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RESEARCH ARTICLE

Comparative analysis of impact of human occupancy on indoor microbiomes

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HIGHLIGHTS

- Exposure to indoor microbiomes is a public health concern in educational facilities.
- Indoor microbiomes were characterized in two multifunctional university buildings.
- Human occupancy had significant impact on the composition of indoor microbiomes.
- The skin microbiota of occupants represented important sources of indoor microbiomes.

GRAPHIC ABSTRACT

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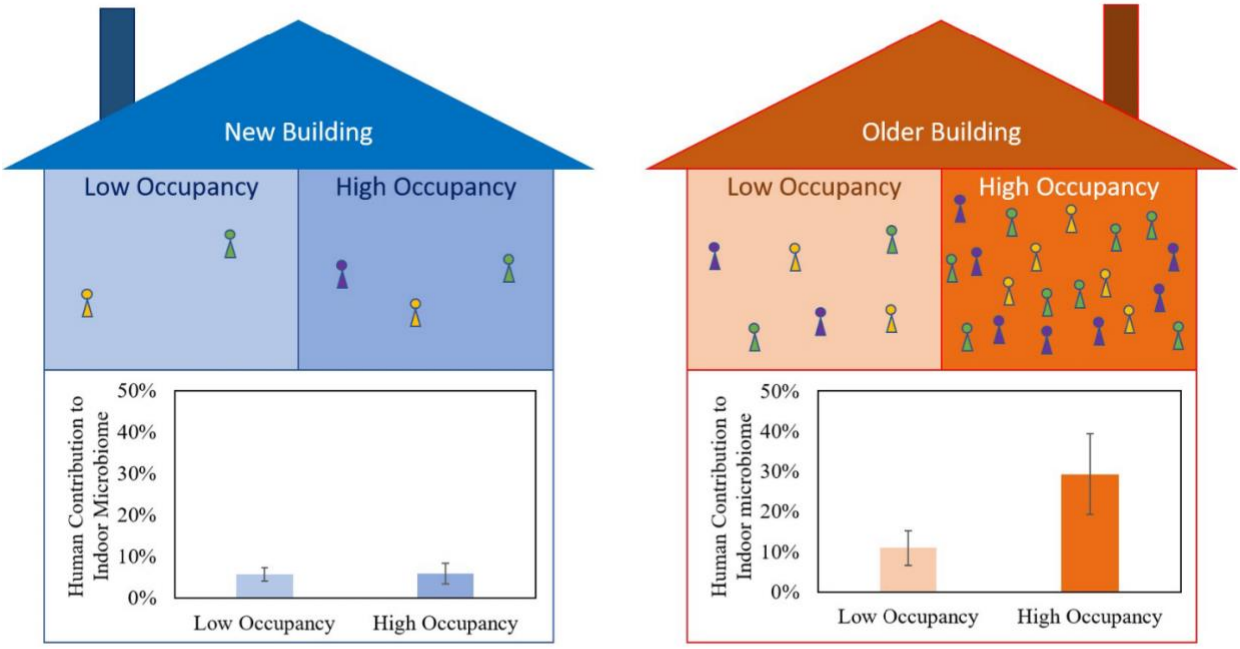

ABSTRACT

Educational facilities serve as community hubs and consequently hotspots for exposure to pathogenic microorganisms. Therefore, it is of critical importance to understand processes shaping the indoor microbiomes in educational facilities to protect public health by reducing potential exposure risks of students and the broader community. In this study, the indoor surface bacterial microbiomes were characterized in two multifunctional university buildings with contrasting levels of human occupancy, of which one was recently constructed with minimal human occupancy while the other had been in full operation for six years. Higher levels of human occupancy in the older building were shown to result in greater microbial abundance in the indoor environment and greater proportion of the indoor surface bacterial microbiomes contributed from human-associated microbiota, particularly the skin microbiota. It was further revealed that human-associated microbiota had greater influence on the indoor surface bacterial microbiomes in areas of high occupancy than areas of low occupancy. Consistent with minimal impact from human occupancy in a new construction, the indoor microbiomes in the new building exhibited significantly lower influence from human-associated microbiota than in the older building, with microbial taxa originating from soil and plants representing the dominant constituents of the indoor surface bacterial microbiomes. In contrast, microbial taxa in the older building with extensive human occupancy were represented by constituents of the human microbiota, likely from occupants. These findings provide insights into processes shaping the indoor microbiomes which will aid

the development of effective strategies to control microbial exposure risks of occupants in educational facilities.

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1 Introduction

It has been estimated that more than 85% of human activities occur in enclosed buildings (Klepeis et al., 2001; Schweizer et al., 2007). Evidently, the built environment has a significant impact on the wellbeing of humans. Given that microorganisms are closely associated with human health, exposure to the microbial populations inhabiting the built environment, i.e. the indoor microbiome, has been linked to various health outcomes of the occupants, such as the regulation of immune functions (Lax et al., 2015), development of allergic diseases (Sbihi et al., 2019), spread of antibiotics resistance (Ben Maamar et al., 2020; Song et al., 2020), and particularly transmission of pathogens (Lax and Gilbert, 2015) which is an urgent concern due to the rapid spread of severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) in indoor environments (Allen and Marr, 2020).

To develop effective management strategies for the built environment to protect public health, indoor microbiomes have been characterized in a variety of built environments, including residential homes, healthcare facilities, office buildings, retail stores, public restrooms, and transit systems (Adams et al., 2015). However, there remains a dearth of studies focusing on the indoor microbiome in educational facilities, which typically serve as community hubs and subsequently hotspots for pathogen exposure. Indeed, more than 76 million students are enrolled in schools and colleges in the US alone (US Census Bureau, 2018). The mass flux of students, parents, and other community members are directly or indirectly exposed to the indoor microbiomes in these educational facilities. It is of critical importance to gain a systematic understanding of the indoor microbiomes in these educational facilities, which are frequently densely-populated with high risks of microbial exposure, for the development of effective control and management practices to reduce exposure risks.

Therefore, the main objective of this study was to characterize the indoor microbiomes in two multifunctional university buildings, of which one was a new construction while the other had been in use for six years. It was found that human occupancy had contributed to the marked contrasts between the indoor microbiomes in the two buildings, providing valuable insights into the processes shaping the indoor microbiomes in educational facilities.

2 Materials and methods

2.1 Sample collection

Two multifunctional buildings, Buildings T and M, on a university campus were selected for sampling. The two buildings shared similarities in function, management practice, and layout, having classrooms, graduate student offices, faculty offices, administrative offices, research laboratories, and student lounges. The two buildings were controlled by identical HVAC systems with the room temperature set at 72°F and relative humidity levels between 40% and 60% during the month of August when sampling occurred. However, the two buildings differed in the level of human occupancy. Building M was recently constructed with minimal human occupancy at the time of sampling. In contrast, Building T had been in use for six years when sampled.

A total of 20-five desk surfaces were selected for sampling in each building according to the level of human occupancy. Rooms with seating capacity less than 0.25 persons/m^2 were classified as low-occupancy areas, which included offices and research laboratories. In contrast, rooms with seating capacity greater than 0.50 persons/m^2 were classified as high-occupancy areas, which included classrooms. In addition, lobbies, located at the main entrances of the buildings, were also classified as high-occupancy areas due to high traffic of occupants. Sampling was conducted simultaneously in both buildings during the lunch break (12:00 – 1:00 PM) when classes were dismissed, which was intended to minimize interferences from occupant traffic during sampling. Two samples were collected from each of the 20-five surface sampling sites, one for heterotrophic plate count (HPC) and the other for 16S rRNA gene amplicon sequencing. Individually packed sterile Puritan polyester-tipped swabs (Puritan, Guilford, Maine, USA) were used to collect both samples. The swabs were pre-moistened with sterile phosphate-buffered saline-tween (PBST, pH = 7.2) solution, pressed against the side of the PBST container to remove excess liquid, then pressed onto the surface, and moved back and forth to cover a 2.25-dm^2 surface area using previously described protocols (Rose et al., 2004). Sample collection was completed within four hours for both buildings. Following surface sampling, one swab was preserved in 50 mL of PBST for HPC analysis within 6 h of sampling. The second swab was preserved at −20℃ until DNA extraction for amplicon sequencing.

