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Cohabitation impacts the microbial diversity and taxonomic makeup of the biotic and abiotic environments in a college dormitory

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SUMMARY Individuals carry a unique collection of microbes on their skin, known as their microbiome, which may impact the microbial makeup of their surrounding environment. The skin microbiome is also influenced by individuals cohabitating the same space. Using data collected by Richardson *et al*., this study aims to understand the influence of roommates on one's skin microbiome and on the microbiota of their abiotic living environment. This will provide insight into how the human microbiota is influenced, and the role that those around us have in shaping the microbiome. Given the changes to the skin microbiome of cohabiting families and the microbial imprint humans tend to leave on the built environment in which they live, we hypothesized that having roommates would affect the microbial diversity of both environments. To address this hypothesis, we generated diversity metrics using QIIME2, which revealed significant differences between single- and multiple-occupancy dorm types. Subsequently, separate analyses of differential abundance and indicator taxa were conducted on the abiotic and biotic samples, which revealed significant differences in the abiotic samples between single and multiple occupancy dorms. This study revealed that not only are differentially abundant species occupying the abiotic environment of those with roommates, but that particular taxa may be used as indicators of individuals who live with or without roommates. It gives new insights into the effects of cohabitation with unrelated individuals and inspires further research into the biological causes behind the observed differences.

INTRODUCTION

It is well known that distinct microbial species inhabit individuals, imparting a unique environment of microbes that collectively make up one's microbiota (1, 2). In particular, the skin microbiome serves as an exterior interface between one's body and their environment, protecting from invading pathogens, priming the immune system, and breaking down natural products (3). These organisms maintain a unique signature, which is stable over time, and is likely a result of both one's experiences and environment (2). Research in this area suggests that microbes from one's skin are continually shed into the surrounding environment (1). This understanding led to subsequent investigation of the similarity between individuals who share living spaces. Previous research revealed that cohabiting individuals significantly influence the composition of each other's skin microbiome, making them more similar to one another when compared to other, non-cohabiting individuals (1). Additionally, it has been found that the human skin microbiome is the greatest contributor to the microbiota of the built environment, which encompasses all structures built by humans including our homes and workspaces (4).

The skin microbiome imprints on the abiotic surfaces within living spaces. Microbial communities associated with family members were shown to readily inhabit the abiotic surfaces of the family's home and converge on the microbial community of their previous home, even after they had moved houses (5). Other research has shown that families, and especially couples, had a greater level of microbial similarity in their microbiota than individuals from different households, highlighting microbial exchange within shared living environments (6). Despite this exchange within shared environments, the skin microbiome of individuals has shown to be relatively stable over time, comprising an individualist microbial signature (7). Previous research has posited that humans can disperse microbes which they harbor through direct contact with surfaces or through airborne release. The latter method

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may even allow individuals to be identified through a distinct combination of microbes present in a "microbial cloud" (8).

This individuality of one's microbiome combined with its ability to imprint on the environment and be influenced by cohabitation with other individuals was investigated by Richardson *et al*. Previous research has shown that the microbiota of the built environment is influenced by human sources and that living with family members or couples modifies one's microbiome. However, changes in the microbiome have not been studied in large groups of unrelated cohabiting individuals, such as students in a college dormitory. In their study, Richardson *et al*. addressed this gap by collecting environmental and biological samples over various time points from students in one dormitory building with both private spaces and shared common areas (9). Their goal in collecting this data was to assign microbial "signatures" to each individual, from which they could predict the rooms they inhabit based on shared microbial composition (9). The study confirmed previous research suggesting that one's microbial signature is stable over time and demonstrated the mixing of these signatures in spaces occupied by multiple individuals (5–7, 9). In addition, their model was able to attribute individuals to their bedroom based on shared characteristics between their skin and their room's microbiome. However, they observed that the presence of a roommate was a significant confounding factor in their model's ability to successfully predict which room an individual belonged to (9). Thus, we wanted to investigate specifically how the presence of a roommate affected the microbiome.

We sought to understand how having a roommate might influence the diversity and composition of both the skin microbiome and abiotic environment of students living in a common dormitory. Previous studies suggest that cohabitation facilitates the exchange of one's microbiome with their roommates and their surrounding environment. Therefore, we hypothesized that having roommates would affect the microbial diversity of both the skin microbiome and abiotic environment. Using Richardson *et al.*'s data, we analyzed diversity metrics for both the biotic and abiotic samples from single and multiple occupancy rooms using Quantitative Insights Into Microbial Ecology (QIIME2) (10). We then used differential abundance and indicator taxa analysis in QIIME2 to provide further insights into the abiotic environment, which showed significant differences between single and multiple occupancy environments.

