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High temporal variability in airborne bacterial diversity and abundance inside single-family residences

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Abstract

Our homes are microbial habitats, and although the amounts and types of bacteria in indoor air have been shown to vary substantially across residences, temporal variability within homes has rarely been characterized. Here, we sought to quantify the temporal variability in the amounts and types of airborne bacteria in homes, and what factors drive this variability. We collected filter samples of indoor and outdoor air in 15 homes over 1 year (approximately eight time points per home, two per season), and we used culture-independent DNA sequencing approaches to characterize bacterial community composition. Significant differences in indoor air community composition were observed both between homes and within each home over time. Indoor and outdoor air community compositions were not significantly correlated, suggesting that indoor and outdoor air communities are decoupled. Indoor air communities from the same home were often just as different at adjacent time points as they were across larger temporal distances, and temporal variation correlated with changes in environmental conditions, including temperature and relative humidity. Although all homes had highly variable indoor air communities, homes with the most temporally variable communities had more stable, lower average microbial loads than homes with less variable communities.

KEYWORDS

bacteria, family residences, homes, microbial ecology, qPCR, temporal variability

1 | INTRODUCTION

Microorganisms are ubiquitous in indoor air, yet we know little about the composition and dynamics of microbial communities in the built environment. On average, humans spend ~90% of their time inside,[1,2] so it is important to learn what benign, beneficial, and/or harmful microbes we could be inhaling indoors, along with the ecological factors that help to shape indoor air microbial community composition. It is well known that some airborne microorganisms can have negative influences on human health, not only through pathogenesis, but also by serving as allergens capable of triggering respiratory ailments, such as asthma and allergies,[3–8] and reduced bacterial loads in indoor environments can be associated with better health outcomes.[9]

Until recently, microscopy and culture-based studies have been the primary means of studying microorganisms in indoor air.[10,11] Microscopy is useful for obtaining estimates of total microbial concentrations in indoor air, but cannot offer high-resolution insight into the taxonomic composition of these bacterial communities, owing to the morphological similarity of many microorganisms. Culturing techniques cannot detect most of the bacteria found inside homes because the vast majority of microorganisms remain uncultivated.[11–13] However, high-throughput DNA sequencing approaches avoid cultivation biases and allow for microbial community characterizations with high taxonomic resolution (at the species level in many cases). As such, DNA sequencing is now widely used in microbial ecology, including in studies of the built environment (e.g.,[14–22]).

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Although there have been relatively few culture-independent characterizations of microbial communities in the air in single-family residences, recent studies have characterized microbial communities in a variety of indoor environments, including university and elementary school classrooms, office buildings, healthcare settings, apartment buildings, daycare centers, the retail environment, and public restrooms.[18–20,23–34] These studies have indicated that the diversity and composition of indoor microbial communities can be strongly influenced by numerous factors, including architectural design, ventilation rates, occupants (e.g., humans and pets), and outdoor air community composition.[15,16,19,20,23,27,31,33,35–37] Changes in the human microbiome signal on home surfaces have been observed over the timescale of days,[21] and temporal variation in indoor air communities has been observed over timescales of approximately 1 week.[33] Over the course of ~300 days, regional temporal variation of floor dust microbial communities was inferred from single samples collected from many homes.[38] However, few studies have evaluated indoor air microbial community composition by resampling the same homes over timescales of months or longer.

In this study, we sought to answer three primary questions: (i) Are airborne bacterial communities in family residences so temporally variable that a single sample is unlikely to adequately capture the amounts and types of bacteria found in the air inside a home? (ii) Do any of the measured ecological and/or environmental variables correlate with indoor air bacterial community composition? and (iii) Does the magnitude of temporal variability in any of the measured environmental variables correlate with the magnitude of temporal variability in the abundance and types of bacteria found in indoor air? To address these questions, we collected indoor and outdoor air bacterial communities from 15 homes over the course of 1 year and compared airborne bacterial community composition and abundance over time with measured environmental parameters.

Practical Implications

- We observed a high degree of temporal variability in indoor air bacterial community composition in single-family residences, suggesting that multiple, temporally resolved measurements are required to capture indoor air microbial community composition in future studies. Because microbial exposures within the built environment can affect occupants' health, the potential to directly or indirectly manipulate microbial community composition by altering the indoor environment is one promising avenue toward promoting healthier buildings and occupants. To this end, we have identified some of the environmental factors that correlate with microbial community composition in homes, including temperature and relative humidity. Results of our comparisons between microbial community variability and the variability in environmental parameters over time suggest a greater degree of resuspension and higher microbial loads in homes with less variable microbial communities. Overall, results suggest that the highly variable microbial communities found within homes are influenced by complex interactions between physical, environmental, and ecological characteristics of the home environment.

2 | METHODS

2.1 | Sample and metadata collection

Volunteer homeowners were recruited for this study through personal contacts and e-mail inquiries. Requirements for participation in the study were as follows: a home within reasonable driving distance of the University of Colorado, Boulder campus (<2 hours), between 2 and 5 non-smoking occupants who had lived in the home for at least 1 year, and a living space between 100 and 500 m^2. All participants gave approval to participate in the study, in accordance with University of Colorado, Boulder IRB Protocol # 12-0624. All 15 homes were located in the vicinity of Boulder, CO, USA, including 10 homes in the city of Boulder, two homes in Fort Collins, and one home each in Niwot, Longmont, and Lyons. Exact home locations are not provided to protect the privacy of the occupants. The homes are labeled at random with alphabetical letters A-R, skipping letters I, L, and O to avoid confusion with the numbers 0 and 1 on sample labels.

