

REVIEW

Studying the microbiology of the indoor environment

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Abstract

The majority of people in the developed world spend more than 90% of their lives indoors. Here, we examine our understanding of the bacteria that co-inhabit our artificial world and how they might influence human health.

The need for culture-independent analysis of microbial diversity

The global trend towards industrialization and urbanization has led to ever more people living and working indoors. Some studies estimate that humans in industrialized countries spend as much as 90% of their lives inside [1,2]. They are born in hospitals, raised in homes or apartments, work in office buildings or factories and move to nursing homes in their old age. This trend towards indoor living is also occurring in developing nations. Thirty years ago, the Chinese city of Shenzhen was a small city of 300,000 inhabitants. Since then, it has grown into a massive industrial megalopolis housing over 10 million people, many of whom have moved there from rural areas [3]. Thus, for billions of humans, the built environment (BE) now represents the modern ecological habitat of *Homo sapiens sapiens*.

In the process of creating BEs, we have also inadvertently created an incredible diversity of microhabitats that have been colonized by thousands of different microorganisms. Indeed, BEs contain many potential novel habitats for microbial life that have chemical and physical properties not found in the natural world. Humans convey a large fraction of the colonizing microbes into these habitats by shedding them from their bodies or transporting them by means of their clothes and shoes, whereas the rest originate from water or other

environmental sources (for example, soil) [4-7]. Depending on the environment, microbes can also be transported indoors by pets, insects and other animals [8].

While culture-based studies have found microbes to be ubiquitous in the BE, recent culture-independent molecular studies have shown that our understanding of indoor microbial diversity remains extremely sparse [9-13]. These molecular methods are also an integral part of the tool kit for expanding our understanding of what species of microorganisms live in these ecosystems, how they function and how they migrate among humans, physical surfaces and other environments [6,14,15].

The most commonly used molecular approach for exploring microbial diversity is based on the PCR amplification and sequencing of genes encoding small subunit ribosomal RNA (16S rRNA) directly from environmental sources [16]. In this approach, DNA is directly extracted from all the microbial cells present in a sample and 'universal' PCR primers designed from conserved regions of the 16S rRNA gene are used to amplify this gene from all the microbial genomes in the sample [16,17]. This approach readily determines several orders of magnitude more microbial diversity than culture-based methods, often discovering novel organisms with no known growth conditions, and has dramatically altered our understanding of global microbial diversity [14]. Although powerful, there are two obvious sources of bias in this method. First, different DNA extraction techniques allow access to different parts of the microbial diversity to varying extents, through uneven cell lysis [18,19]. Second, there are no truly universal PCR primers, with even the most comprehensive pair covering approximately 85% of the known taxa [20]. Despite these limitations, this technology, when combined with a rigorous experimental design, is extremely useful for characterizing the dynamics and ecological patterns of microbial ecosystems [21,22]. The initial 16S rRNA diversity studies performed in the BE cloned PCR amplicons into plasmid vectors before transformation, colony isolation and sequencing [9,10,12]. However, the application of direct emulsion-PCR-based sequencing in 2006 [23] dramatically increased the depth and breadth of analysis; similar technologies now enable the simultaneous processing of thousands of samples at a

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depth of hundreds of thousands of sequence reads each [24,25].

Here, we review the literature on culture-independent analysis of microbial diversity in the BE and explore what it tells us about the dynamics and distribution of bacteria and fungi in these environments. So, why is the BE even worth exploring? First, as already stated, the BE is the primary habitat for a large and growing percentage of humanity, whose health might depend upon our firm comprehension of the interaction between humans and the microbiology of buildings. Second, the climate, materials and design of artificial environments could have unexpected and interesting consequences for the selection and growth of microbes that might help us to design healthier buildings. Finally, a deeper understanding of indoor microbial diversity should help inform public health policy, particularly in settings with many immune-compromised individuals (for example, hospitals, intensive care units and nursing homes). The renewed interest in microbial ecology, and the application of next-generation sequencing technology, presents exciting opportunities to uncover hidden diversity and interactions in these environments. As we will show, these methods have already revealed the BE to be a surprisingly complex and highly dynamic microbial ecosystem that collects and selects for a remarkable array of novel diversity.

