceae | Brevundimonas | Unclassified |
| ASV_0000000024 | 0.051578947 | 0.383636364 | 0.0113787 | Increase | Bacteroidota | Bacteroidia | Flavobacteriales | Weeksellaceae | Chryseobacterium | hominis |
| ASV_0000000025 | 0.018947368 | 0.079090909 | 0.0387379 | Increase | Proteobacteria | Alphaproteobacteria | Sphingomonadales | Sphingomonadaceae | Sphingomonas | Unclassified |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ASV_00000027 | 0 | 0.076363636 | 0.02328 | Increase | Proteobacteria | Alphaproteobacteria | Sphingomonadales | Sphingomonadaceae | Unclassified | Unclassified |
| ASV_00000030 | 0.016842105 | 0.180909091 | 0.00422 | Increase | Proteobacteria | Alphaproteobacteria | Rhizobiales | Beijerinckiaceae | Methylobacterium-Methylorubrum | Unclassified |
| ASV_00000034 | 0.013684211 | 0.249090909 | 0.00188 | Increase | Proteobacteria | Gammaproteobacteria | Enterobacterales | Alteromonadaceae | Rheinheimera | Unclassified |
| ASV_00000035 | 0 | 0.968181818 | 0.00023 | Increase | Proteobacteria | Gammaproteobacteria | Pseudomonadales | Pseudomonadaceae | Pseudomonas | Unclassified |
| ASV_00000042 | 0 | 0.062727273 | 0.03873 | Increase | Proteobacteria | Gammaproteobacteria | Burkholderiales | Rhodocyclaceae | Unclassified | Unclassified |
| ASV_00000051 | 0 | 0.078181818 | 0.00077 | Increase | Proteobacteria | Alphaproteobacteria | Rhizobiales | Xanthobacteraceae | Bradyrhizobium | Unclassified |
| ASV_00000067 | 0.127368421 | 0.33 | 0.02328 | Increase | Bacteroidota | Bacteroidia | Flavobacteriales | Weeksellaceae | Cloacibacterium | haliotis |
| ASV_00000075 | 0 | 0.434545455 | 0.00077 | Increase | Proteobacteria | Gammaproteobacteria | Enterobacterales | Aeromonadaceae | Aeromonas | Unclassified |
| ASV_00000077 | 0 | 0.522727273 | 0.00023 | Increase | Proteobacteria | Alphaproteobacteria | Sphingomonadales | Sphingomonadaceae | Novosphingobium | Unclassified |
| ASV_00000102 | 0.004210526 | 0.135454545 | 0.00105 | Increase | Firmicutes | Bacilli | Lactobacillales | Streptococcaceae | Streptococcus | Unclassified |
| ASV_00000161 | 0.014736842 | 0.151818182 | 0.00492 | Increase | Proteobacteria | Gammaproteobacteria | Enterobacterales | Enterobacteriaceae | Unclassified | Unclassified |
| ASV_00000274 | 0.335789474 | 0.000909091 | 0.03144 | Decrease | Proteobacteria | Gammaproteobacteria | Burkholderiales | Rhodocyclaceae | Unclassified | Unclassified |
| ASV_00000285 | 0.083157895 | 0.006363636 | 0.04731 | Decrease | Proteobacteria | Gammaproteobacteria | Burkholderiales | Rhodocyclaceae | Azospira | Unclassified |
| ASV_00000305 | 0.010526316 | 0.130909091 | 0.02328 | Increase | Proteobacteria | Gammaproteobacteria | Burkholderiales | Rhodocyclaceae | Azospira | Unclassified |

**Table C.3**. Significant ASVs between WKS with increased peak in ASV richness. (A) WK39 vs WK49, (B) WK68 vs WK74, (C) WK93 vs WK98. Mean relative abundances of ASVs at WKs being compared and p-adjusted values from Wilcox test. P values adjusted with Benjamini-Hochberg (BH). Highlighted cells indicate ASVs shared between the peaks. Taxonomy of ASVs included.

| Pairwise Comparison | F | R2 | p-value | p-value (BH corrected) |
|---|---|---|---|---|
| WK1 <-> WK68 | 16.73797 | 0.295005 | 0.001 | 0.001494505 |
| WK1 <-> WK74 | 17.21606 | 0.300896 | 0.001 | 0.001494505 |
| WK1 <-> WK76 | 15.6223 | 0.280864 | 0.001 | 0.001494505 |
| WK1 <-> WK86 | 15.88807 | 0.294835 | 0.001 | 0.001494505 |
| WK1 <-> WK93 | 18.39703 | 0.332094 | 0.001 | 0.001494505 |
| WK1 <-> WK98 | 18.60311 | 0.317442 | 0.001 | 0.001494505 |
| WK1 <-> WK107 | 16.70981 | 0.294655 | 0.001 | 0.001494505 |
| WK1 <-> WK13 | 4.263949 | 0.09633 | 0.001 | 0.001494505 |
| WK1 <-> WK21 | 5.789699 | 0.126441 | 0.001 | 0.001494505 |
| WK1 <-> WK27 | 9.728388 | 0.19563 | 0.001 | 0.001494505 |
| WK1 <-> WK33 | 10.58741 | 0.209289 | 0.001 | 0.001494505 |
| WK1 <-> WK39 | 9.65515 | 0.19844 | 0.001 | 0.001494505 |
| WK1 <-> WK49 | 15.91851 | 0.284673 | 0.001 | 0.001494505 |
| WK1 <-> WK55 | 17.32599 | 0.302236 | 0.001 | 0.001494505 |
| WK1 <-> WK61 | 17.39464 | 0.308445 | 0.001 | 0.001494505 |
| WK7 <-> WK68 | 12.53712 | 0.234176 | 0.001 | 0.001494505 |
| WK7 <-> WK74 | 13.31768 | 0.245181 | 0.001 | 0.001494505 |
| WK7 <-> WK76 | 12.3965 | 0.232159 | 0.001 | 0.001494505 |
| WK7 <-> WK86 | 13.04361 | 0.250628 | 0.001 | 0.001494505 |
| WK7 <-> WK93 | 15.38396 | 0.288176 | 0.001 | 0.001494505 |
| WK7 <-> WK98 | 15.22992 | 0.270851 | 0.001 | 0.001494505 |
| WK7 <-> WK107 | 13.97673 | 0.25423 | 0.001 | 0.001494505 |
| WK7 <-> WK27 | 6.991765 | 0.145687 | 0.001 | 0.001494505 |
| WK7 <-> WK33 | 7.392485 | 0.152761 | 0.001 | 0.001494505 |
| WK7 <-> WK39 | 6.772136 | 0.14479 | 0.001 | 0.001494505 |
| WK7 <-> WK49 | 11.69943 | 0.222003 | 0.001 | 0.001494505 |
| WK7 <-> WK55 | 12.72807 | 0.236898 | 0.001 | 0.001494505 |
| WK7 <-> WK61 | 13.33673 | 0.250048 | 0.001 | 0.001494505 |
| WK68 <-> WK98 | 5.423883 | 0.11437 | 0.001 | 0.001494505 |
| WK68 <-> WK107 | 6.447961 | 0.13309 | 0.001 | 0.001494505 |
| WK68 <-> WK13 | 6.348626 | 0.131309 | 0.001 | 0.001494505 |
| WK68 <-> WK21 | 5.076403 | 0.107833 | 0.001 | 0.001494505 |
| WK68 <-> WK27 | 8.449328 | 0.167481 | 0.001 | 0.001494505 |
| WK68 <-> WK33 | 6.822522 | 0.139741 | 0.001 | 0.001494505 |
| WK74 <-> WK98 | 8.317885 | 0.165307 | 0.001 | 0.001494505 |
| WK74 <-> WK107 | 8.812343 | 0.173429 | 0.001 | 0.001494505 |
| WK74 <-> WK13 | 8.176745 | 0.162959 | 0.001 | 0.001494505 |
| WK74 <-> WK21 | 6.602662 | 0.13585 | 0.001 | 0.001494505 |
| WK74 <-> WK27 | 13.05917 | 0.237184 | 0.001 | 0.001494505 |
| WK74 <-> WK33 | 11.62423 | 0.216772 | 0.001 | 0.001494505 |
| WK74 <-> WK39 | 7.479216 | 0.154277 | 0.001 | 0.001494505 |
| WK74 <-> WK49 | 6.847661 | 0.140184 | 0.001 | 0.001494505 |
| WK76 <-> WK98 | 5.612926 | 0.117887 | 0.001 | 0.001494505 |
| WK76 <-> WK107 | 5.83821 | 0.122041 | 0.001 | 0.001494505 |
| WK76 <-> WK13 | 7.244369 | 0.147111 | 0.001 | 0.001494505 |
| WK76 <-> WK21 | 5.985482 | 0.124735 | 0.001 | 0.001494505 |
| WK76 <-> WK27 | 10.79677 | 0.204497 | 0.001 | 0.001494505 |

| | | | | |
|---|---|---|---|---|
| WK76 <-> WK33 | 9.352544 | 0.182124 | 0.001 | 0.001494505 |
| WK76 <-> WK39 | 5.751007 | 0.123014 | 0.001 | 0.001494505 |
| WK76 <-> WK49 | 5.405528 | 0.114027 | 0.001 | 0.001494505 |
| WK86 <-> WK13 | 7.16036 | 0.15183 | 0.001 | 0.001494505 |
| WK86 <-> WK21 | 6.235371 | 0.134861 | 0.001 | 0.001494505 |
| WK86 <-> WK27 | 9.85343 | 0.197648 | 0.001 | 0.001494505 |
| WK86 <-> WK33 | 8.545432 | 0.17603 | 0.001 | 0.001494505 |
| WK86 <-> WK39 | 5.338289 | 0.120399 | 0.001 | 0.001494505 |
| WK86 <-> WK49 | 5.567526 | 0.122182 | 0.001 | 0.001494505 |
| WK93 <-> WK98 | 8.307988 | 0.175615 | 0.001 | 0.001494505 |
| WK93 <-> WK107 | 8.045862 | 0.171022 | 0.001 | 0.001494505 |
| WK93 <-> WK13 | 9.025514 | 0.187932 | 0.001 | 0.001494505 |
| WK93 <-> Wk21 | 9.604729 | 0.197609 | 0.001 | 0.001494505 |
| WK93 <-> WK27 | 13.3613 | 0.255175 | 0.001 | 0.001494505 |
| WK93 <-> WK33 | 11.23599 | 0.223664 | 0.001 | 0.001494505 |
| WK93 <-> WK39 | 8.275405 | 0.178829 | 0.001 | 0.001494505 |
| WK93 <-> WK49 | 12.7375 | 0.246195 | 0.001 | 0.001494505 |
| WK93 <-> WK55 | 9.041611 | 0.188204 | 0.001 | 0.001494505 |
| WK93 <-> WK61 | 5.549203 | 0.127424 | 0.001 | 0.001494505 |
| WK98 <-> WK13 | 7.188149 | 0.146136 | 0.001 | 0.001494505 |
| WK98 <-> WK21 | 7.985398 | 0.159755 | 0.001 | 0.001494505 |
| WK98 <-> WK27 | 7.666728 | 0.154363 | 0.001 | 0.001494505 |
| WK98 <-> WK33 | 6.391885 | 0.132086 | 0.001 | 0.001494505 |
| WK98 <-> WK39 | 4.770288 | 0.104222 | 0.001 | 0.001494505 |
| WK98 <-> WK49 | 5.236448 | 0.110856 | 0.001 | 0.001494505 |
| WK98 <-> WK55 | 5.421358 | 0.114323 | 0.001 | 0.001494505 |
| WK107 <-> WK13 | 6.709633 | 0.137748 | 0.001 | 0.001494505 |
| WK107 <-> WK21 | 7.29461 | 0.14798 | 0.001 | 0.001494505 |
| WK107 <-> WK27 | 7.196389 | 0.146279 | 0.001 | 0.001494505 |
| WK107 <-> WK33 | 6.099104 | 0.126803 | 0.001 | 0.001494505 |
| WK107 <-> WK39 | 4.400009 | 0.096916 | 0.001 | 0.001494505 |
| WK107 <-> WK55 | 5.623601 | 0.118084 | 0.001 | 0.001494505 |
| WK13 <-> WK49 | 5.141575 | 0.109067 | 0.001 | 0.001494505 |
| WK13 <-> WK55 | 6.017066 | 0.125311 | 0.001 | 0.001494505 |
| WK13 <-> WK61 | 5.843955 | 0.124754 | 0.001 | 0.001494505 |
| WK21 <-> WK49 | 4.262777 | 0.092143 | 0.001 | 0.001494505 |
| WK21 <-> WK55 | 5.015723 | 0.106682 | 0.001 | 0.001494505 |
| WK21 <-> WK61 | 6.055262 | 0.128684 | 0.001 | 0.001494505 |
| WK27 <-> WK49 | 6.457525 | 0.133262 | 0.001 | 0.001494505 |
| WK27 <-> WK55 | 7.768238 | 0.156088 | 0.001 | 0.001494505 |
| WK27 <-> WK61 | 6.012536 | 0.127892 | 0.001 | 0.001494505 |
| WK33 <-> WK49 | 5.423208 | 0.114358 | 0.001 | 0.001494505 |
| WK33 <-> WK55 | 6.801147 | 0.139364 | 0.001 | 0.001494505 |
| WK39 <-> WK49 | 3.308372 | 0.074667 | 0.001 | 0.001494505 |
| WK1 <-> WK7 | 2.341813 | 0.056645 | 0.002 | 0.002804124 |
| WK7 <-> WK21 | 3.271064 | 0.073887 | 0.002 | 0.002804124 |
| WK74 <-> WK61 | 5.261664 | 0.113737 | 0.002 | 0.002804124 |
| WK107 <-> WK49 | 5.196732 | 0.110108 | 0.002 | 0.002804124 |

| | | | | |
|---|---|---|---|---|
| WK33 <-> WK61 | 4.925192 | 0.107244 | 0.002 | 0.002804124 |
| WK39 <-> WK55 | 4.12133 | 0.091339 | 0.002 | 0.002804124 |
| WK68 <-> WK93 | 4.433064 | 0.102067 | 0.003 | 0.003961165 |
| WK68 <-> WK39 | 4.460308 | 0.098114 | 0.003 | 0.003961165 |
| WK76 <-> WK61 | 3.635245 | 0.081443 | 0.003 | 0.003961165 |
| WK86 <-> WK107 | 4.535934 | 0.101849 | 0.003 | 0.003961165 |
| WK39 <-> WK61 | 3.237588 | 0.074879 | 0.003 | 0.003961165 |
| WK49 <-> WK61 | 3.652051 | 0.081789 | 0.003 | 0.003961165 |
| WK74 <-> WK93 | 4.29653 | 0.099235 | 0.004 | 0.005180952 |
| WK86 <-> WK98 | 4.445496 | 0.100021 | 0.004 | 0.005180952 |
| WK107 <-> WK61 | 3.910899 | 0.087081 | 0.005 | 0.006415094 |
| WK76 <-> WK55 | 3.234693 | 0.071509 | 0.006 | 0.007626168 |
| WK68 <-> WK49 | 4.110368 | 0.089142 | 0.007 | 0.008733945 |
| WK74 <-> WK55 | 4.103967 | 0.089015 | 0.007 | 0.008733945 |
| WK98 <-> WK61 | 3.183823 | 0.072059 | 0.008 | 0.009890909 |
| WK86 <-> WK55 | 3.223608 | 0.07458 | 0.01 | 0.012035398 |
| WK21 <-> WK27 | 2.568024 | 0.05762 | 0.01 | 0.012035398 |
| WK21 <-> WK33 | 2.539984 | 0.057027 | 0.01 | 0.012035398 |
| WK76 <-> WK93 | 3.112666 | 0.073913 | 0.012 | 0.014315789 |
| WK7 <-> WK13 | 2.063697 | 0.047922 | 0.019 | 0.022469565 |
| WK86 <-> WK61 | 2.693311 | 0.064598 | 0.024 | 0.028137931 |
| WK86 <-> WK93 | 2.655923 | 0.066974 | 0.029 | 0.033709402 |

**Table C.4.** Pairwise comparisons for all significant pairs of levels of sampling time point (week) by using PERMANOVA. P values corrected with Benjamini-Hochberg (BH) are shown. The R2 values indicated the amount of variation explained.

| ASV ID | Total Count | Total Count (%) | Prevalence | Prevalence (%) | Domain | Phylum | Class | Order | Family | Genus | Species |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ASV_00000 00001 | 630036 | 34.52 | 354 | 97.25 | Bacteria | Proteobacteria | Gammaproteobacteria | Burkholderiales | Rhodocyclaceae | Azospira | oryzae |
| ASV_00000 00004 | 114099 | 6.25 | 349 | 95.88 | Bacteria | Proteobacteria | Alphaproteobacteria | Sphingomonadales | Sphingomonadaceae | Sphingobium | yanoikuyae |
| ASV_00000 00002 | 470522 | 25.78 | 347 | 95.33 | Bacteria | Bacteroidota | Bacteroidia | Flavobacteriales | Flavobacteriaceae | Flavobacterium | Unclassified |
| ASV_00000 00008 | 42058 | 2.3 | 301 | 82.69 | Bacteria | Proteobacteria | Gammaproteobacteria | Xanthomonadales | Xanthomonadaceae | Pseudoxanthomonas | mexicana |
| ASV_00000 00009 | 35097 | 1.92 | 295 | 81.04 | Bacteria | Proteobacteria | Alphaproteobacteria | Caulobacterales | Caulobacteraceae | Brevundimonas | Unclassified |
| ASV_00000 00003 | 68508 | 3.75 | 291 | 79.95 | Bacteria | Proteobacteria | Gammaproteobacteria | Burkholderiales | Comamonadaceae | Acidovorax | Unclassified |
| ASV_00000 00015 | 9682 | 0.53 | 270 | 74.18 | Bacteria | Proteobacteria | Alphaproteobacteria | Rhizobiales | Beijerinckiaceae | Bosea | Unclassified |
| ASV_00000 00017 | 15677 | 0.86 | 262 | 71.98 | Bacteria | Proteobacteria | Gammaproteobacteria | Burkholderiales | Comamonadaceae | Unclassified | Unclassified |
| ASV_00000 00010 | 22993 | 1.26 | 258 | 70.88 | Bacteria | Proteobacteria | Gammaproteobacteria | Pseudomonadales | Moraxellaceae | Enhydrobacter | aerosacus |

**Table C.5.** ASVs classified as core (>70% prevalence). Overall abundance (counts), prevalence and classification are shown.

| Term | Sum Sq | Mean Sq | Num DF | Den DF | F Value | P Value | Significance |
|---|---|---|---|---|---|---|---|
| ***Shannon*** | | | | | | | |
| Week | 2.39032 | 2.39032 | 1 | 45.644 | 23.4437 | 1.51E-05 | *** |
| Treatment | 0.06037 | 0.06037 | 1 | 18.139 | 0.5921 | 0.4515 | NS |
| ***ASV Richness*** | | | | | | | |
| Week | 30.517 | 30.517 | 1 | 48.31 | 0.4383 | 0.5111 | NS |
| Treatment | 78.571 | 78.571 | 1 | 15.927 | 1.1286 | 0.3039 | NS |
| ***Pielou Evenness*** | | | | | | | |
| Week | 0.226209 | 0.226209 | 1 | 46.016 | 36.3099 | 2.65E-07 | *** |
| Treatment | 0.003342 | 0.003342 | 1 | 18.245 | 0.5364 | 0.4732 | NS |

**Table C.6**. Results table from ANOVA of linear mixed effects model for the alpha diversity indices for phase 2. Sum Sq, sum of squares; Mean Sq, mean square; Num DF, degrees of freedom, DEN DF, denominator degrees of freedom. Stars indicate the p-value significance $p < 0.05$; *, $p < 0.01$; **, $P < 0.001$; ***.

# Chapter 5. Microbial Landscape of Public Urinals: a 16S rRNA Survey of the Bacterial Communities in Urinal P-traps and the Discovery of Their Most Abundant and Prevalent Species

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## 5.1 Abstract

Efforts to characterise the surfaces of the built environment (BE) have significantly increased, revealing the colonization of microorganisms associated with humans and the outdoor environment. Indoor spaces exhibit higher levels of pathogenic microbes, emphasising the need to understand microbial communities in the BE, given humans' predominant indoor presence. Shared public restrooms are a unique environment for potential microorganism transmission, with distinct microbial patterns observed in different areas. Notably, the plumbing and drainage pipes found within restrooms, may serve as reservoirs for pathogenic bacteria and antibiotic-resistant strains. Urinals, designed for operational efficiency, share commonalities with sinks in their exposure to wetting and potential microbial contamination. Urine, a variable composition fluid, introduces enzymes, pharmaceuticals, and antibiotics into urinals, raising concerns about bacterial resistance. Despite the critical role of urinals in potential microbial transmission, there has been very little study on the urinal microbiome. Recognising this gap and the need for a comprehensive investigation into the microbial community composition of the urinal environment, we investigated over 100 urinal P-trap bacterial communities using 16S rRNA sequencing from across a university campus and a train station. The focus of our research was on bacterial communities in urinal P-traps across a university campus, aiming to analyse the impact of different buildings, understand composition and identify core bacterial families. The study revealed considerable variability in community composition and structure between buildings and individual sinks. Despite these differences, Proteobacteria and Firmicutes were the predominant phyla in urinal communities. Notably, a species from the genus *Dolosicoccus* highly dominated the urinal P-traps in terms of both prevalence and abundance. Further investigation indicated significant differences from the only known species in the genus, *Dolosicoccus paucivorans*. Several top genera identified in the study had been previously detected in urine, although studies referencing *Dolosicoccus* were scarce. This research provides valuable insights into bacterial community members and highlights the need for further exploration of specific bacteria to better understand potential risks posed by urinals to human occupants.

## 5.2 Introduction

The efforts to characterise the surfaces of the built environment (BE) have increased dramatically over the years. The BE consistently undergoes colonisation by microorganisms associated with humans and the outdoor environment (Kembel et al., 2012; Rintala et al., 2008). It has been observed that the relative abundances of human pathogenic microbes are higher indoors than outdoors (Carrazana et al., 2023; Kembel et al., 2014). Given that humans spend the majority of their lives indoors, understanding microbial communities within the BE is crucial for gaining insights into their potential impact on our lives. This understanding can aid in the development of strategies to minimize associated risks.

Shared public spaces, such as restrooms, provide a unique setting for the potential transmission of microorganisms, including pathogens (Flores et al., 2011; Fouquier et al., 2016). This is due to the substantial number and diversity of users. Moreover, individual variations in hygiene practices contribute to the potential transmission of viable pathogens from surfaces (Gibbons et al., 2015; Lee et al., 2015; Wu et al., 2019). Previous studies have investigated the bacterial communities of surfaces in public restrooms have revealed a prevalence of human-associated bacteria on most surfaces, with distinct patterns of microorganisms associated with specific areas (Flores et al., 2011). For example, surfaces or areas near toilets had higher levels of gut-associated bacteria. Contact with these surfaces could facilitate the spread and transmission of bacterial enteropathogens. Furthermore, an analysis of restroom dust identified that two of the most common Operational Taxonomic Units (OTUs) in men's restrooms were assigned to the family *Enterobacteriaceae* (Dobbler et al., 2018). Both studies identified gender-specific microbial signatures, such as *Lactobacillaceae* in women's restrooms and *Corynebacterium* in men's restrooms (Dobbler et al., 2018; Flores et al., 2011). These genera are typically part of the healthy urinary tract microbiota (Fouts et al., 2012; Modena et al., 2017).

Some pathogenic bacteria are able to endure on surfaces for extended periods, suggesting that restrooms might serve as "hot spots" for bacterial contamination (Barker & Bloomfield, 2000; Bures et al., 2000; Islam et al., 2001; Noskin et al., 1995; Webster et al., 2000). Additionally, non-healthcare restrooms have been identified as sources of antibiotic-resistant bacteria, potentially forming bacterial resistomes (Mkrtchyan et al., 2013). The prevalence of skin and outdoor-associated microorganisms was observed after a decontamination event on restroom surfaces, alongside the presence and persistence of faecal matter (Gibbons et al., 2015). This indicates the significance of external sources in determining the microbial composition within the restroom environment.

Within restroom environments, various potential microhabitats exist, including those found in plumbing and drainage pipes. Many studies have investigated microbial communities of Drinking

Water Distribution Systems (DWDS) due to the direct impact they can have on human health (Bitton, 2014; Douterelo et al., 2016; Perrin et al., 2019. Waste drains and associated pipes, such as P-traps, have received less attention, particularly in public areas, due to the misconception that they operate as one-way systems. Biofilms in P-traps have been shown to grow vertically and spread to the surrounding area, with instances of being responsible for nosocomial outbreaks in clinical settings (Cholley et al., 2008; Gillespie et al., 2000; Kotay et al., 2017; Lowe et al., 2012). There is a notable scarcity of research conducted on P-traps in non-clinical settings (Lim et al., 2022; Mcbain et al., 2003; Withey et al., 2021, 2023), particularly those associated with urinals.

In the majority of public restrooms designated for males, the installation of urinals is implemented to enhance operational efficiency. When contrasted with conventional toilets, the use of urinals presents advantages such as space saving, ease of use and reduced water usage. These urinals, akin to sinks, share a design principle that exposes them to regular wetting through urine or automatic flushing with tap water. In recent years, advancements in bacterial assessment have demonstrated that the bladder is not inherently sterile (Thomas-White et al., 2016). Notably, contemporary techniques and technologies such as 16S rRNA gene sequencing, have enabled the identification of previously uncultured microorganisms and highlighted the existence of a urinary microbiome. Despite ongoing investigations into the composition, characteristics, and functional role of microbiota in the urinary tract (Ackerman & Chai, 2019; Li et al., 2019), it is evident that urine can potentially serve as a source of microbiota within the urinal environment, some of which may have pathogenic implications.

While both tap water and urine exhibit low bacterial biomass (Neugent et al., 2020; Putri et al., 2021), the P-trap of urinals may create conducive conditions for the establishment of stable bacterial communities, as observed in model sinks and water pipes (Douterelo et al., 2016, 2018; Ledwoch et al., 2020). However, given the comparatively less diverse input source of bacteria to urinals than sinks, nutrients availability may be limited, creating potentially more competitive microbe environment.

Apart from its potential as a source of microbiota, human-produced urine, amounting to approximately 1.2 liters per day, exhibits a variable composition that includes enzymes, organic substances, heavy metals, pharmaceuticals, and hormones (Jia et al., 2012; Zhou et al., 2013). Notably, antibiotics, crucial in treating various human diseases, are only partially metabolized, with 30%–90% being excreted through urine and faeces (Frade et al., 2014). This disposal process raises the possibility that certain bacteria may develop resistance to specific chemicals or adapt to utilizing these chemicals as nutrients for growth under challenging conditions (Boyle et al., 2020; Nizer et al., 2020). Moreover, due to urinal design, bacteria could proliferate along pipe walls and disseminate through droplet transmission. A recent study indicates that urinal flushing generates a large number of droplets,

reaching heights of 1.2 metre above the urinal bowl and remaining suspended in the air for extended periods due to their small size (<3µm) (Schreck et al., 2021). The generation of these droplets during flushing poses a significant transmission risk if they contain infectious or pathogenic microorganisms. A simulation of particle movement from urinal flushing revealed that over 57% of particles travelled away from the urinal, reaching the height of a man's thigh in only 5.5 seconds, showcasing higher diffusion performance compared to toilet-induced diffusion (Wang et al., 2020). While previous studies have mainly investigated toilet flushing mechanisms, which share similarities with urinals, there is evidence that airborne microbes can be disseminated through flushing, leading to potential surface contamination (Barker & Jones, 2005). In a previous investigation into hand hygiene practices, it was found that 57.5% of males said that they washed their hands prior to leaving the toilet, with fewer washing with soap (29.5%). Given that person-to-person transmission via contact surfaces is a recognised route in public toilets, there exists a potential for microorganisms from the urinal disseminate into the wider BE (Gerhardts et al., 2012; Lee et al., 2015). Under the right conditions, the infection risks from these disseminating microorganisms could be heightened.

Public restrooms may not conform to the perceived microbial wasteland within the BE and could pose health challenges for regular users, especially in inadequately ventilated, confined, irregularly cleaned, and frequently used restrooms (Lee & Tham, 2021). In such environments, bacteria and fungi may contribute to the occurrence of disease or allergic reactions (Douglas & Lumati, 2018). It is, therefore, imperative to identify the reservoirs and enhance our understanding of bacterial communities and their diversity within the BE, particularly in areas with potential transmission of pathogenic microbes. In this study, we utilised 16S rRNA amplicon sequencing to study bacterial communities of urinal P-traps across a university campus. The primary objectives were to (1) analyse the effect of different buildings and their use on bacterial populations, (2) understand the composition of bacterial communities in urinal P-Traps, (3) identify core bacterial families and genera, and (4) determine if urinal P-Traps reflect human associated bacterial signatures especially those related to the urogenital tract or urine.

## 5.3 Methods

### *5.3.1 Sample Collection and Isolation of genomic DNA*

Sampling was conducted during the period of June to July 2021. Swab samples from the P-trap of urinals were collected from 43 male restrooms situated across 15 different buildings. The selected buildings primarily consisted mainly of those located on the University of Reading's main Whiteknights campus (13 buildings), one building from the University of Reading London Road Campus, and a public

train station (Reading West). This resulted in a total of 107 urinal P-trap samples that were processed for amplicon sequencing (Table D.1). The methods for collecting P-trap samples followed procedure employed in previous studies (Withey et al., 2021, 2023). Briefly, prior to sampling, each urinal was flushed with two litres of water. Subsequently, sterile cotton swabs were inserted into the P-traps using a sampling rod, and the circumference of the pipe was swabbed for 10 seconds. The swabs were then stored in 1.5 ml tubes within a freezer at -20°C until required for DNA extraction. Genomic DNA isolation from the samples, along with three unused swabs as negative controls was performed using the HigherPurity Soil DNA Isolation kit (Canvax Biotech), following the manufacturers protocol. The DNA was eluted in 50 µl of UltraPure Dnase/Rnase-Free Distilled Water (Invitrogen) in the final step, and the extracted genomic DNA was stored at -20°C until required.

### *5.3.2 Library Preparation and Sequencing*

The V4 region of the 16S rRNA gene were amplified by the recommended Earth Microbiome Project (EMP, https://earthmicrobiome.org; Thompson et al., 2017; Walters et al., 2016): 515F (Forward: GTGYCAGCMGCCGCGGTAA) and 806R (Reverse: GGACTACNVGGGTWTCTAAT) primers. The PCR reaction mixtures (50 µl) contained 0.25 µl Sigma JumpStart$_{TM}$ REDTaq® DNA Polymerase, 5 µl Sigma 10X PCR Buffer with $MgCL_2$, 1 µl Sigma Deoxynucleotide Mix (10mM), 0.5 µl forward primer (10 µM), 0.5 µl reverse primer (10 µM), 37.75 µl Nuclease-free water and 5 µl of genomic DNA (<10ng/ µl). Negative template controls (NTCs, Nuclease-free water) were used in all PCR reactions. The PCR thermocycling conditions were followed as described by the EMP protocol (Initial denature at 94°C for 3 minutes, followed by 35 cycles of 45 s denature at 94°C, 60 s annealing at 50°C, 90 s extension at 72°C, then final extension at 72°C for 10 minutes). Post PCR clean-up was done with AMPure XP beads (Beckman Coulter) in accordance with manufacturers PCR purification workflow. Purified PCR products underwent a second PCR rection to add Illumina-specific adapters and unique barcodes as described in Withey et al., 2021. Briefly, thermocycle conditions for the second round of PCR were 95°C for 2 minutes 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 minutes. NGS Normalisation 96-Well Kit (Norgen) was used to purify and normalise samples before being pooled. The library was sequenced at a concentration of 10pM and merged with 5% PhiX on an Illuminia MiSeq Platform (2x 250PE) at UK Centre for Ecology & Hydrology.

### *5.3.3 Bioinformatics and Statistical Analysis*

The Illumina raw paired-end sequences were quality filtered using FASTP (v.0.23.2, Chen et al., 2018), and CUTADAPT (v.4.2, Martin, 2011), https://cutadapt.readthedocs.io/en/stable/) was used to

remove sequencing adaptors and primers. Sequences were further dereplicated, denoised, merged and assessed for chimeras to produce amplicon sequence variants (ASVs) using DADA2 (v.1.26.0, Callahan et al., 2016). Taxonomy was then assigned to ASVs using a naïve Bayesian classifier against SILVA database (v.138, Quast et al., 2013). Based on the generated taxonomy, the ASV table was filtered to exclude all ASVs not assigned to the bacterial domain. Further filtering of the ASV table was implemented to remove ASVs with low abundance (less than 10 counts, 0.67% of reads were removed). One building had only one urinal sample associated with it, so this was removed from subsequent analysis.