Building and occupant characteristics for each home are provided in Tables S1 and S2, and occupant-reported times spent on various activities inside each home during the sampling events are provided in Table S3. Methods text for those measurements is in the Supplementary Material. Briefly, homes were in both urban and rural settings, eight homes included families with children (seven were only adult-occupied), 11 homes had pets (e.g., eight homes had at least one dog and five homes had at least one cat), and homes had a variety of heating, ventilation, and air-conditioning (HVAC) systems. A blower door test was used to measure the envelope tightness of each home, and across homes, the air change rates at 50 Pa ranged from 4.2 to 16.4 per hour.

Each home was sampled at eight time points, approximately twice per season for approximately 1 year (November 2012 - December 2013). Home D was not sampled after the fifth time point because that home experienced significant flooding during the September 2013 Colorado Front Range flood.[39] During each home visit, particulate matter (in $\mu g/m^3$ for both PM_{10}, particles with diameters <10 μm, and $PM_{2.5}$, particles with diameters <2.5 μm), total suspended particles (TSP) for microbial analysis, temperature, relative humidity (RH), and dew point were monitored inside the home for 24 hours. Outside the home, we monitored particulate matter ($PM_{2.5}$), TSP, temperature, and RH. These environmental measurements are reported in Table S4.

The microbial air filter samples were collected by sampling total suspended particles onto open-faced 0.45-μm nitrocellulose filters (4.15 cm in diameter) with a vacuum pump (KNF Neuberger, Inc., UN816.1.2 KTP Mini Diaphragm Vacuum Pump). The flow rate was 10 L per minute for both indoor and outdoor air samples, resulting

in a total volume of approximately 13.2 m^3 of air per 24-hour sample pulled through each filter. To determine whether microbial communities collected on these air filters (24-hour samples) were more variable than airborne microbial communities collected via other common techniques, swabs of an interior door frame surface ("door trim," presumed to be a passive dust collection surface) and dust from the home's HVAC filter (if available) were also collected. Door trim swabs were collected, processed, and sequenced as in Barberán et al.[40] and Barberán et al.,[41] and HVAC filter swabs were collected, processed, and sequenced as in Emerson et al.[39] We installed new HVAC filters in each home at the start of the study, and we provided new HVAC filters to each home once per season (four times throughout the study).

2.2 | DNA extraction, PCR, and sequencing

For DNA extraction from the air filters, four circular punches (each 0.6 cm in diameter) were collected from each filter under sterile conditions and placed in a single well of a MoBio PowerSoil-htp 96-well Soil DNA Isolation Kit (MoBio, Inc., Carlsbad, CA, USA). Negative DNA extraction controls were included for subsequent contamination assessment and removal of likely contaminant taxa, as per Emerson et al.[39] DNA was extracted according to the manufacturer's instructions (MoBio Inc., Carlsbad, CA, USA), with previously described modifications.[42] PCR amplification was performed in triplicate for each DNA sample, as described previously.[39,43,44] Briefly, barcoded 515F and 806R primers were used to amplify the V4 region of bacterial and archaeal 16S rRNA genes.[45] As such, note that "microbial" throughout this text means "bacterial and archaeal," unless otherwise indicated. In addition, because the vast majority of recovered OTUs were bacterial in all samples, we often use "bacterial" instead of "microbial" to avoid confusion with other microbial studies that also consider other microbes, for example, fungi, which were not analyzed in this study. The 1×5 PRIME Hot Master Mix (5 PRIME Inc., Bethesda, MD, USA) was used for PCR reactions. Negative controls were included to test for contamination (no sequences were recovered from negative PCR controls). Triplicate PCRs were pooled for each sample, and amplicon concentrations were measured with a PicoGreen dsDNA assay (Life Technologies, Grand Island, NY, USA). Amplicons were pooled by plate at equimolar concentrations for each sample and then cleaned with the UltraClean PCR Clean-Up Kit (MoBio Inc., Carlsbad, CA, USA). The cleaned pools were combined at a final yield of 2 μg of DNA and sequenced on the Illumina MiSeq platform at the University of Colorado Next Generation Sequencing Facility. All sequencing data will be available at datadryad.org upon publication, searchable by the citation for this article.

2.3 | Sequence processing

Sequences were processed as previously described.[39] Briefly, sequences were demultiplexed, and forward and reverse 16S rRNA gene reads were merged. All resulting sequences were quality-filtered, and singletons were removed with QIIME[46] and UPARSE.[47] Sequences were then dereplicated, and a database containing one sequence for each operational taxonomic unit (OTU) was generated using UCLUST v7[48] at the 97% nucleotide identity level. Sequencing reads from the full dataset were then clustered to the database to generate an OTU table. Taxonomy was assigned to each OTU using the Ribosomal Database Project taxonomic classifier.[49] The OTU table was rarefied to 1000 sequences per sample to achieve equal sampling depth, a practice commonly used in comparable studies (e.g.,[16,50–53]) and recommended in Weiss et al.[54] OTUs at 10% or higher relative abundance in the DNA extraction controls were removed prior to downstream analyses, as were all mitochondrial and chloroplast OTUs. Less than 10 samples had >20% contaminating sequences (i.e., <80% of sequences remaining after contaminant OTUs were removed), and these samples were not included in downstream analyses.