METHODS AND MATERIALS

The dataset. The data in this paper originated from a study by Richardson *et al*. in which samples were collected from 37 students living in one dormitory at the University of Chicago (9). Biotic samples were collected from the students' dominant hand (categorized in the metadata as "skin") and abiotic samples were collected from their bed sheets, bedroom door handles, floors, and desks (categorized in the metadata as "surface"). The V4 region of the 16s rRNA gene was amplified and sequenced using the Illumina® MiSeq™ system (11). The metadata categories of interest to our project included sample type, roommate status, and sample-ID-associated roommate pairs (or groups). The metadata and the raw sequences are available from Qiita (ID 12470) and from the European Nucleotide Archive (ENA) of the European Bioinformatics Institute (EBI) (project number PRJEB33050/ERP115809, at https://www.ebi.ac.uk/ena/browser/view/PRJEB33050?show=reads) (12).

Data processing in QIIME2. Prior to data processing, an "occupancy" column describing roommate status was added to the metadata file using R Studio version 4.1.2 and the tidyverse package (13, 14). Individuals were categorized as "single-occupants" if they had 0 roommates or categorized as "multiple-occupants" if they had 1, 2, or 3 roommates. Data processing was performed using QIIME2 (10). Raw sequence reads were imported to QIIME2 and demultiplexed. Sequence quality control was performed using DADA2 (15) to correct (or discard) sequencing errors and define Amplicon Sequencing Variants (ASVs). To maintain high sequence quality, the reads were truncated at 150 base pairs (bp), after which there was a drop in the median Phred Quality Score. Taxonomy was assigned to the ASVs using a trained classifier with reference to the SILVA 16s rRNA database (16), and a bar plot was created to visualize taxonomy resolved from taxonomic levels 1-7. Rooted and unrooted phylogenetic relationships were then established using MAFFT sequence alignment (17).

Feature table filtering and rarefaction. The feature table was first filtered to eliminate all samples that were not useful for our analysis (mitochondrial RNA sequences, dormitory common area samples, samples categorized as "NA"). The feature table was used to generate an alpha rarefaction plot from which an optimal sampling depth of 5195 was chosen. At this depth, representative sample richness is maintained while preserving all 594 samples from the 37 students and their bedrooms. To first determine if biotic and abiotic samples were significantly different (and therefore should be kept separate for the main analysis), the feature table was filtered to produce two separate tables containing only single-occupancy or only multiple-occupancy samples. For our main analysis, to assess differences between single and multiple occupancy dorms, the feature table was filtered to produce two separate tables containing only biotic or only abiotic samples.

Alpha and beta diversity analysis. Alpha and beta diversity metrics were evaluated and visualized in QIIME2 (10). To first determine if biotic and abiotic samples were significantly different, Kruskal-Wallis pairwise testing (18) was used to evaluate Shannon's diversity (19), Faith's Phylogenetic diversity (20), and Pielou's evenness (21) within biotic and abiotic samples (significance level $p < 0.05$). Subsequently, differences between single and multiple occupancy dorms were assessed by Weighted Unifrac and Unweighted Unifrac (22), Jaccard Index (23), and Bray-Curtis Dissimilarity (24). The results of beta diversity analysis were then evaluated by pairwise permutational multivariate analysis of variance (PERMANOVA, significance level $q < 0.05$) (25). The false discovery rate approach was used to correct for multiple comparisons, producing a q-value (adjusted p-value).

Unique taxa. To determine the number of taxa unique to single or unique to multiple-occupancy dorms, a taxa bar plot was generated as described above then visualized at the species level in QIIME2 (10). A comma-separated values (CSV) file containing the relative abundance of each organism in each unique sample was exported from QIIME2 and analyzed in Excel (v. 16.60). Samples were grouped by occupancy status (single or multiple) then the total abundance of each taxon in both groups was determined using the sum function in Excel. Taxa were considered unique to one group if their total abundance in the opposite group was 0, and this was summarized using the countif function. The number of shared taxa was obtained by subtracting the number of taxa unique to each group from the total number of taxa in the entire dataset. Unique and shared taxa between single and multiple-occupancy rooms for both biotic and abiotic groups were visualized using the VennDiagram package in R (v. 4.1.2) (13, 26).