Statistical analyses were performed in R (v. 4.3.1, R Core Team, 2022). The diversity and richness of all samples across all buildings were compared using alpha (ASV richness, Shannon diversity and Pielou's evenness) and beta (Bray-Curtis dissimilarity) diversity indices using the vegan package (v.2.6-4, Oksanen et al., 2020). For all analyses, the samples were rarefied to a depth of 9,314. This threshold was selected to avoid sample loss (lowest reads in a sample), and the rarefaction curves had plateaued, indicating sufficient sequencing depth. Negative controls, which did not yield quantifiable DNA, were excluded from subsequent analysis. Differences between buildings were tested with Kruskal-Wallis and permutational multivariate analysis of variance (PERMANOVA) for alpha and beta diversities, respectively. Non-metric Multidimensional Scaling (NMDS) based on Bray-Curtis was used to visualise beta diversity. Pairwise comparisons between groups were tested using Dunns test for alpha diversity and Adonis.pair() from the package EcolUtils (Salazar, 2023) for beta diversity. The p-values for multiple comparisons were adjusted using the Benjamini-Hochberg method. Further, Betadisper() tested homogeneity of dispersions among buildings and ANOVA assessed significance.

Core ASVs were determined by setting a prevalence threshold of 70%. The ASVs that made up the most abundant genera, *Dolosicoccus*, were further investigated. The ASVs classified to the genus *Dolosicoccus* were aligned in Geneious Prime (v.2023.2, https://www.geneious.com) based using Clustal Omega (Goujon et al., 2010; Sievers et al., 2011) against each other and then the most abundant ASVs aligned to the 16S gene of *Dolosicoccus Paucivorans* (15742, Type Strain).

## 5.4 Results

### *5.4.1 Sequencing information*

The sequencing of 106 samples yielded 2,562 Amplicon Sequence Variants (ASVs) obtained from 2,335,073 paired-end sequences, with an average and median of 22,029 and 20,812 sequences per sample, respectively. Reads unclassified at the phylum level were excluded, and low abundance ASVs with fewer than 10 total counts were filtered out, leading to the removal of 338 ASVs (this accounted

for 0.3% of the sequences, indicating minimal data loss). Following rarefaction, a dataset of 2,137 ASVs was retained across 105 samples. On average, each sample exhibited 66 ASVs, with a range from 12 to 203 ASVs. Taxonomic profiles and relative abundances (RA) were determined at the phylum, class, order, family, and genus levels (Table D.2).

### *5.4.2 Diversity and composition of urinal P-trap microbiome*

#### *5.4.2.1 Beta and alpha diversities*

The ASVs richness differed significantly between buildings (Kruskal-Wallis: P = 0.0104). Pairwise comparisons showed that samples from Polly Vacher building had a significantly higher ASV richness compared to Agriculture building, Mathematics building, Park Eat building and the Train Station (Kruskal-Wallis: P = 0.0423, P = 0.414, P = 0.0131, P = 0.0139, respectively). No significant differences in ASV richness were detected between other combinations of buildings (Figure 5.1a). Pielou's evenness (Figure 5.1b) did not differ significantly between all samples (Kruskal-Wallis: P = 0.106), while Shannon diversity differed significantly across all samples (Kruskal-Wallis: P = 0.0349). Pairwise comparisons showed significantly higher Shannon diversity in samples from Polly Vacher building compared to those from the Train Station (Kruskal-Wallis: P = 0.0426). No significant differences in Shannon diversity were recorded between other pairs of buildings (Figure 5.1c). Notably, the rank abundance curve based on ASVs showed very few ASVs with high relative abundance and many with abundances less than 0.1% (2,006 ASVs, Figure 5.2a).

The beta diversity analysis based on Bray-Curtis dissimilarity showed significant overall differences in the structure and composition of bacterial communities across all buildings (PERMANOVA: F.model = 2.0006, R2 = 0.22228, P = 0.001). NMDS did not show obvious clustering between buildings (Figure 1.1d), and pairwise comparisons for the PERMANOVA of buildings confirmed significant differences in all pairs of buildings (Table D.3). Additionally, there was a significant difference in the homogeneity of group dispersions (variances) between the buildings (ANOVA, DF = 13, F = 5.0594, $p < 0.001$, Figure 5.1e). Pairwise comparison of mean dispersions for buildings showed that the Agriculture building significantly differed from most of the other buildings (Table D.4). These observations suggest significant variability in the bacterial communities of urinals across different buildings.

#### *5.4.2.2 Taxonomic Composition and Core Bacterial Taxa*

Three phyla constituted the majority of sequences: Proteobacteria (70.72%), Firmicutes (22.24%) and Bacteroidota (2.6%), and the remaining reads (4.44%) were classified to 21 other Phyla. Proteobacteria

dominated the sink communities in most buildings, accounting for more than 50% of the total relative abundance (Figure 5.2b). The ASVs present in the Train Station were mainly classified as Proteobacteria (96.61%).

The 24 phyla were further classified into classes (n = 42), orders (n =95) and families (n = 174). The major classes were Gammaproteobacteria (54.39%), Bacilli (20.1%) and Alphaproteobacteria (16.33%). The main orders were Burkholderiales (23.84%), Lactobacillales (19.72%), Pseudomonadales (12.28%) and Enterobacterales (9.94%). At the family level, the top six families accounted for >50% of the reads, with *Comamonadaceae* (13.7%), *Aerococcaceae* (12.52%), *Pseudomonadaceae* (10.35%), *Carnobacteriaceae* (6.41%), *Alcaligenaceae* (5.65%) and *Xanthomonadaceae* (5.58%).

At the genus level, taxonomic classification identified 314 known genera. The most abundant genera were *Dolosicoccus* (11.65%) and *Pseudomonas* (7.15%), with 19 classified genera having a relative abundance greater than 1% (Table D.2E). The relative abundances of these top genera varied between buildings (Figure 5.2c). For example, *Dolosicoccus* dominated in most buildings including Agriculture building (17.09%), Henley Business School building (22.57%), Meteorology building (18.9%), Park Eat building (13.77%), The Dairy building (39.37%), while *Pseudomonas* was most abundant in Chemistry building (11.89%) and Edith Morley building (23.39%). *Acinetobacter* was most abundant in the Train Station (mean 36.71%).

In terms of prevalence, *Pseudomonas* (84%), *Stenotrophomonas* (79%), *Achromobacter* (73%), *Brevundimonas* (70%) and *Dolosicoccus* (70%) were the most commonly occurring genera, with the remaining genera exhibiting prevalence below 70% (Table D.2E). Notably, the most abundant genera, *Dolosicoccus* and *Pseudomonas*, were particularly prevalent, indicating widespread presence in urinal P-traps. *Dolosicoccus*, with the highest mean relative abundance (11.7%) across all samples, also displayed the largest maximum relative abundance in a single sample at 93.9% (Figure D.1). Its occurrence was noted in at least two urinals per building (Table D.5, Figure D.1).

No individual ASV was present in more than 70% of samples. Notably, however, three ASVs had a prevalence greater than 60%. These ASVs belonged to the genera *Dolosicoccus* (family *Aerococcaceae*) at 69% (ASV_0000000004), and the families *Comamonadaceae* at 67% (ASV_0000000008), and *Carnobacteriaceae* at 63% (ASV_0000000013). Cumulatively, these three ASVs, among a total of 2,137 ASVs, represented 18.32% of total reads.

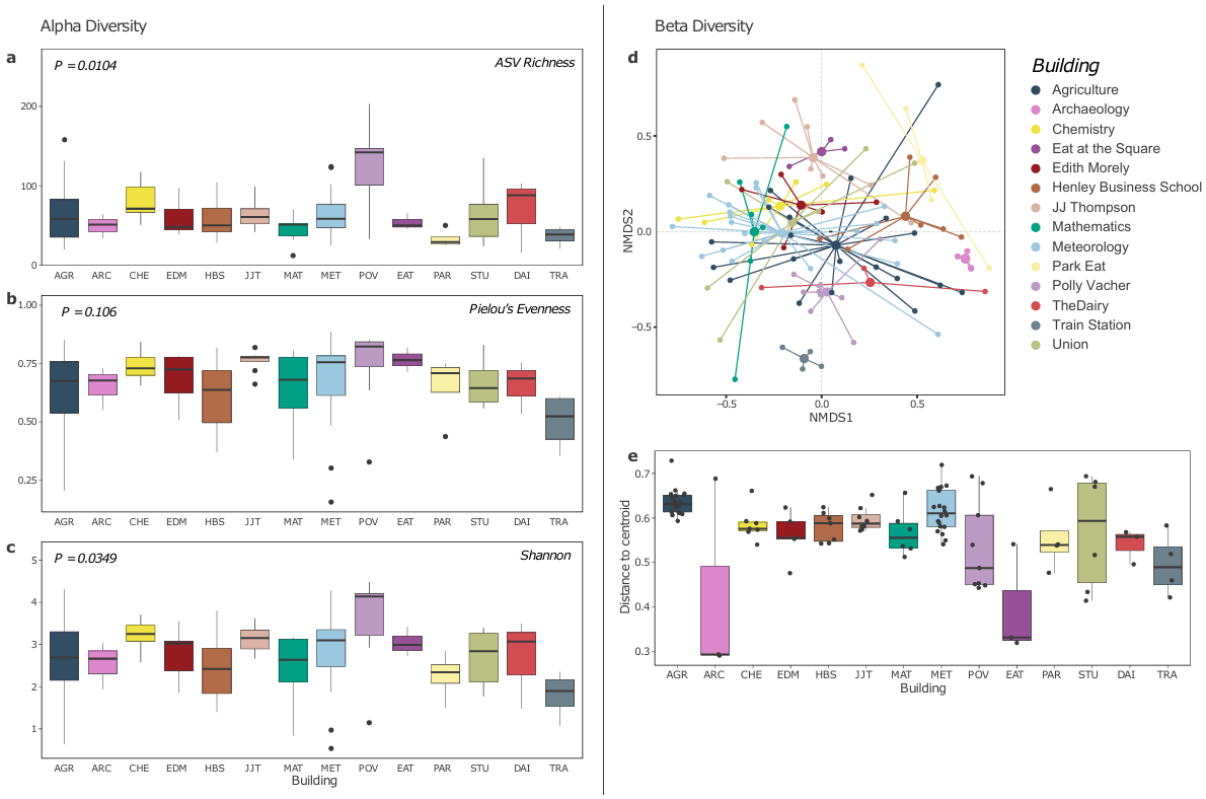


**Figure 5.1.** Alpha and Beta Diversity. (a-c) Alpha diversity measurements (ASV richness (a), Pielou's evenness (b) and Shannon diversity (c)) in urinal communities across buildings sampled. ASV richness differed significantly between buildings. (d) NMDS resulting from Bray-Curtis dissimilarity matrices of community composition between the different buildings samples. (e) Distances to centroid in multivariate homogeneity of group variance analysis for urinal bacterial communities for each building.

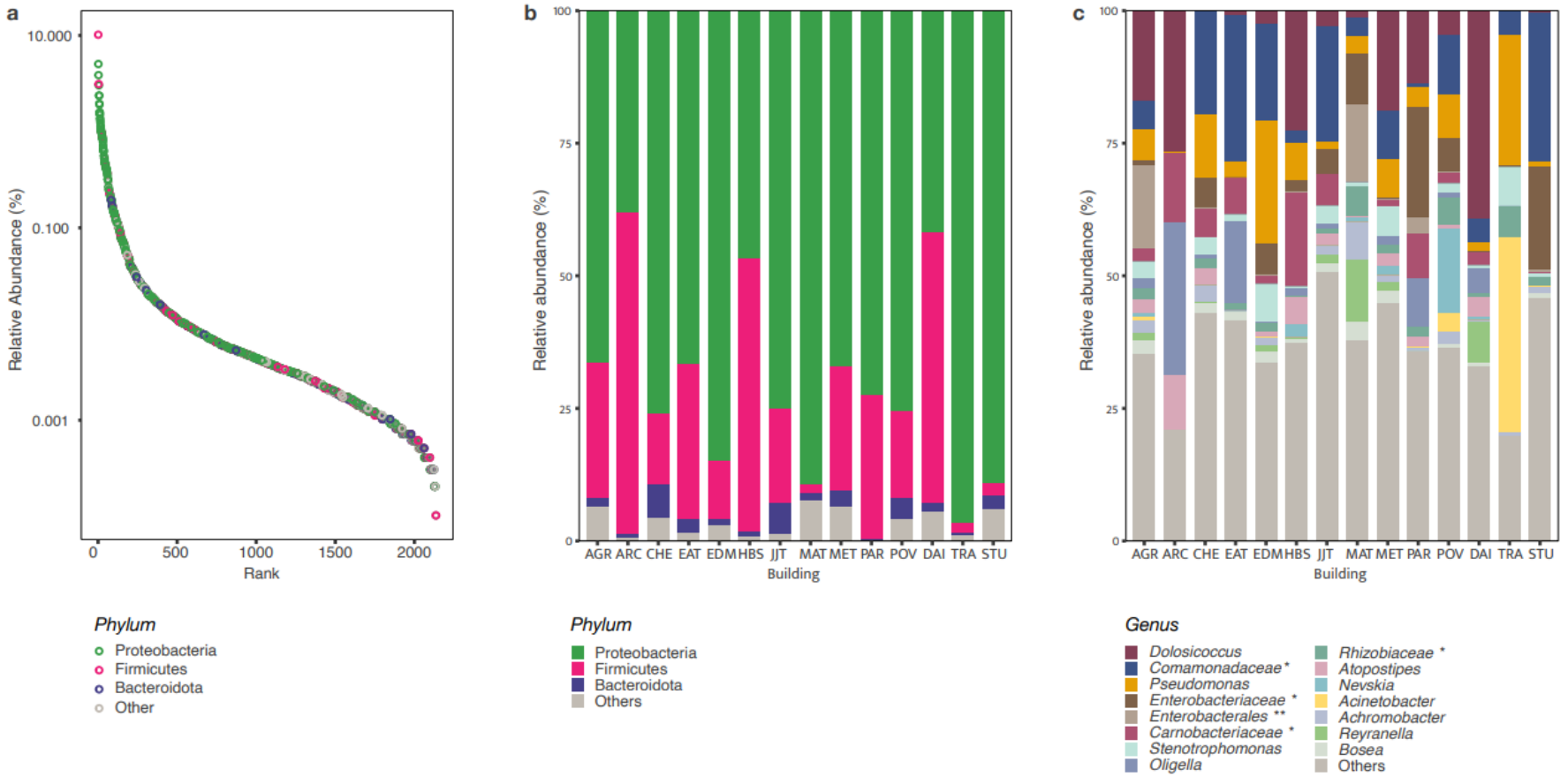


**Figure 5.2.** (a) Rank abundance curve of bacterial ASVs derived from all urinal samples. x-axis indicates the richness of urinals (number of ASVs), slope of curve indicates evenness. (b) Average relative abundance of the top phyla and (b) top 15 taxa at the genus level by building. The phylum Proteobacteria dominate across most buildings. There are more variations between building in relative abundance at the genus level. *Dolosicoccus* is one of the more prevalent and abundant genera.

### *5.4.3 Comparison of Dolosicoccus ASVs*

To further explore the most abundant and prevalent species of the urinals, we examined all 25 ASVs classified to the genus *Dolosicoccus* (Table D.6). The most abundant ASV (ASV_0000000004) constituted the majority of reads representing 87.2% of the total ASVs classified to *Dolosicoccus*. ASV_0000000038 and ASV_0000000061 were the next most abundant, representing 7.7% and 3.9% of all reads, respectively. As noted above, ASV_0000000004 was the most prevalent and abundant ASV. ASV_0000000038 was present in 30% of urinals sampled, while ASV_0000000061 occurred in 20%. These two ASVs exhibited a diverse presence across various buildings, as illustrated by ASV_0000000038 exclusively appearing in 100% of samples from the Dairy building but not in the Student Union building (Figure 5.3). The 16S rRNA V4 sequences of the 25 *Dolosicoccus* ASVs were aligned using Clustal Omega (Figure D.3). The sequence percentage identity among the top three *Dolosicoccus* ASVs was greater than 99%. It is worth noting that if sequences had been clustered based on a similarity threshold (97%) during data processing, they would have been clustered as the same operational taxonomic unit (OTU). However, since DADA2 corrects errors, these observed differences in bases may indeed reflect genuine biological differences. The top three *Dolosicoccus* ASVs, which accounted for 98.8% of all *Dolosicoccus* ASVs, were aligned to the 16S rRNA of *Dolosicoccus Paucivorans* retrieved from NCBI accession number AJ012666.1. Based in the V4 region alone, clear differences were evident between the sequences from the urinals and *Dolosicoccus Paucivorans* (Figure D.4).

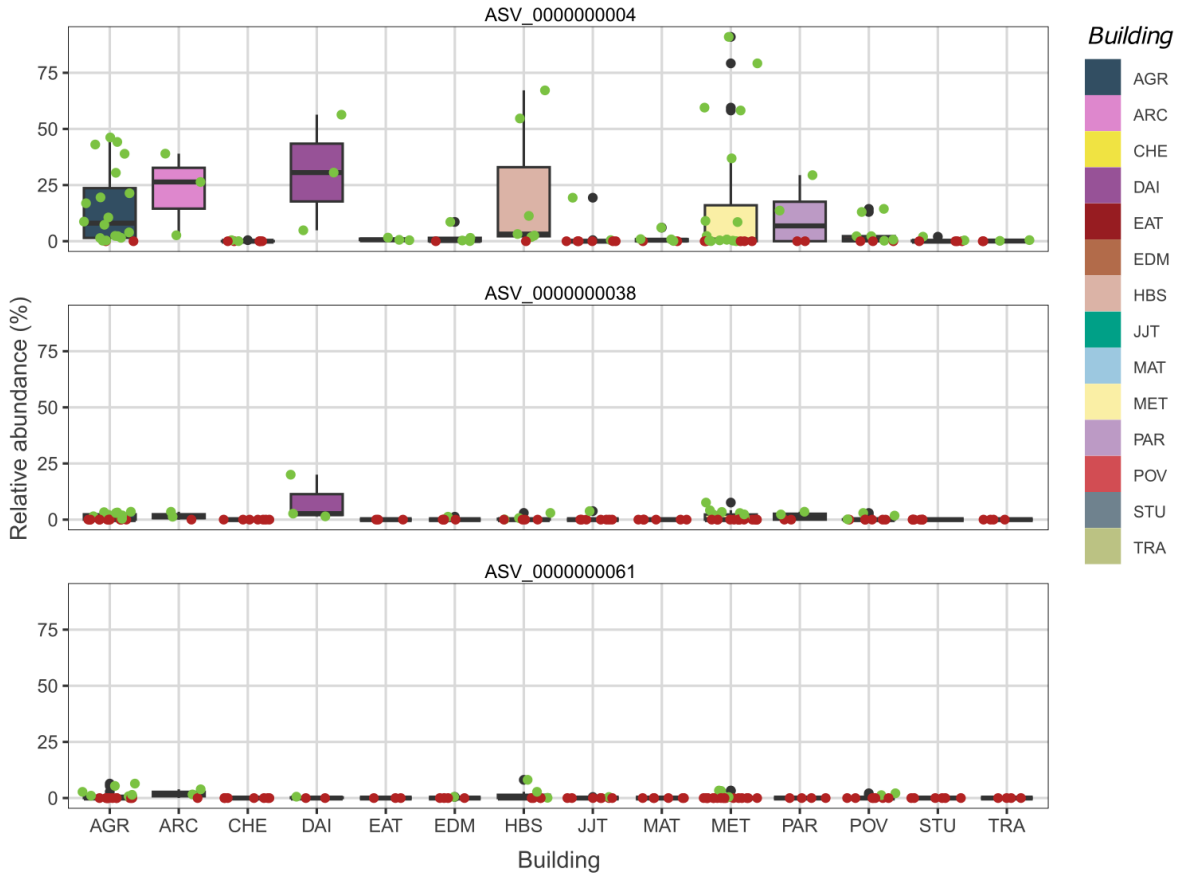


**Figure 5.3.** Relative abundances (%) of the top 3 *Dolosicoccus* ASVs by building. Points represent individual urinal samples. Red dots indicate relative abundance of zero, green indicate urinals with *Dolosicoccus* present. The top ASV (ASV_0000000004) is observed in all buildings, with the Meteorology building having the highest relative abundance of ASV_00000000004 in an individual urinal.

## 5.5 Discussion

This study provides the insight into the bacterial community structure and composition of urinal P-traps. Over 100 urinals located in various public restrooms within university buildings and a train station were analysed through amplicon sequencing of the 16S rRNA gene. Given the limited existing research on the bacterial communities specific to urinals, only limited comparative information was available. However, studies on the urinary tract microbiome enable us to draw comparisons between bacterial taxa identified in urine and those present in urinals for discussion. Our results demonstrate that the bacterial communities of urinal P-traps are diverse and vary significantly both within and between urinals situated in different buildings. The most abundant ASV identified also the had the highest prevalence and belonged to a genus with little reference in current literature.

### *5.5.1 Diversity and Composition of Urinal P-traps*

The bacterial community of urinal P-traps was less diverse in terms of alpha diversity in comparison to other samples from outdoor environments such as soil, high-touch surfaces in built environments, and skin (Banerjee & van der Heijden, 2023; Kim et al., 2022; Ross & Neufeld, 2015; Wetzels et al., 2021). However, the number of ASVs obtained from urinals was comparable to those found in sink P-traps (Withey et al., 2021). Although not as rich as other environments, P-traps still harboured a diverse number of bacterial taxa. Proteobacteria was the dominant phylum, followed by Firmicutes (Figure 5.2b). Proteobacteria has the largest phylogenetic composition and are found in various environments including the built environment and water-associated indoor areas (Chase et al., 2016; Douterelo et al., 2018; Park et al., 2021; Shin et al., 2015). Firmicutes are one of the two major phyla in the human gut, but they are also found widely e.g., households, hospital environments, schools, river sediments and marine sites (Rinninella et al., 2019; Rosenberg et al., 2011). Many Firmicutes can form spores that are highly resistant to environmental stresses such as desiccation (Galperin, 2013; Paredes-Sabja et al., 2014). Moreover, depending on the study, both Proteobacteria and Firmicutes have been reported to the most abundant bacteria in urine (Karstens et al., 2016; Ning et al., 2020; Perez-Carrasco et al., 2021; Siddiqui et al., 2011, 2012; Thomas-White et al., 2017).

Of the top ten identifiable genera, seven have been detected in urine from previous studies (Figure 5.2c, Table D.2E). The genera *Pseudomonas*, *Oligella* and *Atopostipes* were identified in healthy male urine (Bajic et al., 2020; Lewis et al., 2013; Perez-Carrasco et al., 2021). From female urine *Pseudomonas*, *Oligella*, *Acinetobacter* and *Dolosicoccus* species have been isolated (Miller-Ensminger et al., 2018). *Stenotrophomonas* has been detected in healthy female urine using amplicon pyrosequencing (Lewis et al., 2013). The remaining genera, *Reyranella* and *Bosea* have been isolated

from hospital water and water piping (Li et al., 2022; Nisar et al., 2023) and *Nevskia* found in water i.e., aquifers, lakes and aquariums (Cui et al., 2019; Leandro et al., 2012; Sturmeyer et al., 1998). Moreover, in some cases these bacterial genera can be pathogenic namely, *Acinetobacter*, *Bosea*, *Pseudomonas*, *Stenotrophomonas*, *Oligella* and *Achromobacter* (Nisar et al., 2023). *Achromobacter* species which have been previously isolated from hospitals, washing sinks and showers (Amoureux et al., 2013; Franco et al., 2020; Marion-Sanchez et al., 2020) can, although rare, cause urinary tract infections (Elston & Hoffman, 1966; Sarı et al., 2018; Tena et al., 2008). *Stenotrophomonas* has also been associated with urinary tract infections (UTI) (Vartivarian et al., 1996), and in rare cases *Oligella* was an infectious agent related with bacteraemia (Pagotto et al., 2016; Simmons et al., 2015). Research of the male urinary microbiota has found *Corynebacterium* to be the main genus (Fouts et al., 2012). However, in the present study, *Corynebacterium* was detected in urinal P-traps at very low abundances and prevalence (< 0.1% total relative abundance, 15% prevalence). This suggests that *Corynebacterium* is unable to proliferate in the P-trap environment and what is observed is a result of *Corynebacterium* cells, dead or alive, passing through the system. Whereas the top genera, particularly those with greater prevalence such as *Pseudomonas*, *Stenotrophomonas*, *Achromobacter*, *Dolosicoccus* are probably able to persist and tolerate the hostile environment of the P-trap. The elevated levels of ammonia and variation in pH of the P-trap will select for bacteria that can survive. A study by Lim and colleagues, 2021, characterising the ureolytic biomineralization from public restrooms identified *Oligella* in low-flow and waterless urinals, and *Atopostipes* and *Dolosicoccus* in waterless urinals. Compared to the current study they had a very limited sample size (n=11) and the focused on different types of urinals whereas on the university campus all urinals are conventional washdown urinals that experience flushing at regular timed intervals.

For all alpha diversity indices, the highest values were observed in Polly Vacher building. Since the building had a high Pielou's evenness and the highest ASV richness this could mean the bacterial community in this building is made up of many ASVs at relatively equally small abundances. When investigating the individual urinal P-traps from Polly Vacher, compositionally they appear similar and around 50% of genera are grouped as other due to low abundances except for one urinal which is dominated by *Nevskia*: thus, the reason for the highest mean relative abundance of *Nevskia* in this building (Figure 5.2c). Considering the samples in this building are from one of two restrooms it is unclear as to why the great abundance of *Nevskia* without monitoring patterns of behaviour in the urinals beforehand. In comparison the Train Station had the lowest Shannon diversity, Pielou's evenness values, and one of lowest ASV richness. The lower richness observed in the Train station could be explained by patterns of human usage. The Train Station when compared to university buildings will experience a higher traffic of human occupants therefore, the urinal although potentially

exposed to a wider variety of bacteria, will experience more frequent flushing after use thus, increased disturbance and turnover of bacteria in the P-traps. Moreover, the Train Station was clustered together on NMDS quite distinct from the other buildings and had a relatively homogeneous community (Figure 5.1d, e). As this building was at a different location, served a different purpose and may experience a more diverse set of users with differing behaviours, it is logical to infer that the bacterial community may differ. Additionally, three out of the four urinal P-traps located in the train station were dominated by the genus *Acinetobacter* and no other urinal from the campus showed relative abundances comparable to those from the Train station (Figure D.1). As noted, members of this genus can be pathogenic, but they can also be part of the human skin microflora and present in urine (Badave & Dhananjay, 2015; Powell et al., 2008). This increased signature of *Acinetobacter* could be again related to increased use but without occupancy numbers, this is speculation. Also, the cleaning practices of the Train station will differ compared to those of the university which are managed and cleaned consistently. Unfortunately, no data was obtained regarding cleaning practices, however in our previous study (Chapter 4) we showed that after bleach intervention in sink traps there was an increase in *Acinetobacter*. In the case of these urinals, they potentially may have been bleached prior to sampling hence the high relative abundance of *Acinetobacter* observed.

Overall, the results indicated building sampled had some effect on bacterial community structure and pairwise comparisons showed the majority of buildings were compositionally significantly different from one another with variable effect sizes ($R^2$ values ranging from 0.04 to 0.45, Table D.3). The smaller study of urinals and associated pipe biomineral deposits also found strong significant difference between samples grouped by location when using PERMANOVA. However, they elucidate to an interaction between sampling location and urinal type, and the presence of water in some urinals and its effects on nutrient concentrations. Therefore, the observed effects on biomineral microbial communities from sampling locations may depend on the urinal type (Lim et al., 2022). In the present study we demonstrate the effect of sampling location on bacterial community and the high variability across individual urinal P-traps. Thus, that urinal P-traps bacterial communities are shaped by restroom users, as is the case for many indoor built environment microbial communities (Hospodsky et al., 2012; Meadow et al., 2015). Generally, Pielou's evenness was high across all P-traps and buildings did not significantly differ. Ecosystems under high environmental stress often exhibit low species evenness as certain resilient species dominate (Scrosati et al., 2011). Urinal P-traps could be considered a difficult environment to proliferate due to chemical properties and differing microbial profiles of urine as this would be the primary source of microorganisms and nutrients to the environment (Pohl et al., 2020). In the case of the Train station, with the lowest evenness, this could be observed as *Acinetobacter* dominated and these urinals may experience more frequent

disturbances and increased diversity of microorganisms. For the buildings from campus, there was large variation in evenness within some buildings i.e., Agriculture building. Further investigation of individual urinal P-traps show domination by some bacterial taxa such as *Dolosicoccus*. The variations observed could be related to human use and behaviour. For example, preferential selection over certain urinals or incorrect use such as spitting (Wu et al., 2019). Many ASVs were observed at low relative abundances, these bacterial ASVs are possibly transient, passing through the system and a signature of the last user.

### *5.5.2 Core bacterial taxa of urinal P-traps*

Despite the high number of low abundant ASVs a core microbiome was observed in urinals. Five genera were classified as part of the core urinal P-trap microbiome due to their high prevalence. Phenotypic features that enable bacteria to tolerate the elevated pH and ammonia levels or utilise nutrients from urine will facilitate their establishment in urinal P-traps. The most prevalent genera identified was *Pseudomonas*. Some *Pseudomonas* spp. can be ureolytic and can increase in relative abundance in the presence of urea, therefore able to exploit this environment (Goswami et al., 2015; Jin et al., 2016; Jyothi & Rao, 2013; Subramaniyan et al., 2023). Furthermore, species of *Stenotrophomonas* and *Achromobacter* can also be ureolytic and can form biofilms (Jalilvand et al., 2020; Konstantinovic et al., 2017; Prasad, 1978; Umar et al., 2022). Additionally, *Achromobacter* has shown tolerance to stressful environments, a study demonstrated *Achromobacter* entered a viable but non-culturable (VBNC) state after chlorine disinfection and after approximately 25h the injured VBNC *Achromobacter* were resuscitated (Hu & Bai, 2023). *Brevundimonas* although not one of the top ten most abundant genera was present in 73% of urinal P-traps. Like the other highly prevalent genera, *Brevundimonas* spp. have been isolated from urine as well as from numerous aquatic habitats and can grow in nutrient limited conditions and form biofilms (Gricajeva et al., 2022; Karstens et al., 2016; Ryan & Pembroke, 2018). The final core genus, *Dolosicoccus* will be discussed in the following section. It is expected that the microbial communities present in urinals are also influenced by the surrounding environment and its users and in the case of the core bacterial genera many are associated with urine and have properties that enable their exploitation of this niche. Note, identification to species of these bacteria would be required to confirm their properties and would provide additional information on why these bacteria survive and proliferate in urinals.

### *5.5.3 The presence of* Dolosicoccus

One of the most notable findings from this study was the high prevalence and abundance of the genus *Dolosicoccus*. *Dolosicoccus* is one of the "minor" genera of the gram-stain positive family *Aerococcaceae* and only one species, *Dolosicoccus paucivorans* has been documented (Collins et al., 1999; Huch et al., 2014). *Dolosicoccus paucivorans* was originally isolated in 1995 from human blood, yet further research since then has been limited with few references to this genus in the literature (Collins et al., 1999). Using 16S rRNA sequencing, *Dolosicoccus* has been found in a variety of sample types including, human skin samples, rat guts, salvia from ticks, shrimp gut and, sediment from shrimp ponds (Lin et al., 2022; Qi et al., 2017; XiaoMing et al., 2016; Zhao et al., 2018). In these studies, the relative abundances of *Dolosicoccus* were consistently very low, with one notable exception found in a study of tick saliva where the dominant bacteria had highly similar sequences to *Dolosicoccus paucivorans* (XiaoMing et al., 2016). Interestingly, *Dolosicoccus* has been reported more frequently in samples associated with the urinary tract. Specifically, vaginal swabs from beagles and human urine samples taken directly from the bladder have identified *Dolosicoccus paucivorans* (Du et al., 2023; Hu et al., 2022; Miller-Ensminger et al., 2018). However, neither study identified *Dolosicoccus* as a majorly abundant genus. The sole urinal microbial community study that did identify *Dolosicoccus* reported its presence in waterless urinals, with a relative abundance of around 12% (Lim et al., 2022). This information was inferred from a figure in the paper, as there is no other explicit mention of the genus in the text. In contrast to the findings of these studies, our research demonstrates the presence of *Dolosicoccus* in conventional urinals, and notably, at varying yet high abundances depending on the building. The disparities observed could be attributed to the limited sampling size of conventional urinals (n=2), resulting in insufficient data was collection. In the present study, *Dolosicoccus* was detected at low prevalence (25% of urinals) in certain buildings, whereas in others, it was found in all urinals. This variability underscores the importance of a more extensive sampling approach to capture the nuanced distribution of *Dolosicoccus* in different environments. By solely comparing the 16S rRNA V4 region of the *Dolosicoccus* ASV found in urinals to *Dolosicoccus paucivorans,* notable differences in the sequences were observed, surpassing what would typically be expected from sequencing and data processing errors alone. This suggests the possibility of genuine biological distinctions; however, further investigation is essential to solidify this conclusion. Subsequent research efforts could be directed towards obtaining the metagenome-assembled genome (MAG) of the *Dolosicoccus* strain present in urinals from metagenomic samples. Successful recovery of the MAG would pave the way for selective isolation from fresh samples. This comprehensive approach would allow for a thorough comparison of the urinal *Dolosicoccus* genome to the complete genome of the *Dolosicoccus paucivorans* type strain, which has been assembled as part of this study. Additionally, this investigation

could involve the exploration of genes that contribute to the survival and proliferation of *Dolosicoccus* within the urinal environment.

