



## 10 Questions

## Ten questions concerning the microbiomes of buildings



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## ABSTRACT

Buildings represent habitats for microorganisms that can have direct or indirect effects on the quality of our living spaces, health, and well-being. Over the last ten years, new research has employed sophisticated tools, including DNA sequencing-based approaches, to study microbes found in buildings and the overall built environment. These investigations have catalyzed new insights into and questions about the microbes that surround us in our daily lives. The emergence of the “microbiology of the built environment” field has required bridging disciplines, including microbiology, ecology, building science, architecture, and engineering. Early insights have included a fuller characterization of sources of microbes within buildings, important processes that structure the distributions and abundances of microbes, and a greater appreciation of the role that occupants can have on indoor microbiology. This ongoing work has also demonstrated that traditional culture- and microscopy-based approaches for studying microbiology vastly underestimate the types and quantity of microbes present in environmental samples. We offer ten questions that highlight important lessons learned regarding the microbiology of buildings and suggest future areas of investigation.

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## 1. Introduction

Microorganisms are fundamentally important to the

functioning of ecosystems, including that of the human body itself. The built environment is an ecosystem of great interest because people in the developed world spend nearly 90% of their lives in buildings [1]. Studying the role of the built environment in exposing humans to specific microbes (e.g. pathogens or allergens) and the role of microbes responsible for the deterioration of building materials has a rich history. Recently, partly spurred by a research initiative sponsored by the Alfred P. Sloan Foundation [2], research efforts have expanded to include the “microbiomes” of indoor environments and the processes that shape these microbiomes. Here, we use the term microbiome to refer to the collection

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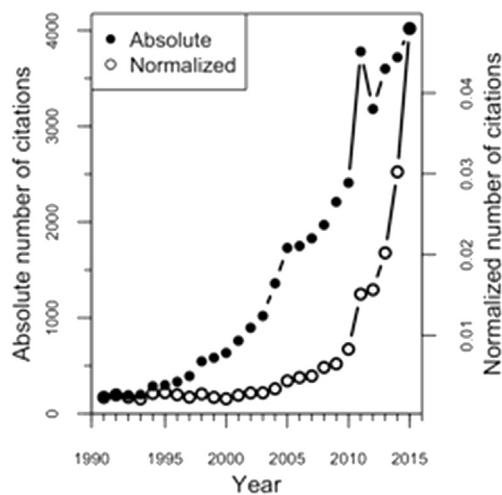
E-mail addresses: [adamsri@berkeley.edu](mailto:adamsri@berkeley.edu) (R.I. Adams), [seema.bhangar@aclima.io](mailto:seema.bhangar@aclima.io) (S. Bhangar), [dannemiller.70@osu.edu](mailto:dannemiller.70@osu.edu) (K.C. Dannemiller), [jaeisen@ucdavis.edu](mailto:jaeisen@ucdavis.edu) (J.A. Eisen), [noah.fierer@colorado.edu](mailto:noah.fierer@colorado.edu) (N. Fierer), [gilbertjack@uchicago.edu](mailto:gilbertjack@uchicago.edu) (J.A. Gilbert), [jlgreen@uoregon.edu](mailto:jlgreen@uoregon.edu) (J.L. Green), [lmarr@vt.edu](mailto:lmarr@vt.edu) (L.C. Marr), [shelly.miller@colorado.edu](mailto:shelly.miller@colorado.edu) (S.L. Miller), [jeffrey.siegel@utoronto.ca](mailto:jeffrey.siegel@utoronto.ca) (J.A. Siegel), [brent@iit.edu](mailto:brent@iit.edu) (B. Stephens), [m5w59@drexel.edu](mailto:m5w59@drexel.edu) (M.S. Waring), [bibbykj@pitt.edu](mailto:bibbykj@pitt.edu) (K. Bibby).

of microorganisms inhabiting a particular environment and, in this case, those found in structures built primarily for human occupancy. Research interest in the microbiology of built environments is high (the number of publications on this topic continues to grow [Fig. 1]), and the research area is increasingly emphasized within basic microbiology [3] and indoor air quality [4] scientific societies. In order to summarize ongoing research – specifically focusing on efforts that rely on DNA-based research methods – and to propose future endeavors, we present ten questions and answers regarding our understanding of the built environment microbiome.

### Q1) What does the microbiome of a typical indoor environment look like?

The microbiome of indoor environments comprise a large number of different taxonomic groups. For example, a survey of homes across the United States revealed on average approximately 7000 different types (operationally defined as operational taxonomic units (OTUs) based on sequence similarity) of bacteria and 2000 types of fungi per house in the dust on the upper trim of an inside door [5]. Another study of a neonatal intensive care unit (NICU) in a hospital identified an average of approximately 12,000 bacterial OTUs on various surfaces per room [6]. Common bacterial genera in indoor environments include *Staphylococcus*, *Corynebacterium*, *Lactococcus*, *Firmicutes*, and *Actinobacteria*, while common fungi are *Cladosporium*, *Penicillium*, and *Aspergillus* [5,7,8]. While there are a variety of microorganisms in indoor (and other) environments, methodological hurdles have largely limited work to bacteria and fungi. For instance, studies considering viruses have typically targeted specific viruses in particular indoor settings, such as daycares [8–10]. As such, a comprehensive understanding of the community of viruses and their effects on other microbes, as well potential implications for human health, is still lacking. Similarly, little data exists on the activity and viability of microorganisms identified by DNA sequencing methods. Previous investigations in cleanrooms have suggested that as little as 1–10% of identified sequences and 1% of the overall microbial concentration corresponds to microbes with intact membranes [11,12].