Differential abundance. Differential abundance at the genus level was assessed in R (v. 4.1.2) using the following packages: qiime2R, phyloseq, and DEseq2 (27–29). Rare genera were pruned if relative abundance was less than 0.0005 and significance was assigned using an adjusted p-value of $p < 0.05$. Using the ggplot2 package (30) in R (v. 4.1.2), differential abundance was visualized as $\log_2$ fold change from single-occupancy.

Indicator taxa. Indicator species analysis was conducted in R (v. 4.1.2) using the following packages: qiime2R, phyloseq, and indicspecies (27, 28, 31). Taxonomic data was grouped at the species level using a custom-made function in R. Multipattern analysis was performed to determine indicator species from single and multiple-occupancy samples. Only indicator species with $p < 0.01$ were reported to narrow down the most relevant and high-confidence species.

RESULTS

Composition drives differences between single and multiple occupancy dorms. To investigate differences in composition between and evaluate diversity within single and multiple-occupancy dorms, alpha and beta diversity metrics were performed twice using QIIME2, once for biotic and once for abiotic samples. Biotic and abiotic samples were kept as separate groups because they were found to be significantly different according to all alpha and beta diversity metrics (Supplemental Table 1). Kruskal-Wallis testing and Pairwise PERMANOVA were used to assess significance as described in the methods (18, 25). Our

results showed that the composition of the biotic microbiome significantly differed between single- and multiple-occupancy rooms according to Jaccard, Bray-Curtis, and Weighted Unifrac metrics (**Table 1**, q = 0.006, q = 0.035, q = 0.024, respectively) (22–24). For the abiotic microbiome, Jaccard, Bray-Curtis, and Unweighted Unifrac were significant (**Table 1**, q = 0.0015, q = 0.009, q = 0.003, respectively) (22–24). Faith's Phylogenetic Diversity (20) of abiotic samples was the only alpha diversity metric that significantly differed between single- and multiple-occupancy rooms (**Table 1**, q = 0.028), indicating that multiple-occupancy samples tend to have more phylogenetic variation than single-occupancy samples. No other alpha diversity metrics were significant for either sample group. The significant beta diversity metrics indicate that single- and multiple-occupancy dorms are compositionally dissimilar, and this dissimilarity is more likely driven by abundance (Bray-Curtis, (24)) and observed ASVs (Jaccard, (23)) rather than phylogeny because the two metrics that incorporate phylogenetic distance (Weighted and Unweighted Unifrac, (22)) were inconsistently significant.

Table 1. Single and multiple-occupancy rooms significantly differ in both their biotic and abiotic microbiomes. Numbers represent q-values. * indicates statistical significance (q < 0.05) by Kruskal-Wallis testing (for alpha diversity metrics) or by Pairwise PERMANOVA (for beta diversity metrics).

	Diversity Metric	Biotic	Abiotic
Alpha	Observed Features	0.492	0.060
	Shannon's Diversity Index	0.930	0.357
	Faith's Phylogenetic Diversity	0.552	0.028*
	Pielou's Evenness	0.995	0.943
Beta	Weighted Unifrac	0.024*	0.204
	Unweighted Unifrac	0.078	0.003*
	Jaccard Distance	0.006*	0.0015*
	Bray-Curtis Dissimilarity	0.035*	0.009*

In both biotic and abiotic samples, multiple-occupancy dorms have more unique taxa than single-occupancy dorms. To better understand the compositional differences between single- and multiple-occupancy rooms, we calculated the number of taxa unique to each room type using the taxa bar plot generated in QIIME2. We found that multiple-occupancy dorms had more unique taxa than single-occupancy dorms in both abiotic and biotic sample groups (**Figure 1**). Despite the large number of taxa unique to each room, single- and multiple-occupancy dorms shared 56.2% of taxa from abiotic samples and 46.7% of taxa from biotic samples (**Figure 1**).