2.2 Heterotrophic plate count (HPC) analysis

HPC analysis was used as a measure of the abundance of heterotrophic bacterial populations in the surface samples. The HPC agar plates containing the solid growth medium were prepared one day prior to sampling according to established standard procedures (Reasoner, 2004). The agar plates were made by first preparing a liquid medium containing (per liter) 5.0 g tryptone, 2.5 g yeast extract, 1.0 g glucose, and 15.0 g agar. After autoclaving at 121℃ for 30 min, the medium was poured into sterile plates to a thickness of 1–2 mm and cooled at room temperature to solidify. The agar plates were then sealed with parafilm and stored at 4℃ before use.

Swab samples kept in 50 mL of PBST were vortexed for 5 min and the resulting suspensions were filtered through 0.22 μm (pore size) Isopore polycarbonate membrane filters (Merck KGaA, Darmstadt, Germany). The filters were then placed onto pre-prepared HPC agar plates and incubated for 48 h before the colonies were counted. Results from HPC analysis were shown in CFU/dm^3 which was normalized by the sampling area.

2.3 DNA extraction, amplification and sequencing

DNA was extracted directly from each swab sample using the FastDNA Spin Kit for Soil (MP Biomedicals, Irvine, California, USA) according to the manufacturer's instructions. The DNA extracts were then purified

with the Genomic DNA Clean & Concentrator™-10 kit (Zymo Research, Irvine, California, USA) following the manufacturer's instructions. DNA concentrations were determined using the NanoDrop ND-3300 fluorospectrometer (Thermo Scientific, Waltham, Massachusetts, USA).

Purified DNA extracts were amplified targeting the V4 region of the 16S rRNA gene with primers 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT) as previously described (Wyckoff et al., 2017). The polymerase chain reaction (PCR) was prepared with a cocktail mix containing 12.5 µL Phusion Flash High-Fidelity PCR Master Mix (Thermo Scientific, Waltham, Massachusetts, USA), 10 µL ultra-pure water, 1 µL forward primer, 1 µL reverse primer, and 2 µL (100 to 150 ng) DNA template. The PCR program included one cycle at 94℃ for 3 min, followed by 35 cycles at 94℃ for 45 s, 55℃ for 60 s, and 72℃ for 90 s, with a final extension at 72℃ for 10 min. PCR products were examined by an Agilent 2100 Bioanalyzer instruments with Agilent DNA 7500 kits (Santa Clara, California, USA) for amplicon quantification and quality verification.

Subsequently, amplicons were pooled and amplicon library concentrations were measured using the KAPA Illumina Library Quantification Kit (Kapa Biosystems, Wilmington, Massachusetts, USA). The PhiX control library (Illumina, San Diego, California, USA) was added to the amplicon libraries to increase diversity. Sequencing of the amplicon libraries was performed with an Illumina MiSeq System (Illumina, San Diego, California, USA) at the University of Tennessee Genomics Core Facilities.

2.4 Data processing

Raw amplicon sequences were processed using the CLC Genomics Workbench 10 (Qiagen, Aarhus, Denmark) with the microbial genomics module following previously described protocols (Treu et al., 2018). Briefly, forward and reverse sequence reads were aligned and sequence pairs with more than 3 mismatches were removed. Forward and reverse sequence reads were then merged and trimmed with sequences shorter than 250 bp discarded. Further sequence processing was executed with the reference-based OTU clustering module using the SILVA 16S v 128 97% database and the following setting: similarity threshold of 97%, minimum occurrence of 2, chimera crossover cost at 3, and maximum unaligned end mismatches at 5. Raw sequence reads were deposited at the sequence read archive database (SRA) of NCBI under accession numbers SAMN14939471-SAMN14939499.

2.5 Identification of sources contributing to the indoor microbiomes

The contributions of potential microbial sources to the indoor microbiomes were determined with the SourceTracker software package (Knights et al., 2011), which estimates the proportion of the indoor microbiome derived from each of the potential sources using a Bayesian approach. Human skin and oral microbiota were chosen as the potential sources to be evaluated by SourceTracker analysis. Human skin and oral microbiota sequence data used in this study were retrieved from the Earth Microbiome Project and the European Nucleotide Archive (ENA), including 594 samples from the human skin (accession number ERR1867196-ERR1867464 and ERR1867837-ERR1868161) and 326 samples from the human mouth (accession number ERR1868427-ERR1868749). These skin and oral samples were processed with the same primer sets used in this study (515F and 806R), which facilitated sequence analysis. When a portion of the indoor microbiome did not match either of the potential sources, i.e. human skin or oral microbiota, this portion of the indoor microbiome would be assigned as being contributed from unknown sources.

3 Results and discussion

The indoor microbiome could be influenced by various factors, including occupant activities, design features, and management practices. The opening of new Building M on campus provided a rare opportunity to investigate the indoor microbiome when the impact of occupant activities was at a minimum. In contrast, Building T, as another building on campus with identical design features and management practices, had been in full operation for six years. Thus, the comparisons between Buildings M and T could readily illustrate the impact of human occupancy on the indoor microbiomes.

3.1 Microbial abundance in the indoor environment

Heterotrophic plate count (HPC) was used as a measure of microbial abundance at 25 indoor surface sites in both Buildings M and T. HPC analysis showed that culturable heterotrophic bacteria were present at an average concentration of 18 ± 19 CFU/ft^2 in Building M (Fig. 1). In comparison, the abundance of culturable heterotrophic bacteria was 207 ± 210 CFU/ft^2 in Building T (Fig. 1). Despite considerable variations in HPC results, microbial abundance was significantly greater in Building T than in Building M, suggesting a potential correlation between increased occupant activities and greater levels of microbial presence in the indoor environment.

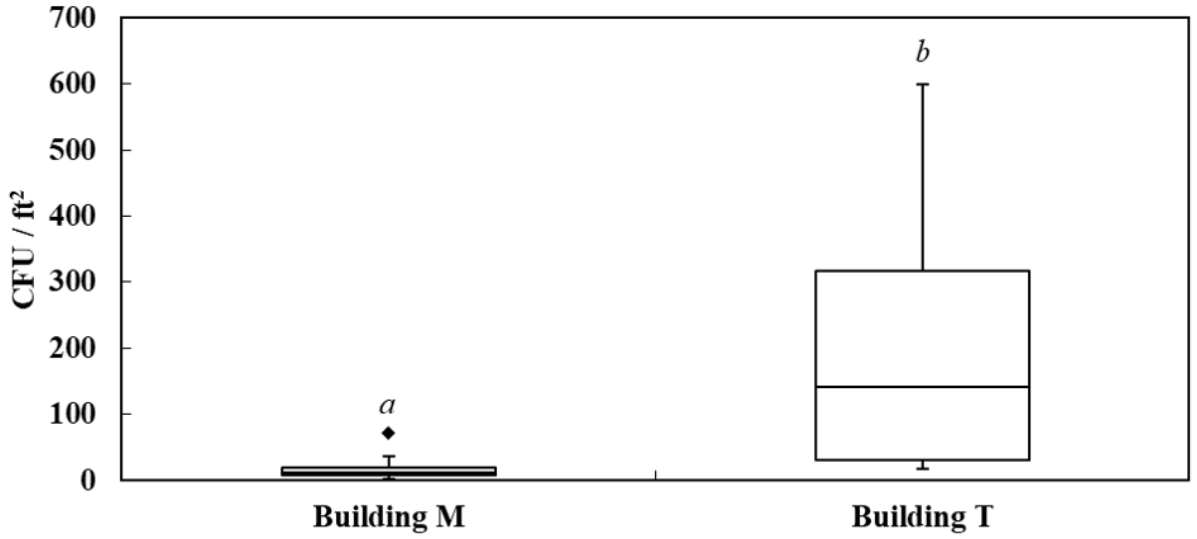

Fig. 1 HPC analysis of microbial abundance in the indoor environments with the box plots showing data from 25 surface samples in each building and the symbol "♦" indicating an outlier. The means are not significantly different from each other in boxes labeled with the same italicized lowercase letters (Student's *t*-test, $p < 0.05$)

3.2 Structure of indoor microbiomes

Since HPC captured only a small subset of the indoor microbiome that could grow on a specific solid medium, more comprehensive profiles of the indoor microbiome were achieved with high-throughput sequencing of

bacterial 16S rRNA gene amplicon libraries constructed for the same indoor surface samples from both buildings, resulting in a total of 13348 bacterial operational taxonomic units (OTUs) with > 97% sequence similarity, representing 88% of all clean reads.