2.4 | Quantitative PCR (qPCR)

The DNA extracted from filter samples was also used for qPCR with universal bacterial primers to determine bacterial abundance, as described in Emerson et al.[39] Briefly, we used the 515F/806R bacterial 16S rRNA gene primer pair for the qPCR assays (the same primer pair used for DNA sequencing, but without the adapters required for Illumina library construction). Seven 10-fold dilutions of genomic DNA from *E. coli* K-12 were used to make a standard curve for each 96-well qPCR plate. As such, our results are reported in *E. coli* genome equivalents, but they can be interpreted as approximate estimates of the total number of bacterial cells per m^3 of air. Each standard dilution, negative control, and sample was run in triplicate qPCR reactions on a Mastercycler ep realplex thermocycler (Eppendorf, Hamburg, Germany) in 96-well plates (40 cycles per reaction). Comparisons with the standard curve gave the estimated genome copy number in each qPCR reaction, and we report the estimated genome copy number per m^3 of air, calculated by accounting for reaction volumes, area of the filter analyzed, flow rate, and sampling time.

2.5 | Statistical analyses

Statistical analyses were conducted in R,[55] unless otherwise indicated. In accordance with some previous studies (e.g.,[56–58]) rarefied OTU tables were square-root-transformed, and Bray–Curtis dissimilarity matrices were generated for sample comparisons. We used permutational MANOVA (PERMANOVA) as implemented in PRIMER v6[59,60] to test for significant differences in bacterial community composition between groups of samples (e.g., within vs. between homes, in indoor vs. outdoor air, and for categorical home characteristics). We also used Mantel tests as implemented in PRIMER v6[59] to compare bacterial community composition (Bray–Curtis dissimilarity matrices) to Euclidean distance matrices of environmental variables, qPCR abundance data, and numerical measures of home characteristics (i.e., home surface area, home volume, the number of residents, the number of plants, and ventilation potential score) and to compare qPCR abundance data with environmental variables and numerical home characteristics. Unless otherwise reported, all *P*-values are the result of 1000 random permutations of the data. We used the BIOENV function in R to identify the factors most significantly correlated with

indoor air microbial community composition across the dataset. Using PRIMER v6,[59] we performed Spearman's rank correlations between average indoor air microbial community Bray–Curtis dissimilarities by home and average Euclidean distances by home for each environmental variable, qPCR abundance data, and each numerical metric of home characteristics, in order to identify factors correlated with degrees of variability in microbial community composition. Similarly, we performed Spearman's rank correlations between qPCR data and environmental variables and home characteristics to identify factors correlated with variability in microbial abundance. Results from all of these statistical analyses are reported in Table S5.

A source-tracking analysis was performed as described in Dunn et al.[16] and Barberán et al.[41] Briefly, microbial taxa known to be associated with specific source environments, including human skin, the human oral cavity, human stool, leaf surfaces, and soil, were identified, and their relative abundances were summed for each sample (Table S6). Comparisons of the relative abundances of these groups of taxa were used to estimate the relative importance of the contributions from different source environments to the airborne microbial communities.

3 | RESULTS AND DISCUSSION

3.1 | Composition of indoor air microbial communities as compared to outdoor air

After DNA sequence processing, 104 indoor air and 94 outdoor air samples were included in downstream 16S rRNA gene sequencing analyses. The focus of this study is on the airborne microbial communities inside 15 family residences and the factors that contribute to microbial community composition and variability in indoor air. As such, our analyses focus primarily on the indoor air samples, with information from outdoor air used for comparative purposes and to determine how the bacteria found in outdoor air may be influencing indoor air community composition (Figures 1 and 2A).

Across the full dataset, microbial community composition was significantly different in indoor, relative to outdoor air (PERMANOVA P=.001). Indoor and outdoor air had similar richness (i.e., a similar number of microbial operational taxonomic units, OTUs, or "species" identified), ranging from 30 to 394 (average 159) OTUs per sample for indoor air and from 46 to 427 (average 170) for outdoor air. There was no significant difference in richness between indoor and outdoor air (Student's t-test P=.305), consistent with previous work showing similarities in diversity between indoor and outdoor air microbial communities associated with university classrooms over a ~1-week period.[33] Average bacterial abundance, as measured by qPCR (Figure 3A), was slightly higher in outdoor air, relative to indoor air across the study (Student's t-test P=.015), although some homes showed higher average abundance in indoor air (Figure 3B).

Interestingly, despite significant differences in overall community composition between indoor and outdoor air, many of the most abundant taxa were found both indoors and outdoors, with members of the Actinomycetales, Bacillales, and Pseudomonadales orders at particularly high abundance in both environments (Figure 1). These groups of bacteria have been shown to be abundant in previous studies of indoor and outdoor air microbial communities,[14,33,42,61–63] and members of these bacterial groups are found in a variety of environments, including soil, water, human skin, and household surfaces.[17,28,35,64–66]

Across the dataset (with some exceptions for individual homes), the relative contributions of taxa predicted to be sourced from human skin were significantly higher in indoor air microbial communities (Student's t-test P=7e−7), and taxa predicted to be sourced from soil tended to be higher in outdoor air communities (P=.011) (Figure 1, Table S6). Taxa sourced from the human oral cavity were also significantly more abundant in indoor air (P=2e−9), but interestingly, taxa predicted to be sourced from human stool were at slightly higher relative abundance in outdoor air (P=.002), which we speculate may be due to contributions from related but different sources, such as dog feces, which has previously been shown to contribute significantly to outdoor air communities in the winter.[63] Also somewhat surprisingly, taxa predicted to be sourced from leaf surfaces were at significantly higher relative abundance in indoor air (P=6e−7), perhaps due to contributions from indoor plants and/or produce, which has previously been shown to have a strong signal on kitchen surfaces.[17]