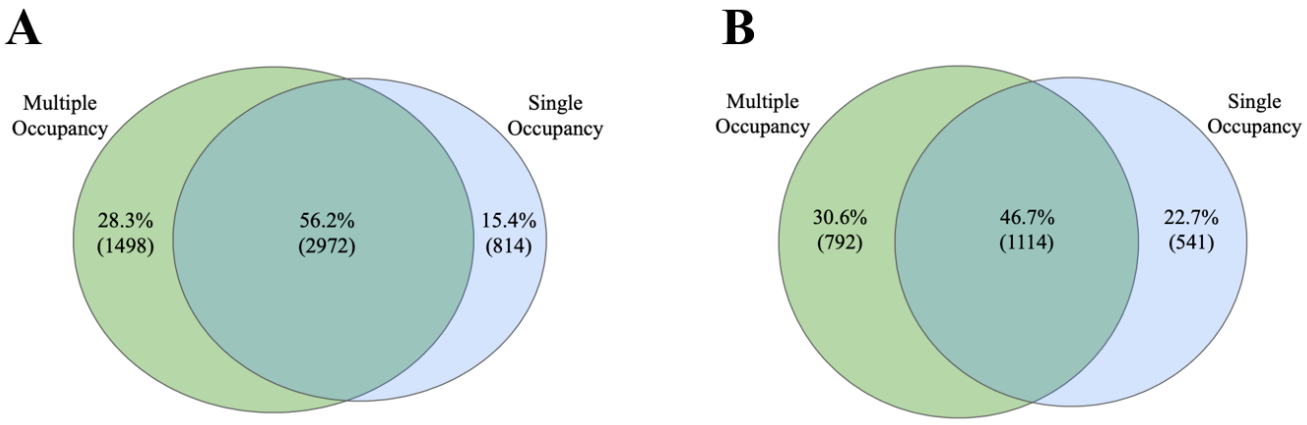

FIG. 1 Multiple-occupancy dorms have more unique taxa than single-occupancy dorms. (a) Abiotic unique and shared taxa. (b) Biotic unique and shared taxa. Numbers of shared and unique taxa were determined using taxa bar plots generated by QIIME2. Bracketed numbers reveal raw values for taxa within multiple- or single-occupancy categories. Overlap represents the shared taxa between both room types.

Common taxa decrease in abundance from single to multiple occupancy dorms. Although we discovered a large number of taxa unique to each room type, we sought to further investigate the taxa that were shared between single- and multiple-occupancy rooms using differential abundance analysis. This analysis aims to detect differences in taxonomic composition between two conditions. For our study, this analysis was done at the genus level using DESeq2 (29). Genera whose relative abundance changed significantly between single and multiple occupancy rooms were included in **Figure 1**, as defined by a p-value of less than 0.05. Overall, genera which showed significant changes in abundance mostly decreased when moving from single to multiple occupancy dorms. In particular, only the *Lactobacillus* and *Turicella* genera showed an increase in abundance in the multiple occupancy dorms, and these changes were relatively small. The *Corynebacterium* genus showed the largest decrease in abundance in our analysis, with over a 30-fold decrease in abundance between single and multiple occupancy dorms. Overall, in performing differential abundance analysis we found that fifteen genera showed significant changes in abundance, most of which were decreases, between single and multiple occupancy dorms.

Indicator species delineate single and multiple occupancy dorms. Our taxonomic analysis of single and multiple-occupancy samples revealed that each group has its own subset of unique taxa. To further examine what species are unique to single and multiple-occupancy samples, we performed indicator taxa analysis. This analysis was performed separately on biotic and abiotic samples. Only the indicator taxa resolved to the species level with a p-value equal to or less than 0.01 were included in this analysis to return the most biologically informative and significant species. The a-value measures the likelihood that a sample containing the indicator species truly belongs to the given group. The b-value reflects the likelihood that all samples in the given group contain the indicator species. Indicator values combine the a- and b-values and measure how likely it is that the indicator species will be present in all the samples of only one specific group. We consistently observed that a-values were lower than b-values for all sample groups (**Table 2**). This suggests that these indicator species represent organisms that are unique to their group but are only found in a minority of total samples. The samples with the largest number of indicator species came from single-occupancy dorms (**Table 2b, c**). Fewer species were found for abiotic multiple-occupancy dorms, and no indicator species were identified for biotic multiple-occupancy dorms (**Table 2a**). These indicator species delineate single- and multiple-occupancy samples and allow further exploration of the biological relevance of the organisms unique to a specific group.
Single occupancy dorms share indicator species between biotic and abiotic samples. During our analysis of indicator taxa, we observed that the same indicator species were present in both biotic and abiotic samples for the single occupancy setting. The four indicator species common to both abiotic and biotic single-occupancy samples were: *Corynebacterium matruchotii, Prevotella loescheii, Prevotella oulorum,* and *Prevotella saccharolytica* (**Table 2d**). Among these species, *Corynebacterium matruchotii* had the highest indicator value across both biotic and abiotic samples (**Table 2d**). The presence of indicator species common to both biotic and abiotic samples illustrates how an individual's microbiome can shape the microbiome of their physical environment and vice versa. These results indicate that significant indicator species delineate single-occupancy settings from multiple-occupancy and that this delineation is independent of sample type.