### *5.5.4 Limitations and concluding remarks*

Limitations of this study include that environmental variables were not measured which could potentially influence bacterial community composition. However, within the BE conditions are kept relatively consistent for comfort of the occupants but this will be dependent on the BE purpose. Furthermore, collection of data regarding activities in restrooms and frequency of urinal use would provide greater insight to why we might observe some differences. Factors such as cleaning strategies and intensity will impact communities. While this should be consistent across the university managed sites, we were unable to obtain information regarding cleaning practices of the Train station urinals. Further, age of P-traps and associated pipes could affect the physical and community composition. Moreover, although our results show high abundances of certain bacterial genera, further work could be included to establish if the communities particularly the transient bacteria are live or dead. Either by applying additional high throughput "omics" approaches to detect what genes are active or metabolites being produced, or by using a viability dye such as PMA (Nocker & Camper, 2008; Shaffer et al., 2022).

Restrooms are a dynamic environment that host a diverse microbial community even within the P-traps of urinals. The perception that urine is sterile has been disproven and the microorganisms that pass through the urogenital tract may be able to form biofilms in the p-traps of urinals. Yet the species that can survive must tolerate elevated ammonia concentrations and the conditions of the P-trap. As a result, the urinal P-trap is a selective environment. Our results showed differences in bacterial communities from different buildings and large variations in the bacterial communities between individual sinks possibly due to the pressures of this environment and the differences due to human users and their behaviours. Despite this, core bacterial taxa were observed across urinals sampled and previously unexplored genera were found to be abundant. Further studies should aim to investigate bacterial members of the community in depth so we can begin to understand the possible risk urinals could pose and develop strategies mitigate their spread.

## 5.6 References

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## Appendix D

## Supplementary material for Chapter 5

**Microbial Landscape of University Urinals: a Metagenomic Perspective on Urinal P-traps and the Discovery of Their Most Abundant and Prevalent Species**

This appendix includes:

- **Figure D.1** - Bacterial composition of the top 15 taxa at genera level in all urinal samples, ordered by building. All less abundant genera are grouped as "Other".
- **Figure D.2** - Relative abundances (%) of the genus Dolosicoccus by building. Archaeology and The Dairy have the highest median of all the buildings.
- **Figure D.3** - Alignment of all the Dolosicoccus ASVs. Differences in bases between ASVs are shown (left). Heatmap based on the percentage identity generated from the alignment of the Dolosicoccus ASVs using Clustal Omega. Lighter purple represents less similarity between sequences (right).
- **Figure D.4** - Alignment of the top 3 Dolosicoccus ASVS (ASV_0000000004, ASV_0000000038, ASV_0000000061) and the 16S rRNA sequence of Dolosicoccus Paucivorans (J012666.1). Differences in bases between ASVs are shown (left). Heatmap based on the percentage identity generated from the alignment of the Dolosicoccus ASVs using Clustal Omega. Lighter purple represents less similarity between sequences (right).

- **Table D.1 -** Metadata collected for each urinal P-trap sample, including building sample collected and its main purpose.
- **Table D.2 -** Taxonomic profiling of the bacterial community of P-traps of urinals located on a university campus and train station (A) Phylum, (B) Class, (C) Order, (D) Family, (E) Genus.
- **Table D.3 -** Pairwise comparisons for all pairs of levels of the factor "Building" by using PERMANOVA. Benjamini-Hochberg corrected p-values shown. The R2 values indicate the amount of variation explained by the comparisons in the model.
- **Table D.4 -** Pairwise comparison for all significant pairs of levels of building by using permutest(). Permuatation-based test of multivariate homogeneity of group dispersions (variance). P-values based on 999 permutations and corrected with Benjamini-Hochberg (BH).

- **Table D.5** - The prevalence of Dolosicoccus within a building and the mean/median relative abundance (%) of Dolosicoccus in each building. Maximum relative abundance (%) and lowest relative abundance (%) of Dolosicoccus within a building shown.
- **Table D.6** - Sequences of the 25 Dolosicoccus ASVs and their mean relative abundance (%) across all urinal samples and their maximum relative abundance (%) observed in a urinal sample.

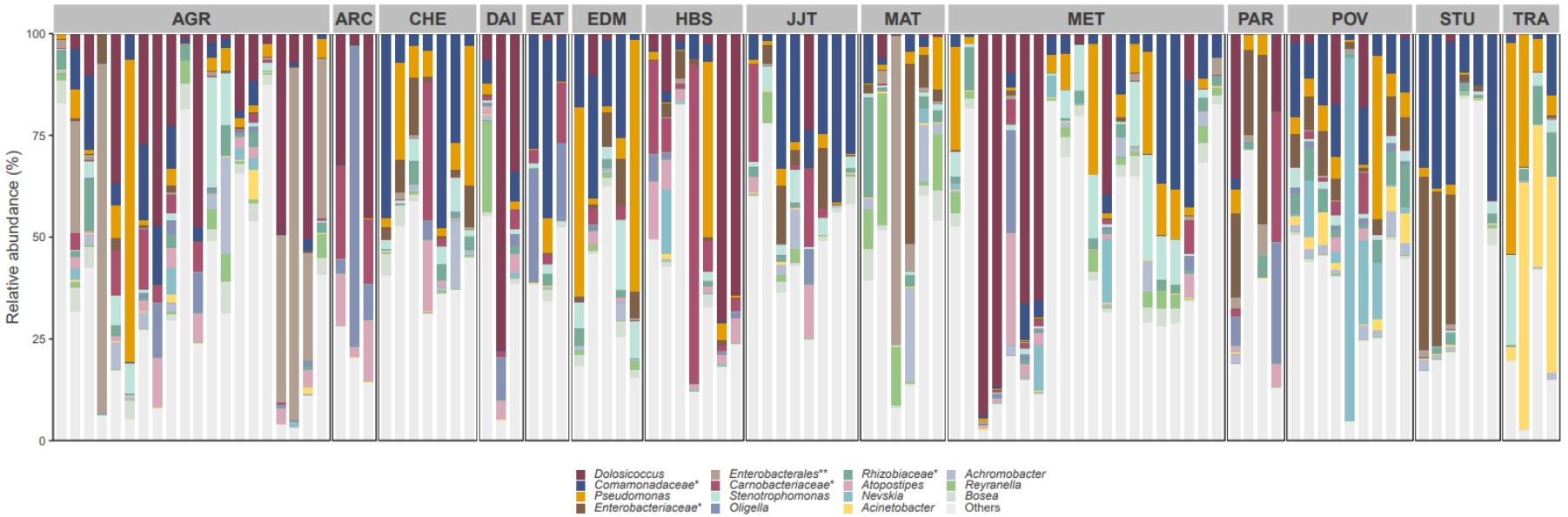


**Figure D.1**. Bacterial composition of the top 15 taxa at genera level in all urinal samples, ordered by building. All less abundant genera are grouped as “Other”.

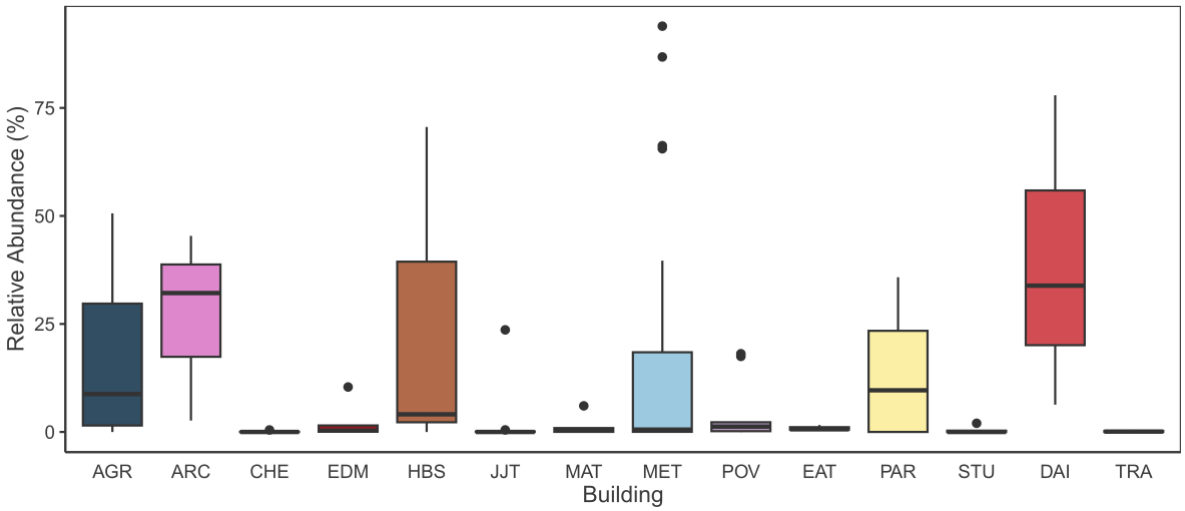


**Figure D.2**. Relative abundances (%) of the genus Dolosicoccus by building. Archaeology and The Dairy have the highest median of all the buildings.

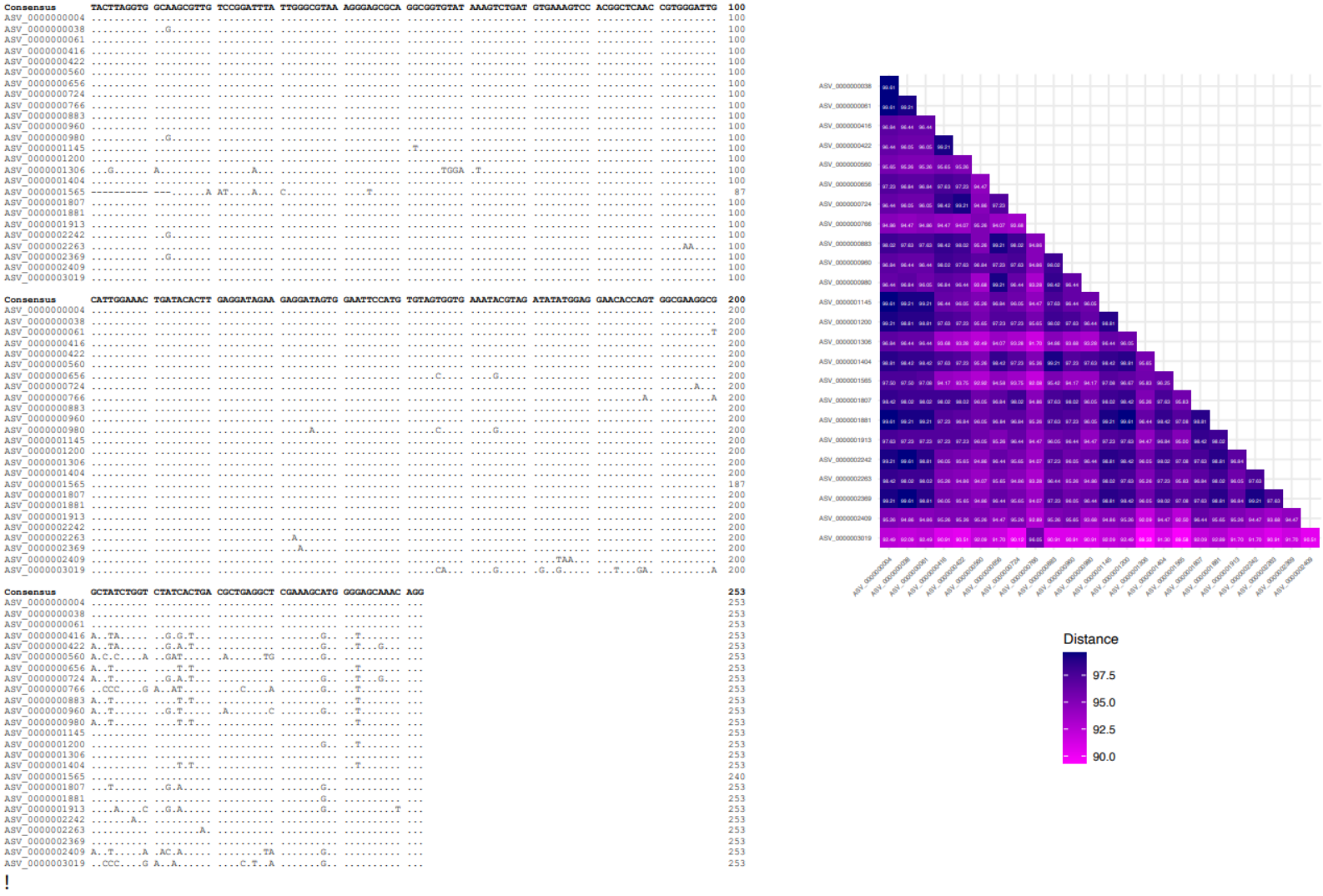


**Figure D.3**. Alignment of all the Dolosicoccus ASVs. Differences in bases between ASVs are shown (left). Heatmap based on the percentage identity generated from the alignment of the Dolosicoccus ASVs using Clustal Omega. Lighter purple represents less similarity between sequences (right).

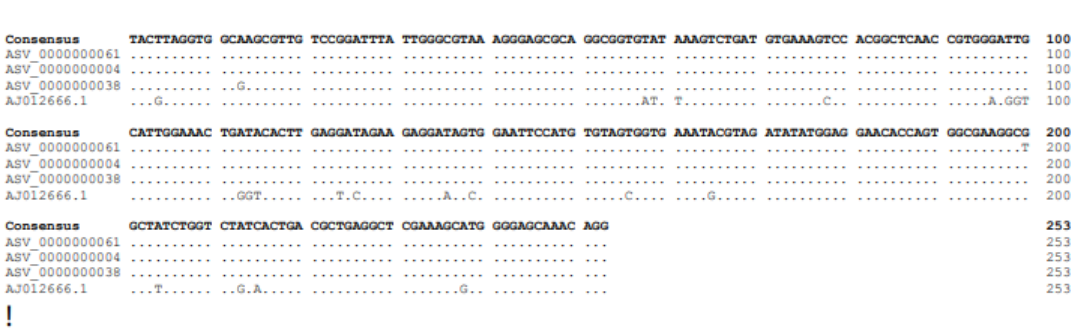


**Figure D.4** - Alignment of the top 3 Dolosicoccus ASVS (ASV_0000000004, ASV_0000000038, ASV_0000000061) and the 16S rRNA sequence of Dolosicoccus Paucivorans (J012666.1). Differences in bases between ASVs are shown (left). Heatmap based on the percentage identity generated from the alignment of the Dolosicoccus ASVs using Clustal Omega. Lighter purple represents less similarity between sequences (right).

| sample_id | sequencer | date_sampled | building | floor | restroom | urinal_id | urinal_type | slow_drain | location | building_purpose |
|---|---|---|---|---|---|---|---|---|---|---|
| AFS.E01.AgU1 | CEH | 04/06/2021 | Agriculture | Basement West | B1 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.AgU2 | CEH | 04/06/2021 | Agriculture | Basement West | B1 | U2 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.AgU2R | CEH | 04/06/2021 | Agriculture | Basement West | B1 | U2 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.AgU3 | CEH | 04/06/2021 | Agriculture | Basement West | B1 | U3 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.AgU4 | CEH | 04/06/2021 | Agriculture | Basement West | B1 | U4 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.AgU5 | CEH | 04/06/2021 | Agriculture | Basement West | B1 | U5 | Bowl | Y | WhiteKnights | Teaching |
| AFS.E01.AgU6 | CEH | 04/06/2021 | Agriculture | Basement West | B1 | U6 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.AgU7 | CEH | 04/06/2021 | Agriculture | 1st Floor West | B2 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.AgU9 | CEH | 04/06/2021 | Agriculture | 1st Floor West | B2 | U3 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.AgU10 | CEH | 04/06/2021 | Agriculture | 1st Floor West | B2 | U4 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.AgU11 | CEH | 04/06/2021 | Agriculture | 1st Floor West | B2 | U5 | Bowl | N | WhiteKnights | Teaching |

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| AFS.E01.AgU12 | CEH | 04/06/2021 | Agriculture | 1st Floor West | B2 | U6 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.AgU13 | CEH | 04/06/2021 | Agriculture | 1st Floor East | B3 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.AgU13R | CEH | 04/06/2021 | Agriculture | 1st Floor East | B3 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.AgU14 | CEH | 04/06/2021 | Agriculture | 1st Floor East | B3 | U2 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.AgU15 | CEH | 04/06/2021 | Agriculture | 2nd Floor East | B4 | U1 | Bowl | Y | WhiteKnights | Teaching |
| AFS.E01.AgU16 | CEH | 04/06/2021 | Agriculture | 2nd Floor East | B4 | U2 | Bowl | Y | WhiteKnights | Teaching |
| AFS.E01.AgU17 | CEH | 04/06/2021 | Agriculture | 3rd Floor West | B6 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.AgU17R | CEH | 04/06/2021 | Agriculture | 3rd Floor West | B6 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.AgU18 | CEH | 04/06/2021 | Agriculture | 3rd Floor West | B6 | U2 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.AgU20 | CEH | 04/06/2021 | Agriculture | 2nd Floor West | B7 | U2 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.AgU21 | CEH | 04/06/2021 | Agriculture | Ground Floor East | B7 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.AgU22 | CEH | 04/06/2021 | Agriculture | Ground Floor East | B7 | U2 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.ArU1 | CEH | 08/06/2021 | Archaeology | 1st Floor Mid | B1 | U1 | Bowl | Y | WhiteKnights | Teaching |
| AFS.E01.ArU2 | CEH | 08/06/2021 | Archaeology | 1st Floor Mid | B1 | U2 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.ArU3 | CEH | 08/06/2021 | Archaeology | 1st Floor Mid | B1 | U3 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.ChU1 | CEH | 10/06/2021 | Chemistry | 1st Floor | B1 | U1 | Bowl | Y | WhiteKnights | Teaching |
| AFS.E01.ChU3 | CEH | 10/06/2021 | Chemistry | 1st Floor | B1 | U3 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.ChU4 | CEH | 10/06/2021 | Chemistry | 1st Floor | B1 | U4 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.ChU5 | CEH | 10/06/2021 | Chemistry | 2nd Floor | B2 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.ChU6 | CEH | 10/06/2021 | Chemistry | 2nd Floor | B2 | U2 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.ChU7 | CEH | 10/06/2021 | Chemistry | 2nd Floor | B2 | U3 | Bowl | N | WhiteKnights | Teaching |

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| AFS.E01.ChU8 | CEH | 10/06/2021 | Chemistry | 2nd Floor | B2 | U4 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.DaU1 | CEH | 05/07/2021 | The Dairy | Ground Floor | B1 | U1 | Bowl | N | London Rd | Recreational |
| AFS.E01.DaU2 | CEH | 05/07/2021 | The Dairy | Ground Floor | B1 | U2 | Bowl | N | London Rd | Recreational |
| AFS.E01.DaU3 | CEH | 05/07/2021 | The Dairy | Ground Floor | B1 | U3 | Bowl | N | London Rd | Recreational |
| AFS.E01.EMU1 | CEH | 01/06/2021 | Edith Morely | Ground Floor Tower | B1 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.EMU2 | CEH | 01/06/2021 | Edith Morely | Ground Floor Tower | B1 | U2 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.EMU3 | CEH | 02/06/2021 | Edith Morely | 1st Floor Tower | B2 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.EMU4 | CEH | 03/06/2021 | Edith Morely | 1st Floor Horseshoe South | B3 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.EMU5 | CEH | 04/06/2021 | Edith Morely | 2nd Floor Horseshoe South | B4 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.ESU1 | CEH | 08/06/2021 | Eat at the Square | Ground Floor | B1 | U1 | Bowl | N | WhiteKnights | Recreational |
| AFS.E01.ESU2 | CEH | 08/06/2021 | Eat at the Square | Ground Floor | B1 | U2 | Bowl | N | WhiteKnights | Recreational |
| AFS.E01.ESU3 | CEH | 08/06/2021 | Eat at the Square | Ground Floor | B1 | U3 | Bowl | N | WhiteKnights | Recreational |
| AFS.E01.HeU1 | CEH | 08/06/2021 | Henley Business School | Ground Floor North East | B1 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.HeU2 | CEH | 08/06/2021 | Henley Business School | Ground Floor North East | B1 | U2 | Bowl | Y | WhiteKnights | Teaching |
| AFS.E01.HeU4 | CEH | 08/06/2021 | Henley Business School | 1st Floor North East | B2 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.HeU5 | CEH | 08/06/2021 | Henley Business School | 2nd Floor North East | B3 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.HeU6 | CEH | 08/06/2021 | Henley Business School | 2nd Floor North East | B3 | U2 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.HeU7 | CEH | 08/06/2021 | Henley Business School | 2nd Floor Mid | B4 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.HeU7R | CEH | 08/06/2021 | Henley Business School | 2nd Floor Mid | B4 | U1 | Bowl | Y | WhiteKnights | Teaching |
| AFS.E01.HeU8 | CEH | 08/06/2021 | Henley Business School | 2nd Floor Mid | B4 | U2 | Bowl | Y | WhiteKnights | Teaching |
| AFS.E01.HNU1 | CEH | 05/07/2021 | Harry Nursten | Ground Floor | B1 | U1 | Bowl | N | WhiteKnights | Teaching |

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| AFS.E01.JJU1 | CEH | 02/06/2021 | JJ Thompson | 2nd Floor East | B2 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.JJU2 | CEH | 02/06/2021 | JJ Thompson | 2nd Floor East | B2 | U2 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.JJU3 | CEH | 02/06/2021 | JJ Thompson | 2nd Floor East | B2 | U3 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.JJU4 | CEH | 02/06/2021 | JJ Thompson | 2nd Floor West | B3 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.JJU5 | CEH | 02/06/2021 | JJ Thompson | 2nd Floor West | B3 | U2 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.JJU6 | CEH | 02/06/2021 | JJ Thompson | Ground Floor | B4 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.JJU7 | CEH | 02/06/2021 | JJ Thompson | Ground Floor | B4 | U2 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.JJU8 | CEH | 02/06/2021 | JJ Thompson | Ground Floor | B4 | U3 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.MaU1 | CEH | 02/06/2021 | Maths | 1st Floor | B1 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.MaU2 | CEH | 02/06/2021 | Maths | 1st Floor | B1 | U2 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.MaU3 | CEH | 02/06/2021 | Maths | 1st Floor | B1 | U3 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.MaU4 | CEH | 02/06/2021 | Maths | 2nd Floor | B2 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.MaU5 | CEH | 02/06/2021 | Maths | 2nd Floor | B2 | U2 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.MaU6 | CEH | 02/06/2021 | Maths | 2nd Floor | B2 | U3 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.MeU1 | CEH | 07/06/2021 | Meteorology | Basement West | B1 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.MeU2 | CEH | 07/06/2021 | Meteorology | Basement West | B1 | U2 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.MeU3 | CEH | 07/06/2021 | Meteorology | Basement West | B1 | U3 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.MeU4 | CEH | 07/06/2021 | Meteorology | Basement West | B1 | U4 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.MeU5 | CEH | 07/06/2021 | Meteorology | 2nd Floor West | B2 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.MeU6 | CEH | 07/06/2021 | Meteorology | 2nd Floor West | B2 | U2 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.MeU7 | CEH | 07/06/2021 | Meteorology | 2nd Floor West | B2 | U3 | Bowl | Y | WhiteKnights | Teaching |
| AFS.E01.MeU8 | CEH | 07/06/2021 | Meteorology | 2nd Floor West | B2 | U4 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.MeU9 | CEH | 07/06/2021 | Meteorology | Ground Floor Mid | B3 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.MeU10 | CEH | 07/06/2021 | Meteorology | 1st Floor Mid | B4 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.MeU11 | CEH | 07/06/2021 | Meteorology | 1st Floor North East | B5 | U1 | Bowl | Y | WhiteKnights | Teaching |
| AFS.E01.MeU12 | CEH | 07/06/2021 | Meteorology | 1st Floor North East | B5 | U2 | Bowl | Y | WhiteKnights | Teaching |

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|---|---|---|---|---|---|---|---|---|---|---|
| AFS.E01.MeU14 | CEH | 07/06/2021 | Meteorology | 1st Floor Further North East | B6 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.MeU15 | CEH | 07/06/2021 | Meteorology | 1st Floor Further North East | B6 | U2 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.MeU16 | CEH | 07/06/2021 | Meteorology | 2nd Floor North East | B7 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.MeU17 | CEH | 07/06/2021 | Meteorology | 2nd Floor North East | B7 | U2 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.MeU18 | CEH | 07/06/2021 | Meteorology | 2nd Floor North East | B7 | U3 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.MeU19 | CEH | 07/06/2021 | Meteorology | 2nd Floor Further North East | B8 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.MeU20 | CEH | 07/06/2021 | Meteorology | Ground Floor North East | B9 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.MeU20R | CEH | 07/06/2021 | Meteorology | Ground Floor North East | B9 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.MeU21 | CEH | 07/06/2021 | Meteorology | Ground Floor North East | B9 | U2 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.MeU22 | CEH | 07/06/2021 | Meteorology | Ground Floor North East | B9 | U3 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.Neg03 | CEH | Control | Negative Control | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| AFS.E01.Neg04 | CEH | Control | Negative Control | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| AFS.E01.Neg05 | CEH | Control | Negative Control | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| AFS.E01.PEU1 | CEH | 05/07/2021 | Park Eat | Ground Floor | B1 | U1 | Bowl | N | WhiteKnights | Recreational |
| AFS.E01.PEU2 | CEH | 05/07/2021 | Park Eat | Ground Floor | B1 | U2 | Bowl | N | WhiteKnights | Recreational |
| AFS.E01.PEU3 | CEH | 05/07/2021 | Park Eat | Ground Floor | B1 | U3 | Bowl | N | WhiteKnights | Recreational |
| AFS.E01.PEU4 | CEH | 05/07/2021 | Park Eat | Ground Floor | B1 | U4 | Bowl | N | WhiteKnights | Recreational |
| AFS.E01.PVU1 | CEH | 01/06/2021 | Polly Vacher | Ground Floor East | B1 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.PVU2 | CEH | 01/06/2021 | Polly Vacher | Ground Floor East | B1 | U2 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.PVU3 | CEH | 01/06/2021 | Polly Vacher | Ground Floor East | B1 | U3 | Bowl | N | WhiteKnights | Teaching |

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|---|---|---|---|---|---|---|---|---|---|---|
| AFS.E01.PVU5 | CEH | 01/06/2021 | Polly Vacher | Ground Floor West | B2 | U2 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.PVU6 | CEH | 01/06/2021 | Polly Vacher | Ground Floor West | B2 | U3 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.PVU7 | CEH | 01/06/2021 | Polly Vacher | Ground Floor West | B2 | U4 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.PVU7R | CEH | 01/06/2021 | Polly Vacher | Ground Floor West | B2 | U4 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.PVU8 | CEH | 01/06/2021 | Polly Vacher | 1st Floor | B3 | U1 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.PVU9R | CEH | 01/06/2021 | Polly Vacher | 1st Floor | B3 | U2 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.PVU11 | CEH | 01/06/2021 | Polly Vacher | 1st Floor | B3 | U4 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.PVU11R | CEH | 01/06/2021 | Polly Vacher | 1st Floor | B3 | U4 | Bowl | N | WhiteKnights | Teaching |
| AFS.E01.TSU1 | CEH | 03/07/2021 | Train Station | Ticket Hall | B1 | U1 | Bowl | N | Public | Other |
| AFS.E01.TSU2 | CEH | 03/07/2021 | Train Station | Ticket Hall | B1 | U2 | Bowl | N | Public | Other |
| AFS.E01.TSU3 | CEH | 03/07/2021 | Train Station | Ticket Hall | B1 | U3 | Bowl | N | Public | Other |
| AFS.E01.TSU4 | CEH | 03/07/2021 | Train Station | Ticket Hall | B1 | U4 | Bowl | N | Public | Other |
| AFS.E01.UnU1 | CEH | 02/06/2021 | Union | Corridor outside Mojos | B1 | U1 | Trench | N | WhiteKnights | Recreational |
| AFS.E01.UnU2 | CEH | 02/06/2021 | Union | Corridor outside Mojos | B1 | U2 | Trench | N | WhiteKnights | Recreational |
| AFS.E01.UnU3 | CEH | 02/06/2021 | Union | Ground Floor Main Room | B2 | U1 | Trench | N | WhiteKnights | Recreational |
| AFS.E01.UnU4 | CEH | 02/06/2021 | Union | Ground Floor Main Room | B2 | U2 | Trench | N | WhiteKnights | Recreational |
| AFS.E01.UnU5 | CEH | 02/06/2021 | Union | First Floor Main Room | B3 | U1 | Trench | N | WhiteKnights | Recreational |
| AFS.E01.UnU6 | CEH | 02/06/2021 | Union | First Floor Main Room | B3 | U2 | Trench | N | WhiteKnights | Recreational |
| AFS.E02.Flc1 | CEH | Falcon | Falcon Tube | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| AFS.E02.Flc2 | CEH | Falcon | Falcon Tube | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| ZW.E03.URI01 | Novogene | 01/02/2023 | Palmer | Ground Floor | Pa1 | Pa1 | Bowl | unknown | WhiteKnights | Teaching |
| ZW.E03.URI02 | Novogene | 01/02/2023 | Palmer | Ground Floor | Pa2 | Pa2 | Bowl | unknown | WhiteKnights | Teaching |
| ZW.E03.URI03 | Novogene | 01/02/2023 | Palmer | Ground Floor | Pa4 | Pa4 | Bowl | unknown | WhiteKnights | Teaching |
| ZW.E03.URI04 | Novogene | 01/02/2023 | Palmer | Ground Floor | Pa5 | Pa5 | Bowl | unknown | WhiteKnights | Teaching |

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|---|---|---|---|---|---|---|---|---|---|---|
| ZW.E03.URI05 | Novogene | 01/02/2023 | Henley Business School | Ground Floor | HBS2 | HBS2 | Bowl | unknown | WhiteKnights | Teaching |
| ZW.E03.URI06 | Novogene | 01/02/2023 | Henley Business School | Ground Floor | HBS3 | HBS3 | Bowl | unknown | WhiteKnights | Teaching |
| ZW.E03.URI07 | Novogene | 01/02/2023 | Henley Business School | Ground Floor | HBS4 | HBS4 | Bowl | unknown | WhiteKnights | Teaching |
| ZW.E03.URI08 | Novogene | 01/02/2023 | Henley Business School | Ground Floor | HBS5 | HBS5 | Bowl | unknown | WhiteKnights | Teaching |
| ZW.E03.URI09 | Novogene | 01/02/2023 | Henley Business School | Ground Floor | HBS6 | HBS6 | Bowl | unknown | WhiteKnights | Teaching |

**Table D.1.** Metadata collected for each urinal P-trap sample, including building sample collected and its main purpose.