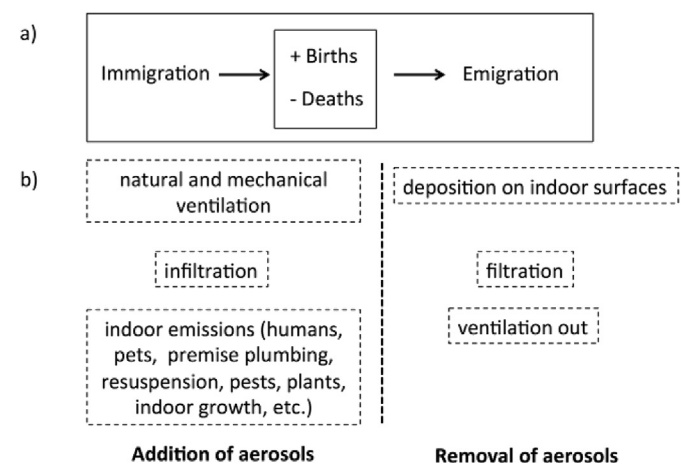
The multitude of recent studies examining various indoor microbiomes reveals that microbial communities in indoor environments are complex and highly variable. To help interpret the



**Fig. 1.** The absolute number of citations that are flagged in Google Scholar by the keywords: 'microbiology OR microbiome OR bioaerosol AND indoor' (left axis), and that number of citations normalized by 'microbiology OR microbiome OR bioaerosol' (right axis).

different studies, we propose a mechanistic framework that unites a material-balance approach of engineering with the ecological concept of metacommunities, which both seek to track the sources and sinks of a constituent in a system (Fig. 2). A material-balance approach draws on the principle of conservation of mass to track the material (typically a pollutant) entering and leaving a system, while in ecological theory, metacommunities are considered sets of local communities linked by the dispersal of organisms. Along with environmental heterogeneity, there are demographic parameters that structure metacommunities, and these demographic parameters have direct analogs in the material-balance approach. Adopting the mass-balance framework of aerosols [13–15], inputs to the system arrive from ventilation, infiltration, and indoor emissions, while removal comes about through deposition, exfiltration, and ventilation (Fig. 2b). Analogously, within a biological system inputs to the system come as immigrants or originate in the system through births, and loss from the system results from emigration (Fig. 2a). When linking the abiotic and inactive nature of particles typically considered in aerosol models with active biological organisms that appear in aerosol form (bioaerosols), additional considerations need to be made. For instance, the pool of microbes could self-propagate and expand in population size, should favorable growth conditions exist; likewise, the death of an organism within the environment is not necessarily a loss to the system, because dead organisms can persist in the indoor environment and be resuspended as an aerosol. Similarly, not all microbes should be considered as pollutants or contaminants that warrant efforts to limit exposure in the indoor environment.

We propose this integrated framework, which combine principles of particle transport and microbial demographics, to inform how microbiomes of indoor environments assemble to generate indoor microbiome patterns observed across a variety of settings. Understanding the source strengths of the different processes aids interpretation and generalization of findings from vastly different indoor environments, from transit systems [16–18] to homes [5,19] to hospitals [6] and the International Space Station [20], and across geographic areas where the outdoor environment and building design, operation, and use vary. For example, different rates and types of bioaerosol immigration comes about through different forms of ventilation [21], and different surfaces are expected to have different rates of microbial immigration through the nature and extent of human contact [22–25]. Similarly, the likelihood of



**Fig. 2.** Demographic processes that structure metacommunities (a) have parallel processes when considering the concentration and composition of bioaerosols in buildings (b). Immigrants are analogous to inputs from ventilation and infiltration, while births are inputs to the system from indoor emissions. Likewise, deaths and emigration out of the system can result from deposition, filtration, and ventilation out.

propagation (or birth) will likely depend on the water and nutrient context where the microorganism is deposited, with important implications for the source pool for indoor emissions. As such, growth in indoor environments likely does not contribute greatly to indoor microbial communities, except on surfaces with intentional (sinks, for example) and unintentional (water damage) water use. Microbial quantity can also be incorporated into this framework, as has been done showing that human occupancy contributes ~14 to ~37 million bacterial genome copies per person per hour to air [26,27]. This could similarly be done with temporal dynamics, as the strength of different immigration rates are known to vary with outdoor and building conditions.

### **Q2) How do building characteristics, including occupants and their behaviors, influence the indoor microbiome?**

The abundance, composition, and diversity of microbial communities found in buildings are the products of dynamic interactions between outdoor air, the building itself (including ventilation strategies, moisture levels, and – perhaps – building materials), and occupants (humans and animals) [28]. Using the framework developed in Q1 (Fig. 2), we discuss how (I) building location, operation, and design (II) human occupants and their activities, and (III) indoor environmental conditions each contribute to structuring the microbiomes of buildings. We should note that while this review focuses primarily on findings from recent studies using DNA-based methods, some of the same conclusions have also been drawn from decades of applying culture-based methods to study indoor microbes [29].

#### **Building location, operation, and design**

The microbes in outdoor air are geographically patterned [30], and this structure transfers to indoor environments [5,31,32]. Spatial variation in the outdoors likely results from differences in land use and vegetation type which in turn host different microbial communities that get entrained in the passing air [33,34], and temporal variation in sources can result from varying seasonal and climatic variables [35].