Unnatural selection

It goes without saying that the BE contains chemical compounds and physical conditions entirely unlike the natural world where microbes evolved. Drywall, floor wax, polyester, carpeting, constant temperature and humidity, novel hydrocarbons and polymers - these are just some of the many features found in BEs that potentially provide unusual growth conditions and niches for bacteria and fungi. While natural microbial evolution might not have originally selected for phenotypes able to exploit these conditions or materials, the incredible diversity and adaptability of the microbial world means that there probably exists a microbe that will survive on almost any BE surface or condition. Moreover, the short generation times and rapid evolutionary mechanisms (for example, horizontal gene transfer) found in the microbial world mean that many microbes might have already adapted to the BE over the past few hundred years.

Culture-based studies have repeatedly discovered conditions in the BE that enrich for particular bacteria and fungi [26-28]. However, while culture isolation focuses on the presence of particular bacteria or fungi, molecular studies of BEs typically uncover polymicrobial communities comprising hundreds or thousands of uncultured microbes. When conditions in the BE enrich strongly for particular traits, these polymicrobial communities tend

to be dominated by a few microbial groups (Table 1). For instance, an analysis of bacterial 16S rRNA sequence diversity in hospital therapy pools and showerheads found they enriched for diverse, primarily uncultured, soil-dwelling *Mycobacteria*, as well as many Proteobacteria and other, rarer groups [9,29]. Shower curtains tend to be dominated by dozens of different *Sphingomonas* and *Methylobacterium* species, many of them uncultured [10]. The latter are particularly interesting as some *Methylobacterium* species have been shown to grow on an enormous diversity of different carbon compounds (that is, C2, C3 and C4 compounds) [30], and some species thrive on the decay products of human skin cells [31]. Studies of showerheads and hospital shower stalls find them, like therapy pools, to be hospitable environments for Actinobacteria and Proteobacteria [29,32]. Other highly selective environments include the tubing of catheters [33,34], which maintain a significantly different microbial community internally compared with externally, and NASA clean-rooms used for spacecraft assembly [35,36]. In the latter case, the extensive sealing and sterilization protocols appeared to select for only the hardiest of microbes that also survive space journeys [35].

Colonization and dispersal within the built environment

Molecular studies have also enhanced our understanding of how indoor microbes colonize surfaces and are transmitted between microenvironments. Studies of keyboards [37], restrooms [4] and offices [5] show that humans are the main source of the microbial diversity in many indoor environments and are also its principle vector. Perhaps unsurprisingly, a great number of surface microbes can be traced back to human skin, although gut and nasal/oral environments also contribute [5,6]. Molecular studies of bacterial diversity in offices [5,12], hospitals [11], airplanes [7,38], kitchens [39] and restrooms [4] have consistently found a predominance of human skin-associated, and gastrointestinal or urogenital-associated, bacteria on most physical surfaces. In fact, Tringe and colleagues showed that the bacteria living on the skin of individuals inhabiting a particular space define the microbial community observed in air samples taken from that same space [13]. Given that the average human sheds approximately 1.5 million skin cells an hour, carrying with them approximately 15 million bacterial cells [40], humans clearly contribute substantially, and perhaps predominantly, to indoor microbial diversity.