**(A)**

| Phylum | Total Reads | Total Reads (%) | Prevalence (number of samples) | Prevalence (%) |
|---|---|---|---|---|
| Proteobacteria | 691653 | 70.72 | 105 | 100 |
| Firmicutes | 217503 | 22.24 | 96 | 91.43 |
| Bacteroidota | 25406 | 2.6 | 98 | 93.33 |
| Actinobacteriota | 15810 | 1.62 | 68 | 64.76 |
| Cyanobacteria | 12220 | 1.25 | 50 | 47.62 |
| Planctomycetota | 5165 | 0.53 | 45 | 42.86 |
| Verrucomicrobiota | 2101 | 0.21 | 29 | 27.62 |
| Acidobacteriota | 1923 | 0.2 | 18 | 17.14 |
| Bdellovibrionota | 1194 | 0.12 | 36 | 34.29 |
| Dependentiae | 1027 | 0.11 | 23 | 21.9 |
| Desulfobacterota | 1102 | 0.11 | 19 | 18.1 |
| Gemmatimonadota | 951 | 0.1 | 21 | 20 |
| Campylobacterota | 732 | 0.07 | 15 | 14.29 |
| Nitrospirota | 249 | 0.03 | 5 | 4.76 |
| Synergistota | 257 | 0.03 | 8 | 7.62 |
| WPS-2 | 270 | 0.03 | 12 | 11.43 |
| Chloroflexi | 102 | 0.01 | 6 | 5.71 |
| Deinococcota | 50 | 0.01 | 3 | 2.86 |
| Myxococcota | 145 | 0.01 | 6 | 5.71 |
| Fusobacteriota | 43 | 0 | 3 | 2.86 |
| Hydrogenedentes | 45 | 0 | 1 | 0.95 |
| Patescibacteria | 5 | 0 | 1 | 0.95 |
| Spirochaetota | 12 | 0 | 2 | 1.9 |
| Thermotogota | 5 | 0 | 1 | 0.95 |

**(B)**

| Phylum | Class | Total Reads | Total Reads (%) | Prevalence (number of samples) | Prevalence (%) |
|---|---|---|---|---|---|
| Acidobacteriota | Acidobacteriae | 85 | 0.01 | 3 | 2.86 |
| Acidobacteriota | Blastocatellia | 1748 | 0.18 | 16 | 15.24 |
| Acidobacteriota | Vicinamibacteria | 90 | 0.01 | 3 | 2.86 |
| Actinobacteriota | Actinobacteria | 15694 | 1.6 | 68 | 64.76 |
| Actinobacteriota | Coriobacteriia | 105 | 0.01 | 6 | 5.71 |
| Actinobacteriota | Thermoleophilia | 11 | 0 | 1 | 0.95 |
| Bacteroidota | Bacteroidia | 25406 | 2.6 | 98 | 93.33 |
| Bdellovibrionota | Bdellovibrionia | 1194 | 0.12 | 36 | 34.29 |
| Campylobacterota | Campylobacteria | 732 | 0.07 | 15 | 14.29 |
| Chloroflexi | Chloroflexia | 14 | 0 | 1 | 0.95 |
| Chloroflexi | JG30-KF-CM66 | 10 | 0 | 1 | 0.95 |
| Chloroflexi | KD4-96 | 15 | 0 | 2 | 1.9 |
| Chloroflexi | TK10 | 63 | 0.01 | 2 | 1.9 |
| Cyanobacteria | Cyanobacteriia | 766 | 0.08 | 22 | 20.95 |
| Cyanobacteria | Vampirivibrionia | 11454 | 1.17 | 42 | 40 |
| Deinococcota | Deinococci | 50 | 0.01 | 3 | 2.86 |
| Dependentiae | Babeliae | 1027 | 0.11 | 23 | 21.9 |
| Desulfobacterota | Desulfovibrionia | 1079 | 0.11 | 17 | 16.19 |
| Desulfobacterota | Desulfuromonadia | 9 | 0 | 1 | 0.95 |
| Desulfobacterota | Unclassified | 14 | 0 | 2 | 1.9 |
| Firmicutes | Bacilli | 196534 | 20.1 | 96 | 91.43 |
| Firmicutes | Clostridia | 16166 | 1.65 | 75 | 71.43 |
| Firmicutes | Desulfitobacteriia | 53 | 0.01 | 2 | 1.9 |
| Firmicutes | Limnochordia | 14 | 0 | 2 | 1.9 |
| Firmicutes | Negativicutes | 229 | 0.02 | 20 | 19.05 |
| Firmicutes | Unclassified | 4507 | 0.46 | 34 | 32.38 |
| Fusobacteriota | Fusobacteriia | 43 | 0 | 3 | 2.86 |
| Gemmatimonadota | Gemmatimonadetes | 951 | 0.1 | 21 | 20 |
| Hydrogenedentes | Hydrogenedentia | 45 | 0 | 1 | 0.95 |
| Myxococcota | Myxococcia | 101 | 0.01 | 4 | 3.81 |
| Myxococcota | Polyangia | 44 | 0 | 2 | 1.9 |
| Nitrospirota | Nitrospiria | 249 | 0.03 | 5 | 4.76 |
| Patescibacteria | Saccharimonadia | 5 | 0 | 1 | 0.95 |
| Planctomycetota | OM190 | 264 | 0.03 | 7 | 6.67 |
| Planctomycetota | Phycisphaerae | 322 | 0.03 | 8 | 7.62 |
| Planctomycetota | Planctomycetes | 4541 | 0.46 | 42 | 40 |
| Planctomycetota | Unclassified | 23 | 0 | 2 | 1.9 |
| Planctomycetota | vadinHA49 | 15 | 0 | 1 | 0.95 |
| Proteobacteria | Alphaproteobacteria | 159667 | 16.33 | 97 | 92.38 |
| Proteobacteria | Gammaproteobacteria | 531958 | 54.39 | 105 | 100 |

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|---|---|---|---|---|---|
| Proteobacteria | Unclassified | 28 | 0 | 3 | 2.86 |
| Spirochaetota | Leptospirae | 12 | 0 | 2 | 1.9 |
| Synergistota | Synergistia | 257 | 0.03 | 8 | 7.62 |
| Thermotogota | Thermotogae | 5 | 0 | 1 | 0.95 |
| Verrucomicrobiota | Chlamydiae | 437 | 0.04 | 15 | 14.29 |
| Verrucomicrobiota | Verrucomicrobiae | 1664 | 0.17 | 22 | 20.95 |
| WPS-2 | Unclassified | 270 | 0.03 | 12 | 11.43 |

**(C)**

| Phylum | Class | Order | Total Reads | Total Reads (%) | Prevalence (number of samples) | Prevalence (%) |
|---|---|---|---|---|---|---|
| Acidobacteriota | Acidobacteriae | Bryobacterales | 30 | 0 | 1 | 0.95 |
| Acidobacteriota | Acidobacteriae | Paludibaculum | 35 | 0 | 1 | 0.95 |
| Acidobacteriota | Acidobacteriae | Unclassified | 20 | 0 | 1 | 0.95 |
| Acidobacteriota | Blastocatellia | Blastocatellales | 1662 | 0.17 | 15 | 14.29 |
| Acidobacteriota | Blastocatellia | Chloracidobacteriales | 6 | 0 | 1 | 0.95 |
| Acidobacteriota | Blastocatellia | Nov-24 | 80 | 0.01 | 2 | 1.9 |
| Acidobacteriota | Vicinamibacteria | Vicinamibacterales | 90 | 0.01 | 3 | 2.86 |
| Actinobacteriota | Actinobacteria | Actinomycetales | 702 | 0.07 | 24 | 22.86 |
| Actinobacteriota | Actinobacteria | Bifidobacteriales | 52 | 0.01 | 1 | 0.95 |
| Actinobacteriota | Actinobacteria | Corynebacteriales | 5886 | 0.6 | 45 | 42.86 |
| Actinobacteriota | Actinobacteria | Frankiales | 65 | 0.01 | 6 | 5.71 |
| Actinobacteriota | Actinobacteria | Micrococcales | 8776 | 0.9 | 44 | 41.9 |
| Actinobacteriota | Actinobacteria | Unclassified | 213 | 0.02 | 6 | 5.71 |
| Actinobacteriota | Coriobacteriia | Coriobacteriales | 105 | 0.01 | 6 | 5.71 |
| Actinobacteriota | Thermoleophilia | Gaiellales | 11 | 0 | 1 | 0.95 |
| Bacteroidota | Bacteroidia | Bacteroidales | 5992 | 0.61 | 60 | 57.14 |
| Bacteroidota | Bacteroidia | Chitinophagales | 3244 | 0.33 | 40 | 38.1 |
| Bacteroidota | Bacteroidia | Cytophagales | 3471 | 0.35 | 25 | 23.81 |
| Bacteroidota | Bacteroidia | Flavobacteriales | 10320 | 1.06 | 78 | 74.29 |
| Bacteroidota | Bacteroidia | Sphingobacteriales | 2141 | 0.22 | 47 | 44.76 |
| Bacteroidota | Bacteroidia | Unclassified | 238 | 0.02 | 8 | 7.62 |
| Bdellovibrionota | Bdellovibrionia | Bacteriovoracales | 478 | 0.05 | 25 | 23.81 |
| Bdellovibrionota | Bdellovibrionia | Bdellovibrionales | 716 | 0.07 | 19 | 18.1 |

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|---|---|---|---|---|---|---|
| Campylobacterota | Campylobacteria | Campylobacterales | 732 | 0.07 | 15 | 14.29 |
| Chloroflexi | Chloroflexia | Thermomicrobiales | 14 | 0 | 1 | 0.95 |
| Chloroflexi | JG30-KF-CM66 | Unclassified | 10 | 0 | 1 | 0.95 |
| Chloroflexi | KD4-96 | Unclassified | 15 | 0 | 2 | 1.9 |
| Chloroflexi | TK10 | Unclassified | 63 | 0.01 | 2 | 1.9 |
| Cyanobacteria | Cyanobacteriia | Chloroplast | 633 | 0.06 | 22 | 20.95 |
| Cyanobacteria | Cyanobacteriia | Cyanobacteriales | 106 | 0.01 | 6 | 5.71 |
| Cyanobacteria | Cyanobacteriia | Leptolyngbyales | 27 | 0 | 1 | 0.95 |
| Cyanobacteria | Vampirivibrionia | Obscuribacterales | 11190 | 1.14 | 41 | 39.05 |
| Cyanobacteria | Vampirivibrionia | Vampirovibrionales | 264 | 0.03 | 3 | 2.86 |
| Deinococcota | Deinococci | Deinococcales | 49 | 0.01 | 2 | 1.9 |
| Deinococcota | Deinococci | Thermales | 1 | 0 | 1 | 0.95 |
| Dependentiae | Babeliae | Babeliales | 1027 | 0.11 | 23 | 21.9 |
| Desulfobacterota | Desulfovibrionia | Desulfovibrionales | 1079 | 0.11 | 17 | 16.19 |
| Desulfobacterota | Desulfuromonadia | Geobacterales | 9 | 0 | 1 | 0.95 |
| Desulfobacterota | Unclassified | Unclassified | 14 | 0 | 2 | 1.9 |
| Firmicutes | Bacilli | Acholeplasmatales | 7 | 0 | 1 | 0.95 |
| Firmicutes | Bacilli | Bacillales | 51 | 0.01 | 5 | 4.76 |
| Firmicutes | Bacilli | Erysipelotrichales | 3129 | 0.32 | 39 | 37.14 |
| Firmicutes | Bacilli | Lactobacillales | 192813 | 19.72 | 95 | 90.48 |
| Firmicutes | Bacilli | RF39 | 23 | 0 | 2 | 1.9 |
| Firmicutes | Bacilli | Staphylococcales | 466 | 0.05 | 17 | 16.19 |
| Firmicutes | Bacilli | Unclassified | 45 | 0 | 2 | 1.9 |
| Firmicutes | Clostridia | Christensenellales | 35 | 0 | 1 | 0.95 |
| Firmicutes | Clostridia | Clostridia UCG-014 | 119 | 0.01 | 4 | 3.81 |
| Firmicutes | Clostridia | Clostridiales | 1326 | 0.14 | 28 | 26.67 |
| Firmicutes | Clostridia | Eubacteriales | 163 | 0.02 | 9 | 8.57 |
| Firmicutes | Clostridia | Lachnospirales | 309 | 0.03 | 13 | 12.38 |
| Firmicutes | Clostridia | Oscillospirales | 1310 | 0.13 | 26 | 24.76 |

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|---|---|---|---|---|---|---|
| Firmicutes | Clostridia | Peptococcales | 116 | 0.01 | 5 | 4.76 |
| Firmicutes | Clostridia | Peptostreptococcales-Tissierellales | 12405 | 1.27 | 73 | 69.52 |
| Firmicutes | Clostridia | Unclassified | 383 | 0.04 | 17 | 16.19 |
| Firmicutes | Desulfitobacteriia | Desulfitobacteriales | 53 | 0.01 | 2 | 1.9 |
| Firmicutes | Limnochordia | MBA03 | 14 | 0 | 2 | 1.9 |
| Firmicutes | Negativicutes | Veillonellales-Selenomonadales | 229 | 0.02 | 20 | 19.05 |
| Firmicutes | Unclassified | Unclassified | 4507 | 0.46 | 34 | 32.38 |
| Fusobacteriota | Fusobacteriia | Fusobacteriales | 43 | 0 | 3 | 2.86 |
| Gemmatimonadota | Gemmatimonadetes | Gemmatimonadales | 951 | 0.1 | 21 | 20 |
| Hydrogenedentes | Hydrogenedentia | Hydrogenedentiales | 45 | 0 | 1 | 0.95 |
| Myxococcota | Myxococcia | Myxococcales | 101 | 0.01 | 4 | 3.81 |
| Myxococcota | Polyangia | Blfdi19 | 37 | 0 | 1 | 0.95 |
| Myxococcota | Polyangia | Haliangiales | 7 | 0 | 1 | 0.95 |
| Nitrospirota | Nitrospiria | Nitrospirales | 249 | 0.03 | 5 | 4.76 |
| Patescibacteria | Saccharimonadia | Saccharimonadales | 5 | 0 | 1 | 0.95 |
| Planctomycetota | OM190 | Unclassified | 264 | 0.03 | 7 | 6.67 |
| Planctomycetota | Phycisphaerae | Phycisphaerales | 322 | 0.03 | 8 | 7.62 |
| Planctomycetota | Planctomycetes | Gemmatales | 1710 | 0.17 | 29 | 27.62 |
| Planctomycetota | Planctomycetes | Isosphaerales | 108 | 0.01 | 5 | 4.76 |
| Planctomycetota | Planctomycetes | Pirellulales | 1470 | 0.15 | 19 | 18.1 |
| Planctomycetota | Planctomycetes | Planctomycetales | 1154 | 0.12 | 20 | 19.05 |
| Planctomycetota | Planctomycetes | Unclassified | 99 | 0.01 | 7 | 6.67 |
| Planctomycetota | Unclassified | Unclassified | 23 | 0 | 2 | 1.9 |
| Planctomycetota | vadinHA49 | Unclassified | 15 | 0 | 1 | 0.95 |
| Proteobacteria | Alphaproteobacteria | Acetobacterales | 93 | 0.01 | 6 | 5.71 |
| Proteobacteria | Alphaproteobacteria | Azospirillales | 268 | 0.03 | 4 | 3.81 |
| Proteobacteria | Alphaproteobacteria | Caedibacterales | 598 | 0.06 | 18 | 17.14 |
| Proteobacteria | Alphaproteobacteria | Caulobacterales | 13384 | 1.37 | 78 | 74.29 |
| Proteobacteria | Alphaproteobacteria | Holosporales | 88 | 0.01 | 6 | 5.71 |

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|---|---|---|---|---|---|---|
| Proteobacteria | Alphaproteobacteria | Micavibrionales | 324 | 0.03 | 6 | 5.71 |
| Proteobacteria | Alphaproteobacteria | Paracaedibacterales | 414 | 0.04 | 13 | 12.38 |
| Proteobacteria | Alphaproteobacteria | Reyranellales | 16853 | 1.72 | 59 | 56.19 |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | 81506 | 8.33 | 95 | 90.48 |
| Proteobacteria | Alphaproteobacteria | Rhodobacterales | 4612 | 0.47 | 39 | 37.14 |
| Proteobacteria | Alphaproteobacteria | Rhodospirillales | 186 | 0.02 | 9 | 8.57 |
| Proteobacteria | Alphaproteobacteria | Rickettsiales | 3612 | 0.37 | 45 | 42.86 |
| Proteobacteria | Alphaproteobacteria | Sphingomonadales | 36000 | 3.68 | 78 | 74.29 |
| Proteobacteria | Alphaproteobacteria | Unclassified | 1729 | 0.18 | 20 | 19.05 |
| Proteobacteria | Gammaproteobacteria | Beggiatoales | 253 | 0.03 | 9 | 8.57 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | 233158 | 23.84 | 105 | 100 |
| Proteobacteria | Gammaproteobacteria | Cardiobacteriales | 174 | 0.02 | 6 | 5.71 |
| Proteobacteria | Gammaproteobacteria | Coxiellales | 32 | 0 | 2 | 1.9 |
| Proteobacteria | Gammaproteobacteria | Diplorickettsiales | 485 | 0.05 | 10 | 9.52 |
| Proteobacteria | Gammaproteobacteria | Enterobacterales | 97220 | 9.94 | 91 | 86.67 |
| Proteobacteria | Gammaproteobacteria | Gammaproteobacteria Incertae Sedis | 691 | 0.07 | 14 | 13.33 |
| Proteobacteria | Gammaproteobacteria | Legionellales | 1128 | 0.12 | 28 | 26.67 |
| Proteobacteria | Gammaproteobacteria | Pseudomonadales | 120119 | 12.28 | 99 | 94.29 |
| Proteobacteria | Gammaproteobacteria | Salinisphaerales | 20207 | 2.07 | 35 | 33.33 |
| Proteobacteria | Gammaproteobacteria | Steroidobacterales | 19 | 0 | 1 | 0.95 |
| Proteobacteria | Gammaproteobacteria | Tenderiales | 5 | 0 | 1 | 0.95 |
| Proteobacteria | Gammaproteobacteria | Unclassified | 3111 | 0.32 | 45 | 42.86 |
| Proteobacteria | Gammaproteobacteria | Xanthomonadales | 55356 | 5.66 | 90 | 85.71 |
| Proteobacteria | Unclassified | Unclassified | 28 | 0 | 3 | 2.86 |
| Spirochaetota | Leptospirae | Leptospirales | 12 | 0 | 2 | 1.9 |
| Synergistota | Synergistia | Synergistales | 257 | 0.03 | 8 | 7.62 |
| Thermotogota | Thermotogae | Thermotogales | 5 | 0 | 1 | 0.95 |
| Verrucomicrobiota | Chlamydiae | Chlamydiales | 437 | 0.04 | 15 | 14.29 |
| Verrucomicrobiota | Verrucomicrobiae | Chthoniobacterales | 682 | 0.07 | 12 | 11.43 |

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|---|---|---|---|---|---|---|
| Verrucomicrobiota | Verrucomicrobiae | Opitutales | 464 | 0.05 | 11 | 10.48 |
| Verrucomicrobiota | Verrucomicrobiae | Unclassified | 5 | 0 | 1 | 0.95 |
| Verrucomicrobiota | Verrucomicrobiae | Verrucomicrobiales | 513 | 0.05 | 9 | 8.57 |
| WPS-2 | Unclassified | Unclassified | 270 | 0.03 | 12 | 11.43 |

**(D)**

| Phylum | Class | Order | Family | Total Reads | Total Reads (%) | Prevalence (number of samples) | Prevalence (%) |
|---|---|---|---|---|---|---|---|
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Comamonadaceae | 133995 | 13.7 | 98 | 93.33 |
| Firmicutes | Bacilli | Lactobacillales | Aerococcaceae | 122440 | 12.52 | 84 | 80 |
| Proteobacteria | Gammaproteobacteria | Pseudomonadales | Pseudomonadaceae | 101194 | 10.35 | 98 | 93.33 |
| Firmicutes | Bacilli | Lactobacillales | Carnobacteriaceae | 62648 | 6.41 | 78 | 74.29 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Alcaligenaceae | 55270 | 5.65 | 101 | 96.19 |
| Proteobacteria | Gammaproteobacteria | Xanthomonadales | Xanthomonadaceae | 54603 | 5.58 | 88 | 83.81 |
| Proteobacteria | Gammaproteobacteria | Enterobacterales | Enterobacteriaceae | 42209 | 4.32 | 67 | 63.81 |
| Proteobacteria | Gammaproteobacteria | Enterobacterales | Unclassified | 40731 | 4.16 | 52 | 49.52 |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Rhizobiaceae | 40341 | 4.12 | 90 | 85.71 |
| Proteobacteria | Alphaproteobacteria | Sphingomonadales | Sphingomonadaceae | 36000 | 3.68 | 78 | 74.29 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Rhodocyclaceae | 29197 | 2.99 | 74 | 70.48 |

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|---|---|---|---|---|---|---|---|
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Beijerinckiaceae | 27316 | 2.79 | 75 | 71.43 |
| Proteobacteria | Gammaproteobacteria | Salinisphaerales | Solimonadaceae | 20207 | 2.07 | 35 | 33.33 |
| Proteobacteria | Gammaproteobacteria | Pseudomonadales | Moraxellaceae | 18569 | 1.9 | 46 | 43.81 |
| Proteobacteria | Alphaproteobacteria | Reyranellales | Reyranellaceae | 16853 | 1.72 | 59 | 56.19 |
| Proteobacteria | Alphaproteobacteria | Caulobacterales | Caulobacteraceae | 13105 | 1.34 | 78 | 74.29 |
| Proteobacteria | Gammaproteobacteria | Enterobacterales | Morganellaceae | 12310 | 1.26 | 45 | 42.86 |
| Cyanobacteria | Vampirivibrionia | Obscuribacterales | Obscuribacteraceae | 11190 | 1.14 | 41 | 39.05 |
| Firmicutes | Clostridia | Peptostreptococcales-Tissierellales | Family XI | 10092 | 1.03 | 68 | 64.76 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Methylophilaceae | 7411 | 0.76 | 29 | 27.62 |
| Actinobacteriota | Actinobacteria | Micrococcales | Microbacteriaceae | 7138 | 0.73 | 33 | 31.43 |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Xanthobacteraceae | 5984 | 0.61 | 59 | 56.19 |
| Bacteroidota | Bacteroidia | Flavobacteriales | Flavobacteriaceae | 5821 | 0.6 | 65 | 61.9 |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Hyphomicrobiaceae | 5865 | 0.6 | 40 | 38.1 |
| Proteobacteria | Alphaproteobacteria | Rhodobacterales | Rhodobacteraceae | 4612 | 0.47 | 39 | 37.14 |
| Firmicutes | Unclassified | Unclassified | Unclassified | 4507 | 0.46 | 34 | 32.38 |
| Bacteroidota | Bacteroidia | Flavobacteriales | Weeksellaceae | 4372 | 0.45 | 51 | 48.57 |
| Firmicutes | Bacilli | Lactobacillales | Unclassified | 4323 | 0.44 | 33 | 31.43 |
| Bacteroidota | Bacteroidia | Bacteroidales | Dysgonomonadaceae | 3893 | 0.4 | 46 | 43.81 |

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|---|---|---|---|---|---|---|---|
| Actinobacteriota | Actinobacteria | Corynebacteriales | Mycobacteriaceae | 3808 | 0.39 | 39 | 37.14 |
| Bacteroidota | Bacteroidia | Chitinophagales | Chitinophagaceae | 3199 | 0.33 | 39 | 37.14 |
| Proteobacteria | Gammaproteobacteria | Unclassified | Unclassified | 3111 | 0.32 | 45 | 42.86 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Unclassified | 3169 | 0.32 | 27 | 25.71 |
| Firmicutes | Bacilli | Erysipelotrichales | Erysipelotrichaceae | 3043 | 0.31 | 39 | 37.14 |
| Proteobacteria | Alphaproteobacteria | Rickettsiales | Mitochondria | 3068 | 0.31 | 38 | 36.19 |
| Firmicutes | Bacilli | Lactobacillales | Lactobacillaceae | 2698 | 0.28 | 22 | 20.95 |
| Bacteroidota | Bacteroidia | Cytophagales | Microscillaceae | 2776 | 0.28 | 14 | 13.33 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Oxalobacteraceae | 2036 | 0.21 | 24 | 22.86 |
| Proteobacteria | Alphaproteobacteria | Unclassified | Unclassified | 1729 | 0.18 | 20 | 19.05 |
| Bacteroidota | Bacteroidia | Sphingobacteriales | Sphingobacteriaceae | 1617 | 0.17 | 38 | 36.19 |
| Planctomycetota | Planctomycetes | Gemmatales | Gemmataceae | 1710 | 0.17 | 29 | 27.62 |
| Acidobacteriota | Blastocatellia | Blastocatellales | Blastocatellaceae | 1662 | 0.17 | 15 | 14.29 |
| Actinobacteriota | Actinobacteria | Corynebacteriales | Corynebacteriaceae | 1519 | 0.16 | 22 | 20.95 |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Rhizobiales Incertae Sedis | 1537 | 0.16 | 17 | 16.19 |
| Planctomycetota | Planctomycetes | Pirellulales | Pirellulaceae | 1470 | 0.15 | 19 | 18.1 |
| Firmicutes | Clostridia | Peptostreptococcales-Tissierellales | Peptostreptococcaceae | 1329 | 0.14 | 26 | 24.76 |
| Firmicutes | Clostridia | Clostridiales | Clostridiaceae | 1314 | 0.13 | 28 | 26.67 |
| Bacteroidota | Bacteroidia | Bacteroidales | Muribaculaceae | 1269 | 0.13 | 19 | 18.1 |

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|---|---|---|---|---|---|---|---|
| Proteobacteria | Gammaproteobacteria | Legionellales | Legionellaceae | 1128 | 0.12 | 28 | 26.67 |
| Desulfobacterota | Desulfovibrionia | Desulfovibrionales | Desulfovibrionaceae | 1079 | 0.11 | 17 | 16.19 |
| Gemmatimonadota | Gemmatimonadetes | Gemmatimonadales | Gemmatimonadaceae | 951 | 0.1 | 21 | 20 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Nitrosomonadaceae | 924 | 0.09 | 16 | 15.24 |
| Firmicutes | Clostridia | Peptostreptococcales-Tissierellales | Gottschalkia | 742 | 0.08 | 20 | 19.05 |
| Proteobacteria | Gammaproteobacteria | Enterobacterales | Shewanellaceae | 787 | 0.08 | 2 | 1.9 |
| Actinobacteriota | Actinobacteria | Actinomycetales | Actinomycetaceae | 702 | 0.07 | 24 | 22.86 |
| Bdellovibrionota | Bdellovibrionia | Bdellovibrionales | Bdellovibrionaceae | 716 | 0.07 | 19 | 18.1 |
| Proteobacteria | Gammaproteobacteria | Gammaproteobacteria Incertae Sedis | Unknown Family | 691 | 0.07 | 14 | 13.33 |
| Campylobacterota | Campylobacteria | Campylobacterales | Arcobacteraceae | 711 | 0.07 | 13 | 12.38 |
| Planctomycetota | Planctomycetes | Planctomycetales | Schlesneriaceae | 667 | 0.07 | 13 | 12.38 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Burkholderiaceae | 578 | 0.06 | 24 | 22.86 |
| Cyanobacteria | Cyanobacteriia | Chloroplast | Unclassified | 633 | 0.06 | 22 | 20.95 |
| Firmicutes | Bacilli | Lactobacillales | Streptococcaceae | 630 | 0.06 | 19 | 18.1 |
| Proteobacteria | Alphaproteobacteria | Caedibacterales | Caedibacteraceae | 598 | 0.06 | 18 | 17.14 |
| Proteobacteria | Gammaproteobacteria | Xanthomonadales | Rhodanobacteraceae | 538 | 0.06 | 15 | 14.29 |

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|---|---|---|---|---|---|---|---|
| Actinobacteriota | Actinobacteria | Corynebacteriales | Nocardiaceae | 559 | 0.06 | 12 | 11.43 |
| Verrucomicrobiota | Verrucomicrobiae | Chthoniobacterales | Terrimicrobiaceae | 632 | 0.06 | 10 | 9.52 |
| Firmicutes | Clostridia | Oscillospirales | Hungateiclostridiaceae | 605 | 0.06 | 9 | 8.57 |
| Proteobacteria | Gammaproteobacteria | Enterobacterales | Yersiniaceae | 607 | 0.06 | 9 | 8.57 |
| Bdellovibrionota | Bdellovibrionia | Bacteriovoracales | Bacteriovoracaceae | 478 | 0.05 | 25 | 23.81 |
| Actinobacteriota | Actinobacteria | Micrococcales | Micrococcaceae | 529 | 0.05 | 18 | 17.14 |
| Firmicutes | Bacilli | Staphylococcales | Staphylococcaceae | 459 | 0.05 | 16 | 15.24 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | TRA3-20 | 450 | 0.05 | 13 | 12.38 |
| Dependentiae | Babeliae | Babeliales | Vermiphilaceae | 486 | 0.05 | 12 | 11.43 |
| Proteobacteria | Gammaproteobacteria | Diplorickettsiales | Diplorickettsiaceae | 485 | 0.05 | 10 | 9.52 |
| Planctomycetota | Planctomycetes | Planctomycetales | Rubinisphaeraceae | 487 | 0.05 | 8 | 7.62 |
| Bacteroidota | Bacteroidia | Bacteroidales | Prevotellaceae | 425 | 0.04 | 19 | 18.1 |
| Firmicutes | Clostridia | Unclassified | Unclassified | 383 | 0.04 | 17 | 16.19 |
| Proteobacteria | Alphaproteobacteria | Paracaedibacterales | Paracaedibacteraceae | 414 | 0.04 | 13 | 12.38 |
| Proteobacteria | Alphaproteobacteria | Rickettsiales | SM2D12 | 353 | 0.04 | 13 | 12.38 |
| Bacteroidota | Bacteroidia | Cytophagales | Spirosomaceae | 439 | 0.04 | 12 | 11.43 |
| Verrucomicrobiota | Verrucomicrobiae | Opitutales | Opitutaceae | 424 | 0.04 | 11 | 10.48 |
| Dependentiae | Babeliae | Babeliales | Babeliaceae | 426 | 0.04 | 10 | 9.52 |

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|---|---|---|---|---|---|---|---|
| Proteobacteria | Gammaproteobacteria | Pseudomonadales | Unclassified | 356 | 0.04 | 9 | 8.57 |
| Proteobacteria | Gammaproteobacteria | Enterobacterales | Alteromonadaceae | 393 | 0.04 | 8 | 7.62 |
| Firmicutes | Clostridia | Oscillospirales | Unclassified | 300 | 0.03 | 14 | 13.33 |
| Firmicutes | Clostridia | Lachnospirales | Lachnospiraceae | 303 | 0.03 | 13 | 12.38 |
| WPS-2 | Unclassified | Unclassified | Unclassified | 270 | 0.03 | 12 | 11.43 |
| Firmicutes | Clostridia | Oscillospirales | Ruminococcaceae | 263 | 0.03 | 10 | 9.52 |
| Proteobacteria | Gammaproteobacteria | Beggiatoales | Beggiatoaceae | 253 | 0.03 | 9 | 8.57 |
| Planctomycetota | Phycisphaerae | Phycisphaerales | Phycisphaeraceae | 322 | 0.03 | 8 | 7.62 |
| Synergistota | Synergistia | Synergistales | Synergistaceae | 257 | 0.03 | 8 | 7.62 |
| Planctomycetota | OM190 | Unclassified | Unclassified | 264 | 0.03 | 7 | 6.67 |
| Proteobacteria | Alphaproteobacteria | Caulobacterales | Hyphomonadaceae | 279 | 0.03 | 6 | 5.71 |
| Proteobacteria | Alphaproteobacteria | Micavibrionales | Unclassified | 324 | 0.03 | 6 | 5.71 |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Unclassified | 305 | 0.03 | 6 | 5.71 |
| Nitrospirota | Nitrospiria | Nitrospirales | Nitrospiraceae | 249 | 0.03 | 5 | 4.76 |
| Verrucomicrobiota | Verrucomicrobiae | Verrucomicrobiales | Rubritaleaceae | 334 | 0.03 | 5 | 4.76 |
| Cyanobacteria | Vampirivibrionia | Vampirovibrionales | Vampirovibrionaceae | 264 | 0.03 | 3 | 2.86 |
| Actinobacteriota | Actinobacteria | Micrococcales | Promicromonosporaceae | 267 | 0.03 | 1 | 0.95 |
| Firmicutes | Negativicutes | Veillonellales-Selenomonadales | Veillonellaceae | 184 | 0.02 | 17 | 16.19 |

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|---|---|---|---|---|---|---|---|
| Verrucomicrobiota | Chlamydiae | Chlamydiales | Parachlamydiaceae | 189 | 0.02 | 11 | 10.48 |
| Bacteroidota | Bacteroidia | Sphingobacteriales | env.OPS 17 | 239 | 0.02 | 9 | 8.57 |
| Proteobacteria | Alphaproteobacteria | Rhodospirillales | Unclassified | 186 | 0.02 | 9 | 8.57 |
| Bacteroidota | Bacteroidia | Cytophagales | Cytophagaceae | 239 | 0.02 | 8 | 7.62 |
| Bacteroidota | Bacteroidia | Unclassified | Unclassified | 238 | 0.02 | 8 | 7.62 |
| Proteobacteria | Gammaproteobacteria | Xanthomonadales | Unclassified | 215 | 0.02 | 8 | 7.62 |
| Verrucomicrobiota | Chlamydiae | Chlamydiales | Simkaniaceae | 220 | 0.02 | 7 | 6.67 |
| Actinobacteriota | Actinobacteria | Micrococcales | Dermabacteraceae | 238 | 0.02 | 6 | 5.71 |
| Actinobacteriota | Actinobacteria | Unclassified | Unclassified | 213 | 0.02 | 6 | 5.71 |
| Proteobacteria | Gammaproteobacteria | Cardiobacteriales | Wohlfahrtiimonadaceae | 174 | 0.02 | 6 | 5.71 |
| Verrucomicrobiota | Verrucomicrobiae | Verrucomicrobiales | Verrucomicrobiaceae | 179 | 0.02 | 5 | 4.76 |
| Actinobacteriota | Actinobacteria | Micrococcales | Brevibacteriaceae | 165 | 0.02 | 3 | 2.86 |
| Bacteroidota | Bacteroidia | Bacteroidales | Rikenellaceae | 203 | 0.02 | 3 | 2.86 |
| Proteobacteria | Alphaproteobacteria | Azospirillales | Azospirillaceae | 149 | 0.02 | 3 | 2.86 |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Devosiaceae | 156 | 0.02 | 3 | 2.86 |
| Actinobacteriota | Actinobacteria | Micrococcales | Intrasporangiaceae | 213 | 0.02 | 1 | 0.95 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Neisseriaceae | 122 | 0.01 | 13 | 12.38 |