Building operation – specifically, the ventilation strategy used – has been shown to influence the inputs of microbial communities from these outdoor sources through ventilation and infiltration, or immigrants to the system. The source strength of outdoor air varies by ventilation type: within mechanically or naturally ventilated buildings, the magnitude and source of the ventilation air delivery rate affects the relative contribution of outdoor air, such that rooms with natural ventilation (i.e., open windows) or modest supply air filtration show microbial profiles that are similar to outdoor air and a weaker influence from other sources [21,36–38]. Accordingly, Ruiz-Calderon et al. [39] recently showed that houses along an intensifying urbanization gradient showed a decrease in outdoor-associated bacteria, such as Intrasporangiaceae and Rhodobacteraceae, and an increase of human-associated bacteria, for example Streptococcaceae, Lactobacillaceae, and Pseudomonadaceae.

In addition, architectural and interior building design have been shown to influence the types of bacteria that accumulate indoors, in part because variations in building form and interior spatial arrangements can alter the way occupants utilize the built spaces and impact the magnitude and directionality of human-mediated microbial transport indoors [40].

#### **Occupancy and activity**

Humans are an important source of microbial inputs into built environments, typically accounting for between 5% and 40% of sequence reads (Table 1). Humans contribute to the indoor microbiome via two major routes. First, the microbiome of occupants, including people and pets, has been identified in air and on surfaces in the indoor environment [e.g. 5, 26, 41, 42]. Higher levels of

occupancy and activity will influence the abundance and composition of bacteria found indoors (including that of the microbial reservoir left indoors) because we shed a large quantity of microbe-laden particles from our bodies [26,43]. The rate of direct and indirect contact between people and surfaces will also influence the structure and diversity of bacterial communities found on surfaces [23,25,44]. The second route by which occupants generate particles indoors is through their movements, which causes resuspension of settled particles even if they are not the original source of those microbes [37,45]. For example, Yamamoto et al. [45] showed that occupant-generated emissions contributed approximately 80% of the allergenic fungi in the aerosols of university classrooms, thus contributing more substantially than outdoor contributions from ventilation. The type of activity and flooring can also influence resuspension amounts [46], demonstrating an interaction between human occupancy and specific building parameters.

#### **Environmental surface characteristics**

Indoor surfaces create unique ecosystems in the indoor environment. The microbes on surfaces could be considered inputs if they lead to indoor emissions, or they could be losses resulting from deposition (Fig. 2). Different building materials and environmental conditions (e.g., temperature, available water, cleaning chemicals and frequency, light intensity at certain wavelengths, and carbon sources) can create different selective pressures for microorganisms if varied over wide ranges, which can result in differential survival and persistence rates [53–57]. However, much of the previous work investigating the impact of environmental conditions on microorganism survival has focused on infectious organisms. For the vast majority of building operating conditions, more recent evidence suggests that the majority of bacteria and fungi found on surfaces are not actually growing in what are mostly inhospitable environments [52,58–60]. However, it is likely that many of the microbes identified in areas of the home with periodic water exposure (e.g. sinks, drains, showers) are alive; of course many cleaning events also introduce water, but they also introduce chemicals that are designed to remove or reduce microbes. Surprisingly, while studies have shown the impact of cleaning products on specific microbial groups such as fecal coliforms [61,62], no published studies have characterized how they impact diversity or community structure within buildings. Approaches for studying the active portion of microbial assemblages while still culture-independent are beginning to be applied to indoor environments, and future work is likely to inform the extent of microbial activity and persistence in the indoor environment. Importantly, while it is likely that most microbes deposited onto surfaces become inactive or die, these microbes may remain possible sources of allergens.

### **Q3) How do moisture problems alter typical indoor microbiomes?**

The effects of moisture problems on the growth of indoor microorganisms have long been examined due to associations between indoor dampness and ill health outcomes [63,64]. Moisture is the limiting factor for microbial growth in the indoor environment, and fungi are more tolerant of low-moisture conditions than bacteria [7]. Aside from direct input of bulk-phase water, either intentionally or unintentionally, levels of adsorbed water may be sufficient to support growth. For instance, growth has been observed on wood at an air relative humidity of 78%, on gypsum board at 86%, and in floor dust at 80% [65,66]. While water availability is generally thought to be the limiting growth factor, critical surface moisture levels are challenging to define [67]. Growth can occur directly on a wide range of building materials, such as insulation, concrete, paper, paints, and glues [65,68,69], and some building materials may come pre-contaminated with degrading

**Table 1**The percentage of sequence is indoor air studies that are derived from human sources (predominately skin).<sup>#</sup>

Study	Environment	Location	Approach	Percent associated with human body
Hospodsky et al., 2012 [47]	University classroom	Northeastern United States	Sequences associated with five taxonomic groups (a,b,c,d,e)	17–20%
Gaüzère et al., 2013 [48]	Museum	Paris, France	Sequences associated with six genera (b,c,f,g,h,i)	10%
Meadow et al., 2013 [25]	University classrooms	Eugene, Oregon	Sequences associated with three groups (b,f,h)	7.8% (max of 38%)
Adams et al., 2014 [49]	Residences	Albany, California	Sequences associated with six groups (a,b,c,d,f,h)	32%
Adams et al., 2015 [37]	Environmental Chamber (conference room)	Berkeley, California	Sequences associated with five groups (a,b,c,d,e)	4%
Barberán et al., 2015 [5]	Residences	Throughout United States	Sequences associated with ten groups (b,c,l,j,k,l,m,n,o,p)	11%
Miletto & Lindow 2015 [50]	Residences	San Francisco Bay Area, California	Sequences associated with five groups (b,f,h,k,q)	23%
Shin et al., 2015 [51]	Childcare facilities	Seoul, South Korea	Sequences associated with five groups (b,c,f,k,r)	26%
Wilkins et al., 2015 [38]	Residences	Hong Kong	Sequences associated with five groups (b,c,h,j,k)	11%
Chase et al., 2016 [52]	Offices	Flagstaff, Arizona; San Diego, California; Toronto, Ontario	SourceTracker2, with human microbiome samples as "sources"	25–30%