To compare the structure of the indoor microbiomes between Buildings T and M, results from 16S rRNA gene amplicon library sequencing of indoor surface samples were examined by principle coordinated analysis (PCoA) with weighted UniFrac distance (Fig. 2). It is evident that Buildings M and T accommodated distinct indoor microbiomes, as the microbial communities from the two buildings formed two distant clusters. It is noted that the cluster formed by indoor surface samples from Building T was much more scattered than that from Building M (Fig. 2), suggesting the greater heterogeneity in the indoor microbiome of Building T than that of Building M. One major difference between Buildings M and T was the level of human occupancy at the time of sampling. Building M was more homogenous in terms of occupancy because all rooms in the building had similarly low levels of occupancy as a new building with minimal use. In contrast, the levels of occupancy differed considerably between rooms in Building T, which had been subjected to extensive use for six years. Thus, the linkage between occupancy and indoor microbiome needed further investigation.

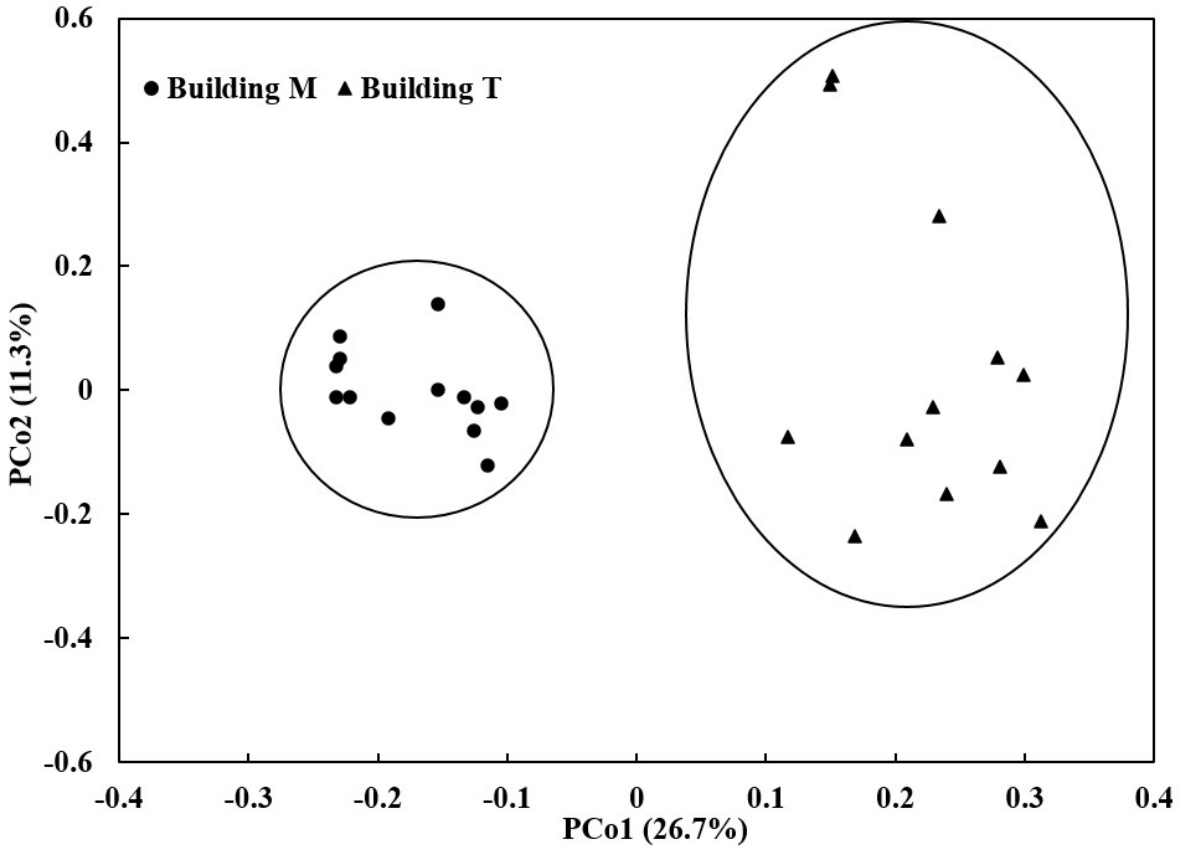

Fig. 2 Weighted principal coordinates analysis (PCoA) of the indoor microbiomes in Buildings M and T. Data points represent microbial community composition in indoor surface samples of Buildings M and T defined according to OTUs

Analysis of similarities (ANOSIM) was conducted for the 16S rRNA gene sequences from the indoor surface samples to further illustrate the differences in the indoor microbiomes between the two buildings (Fig. 3). ANOSIM results (R = 0.597 and P = 0.001) indicated that the dissimilarity in the indoor microbiomes between Buildings M and T was much greater than the dissimilarity within either Building M or T (Fig. 3),

which is consistent with results from PCoA showing two distinct clusters between Buildings M and T (Fig. 2). ANOSIM further revealed that the dissimilarity in the indoor microbiomes within Building T was considerably higher than that within Building M (Fig. 3), in line with the greater indoor microbial heterogeneity identified by PCoA in Building T than in Building M (Fig. 2).

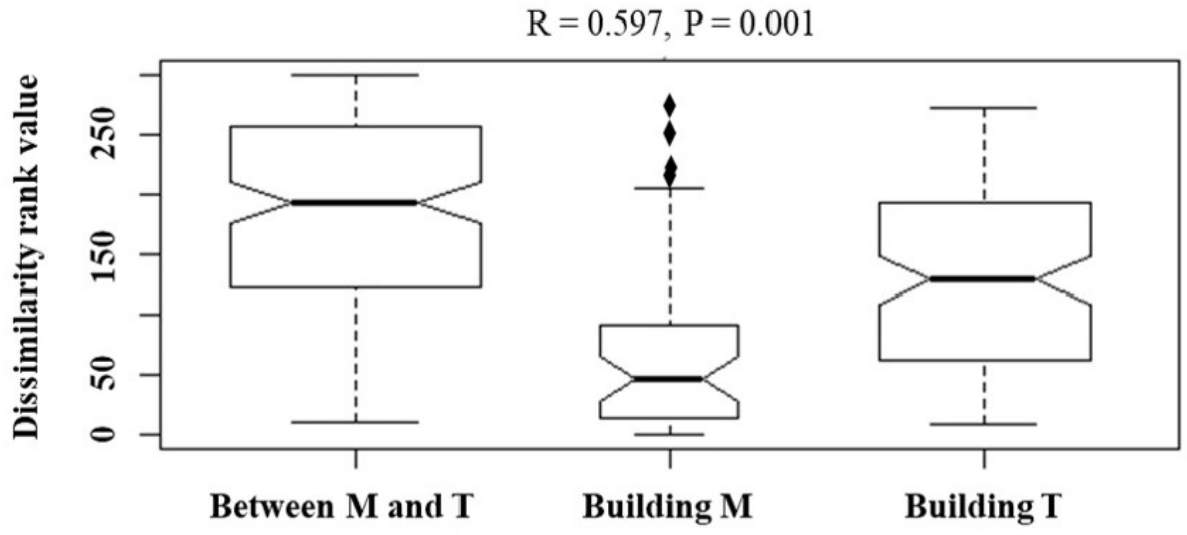

Fig. 3 Dissimilarity in indoor microbiome composition between and within Buildings M and T according to analysis of similarity (ANOSIM) with the box plots showing dissimilarity rank distribution sand the symbol "♦" indicating an outlier. The microbial community composition of indoor surface samples is defined according to OTUs

3.3 Composition of indoor microbiomes

To identify the microbial populations potentially contributing to the differences in the indoor microbiomes between Buildings M and T, the composition of the indoor surface microbiomes was examined in more detail.