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|---|---|---|---|---|---|---|---|
| Proteobacteria | Gammaproteobacteria | Enterobacterales | Pasteurellaceae | 85 | 0.01 | 8 | 7.62 |
| Planctomycetota | Planctomycetes | Unclassified | Unclassified | 99 | 0.01 | 7 | 6.67 |
| Bacteroidota | Bacteroidia | Bacteroidales | Porphyromonadaceae | 76 | 0.01 | 6 | 5.71 |
| Proteobacteria | Alphaproteobacteria | Acetobacterales | Acetobacteraceae | 93 | 0.01 | 6 | 5.71 |
| Proteobacteria | Alphaproteobacteria | Holosporales | Holosporaceae | 88 | 0.01 | 6 | 5.71 |
| Proteobacteria | Alphaproteobacteria | Rickettsiales | Rickettsiaceae | 106 | 0.01 | 6 | 5.71 |
| Actinobacteriota | Coriobacteriia | Coriobacteriales | Atopobiaceae | 57 | 0.01 | 5 | 4.76 |
| Bacteroidota | Bacteroidia | Flavobacteriales | Crocinitomicaceae | 106 | 0.01 | 5 | 4.76 |
| Bacteroidota | Bacteroidia | Sphingobacteriales | NS11-12 marine group | 103 | 0.01 | 5 | 4.76 |
| Cyanobacteria | Cyanobacteriia | Cyanobacteriales | Chroococcidiopsaceae | 102 | 0.01 | 5 | 4.76 |
| Dependentiae | Babeliae | Babeliales | Unclassified | 85 | 0.01 | 5 | 4.76 |
| Firmicutes | Clostridia | Eubacteriales | Eubacteriaceae | 72 | 0.01 | 5 | 4.76 |
| Firmicutes | Clostridia | Oscillospirales | Oscillospiraceae | 77 | 0.01 | 5 | 4.76 |
| Firmicutes | Clostridia | Peptococcales | Peptococcaceae | 116 | 0.01 | 5 | 4.76 |
| Firmicutes | Clostridia | Peptostreptococcales-Tissierellales | Sedimentibacteraceae | 57 | 0.01 | 5 | 4.76 |
| Planctomycetota | Planctomycetes | Isosphaerales | Isosphaeraceae | 108 | 0.01 | 5 | 4.76 |
| Actinobacteriota | Actinobacteria | Micrococcales | Cellulomonadaceae | 71 | 0.01 | 4 | 3.81 |
| Firmicutes | Clostridia | Clostridia UCG-014 | Unclassified | 119 | 0.01 | 4 | 3.81 |
| Firmicutes | Clostridia | Peptostreptococcales-Tissierellales | Guggenheimella | 91 | 0.01 | 4 | 3.81 |

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|---|---|---|---|---|---|---|---|
| Myxococcota | Myxococcia | Myxococcales | Myxococcaceae | 101 | 0.01 | 4 | 3.81 |
| Acidobacteriota | Vicinamibacteria | Vicinamibacterales | Vicinamibacteraceae | 90 | 0.01 | 3 | 2.86 |
| Actinobacteriota | Actinobacteria | Micrococcales | Unclassified | 135 | 0.01 | 3 | 2.86 |
| Bacteroidota | Bacteroidia | Bacteroidales | Paludibacteraceae | 91 | 0.01 | 3 | 2.86 |
| Bacteroidota | Bacteroidia | Sphingobacteriales | Lentimicrobiaceae | 99 | 0.01 | 3 | 2.86 |
| Bacteroidota | Bacteroidia | Sphingobacteriales | Unclassified | 69 | 0.01 | 3 | 2.86 |
| Acidobacteriota | Blastocatellia | Nov-24 | Unclassified | 80 | 0.01 | 2 | 1.9 |
| Chloroflexi | TK10 | Unclassified | Unclassified | 63 | 0.01 | 2 | 1.9 |
| Firmicutes | Bacilli | Erysipelotrichales | Erysipelatoclostridiaceae | 86 | 0.01 | 2 | 1.9 |
| Firmicutes | Bacilli | Lactobacillales | Vagococcaceae | 63 | 0.01 | 2 | 1.9 |
| Firmicutes | Desulfitobacteria | Desulfitobacteriales | TC1 | 53 | 0.01 | 2 | 1.9 |
| Actinobacteriota | Actinobacteria | Bifidobacteriales | Bifidobacteriaceae | 52 | 0.01 | 1 | 0.95 |
| Firmicutes | Clostridia | Oscillospirales | [Eubacterium] coprostanoligenes group | 49 | 0.01 | 1 | 0.95 |
| Firmicutes | Clostridia | Peptostreptococcales-Tissierellales | Unclassified | 58 | 0.01 | 1 | 0.95 |
| Proteobacteria | Alphaproteobacteria | Azospirillales | Inquilinaceae | 119 | 0.01 | 1 | 0.95 |
| Proteobacteria | Alphaproteobacteria | Rickettsiales | Anaplasmataceae | 69 | 0.01 | 1 | 0.95 |
| Proteobacteria | Gammaproteobacteria | Enterobacterales | Aeromonadaceae | 98 | 0.01 | 1 | 0.95 |
| Actinobacteriota | Actinobacteria | Frankiales | Geodermatophilaceae | 35 | 0 | 4 | 3.81 |
| Actinobacteriota | Coriobacteriia | Coriobacteriales | Coriobacteriaceae | 35 | 0 | 3 | 2.86 |

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|---|---|---|---|---|---|---|---|
| Actinobacteriota | Coriobacteriia | Coriobacteriales | Eggerthellaceae | 13 | 0 | 3 | 2.86 |
| Firmicutes | Bacilli | Bacillales | Bacillaceae | 14 | 0 | 3 | 2.86 |
| Firmicutes | Negativicutes | Veillonellales-Selenomonadales | Selenomonadaceae | 27 | 0 | 3 | 2.86 |
| Proteobacteria | Alphaproteobacteria | Rickettsiales | Unclassified | 16 | 0 | 3 | 2.86 |
| Proteobacteria | Unclassified | Unclassified | Unclassified | 28 | 0 | 3 | 2.86 |
| Actinobacteriota | Actinobacteria | Frankiales | Sporichthyaceae | 30 | 0 | 2 | 1.9 |
| Actinobacteriota | Actinobacteria | Micrococcales | Bogoriellaceae | 20 | 0 | 2 | 1.9 |
| Bacteroidota | Bacteroidia | Cytophagales | Unclassified | 10 | 0 | 2 | 1.9 |
| Campylobacterota | Campylobacteria | Campylobacterales | Campylobacteraceae | 21 | 0 | 2 | 1.9 |
| Chloroflexi | KD4-96 | Unclassified | Unclassified | 15 | 0 | 2 | 1.9 |
| Dependentiae | Babeliae | Babeliales | UBA12409 | 30 | 0 | 2 | 1.9 |
| Desulfobacterota | Unclassified | Unclassified | Unclassified | 14 | 0 | 2 | 1.9 |
| Firmicutes | Bacilli | Bacillales | Planococcaceae | 37 | 0 | 2 | 1.9 |
| Firmicutes | Bacilli | Lactobacillales | Enterococcaceae | 11 | 0 | 2 | 1.9 |
| Firmicutes | Bacilli | RF39 | Unclassified | 23 | 0 | 2 | 1.9 |
| Firmicutes | Bacilli | Staphylococcales | Gemellaceae | 7 | 0 | 2 | 1.9 |
| Firmicutes | Bacilli | Unclassified | Unclassified | 45 | 0 | 2 | 1.9 |
| Firmicutes | Clostridia | Eubacteriales | Alkalibaculum | 26 | 0 | 2 | 1.9 |
| Firmicutes | Clostridia | Eubacteriales | Anaerofustaceae | 39 | 0 | 2 | 1.9 |
| Firmicutes | Clostridia | Peptostreptococcales-Tissierellales | Anaerovoracaceae | 36 | 0 | 2 | 1.9 |
| Firmicutes | Limnochordia | MBA03 | Unclassified | 14 | 0 | 2 | 1.9 |

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|---|---|---|---|---|---|---|---|
| Fusobacteriota | Fusobacteriia | Fusobacteriales | Fusobacteriaceae | 38 | 0 | 2 | 1.9 |
| Planctomycetota | Unclassified | Unclassified | Unclassified | 23 | 0 | 2 | 1.9 |
| Proteobacteria | Gammaproteobacteria | Coxiellales | Coxiellaceae | 32 | 0 | 2 | 1.9 |
| Spirochaetota | Leptospirae | Leptospirales | Leptospiraceae | 12 | 0 | 2 | 1.9 |
| Verrucomicrobiota | Chlamydiae | Chlamydiales | Unclassified | 28 | 0 | 2 | 1.9 |
| Acidobacteriota | Acidobacteriae | Bryobacterales | Bryobacteraceae | 30 | 0 | 1 | 0.95 |
| Acidobacteriota | Acidobacteriae | Paludibaculum | Unclassified | 35 | 0 | 1 | 0.95 |
| Acidobacteriota | Acidobacteriae | Unclassified | Unclassified | 20 | 0 | 1 | 0.95 |
| Acidobacteriota | Blastocatellia | Chloracidobacteriales | Chloracidobacteriaceae | 6 | 0 | 1 | 0.95 |
| Actinobacteriota | Thermoleophilia | Gaiellales | Unclassified | 11 | 0 | 1 | 0.95 |
| Bacteroidota | Bacteroidia | Bacteroidales | Unclassified | 35 | 0 | 1 | 0.95 |
| Bacteroidota | Bacteroidia | Chitinophagales | Unclassified | 45 | 0 | 1 | 0.95 |
| Bacteroidota | Bacteroidia | Cytophagales | Hymenobacteraceae | 7 | 0 | 1 | 0.95 |
| Bacteroidota | Bacteroidia | Flavobacteriales | Cryomorphaceae | 3 | 0 | 1 | 0.95 |
| Bacteroidota | Bacteroidia | Flavobacteriales | Unclassified | 18 | 0 | 1 | 0.95 |
| Bacteroidota | Bacteroidia | Sphingobacteriales | KD3-93 | 14 | 0 | 1 | 0.95 |
| Chloroflexi | Chloroflexia | Thermomicrobiales | JG30-KF-CM45 | 14 | 0 | 1 | 0.95 |
| Chloroflexi | JG30-KF-CM66 | Unclassified | Unclassified | 10 | 0 | 1 | 0.95 |
| Cyanobacteria | Cyanobacteriia | Cyanobacteriales | Coleofasciculaceae | 4 | 0 | 1 | 0.95 |

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|---|---|---|---|---|---|---|---|
| Cyanobacteria | Cyanobacteriia | Leptolyngbyales | Leptolyngbyaceae | 27 | 0 | 1 | 0.95 |
| Deinococcota | Deinococci | Deinococcales | Deinococcaceae | 15 | 0 | 1 | 0.95 |
| Deinococcota | Deinococci | Deinococcales | Trueperaceae | 34 | 0 | 1 | 0.95 |
| Deinococcota | Deinococci | Thermales | Thermaceae | 1 | 0 | 1 | 0.95 |
| Desulfobacterota | Desulfuromonadia | Geobacterales | Geobacteraceae | 9 | 0 | 1 | 0.95 |
| Firmicutes | Bacilli | Acholeplasmatales | Acholeplasmataceae | 7 | 0 | 1 | 0.95 |
| Firmicutes | Clostridia | Christensenellales | Christensenellaceae | 35 | 0 | 1 | 0.95 |
| Firmicutes | Clostridia | Clostridiales | Unclassified | 12 | 0 | 1 | 0.95 |
| Firmicutes | Clostridia | Eubacteriales | Alkalibacteraceae | 7 | 0 | 1 | 0.95 |
| Firmicutes | Clostridia | Eubacteriales | Unclassified | 19 | 0 | 1 | 0.95 |
| Firmicutes | Clostridia | Lachnospirales | Unclassified | 6 | 0 | 1 | 0.95 |
| Firmicutes | Clostridia | Oscillospirales | Hydrogenoanaerobacterium | 16 | 0 | 1 | 0.95 |
| Firmicutes | Negativicutes | Veillonellales-Selenomonadales | Sporomusaceae | 18 | 0 | 1 | 0.95 |
| Fusobacteriota | Fusobacteriia | Fusobacteriales | Leptotrichiaceae | 5 | 0 | 1 | 0.95 |
| Hydrogenedentes | Hydrogenedentia | Hydrogenedentiales | Hydrogenedensaceae | 45 | 0 | 1 | 0.95 |
| Myxococcota | Polyangia | Blfdi19 | Unclassified | 37 | 0 | 1 | 0.95 |
| Myxococcota | Polyangia | Haliangiales | Haliangiaceae | 7 | 0 | 1 | 0.95 |
| Patescibacteria | Saccharimonadia | Saccharimonadales | Saccharimonadaceae | 5 | 0 | 1 | 0.95 |
| Planctomycetota | vadinHA49 | Unclassified | Unclassified | 15 | 0 | 1 | 0.95 |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Methylopilaceae | 2 | 0 | 1 | 0.95 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | SC-I-84 | 6 | 0 | 1 | 0.95 |

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|---|---|---|---|---|---|---|---|
| Proteobacteria | Gammaproteobacteria | Steroidobacterales | Steroidobacteraceae | 19 | 0 | 1 | 0.95 |
| Proteobacteria | Gammaproteobacteria | Tenderiales | Tenderiaceae | 5 | 0 | 1 | 0.95 |
| Thermotogota | Thermotogae | Thermotogales | Fervidobacteriaceae | 5 | 0 | 1 | 0.95 |
| Verrucomicrobiota | Verrucomicrobiae | Chthoniobacterales | Chthoniobacteraceae | 4 | 0 | 1 | 0.95 |
| Verrucomicrobiota | Verrucomicrobiae | Chthoniobacterales | Xiphinematobacteraceae | 46 | 0 | 1 | 0.95 |
| Verrucomicrobiota | Verrucomicrobiae | Opitutales | Puniceicoccaceae | 40 | 0 | 1 | 0.95 |
| Verrucomicrobiota | Verrucomicrobiae | Unclassified | Unclassified | 5 | 0 | 1 | 0.95 |

**(E)**

| Phylum | Class | Order | Family | Genus | Total Reads | Total Reads (%) | Prevalence (number of samples) | Prevalence (%) |
|---|---|---|---|---|---|---|---|---|
| Proteobacteria | Gammaproteobacteria | Pseudomonadales | Pseudomonadaceae | Pseudomonas | 69914 | 7.15 | 88 | 83.81 |
| Proteobacteria | Gammaproteobacteria | Xanthomonadales | Xanthomonadaceae | Stenotrophomonas | 30990 | 3.17 | 83 | 79.05 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Alcaligenaceae | Achromobacter | 18162 | 1.86 | 77 | 73.33 |
| Proteobacteria | Alphaproteobacteria | Caulobacterales | Caulobacteraceae | Brevundimonas | 10453 | 1.07 | 74 | 70.48 |
| Firmicutes | Bacilli | Lactobacillales | Aerococcaceae | Dolosicoccus | 113946 | 11.65 | 73 | 69.52 |

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|---|---|---|---|---|---|---|---|---|
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Beijerinckiaceae | Bosea | 16506 | 1.69 | 70 | 66.67 |
| Bacteroidota | Bacteroidia | Flavobacteriales | Flavobacteriaceae | Flavobacterium | 5735 | 0.59 | 63 | 60 |
| Proteobacteria | Gammaproteobacteria | Xanthomonadales | Xanthomonadaceae | Pseudoxanthomonas | 12823 | 1.31 | 62 | 59.05 |
| Proteobacteria | Alphaproteobacteria | Reyranellales | Reyranellaceae | Reyranella | 16853 | 1.72 | 59 | 56.19 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Comamonadaceae | Delftia | 13421 | 1.37 | 59 | 56.19 |
| Proteobacteria | Alphaproteobacteria | Sphingomonadales | Sphingomonadaceae | Sphingopyxis | 10543 | 1.08 | 57 | 54.29 |
| Proteobacteria | Alphaproteobacteria | Sphingomonadales | Sphingomonadaceae | Sphingobium | 12606 | 1.29 | 56 | 53.33 |
| Firmicutes | Bacilli | Lactobacillales | Carnobacteriaceae | Atopostipes | 21235 | 2.17 | 53 | 50.48 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Alcaligenaceae | Oligella | 26503 | 2.71 | 49 | 46.67 |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Rhizobiaceae | Shinella | 8304 | 0.85 | 49 | 46.67 |
| Bacteroidota | Bacteroidia | Flavobacteriales | Weeksellaceae | Chryseobacterium | 4150 | 0.42 | 49 | 46.67 |
| Proteobacteria | Gammaproteobacteria | Pseudomonadales | Pseudomonadaceae | Thiopseudomonas | 15867 | 1.62 | 47 | 44.76 |
| Firmicutes | Clostridia | Peptostreptococcales-Tissierellales | Family XI | Tissierella | 4198 | 0.43 | 47 | 44.76 |
| Proteobacteria | Gammaproteobacteria | Xanthomonadales | Xanthomonadaceae | Thermomonas | 6247 | 0.64 | 45 | 42.86 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Alcaligenaceae | Alcaligenes | 5674 | 0.58 | 45 | 42.86 |
| Proteobacteria | Gammaproteobacteria | Pseudomonadales | Moraxellaceae | Acinetobacter | 18170 | 1.86 | 43 | 40.95 |

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|---|---|---|---|---|---|---|---|---|
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Beijerinckiaceae | Methylobacterium-Methylorubrum | 10361 | 1.06 | 43 | 40.95 |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Rhizobiaceae | Pseudochrobactrum | 4717 | 0.48 | 40 | 38.1 |
| Actinobacteriota | Actinobacteria | Corynebacteriales | Mycobacteriaceae | Mycobacterium | 3808 | 0.39 | 39 | 37.14 |
| Proteobacteria | Gammaproteobacteria | Enterobacterales | Morganellaceae | Morganella | 4346 | 0.44 | 38 | 36.19 |
| Proteobacteria | Gammaproteobacteria | Salinisphaerales | Solimonadaceae | Nevskia | 20105 | 2.06 | 35 | 33.33 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Rhodocyclaceae | Methyloversatilis | 15628 | 1.6 | 35 | 33.33 |
| Firmicutes | Bacilli | Erysipelotrichales | Erysipelotrichaceae | Erysipelothrix | 2350 | 0.24 | 35 | 33.33 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Rhodocyclaceae | Thauera | 10655 | 1.09 | 33 | 31.43 |
| Bacteroidota | Bacteroidia | Bacteroidales | Dysgonomonadaceae | Proteiniphilum | 1710 | 0.17 | 32 | 30.48 |
| Firmicutes | Clostridia | Peptostreptococcales-Tissierellales | Family XI | W5053 | 2350 | 0.24 | 30 | 28.57 |
| Cyanobacteria | Vampirivibrionia | Obscuribacterales | Obscuribacteraceae | Candidatus Obscuribacter | 5925 | 0.61 | 28 | 26.67 |
| Proteobacteria | Gammaproteobacteria | Legionellales | Legionellaceae | Legionella | 1128 | 0.12 | 28 | 26.67 |
| Bacteroidota | Bacteroidia | Sphingobacteriales | Sphingobacteriaceae | Sphingobacterium | 888 | 0.09 | 27 | 25.71 |
| Bacteroidota | Bacteroidia | Chitinophagales | Chitinophagaceae | Sediminibacterium | 2228 | 0.23 | 26 | 24.76 |
| Firmicutes | Clostridia | Clostridiales | Clostridiaceae | Proteiniclasticum | 1219 | 0.12 | 26 | 24.76 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Rhodocyclaceae | Azospira | 1353 | 0.14 | 25 | 23.81 |

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|---|---|---|---|---|---|---|---|---|
| Bdellovibrionota | Bdellovibrionia | Bacteriovoracales | Bacteriovoracaceae | Peredibacter | 474 | 0.05 | 25 | 23.81 |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Rhizobiaceae | Brucella | 4528 | 0.46 | 24 | 22.86 |
| Firmicutes | Bacilli | Lactobacillales | Carnobacteriaceae | Lacticigenium | 2367 | 0.24 | 24 | 22.86 |
| Proteobacteria | Alphaproteobacteria | Sphingomonadales | Sphingomonadaceae | Sphingomonas | 976 | 0.1 | 24 | 22.86 |
| Planctomycetota | Planctomycetes | Gemmatales | Gemmataceae | Gemmata | 1201 | 0.12 | 23 | 21.9 |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Xanthobacteraceae | Bradyrhizobium | 1417 | 0.14 | 21 | 20 |
| Gemmatimonadota | Gemmatimonadetes | Gemmatimonadales | Gemmatimonadaceae | Gemmatimonas | 806 | 0.08 | 21 | 20 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Burkholderiaceae | Ralstonia | 407 | 0.04 | 21 | 20 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Methylophilaceae | Methylotenera | 7100 | 0.73 | 20 | 19.05 |
| Proteobacteria | Alphaproteobacteria | Sphingomonadales | Sphingomonadaceae | Blastomonas | 2631 | 0.27 | 20 | 19.05 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Oxalobacteraceae | Herminiimonas | 1932 | 0.2 | 20 | 19.05 |
| Bdellovibrionota | Bdellovibrionia | Bdellovibrionales | Bdellovibrionaceae | Bdellovibrio | 716 | 0.07 | 19 | 18.1 |
| Firmicutes | Bacilli | Lactobacillales | Streptococcaceae | Streptococcus | 592 | 0.06 | 19 | 18.1 |
| Proteobacteria | Alphaproteobacteria | Rhodobacterales | Rhodobacteraceae | Rhodobacter | 1816 | 0.19 | 18 | 17.14 |
| Firmicutes | Bacilli | Lactobacillales | Lactobacillaceae | Lactobacillus | 1103 | 0.11 | 18 | 17.14 |
| Firmicutes | Bacilli | Lactobacillales | Carnobacteriaceae | Jeotgalibaca | 1074 | 0.11 | 18 | 17.14 |

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|---|---|---|---|---|---|---|---|---|
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Rhizobiales Incertae Sedis | Nordella | 1537 | 0.16 | 17 | 16.19 |
| Planctomycetota | Planctomycetes | Pirellulales | Pirellulaceae | Pir4 lineage | 1037 | 0.11 | 17 | 16.19 |
| Proteobacteria | Alphaproteobacteria | Sphingomonadales | Sphingomonadaceae | Sphingorhabdus | 1402 | 0.14 | 16 | 15.24 |
| Actinobacteriota | Actinobacteria | Corynebacteriales | Corynebacteriaceae | Corynebacterium | 820 | 0.08 | 16 | 15.24 |
| Firmicutes | Clostridia | Peptostreptococcales-Tissierellales | Peptostreptococcaceae | Romboutsia | 429 | 0.04 | 16 | 15.24 |
| Firmicutes | Bacilli | Lactobacillales | Aerococcaceae | Ignavigranum | 963 | 0.1 | 15 | 14.29 |
| Desulfobacterota | Desulfovibrionia | Desulfovibrionales | Desulfovibrionaceae | Desulfovibrio | 947 | 0.1 | 15 | 14.29 |
| Bacteroidota | Bacteroidia | Bacteroidales | Dysgonomonadaceae | Dysgonomonas | 753 | 0.08 | 15 | 14.29 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Rhodocyclaceae | Georgfuchsia | 567 | 0.06 | 15 | 14.29 |
| Proteobacteria | Alphaproteobacteria | Caedibacterales | Caedibacteraceae | Candidatus Nucleicultrix | 523 | 0.05 | 15 | 14.29 |
| Firmicutes | Bacilli | Staphylococcales | Staphylococcaceae | Staphylococcus | 387 | 0.04 | 15 | 14.29 |
| Actinobacteriota | Actinobacteria | Corynebacteriales | Corynebacteriaceae | Lawsonella | 362 | 0.04 | 15 | 14.29 |
| Proteobacteria | Alphaproteobacteria | Rhodobacterales | Rhodobacteraceae | Tabrizicola | 750 | 0.08 | 14 | 13.33 |
| Proteobacteria | Gammaproteobacteria | Gammaproteobacteria Incertae Sedis | Unknown Family | Candidatus Berkiella | 670 | 0.07 | 14 | 13.33 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Alcaligenaceae | Parapusillimonas | 369 | 0.04 | 14 | 13.33 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Comamonadaceae | Ottowia | 5055 | 0.52 | 13 | 12.38 |

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|---|---|---|---|---|---|---|---|---|
| Firmicutes | Clostridia | Peptostreptococcales-Tissierellales | Family XI | Gallicola | 1438 | 0.15 | 13 | 12.38 |
| Planctomycetota | Planctomycetes | Planctomycetales | Schlesneriaceae | Planctopirus | 666 | 0.07 | 13 | 12.38 |
| Actinobacteriota | Actinobacteria | Actinomycetales | Actinomycetaceae | Flaviflexus | 459 | 0.05 | 13 | 12.38 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Nitrosomonadaceae | DSSD61 | 438 | 0.04 | 13 | 12.38 |
| Bacteroidota | Bacteroidia | Chitinophagales | Chitinophagaceae | Lacibacter | 389 | 0.04 | 13 | 12.38 |
| Proteobacteria | Gammaproteobacteria | Pseudomonadales | Moraxellaceae | Enhydrobacter | 273 | 0.03 | 13 | 12.38 |
| Firmicutes | Negativicutes | Veillonellales-Selenomonadales | Veillonellaceae | Veillonella | 151 | 0.02 | 13 | 12.38 |
| Firmicutes | Bacilli | Lactobacillales | Aerococcaceae | Facklamia | 866 | 0.09 | 12 | 11.43 |
| Firmicutes | Clostridia | Peptostreptococcales-Tissierellales | Peptostreptococcaceae | Proteocatella | 854 | 0.09 | 12 | 11.43 |
| Actinobacteriota | Actinobacteria | Corynebacteriales | Nocardiaceae | Rhodococcus | 559 | 0.06 | 12 | 11.43 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Alcaligenaceae | Eoetvoesia | 500 | 0.05 | 12 | 11.43 |
| Proteobacteria | Gammaproteobacteria | Xanthomonadales | Rhodanobacteraceae | Dokdonella | 452 | 0.05 | 12 | 11.43 |
| Campylobacterota | Campylobacteria | Campylobacterales | Arcobacteraceae | Arcobacter | 684 | 0.07 | 11 | 10.48 |
| Proteobacteria | Alphaproteobacteria | Sphingomonadales | Sphingomonadaceae | Novosphingobium | 525 | 0.05 | 11 | 10.48 |
| Proteobacteria | Alphaproteobacteria | Caulobacterales | Caulobacteraceae | Caulobacter | 210 | 0.02 | 11 | 10.48 |
| Firmicutes | Clostridia | Peptostreptococcales-Tissierellales | Family XI | Peptoniphilus | 131 | 0.01 | 11 | 10.48 |

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|---|---|---|---|---|---|---|---|---|
| Firmicutes | Clostridia | Peptostreptococcales-Tissierellales | Family XI | Anaerococcus | 88 | 0.01 | 11 | 10.48 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Comamonadaceae | Acidovorax | 6113 | 0.63 | 10 | 9.52 |
| Verrucomicrobiota | Verrucomicrobiae | Chthoniobacterales | Terrimicrobiaceae | Terrimicrobium | 632 | 0.06 | 10 | 9.52 |
| Proteobacteria | Alphaproteobacteria | Caulobacterales | Caulobacteraceae | Phenylobacterium | 347 | 0.04 | 10 | 9.52 |
| Actinobacteriota | Actinobacteria | Micrococcales | Micrococcaceae | Kocuria | 283 | 0.03 | 10 | 9.52 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Neisseriaceae | Neisseria | 83 | 0.01 | 10 | 9.52 |
| Proteobacteria | Gammaproteobacteria | Xanthomonadales | Xanthomonadaceae | Lysobacter | 2208 | 0.23 | 9 | 8.57 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Alcaligenaceae | Paenalcaligenes | 1289 | 0.13 | 9 | 8.57 |
| Bacteroidota | Bacteroidia | Cytophagales | Spirosomaceae | Dyadobacter | 393 | 0.04 | 9 | 8.57 |
| Bacteroidota | Bacteroidia | Chitinophagales | Chitinophagaceae | Flavihumibacter | 260 | 0.03 | 9 | 8.57 |
| Bacteroidota | Bacteroidia | Bacteroidales | Prevotellaceae | Prevotella_9 | 250 | 0.03 | 9 | 8.57 |
| Proteobacteria | Alphaproteobacteria | Sphingomonadales | Sphingomonadaceae | Plot4-2H12 | 246 | 0.03 | 9 | 8.57 |
| Proteobacteria | Alphaproteobacteria | Sphingomonadales | Sphingomonadaceae | Porphyrobacter | 818 | 0.08 | 8 | 7.62 |
| Planctomycetota | Planctomycetes | Planctomycetales | Rubinisphaeraceae | SH-PL14 | 487 | 0.05 | 8 | 7.62 |
| Firmicutes | Bacilli | Lactobacillales | Aerococcaceae | Aerococcus | 310 | 0.03 | 8 | 7.62 |
| Planctomycetota | Phycisphaerae | Phycisphaerales | Phycisphaeraceae | SM1A02 | 281 | 0.03 | 8 | 7.62 |

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|---|---|---|---|---|---|---|---|---|
| Proteobacteria | Alphaproteobacteria | Sphingomonadales | Sphingomonadaceae | Qipengyuania | 272 | 0.03 | 8 | 7.62 |
| Proteobacteria | Alphaproteobacteria | Paracaedibacterales | Paracaedibacteraceae | Candidatus Paracaedibacter | 263 | 0.03 | 8 | 7.62 |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Hyphomicrobiaceae | Hyphomicrobium | 192 | 0.02 | 8 | 7.62 |
| Proteobacteria | Gammaproteobacteria | Enterobacterales | Pasteurellaceae | Haemophilus | 85 | 0.01 | 8 | 7.62 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Comamonadaceae | Pseudorhodoferax | 1263 | 0.13 | 7 | 6.67 |
| Proteobacteria | Gammaproteobacteria | Xanthomonadales | Xanthomonadaceae | Luteimonas | 766 | 0.08 | 7 | 6.67 |
| Firmicutes | Clostridia | Oscillospirales | Hungateiclostridiaceae | Fastidiosipila | 443 | 0.05 | 7 | 6.67 |
| Actinobacteriota | Actinobacteria | Micrococcales | Microbacteriaceae | Agromyces | 382 | 0.04 | 7 | 6.67 |
| Proteobacteria | Gammaproteobacteria | Enterobacterales | Alteromonadaceae | Rheinheimera | 314 | 0.03 | 7 | 6.67 |
| Firmicutes | Bacilli | Lactobacillales | Lactobacillaceae | Ligilactobacillus | 297 | 0.03 | 7 | 6.67 |
| Planctomycetota | Planctomycetes | Gemmatales | Gemmataceae | Fimbriiglobus | 220 | 0.02 | 7 | 6.67 |
| Bacteroidota | Bacteroidia | Cytophagales | Cytophagaceae | Cytophaga | 199 | 0.02 | 7 | 6.67 |
| Actinobacteriota | Actinobacteria | Corynebacteriales | Corynebacteriaceae | Turicella | 167 | 0.02 | 7 | 6.67 |
| Actinobacteriota | Actinobacteria | Micrococcales | Micrococcaceae | Rothia | 84 | 0.01 | 7 | 6.67 |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Rhizobiaceae | Aminobacter | 441 | 0.05 | 6 | 5.71 |
| Proteobacteria | Alphaproteobacteria | Rhodobacterales | Rhodobacteraceae | Pseudorhodobacter | 294 | 0.03 | 6 | 5.71 |

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|---|---|---|---|---|---|---|---|---|
| Actinobacteriota | Actinobacteria | Micrococcales | Dermabacteraceae | Brachybacterium | 238 | 0.02 | 6 | 5.71 |
| Bacteroidota | Bacteroidia | Cytophagales | Microscillaceae | OLB12 | 213 | 0.02 | 6 | 5.71 |
| Synergistota | Synergistia | Synergistales | Synergistaceae | Syner-01 | 210 | 0.02 | 6 | 5.71 |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Xanthobacteraceae | Pseudorhodoplanes | 110 | 0.01 | 6 | 5.71 |
| Bacteroidota | Bacteroidia | Flavobacteriales | Weeksellaceae | Cloacibacterium | 99 | 0.01 | 6 | 5.71 |
| Bacteroidota | Bacteroidia | Bacteroidales | Porphyromonadaceae | Porphyromonas | 76 | 0.01 | 6 | 5.71 |
| Bacteroidota | Bacteroidia | Bacteroidales | Prevotellaceae | Prevotella | 37 | 0 | 6 | 5.71 |
| Firmicutes | Bacilli | Lactobacillales | Aerococcaceae | Aerosphaera | 388 | 0.04 | 5 | 4.76 |
| Verrucomicrobiota | Verrucomicrobiae | Verrucomicrobiales | Rubritaleaceae | Luteolibacter | 334 | 0.03 | 5 | 4.76 |
| Verrucomicrobiota | Verrucomicrobiae | Opitutales | Opitutaceae | Lacunisphaera | 304 | 0.03 | 5 | 4.76 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Comamonadaceae | Aquabacterium | 279 | 0.03 | 5 | 4.76 |
| Actinobacteriota | Actinobacteria | Micrococcales | Microbacteriaceae | Leucobacter | 274 | 0.03 | 5 | 4.76 |
| Firmicutes | Bacilli | Erysipelotrichales | Erysipelotrichaceae | Allobaculum | 269 | 0.03 | 5 | 4.76 |
| Proteobacteria | Gammaproteobacteria | Diplorickettsiales | Diplorickettsiaceae | Aquicella | 253 | 0.03 | 5 | 4.76 |
| Nitrospirota | Nitrospiria | Nitrospirales | Nitrospiraceae | Nitrospira | 249 | 0.03 | 5 | 4.76 |
| Verrucomicrobiota | Verrucomicrobiae | Verrucomicrobiales | Verrucomicrobiaceae | Prosthecobacter | 179 | 0.02 | 5 | 4.76 |
| Acidobacteriota | Blastocatellia | Blastocatellales | Blastocatellaceae | Blastocatella | 137 | 0.01 | 5 | 4.76 |