DISCUSSION

Given the large impact of the microbiome on human development and its ability to imprint on abiotic environments, further research was needed to elucidate how microbiome composition is influenced (3, 4). Previous research has shown that cohabitation has an impact on the microbial composition of biotic and abiotic environments (1, 4). However, no study had yet examined the effect of cohabitation on microbiome composition in a living environment where a large population of diverse individuals share the same space.

To increase the current understanding of how the microbiome changes in shared settings, we analyzed data collected by Richardson *et al.* to explore differences in the biotic and abiotic microbiomes between single and multiple-occupancy dormitories (9). Our findings demonstrate that cohabitation in the college dormitory setting alters the microbial

Table 2. Indicator species are present in single- and multiple-occupancy abiotic samples and single-occupancy biotic samples. (a) Abiotic multiple-occupancy indicator species. (b) Abiotic single-occupancy indicator species. (c) Biotic single-occupancy indicator species. (d) Shared single-occupancy indicator species. Only taxa resolved to the species level with $p \leq 0.01$ are shown. A-value is the positive prediction value and indicates the likelihood of a sample with this organism truly belonging to the given group. B-value is the specificity value and estimates the likelihood that every sample within the given group will contain the indicator species. Indicator value is a measure of how likely it is that a given organism will be present in all samples of only one specific group.

Organism	A-value	B-value	Indicator value	p-value
(A) Abiotic Multi-occupancy indicator species				
Actinomyces graeventizii	0.80266	0.22596	0.426	0.005
Bdellovibrio bacteriovours	0.78787	0.22115	0.417	0.005
Corynebacterium mycetoides	0.94533	0.0625	0.243	0.005
Roseomonas frigidaquae	0.9024	0.05769	0.228	0.01
(B) Abiotic single occupancy indicator species				
Corynebacterium matruchotii	0.8401	0.43646	0.606	0.005
Kocuria marina	0.81486	0.42541	0.589	0.005
Prevotella loescheii	0.86983	0.20994	0.427	0.005
Peptoniphilus duerdenii	0.77195	0.22099	0.413	0.005
Actinomyces dentalis	0.83883	0.1768	0.385	0.005
Prevotella oulorum	0.8047	0.17127	0.371	0.01
Selenomonas sputigena	0.86482	0.13812	0.346	0.005
Prevotella oris	0.72893	0.14917	0.33	0.01
Prevotella intermedia	0.99634	0.09945	0.315	0.005
Streptococcus agalactiae	0.96404	0.09945	0.31	0.005
Prevotella saccharolytica	0.81439	0.1105	0.3	0.005
Anaerococcus lactolyticus	0.99263	0.0884	0.296	0.005
Campylobacter concisus	0.9696	0.08287	0.283	0.005
Filifactor alocis	1	0.07735	0.278	0.005
Prevotella micans	0.90744	0.07735	0.265	0.01
Johnsonella ignava	0.97226	0.07182	0.264	0.005
Alloprevotella rava	0.93492	0.04972	0.216	0.01
Phocaeicola abscessus	1	0.03867	0.197	0.005
Catonella morbi	1	0.03867	0.197	0.01
Prevotella marshii	1	0.02762	0.166	0.01
(C) Biotic single-occupancy indicator species				
Corynebacterium matruchotii	0.8203	0.7083	0.762	0.005
Leptotrichia buccalis	0.8652	0.4167	0.6	0.005
Prevotella loesiceii	0.824	0.4167	0.586	0.01
Prevotella nigrescens	0.7703	0.375	0.537	0.01
Alloprevotella tannerae	0.937	0.2917	0.523	0.005
Prevotella denticola	0.9255	0.2917	0.52	0.01
Prevotella oulorum	0.8761	0.2917	0.506	0.005
Leptotrichia hongkongensis	0.7864	0.2917	0.479	0.005
Prevotella saccharolytica	0.9352	0.2292	0.463	0.005
Streptococcus anginosus	0.7272	0.2917	0.461	0.005
Prevotella veroalis	0.9541	0.2083	0.446	0.005
Streptococcus mutans	0.8566	0.2292	0.443	0.005
Porphyromonas uenonis	0.9196	0.125	0.339	0.01