3.3.1 Composition at phylum level

Sequence analysis identified 44 and 55 bacterial phyla in Buildings M and T, respectively, indicating the greater population richness in Building T. The most abundant phylum in Building T was *Proteobacteria*, representing 39% of the sequences (Fig. 4), which was followed by *Actinobacteria* and *Firmicutes*, accounting for 25% and 21% of the indoor microbial sequences, respectively (Fig. 4). Other significant populations in Building T included *Cyanobacteria*, *Bacteroidetes*, and *Fusobacteria*, with no other bacterial phyla representing > 1% of the indoor microbiome (Fig. 4).

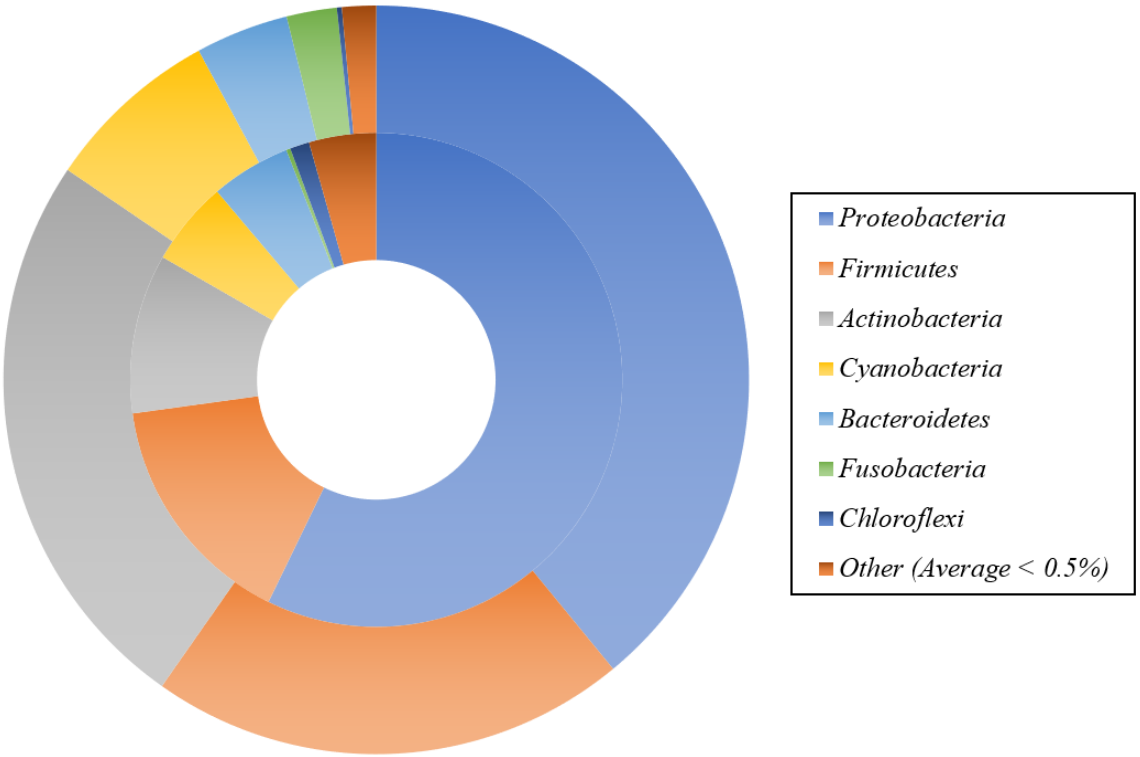

Fig. 4 Comparison of indoor microbiome composition between Buildings T and M at the phylum level. Outer ring: Building T; inner ring: Building M

For Building M, *Proteobacteria* was the most abundant phylum as well, accounting for 57% of the sequences, which however was much greater than its abundance in Building T (Fig. 4). Similar to Building T, *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, and *Cyanobacteria* represented other dominant populations at the phylum level in Building M, with relative abundance of 16%, 10%, 6%, and 5%, respectively. The only other phylum in Building M with relative abundance > 1% was *Chloroflexi*, which in comparison accounted for < 0.1% of the sequences in Building T (Fig. 4).

While the phylum-level composition of the indoor microbiomes differed slightly between Buildings M and T, the most abundant populations were identical in the two buildings, including *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, and *Cyanobacteria*. These results are consistent with previous indoor microbiome studies, where populations associated with these phyla have been frequently identified in a variety of indoor environments (Hewitt et al., 2012; Kembel et al., 2012; Kembel et al., 2014; Hoisington et al., 2016).

3.3.2 Composition at genus level

The indoor microbiomes exhibited more distinctions at the genus level between Buildings M and T. The most frequently detected genus in Building M was *Rhizobium*, accounting for 18% of the sequences on average (Fig. 5). In contrast, *Rhizobium* was identified with a much lower relative abundance in Building T, averaging 3.7% only. Given that *Rhizobium* populations are known to be soil bacteria specialized in nitrogen fixation (Sawada et al., 2003), the higher abundance of *Rhizobium* found in Building M is indicative of a greater exposure to the outdoor environment, which is consistent with the fact that construction of Building M was only recently completed.

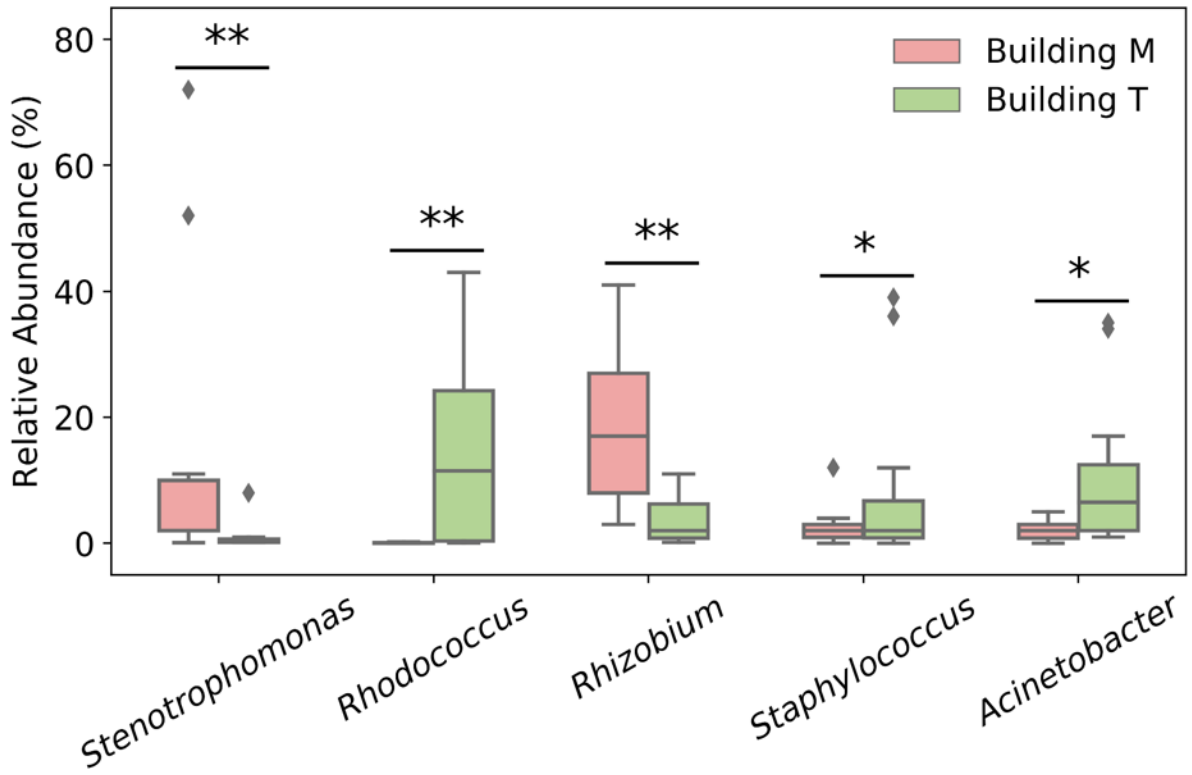

Fig. 5 The relative abundance of bacterial genera with average relative abundance greater than 5% in indoor microbiomes. The asterisks on top of box plots show the level of significance in the difference between Buildings T and M, with * indicating $p < 0.05$ and ** indicating $p < 0.01$ according to the Wilcoxon rank-sum test. The symbol "♦" indicates an outlier

Stenotrophomonas was another bacterial genus frequently detected in Building M at an average abundance of 12.7%, accounting for more than 50% of the sequences at two of the sampling locations in Building M (Fig. 5). In comparison, *Stenotrophomonas* was present at a significantly lower relative abundance in Building T, only contributing 1.0% on average to the indoor microbiome. Populations of *Stenotrophomonas* are found throughout the environment, particularly in close association with plants (Ryan et al., 2009). Thus, the higher abundance of these bacteria in Building M could again be attributed to greater influences from the outdoor environment as a result of recent construction activities in the new building.