<sup>#</sup>The specific approach of identifying human-associated taxa were set by each study and are not consistent across the studies listed here. <sup>a</sup>Propionibacteriaceae, <sup>b</sup>Staphylococcus, <sup>c</sup>Streptococcus, <sup>d</sup>Enterobacteriaceae, <sup>e</sup>Corynebacterineae, <sup>f</sup>Corynebacterium, <sup>g</sup>Propionibacter, <sup>h</sup>Acinetobacter, <sup>i</sup>Lactobacillus, <sup>j</sup>Corynebacterium, <sup>k</sup>Propionibacterium, <sup>l</sup>Bifidobacterium, <sup>m</sup>Lactococcus, <sup>n</sup>Bacteroides, <sup>o</sup>Faecalibacterium, <sup>p</sup>Ruminococcus, <sup>q</sup>Kocuria, <sup>r</sup>Micrococcus.

fungi [70]. Interestingly, while high relative humidity can support microbial growth, experiments indicate that spore release for some fungi can be higher under lower relative humidity [71,72]. Often saprophytic fungi that are also abundant as aerosols are commonly found on damp building materials [7]. The most common genera in moisture-damaged buildings include *Aspergillus*, *Penicillium*, *Cladosporium*, *Eurotium*, and *Chaetomium*, among others [7,69].

Historically, most research has relied on culture-dependent, microscopic, and biochemical assays of microbial presence in buildings, while new DNA sequence-based approaches are beginning to be applied (see Q5). Regardless of the methodological tool, there are analytical issues that persist independent of the specific approach when studying aerosols, namely identifying an indoor source of microbial contamination rather than simply detecting the presence of a microbe indoors [8]. For aerosols, two approaches have typically been taken. In one approach, the microbial composition of aerosols in moldy homes is compared to dry homes; in another, indoor and outdoor concentrations of taxa are compared [73]. The two approaches have also been used simultaneously [74,75]. The former formed the basis for the Environmental Relative Moldiness Index (ERMI), which sought to identify fungal species that may be informative for determining the mold-burden of a building [76]. For building materials, the taxonomic identification of growing organisms, versus merely present, relies on direct culture and microscopic examinations of tape lifts.

While it is expected that unintended water intrusion would lead to greater microbial growth and detectable microbial biomass (i.e. quantity) when compared to “dry” homes, this pattern is not generalizable [77,78]. In some studies of floor dust, an increase in moisture in the building is associated with an increase in fungal richness [79–82], while other studies conducted at the site of fungal growth have demonstrated dominance of a small number of species with increased moisture, and thus an apparent decrease in richness [58,66,83]. Therefore, the increased overall richness seen in homes with increased moisture may be due to contributions from growth at multiple locations. For composition (the different taxonomic constituents), it might be predicted that moldy homes would have a distinct microbial makeup, as they would support the growth and persistence of certain taxa that would not thrive in a dry home. A recent study of the 2013 flood in Boulder, Colorado demonstrated the lasting effects of moisture in a home. After remediation had been completed, previously flooded homes still retained different microbial communities when compared to nonflooded controls [84]. In particular, fungal concentrations were three times higher in flooded compared to non-flooded homes, and

flooded homes had higher concentrations of *Penicillium*, *Pseudo-monadaceae*, and *Enterobacteriaceae* [84].

#### **Q4) How does the microbiome affect indoor chemistry, and how do chemical processes and the composition of building materials influence the indoor microbiome?**

Indoor chemistry may be affected when fungi, bacteria, and other microbes produce chemical metabolites, especially on wetted building materials. Microbial volatile organic compounds (MVOCs) have been isolated by measuring emissions from microbe-colonized materials, often in laboratory chambers. Common indoor MVOCs are summarized in Table 2 [85–89]. Frequently observed chemical classes include alcohols, carbonyls, furans, terpenes and terpene alcohols, and sulfides. Semivolatile toxins are also produced by mold growing on building materials [90,91]. MVOCs may undergo oxidative chemical reactions indoors with radicals (including the hydroxyl radical) and ozone (O<sub>3</sub>). However, the actual impact of microbes on indoor chemistry may be weak, since MVOCs may only be slightly elevated even in moldy versus non-moldy spaces, if at all [92], and the concentrations may not be that high compared to other VOCs typically present indoors. Also, MVOCs from microbial emissions are difficult to isolate, because no MVOCs are exclusively emitted from any particular species or genera, or even from microbes only [93–95]. That said, the prevalence of sick building syndrome (SBS) symptoms have been previously associated with MVOCs, including 1-octen-3-ol, 2-pentanol, 2-hexanone, 2-pentylfuran, and formaldehyde [96,97].