Individual taxa that were significantly more abundant in indoor, relative to outdoor, air include Mycobacterium (a genus of Actinobacteria) and the Pasteurellales. Non-tuberculous mycobacteria can be opportunistic human pathogens and may be particularly problematic for individuals with compromised respiratory or immune systems, and they are thought to be aerosolized from biofilms inside shower heads.[67] Pasteurellales have been shown to be associated with cats and dogs in indoor environments,[41] and they are also common human respiratory commensals,[68–70] which could be aerosolized in homes through breathing, coughing, sneezing, etc. Other taxa at appreciably higher abundance in indoor air, relative to outdoor air, include Mycoplana, Thermoanaerobacter, Comamonas, Acidaminococcus, Paraprevotella, Methylobacter, Chloroflexi, and Sphingophyxis, several of which are commonly associated with human skin. Only Caulobacter, which is commonly found in freshwater lakes, streams, and soil,[71–74] was significantly more abundant in outdoor air, relative to indoor air. The detection of human-associated taxa indoors is not surprising and is consistent with previous studies that have shown human occupants to be a major source of indoor air bacterial taxa.[19,33,75]

3.2 | Indoor air microbial communities within and among homes

Indoor air microbial community composition was highly variable across the 15 homes that were sampled (PERMANOVA P=.001). Most homes harbored distinct communities, highlighting the high degree of interhome variability in bacterial community composition. Specifically, 87.5% of pairwise comparisons between homes, including all time points, showed a notable difference in bacterial community composition (PERMANOVA P<.1). Differences in microbial community composition between homes are highlighted in Figure 1.

When considering the full dataset (as opposed to separate analyses of individual homes, described later), none of the measured

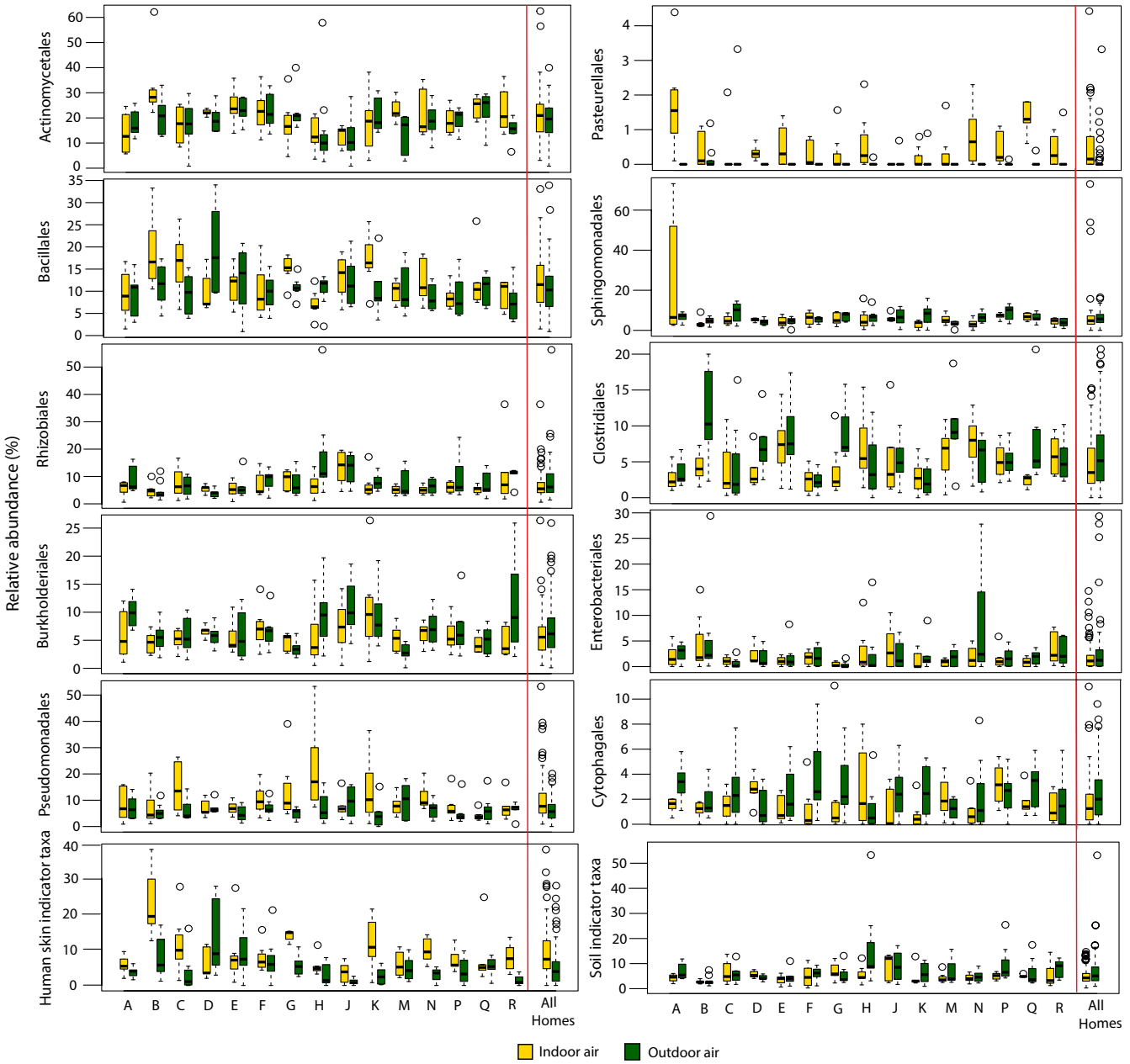

FIGURE 1 Relative abundance of different bacterial taxa in indoor and outdoor air. Ten bacterial orders were selected (each in a separate plot), as they were the most abundant and/or most different in abundance between indoor and outdoor air. The bottom two plots indicate the relative abundance of taxa from human skin and soil source environments, respectively. On the right side of each plot, "all homes" indicates average abundance across the dataset. The line through the middle of each boxplot represents the median, the box is the 25th to 75th percentile, and the whiskers (dashed lines) indicate the maximum and minimum values, apart from outliers, which are indicated with circles beyond the whiskers. Note that the *y*-axis scales differ between plots

environmental variables was significantly correlated with indoor air microbial community composition, but a variety of categorical home characteristics were significantly correlated (PERMANOVA $P<.01$). These included the presence of one or more dogs, fireplace type, how long the family had lived at the residence, heating type, AC type, and whether or not the HVAC system used electrostatic filters (Table S5). Many of these variables are co-correlated, and the fact that these measurements were constant over the course of the study means that each sample from the same home had the same measurement, which could inflate the significance of these correlations. Still, there was a strong within-home signal in microbial community composition, and that signal was partly related to a subset of the measured home characteristics.