An alarming finding in Building T was the abundance of *Staphylococcus* strains, representing on average 8.5% of the indoor microbiome, which was significantly higher than the relative abundance of *Staphylococcus* in Building M, averaging 2.5% (Fig. 5). It is known that members of *Staphylococcus*, considered opportunistic pathogens, frequently colonize the skin and mucous membranes of warm-blooded animals, including humans

(Baird - Parker, 1990). Given that humans are natural hosts of *Staphylococcus*, the greater presence of *Staphylococcus* populations in Building T could be attributed to higher levels of human activities in Building T with six years of operation than those in new Building M, which was yet to experience substantial human occupancy.

Other abundant microbial inhabitants of Building T included members of *Acinetobacter*, accounting for 10.7% of the indoor microbiome (Fig. 5). In comparison, the relative abundance of *Acinetobacter* averaged only 2.1% in Building M, which was significantly lower than that in Building T. Similar to *Staphylococcus*, *Acinetobacter* populations are known to be opportunistic pathogens and constituents of the normal human skin microflora (Towner, 2009). Thus, the greater abundance of *Acinetobacter* in Building T, where human occupancy was much more extensive, again points to the potential importance of human activities in shaping the indoor microbiome.

Besides *Rhizobium*, *Stenotrophomonas*, *Acinetobacter*, and *Staphylococcus*, the only other bacterial genus with the average relative abundance greater than 5% in either building was *Rhodococcus*. Indeed, with its relative abundance averaging 14.8%, *Rhodococcus* was the most abundant genus in Building T. Interestingly, the presence of *Rhodococcus* in Building M, however, was minimal, with less than 0.1% of the indoor microbiome identified as *Rhodococcus* (Fig. 5). This sharp contrast between Buildings T and M was unexpected. Because *Rhodococcus* strains are known to be broadly distributed in the environment (Bell et al., 1998), it would be more reasonable to expect greater abundance of *Rhodococcus* in Building M instead of Building T, as observed for *Rhizobium* and *Stenotrophomonas*, both having broad environmental distributions and consequently greater abundance in Building M rather than Building T (Fig. 5). Further examination of the *Rhodococcus* sequences was needed to shed more light on the unexpected abundance of *Rhodococcus* in Building T.

3.4 Phylogenetic analysis of abundant OTUs

The sequences of the abundant genera in the indoor microbiomes, i.e. relative abundance > 5.0% in either Building T or M, were grouped into OTUs with > 97% sequence similarity (Fig. 6). One important distinction between Buildings M and T was the significantly higher abundance of two genera, *Rhizobium* and *Stenotrophomonas*, in Building M (Fig. 5). *Rhizobium* was represented exclusively by OTU2, which is most closely related to *Rhizobium leguminosarum* (Fig. 6). Similar to all rhizobia, *R. leguminosarum* is an obligate symbiont of legumes carrying out nitrogen fixation in root nodules (Masson-Boivin et al., 2009). The genus *Stenotrophomonas* comprised OTU7, which is most closely related to *Stenotrophomonas maltophilia* (Fig. 6). *S. maltophilia* has broad distribution in the natural environment, particularly abundant in the plant rhizosphere with ecological functions in nutrient cycling (Hayward et al., 2010). The findings that soil is the natural habitat of both OTU2 and OTU7 suggest that populations of *Rhizobium* and *Stenotrophomonas* identified in Buildings M and T were likely originated from the soil. It could be reasoned that, as a new construction, Building M had greater resemblance to the outdoor environment and subsequently greater abundance of soil-associated microbial populations such as *Rhizobium* and *Stenotrophomonas*. It should be noted that *S. maltophilia* has been frequently isolated from clinical environments as an emerging opportunistic pathogen (An and Berg, 2018). It is unlikely that rigorous microbial control practices in these clinical environments would promote the introduction of soil microorganisms from the outdoor environment. Instead, this might be the result of the exceptional metabolic persistence and versatility that enable the adaptation of *S. maltophilia* to these unique environments (Ryan et al., 2009).

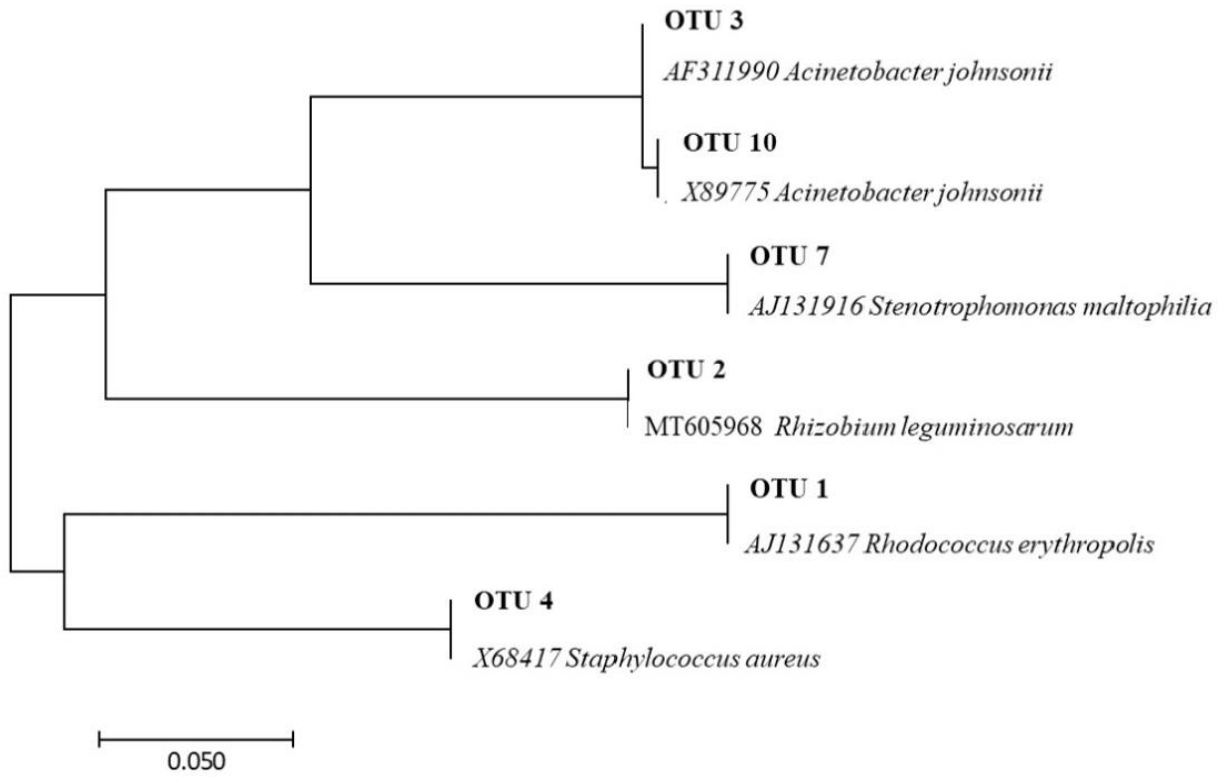

Fig. 6 Maximum-likelihood tree showing abundant 16S rRNA gene sequences in indoor microbiomes (average relative abundance > 5%) in relation to sequences of closest reference strains, which were obtained by SeqMatch as the first perfect match (similarity score = 1.0) in the Ribosomal Database Project (RDP). GenBank accession numbers of the reference strains are shown together with the name of the strains. The scale bar represents the number of substitutions per sequence position