Direct transfer of skin microbiota through surface contact has also been shown to have a considerable and area-specific impact. Depending on the surface, skin contact can transfer millions of microbial cells in each event [41], leading to the rapid and continual inoculation

Table 1. Signature microbes of various built environments

Site	Bacterial species
Office	<i>Streptococcus</i> spp. <i>Corynebacterium</i> spp. <i>Flavimonas</i> spp. <i>Lactobacillus</i> spp. <i>Burkholderia</i> spp. <i>Bacillus</i> spp. <i>Bradyrhizobium</i> spp.
Neonatal intensive care unit	<i>Propionibacterium</i> spp. <i>Enterobacter</i> spp. <i>Neisseria</i> spp. <i>Pseudomonas</i> spp. <i>Streptococcus</i> spp. <i>Staphylococcus</i> spp.
Aquarium filter	<i>Nitrospumilus</i> spp. (archaea) <i>Nitrospira</i> spp. <i>Nitrosomonas</i> spp.
Hospital air	<i>Kytococcus sedentarius</i> <i>Staphylococcus epidermidis</i> <i>S. haemolyticus</i> <i>Ralstonia pickettii</i> <i>Enterobacter</i> spp. <i>Kocuria rhizophila</i> <i>Micrococcus luteus</i> <i>Microcystis aeruginosa</i> <i>Prochlorococcus marinus</i> <i>Methylocella silvestris</i> <i>Methylobacterium extorquens</i>
Catheter	<i>Pseudomonas</i> spp. <i>Staphylococcus epidermidis</i> <i>Enterococcus faecalis</i> <i>Klebsiella</i> spp.
Restroom	<i>Propionibacterium</i> spp. <i>Corynebacterium</i> spp. Micrococcaceae <i>Streptococcus</i> spp. <i>Staphylococcus</i> spp. Bacteroidaceae
Shower curtain	<i>Methylobacterium</i> spp. <i>Sphingomonas</i> spp.
Showerhead	<i>Mycobacterium</i> spp. <i>Pseudomonas aeruginosa</i> <i>Legionella</i> spp.
Therapy pool	<i>Mycobacterium</i> spp. <i>Sphingomonas</i> spp.
Classroom	Propionibacterineae Xanthomonadaceae Micrococcineae <i>Sphingomonas</i> spp. <i>Caenibacterium</i> <i>Staphylococcus</i> spp. Enterobacteriaceae Corynebacterineae

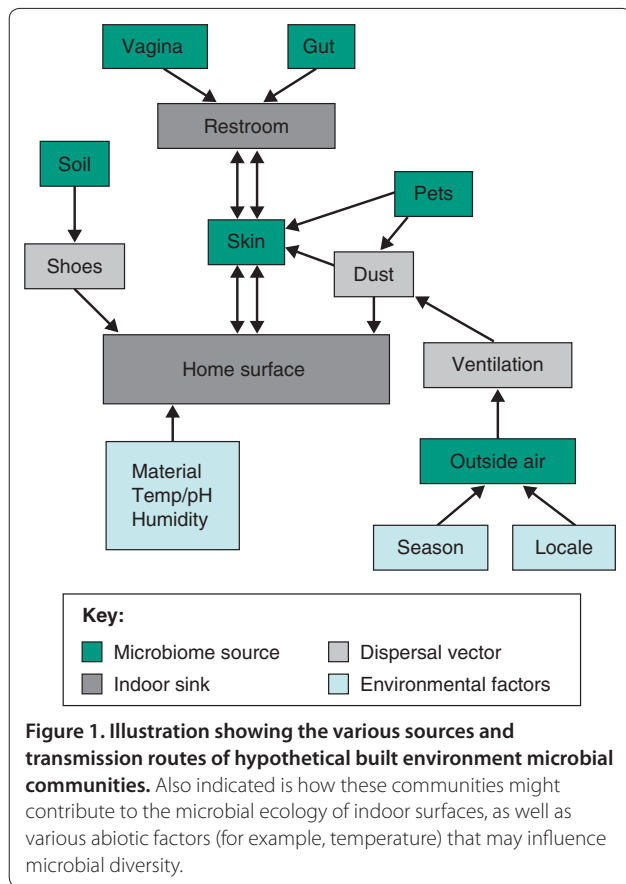
and re-inoculation of surfaces touched repeatedly, such as light switches, door handles, keyboards, soap dispensers and toilet seats [4,5,37]. Furthermore, a number of studies have demonstrated that the dominant microbiota

associated with human skin differs dramatically among individuals [42-46]. Indeed, it might be possible to identify people based on the unique microbial signature they leave behind in a room or on a surface, which has significant potential applications in forensics. Figure 1 diagrams various sources and transmission routes of hypothetical BE microbial communities and indicates how they might contribute to the microbial ecology of indoor surfaces.