Beyond the microbial influence on indoor chemistry, chemical compounds and physicochemical states could also influence the indoor microbiome. Microbes growing on building materials may be influenced by adsorbed water or organic films, as well as

**Table 2**

Some common microbial volatile organic compounds (MVOCs) indoors.

Formaldehyde	4-Methylheptan-3-one	Endo-borneol
Acrolein	1-Octen-2-ol	Fenchone
2-Methyl-1-propanol	1-Octen-3-ol	Geosmine
1-Butanol	3-Octanol	Karveol
3-Methyl-1-butanol	3-Octanone	Limonene
2-Methyl-1-butanol	Nonanal	Terpineol
Ethyl isobutyrate	2-Nonanone	Thujopsene
2-Pentanol	2-Methylfuran	Dimethyl sulfide
2-Hexanone	3-Methylfuran	Dimethyl disulfide
2-Haptanone	2-n-Pentylfuran	Dimethyl sulfoxide



compounds from the nearby air. Though little is known about how these variables impact microbial communities, certain inferences may be drawn. Adsorbed water may be a few monolayers thick, and more than that if the surface is wetted. Most microbes prefer neutral pH ranges [98], and Corsi et al. proposed that changes in the concentration of carbon dioxide, ammonia, or other compounds indoors might lead to pH changes in these surface water films in such a way as to influence microbial growth or diversity [99]. Though organic surface films may resemble each other among surface types across different indoor spaces [100,101], some films could become more toxic over time due to absorption of harmful semivolatiles, such as pesticides. Furthermore, airborne chemicals could influence microbes. Russell et al. demonstrated that bacteria on roots of plants exposed to VOCs change community character in response to the VOC exposure [102], and this effect could conceivably occur with microbes in indoor environments. Microbes might also be inactivated by direct oxidation from hydroxyl radical or ozone on surfaces.

Finally, a related focus of indoor microbiome research and chemical interactions has been on whether different building materials harbor microbial communities of differing composition. Studies with wetted materials do indicate some differences in the microbial composition and metabolite production based on growth substrate [103]. For example, wooden materials show greater fungal diversity than plasterboard or ceramics [69], and cellulose-based materials are more sensitive to contamination by fungal growth than inorganic materials such as gypsum, mortar, and concrete [104,105]. However, field studies in non-wetted buildings have challenged the viewpoint that substrate composition drives microbial community structure by showing that source strength dominates instead, e.g. Refs. [23,49]. Most recently, a study in offices assessed the impacts of geography, material type, location in a room, seasonal variation, and indoor and micro-environmental parameters on bacterial communities of standardized surface materials [52]. Bacterial communities did not depend on the surface material itself, but they did depend on geography and location in the room. Specifically, floor samples of all surface materials showed richer microbial assemblages than other locations within the rooms, a finding also observed in a recent study of public restrooms [60].

#### **Q5) What do DNA sequencing and modern analytical techniques tell us about the indoor environment?**

Many previous studies of the indoor microbiome relied on culture-based methods, microscopic identification, or biochemical assays, such as measuring ergosterol or ATP. More recently, the use of high-throughput DNA sequencing has allowed for a more thorough characterization of microbial communities. Analysis can involve targeted sequencing of specific genes, sometimes called amplicon sequencing or “barcoding” because it uses a common region (e.g., the 16S ribosomal gene in bacteria and the internal transcribed spacer [ITS] region in fungi) to identify the microbes present, or metagenomics, which aims to sequence randomly from all of the genetic material found in a given environmental sample. Sequence-based approaches offer several advances over culture- or microscopy-based techniques in identifying microbes in buildings. In addition to the increased efficiency by which microbes can be detected compared to these previous methods, DNA-based detection often facilitates the refined identification of species. Moreover, culture-based analysis may not detect organisms in a “viable but not culturable” state. On the other hand, sequence-based approaches cannot differentiate the DNA of viable and non-viable organisms or other fragments. A complementary approach would be to combine existing biochemical assays with emerging DNA-

based approaches to provide a fuller view of microbial activity and diversity.

Ironically, the detection of many additional species can result in greater analytical challenges, increasing the difficulty of separating out the “signal” from the “noise.” The vast amount of data generated with high-throughput sequencing can require the use of additional statistical tools such as methods to control for many comparisons in an analysis, and these may be borrowed from other genetic methods [106]. The same sample is not typically analyzed by different methods (e.g. by both microscopy and genetic-based tools), often because of logistical issues surrounding the processing, but studies that have used a combination of approaches have shown that they offer different but complementary views of the indoor microbiome [e.g. Ref. [107]].

Quantitative Polymerase Chain Reaction (qPCR) provides quantitative information on the abundance of a specific taxonomic group of interest. The use of qPCR with universal fungal or bacterial primers can provide a general estimate of total bacterial genomes or fungal spore equivalents in a sample [108–110], although these determinations of biomass based on universal primers are estimates of concentration due to differences in gene copy number and amplification bias across different species. Despite potential biases, qPCR analyses may be done in conjunction with DNA sequencing to improve understanding of microbial exposure [111] and to yield quantitative estimates of the concentrations of individual species [112].

Using these new techniques, the most significant contribution to the literature has arguably been the acknowledgement of the sheer diversity of microorganisms in buildings. Often, hundreds to thousands of OTUs are identified by any given study (see Q1). Since it remains unclear whether overall microbial diversity itself or individual microbial groups are more important to human and building health, current techniques that better capture overall microbial diversity may be positioned to answer long-standing questions in the field. Moreover, there are opportunities for further expansion to broader taxonomic groups, including viruses, and to analyze different targets, such the RNA transcripts (metatranscriptomics) and proteins (metaproteomics) to more fully characterize microbial gene expression and proteins of interest in the indoor environment.