Although the differences in community composition between homes were slightly higher than the differences observed within each home over time, temporal variability within a home was still quite high (average Bray–Curtis dissimilarity between homes=0.83, within

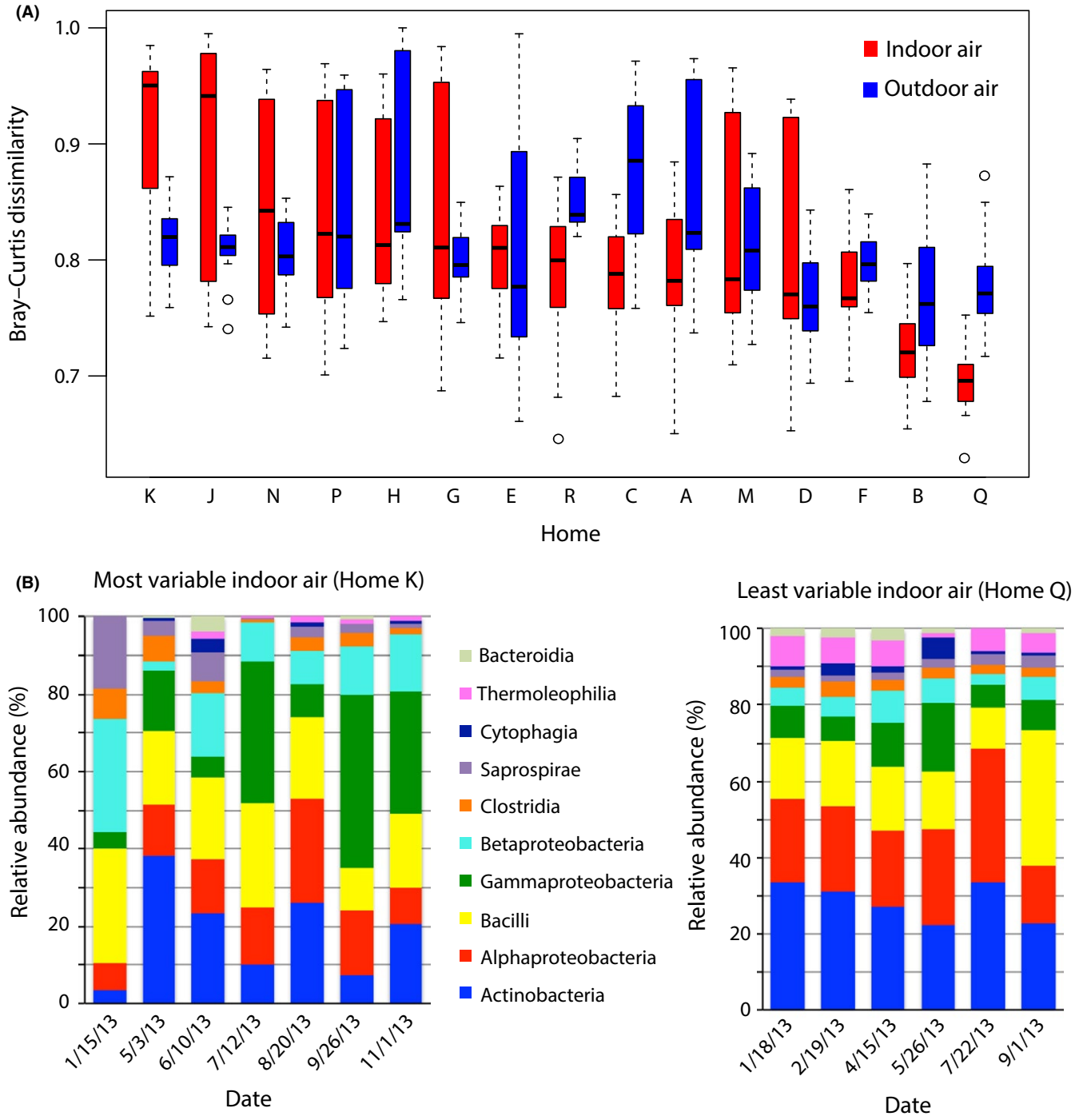

FIGURE 2 Temporal variability of indoor air bacterial community composition. A: Mean Bray–Curtis dissimilarity (a measure of variability in bacterial community composition) across time points within each home, in rank order of most to least temporally variable home. B: Changes in the relative abundance of major taxa in the two homes, in which we identified the most (Home K) and the least (Home Q) temporal variability in bacterial community composition

a home=0.80). To test for a time–decay relationship[76] and/or a seasonal signal in microbial community composition, we compared Bray–Curtis dissimilarities with the temporal distance between sampling time points for each home (Table S5). In other words, we quantified whether samples collected closer together in time from an individual home had more similar microbial communities than samples collected from the same home over longer periods of time. There was no significant correlation with temporal distance for 13 of the 15 homes, or across all indoor air samples in the study considered together, suggesting that most airborne bacterial communities in homes are just as different a few weeks apart as they are many months apart. However, homes R and J did show correlations with the temporal distance between sampling times, indicating that samples collected at those two homes were more similar over shorter timescales. Overall, these