While new Building M was predominated by soil microbial populations represented by *Rhizobium* and *Stenotrophomonas*, Building T was characterized by the significantly higher abundance of *Acinetobacter*, *Staphylococcus*, and *Rhodococcus* (Fig. 5). Sequences of *Acinetobacter* were grouped into two OTUs, i.e. OTU3 and OTU10; however, both OTUs are closely related to *Acinetobacter johnsonii* (Fig. 6). Previous studies have frequently identified humans and human-impacted habitats as reservoirs of *A. johnsonii* and other *Acinetobacter* strains (Al Atrouni et al., 2016). The abundance of *A. johnsonii* in Building T might also be attributable to its ability to survive dry conditions for prolonged periods (Hirai, 1991). Phylogenetics analysis revealed that sequences of *Staphylococcus* formed OTU4, most closely related to *Staphylococcus aureus* (Fig. 6). It has been recognized that *S. aureus* is an endogenous pathogen which colonizes the human nose as its preferred reservoir in 30% of the human population (Krismer et al., 2017). Thus, the higher abundance of human-associated *Acinetobacter* and *Staphylococcus* in Building T is consistent with more extensive human occupancy in Building T than in Building M.

Rhodococcus was another bacterial genus highly abundant in Building T but not in Building M (Fig. 5). Phylogenetic analysis indicates that the *Rhodococcus* sequences in the indoor microbiomes were represented by *Rhodococcus erythropolis* (Fig. 6), which is broadly distributed in diverse natural environments (de Carvalho and da Fonseca, 2005). *R. erythropolis* is known to have hydrophobic cells with the ability to attach to surfaces (Rodrigues and de Carvalho, 2015), providing a potential mechanism contributing to the abundance

of *R. erythropolis* on indoor surfaces in Building T. However, it could not explain the significantly lower abundance of *R. erythropolis* in Building M (Fig. 5). If outdoor environment was the primary source of indoor *R. erythropolis*, its abundance in Building M would be expected to be higher given the greater influence of the outdoor environment on Building M as a new construction. An alternative postulation is that *R. erythropolis* in the indoor environment could have human origins, which is supported by reports that *R. erythropolis* could colonize humans along with other endogenous pathogens such as staphylococci (Graham et al., 2007). This would explain the higher abundance of *R. erythropolis* in Building T due to its longer history of human occupancy than Building M. Further studies are needed to assess the importance of humans as the reservoir of *R. erythropolis*.

3.5 Indoor microbiome source identification

Given the significance of human occupancy in shaping the indoor microbiomes as revealed by phylogenetic analysis, SourceTracker was used to quantify the contribution of human-associated microorganisms as sources to the indoor microbiomes (Knights et al., 2011). The dispersal of human-associated microorganisms in the indoor environment occurs primarily via direct surface contact, desquamation, or emission of bioaerosols and particulates from human breath (Lax et al., 2014; Meadow et al., 2014). Therefore, human skin and oral microbiota were chosen as the sources for SourceTracker analysis.

It was evident that a significantly greater portion of the indoor microbiome in Building T originated from human-associated microorganisms than in Building M (Fig. 7). Specifically, human skin microbiota represented a median contribution of 13.4% to the indoor microbiome in Building T, which was significantly higher than the median contribution of 6.2% in Building M (Fig. 7(a)). Compared with human skin microbiota, the contribution from human oral microbiota to the indoor microbiomes was much smaller, with the median at 2.8% and 0.7% in Buildings T and M, respectively (Fig. 7(b)). Similar to the skin microbiota, the oral microbiota contributed to the indoor microbiome in Building T at significantly higher levels than in Building M. These results show that the impact of human-associated microorganisms was significantly greater on the indoor microbiome in Building T than in Building M, which is consistent with more extensive human occupancy in Building T than Building M.

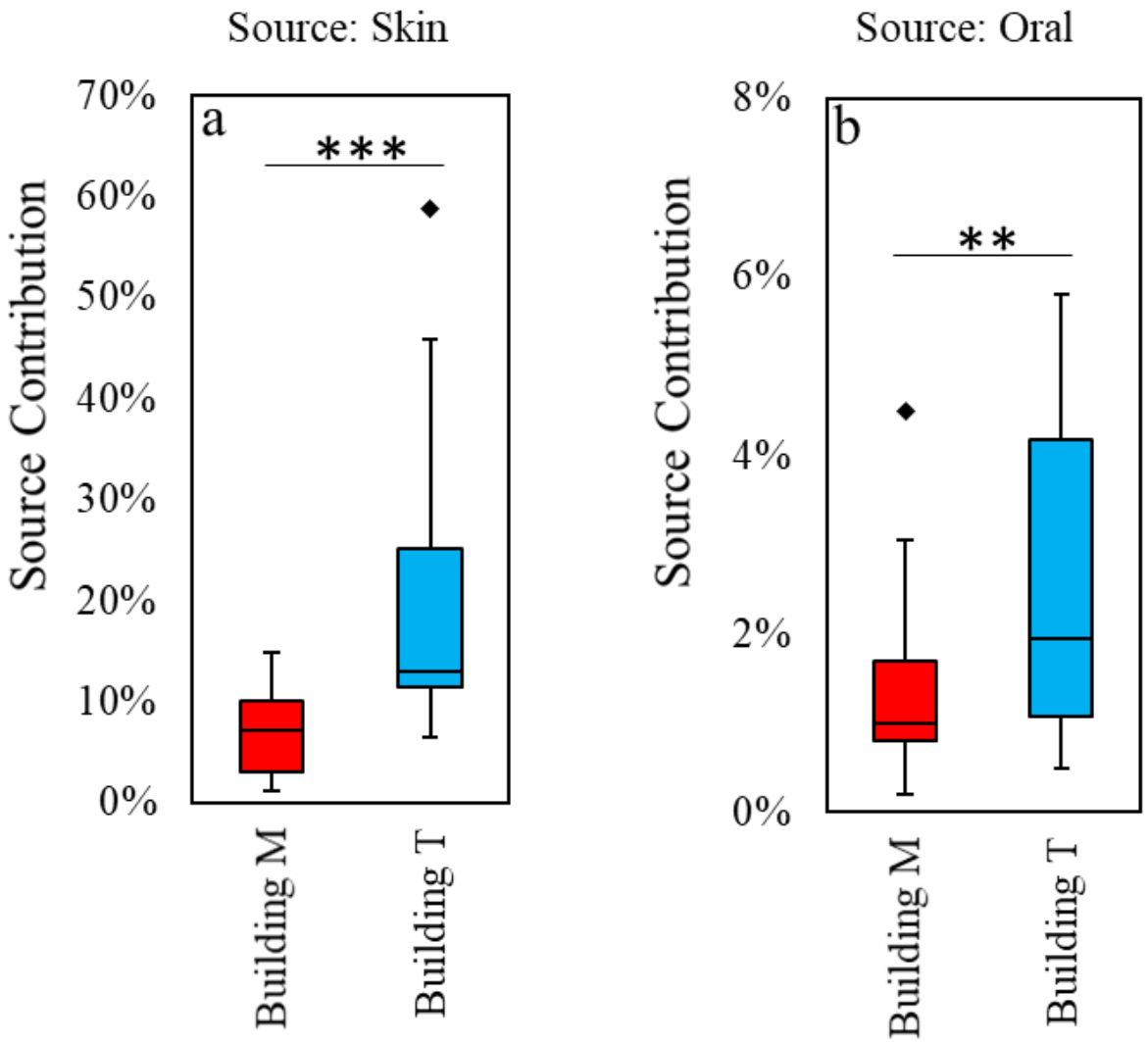

Fig. 7 Percentage of indoor microbiomes contributed by human skin (a) and oral (b) sources in Buildings M and T. The asterisks on top of box plots show the level of significance in the difference between Buildings T and M, with * indicating $p < 0.05$ and ** indicating $p < 0.01$ according to the Student *t*-test. The symbol "♦" indicates an outlier