Despite the evidence suggesting that humans interact microbially with their indoor spaces, the mechanistic relationship enabling this interaction remains poorly characterized. As the BE can also be a source of microbes that colonize people, it is essential that we investigate 'who lives where' in the BE. Thus far, the literature has primarily explored the phenomenon of fungal contamination on damp surfaces [40,47-49]; the role of hygiene in removing microbial communities [46,50]; and the length of time microbes can survive on surfaces [51]. The microbial diversity of dust has also been explored [12,52,53], as has the diversity of indoor air [11,13,54]. One study investigated the temporal succession of microbial communities performed on indoor dust and found building-specific seasonal patterns that were a combination of skin cells shed from inhabitants within the buildings and seasonally defined microbiota from the external environment [12].

In addition to human skin, other aspects of the human microbiome can also contribute significantly to the BE microbiome, although the contribution tends to be more situation specific. As might be expected, human gut and vaginal flora play a major role in shaping the microbial communities associated with surfaces in restrooms [4], daycare centers [55] and neonatal intensive care units [56]. Although few molecular studies have directly investigated the impact of pets, it is expected that animal skin, hair, fecal material (for example, cat litter), saliva and possibly fleas all contribute significantly to microbial communities associated with indoor surfaces and air [1,8]. The impact of fish tanks on the BE microbiome might also be worth considering as these environments have a unique microbial consortia [57,58]. Understanding the contribution of animal inhabitants might be particularly helpful in understanding the causes of childhood asthma [59].

Most indoor settings also obtain a considerable fraction of their microbiota from environmental sources. Dust samples in offices consistently uncovered bacteria associated with soil and the plant rhizosphere [5]. The amount and type appears to depend on the degree of outdoor ventilation and the local climate. Kembel and colleagues found significant differences in the contribution of natural environmental bacteria in buildings that were well ventilated versus those with sealed windows



[11]. Rintala and co-workers showed dramatic changes in dust microflora according to different seasons [12], while Hewitt and colleagues found a strong climatic signal in the office dust of buildings in Tucson, Arizona (desert soil bacteria), compared with dust in New York and San Francisco [5]. The restroom study of Flores *et al.* also found soil bacteria over the restroom floor that was probably transferred from shoes [4].

Clearly, BE microbial communities comprise microbes from a diverse array of human and environmental habitats. The ability to compare vast metagenomic datasets of either ribosomal sequences or whole microbial genome fragments from BE sinks with environmental, animal and human source communities, and the application of bioinformatic tools that estimate the relative contribution of various sources to communities (for example, SourceTracker [6]), will be instrumental in understanding microbial colonization and transmission in the BE.

Open questions and future directions

The molecular studies cited in this review have just begun to inform and advance the understanding of microbial diversity in the BE. As the methods improve in terms of cost, speed and depth, the experimental design possibilities grow proportionally. Analyzing thousands of

samples simultaneously provides a much broader assessment of the role humans and environmental sources play in the development and persistence of the indoor microbiome. These 'faster, better, cheaper' methods will also allow more sophisticated statistical design and lengthy time-series analyses.

Proper study designs and increased sequencing power will make it possible to understand how BEs are colonized, how microbes spread through these environments, and the rate at which microbial communities change in the BE. For example, a recent study funded by the Alfred P Sloan Foundation plans to investigate all these factors in a newly built hospital as it becomes occupied with staff and patients [60]. The relatively low cost of sample processing and sequencing means that this study is examining >15,000 surfaces (both human and built), with daily (35 samples a day) collection for an entire year, providing unparalleled resolution for investigating colonization and transmission of bacteria, fungi and viruses in the hospital infrastructure. This study aims to identify the microbial streets and highways that enable rapid transmission of bacteria through this ecosystem, and it is expected that pathogens will also use these routes. Because the human microbiome contributes the majority of the BE microbes, and humans also probably vector a significant proportion of soil and other environmental microbes through shoes, hands and clothes (for example, see [60]), future studies need to follow and sample the human inhabitants as they enter and move through the BE over time, but to do this it will be necessary to define the rate of change in the microbiome to sample at the appropriate temporal and spatial resolution.