#### **Q6) What are appropriate sampling methods and constraints for studies of the microbiology of the built environment?**

Perhaps the most practical question while investigating the microbiome of buildings is the choice of sampling methodology. It would be ideal if common practices were used to facilitate understanding and comparison across studies. There are many biological sampling methods available, each with distinct advantages and disadvantages. Most require sample collection followed by offline analysis, although several newer on-line techniques are also available. While there is at present no “gold standard” method that meets all requirements for sampling and subsequent analysis for all purposes (see Q7), below we summarize many commonly used methods for biological sampling in indoor environments and discuss considerations on spatial and temporal resolution.

**Surface sampling.** Moistened sterile swabs are widely used for biological sampling directly from surfaces [23,24], although it can be difficult to obtain adequate biomass from some locations [6,58]. Settled dust samples are also collected using wipes or vacuum filter devices, as they represent an integrated record of microbial communities in a space [40,41,113]. It is important to consider the size cutoff of the filter for vacuum collection, since larger particles may dominate the composition analysis but are not likely to contribute significantly to indoor exposure due to rapid settling after

resuspension. More traditional approaches include tape lifts and contact plates for microscopy and culturing. Low-retention swabs have been developed to isolate minute amounts of biological material for subsequent analysis for surface sampling; however, these swab-based techniques are currently incompatible with quantitative approaches, due to interpersonal variation in the strength of swabbing.

**Air sampling.** Airborne microbial sampling involves either active or passive techniques [114,115]. Commonly used active air sampling methods include liquid impingers [16,116], size-resolved [26] and non-size-resolved [21,37,117] impaction-based filter methods (with a variety of filter materials), and wetted wall cyclones [48]. Active air samplers operate at a range of airflow rates (4 L min<sup>-1</sup> [21] to as much 1000 L min<sup>-1</sup> [48]). While the advantage of higher flow rates is that more biomass can be collected over shorter amounts of time, there remain practical size and noise concerns associated with the higher flow rate pumps. A newly developed air-sampler relies on electro-kinetic air ionization to positively charge particles in the air, and then collect them onto a negatively charged surface [118]. Commonly used passive air sampling methods include Petri dishes suspended in air, both with and without a growth medium [19,84,117], dust fall collectors [119,120], and sampling of portions of used HVAC filters from recirculating air handling units [10,121–123].

A few studies have compared the ability of various bioaerosol samplers to deliver repeatable results using molecular analysis techniques [48,124] or for various analysis techniques to deliver repeatable microbial community results from a particular air sampling method [118,125]. Airborne collection methods can vary widely in their collection efficiencies for different sizes of bioaerosols, as well as in their DNA extraction efficiencies from the sample collection media [126]. One recent study suggests that because different air sampling methods can yield such different results, it may be more appropriate to use a variety of techniques to provide a more complete representation of microbial communities present indoors [124], consistent with recommendations before next-generation DNA sequencing [127]. Overall, particle collection techniques involve difficult trade-offs between ease of use, cost, and unobtrusiveness with the amount of biomass collected, the impact of the collection on viability, and the consistency and representativeness of the targeted sample.

**After sample collection.** Once particles have been collected, analysis techniques are structured toward providing physical (e.g., size, shape, morphology, mass), chemical (e.g., biomarker profile), or biological (taxonomic classification) attributes [128,129]. See Q5 for a discussion of current biological techniques.

**Online techniques.** Online methods are emerging that provide high time-resolution and are easy to use, such as those based on laser-induced fluorescence (LIF), chemical marker detection, or other techniques, but specificity is currently limited [130,131]. In spite of this limitation, LIF-based particle counting is a useful choice in studies where the study of dynamic processes (i.e., varying on short timescales) is of interest, or where information on particle size is critical. In studies where processes of interest have longer timescales, or if the schedule of particle collection can be dynamically managed to target conditions of interest, particle collection/analysis offers greater specificity to well-defined outcomes.

**Spatial and temporal resolution.** Aside from the specific method of sampling, there are additional questions of where in a building to sample and how many areas need to be studied to give a spatially and temporally representative outcome [e.g. 114, 128, 132]. For spatial resolution, current research indicates that areas that vary in their degree and nature of human contact and water exposure exhibit greater compositional differences than those accumulating environmental microbes in other ways

[22,25,40,52,58,133]. Temporal variability of microbes indoors can be high, varying on the order of hours for air samples [134] – likely due in part to diurnal activity of outdoor microbes [e.g. 135] and to activity levels in the room [43] – and, of course, across longer time scales of weeks, months, and seasons [19,136–138]. It has been suggested previously that sampling on different days is necessary to obtain a representative sample of aerosol exposure in a home [134] and that sampling time on the order of 5–7 days better captures ergosterol concentrations in homes than <24 h air samples due to the considerable temporal variability in bioaerosols [139]. Since repeated or long-term sampling is not always practical, especially in larger epidemiological studies, settled dust is often used as a surrogate. While it is unclear precisely what portion of exposure originates from floor dust, it is likely to be high, given the strong role that resuspension plays on structuring bioaerosols [45].

### **Q7) What technological developments will enhance our understanding of the microbiology of the built environment?**

There are many opportunities for technological improvements in the way built environments are studied and sampled. Many of these have to do with bridging biological-oriented sampling, particularly those relying on genetic assays, with particle-based sampling.