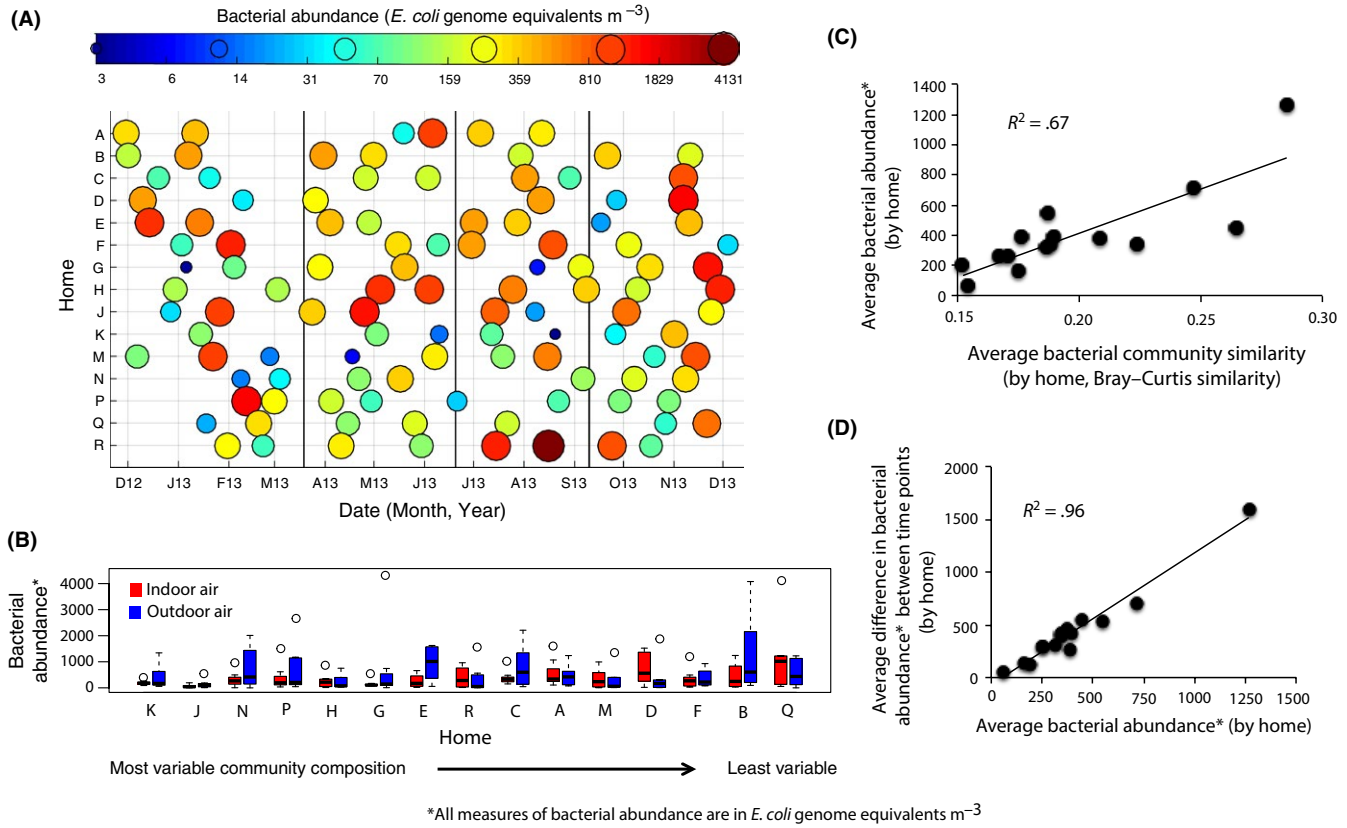

FIGURE 3 Bacterial abundance as determined via qPCR. Abundance measurements are in *E. coli* genome equivalents per m^3 and can be interpreted as bacterial cellular concentrations (see Methods). A: Indoor air bacterial abundance in each home at each time point. Note that this panel also illustrates the sampling scheme for the study, showing each 24-hour sampling point; B: Indoor and outdoor air bacterial abundance for each home; homes are in order of decreasing temporal variability of bacterial community composition (as measured by average Bray–Curtis dissimilarity) from left to right; the line through the middle of each boxplot represents the median, the box is the 25th to 75th percentile, and the whiskers (lines) indicate the maximum and minimum values, apart from outliers, which are indicated with circles beyond the whiskers; C: Average bacterial abundance (*y*-axis) versus average bacterial community similarity (*x*-axis) for each home. Note that Bray–Curtis similarity (i.e., 1 – dissimilarity) is shown; D: Average difference in bacterial abundance (i.e., the average Euclidean distance) between each pair of time points (*y*-axis) versus average bacterial abundance (*x*-axis) for each home

results demonstrate that microbial communities in home air are highly variable over time, and the microbes present in a home on a given date are not generally predictive of which microbes will be found in that home at another time, even if the sampling dates are only a few weeks apart in the same season. These results are consistent with a recent meta-analysis of the time–decay relationship in microbial diversity across 76 different sites, including air, soil, human-, and plant-associated environments, which revealed that only ~26%-40% of microbial communities show discernable time-decay patterns.[76]

3.3 | Factors that contribute to differences in microbial community composition and abundance within a home over time