(D) Shared single-occupancy indicator species

Organism	Abiotic indicator value	Biotic indicator value
Corynebacterium matruchotii	0.606	0.762
Prevotella loescheii	0.427	0.586
Prevotella oulorum	0.371	0.506
Prevotella saccharolytica	0.3	0.463

composition of the biotic and abiotic environment. We have shown that beta diversity significantly differs between single and multiple-occupancy dorms, indicating the two communities are dissimilar. Further analysis of shared and unique taxa between single and multiple-occupancy samples indicated that multiple-occupancy samples tend to have more unique taxa and less abundant shared taxa. Finally, we performed indicator taxa analysis to determine the unique species found in single and multiple-occupancy environments. This analysis revealed that single-occupancy environments have a large number of indicator species and that there are indicator species shared between abiotic and biotic single-occupancy samples. Multiple-occupancy environments had fewer indicator species in general and indicator species were only found for abiotic multiple-occupancy samples. This suggests that it may be more difficult to identify multiple occupancy samples using indicator taxa compared to single occupancy samples.

For both biotic and abiotic sample groups, beta diversity highlights significant compositional differences between single- and multiple-occupancy dorms. We first investigated if there was a significant difference in microbial diversity within and between single- and multiple-occupancy dorms. The results of these analyses revealed several significant differences in beta diversity in both the biotic and abiotic environments after data filtering, which suggested the two room types had distinct microbial compositions (**Table 1**). The lack of significant results in alpha diversity metrics suggested that the biotic and abiotic samples had similar levels of richness and abundance in both single and multiple-occupancy dormitories at the local level and indicated that single samples did not significantly differ in their diversity (**Table 1**). However, both the biotic and abiotic categories displayed several significant beta diversity results, which suggested the two room types as a whole were significantly dissimilar (**Table 1**). This data suggests that having multiple cohabitants within a single dorm alters the diversity of both the biotic and abiotic environments in terms of observed features and abundance. These initial results aligned with our hypothesis that cohabitation affects the microbial diversity of the skin microbiome and the physical environment. As the alpha diversity metrics were inconclusive, we decided to focus our study on determining the underlying causes behind the differing beta diversity.

Multiple-occupancy dorms have more unique taxa and less abundant shared taxa compared to single-occupancy dorms. Taxonomic analysis revealed that despite having a 46.7% overlap in taxa between single and multiple-occupancy dorms in the biotic environment, multiple-occupancy dorms had 251 more unique taxa than single-occupancy dorms (**Figure 1a**). This result was mirrored in the abiotic environment, where multiple occupancy dorms had 684 more unique taxa than single-occupancy dorms, despite a 56.2% overlap (**Figure 1b**). These results suggest a greater variety in bacterial taxonomy in multiple-occupancy dorms, possibly due to a greater number of unique microbiomes between the occupants, as compared to those who live alone. It can be hypothesized that since each individual has a unique microbiome, the presence of more individuals invites the possibility of more unique species. However, due to a lack of research in this area, there are no previous studies that can corroborate or explain these results, therefore this knowledge gap presents an ideal area for future research. Differential abundance analysis revealed that *Lactobacillus* and *Turicella* genera were, to a small degree, relatively more abundant in multiple-occupancy dorms than single-occupancy dorms (**Figure 2**). The *Lactobacillus* genera is an important member of the gut microbiome, and one of the most prominent probiotics (32). They have also been reported as endogenous inhabitants of healthy skin, which would explain why they were present in the skin samples, and may have been transferred to the abiotic environment through touch (33). Further research is required to confirm why these bacteria are more prominent in multiple-occupancy dorms, but it may be due to a greater surface area of skin in the room when there are multiple cohabitants, allowing a denser population of this genera to form. *Turicella* have been previously reported to be among the most common commensal bacteria within the external auditory canal, and may have been found on the skin microbiome due to scratching or touching of the ears (34). The increased abundance of this genera in multiple-occupancy dorms may be due to an increased number of individuals in the room, but further research is necessary to confirm this. The most drastic difference in abundance,