It is noted that results from SourceTracker analysis exhibited large variations (Fig. 7). For example, the median contribution of human skin microbiota to the indoor microbiomes in Building T was 13.4%, with the maximum reaching 54.3% (Fig. 7(a)). Thus, it could be hypothesized that some areas in the indoor environment had much greater influence from the occupants and associated microbiota than others. To test this hypothesis, the sampling sites in the two buildings were divided into two groups—high-occupancy areas, including classrooms and public areas (lobbies), and low-occupancy areas such as offices and research laboratories. In Building T, both human skin and oral microbiota sources were found to contribute to indoor microbiomes at significantly greater levels in high-occupancy areas than in low-occupancy areas (Fig. 8), confirming the important impact of human occupancy on the indoor microbiome. In contrast, in Building M,

no significant differences were detected in human contribution to the indoor microbiomes between high-occupancy areas and low-occupancy areas (Fig. 8), which is likely due to the lack of extensive human occupancy even in the high-occupancy areas of Building M. These results again highlight the significance of human occupancy in shaping the indoor microbiome, which has been suggested in studies of indoor microbiomes in other types of built environments (Gilbert and Stephens, 2018).

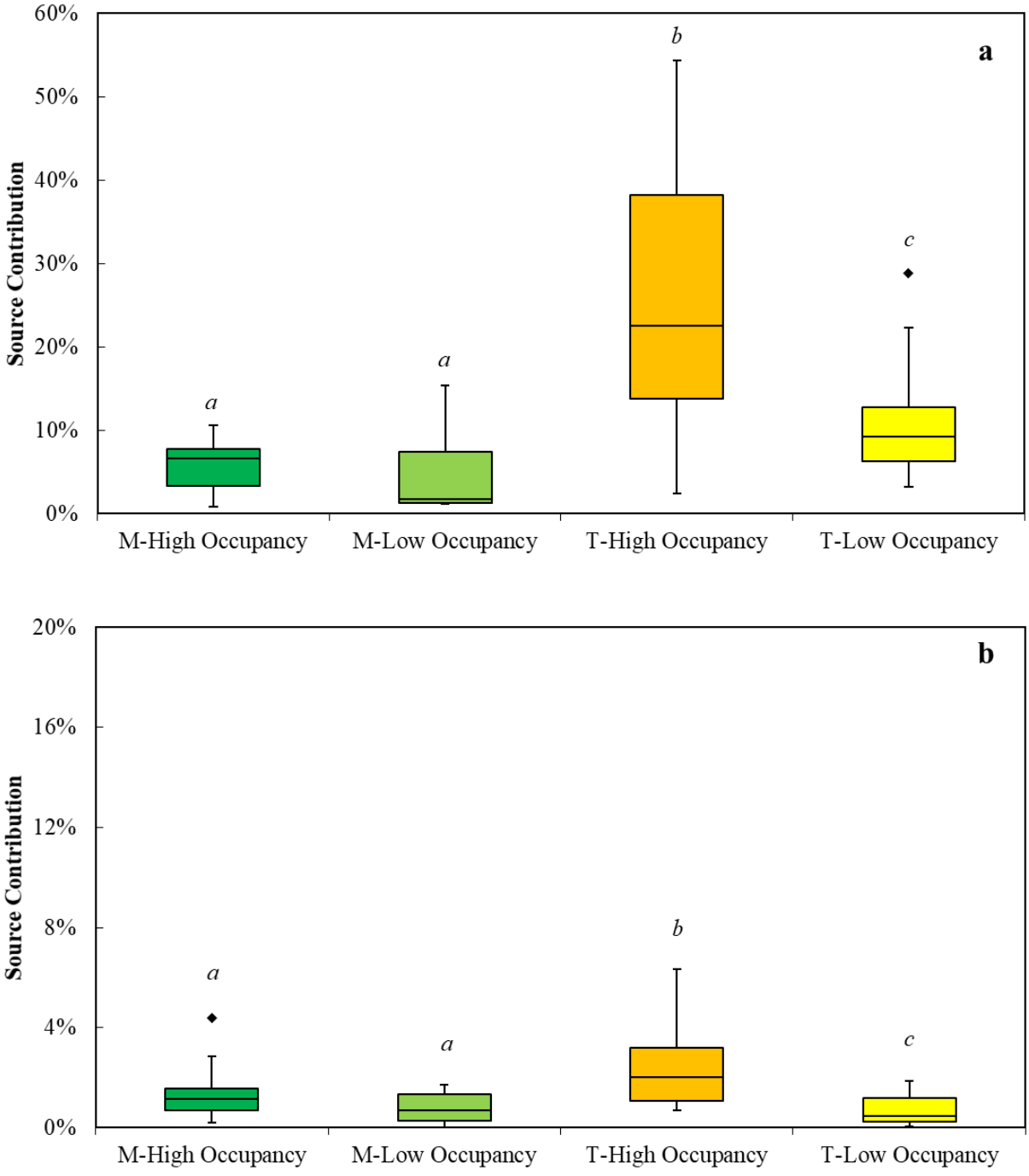

Fig. 8 Percentage of indoor microbiomes contributed by human skin (a) and oral (b) sources in high- and low-occupancy areas of Buildings M and T. M—Building M; T—Building T. High occupancy—samples collected from classrooms and lobbies; low-occupancy—samples taken from offices and research laboratories. The means are not significantly different from each other in boxes labeled with the same italicized lowercase letters (Student's *t*-test, $p < 0.05$). The symbol "♦" indicates an outlier

4 Conclusions

Comparisons were made between the indoor microbiomes of two multifunctional university buildings, of which one was new with minimal human occupancy while the other was older with six years of occupancy. Higher levels of human occupancy in the older building were shown to result in greater microbial abundance in the indoor environment and greater proportion of the indoor microbiome to be contributed from human-associated microbiota, particularly the skin microbiota. It was further revealed that human-associated microbiota had greater influence on the indoor microbiome in areas of high occupancy than areas of low occupancy in the same building. Consistent with minimal impact from human occupancy in a new construction, the indoor microbiomes in the new building exhibited significantly lower influence from human-associated microbiota than in the older building, with microbial taxa originating from soil and plants representing the dominant constituents of the indoor microbiome. In contrast, abundant microbial taxa in the older building were represented by constituents of the human microbiota, likely derived from occupants. These findings provide insights into the processes shaping the indoor microbiomes for the development of effective strategies to manage the microbial exposure of occupants in the built environment.

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References

Adams R I, Bateman A C, Bik H M, Meadow J F (2015). Microbiota of the indoor environment: a meta-analysis. Microbiome, 3: 49 doi:10.1186/s40168-015-0108-3

Al Atrouni A, Joly-Guillou M L, Hamze M, Kempf M (2016). Reservoirs of non-*baumannii Acinetobacter* species. Frontiers in Microbiology, 7: 49 doi:10.3389/fmicb.2016.00049

Allen J G, Marr L C (2020). Recognizing and controlling airborne transmission of SARS‐CoV‐2 in indoor environments. Indoor Air, 30(4): 557–558 doi:10.1111/ina.12697

An S Q, Berg G (2018). Stenotrophomonas maltophilia. Trends in Microbiology, 26(7): 637–638 doi:10.1016/j.tim.2018.04.006

Baird‐Parker A C (1990). The staphylococci: an introduction. Journal of Applied Bacteriology, 69: 1S–8S doi:10.1111/j.1365-2672.1990.tb01793.x

Bell K S, Philp J C, Aw D W J, Christofi N (1998). The genus *Rhodococcus*. Journal of Applied Microbiology, 85(2): 195–210 doi:10.1046/j.1365-2672.1998.00525.x