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|---|---|---|---|---|---|---|---|---|
| Firmicutes | Clostridia | Peptococcales | Peptococcaceae | Desulfonispora | 116 | 0.01 | 5 | 4.76 |
| Bacteroidota | Bacteroidia | Flavobacteriales | Crocinitomicaceae | Fluviicola | 106 | 0.01 | 5 | 4.76 |
| Actinobacteriota | Actinobacteria | Actinomycetales | Actinomycetaceae | Trueperella | 89 | 0.01 | 5 | 4.76 |
| Actinobacteriota | Actinobacteria | Micrococcales | Micrococcaceae | Micrococcus | 88 | 0.01 | 5 | 4.76 |
| Firmicutes | Clostridia | Eubacteriales | Eubacteriaceae | Acetobacterium | 72 | 0.01 | 5 | 4.76 |
| Firmicutes | Clostridia | Peptostreptococcales-Tissierellales | Family XI | Ezakiella | 67 | 0.01 | 5 | 4.76 |
| Firmicutes | Clostridia | Peptostreptococcales-Tissierellales | Sedimentibacteraceae | Sedimentibacter | 57 | 0.01 | 5 | 4.76 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Comamonadaceae | Comamonas | 3779 | 0.39 | 4 | 3.81 |
| Proteobacteria | Gammaproteobacteria | Xanthomonadales | Xanthomonadaceae | Arenimonas | 386 | 0.04 | 4 | 3.81 |
| Bacteroidota | Bacteroidia | Cytophagales | Microscillaceae | Chryseolinea | 323 | 0.03 | 4 | 3.81 |
| Bacteroidota | Bacteroidia | Bacteroidales | Dysgonomonadaceae | Petrimonas | 301 | 0.03 | 4 | 3.81 |
| Proteobacteria | Alphaproteobacteria | Caulobacterales | Hyphomonadaceae | Hirschia | 235 | 0.02 | 4 | 3.81 |
| Firmicutes | Bacilli | Erysipelotrichales | Erysipelotrichaceae | Solobacterium | 184 | 0.02 | 4 | 3.81 |
| Bacteroidota | Bacteroidia | Bacteroidales | Prevotellaceae | Alloprevotella | 107 | 0.01 | 4 | 3.81 |
| Proteobacteria | Alphaproteobacteria | Acetobacterales | Acetobacteraceae | Roseomonas | 82 | 0.01 | 4 | 3.81 |
| Proteobacteria | Alphaproteobacteria | Sphingomonadales | Sphingomonadaceae | Sphingosinicella | 80 | 0.01 | 4 | 3.81 |

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|---|---|---|---|---|---|---|---|---|
| Firmicutes | Clostridia | Peptostreptococcales-Tissierellales | Family XI | Finegoldia | 76 | 0.01 | 4 | 3.81 |
| Proteobacteria | Alphaproteobacteria | Caedibacterales | Caedibacteraceae | Caedibacter | 75 | 0.01 | 4 | 3.81 |
| Proteobacteria | Gammaproteobacteria | Cardiobacteriales | Wohlfahrtiimonadaceae | Ignatzschineria | 74 | 0.01 | 4 | 3.81 |
| Firmicutes | Bacilli | Staphylococcales | Staphylococcaceae | Jeotgalicoccus | 72 | 0.01 | 4 | 3.81 |
| Planctomycetota | Planctomycetes | Isosphaerales | Isosphaeraceae | Paludisphaera | 69 | 0.01 | 4 | 3.81 |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Beijerinckiaceae | Camelimonas | 59 | 0.01 | 4 | 3.81 |
| Bacteroidota | Bacteroidia | Chitinophagales | Chitinophagaceae | Niabella | 53 | 0.01 | 4 | 3.81 |
| Firmicutes | Clostridia | Peptostreptococcales-Tissierellales | Family XI | Fenollaria | 36 | 0 | 4 | 3.81 |
| Verrucomicrobiota | Chlamydiae | Chlamydiales | Parachlamydiaceae | Candidatus Protochlamydia | 35 | 0 | 4 | 3.81 |
| Bacteroidota | Bacteroidia | Cytophagales | Microscillaceae | Hassallia | 1919 | 0.2 | 3 | 2.86 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Nitrosomonadaceae | Nitrosospira | 458 | 0.05 | 3 | 2.86 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Rhodocyclaceae | Dechlorobacter | 194 | 0.02 | 3 | 2.86 |
| Actinobacteriota | Actinobacteria | Micrococcales | Brevibacteriaceae | Brevibacterium | 165 | 0.02 | 3 | 2.86 |
| Proteobacteria | Alphaproteobacteria | Azospirillales | Azospirillaceae | Skermanella | 149 | 0.02 | 3 | 2.86 |
| Bacteroidota | Bacteroidia | Cytophagales | Microscillaceae | Ohtaekwangia | 147 | 0.02 | 3 | 2.86 |
| Bacteroidota | Bacteroidia | Sphingobacteriales | Lentimicrobiaceae | Lentimicrobium | 99 | 0.01 | 3 | 2.86 |

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|---|---|---|---|---|---|---|---|---|
| Bacteroidota | Bacteroidia | Sphingobacteriales | Sphingobacteriaceae | Solitalea | 86 | 0.01 | 3 | 2.86 |
| Verrucomicrobiota | Chlamydiae | Chlamydiales | Parachlamydiaceae | Neochlamydia | 84 | 0.01 | 3 | 2.86 |
| Bacteroidota | Bacteroidia | Flavobacteriales | Weeksellaceae | Elizabethkingia | 76 | 0.01 | 3 | 2.86 |
| Firmicutes | Bacilli | Lactobacillales | Carnobacteriaceae | Granulicatella | 51 | 0.01 | 3 | 2.86 |
| Firmicutes | Clostridia | Peptostreptococcales-Tissierellales | Family XI | Keratinibaculum | 49 | 0.01 | 3 | 2.86 |
| Actinobacteriota | Actinobacteria | Actinomycetales | Actinomycetaceae | Actinomyces | 47 | 0 | 3 | 2.86 |
| Actinobacteriota | Actinobacteria | Micrococcales | Cellulomonadaceae | Actinotalea | 45 | 0 | 3 | 2.86 |
| Bacteroidota | Bacteroidia | Bacteroidales | Paludibacteraceae | H1 | 43 | 0 | 3 | 2.86 |
| Firmicutes | Bacilli | Lactobacillales | Streptococcaceae | Lactococcus | 38 | 0 | 3 | 2.86 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Alcaligenaceae | Pusillimonas | 38 | 0 | 3 | 2.86 |
| Actinobacteriota | Coriobacteriia | Coriobacteriales | Coriobacteriaceae | Collinsella | 35 | 0 | 3 | 2.86 |
| Proteobacteria | Alphaproteobacteria | Sphingomonadales | Sphingomonadaceae | Sandarakinorhabdus | 31 | 0 | 3 | 2.86 |
| Actinobacteriota | Actinobacteria | Actinomycetales | Actinomycetaceae | Actinotignum | 27 | 0 | 3 | 2.86 |
| Actinobacteriota | Actinobacteria | Actinomycetales | Actinomycetaceae | Varibaculum | 27 | 0 | 3 | 2.86 |
| Proteobacteria | Gammaproteobacteria | Enterobacterales | Morganellaceae | Buchnera | 23 | 0 | 3 | 2.86 |
| Firmicutes | Bacilli | Lactobacillales | Aerococcaceae | Abiotrophia | 19 | 0 | 3 | 2.86 |

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|---|---|---|---|---|---|---|---|---|
| Actinobacteriota | Coriobacteriia | Coriobacteriales | Eggerthellaceae | Senegalimassilia | 13 | 0 | 3 | 2.86 |
| Acidobacteriota | Blastocatellia | Blastocatellales | Blastocatellaceae | OLB17 | 1131 | 0.12 | 2 | 1.9 |
| Proteobacteria | Gammaproteobacteria | Enterobacterales | Shewanellaceae | Shewanella | 787 | 0.08 | 2 | 1.9 |
| Proteobacteria | Gammaproteobacteria | Enterobacterales | Enterobacteriaceae | Escherichia-Shigella | 375 | 0.04 | 2 | 1.9 |
| Proteobacteria | Gammaproteobacteria | Enterobacterales | Morganellaceae | Proteus | 357 | 0.04 | 2 | 1.9 |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Rhizobiaceae | Aureimonas | 188 | 0.02 | 2 | 1.9 |
| Cyanobacteria | Vampirivibrionia | Vampirovibrionales | Vampirovibrionaceae | Vampirovibrio | 164 | 0.02 | 2 | 1.9 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Burkholderiaceae | Cupriavidus | 127 | 0.01 | 2 | 1.9 |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Rhizobiaceae | Paenochrobactrum | 122 | 0.01 | 2 | 1.9 |
| Firmicutes | Clostridia | Oscillospirales | Ruminococcaceae | Faecalibacterium | 92 | 0.01 | 2 | 1.9 |
| Firmicutes | Bacilli | Erysipelotrichales | Erysipelatoclostridiaceae | Catenibacterium | 86 | 0.01 | 2 | 1.9 |
| Proteobacteria | Gammaproteobacteria | Enterobacterales | Morganellaceae | Moellerella | 74 | 0.01 | 2 | 1.9 |
| Firmicutes | Bacilli | Lactobacillales | Vagococcaceae | Vagococcus | 63 | 0.01 | 2 | 1.9 |
| Firmicutes | Bacilli | Lactobacillales | Lactobacillaceae | HT002 | 57 | 0.01 | 2 | 1.9 |
| Firmicutes | Bacilli | Lactobacillales | Lactobacillaceae | Lentilactobacillus | 51 | 0.01 | 2 | 1.9 |
| Verrucomicrobiota | Verrucomicrobiae | Opitutales | Opitutaceae | Opitutus | 51 | 0.01 | 2 | 1.9 |

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|---|---|---|---|---|---|---|---|---|
| Synergistota | Synergistia | Synergistales | Synergistaceae | Jonquetella | 47 | 0 | 2 | 1.9 |
| Bacteroidota | Bacteroidia | Sphingobacteriales | Sphingobacteriaceae | Nubsella | 44 | 0 | 2 | 1.9 |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Devosiaceae | Devosia | 40 | 0 | 2 | 1.9 |
| Firmicutes | Clostridia | Eubacteriales | Anaerofustaceae | Anaerofustis | 39 | 0 | 2 | 1.9 |
| Bacteroidota | Bacteroidia | Chitinophagales | Chitinophagaceae | Terrimonas | 38 | 0 | 2 | 1.9 |
| Fusobacteriota | Fusobacteriia | Fusobacteriales | Fusobacteriaceae | Fusobacterium | 38 | 0 | 2 | 1.9 |
| Proteobacteria | Gammaproteobacteria | Coxiellales | Coxiellaceae | Coxiella | 32 | 0 | 2 | 1.9 |
| Bacteroidota | Bacteroidia | Cytophagales | Cytophagaceae | Siphonobacter | 30 | 0 | 2 | 1.9 |
| Bacteroidota | Bacteroidia | Chitinophagales | Chitinophagaceae | Flavitalea | 29 | 0 | 2 | 1.9 |
| Campylobacterota | Campylobacteria | Campylobacterales | Arcobacteraceae | Pseudarcobacter | 27 | 0 | 2 | 1.9 |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Beijerinckiaceae | 28-YEA-48 | 27 | 0 | 2 | 1.9 |
| Bacteroidota | Bacteroidia | Flavobacteriales | Weeksellaceae | Bergeyella | 26 | 0 | 2 | 1.9 |
| Bacteroidota | Bacteroidia | Chitinophagales | Chitinophagaceae | Edaphobaculum | 25 | 0 | 2 | 1.9 |
| Proteobacteria | Alphaproteobacteria | Rhodobacterales | Rhodobacteraceae | Paracoccus | 25 | 0 | 2 | 1.9 |
| Actinobacteriota | Actinobacteria | Frankiales | Geodermatophilaceae | Klenkia | 23 | 0 | 2 | 1.9 |
| Firmicutes | Negativicutes | Veillonellales-Selenomonadales | Veillonellaceae | Dialister | 22 | 0 | 2 | 1.9 |

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|---|---|---|---|---|---|---|---|---|
| Campylobacterota | Campylobacteria | Campylobacterales | Campylobacteraceae | Campylobacter | 21 | 0 | 2 | 1.9 |
| Actinobacteriota | Actinobacteria | Micrococcales | Bogoriellaceae | Georgenia | 20 | 0 | 2 | 1.9 |
| Bacteroidota | Bacteroidia | Bacteroidales | Prevotellaceae | Prevotella_7 | 18 | 0 | 2 | 1.9 |
| Dependentiae | Babeliae | Babeliales | Babeliaceae | Candidatus Babela | 17 | 0 | 2 | 1.9 |
| Firmicutes | Clostridia | Oscillospirales | Ruminococcaceae | Subdoligranulum | 17 | 0 | 2 | 1.9 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Burkholderiaceae | Lautropia | 17 | 0 | 2 | 1.9 |
| Proteobacteria | Gammaproteobacteria | Xanthomonadales | Xanthomonadaceae | Xanthomonas | 17 | 0 | 2 | 1.9 |
| Bacteroidota | Bacteroidia | Cytophagales | Microscillaceae | Flexibacter | 16 | 0 | 2 | 1.9 |
| Actinobacteriota | Actinobacteria | Micrococcales | Micrococcaceae | Renibacterium | 14 | 0 | 2 | 1.9 |
| Bacteroidota | Bacteroidia | Flavobacteriales | Weeksellaceae | Moheibacter | 14 | 0 | 2 | 1.9 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Oxalobacteraceae | Massilia | 14 | 0 | 2 | 1.9 |
| Spirochaetota | Leptospirae | Leptospirales | Leptospiraceae | Turneriella | 12 | 0 | 2 | 1.9 |
| Firmicutes | Negativicutes | Veillonellales-Selenomonadales | Veillonellaceae | Negativicoccus | 11 | 0 | 2 | 1.9 |
| Proteobacteria | Gammaproteobacteria | Xanthomonadales | Rhodanobacteraceae | Pseudofulvimonas | 10 | 0 | 2 | 1.9 |
| Firmicutes | Bacilli | Staphylococcales | Gemellaceae | Gemella | 7 | 0 | 2 | 1.9 |
| Actinobacteriota | Actinobacteria | Micrococcales | Intrasporangiaceae | Knoellia | 213 | 0.02 | 1 | 0.95 |

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|---|---|---|---|---|---|---|---|---|
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Comamonadaceae | Pseudacidovorax | 167 | 0.02 | 1 | 0.95 |
| Proteobacteria | Gammaproteobacteria | Enterobacterales | Morganellaceae | Cosenzaea | 134 | 0.01 | 1 | 0.95 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Comamonadaceae | Hydrogenophaga | 129 | 0.01 | 1 | 0.95 |
| Proteobacteria | Alphaproteobacteria | Azospirillales | Inquilinaceae | Inquilinus | 119 | 0.01 | 1 | 0.95 |
| Bacteroidota | Bacteroidia | Bacteroidales | Rikenellaceae | Rikenellaceae RC9 gut group | 110 | 0.01 | 1 | 0.95 |
| Proteobacteria | Gammaproteobacteria | Enterobacterales | Aeromonadaceae | Aeromonas | 98 | 0.01 | 1 | 0.95 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Rhodocyclaceae | Uliginosibacterium | 93 | 0.01 | 1 | 0.95 |
| Firmicutes | Bacilli | Lactobacillales | Carnobacteriaceae | Carnobacterium | 80 | 0.01 | 1 | 0.95 |
| Proteobacteria | Alphaproteobacteria | Rickettsiales | Anaplasmataceae | Wolbachia | 69 | 0.01 | 1 | 0.95 |
| Proteobacteria | Gammaproteobacteria | Xanthomonadales | Rhodanobacteraceae | Tahibacter | 60 | 0.01 | 1 | 0.95 |
| Firmicutes | Bacilli | Lactobacillales | Carnobacteriaceae | Isobaculum | 58 | 0.01 | 1 | 0.95 |
| Actinobacteriota | Actinobacteria | Bifidobacteriales | Bifidobacteriaceae | Bifidobacterium | 52 | 0.01 | 1 | 0.95 |
| Verrucomicrobiota | Verrucomicrobiae | Chthoniobacterales | Xiphinematobacteraceae | Candidatus Xiphinematobacter | 46 | 0 | 1 | 0.95 |
| Firmicutes | Clostridia | Oscillospirales | Oscillospiraceae | NK4A214 group | 41 | 0 | 1 | 0.95 |
| Proteobacteria | Alphaproteobacteria | Caulobacterales | Hyphomonadaceae | SWB02 | 38 | 0 | 1 | 0.95 |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Rhizobiaceae | Neorhizobium | 37 | 0 | 1 | 0.95 |

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|---|---|---|---|---|---|---|---|---|
| Proteobacteria | Gammaproteobacteria | Pseudomonadales | Moraxellaceae | Perlucidibaca | 37 | 0 | 1 | 0.95 |
| Firmicutes | Clostridia | Christensenellales | Christensenellaceae | Christensenellaceae R-7 group | 35 | 0 | 1 | 0.95 |
| Actinobacteriota | Actinobacteria | Actinomycetales | Actinomycetaceae | Mobiluncus | 34 | 0 | 1 | 0.95 |
| Deinococcota | Deinococci | Deinococcales | Trueperaceae | Truepera | 34 | 0 | 1 | 0.95 |
| Firmicutes | Clostridia | Oscillospirales | Ruminococcaceae | Ruminococcus | 33 | 0 | 1 | 0.95 |
| Cyanobacteria | Cyanobacteriia | Cyanobacteriales | Chroococcidiopsaceae | Aliterella | 32 | 0 | 1 | 0.95 |
| Proteobacteria | Gammaproteobacteria | Pseudomonadales | Moraxellaceae | [Agitococcus] lubricus group | 31 | 0 | 1 | 0.95 |
| Proteobacteria | Gammaproteobacteria | Pseudomonadales | Moraxellaceae | Alkanindiges | 31 | 0 | 1 | 0.95 |
| Acidobacteriota | Acidobacteriae | Bryobacterales | Bryobacteraceae | Bryobacter | 30 | 0 | 1 | 0.95 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Comamonadaceae | Malikia | 28 | 0 | 1 | 0.95 |
| Cyanobacteria | Cyanobacteriia | Leptolyngbyales | Leptolyngbyaceae | Leptolyngbya PCC-6306 | 27 | 0 | 1 | 0.95 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Burkholderiaceae | Polynucleobacter | 27 | 0 | 1 | 0.95 |
| Bacteroidota | Bacteroidia | Cytophagales | Spirosomaceae | Emticicia | 25 | 0 | 1 | 0.95 |
| Firmicutes | Bacilli | Erysipelotrichales | Erysipelotrichaceae | Dubosiella | 25 | 0 | 1 | 0.95 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Neisseriaceae | Kingella | 24 | 0 | 1 | 0.95 |
| Firmicutes | Clostridia | Peptostreptococcales-Tissierellales | Anaerovoracaceae | Mogibacterium | 21 | 0 | 1 | 0.95 |

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|---|---|---|---|---|---|---|---|---|
| Proteobacteria | Alphaproteobacteria | Sphingomonadales | Sphingomonadaceae | Parablastomonas | 21 | 0 | 1 | 0.95 |
| Proteobacteria | Gammaproteobacteria | Gammaproteobacteria Incertae Sedis | Unknown Family | Acidibacter | 21 | 0 | 1 | 0.95 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Nitrosomonadaceae | MND1 | 20 | 0 | 1 | 0.95 |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Rhizobiaceae | Pseudaminobacter | 19 | 0 | 1 | 0.95 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Methylophilaceae | Candidatus Methylopumilus | 19 | 0 | 1 | 0.95 |
| Firmicutes | Negativicutes | Veillonellales-Selenomonadales | Sporomusaceae | Anaerosporomusa | 18 | 0 | 1 | 0.95 |
| Proteobacteria | Alphaproteobacteria | Paracaedibacterales | Paracaedibacteraceae | Candidatus Odyssella | 17 | 0 | 1 | 0.95 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Rhodocyclaceae | Dechloromonas | 17 | 0 | 1 | 0.95 |
| Acidobacteriota | Vicinamibacteria | Vicinamibacterales | Vicinamibacteraceae | Vicinamibacter | 15 | 0 | 1 | 0.95 |
| Deinococcota | Deinococci | Deinococcales | Deinococcaceae | Deinococcus | 15 | 0 | 1 | 0.95 |
| Firmicutes | Clostridia | Peptostreptococcales-Tissierellales | Anaerovoracaceae | Anaerovorax | 15 | 0 | 1 | 0.95 |
| Firmicutes | Clostridia | Clostridiales | Clostridiaceae | Clostridium sensu stricto 1 | 14 | 0 | 1 | 0.95 |
| Actinobacteriota | Actinobacteria | Micrococcales | Micrococcaceae | Enteractinococcus | 13 | 0 | 1 | 0.95 |
| Firmicutes | Negativicutes | Veillonellales-Selenomonadales | Selenomonadaceae | Centipeda | 13 | 0 | 1 | 0.95 |
| Myxococcota | Myxococcia | Myxococcales | Myxococcaceae | P3OB-42 | 13 | 0 | 1 | 0.95 |
| Proteobacteria | Gammaproteobacteria | Enterobacterales | Morganellaceae | Candidatus Hamiltonella | 13 | 0 | 1 | 0.95 |

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|---|---|---|---|---|---|---|---|---|
| Bacteroidota | Bacteroidia | Bacteroidales | Paludibacteraceae | Paludibacter | 12 | 0 | 1 | 0.95 |
| Firmicutes | Bacilli | Erysipelotrichales | Erysipelotrichaceae | Holdemanella | 12 | 0 | 1 | 0.95 |
| Firmicutes | Clostridia | Peptostreptococcales-Tissierellales | Peptostreptococcaceae | Peptostreptococcus | 12 | 0 | 1 | 0.95 |
| Actinobacteriota | Actinobacteria | Frankiales | Sporichthyaceae | Candidatus Planktophila | 11 | 0 | 1 | 0.95 |
| Actinobacteriota | Actinobacteria | Micrococcales | Micrococcaceae | Yaniella | 11 | 0 | 1 | 0.95 |
| Actinobacteriota | Actinobacteria | Micrococcales | Microbacteriaceae | Rathayibacter | 10 | 0 | 1 | 0.95 |
| Firmicutes | Clostridia | Lachnospirales | Lachnospiraceae | Oribacterium | 10 | 0 | 1 | 0.95 |
| Proteobacteria | Gammaproteobacteria | Salinisphaerales | Solimonadaceae | Hydrocarboniphaga | 10 | 0 | 1 | 0.95 |
| Firmicutes | Clostridia | Lachnospirales | Lachnospiraceae | [Eubacterium] fissicatena group | 9 | 0 | 1 | 0.95 |
| Proteobacteria | Alphaproteobacteria | Rhodobacterales | Rhodobacteraceae | Haematobacter | 9 | 0 | 1 | 0.95 |
| Proteobacteria | Alphaproteobacteria | Sphingomonadales | Sphingomonadaceae | Altererythrobacter | 9 | 0 | 1 | 0.95 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Methylophilaceae | Methylobacillus | 8 | 0 | 1 | 0.95 |
| Proteobacteria | Gammaproteobacteria | Burkholderiales | Nitrosomonadaceae | Nitrosomonas | 8 | 0 | 1 | 0.95 |
| Acidobacteriota | Vicinamibacteria | Vicinamibacterales | Vicinamibacteraceae | Luteitalea | 7 | 0 | 1 | 0.95 |
| Bacteroidota | Bacteroidia | Cytophagales | Hymenobacteraceae | Hymenobacter | 7 | 0 | 1 | 0.95 |
| Cyanobacteria | Cyanobacteriia | Cyanobacteriales | Chroococcidiopsaceae | Chroococcidiopsis PCC 7203 | 7 | 0 | 1 | 0.95 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Firmicutes | Bacilli | Acholeplasmatales | Acholeplasmataceae | Acholeplasma | 7 | 0 | 1 | 0.95 |
| Firmicutes | Clostridia | Eubacteriales | Alkalibacteraceae | Alkalibacter | 7 | 0 | 1 | 0.95 |
| Myxococcota | Polyangia | Haliangiales | Haliangiaceae | Haliangium | 7 | 0 | 1 | 0.95 |
| Acidobacteriota | Blastocatellia | Chloracidobacteriales | Chloracidobacteriaceae | Chloracidobacterium | 6 | 0 | 1 | 0.95 |
| Bacteroidota | Bacteroidia | Cytophagales | Spirosomaceae | Leadbetterella | 6 | 0 | 1 | 0.95 |
| Firmicutes | Clostridia | Peptostreptococcales-Tissierellales | Family XI | Helcococcus | 6 | 0 | 1 | 0.95 |
| Bacteroidota | Bacteroidia | Cytophagales | Spirosomaceae | Larkinella | 5 | 0 | 1 | 0.95 |
| Bacteroidota | Bacteroidia | Flavobacteriales | Flavobacteriaceae | Myroides | 5 | 0 | 1 | 0.95 |
| Firmicutes | Clostridia | Lachnospirales | Lachnospiraceae | Blautia | 5 | 0 | 1 | 0.95 |
| Fusobacteriota | Fusobacteriia | Fusobacteriales | Leptotrichiaceae | Sneathia | 5 | 0 | 1 | 0.95 |
| Patescibacteria | Saccharimonadia | Saccharimonadales | Saccharimonadaceae | TM7a | 5 | 0 | 1 | 0.95 |
| Proteobacteria | Gammaproteobacteria | Tenderiales | Tenderiaceae | Candidatus Tenderia | 5 | 0 | 1 | 0.95 |
| Thermotogota | Thermotogae | Thermotogales | Fervidobacteriaceae | Fervidobacterium | 5 | 0 | 1 | 0.95 |
| Cyanobacteria | Cyanobacteriia | Cyanobacteriales | Coleofasciculaceae | Wilmottia Ant-Ph58 | 4 | 0 | 1 | 0.95 |
| Proteobacteria | Alphaproteobacteria | Acetobacterales | Acetobacteraceae | Rhodovastum | 4 | 0 | 1 | 0.95 |
| Verrucomicrobiota | Verrucomicrobiae | Chthoniobacterales | Chthoniobacteraceae | Candidatus Udaeobacter | 4 | 0 | 1 | 0.95 |

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|---|---|---|---|---|---|---|---|---|
| Bacteroidota | Bacteroidia | Flavobacteriales | Flavobacteriaceae | Capnocytophaga | 3 | 0 | 1 | 0.95 |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Xanthobacteraceae | Rhodopseudomonas | 3 | 0 | 1 | 0.95 |
| Deinococcota | Deinococci | Thermales | Thermaceae | Thermus | 1 | 0 | 1 | 0.95 |
| Planctomycetota | Planctomycetes | Planctomycetales | Schlesneriaceae | Schlesneria | 1 | 0 | 1 | 0.95 |

**Table D.2.** Taxonomic profiling of the bacterial community of P-traps of urinals located on a university campus and train station (A) Phylum, (B) Class, (C) Order, (D) Family, (E) Genus.

| Pairwise Comparison | Sums Of Squares | Mean Squares | F Model | R2 | p-value | p-value (BH corrected) |
|---|---|---|---|---|---|---|
| Agriculture <-> Archaeology | 0.746251 | 0.746251 | 1.806156 | 0.079196 | 0.002 | 0.0065 |
| Agriculture <-> Chemistry | 0.774539 | 0.774539 | 1.845297 | 0.068738 | 0.001 | 0.004789474 |
| Agriculture <-> Eat at the Square | 0.888562 | 0.888562 | 2.176425 | 0.093907 | 0.001 | 0.004789474 |
| Agriculture <-> Edith Morely | 0.563362 | 0.563362 | 1.341274 | 0.055103 | 0.027 | 0.0351 |
| Agriculture <-> Henley Business School | 0.668741 | 0.668741 | 1.600708 | 0.060175 | 0.002 | 0.0065 |
| Agriculture <-> JJ Thompson | 0.810152 | 0.810152 | 1.925159 | 0.06894 | 0.001 | 0.004789474 |
| Agriculture <-> Maths | 0.724835 | 0.724835 | 1.734963 | 0.067417 | 0.002 | 0.0065 |
| Agriculture <-> Meteorology | 0.67875 | 0.67875 | 1.641577 | 0.04141 | 0.013 | 0.020050847 |
| Agriculture <-> Park Eat | 0.720101 | 0.720101 | 1.698227 | 0.07166 | 0.004 | 0.008878049 |
| Agriculture <-> Polly Vacher | 1.075715 | 1.075715 | 2.711074 | 0.091248 | 0.001 | 0.004789474 |
| Agriculture <-> The Dairy | 0.437697 | 0.437697 | 1.025576 | 0.046563 | 0.374 | 0.374 |
| Agriculture <-> Train Station | 0.954318 | 0.954318 | 2.312776 | 0.095126 | 0.001 | 0.004789474 |
| Agriculture <-> Union | 0.8195 | 0.8195 | 1.952421 | 0.075231 | 0.001 | 0.004789474 |
| Archaeology <-> Chemistry | 0.863837 | 0.863837 | 2.301973 | 0.22345 | 0.01 | 0.015964912 |
| Archaeology <-> Eat at the Square | 0.891125 | 0.891125 | 3.293595 | 0.451574 | 0.1 | 0.104597701 |
| Archaeology <-> Edith Morely | 0.808211 | 0.808211 | 2.235595 | 0.271455 | 0.029 | 0.035186667 |
| Archaeology <-> Henley Business School | 0.513047 | 0.513047 | 1.389845 | 0.148016 | 0.039 | 0.0443625 |
| Archaeology <-> JJ Thompson | 0.8455 | 0.8455 | 2.205615 | 0.196831 | 0.009 | 0.014890909 |
| Archaeology <-> Maths | 0.930671 | 0.930671 | 2.56949 | 0.268509 | 0.015 | 0.021328125 |
| Archaeology <-> Meteorology | 0.899992 | 0.899992 | 2.299062 | 0.098676 | 0.001 | 0.004789474 |
| Archaeology <-> Park Eat | 0.648697 | 0.648697 | 1.765317 | 0.260936 | 0.057 | 0.063256098 |
| Archaeology <-> Polly Vacher | 1.046461 | 1.046461 | 3.248002 | 0.245169 | 0.008 | 0.014 |
| Archaeology <-> The Dairy | 0.552299 | 0.552299 | 1.501756 | 0.272959 | 0.1 | 0.104597701 |
| Archaeology <-> Train Station | 0.931068 | 0.931068 | 2.934374 | 0.369831 | 0.031 | 0.036636364 |
| Archaeology <-> Union | 0.902035 | 0.902035 | 2.445201 | 0.258883 | 0.018 | 0.024447761 |
| Chemistry <-> Eat at the Square | 0.80476 | 0.80476 | 2.220729 | 0.217277 | 0.008 | 0.014 |
| Chemistry <-> Edith Morely | 0.474073 | 0.474073 | 1.189361 | 0.106294 | 0.135 | 0.138033708 |