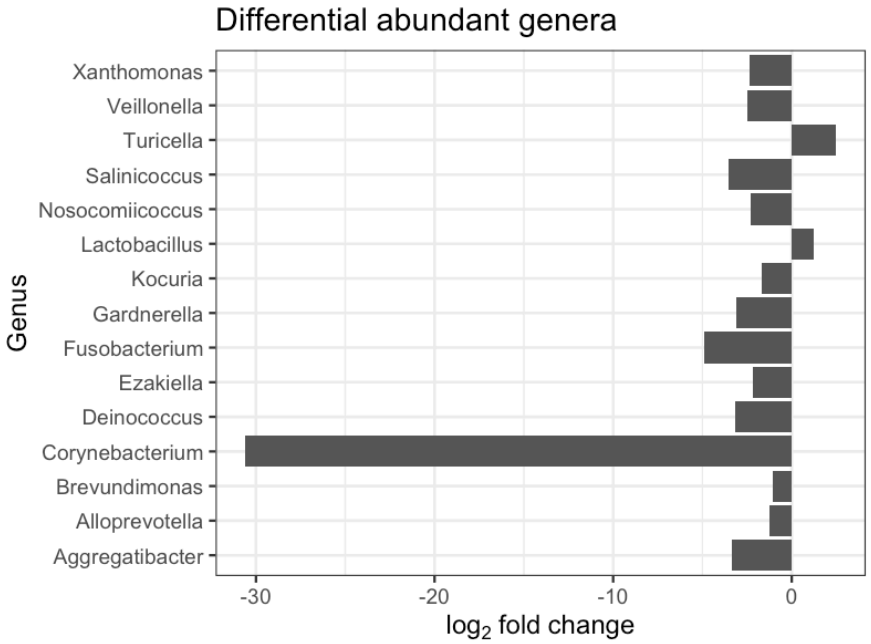

FIG. 2 Fifteen genera show significant changes in abundance, most of which decrease, between abiotic samples from single and multiple occupancy dorms. Relative abundance was calculated for single and multiple occupancy samples. Log-transformed changes in abundance from single to multiple occupancy dorms were plotted along the x-axis. This analysis included only genera with significant changes in abundance denoted by a p-value of less than 0.05.

however, was seen in the 30-fold decrease in *Corynebacterium* abundance when moving from single to multiple-occupancy rooms (Figure 2). Members of the *Corynebacterium* genus have been reported as members of the upper respiratory tract and the normal skin flora, which explains why they were present in skin samples (3, 35). Transference from the skin to abiotic surfaces such as doorknobs and bedsheets would explain the presence of these bacteria in the abiotic environment. However, it is unclear why this genus is underrepresented in multiple-occupancy dorms, and further research is required to fill this gap in knowledge. Overall, the differences in unique taxa and shared abundance between single and multiple-occupancy dorms aligned with our hypothesis that having one or more roommates affects the diversity of the skin microbiome and physical environment.

Biotic and abiotic samples share indicator taxa in a single-occupancy setting. Having established the existence of significantly diverse microbial communities between single and multiple-occupancy dorms, we sought to determine if the presence of any particular taxa could explain the differences seen in the two communities. Interestingly, single-occupancy dorms had the same four indicator species for both the biotic and abiotic environments. One of which, *Corynebacterium matruchotii,* has been previously found in the human microbiome and plays a major role in tooth biofilm formation (36). This bacterium is among the most prevalent species in the oral microbiome, but we have yet to understand how this bacterium was transferred to the skin microbiome and abiotic environment (36). Of note, the other three indicator species which belong to the *Prevotella* genus have also been shown to play a role in oral biofilm formation, and can lead to periodontitis when oral homeostasis is compromised (37). The *Prevotella* genus also inhabits the vaginal and gut microbiotas and may be recovered from respiratory tract infections (38). Since these species are commensal members of the biotic microbiome, it can be hypothesized that they were transferred to the abiotic environment from poor hygienic practices or lack of regular sanitation. The colonization of oral and mucosal sites by all four indicator species suggests transmission into the abiotic environment through sneezing, coughing, or breathing. The presence of these unique indicator taxa in single-occupancy rooms serves to explain how the compositional differences in microbial communities between single and multiple-occupancy rooms may have arisen, and further suggests that cohabitation affects diversity of the microbiome.

Multiple-occupancy dorms have four distinct abiotic indicator species. Multiple-occupancy dorms contained four distinct indicator species, all of which were present in the abiotic samples (**Table 2a**). The lack of indicator species in the biotic environment presents an interesting field of research for future studies to explore, as the dataset for our particular