One major area in need of improvement is how microbes are collected from air for later biological processing. Ideally, samplers would be easy to operate and the sampling protocol would permit consistent use with little to no formal training. This would also allow indoor sampling to be scalable, and enable the sampling of homes or other buildings across the globe that differ in design and operation with minimal cost and logistical hurdles. When using DNA sequencing approaches to survey bioaerosols in buildings, it is critical that the sampling strategy yields sufficient amounts of retrievable DNA for downstream analyses. Current approaches overcome this by taking time-integrated samples, typically over many hours. Time-integrated samples capture a composite view of bioaerosols, which can vary substantially over time. At the same time, time-resolved methods would provide repeated samples continuously over a representative period of time to link specific activities and conditions with the effects on aerosols, as is commonly done with particles. Ideally, the time-resolved methods would also provide information on particle size, which would allow the application of pre-existing understanding of aerosol behavior to better predict and control the dynamics of microorganisms in the built environment. The ideal aerosol sampler would also provide quantitative and reproducible estimates of the amounts and types of bioaerosols found within buildings.

Additional technological developments and availability of low-cost built-environment sensors will enable the appropriate “metadata” to be acquired more easily along with microbiological measurements, to link microbial findings to underlying causes [140]. Spatial mapping (indoors and outdoors), advanced visualization, and other emerging tools will enable the more effective and creative application of the data made available through current molecular and building measurement technologies [141].

Lastly, other areas of technological improvements are related to microbiological analytical methods. Efforts should be extended broadly to include eukaryotes beyond fungi, and also viruses. Approaches are necessary to address the multiple sources of bias that may be present in next-generation sequencing based characterization of microbial communities, including DNA extraction methods, primer bias, and variable gene counts and genome sizes [142–144]. Improved bioinformatic approaches and reference databases will enhance our ability to study the entire microbial community. Improved and validated approaches for discriminating

between dead microbes and those that are alive, and particularly methods that are compatible with current genetic-based microbial detection, would greatly improve our understanding of microbes in buildings. Dead pathogens inside homes and buildings may be of little concern, although allergenic fungal species may still contain allergens regardless of viability. DNA can be remarkably persistent on surfaces and particles [145]. Plus, analytical standards for microbial community analyses would facilitate testing different molecular approaches and comparing results obtained using different strategies (across labs, across sequencing platforms, etc.). Lastly, new tools for studying microbial activity *in situ* would provide a basis to better understand what are the primary microbial processes and in real-world buildings. While many tools focus on DNA, we also need continued advances in metatranscriptomics and metaproteomics to make these techniques more accessible.

### **Q8) What are the connections between indoor microbiomes and occupant health?**

There is a growing appreciation of the impact that microbiomes have on the health of humans (and other organisms) [e.g. 146]. Humans can acquire some components of their own microbiome from their surroundings [147] and are continuously exposed to the indoor microbiome, so it follows that the microbiomes found in the indoor environment could also have a profound effect on human health. Recent research has highlighted this potential connection between the indoor microbiome and health, although many (with a few notable exceptions) of the recently published connections thus far are based on correlation, not causation.

The indoor microbiome could influence health through inhalation, ingestion, and dermal contact, and there are numerous examples of a direct link between specific microbes in the indoor environment and acute infections. Indoor air can serve as a transmission route for pathogens including *Mycobacterium tuberculosis*, influenza, and the fungus *Aspergillus* [148]. One of the most common hospital acquired infections (HAIs) in the United States is caused by the bacterium *Clostridium difficile*, and can lead to lethal diarrhea [149]. *C. difficile* forms spores that can survive on indoor surfaces, even after the use of antimicrobial products [150]. HAIs derived from *Staphylococcus aureus* and the antibiotic resistant strains such as methicillin-resistant *S. aureus* (MRSA) also frequently contaminate environmental surfaces. Water can also serve as a source of infection transmission in the built environment. A widely recognized infectious bacterium that thrives in warm water and can become aerosolized is *Legionella* [151]. While it is well known that building cooling towers can contribute to the spread of Legionnaire's disease [152], other building operational parameters (ventilation, filtration, and plumbing systems) can also influence the transmission of infectious disease [153].

Understanding the link between the microbiome of the indoor environment and non-infectious diseases, such as respiratory ailments, is an active area of research. There is still much work to be done to appreciate the connections between microbial diversity, environmental exposure, and health outcomes across buildings in a variety of settings, especially because for many of the associations the specific causative agents remain unknown. Early on, there were investigations into sick building syndrome (SBS), a syndrome in which occupants experience acute health symptoms while in the building including fatigue, headaches, and irritation in the eyes, nose, and throat [154]. In a similar vein, dampness and mold in buildings are known to be detrimental for respiratory-based diseases, particularly exacerbation of existing asthma [64,155]. It is logical to consider that the ill effects derive from exposure to the microbial agents endogenously growing in these water damaged buildings, but lower fungal diversity has been shown to be

predictive of asthma development [80]. In fact, Dannemiller et al. [80], using next-generation sequencing of fungal DNA, found that no individual fungal taxon was associated with asthma development but overall fungal diversity was. On the other hand, Ege et al. [156], working in farm environments, found that a diverse microbial environment and the presence of bacteria from particular genera (e.g. *Acinetobacter*, *Lactobacillus*, *Neisseria*, *Staphylococcus*, *Jeotgalecoccus*, and *Corynebacterium*) were inversely associated with asthma, atopic sensitization, and hay fever. Similarly, Lynch et al. [157] carried out a longitudinal study in inner-city environments and found that children exposed to specific types of bacteria (including members of the phyla Firmicutes and Bacteroidetes) in combination with well-known allergens at high levels had a reduced risk of allergic disease. The authors suggested that mice and cockroaches were the sources of these bacteria associated with a beneficial health outcome. In addition, even dead cells and cell fragments can have negative health impacts on respiratory health [158], and microbial metabolites may also directly affect human health [91]. Clearly, there is much to learn about the interplay between overall microbial diversity and composition, the presence of particular taxa, and the built environment, and the overall effect of this milieu on immune function.