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|---|---|---|---|---|---|---|
| Chemistry <-> Henley Business School | 0.719321 | 0.719321 | 1.809631 | 0.131041 | 0.006 | 0.012133333 |
| Chemistry <-> JJ Thompson | 0.612349 | 0.612349 | 1.511431 | 0.104154 | 0.014 | 0.020885246 |
| Chemistry <-> Maths | 0.695205 | 0.695205 | 1.757087 | 0.137734 | 0.002 | 0.0065 |
| Chemistry <-> Meteorology | 0.690147 | 0.690147 | 1.718922 | 0.064334 | 0.004 | 0.008878049 |
| Chemistry <-> Park Eat | 0.759854 | 0.759854 | 1.871476 | 0.172146 | 0.003 | 0.007583333 |
| Chemistry <-> Polly Vacher | 1.020766 | 1.020766 | 2.836198 | 0.168458 | 0.001 | 0.004789474 |
| Chemistry <-> The Dairy | 0.572228 | 0.572228 | 1.392322 | 0.14824 | 0.03 | 0.035921053 |
| Chemistry <-> Train Station | 0.880994 | 0.880994 | 2.329771 | 0.205633 | 0.002 | 0.0065 |
| Chemistry <-> Union | 0.67892 | 0.67892 | 1.697635 | 0.133697 | 0.016 | 0.0224 |
| Eat at the Square <-> Edith Morely | 0.67299 | 0.67299 | 1.954354 | 0.245696 | 0.015 | 0.021328125 |
| Eat at the Square <-> Henley Business School | 0.865386 | 0.865386 | 2.429047 | 0.232912 | 0.007 | 0.013553191 |
| Eat at the Square <-> JJ Thompson | 0.66901 | 0.66901 | 1.798914 | 0.166583 | 0.008 | 0.014 |
| Eat at the Square <-> Maths | 0.849925 | 0.849925 | 2.445915 | 0.258939 | 0.008 | 0.014 |
| Eat at the Square <-> Meteorology | 0.860053 | 0.860053 | 2.22491 | 0.095798 | 0.001 | 0.004789474 |
| Eat at the Square <-> Park Eat | 0.83169 | 0.83169 | 2.397703 | 0.324114 | 0.025 | 0.032971014 |
| Eat at the Square <-> Polly Vacher | 0.978818 | 0.978818 | 3.138373 | 0.238871 | 0.009 | 0.014890909 |
| Eat at the Square <-> The Dairy | 0.743636 | 0.743636 | 2.17424 | 0.352147 | 0.1 | 0.104597701 |
| Eat at the Square <-> Train Station | 0.911201 | 0.911201 | 3.071127 | 0.380508 | 0.029 | 0.035186667 |
| Eat at the Square <-> Union | 0.81493 | 0.81493 | 2.300846 | 0.24738 | 0.022 | 0.029441176 |
| Edith Morely <-> Henley Business School | 0.643638 | 0.643638 | 1.634849 | 0.140513 | 0.009 | 0.014890909 |
| Edith Morely <-> JJ Thompson | 0.444114 | 0.444114 | 1.101788 | 0.091043 | 0.259 | 0.261877778 |
| Edith Morely <-> Maths | 0.501454 | 0.501454 | 1.28239 | 0.124717 | 0.017 | 0.023439394 |
| Edith Morely <-> Meteorology | 0.53206 | 0.53206 | 1.329493 | 0.054645 | 0.063 | 0.069072289 |
| Edith Morely <-> Park Eat | 0.653332 | 0.653332 | 1.62105 | 0.188034 | 0.008 | 0.014 |
| Edith Morely <-> Polly Vacher | 0.755935 | 0.755935 | 2.15687 | 0.152355 | 0.005 | 0.010581395 |
| Edith Morely <-> The Dairy | 0.564992 | 0.564992 | 1.380865 | 0.187087 | 0.032 | 0.037333333 |
| Edith Morely <-> Train Station | 0.760408 | 0.760408 | 2.070862 | 0.228298 | 0.01 | 0.015964912 |
| Edith Morely <-> Union | 0.527983 | 0.527983 | 1.332477 | 0.12896 | 0.098 | 0.104597701 |
| Henley Business School <-> JJ Thompson | 0.7321 | 0.7321 | 1.823961 | 0.123041 | 0.001 | 0.004789474 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Henley Business School <-> Maths | 0.873207 | 0.873207 | 2.232083 | 0.168687 | 0.001 | 0.004789474 |
| Henley Business School <-> Meteorology | 0.903125 | 0.903125 | 2.260403 | 0.082919 | 0.002 | 0.0065 |
| Henley Business School <-> Park Eat | 0.632561 | 0.632561 | 1.579115 | 0.149267 | 0.006 | 0.012133333 |
| Henley Business School <-> Polly Vacher | 0.997139 | 0.997139 | 2.797732 | 0.166554 | 0.002 | 0.0065 |
| Henley Business School <-> The Dairy | 0.512264 | 0.512264 | 1.265261 | 0.13656 | 0.038 | 0.043772152 |
| Henley Business School <-> Train Station | 0.925044 | 0.925044 | 2.481962 | 0.216162 | 0.004 | 0.008878049 |
| Henley Business School <-> Union | 0.767618 | 0.767618 | 1.941025 | 0.14999 | 0.001 | 0.004789474 |
| JJ Thompson <-> Maths | 0.71467 | 0.71467 | 1.786592 | 0.129589 | 0.004 | 0.008878049 |
| JJ Thompson <-> Meteorology | 0.805027 | 0.805027 | 1.996159 | 0.071301 | 0.003 | 0.007583333 |
| JJ Thompson <-> Park Eat | 0.693576 | 0.693576 | 1.690761 | 0.144624 | 0.003 | 0.007583333 |
| JJ Thompson <-> Polly Vacher | 1.112246 | 1.112246 | 3.040764 | 0.16855 | 0.001 | 0.004789474 |
| JJ Thompson <-> The Dairy | 0.603294 | 0.603294 | 1.453372 | 0.139034 | 0.012 | 0.018827586 |
| JJ Thompson <-> Train Station | 0.925615 | 0.925615 | 2.403382 | 0.193768 | 0.002 | 0.0065 |
| JJ Thompson <-> Union | 0.678013 | 0.678013 | 1.678556 | 0.122714 | 0.003 | 0.007583333 |
| Maths <-> Meteorology | 0.731607 | 0.731607 | 1.834591 | 0.071013 | 0.005 | 0.010581395 |
| Maths <-> Park Eat | 0.75958 | 0.75958 | 1.906397 | 0.192441 | 0.003 | 0.007583333 |
| Maths <-> Polly Vacher | 1.052954 | 1.052954 | 2.993948 | 0.187193 | 0.001 | 0.004789474 |
| Maths <-> The Dairy | 0.628362 | 0.628362 | 1.55908 | 0.182155 | 0.014 | 0.020885246 |
| Maths <-> Train Station | 0.908323 | 0.908323 | 2.474449 | 0.236237 | 0.003 | 0.007583333 |
| Maths <-> Union | 0.822987 | 0.822987 | 2.092804 | 0.173062 | 0.004 | 0.008878049 |
| Meteorology <-> Park Eat | 0.860553 | 0.860553 | 2.13374 | 0.088413 | 0.001 | 0.004789474 |
| Meteorology <-> Polly Vacher | 1.3304 | 1.3304 | 3.501979 | 0.114812 | 0.001 | 0.004789474 |
| Meteorology <-> The Dairy | 0.544217 | 0.544217 | 1.343509 | 0.06013 | 0.052 | 0.058419753 |
| Meteorology <-> Train Station | 1.028751 | 1.028751 | 2.625001 | 0.106599 | 0.001 | 0.004789474 |
| Meteorology <-> Union | 0.816336 | 0.816336 | 2.037078 | 0.078238 | 0.003 | 0.007583333 |
| Park Eat <-> Polly Vacher | 0.958397 | 0.958397 | 2.721345 | 0.198329 | 0.001 | 0.004789474 |
| Park Eat <-> The Dairy | 0.545018 | 0.545018 | 1.2835 | 0.204265 | 0.111 | 0.114784091 |
| Park Eat <-> Train Station | 0.847464 | 0.847464 | 2.271433 | 0.274612 | 0.028 | 0.035186667 |
| Park Eat <-> Union | 0.670297 | 0.670297 | 1.657922 | 0.171664 | 0.015 | 0.021328125 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Polly Vacher <-> The Dairy | 0.727834 | 0.727834 | 2.074965 | 0.17184 | 0.007 | 0.013553191 |
| Polly Vacher <-> Train Station | 0.910345 | 0.910345 | 2.763872 | 0.200806 | 0.002 | 0.0065 |
| Polly Vacher <-> Union | 0.928817 | 0.928817 | 2.614168 | 0.167423 | 0.001 | 0.004789474 |
| The Dairy <-> Train Station | 0.706449 | 0.706449 | 1.886564 | 0.273948 | 0.028 | 0.035186667 |
| The Dairy <-> Union | 0.645804 | 0.645804 | 1.576159 | 0.183784 | 0.029 | 0.035186667 |
| Train Station <-> Union | 0.780398 | 0.780398 | 2.092541 | 0.207335 | 0.003 | 0.007583333 |

**Table D.3.** Pairwise comparisons for all pairs of levels of the factor “Building” by using PERMANOVA. Benjamini-Hochberg corrected p-values shown. The R2 values indicate the amount of variation explained by the comparisons in the model.

| Building | vs | Building | P Value | P Adjusted Value |
|---|---|---|---|---|
| Agriculture | vs | Archaeology | 0.000197302 | 0.003257809 |
| Agriculture | vs | Chemistry | 0.001314916 | 0.008701213 |
| Agriculture | vs | Eat at the Square | 6.43E-08 | 5.85E-06 |
| Agriculture | vs | Edith Morely | 0.000256472 | 0.003257809 |
| Agriculture | vs | Henley Business School | 0.000322201 | 0.003257809 |
| Agriculture | vs | JJ Thompson | 0.003735664 | 0.022663031 |
| Agriculture | vs | Maths | 0.000369231 | 0.003360004 |
| Agriculture | vs | Park Eat | 0.001278169 | 0.008701213 |
| Agriculture | vs | Polly Vacher | 0.000262025 | 0.003257809 |
| Agriculture | vs | The Dairy | 4.82E-05 | 0.001095709 |
| Agriculture | vs | Train Station | 7.92E-07 | 3.60E-05 |
| Archaeology | vs | Meteorology | 0.001338648 | 0.008701213 |
| Chemistry | vs | Eat at the Square | 0.004597022 | 0.024607589 |
| Eat at the Square | vs | Henley Business School | 0.004945116 | 0.025000309 |
| Eat at the Square | vs | JJ Thompson | 0.00122265 | 0.008701213 |
| Eat at the Square | vs | Meteorology | 6.59E-06 | 0.000199784 |
| JJ Thompson | vs | Train Station | 0.004317743 | 0.024557161 |
| Meteorology | vs | Polly Vacher | 0.005491451 | 0.026301161 |
| Meteorology | vs | Train Station | 0.000304712 | 0.003257809 |

**Table D.4.** Pairwise comparison for all significant pairs of levels of building by using permutest(). Permutation-based test of multivariate homogeneity of group dispersions (variance). P-values based on 999 permutations and corrected with Benjamini-Hochberg (BH).

| Building | Prevelance (Count) | Prevalence (%) | Mean RA (%) | Standard Deviation RA (%) | Median RA (%) | Minimum RA (%) | Maximum RA (%) |
|---|---|---|---|---|---|---|---|
| Agriculture | 18 | 90.00 | 17.09 | 19.14 | 8.77 | 0.00 | 50.59 |
| Archaeology | 3 | 100.00 | 26.73 | 21.88 | 32.16 | 2.64 | 45.38 |
| Chemistry | 2 | 28.57 | 0.07 | 0.17 | 0.00 | 0.00 | 0.46 |
| Eat at the Square | 3 | 100.00 | 0.89 | 0.63 | 0.61 | 0.45 | 1.61 |
| Edith Morely | 4 | 80.00 | 2.45 | 4.49 | 0.30 | 0.00 | 10.40 |
| Henley Business School | 6 | 85.71 | 22.57 | 31.11 | 4.08 | 0.00 | 70.59 |
| JJ Thompson | 2 | 25.00 | 3.01 | 8.33 | 0.00 | 0.00 | 23.63 |
| Maths | 4 | 66.67 | 1.32 | 2.35 | 0.46 | 0.00 | 6.04 |
| Meteorology | 15 | 75.00 | 18.90 | 32.20 | 0.54 | 0.00 | 93.93 |
| Park Eat | 2 | 50.00 | 13.77 | 17.28 | 9.64 | 0.00 | 35.82 |
| Polly Vacher | 7 | 77.78 | 4.63 | 7.52 | 1.23 | 0.00 | 18.11 |
| The Dairy | 3 | 100.00 | 39.37 | 36.13 | 33.86 | 6.31 | 77.94 |
| Train Station | 2 | 50.00 | 0.16 | 0.23 | 0.08 | 0.00 | 0.49 |
| Union | 2 | 33.33 | 0.40 | 0.80 | 0.00 | 0.00 | 2.01 |

**Table D.5**. The prevalence of Dolosicoccus within a building and the mean/median relative abundance (%) of Dolosicoccus in each building. Maximum relative abundance (%) and lowest relative abundance (%) of Dolosicoccus within a building shown.

| Sequence | Mean RA (%) | Max RA (%) |
|---|---|---|
| TACTTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGGGAGCGCAGGCGGTGTATAA AGTCTGATGTGAAAGTCCACGGCTCAACCGTGGGATTGCATTGGAAACTGATACACTTGAGGA TAGAAGAGGATAGTGGAATTCCATGTGTAGTGGTGAAATACGTAGATATATGGAGGAACACC AGTGGCGAAGGCGGCTATCTGGTCTATCACTGACGCTGAGGCTCGAAAGCATGGGGAGCAAA CAGG | 10.1641 | 90.9921 |
| TACTTAGGTGGCGAGCGTTGTCCGGATTTATTGGGCGTAAAGGGAGCGCAGGCGGTGTATAA AGTCTGATGTGAAAGTCCACGGCTCAACCGTGGGATTGCATTGGAAACTGATACACTTGAGGA TAGAAGAGGATAGTGGAATTCCATGTGTAGTGGTGAAATACGTAGATATATGGAGGAACACC AGTGGCGAAGGCGGCTATCTGGTCTATCACTGACGCTGAGGCTCGAAAGCATGGGGAGCAAA CAGG | 0.8981 | 20.0236 |
| TACTTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGGGAGCGCAGGCGGTGTATAA AGTCTGATGTGAAAGTCCACGGCTCAACCGTGGGATTGCATTGGAAACTGATACACTTGAGGA TAGAAGAGGATAGTGGAATTCCATGTGTAGTGGTGAAATACGTAGATATATGGAGGAACACC AGTGGCGAAGGCTGCTATCTGGTCTATCACTGACGCTGAGGCTCGAAAGCATGGGGAGCAAA CAGG | 0.4535 | 8.1168 |
| TACTTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGGGAGCGCAGGCGGTGTATAA AGTCTGATGTGAAAGTCCACGGCTCAACCGTGGGATTGCATTGGAAACTGATACACTTGAGGA TAGAAGAGGATAGTGGAATTCCATGTGTAGTGGTGAAATACGTAGATATATGGAGGAACACC AGTGGCGAAGGCGACTTACTGGTCTGTGATTGACGCTGAGGCTCGAAAGCGTGGGTAGCAAA CAGG | 0.0261 | 0.8697 |
| TACTTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGGGAGCGCAGGCGGTGTATAA AGTCTGATGTGAAAGTCCACGGCTCAACCGTGGGATTGCATTGGAAACTGATACACTTGAGGA TAGAAGAGGATAGTGGAATTCCATGTGTAGTGGTGAAATACGTAGATATATGGAGGAACACC AGTGGCGAAGGCGACTTACTGGTCTGTAATTGACGCTGAGGCTCGAAAGCGTGGGTAGCGAA CAGG | 0.0194 | 0.8160 |
| TACTTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGGGAGCGCAGGCGGTGTATAA AGTCTGATGTGAAAGTCCACGGCTCAACCGTGGGATTGCATTGGAAACTGATACACTTGAGGA TAGAAGAGGATAGTGGAATTCCATGTGTAGTGGTGAAATACGTAGATATATGGAGGAACACC AGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAA ACAGG | 0.0105 | 1.1059 |
| TACTTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGGGAGCGCAGGCGGTGTATAA AGTCTGATGTGAAAGTCCACGGCTCAACCGTGGGATTGCATTGGAAACTGATACACTTGAGGA TAGAAGAGGATAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAACACC AGTGGCGAAGGCGACTTTCTGGTCTATTATTGACGCTGAGGCTCGAAAGCATGGGTAGCAAA CAGG | 0.0114 | 1.1918 |
| TACTTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGGGAGCGCAGGCGGTGTATAA AGTCTGATGTGAAAGTCCACGGCTCAACCGTGGGATTGCATTGGAAACTGATACACTTGAGGA TAGAAGAGGATAGTGGAATTCCATGTGTAGTGGTGAAATACGTAGATATATGGAGGAACACC AGTGGCGAAAGCGACTTTCTGGTCTGTAATTGACGCTGAGGCTCGAAAGCGTGGGTAGCGAA CAGG | 0.0120 | 1.2562 |
| TACTTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGGGAGCGCAGGCGGTGTATAA AGTCTGATGTGAAAGTCCACGGCTCAACCGTGGGATTGCATTGGAAACTGATACACTTGAGGA TAGAAGAGGATAGTGGAATTCCATGTGTAGTGGTGAAATACGTAGATATATGGAGGAACACC AATGGCGAAGGCAGCCCCCTGGGATAATACTGACGCTCAGGCACGAAAGCGTGGGTAGCAAA CAGG | 0.0072 | 0.7516 |

| | | |
|---|---|---|
| TACTTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGGGAGCGCAGGCGGTGTATAA AGTCTGATGTGAAAGTCCACGGCTCAACCGTGGGATTGCATTGGAAACTGATACACTTGAGGA TAGAAGAGGATAGTGGAATTCCATGTGTAGTGGTGAAATACGTAGATATATGGAGGAACACC AGTGGCGAAGGCGACTTTCTGGTCTATTATTGACGCTGAGGCTCGAAAGCATGGGTAGCAAA CAGG | 0.0047 | 0.4939 |
| TACTTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGGGAGCGCAGGCGGTGTATAA AGTCTGATGTGAAAGTCCACGGCTCAACCGTGGGATTGCATTGGAAACTGATACACTTGAGGA TAGAAGAGGATAGTGGAATTCCATGTGTAGTGGTGAAATACGTAGATATATGGAGGAACACC AGTGGCGAAGGCGACTTTCTGGTCTGTTACTGACACTGAGGCCCGAAAGCGTGGGTAGCAAA CAGG | 0.0090 | 0.9448 |
| TACTTAGGTGGCGAGCGTTGTCCGGATTTATTGGGCGTAAAGGGAGCGCAGGCGGTGTATAA AGTCTGATGTGAAAGTCCACGGCTCAACCGTGGGATTGCATTGGAAACTGATACACTTGAGGA TAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAACACC AGTGGCGAAGGCGACTTTCTGGTCTATTATTGACGCTGAGGCTCGAAAGCATGGGTAGCAAA CAGG | 0.0039 | 0.4080 |
| TACTTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGGGAGCGCAGTCGGTGTATAA AGTCTGATGTGAAAGTCCACGGCTCAACCGTGGGATTGCATTGGAAACTGATACACTTGAGGA TAGAAGAGGATAGTGGAATTCCATGTGTAGTGGTGAAATACGTAGATATATGGAGGAACACC AGTGGCGAAGGCGGCTATCTGGTCTATCACTGACGCTGAGGCTCGAAAGCATGGGGAGCAAA CAGG | 0.0030 | 0.3114 |
| TACTTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGGGAGCGCAGGCGGTGTATAA AGTCTGATGTGAAAGTCCACGGCTCAACCGTGGGATTGCATTGGAAACTGATACACTTGAGGA TAGAAGAGGATAGTGGAATTCCATGTGTAGTGGTGAAATACGTAGATATATGGAGGAACACC AGTGGCGAAGGCGGCTATCTGGTCTATCACTGACGCTGAGGCTCGAAAGCGTGGGTAGCAAA CAGG | 0.0040 | 0.4187 |
| TACGTAGGTGACAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGAGCGCAGGCGGTTGGAAT AGTCTGATGTGAAAGTCCACGGCTCAACCGTGGGATTGCATTGGAAACTGATACACTTGAGGA TAGAAGAGGATAGTGGAATTCCATGTGTAGTGGTGAAATACGTAGATATATGGAGGAACACC AGTGGCGAAGGCGGCTATCTGGTCTATCACTGACGCTGAGGCTCGAAAGCATGGGGAGCAAA CAGG | 0.0059 | 0.6227 |
| TACTTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGGGAGCGCAGGCGGTGTATAA AGTCTGATGTGAAAGTCCACGGCTCAACCGTGGGATTGCATTGGAAACTGATACACTTGAGGA TAGAAGAGGATAGTGGAATTCCATGTGTAGTGGTGAAATACGTAGATATATGGAGGAACACC AGTGGCGAAGGCGGCTATCTGGTCTATTATTGACGCTGAGGCTCGAAAGCATGGGTAGCAAA CAGG | 0.0041 | 0.4295 |
| AGCGTTAATCGGAATTACTGGGCGTAAAGGGTGCGCAGGCGGTGTATAAAGTCTGATGTGAA AGTCCACGGCTCAACCGTGGGATTGCATTGGAAACTGATACACTTGAGGATAGAAGAGGATA GTGGAATTCCATGTGTAGTGGTGAAATACGTAGATATATGGAGGAACACCAGTGGCGAAGGC GGCTATCTGGTCTATCACTGACGCTGAGGCTCGAAAGCATGGGGAGCAAACAGG | 0.0044 | 0.4617 |
| TACTTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGGGAGCGCAGGCGGTGTATAA AGTCTGATGTGAAAGTCCACGGCTCAACCGTGGGATTGCATTGGAAACTGATACACTTGAGGA TAGAAGAGGATAGTGGAATTCCATGTGTAGTGGTGAAATACGTAGATATATGGAGGAACACC AGTGGCGAAGGCGGCTTTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAA CAGG | 0.0022 | 0.2362 |
| TACTTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGGGAGCGCAGGCGGTGTATAA AGTCTGATGTGAAAGTCCACGGCTCAACCGTGGGATTGCATTGGAAACTGATACACTTGAGGA TAGAAGAGGATAGTGGAATTCCATGTGTAGTGGTGAAATACGTAGATATATGGAGGAACACC AGTGGCGAAGGCGGCTATCTGGTCTATCACTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAA CAGG | 0.0017 | 0.1825 |

| | | |
|---|---|---|
| TACTTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGGGAGCGCAGGCGGTGTATAA AGTCTGATGTGAAAGTCCACGGCTCAACCGTGGGATTGCATTGGAAACTGATACACTTGAGGA TAGAAGAGGATAGTGGAATTCCATGTGTAGTGGTGAAATACGTAGATATATGGAGGAACACC AGTGGCGAAGGCGGCTAACTGGCCTGTAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCAA ATAGG | 0.0014 | 0.1503 |
| TACTTAGGTGGCGAGCGTTGTCCGGATTTATTGGGCGTAAAGGGAGCGCAGGCGGTGTATAA AGTCTGATGTGAAAGTCCACGGCTCAACCGTGGGATTGCATTGGAAACTGATACACTTGAGGA TAGAAGAGGATAGTGGAATTCCATGTGTAGTGGTGAAATACGTAGATATATGGAGGAACACC AGTGGCGAAGGCGGCTATCTAGTCTATCACTGACGCTGAGGCTCGAAAGCATGGGGAGCAAA CAGG | 0.0021 | 0.2255 |
| TACTTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGGGAGCGCAGGCGGTGTATAA AGTCTGATGTGAAAGTCCACGGCTCAACCGTGAAATTGCATTGGAAACTGATACACTTGAGGA TAGAAGAAGATAGTGGAATTCCATGTGTAGTGGTGAAATACGTAGATATATGGAGGAACACC AGTGGCGAAGGCGGCTATCTGGTCTATCACTAACGCTGAGGCTCGAAAGCATGGGGAGCAAA CAGG | 0.0009 | 0.0966 |
| TACTTAGGTGGCGAGCGTTGTCCGGATTTATTGGGCGTAAAGGGAGCGCAGGCGGTGTATAA AGTCTGATGTGAAAGTCCACGGCTCAACCGTGGGATTGCATTGGAAACTGATACACTTGAGGA TAGAAGAGAATAGTGGAATTCCATGTGTAGTGGTGAAATACGTAGATATATGGAGGAACACC AGTGGCGAAGGCGGCTATCTGGTCTATCACTGACGCTGAGGCTCGAAAGCATGGGGAGCAAA CAGG | 0.0002 | 0.0215 |
| TACTTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGGGAGCGCAGGCGGTGTATAA AGTCTGATGTGAAAGTCCACGGCTCAACCGTGGGATTGCATTGGAAACTGATACACTTGAGGA TAGAAGAGGATAGTGGAATTCCATGTGTAGTGGTGAAATACGTAGATATTAAGAGGAACACC AGTGGCGAAGGCGACTTTCTGGACACTAACTGACGCTGAGGTACGAAAGCGTGGGGAGCAA ACAGG | 0.0013 | 0.1396 |
| TACTTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGGGAGCGCAGGCGGTGTATAA AGTCTGATGTGAAAGTCCACGGCTCAACCGTGGGATTGCATTGGAAACTGATACACTTGAGGA TAGAAGAGGATAGTGGAATTCCATGTGTAGCAGTGAAATGCGTAGAGATGTGGAGGAATACC GATGGCGAAGGCAGCCCCCTGGGATAACACTGACGCTCATGCACGAAAGCGTGGGGAGCAA ACAGG | 0.0001 | 0.0107 |

**Table D.6**. Sequences of the 25 Dolosicoccus ASVs and their mean relative abundance (%) across all urinal samples and their maximum relative abundance (%) observed in a urinal sample.

## Chapter 6. General Discussion

Microbial ecologists over the last decades have challenged the concept that the built environment is a microbial wasteland (Gibbons, 2016) and have provided evidence for the existence of endogenous microbial growth and proliferation (Kanamori et al., 2016; Kotay et al., 2017; Novak Babič et al., 2020). Water-associated environments within the built environment emerge as significant areas where microbial establishment can thrive (Adams et al., 2017; Bruno et al., 2022; Jing et al., 2023). Specifically, sinks and their P-traps in hospital settings have been identified as important reservoirs of pathogenic and antimicrobial resistant bacteria responsible for reoccurring clinical outbreaks (Bourdin et al., 2023; Jones et al., 2020; Lemarié et al., 2021; Regev-Yochay et al., 2018). Yet, despite their potential implications for public health, the microbial community structure and dynamics of communal sink P-traps and other waste traps in public restrooms remains understudied (Hamada & Abe, 2010; Mostafa & Sabra, 2013; Short, 2011), particularly when compared to the extensive research conducted on clinical sinks. In communal restrooms, such as those found on a university campus, there could be health implications for the diverse range of users that frequent the facilities. As communal restrooms are shared by a multitude of individuals, including students, staff, and visitors, there is a pressing need to investigate the microbial ecology of these spaces comprehensively, particularly areas that can harbor microbial communities, such as the P-trap.

This thesis provides comprehensive insights into the microorganisms populating P-traps in communal restrooms, with a primary focus on sink traps, given their critical role in clinical settings. Through in-situ sampling of sink P-traps, bacterial communities (Chapter 2) and mycobial communities (Chapter 3) were characterized, leading to the identification of a core microbiome. In both studies the influence of building and gender on sink communities was found to be marginal or non-existent, suggesting a notable resilience of the core taxa present. The prevalence of microbial taxa associated with humans demonstrated the external influence of human activities as a prominent source of microorganisms to sink environments. In Chapter 4, a long-term dataset, provided a unique opportunity to elucidate trends in bacterial community development in a newly built university building. Over a two-year period, bacterial communities exhibited increasing structural similarity and homogeneity across individual sinks. The bacterial taxa identified in the preceding study (Chapter 2) remained prevalent and abundant in this temporal dataset. Following the establishment of a sink community, an intervention with sodium hypochlorite was implemented. Application of 10% sodium hypochlorite to sinks resulted in “resetting” the sink bacterial community and subsequent increase in relative abundance of *Acinetobacter*. However, this effect was transient, with the bacterial community re-

establishing to a composition similar to the pre-treatment state. In Chapter 5, urinal P-traps were characterized and analysis of this revealed greater variability across urinals when compared to sink bacterial taxonomic profile and identified core bacterial taxa, including the genus *Dolosicoccus*. The additional research included at the end of thesis  demonstrated how molecular methodologies and currently available sequencing technologies could be used to isolate and classify new species. This approach could potentially be extended to identify novel bacterial species from sink and urinal traps, and potentially recover the genome of the urinal *Dolosicoccus*.

In this final chapter I discuss the key results of these studies and explore the implications of my findings. I further discuss the limitations associated with these studies and highlight future directions for further research.

## 6.1 Main Findings and Implications

### *6.1.1 P-traps within university restrooms are reservoirs of successful microorganisms (that can demonstrate resilience)*

Restrooms represent environments characterized by a concentrated microbial presence, with a notable portion possessing pathogenic potential (Gibbons et al., 2015; Lee & Tham, 2021). Within this environment, waste traps, such as the sink P-trap, facilitate the establishment of microorganisms, particularly through the development of biofilms (Franco et al., 2020; Winder & Bonheyo, 2015). Upon use of sinks or taps, these biofilms may undergo disturbance, leading to the dispersion of microorganisms onto surrounding surfaces or potentially exposing the user, thereby presenting an Imminent risk for further transmission (Garvey et al., 2023; Hajar et al., 2019; Kotay et al., 2019). Despite the critical role that sinks play as reservoirs in clinical settings, the microbial community associated with these systems has seldom been the focus of comprehensive investigations. Existing studies have typically concentrated on specific bacterial taxa or groups, leaving a gap in our understanding of microbial composition, especially that of shared public sinks.

Our extensive efforts to characterize the communities in university sink P-traps have unveiled distinct microbial taxa that exhibit spatial and temporal prevalence. Results from Chapter 2 collaborate with those of Chapter 4, with both studies identifying *Moraxellaceae*, *Sphingomonadaceae*, *Rhodocyclaceae* and *Enterobacteriaceae* as core families within the sink P-traps. Regarding fungal communities, striking similarity in composition across diverse buildings was observed, with the mycobial genera *Saccharomyces*, *Fusarium*, and *Exophiala* displaying high prevalence and abundance. The widespread distribution of these microbial taxa highlights the P-trap as an environment that

selectively favors certain taxa, as rooms and buildings that are unconnected consistently had these microbial taxa present. In the literature there are references to these taxa persisting in water-associated BE environments (Eichler et al., 2006; Numberger et al., 2019; Pirzadian et al., 2020; Vaz-Moreira et al., 2013), reinforcing the expectation of their presence and sustainability in sink P-traps. Furthermore, Chapter 4 demonstrates the formation of stable bacterial communities over time, across individual sinks sampled during the study. While an intervention with bleach induced perturbations in the community structure, the bacterial community, after four weeks resembled that of untreated sinks. Previous studies have demonstrated the ineffectiveness of bleach in controlling outbreaks or demonstrated the partial efficacy on sink biofilms (Clarivet et al., 2016; Hota et al., 2009; Ledwoch et al., 2020). However, this chapter provides insights into the comprehensive influence of bleach on bacterial communities, a feature overlooked in the forementioned studies. Interestingly, post-bleach intervention, *Acinetobacter* exhibited a notable increase in relative abundance, briefly becoming one of the most dominant genera. However, by week four post-treatment, *Acinetobacter* had reverted to significantly lower relative abundances. While bleach treatment eradicated bacterial communities immediately after application, in healthcare settings there should be more consideration and management of disinfection protocols. The potential eradication of a stable sink community following disinfection, under improper management, could lead to the establishment of a reservoir for potentially pathogenic bacteria, unhindered by competition for nutrients with “normal” sink bacteria. In Chapter 5, focus shifted to urinal P-traps. Similar to sinks, five bacterial genera were identified as part of the core microbiome, with a prevalence exceeding 70%. However, in contrast to sinks, the bacterial community structure and composition across individual urinals displayed more variability. The only other study on urinal bacterial communities revealed distinctions in structure, composition, and diversity among different types of urinals but did not report structural variations within specific types (Lim et al., 2022).

Understanding the reservoir potential of P-traps is crucial for assessing potential risks to human health and designing effective strategies for microbial control in built environments. Characterizing the microbial communities that constitute a “healthy” P-trap is foundational for implementing monitoring in environments such as hospitals and enabling the identification of factors influencing community changes. Furthermore, investigating temporal changes in sink P-trap microbial communities and their responses to stressors or interventions, such as sodium hypochlorite, provides insights for designing intervention and management strategies to maintain a healthy microbial balance in the BE. This approach allows for the exploration of targeted cleaning or removal of specific microbial taxa. Additionally, there is potential for the development of probiotic cleaners based on the microbial communities identified in public sinks. Designing probiotic cleaners that incorporate non-harmful

microorganisms naturally dominant in sink P-traps could enable them to outcompete pathogens in sink drains, particularly in hospital settings, thereby reducing the risk of outbreaks. 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. This is crucial, as repeated bleach interventions may lead to the selection of persisters and the stimulation of the transformation of plasmid-encoded antibiotic resistance genes (Dai et al., 2020; Jin et al., 2020; Zhang et al., 2021). However, further research is essential to comprehensively understand these occurrences and outcomes in sink environments, particularly in the context of potential public health implications.

### *6.1.2 Human-Built Environment Interactions*

The influence of human occupants on the microbiology of the BE, with a particular emphasis on bacterial communities, is well established (Hospodsky et al., 2012; Leung & Lee, 2016; Meadow et al., 2014a). Human occupants, both directly and indirectly, play a pivotal role in shaping the microbiome of the BE. Occupants serve as sources of microorganisms, which are introduced into the surrounding environment through activities such as shedding (Hospodsky et al., 2015), the release of bioaerosols during respiration (Qian et al., 2012), and direct contact with various surfaces (Flores et al., 2011; Lax et al., 2017; Meadow et al., 2014b). Moreover, routine activities such as bed making (Ferro et al., 2004) or walking can resuspend previously deposited microbial materials (Heo et al., 2017), while lifestyle choices, including pet ownership (Fujimura et al., 2010), contribute to the introduction of exogenous microorganisms from outdoor environments, collectively influencing the composition of the BE microbiome (Adams et al., 2013a; Meadow et al., 2014a). Previous investigations focusing on restroom environments have yielded predictable findings, indicating contamination with bacteria originating from fecal or skin (Barker & Bloomfield, 2000; Flores et al., 2011; Gibbons et al., 2015). The hygienic practice of handwashing is anticipated to remove bacteria present on the skin; consequently, sink P-traps are expected to be contaminated with microorganisms associated with the skin biome.