Ben Maamar S, Hu J, Hartmann E M (2020). Implications of indoor microbial ecology and evolution on antibiotic resistance. Journal of Exposure Science & Environmental Epidemiology, 30(1): 1–15 doi:10.1038/s41370-019-0171-0

de Carvalho C C C R, da Fonseca M M R (2005). The remarkable *Rhodococcus erythropolis.* Applied Microbiology and Biotechnology, 67(6): 715–726 doi:10.1007/s00253-005-1932-3

Gilbert J A, Stephens B (2018). Microbiology of the built environment. Nature Reviews. Microbiology, 16(11): 661–670 doi:10.1038/s41579-018-0065-5

Graham J E, Moore J E, Jiru X U, Moore J E, Goodall E A, Dooley J S, Hayes V E, Dartt D A, Downes C S, Moore T C (2007). Ocular pathogen or commensal: a PCR-based study of surface bacterial flora in normal and dry eyes. Investigative Ophthalmology & Visual Science, 48(12): 5616–5623 doi:10.1167/iovs.07-0588

Hayward A C, Fegan N, Fegan M, Stirling G R (2010). *Stenotrophomonas* and *Lysobacter*: ubiquitous plant - associated *gamma* - proteobacteria of developing significance in applied microbiology. Journal of Applied Microbiology, 108(3): 756–770 doi:10.1111/j.1365-2672.2009.04471.x

Hewitt K M, Gerba C P, Maxwell S L, Kelley S T (2012). Office space bacterial abundance and diversity in three metropolitan areas. PLoS One, 7(5): e37849 doi:10.1371/journal.pone.0037849

Hirai Y (1991). Survival of bacteria under dry conditions; from a viewpoint of nosocomial infection. Journal of Hospital Infection, 19(3): 191–200 doi:10.1016/0195-6701(91)90223-U

Hoisington A, Maestre J P, Kinney K A, Siegel J A (2016). Characterizing the bacterial communities in retail stores in the United States. Indoor Air, 26(6): 857–868 doi:10.1111/ina.12273

Kembel S W, Jones E, Kline J, Northcutt D, Stenson J, Womack A M, Bohannan B J, Brown G Z, Green J L (2012). Architectural design influences the diversity and structure of the built environment microbiome. ISME Journal, 6(8): 1469–1479 doi:10.1038/ismej.2011.211

Kembel S W, Meadow J F, O'Connor T K, Mhuireach G, Northcutt D, Kline J, Moriyama M, Brown G Z, Bohannan B J M, Green J L (2014). Architectural design drives the biogeography of indoor bacterial communities. PLoS One, 9(1): e87093 doi:10.1371/journal.pone.0087093

Klepeis N E, Nelson W C, Ott W R, Robinson J P, Tsang A M, Switzer P, Behar J V, Hern S C, Engelmann W H (2001). The National Human Activity Pattern Survey (NHAPS): a resource for assessing exposure to environmental pollutants. Journal of Exposure Science & Environmental Epidemiology, 11(3): 231–252 doi:10.1038/sj.jea.7500165

Knights D, Kuczynski J, Charlson E S, Zaneveld J, Mozer M C, Collman R G, Bushman F D, Knight R, Kelley S T (2011). Bayesian community-wide culture-independent microbial source tracking. Nature Methods, 8(9): 761–763 doi:10.1038/nmeth.1650

Krismer B, Weidenmaier C, Zipperer A, Peschel A (2017). The commensal lifestyle of *Staphylococcus aureus* and its interactions with the nasal microbiota. Nature Reviews. Microbiology, 15(11): 675–687 doi:10.1038/nrmicro.2017.104

Lax S, Gilbert J A (2015). Hospital-associated microbiota and implications for nosocomial infections. Trends in Molecular Medicine, 21(7): 427–432 doi:10.1016/j.molmed.2015.03.005

Lax S, Nagler C R, Gilbert J A (2015). Our interface with the built environment: immunity and the indoor microbiota. Trends in Immunology, 36(3): 121–123 doi:10.1016/j.it.2015.01.001

Lax S, Smith D P, Hampton-Marcell J, Owens S M, Handley K M, Scott N M, Gibbons S M, Larsen P, Shogan B D, Weiss S, Metcalf J L, Ursell L K, Vazquez-Baeza Y, Van Treuren W, Hasan N A, Gibson M K, Colwell R, Dantas G, Knight R, Gilbert J A (2014). Longitudinal analysis of microbial interaction between humans and the indoor environment. Science, 345(6200): 1048–1052 doi:10.1126/science.1254529

Masson-Boivin C, Giraud E, Perret X, Batut J (2009). Establishing nitrogen-fixing symbiosis with legumes: how many rhizobium recipes? Trends in Microbiology, 17(10): 458–466 doi:10.1016/j.tim.2009.07.004

Meadow J F, Altrichter A E, Kembel S W, Moriyama M, O'Connor T K, Womack A M, Brown G Z, Green J L, Bohannan B J (2014). Bacterial communities on classroom surfaces vary with human contact. Microbiome, 2: 7 doi:10.1186/2049-2618-2-7

Reasoner D J (2004). Heterotrophic plate count methodology in the United States. International Journal of Food Microbiology, 92(3): 307–315 doi:10.1016/j.ijfoodmicro.2003.08.008

Rodrigues C J C, de Carvalho C C C R (2015). *Rhodococcus erythropolis* cells adapt their fatty acid composition during biofilm formation on metallic and non-metallic surfaces. FEMS Microbiology Ecology, 91(12): fiv135 doi:10.1093/femsec/fiv135

Rose L, Jensen B, Peterson A, Banerjee S N, Arduino M J (2004). Swab materials and *Bacillus anthracis* spore recovery from nonporous surfaces. Emerging Infectious Diseases, 10(6): 1023–1029 doi:10.3201/eid1006.030716

Ryan R P, Monchy S, Cardinale M, Taghavi S, Crossman L, Avison M B, Berg G, Van Der Lelie D, Dow J M (2009). The versatility and adaptation of bacteria from the genus *Stenotrophomonas.* Nature Reviews. Microbiology, 7(7): 514–525 doi:10.1038/nrmicro2163

Sawada H, Kuykendall L D, Young J M (2003). Changing concepts in the systematics of bacterial nitrogen-fixing legume symbionts. Journal of General and Applied Microbiology, 49(3): 155–179 doi:10.2323/jgam.49.155

Sbihi H, Boutin R C, Cutler C, Suen M, Finlay B B, Turvey S E (2019). Thinking bigger: How early‐life environmental exposures shape the gut microbiome and influence the development of asthma and allergic disease. Allergy, 74(11): 2103–2115 doi:10.1111/all.13812

Schweizer C, Edwards R D, Bayer-Oglesby L, Gauderman W J, Ilacqua V, Juhani Jantunen M, Lai H K, Nieuwenhuijsen M, Künzli N (2007). Indoor time–microenvironment–activity patterns in seven regions of Europe. Journal of Exposure Science & Environmental Epidemiology, 17(2): 170–181 doi:10.1038/sj.jes.7500490

Song L, Wang C, Wang Y (2020). Optimized determination of airborne tetracycline resistance genes in laboratory atmosphere. Frontiers of Environmental Science and Engineering, 14(6): 95 doi:10.1007/s11783-020-1274-5

Towner K J (2009). *Acinetobacter*: an old friend, but a new enemy. Journal of Hospital Infection, 73(4): 355–363 doi:10.1016/j.jhin.2009.03.032

Treu L, Kougias P G, de Diego-Díaz B, Campanaro S, Bassani I, Fernández-Rodríguez J, Angelidaki I (2018). Two-year microbial adaptation during hydrogen-mediated biogas upgrading process in a serial reactor configuration. Bioresource Technology, 264: 140–147 doi:10.1016/j.biortech.2018.05.070

US Census Bureau (2018). Classrooms More Racially and Ethnically Diverse. Suitland, USA: US Census Bureau

Wyckoff K N, Chen S, Steinman A J, He Q (2017). Impact of roadway stormwater runoff on microbial contamination in the receiving stream. Journal of Environmental Quality, 46(5): 1065–1071 doi:10.2134/jeq2017.03.0116