We next sought to determine whether the temporal changes in the composition of the indoor air bacterial communities could be predicted from measured ecological factors (i.e., measured environmental variables and/or outdoor air microbial community composition). For this analysis, we used Spearman's rank correlations to compare Bray–Curtis dissimilarities for indoor air microbial community composition with Bray–Curtis dissimilarities of outdoor air microbial community composition and with Euclidean distances of various environmental variables, including indoor PM_{10}, indoor $PM_{2.5}$, indoor bacterial abundance via qPCR, indoor relative humidity (RH, %), indoor temperature (T, °C), dewpoint, outdoor temperature (T, °C), and outdoor relative humidity (RH, %). The full range of measured indoor temperatures across the study was 10.6 to 31.4°C (average 21.2°C), and indoor RH ranged from 7.2% to 73.2% (average 34.2%) (Table S4). Environmental measurements showed a range in the temporal variability of these factors across homes, with some homes varying quite a bit over the course of the study (e.g., by 10 or more °C in average temperature) and others being more stable (e.g., average temperatures within a ~3-4°C range). Similar trends were observed for other variables (Table S4).

For 10 homes, we observed significant correlations between microbial community composition and at least one of the measured variables (Table S5). When considering all homes together, combinations of variables did not significantly improve predictive power over single

TABLE 1 Spearman's rho and *P*-values for the four environmental variables best correlated with indoor air microbial community composition

Home	Indoor RH, rho	Indoor RH, *P*	Indoor T (deg C), rho	Indoor T (deg C), *P*	Dew point, rho	Dew point, *P*	Outdoor RH, rho	Outdoor RH, *P*
A	**0.461**	**.019**	0.037	.43	**0.425**	**.031**	**0.482**	**.013**
B	0.226	.101	0.125	.276	**0.289**	**.077**	0.137	.191
C	0.135	.214	**0.383**	**.043**	0.037	.363	−0.01	.457
D	0.418	.242	−0.115	.551	0.2	.308	0.018	.415
E	0.104	.278	−0.129	.7	0.186	.218	0.129	.292
F	**0.392**	**.04**	0.148	.195	**0.285**	**.067**	**0.299**	**.098**
G	**0.281**	**.066**	0.036	.369	0.3	.105	0.401	.142
H	−0.381	.961	−0.223	.721	−0.322	.925	−0.013	.511
J	0.6	.155	0.543	.249	0.6	.17	0.6	.148
K	0.104	.28	−0.284	.926	0.043	.358	**0.407**	**.064**
M	0.199	.15	**0.326**	**.089**	0.23	.125	−0.055	.562
N	0.024	.367	0.134	.174	−0.043	.517	0.038	.324
P	−0.049	.573	0.017	.398	0.056	.387	0.004	.487
Q	0.025	.423	0.275	.14	−0.064	.568	−0.375	.85
R	0.382	.172	**0.794**	**.039**	0.455	.217	0.467	.111

Bolded values indicate $P<.1$.

variables. Interestingly, different variables or combinations of variables were best correlated with indoor air microbial community composition in individual homes, and no variable was significantly correlated with community composition for more than three homes. Five homes (D, E, J, P, and Q) had no variables correlated with community composition, suggesting that none of our measured variables were significant predictors of the changes in microbial community composition observed in those homes. Correlation coefficients and *P*-values for the four variables—specifically, indoor RH, indoor T, dewpoint, and outdoor RH—that correlated significantly with indoor air microbial community composition in at least three homes are reported in Table 1 (comparisons of all homes with all measured variables are in Table S5). Together, these analyses suggest that the temporal variability within a home can be difficult to predict from these commonly measured variables, but that a few variables (most notably indoor RH and temperature) are most strongly associated with the observed temporal variation within homes. For all of our results, we cannot rule out the possibility that factors that were not measured, such as differences in cleaning regimes, might also contribute to changes in community composition and/or abundance.

We performed the same Spearman's rank correlation analyses to identify environmental factors correlated with microbial abundance, as measured via qPCR (Table S5). When considering the full dataset together (as opposed to separate analyses for each home), air-conditioning (AC) system type, PM_{10}, and $PM_{2.5}$ were significantly correlated with bacterial abundance. Interestingly, when separating the analyses by home, the only factors that correlated significantly with microbial abundance in any of the homes were PM_{10} (four homes: C, D, M, and N) and $PM_{2.5}$ (two homes: C and D). Although not statistically significant, abundance for three additional homes (A, E, and H) also showed correlations ($\rho>0.3$) with PM_{10}, and abundance for four additional homes (A, H, J, and N) showed correlations with $PM_{2.5}$. Together, these results suggest that although PM_{10} and $PM_{2.5}$ can be useful proxies for inferring microbial abundance in indoor air in some homes, the two are not always correlated with microbial abundance.

3.4 | Differences in the degree of indoor air microbial community variability among homes

Although microbial community composition was highly variable in all homes, we sought to determine whether some homes had more temporally variable microbial communities than others. In other words, we identified those homes with microbial communities that were highly variable over time and compared them to homes with more stable microbial community composition. The degree of variability differed across homes, with home K as the most variable and home Q as the least variable (Figure 2). Indoor and outdoor air microbial communities had similar average variability (average Bray–Curtis dissimilarity within a home for indoor air=0.81 and for outdoor air=0.82), highlighting that both indoor and outdoor airborne bacterial communities changed substantially over time in this study.

To determine whether any of our ecological data could explain differences in the degree of variability in microbial community composition across homes, we calculated Spearman's correlations of the average Bray–Curtis dissimilarity for each home with variability in environmental parameters and numerical measures of home characteristics (e.g., surface area, ventilation potential score, the number of residents), as measured by the average Euclidean distance between measurements for each home. Although variability in most environmental parameters and home characteristics did not correlate with variability in community composition, a highly significant correlation was observed between microbial community variability and variability in microbial abundance, as measured by qPCR ($\rho=0.63$, $P=.001$, Table S5).