study was unable to provide an explanation for this observation. Of the listed indicator species, *Actinomyces graeventizii* has been previously documented as a member of the oropharyngeal flora and has been described in several clinical cases of infections (**Table 2a**) (39). The role of this bacteria as an indicator species in multiple-occupancy dorms suggests possible increases in respiratory infections in areas of cohabitation. This has previously been discussed by Yang *et al*., who observed a strong association between multiple-occupancy rooms and an increased rate of respiratory infections in a college dormitory in China (40). *Bdellovibrio bacteriovours,* while not previously described as members of the biotic or abiotic microbiomes, have been shown to be potential alternatives to antibiotics due to their ability to prey on Gram-negative bacteria (41). The biological reasoning behind their role as an indicator species for multiple-occupancy dorms cannot be explained by the scope of this study. While *Corynebaterium mycetoides* itself has not been well described as a member of the human microbiome or the physical environment, the *Corynebacterium* genus as a whole has been reported to make up a large portion of the skin flora and upper respiratory tract (3, 35). *Roseomonas frigidaquae* has only previously been isolated from a water-cooling system, so its role as an indicator species in multiple-occupancy dorms for the abiotic environment must be explored further in future studies (42). In terms of our research question, the presence of indicator taxa for multiple-occupancy dorms suggests unique differences in the microbial communities between single and multiple-occupancy dorms.

Limitations Our selected data was taken from a single college dormitory, therefore any conclusions we make cannot easily be extrapolated to other age cohorts or environments. The small sample size of this dataset makes it difficult to apply our results to the general population, and further research is required to determine if these findings hold when more diverse environments and individuals are included. Since all data was collected from a college dormitory, all respondents likely belong to a similar socioeconomic class, therefore making it difficult to apply our results to a greater diversity of social statuses. Further, the metadata contained many other pieces of information (such as sex, type of abiotic surface, frequency of sheet washing, length of time with windows open) that could be contributing to the observed differences. While we have attempted to control for confounding factors by filtering the metadata, these distinct categories may be contributing to the observed effects. In addition, our study focused on beta diversity rather than alpha diversity, so all conclusions are based on whole population differences. Lastly, our study is unable to explain why certain taxa are present in single or multiple-occupancy dorms and can only describe that these differences exist. Further research is required to explain why certain genera are more present in one environment than the other, as this could not be answered by our particular dataset.

Conclusions Since the human microbiome has such a large impact on human life, it is critical to gain a deeper understanding of how the microbiome is formed and what aspects of a given environment can influence its composition. Living with roommates has previously been found to be a confounding factor in creating an effective prediction model that matches a microbiome to an individual (9). By using microbiome data collected in college dormitories to conduct diversity and taxa analyses, this study established that having one or more roommates induces significant changes on the diversity of the microbiome, and distinct indicator taxa are present in both single and multiple occupancy rooms. This data suggests that certain microbial taxa have the potential to be used as indicators to determine whether an individual lives alone. In addition, our data contributes to the idea that people leave behind microbial signatures that are unique to their specific person. These findings provide the information necessary to inspire further research into the critical factors influencing the microbial composition of the biotic and abiotic environments within college dormitories.

Future Directions Our investigation into the effect of cohabitation on the biotic and abiotic microbiomes came to its conclusions by aggregating all multiple-occupancy dormitories together, disregarding differences in the number of inhabitants. Therefore, the design of this study prevented comparisons between individual rooms, and further studies should explore the differences in microbial diversity in each room and elucidate potential trends in increasing numbers of roommates. Considering previous findings on the role of the unique microbiome

in profiling individuals who live alone, further research may also seek to predict which type of room an individual inhabits by looking for the presence of specific taxa on their skin or in their environment. Our study determined significant differences in beta diversity between biotic and abiotic environments, however the scope of our study did not extend to differences between the different types of sampled abiotic surfaces. It would therefore be worthwhile to examine if there are significant differences between each surface type. Further, possible confounding factors in our results may be elucidated by further analysis of other metadata categories, including sex of the inhabitant and frequency of sheet washing.

Differential abundance analysis revealed changes in shared taxa between single and multiple-occupancy dorms, however the biological reasoning behind these differences could not be elucidated using our particular dataset. Therefore, future studies could evaluate the conditions that lead to the observed differences in abundance. In addition, indicator bacterial taxa were identified for single and multiple-occupancy dorms, but the reasoning behind their presence in certain environments has not yet been explained. Since many of the indicator taxa have not been thoroughly described as being part of the skin microbiome or abiotic environment in previous literature, future studies may seek to address this knowledge gap. Future studies may also strive to explain the mechanism behind altered microbiomes in the presence of roommates and understand how increased microbial competition may play a role in changing the microbiome.

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CONTRIBUTIONS

Co-authorship should be considered equal for this manuscript. AL contributed to the abstract, introduction, results, and figure captions. KM contributed to the methods, results, and discussion. CM contributed to the methods, table captions, results, and overall formatting. SR contributed to the discussion, limitations, conclusion, and future directions. All authors contributed equally to the analyses, ideas, and conclusions outlined in this manuscript.

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