In what may be the only study showing a direct health benefit from an indoor microbe, Fujimura et al. [159] showed that exposure to dog-associated bacteria from house dust in a mouse model was protective against airway allergen challenge. Moreover, the researchers isolated a single species associated with the dog-associated house dust, *Lactobacillus johnsonii*, and found that intentional supplement with this bacterial species conferred airway protection in mice.

In addition to the inhalation and ingestion routes of environmental exposure, direct contact between surfaces and an occupant could alter the skin microbiome. While the skin microbiome of diseased states is distinct from that of a healthy individual with some ailments [160,161], it is unclear whether this arises through contact with the built environment and whether the skin microbiome influences the body's larger immune system.

### **Q9) What are the implications of recent work for building design and maintenance?**

Decisions that are made during building design have the potential to drive the indoor microbiome regardless of their intention or motivation. As a sterile indoor environment is not possible, nor likely to be desirable (except perhaps in certain health care settings), it has been suggested to move from treating all microorganisms as contaminants towards a more bioinformed design that considers impacts of the microbiome in design decisions [162,163]. However, it is not currently clear what constitutes a healthy (i.e. 'good') indoor microbiome, nor what are the necessary design parameters to drive the microbiome to a healthy microbiome.

With regards to infrastructure health and maintenance, plumbing systems have received the most research attention. Altering the operation of a drinking water system, for example reducing flow and moving towards green building design or using onsite drinking water disinfection, has previously been shown to alter both the microbiome as well as potential pathogens [164,165]. Accordingly, a probiotic approach to the control of drinking water borne opportunistic pathogens has previously been suggested [162]. Additionally, we know that corrosion of other critical infrastructure systems, e.g. sewers, is driven by their microbiome [166].

### **Q10) What do all these recent studies NOT tell us?**

Early studies of the building microbiome have been



illuminating, but there are many opportunities for expanding on the existing approaches and study goals for furthering our understanding of the microbiology of built environments. We suggest the following points as areas of ongoing inquiry.

#### **Predictive power for the microbiome based on building conditions.**

Important factors in the building microbiome are geographic location, occupancy, ventilation rate, and ventilation type (see Q2), but there are many uncertainties within these factors. For example, while ventilation has been suggested to be a primary driver of the built environment microbial community as a source of microorganisms from outdoor air, the precise influence of ventilation type and operation warrants further investigation. Similarly, the roles of temperature, relative humidity, and light intensity in structuring the microbiome remain unclear. Further, we know much more about the impact of these factors on the relative abundances of particular taxa (not necessarily at the species level) than we do about absolute abundances of individual species, their viability, and their function in indoor settings. It would be powerful to be able to predict the microbiome of indoor spaces and their community dynamics based on knowledge of building factors.

#### **The role of building materials in the building microbiome.**

While water availability is likely to be a prominent factor driving the microbiome in particular building locations, the precise role of building materials in shaping the building microbiome is unknown. A recent study controlled for building material and found no association with the microbial community composition when accounting for location and sampling frequency [52]. An exception may be flooring material, which plays an important role in microbiome resuspension [167], likely via altering resuspension rates and not by structuring the microbiome.

#### **The relationship between indoor air pollutants and the building microbiome.**

No known relationship has been demonstrated between the building microbiome and well-established indoor pollutants, such as CO<sub>2</sub>, PM<sub>2.5</sub>, PM<sub>10</sub>, or CO. While microorganisms are known to produce volatile organic compounds indoors with potential human health implications [168,169], linkages between the production of VOCs and the microbial community structure remain elusive (see Q4).

#### **The role of the building microbiome in occupant health.**

A desirable goal is the identification of a “healthy building microbiome.” An ideal scenario is to eliminate the components of the indoor microbiome that are detrimental to health, while promoting the components that are beneficial. There are many intermediate hurdles we still need to overcome to get to that scenario. For one, sampling strategies of indoor microbes need to reflect human exposure. Plus, understanding whether and how the indoor microbiome plays a role in some non-infectious diseases (see Q8) would inform what about the indoor microbiome could be manipulated to bring about a desired outcomes.

## **2. Conclusion**

The microbiomes of buildings are diverse, dynamic, and one component of the larger indoor environment about which many fundamental questions remain. Understanding how building design and operation influence the indoor microbiome will strengthen our knowledge of relevant physical systems and microbial processes in built environments. Improved knowledge will increase opportunities to make actionable recommendations, which may result from fusing microbial-, building practitioner- and health-related datasets. Both improvements in understanding the human microbiome and work already completed in buildings give a basis to better understand what microbes or microbial products

and features in the built environment should be sampled. We can now more strategically target aspects of the built environment that matter to humans, potentially one day influencing how we manage buildings.

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