Interestingly, these results show that homes with the highest variability in microbial community composition (i.e., the lowest average Bray–Curtis similarity) actually had more stable, lower average microbial abundance than homes with less variable communities (Figure 3C). Although more variable microbial abundance might not necessarily equate to higher microbial abundance (a stable high abundance could conceivably be less variable than a varying low abundance), in our study, homes with higher average microbial abundance had more variable abundance over time (Figure 3D). None of the measured variables was found to correlate with the variability in bacterial abundance (Table S5).

We hypothesize that the association between high (and more variable) indoor air microbial concentrations and reduced variability in microbial community composition may be a product of differing degrees of particle resuspension (e.g., from floor dust through occupant movement or vacuuming). For example, if repeated resuspension from the same source were to occur in a particular home, then the airborne microbial communities might be consistently dominated by microbes from that single source environment, resulting in lower variability in community composition over time. If the absolute contributions (in terms of the number of cells) from that single source were variable, then more variable abundance would be observed over time, as we see in this study. Conversely, homes with less resuspension would have lower (and relatively more stable) microbial concentrations, but those airborne communities might be more sensitive to small contributions from different source environments, resulting in higher temporal variability in community composition. This is just one possibility, and we would need more information (e.g., vacuuming frequency, vacuuming proximity to the sampler, and samples from potential source environments) to test this hypothesis. Another related possibility might be that homes with more variable airborne communities correspond to homes in which the human microbial sources are also more variable. Previous studies have shown that there are varying degrees of temporal variability in the human microbiome, with some individuals harboring rapidly changing microbiomes and others having more stable communities,[64] and the human microbiome signal on home surfaces has been shown to shift on the timescale of days.[21] Again, a more detailed sampling of source environments would be required to evaluate possible links between occupant microbiome temporal variability and home air variability.

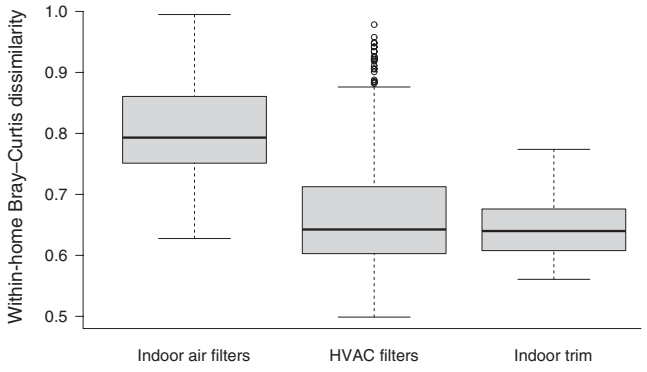

FIGURE 4 Comparison of temporal variability results from three different sampling methods. Temporal variability in indoor air bacterial community composition (average Bray–Curtis dissimilarity within each home), as measured from analyses of indoor air filters, HVAC filter swabs, and indoor door trim swab samples. The line through the middle of each boxplot represents the median, the box is the 25th to 75th percentile, and the whiskers (lines) indicate the maximum and minimum values, apart from outliers, which are indicated with circles beyond the whiskers

3.5 | Comparisons of microbial community temporal variability on indoor air filters, indoor door trim samples, and HVAC filters

With our microbial community measurements of air filters (all analyses described to this point), we captured 24-hour integrated samples of indoor air and compared those results with 24-hour integrated measurements of environmental variables and home characteristics. To place our microbial filter results in the context of other types of culture-independent sampling methods for airborne microbial communities (e.g., swabs of indoor door trim, which acts as a passive dust sampler,[41] and HVAC filter swabs, which capture whole home air[39,77]), we compared the variability in microbial community composition within each home throughout the study across these three sample types to determine how sample collection strategy may impact estimates of temporal variability (Figure 4, Figure S1). Although a more ideal comparison would have included 24-hour sampling of the door trim (i.e., sterilization of the trim surface, followed by dust accumulation for 24 hours) and of the HVAC filters, we did not attempt this, as we were concerned about the recovery of sufficient biomass on that timescale.

As we hypothesized, microbial community composition from direct aerosol collections onto filters was much more variable within each home over time than the communities sampled by the HVAC filters and door trims, suggesting that we are better able to collect a "snapshot" in time with the 24-hour filter samples. This high degree of temporal resolution is lost when analyzing samples from HVAC filters and indoor door trims, a result that is not surprising, given that these samples represent the accumulation of microbes over longer periods of time. If the goal of a study is to provide an integrated assessment of microbial community composition over time, analyzing samples from settled dust or HVAC filters may be preferred. However, if knowledge of the variability in microbial exposures on short timescales is important, then direct air sampling, as conducted here, may be more appropriate and can help to delineate how short-term changes in home environmental conditions may alter microbial exposures.

4 | CONCLUSIONS

Microbial communities in the indoor air of family residences were found to be highly variable both within and between homes over short (a few weeks) and long (up to 1 year) timescales. In general, of the variables that were measured, changes in indoor RH and indoor T were most strongly correlated with the observed temporal variability in indoor microbial community composition. The homes with higher bacterial loads in indoor air were also the homes with the least variable microbial communities over time, possibly suggesting a

greater degree of resuspension from point sources in those homes. Overall, our results suggest that indoor air harbors highly variable microbial communities assembled from complex interactions between physical, environmental, and ecological characteristics of the home environment. Due to the observed high degree of variability, certain research questions will require collecting multiple samples over time from an individual home, as the microbes found in a single sample collected over a 24-hour period are not likely to be informative for predicting microbial exposures inside homes over months to years.

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