Chapters 2 to 5 presents evidence regarding the impact of human activities on the microbial communities inhabiting P-traps, with recurrent identification of human-associated microorganisms within these environments. In Chapter 2, the implementation of SourceTracker (Knights et al., 2013) served to elucidate the potential sources of bacteria in university restroom sinks. Human skin emerged as the predominant source in below-strainer samples, while also contributing significantly to the microbial composition within P-traps. Chapter 3, focusing on fungal communities, revealed the frequent presence of *Malassezia*, a common skin commensal (Adams et al., 2013b; Findley et al.,

2013), in sinks (observed in 91% of sink samples). The families identified in Chapter 4 displayed potential human association, further underlining the influence of human occupants on the microbial landscape. Moreover, Chapter 5, examining bacterial taxa in urinal P-traps, identified genera like *Oligella* and *Atopstipes* that could be linked to urine (Perez-Carrasco et al., 2021). Despite the detection of human-associated microorganisms in P-traps, a notable presence of microorganisms commonly found in water distribution systems was observed. This phenomenon can be attributed to human occupants regularly using taps, thereby contributing water and associated microorganisms to the system. These results add to the large body of literature that underscores the intricate relationship between human occupants and microbial communities within the BE.

Occupant actions can have potential implications on the microbial communities within the P-trap, thereby influencing the overall restroom environment and potentially impacting users. In hospital settings, improper sink usage, such as the inadequate disposal of patient secretions and the cleaning of reusable patient care items in hand hygiene sinks, has been identified as a significant contributor to outbreaks in hospitals (Balm et al., 2013). While sinks outside of hospital settings, may not experience the same pressures or inputs as those in hospitals, the microbial communities and practices of users will differ (Grabowski et al., 2018; Grice et al., 2009; Wu et al., 2019). Throughout Chapters 2-5, variations were observed across buildings, reflecting differences in individual waste P-trap bacterial community structures and compositions. These differences may be attributed to differing user behaviors or variations in the frequency of sink usage. Unfortunately, data regarding individual usage patterns of sink was not obtained. Apart from the presence of human pathogens in restrooms, there is a possibility that the environment could serve as a reservoir for antibiotic resistant bacteria. Mkrtchyan and colleagues have demonstrated that non-healthcare restrooms are a source of antibiotic resistant bacteria highlighting potential for resistome to exist (Mkrtchyan et al., 2013). From the diverse bacteria identified in the university sinks, follow up studies on antimicrobial resistance genes in this environment is required.

Overall, acknowledging that achieving sterility in sinks is neither reasonable nor feasible, the emphasis should be placed on implementing best practices and behaviors to prevent the transmission of potentially dangerous pathogens from sinks. The pivotal role of human occupants and their behaviors in shaping the composition and dynamics of sink trap microbial communities is evident across these chapters. This further emphasizes the need to consider human-environment interactions in microbial ecology studies. Recognizing and understanding the impact of human activities on microbial communities is essential for developing targeted strategies to mitigate the risks associated with microbial proliferation and transmission in shared spaces.

### *6.1.3 Application of combined sequencing techniques enhances our understanding of microbial environments and enables discovery of potentially new microorganisms.*

The combination of sequencing technologies, including short- (i.e., amplicon sequencing and shotgun sequencing) and long- (i.e., Oxford Nanopore) reads enable a more in-depth analysis of microbial community constituents. From diverse microbiomes, reference-quality genomes have been reconstructed due to hybrid assembly of both short- and long- reads (Bertrand et al., 2019; Jin et al., 2022; Singleton et al., 2021).

As part of additional research undertaken during this thesis, , a novel species, *Chitinophaga spargani*, was isolated from an environmental sample (rhizosphere of *Sparagnium erectum*) and the whole genome sequenced and assembled using a combination of short- and long- reads. This work demonstrates that culture-based methods remain valuable for isolating novel species. In Chapter 5, the identification of *Dolosicoccus* as a highly abundant and prevalent genus raised questions about potential variations within the genus. By comparing the top *Dolosicoccus* amplicon sequence variant (ASV) to *Dolosicoccus Paucivorans* 16S V4 region, differences were observed, suggesting the possibility of an alternative species. Similar to the approach used in the Additional Research Chapter of this thesis, culture-based techniques, such as selective Lactobacillales media or using dilution to extinction methods (Bonnet et al., 2020; Stingl et al., 2008; Zhang & Eiler, 2012), could be explored to isolate the *Dolosicoccus* observed in urinals. Alternatively, the use of long-read sequencing, either independently or in conjunction with deep metagenomic sequencing, could facilitate the reconstruction of metagenomic-assembled genomes (MAGs). With the continuous advancement of technologies, Oxford Nanopore Technologies sequencing has demonstrated the capability to recover reference-quality genomes from complex metagenomes using only long reads (Liu et al., 2022).

For future studies, it is advisable to encourage the integration of diverse sequencing techniques whenever feasible. This approach offers a more comprehensive analysis of microbial communities, enabling a deeper understanding of their constituents and their potential functional capabilities.

## 6.2 Limitations

Although Chapters 2-5 each possess specific aims and focus on a different aspect of the public restroom P-trap microbial communities, they all suffer from some of the same limitations. Foremost among these is the limitation in metadata availability. Reporting on indoor physio chemical conditions, human occupancy and cleaning procedures may help to explain some of the variability among

sampling locations (Ramos & Stephens, 2014). However, by selecting the university as a study site, some of variability will be mitigated. For example, cleaning practices and procedures across the university were consistent and the building conditions such as temperature are systematically monitored and sustained within defined thresholds suitable for occupants. In Chapter 4, a year of building occupancy data was acquired, but for other buildings across the university campus (Chapters 1 and 2), the absence of card access entry requirements precluded the provision of occupancy figures. While alternative options, such as employing unidirectional beams for recording the number of individuals entering restrooms (SenSource, http://www.sensourceinc.com/peoplecounters.htm), were considered, they did not offer insights into which sinks were used, nor did they allow for the documentation of specific user behaviors toward the sink. Insufficiently described built environment data can limit our ability to understand microbial communities within and assess strategies to control as well as hinders capacity for comparison of different indoor microbial communities. However, obtaining detailed metadata associated with P-traps in-situ is challenging and often restricted due to the nature of restrooms. Nevertheless, the results obtained from this approach are reflective of real-world P-traps. Furthermore, the use of large sample sizes across the studies enables more general conclusions to be drawn.

Secondly, priority was given to characterizing the bacterial communities of P-traps. Eukaryotic microorganisms such as fungi and protozoa will also play a role in shaping microbial communities that develop in P-traps. In studies focusing on water distribution systems, the importance of these communities has been demonstrated (Inkinen et al., 2019; Paranjape et al., 2020; Wang et al., 2014). In Chapter 3, fungal communities were characterized in sink P-traps providing some insight into their structure. Attempts were made to understand fungal development in HLS (Chapter 4), however amplification of the ITS region was unsuccessful for samples collected earlier in the phase 1 sampling (data not shown), preventing subsequent sequencing. While this thesis did not specifically focus on interactions between different microorganisms in biofilms, it presents a potential avenue for future research that could offer valuable insights into the complexities of microbial communities in P-traps.

While amplicon sequencing facilitates high throughput of samples, it has inherent limitations, particularly in its ability to identify sequences only to the genus level. Consequently, it proves less useful in distinguishing between closely related prokaryotes and has difficulties in confirming the presence of pathogenic species. Additionally, it cannot differentiate between dead, inactive or active cells. However, the identification of highly prevalent and abundant core taxa in the results, observed both temporally and spatially, suggests their integral role as components of the sink P-trap biofilm.

Lastly, while beyond the scope of this thesis, providing source data from the immediate surrounding environment and occupants would have strengthened the analysis. In Chapter 1, “source” sequences supplied to SourceTracker were taken from publicly available datasets resulting in many of the sequences being from unknown sources. If extensive sampling of surrounding sources (i.e., skin of building occupants, tap water, and soil) was implemented, the sources of microorganisms to sink P-traps will be more clearly defined.

## 6.3 Conclusions and Future Research Priorities

This thesis aimed to investigate the microbial communities within P-traps located in public restrooms, observing their development, responses to perturbations, and potential impact on occupants. This aim has been achieved through extensive sampling across a university campus and over a two-and-a-half year sampling regime. The core microbiome of P-traps was revealed and influences between human occupants and sink microbiome demonstrated. While the thesis has provided answers to its primary aims, further investigation is warranted. This thesis focused on P-traps located on a university campus yet, additional research of diverse public buildings is required to ascertain the presence of similar microbial communities. Exploring locations such as airports or large train stations, with exposure to an even broader range of people, could yield valuable insights. Integrating other omics approaches would provide additional information on community functionality and potentially be used to mine novel species, as well as identifying persisters within the communities and antimicrobial resistance genes.

As molecular costs decrease, microbial databases expand, and bioinformatics tools advance to handle complex datasets, a more comprehensive analysis of microbial communities is on the horizon. Understanding the structure, dynamics, and resilience of microbial communities within communal sink P-traps has the potential to offer crucial insights into the microbial ecology of built environments and the intricate interactions between humans and their surroundings. Continued research in this domain is essential for advancing our understanding of the complex interplay between microbial communities and the built environment, ultimately contributing to the development of informed strategies for maintaining a healthy and resilient indoor environment.

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# *Chitinophaga spargani* sp. nov., isolated from rhizosphere of *Sparganium erectum*

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## Abstract

The bacterial strain LS1$^T$ was isolated on 21 July 2022 from the rhizosphere of a shallow water plant *Sparganium erectum* taken from the River Loddon, Reading, United Kingdom (51°24'33.2" N 0°55'27.2" W). Strain LS1$^T$ was found to be Gram-negative and facultative anaerobic, with a genome length of 8,665,338 bp and a G+C content of 43.4%. 16S rRNA phylogenetic analysis identified strain LS1$^T$ as belonging to the genus *Chitinophaga*, having the highest sequence similarity to *Chitinophaga sancti* BA-3$^T$ (98.4%) and *Chitinophaga silvisoli* K20C18050901$^T$ (98.3%) and *Chitinophaga tropicalis* ysch24$^T$ (96.3%). A complete and circularised genome was sequenced and assembled using both long-read (Oxford Nanopore Technologies) and short-read (Illumina) platforms. Average Nucleotide Identity scores between strain LS1$^T$ and previously published complete *Chitinophaga* genomes ranged between 72.75% and 69.16%. Digital DNA-DNA hybridisation analysis produced scores between strain LS1$^T$ and the genomes of the most closely related *Chitinophaga* species in the range of 28.9% to 18.7%. The phylogenetic, genomic and phenotypic analyses show that strain LS1$^T$ represents a novel species of the genus *Chitinophaga*, for which the name *Chitinophaga spargani* sp. nov. is proposed.

**Keywords**

*Chitinophaga spargani* sp. nov., Chitinophagaceae, Sparganium erectum, aquatic rhizosphere

**Author Notes**

The GenBank accession numbers for the complete genome sequence and 16S rRNA gene of strain LS1$^T$ are CP128362 and OR083331, respectively.

## Brief Introduction

The genus *Chitinophaga* is widely considered to be a difficult to culture group of chitinolytic myxobacteria (Sangkhobol & Skerman, 1981). The species within the *Chitinophaga* genus are typically isolated from the soil or the rhizosphere of several plants (Chung et al., 2012; He et al., 2022; Lin et al., 2014), but has also been found within arsenic-contaminated soil (Zong et al., 2019) and vermicompost (Yasir et al., 2011). Members of this genus are typically chemoorganotrophic, Gram-negative, flexible rods with rounded ends and have the ability to germinate spherical myxospheres when on an agar surface (Sangkhobol & Skerman, 1981). Like other myxobacteria (Zhou et al., 2020), members of the *Chitinophaga* genus have the ability to digest biological macromolecules such as chitin (Sangkhobol & Skerman, 1981). While species belonging to this genus have been isolated from the rhizosphere of, to date they have not been isolated from *Sparganium erectum* or aquatic plant rhizosphere.

## Isolation and Ecology

A strain, designated LS1$^T$, was isolated from the rhizosphere of a *Sparganium erectum* plant taken from the River Loddon, Reading, United Kingdom (51°24'33.2" N 0°55'27.2" W). First, 10 ml of river water was filtered with a 0.22 µM filter and collected into a sterile 50 ml falcon tube. A single *Sparganium erectum* reed was then extracted from the riverbank and an approximately 3 cm root, together with the soil layer immediately surrounding the root hair and placed into the filtered water for transport. On the same day within a laboratory setting the sample was vortexed and passed through a 40 µM filter into a sterile 1.5 ml microtube. 1 ml of the re-filtered sample was diluted 10 times in phosphate-buffered saline, from which 20 µl was spread on 10 times diluted tryptic soy agar media (DTSA) plates. These plates were then placed and sealed into a plastic bag along with an Anaero-Gel Compact sachet (Oxoid, Thermo Scientific) and a Resazurin Anaerobic Indicator (Oxoid, Thermo Scientific). The sealed bag was then placed into an incubator at approximately 20 °C for 4 weeks. Individual colonies from these plates were then sub-cultured onto fresh DTSA media, then incubated under the same conditions. Strain LS1$^T$ was then preserved in a cryovial (TS/80-MX, Technical Service Consultants Ltd) at -80 °C for long-term storage and use.

## 16S rRNA phylogeny

Genomic DNA from strain LS1$^T$ was extracted using the GeneJET Genomic DNA Purification Kit (Thermo Scientific) following the manufacturer's Gram-negative extraction protocol. All genomic DNA was purified through AMPure XP bead cleaning (Beckmann-Coulter) following the manufacturer's

protocol. The 16S rRNA gene from strain LS1$^T$ underwent PCR amplification using the universal primers 27F (5' - AGAGTTTGATCCTGGCTCAG - 3') and 1492R (5' - GGTACCTTGTTACGACTT - 3') (Dos Santos et al., 2019). The primer reaction mixture was comprised of 1X JumpStart REDTaq ReadyMix Reaction Mix (New England Biolabs), 0.2 µM 27F primer, 0.2 µM 1492R primer, 2.0 µl genomic DNA with sterile $H_2O$ to make the final volume of 50 µl. The PCR cycling conditions were an initial denaturation of 95 °C for 5 minutes, followed by 30 cycles of denaturation at 95 °C for 90 seconds, annealing at 55 °C for 90 seconds and elongation at 72°C for 5 minutes, then a final elongation at 72 °C for 10 minutes. The amplicons were then purified through AMPure XP bead cleaning (Beckmann-Coulter) following the manufacturer's protocol. Subsequently, the purified amplicons underwent Sanger sequencing for initial genus determination and the resulting sequences were analysed using BLASTn (Altschul et al., 1990), which identified strain LS1$^T$ as a member of the *Chitinophaga* genus. 16S rRNA sequences of all 50 type strains from the *Chitinophaga* genus were retrieved from EZBioCloud (Yoon et al., 2017a) and aligned using Clustal Omega (Goujon et al., 2010). Strain LS1$^T$ exhibited the greatest similarities with the *Chitinophaga* genus, where it shared the highest 16S rRNA similarity with *Chitinophaga sancti* BA-3$^T$ (98.4%) and *Chitinophaga silvisoli* K20C18050901$^T$ (98.3%) and *Chitinophaga tropicalis* ysch24$^T$ (96.3%). The calculated 16S rRNA sequence similarities of all closely related *Chitinophaga* species to strain LS1$^T$ were lower than 98.65%, the suggested cut-off value for delineating novel species (Kim et al., 2014). PhyML (v.3.3) was used to reconstruct a maximum-likelihood tree using the GTR model with 1000 bootstrap replicates (Guindon et al., 2010) where *Flavisolibacter tropicus* LCS9$^T$ was chosen as an outgroup (Figure 1). Phylogenetic analysis based on 16S rRNA gene sequences showed that LS1$^T$ was included in the clusters of species of the genus *Chitinophaga*, forming a stable monophyletic clade with *C. sancti.*

## Genomic features

The purified genomic DNA was sequenced on the MinION by Oxford Nanopore Technologies and Illumina platforms (sequenced by Novogene, China). A hybrid *de novo* assembled genome was produced using Raven (v.1.8.1) (Vaser & Šikić, 2021) resulting in a closed (circularised) genome. The assembled genome was polished with Pilon (Walker et al., 2014) and Polypolish (Wick & Holt, 2022), and annotated using Prokka (Seemann, 2014). Strain LS1$^T$ was found to have a genome composed of 8,665,338 bp, five copies of 16S, 23S and 5S rRNAs, 78 tRNAs, 7286 CDS, with a DNA G+C content of 43.4 mol%. The genome size of strain LS1$^T$ is large in comparison to other bacterial species (Land et al., 2015), but relatively close to the average genome size of a *Chitinophaga* species which is at 7.51 Mbp (Brinkmann et al., 2022). The large genome of strain LS1$^T$ is reflective of the fact that *Chitinophaga* species are myxobacteria which have genomes in the range of 9 Mbp to 14.8 Mbp

(Muñoz-Dorado et al., 2016). The DNA G+C content of strain LS1$^T$ falls within the range commonly seen within the *Chitinophaga* genus (42.8% - 55.4%) (He et al., 2022). This is within the average G+C content in prokaryotes (Lightfield et al., 2011) suggesting that strain LS1$^T$ has a relatively stable genome and has a broad temperature tolerance range for growth (Šmarda et al., 2014).

Digital DNA-DNA hybridization (dDDH) scores and ANI values were calculated between LS1$^T$ and 10 most closely related *Chitinophaga* type strains, namely *Chitinophaga sancti* BA-3$^T$ and *Chitinophaga silvisoli* K20C18050901$^T$, *Chitinophaga tropicalis* ysch24$^T$, *Chitinophaga oryziterrae* YC7001$^T$, *Chitinophaga tropicalis* ysch24$^T$, *Chitinophaga ginsengisoli* M1-22$^T$, *Chitinophaga filiformis* Fx e1$^T$, *Chitinophaga pinensis* DSM 2588$^T$, *Chitinophaga rhizophila* B61$^T$ and *Chitinophaga agri* H33E-04$^T$; also included are five type strains from neighbouring clades (Table 1). dDDH was performed by using Genome-to-Genome Distance Calculator (GGDC, version 3.0, http://ggdc.dsmz.de/)(21), while ANI scores were calculated using OrthoANI (Yoon et al., 2017b). All calculated dDDH scores between strain LS1$^T$ and *Chitinophaga* type strains were between 28.9% and 18.7%, which were well below the 70% delineation for species boundaries (Meier-Kolthoff et al., 2013). ANI scores ranged between 72.75% and 69.16% which are lower than the 95-96% delineation for a novel prokaryotic species (Chun et al., 2018; Richter & Rosselló-Móra, 2009).

## Physiology, optimum growth temperature and carbon utilisation

For transmission electron microscopy observations (Fig 6.2), cells were incubated at 22 °C on tryptic soy agar. Strain LS1$^T$'s growth characteristics were tested on tryptic soy broth at varying temperatures (10 °C, 15 °C, 20 °C and 30 °C) over three days. The growth curves were obtained for each selected temperature by taking 600 nm optical density measurements at 10 minute intervals. After two days, all temperatures had reached their maximum growth and stationary phase. Strain LS1$^T$ was found to be able to grow at all temperatures tested, however maximum growth was found to occur at 20 °C, closely followed by 10 °C. The phenotypic characteristics of strain LS1$^T$ were investigated using BIOLOG GEN III MicroPlates (Biolog Inc) (Table 2). Experiments were performed simultaneously in duplicate to analyse the strain in 94 phenotypic tests including 71 carbon source utilisation assays and 23 chemical sensitivity assays. Plates were then prepared according to the manufacturer's protocol.

Based on the results from the phylogenetic, genomic and phenotypic consensus, strain LS1$^T$ represents a novel species of the genus *Chitinophaga*, for which the name *Chitinophaga spargani* is proposed.

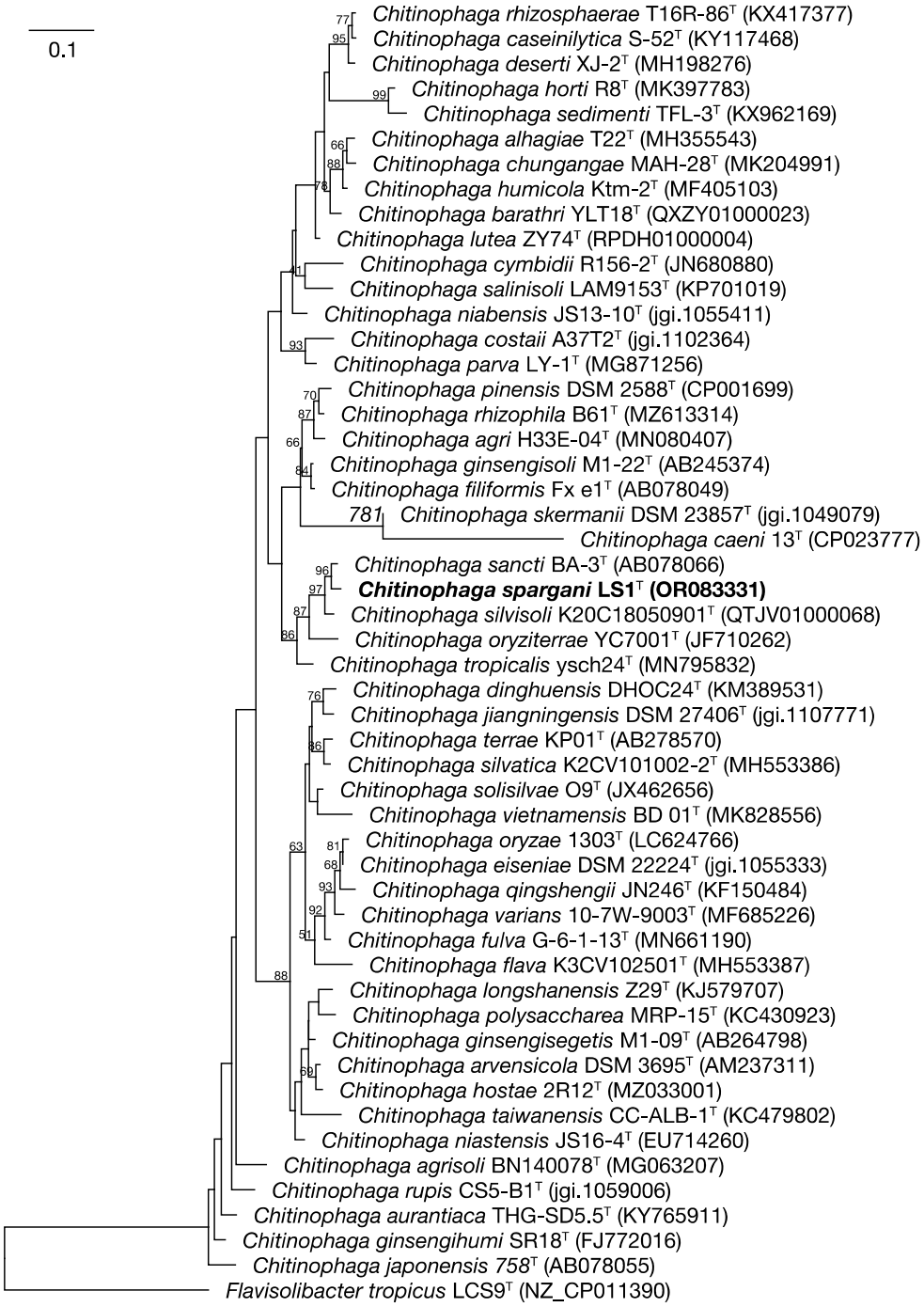


**Figure 1. Phylogenetic tree based on 16S rRNA sequences**: 16S rRNA sequence phylogenetic tree showing the relationship between strain LS1$^T$ and 50 other type strains within the genus *Chitinophaga*. The tree was constructed using PhyML (a maximum-likelihood with GTR model, 1000 bootstrap replicates). Boostrap values are expressed as percentages of 1000 replications. Only boostrap values of more than 50% are shown. Scale shows 0.1 substitutions per nucleotide position. *Flavisolibacter tropicus* LCS9$^T$ was used as an outgroup.

| Species | DB accession number | dDDH (%) | ANI (%) |
|---|---|---|---|
| *Chitinophaga sancti* BA-3$^T$ | GCA_900119105.1 | 28.5 | 83.9 |
| Chitinophaga silvisoli K20C18050901$^T$ | GCA_003412465.1 | 28.9 | 84.5 |
| *Chitinophaga rupis* CS5-B1$^T$ | jgi.1059006.1 | 19.8 | 70.1 |
| *Chitinophaga oryziterrae* YC7001$^T$ | GCA_009758125.1 | 19.2 | 72.7 |
| *Chitinophaga tropicalis* ysch24$^T$ | GCA_009758205.1 | 19.2 | 72.5 |
| *Chitinophaga ginsengisoli* M1-22$^T$ | GCA_003014595.1 | 19.1 | 72.5 |
| *Chitinophaga filiformis* Fx e1$^T$ | jgi.1055216.1 | 19.0 | 72.3 |
| *Chitinophaga pinensis* DSM 2588$^T$ | GCA_000024005.1 | 19.0 | 72.4 |
| *Chitinophaga rhizophila* B61$^T$ | GCA_019492185.1 | 18.6 | 71.9 |
| *Chitinophaga agri* H33E-04$^T$ | GCA_010093065.1 | 19.3 | 72.3 |
| *Chitinophaga parva* LY-1$^T$ | GCA_003071345.1 | 19.7 | 68.9 |
| *Chitinophaga niabensis* JS13-10$^T$ | GCA_900129465.1 | 19.1 | 69.4 |
| *Chitinophaga japonensis* 758$^T$ | GCA_007830125.1 | 19.0 | 69.9 |
| *Chitinophaga barathri* YLT18$^T$ | GCA_003614855.1 | 18.8 | 69.9 |
| *Chitinophaga ginsengisegetis* M1-09$^T$ | jgi.1048998.1 | 18.7 | 69.8 |

**Table 1.** digital DNA-DNA hybridisation (dDDH) scores using Type Strain Genome Server (GGDC) between strain LS1$^T$ and 10 most closely related *Chitinophaga* type strains including *Chitinophaga sancti* BA-3$^T$ and *Chitinophaga silvisoli* K20C18050901$^T$, *Chitinophaga tropicalis* ysch24$^T$, *Chitinophaga oryziterrae* YC7001$^T$, *Chitinophaga tropicalis* ysch24$^T$, *Chitinophaga ginsengisoli* M1-22$^T$, *Chitinophaga filiformis* Fx e1$^T$, *Chitinophaga pinensis* DSM 2588$^T$, *Chitinophaga rhizophila* B61$^T$, *Chitinophaga agri* H33E-04$^T$. Also included are five type strains of *Chitinophaga* from the neighbouring clades. All dDDH and ANI values are lower than the 70% and 90% delineation for species boundaries, respectively. dDDH and ANI values were calculated using Genome-to-Genome Distance Calculator and OrthoANI, respectively.

## Protologue

*Chitinophaga spargani* [spar.ga.ni., L. gen. n. *spargani* of the plant genus *Sparganium*].

Bacterial cells of strain LS1$^T$ are Gram-negative, facultatively anaerobic and long, filamentous, rod-shaped, 0.4 – 0.6 µm wide and > 7.0 µm long. When grown on TSA, colonies appear small and orange in colour with a circular form, a raised elevation and an entire margin. Cell growth can be seen at temperatures 10 °C - 30 °C (optimum, 20 °C). Strain LS1$^T$ (DSM 116439, NCIMB 15476), was isolated from a *Sparganium erectum* plant taken from the River Loddon, Reading, United Kingdom (51°24'33.2" N 0°55'27.2" W). Strain LS1$^T$ has a genomic size of 8.6 Mb with a G+C content of 43.4 mol%. The GenBank accession number of the complete genome assembly and 16S rRNA of strain LS1$^T$ is CP128362 and OR083331, respectively.

*Conflict of interest statement*

The authors declare that there are no conflicts of interest.

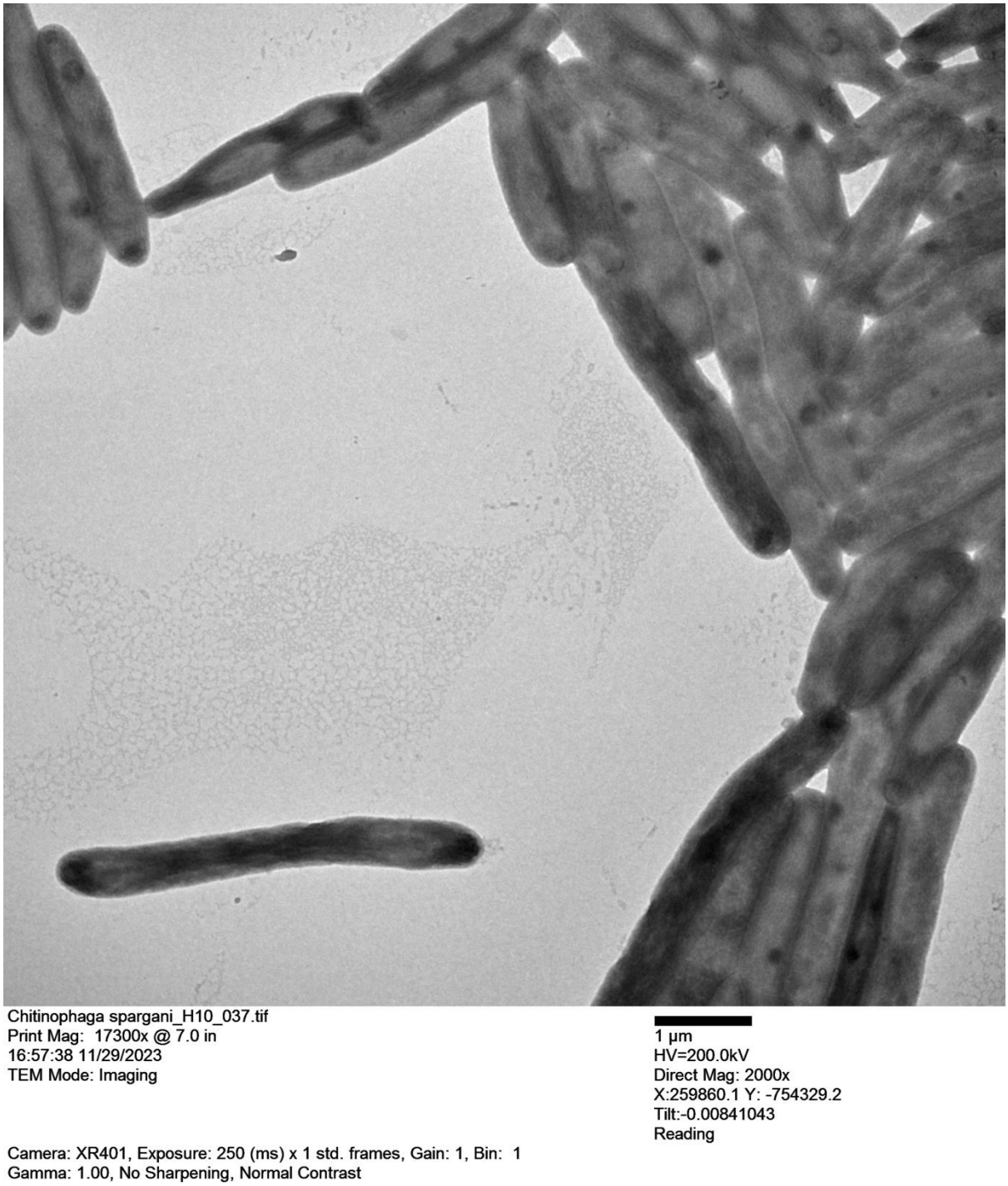


**Figure 2**. A transmission electron microscope image of strain LS1$^{T}$ at 17,300x magnification. Metadata associated with the imaging is shown below the image.

| **Characteristic** | 1: LS1$^T$ | 2: *C. sancti* BA-3$^T$ | 3: *C. silvisoli* K20C18050901$^T$ |
|---|---|---|---|
| Max. NaCl for growth (% w/v) | 4% | 1.50% | 2% |
| Growth on R2A | Postive | Negative | Positive |
| **Hydrolysis of:** | | | |
| Tween 40 | Positive | Positive | Negative |
| Gelatin | Positive | Negative | Positive |
| **Assimilation of:** | | | |
| N-Acetyl-D-Glucosamine | Postive | Negative | Negative |
| N-Acetyl-D-Galactosamine | Postive | Negative | Positive |
| 3-Methyl-D-Glucoside | Postive | Negative | Negative |
| D-Trehalose | Postive | Negative | Positive |
| D-Raffinose | Postive | Negative | Positive |
| Glucuronamide | Postive | Negative | Positive |
| Glycyl-L-Proline | Postive | Negative | Positive |
| D-Serine | Negative | Negative | Positive |
| **Genome features:** | | | |
| DNA G+C content (mol%) | 43.4 | 43.3 | 44.7 |
| Genome size (Mb) | 8.67 | 8.24 | 8.36 |

**Table 2**. Differential characteristics between strain LS1$^T$ and two closely related species of the genus *Chitinophage* strains: 1. LS1$^T$; 2. *C. sancti* BA-3$^T$; 3. *C. silvisoli* K20C18050901$^T$. Data for *C. sancti* and *C. silvisoli* were obtained from another study (Wang et al., 2019).

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