A number of other key questions concerning the BE will need to be addressed, such as the role of surface materials, ventilation, local climatic conditions and the physical conditions of the building in shaping the microbial diversity. Answers to these questions will require thorough and systematic sampling, rigorous statistical design and collaboration with experts in building science and engineering. The issue of ventilation and airflow appears to deserve special attention as these affect the rate and direction of bioaerosol transmission to sensitive respiratory mucosal surfaces. Studies by Kembel *et al.* [11] and Poza *et al.* [61] provide tantalizing evidence that the amount of external air makes a significant difference in the microbial diversity in a hospital setting, perhaps even affecting overall pathogen loads, and the usage of different environments (for example, operating room versus waiting room) also impacts the microbial profiles of these spaces. Collaborations between microbiologists and building scientists will be especially important for understanding the impact of materials and machine-aided ventilation, especially given their variability and complexity between different BEs.

Thus far, the molecular studies of the BE have primarily investigated bacterial diversity through the sequencing of community 16S rRNA gene amplicons. The advantages of using this gene for surveying microbial diversity are well known [62]. However, the limits of amplicon sequencing for both diversity and functional studies are also familiar, including PCR bias [63], variable copy numbers per genome [64], limited taxonomic resolution [65] and indirect functional knowledge (that is, inference of community function through phylogenetic relationships). Higher throughput and sensitivity (for example, lower DNA concentrations required to generate sequence data), lower sequencing costs and advances in computation should enable the future application of shotgun metagenomics (that is, sequencing of all genomic DNA in a given sample) and whole-genome sequencing in the indoor environment [66,67]. This will allow the simultaneous analysis of microbial diversity, including deeper taxonomic resolution (strains and subspecies) and more direct functional analysis at the genomic level. For example, the Hospital Microbiome Project is implementing Illumina Nextera library generation to sequencing thousands of microbial and viral metagenomes. Deeper taxonomic resolution and determination of gene function will be important for verifying pathogens in indoor settings by allowing the differentiation between commensals and pathogens (for example, *Escherichia coli* K12 versus *E. coli* OH157:H7) and for detecting pathogenic islands or antibiotic resistance markers on chromosomal and extra-chromosomal elements. Table 2 compares and contrasts shotgun metagenomics and other methods (amplicon sequencing, metatranscriptomics, metaproteomics and metabolomics) used in microbial community research that could potentially be applied to the BE.

Methods such as shotgun metagenomics will also be necessary to analyze the diversity of viruses (bacteriophage and eukaryotic viral diversity, also known as the 'virome' [68]) in the BE, which is very poorly understood. Bacteriophage, in particular, play a major role in the dynamics of natural microbial populations (predator-prey relationships) [69] and also facilitate the horizontal transfer of crucial genes (for example, toxin and metabolic genes) among bacteria [70]. Models of BE microbial ecology will certainly be incomplete without a parallel understanding of viral community diversity, and understanding the contribution of bacteriophage to bacterial pathogenicity in places such as hospitals could be an extremely important contribution to public health. Recent studies have used deep-sequencing approaches to characterize viral diversity and gene content for bacteriophage [71,72] and eukaryotic viruses [73] in the human microbiome, and similar approaches could be applied in the BE. In fact, we ourselves have been funded by the Sloan Foundation to study viral metagenomics in the BE.

Although we have found evidence of viral-like particles (VLPs) in all of our samples, the low biomass on indoor surface biofilms presents a significant challenge to this work, particularly with short-lived and low-abundance eukaryotic viruses.

Molecular studies of fungal community diversity, clearly of great interest in the BE, have also been limited by choice of marker gene and taxonomic breadth of primer amplification [53,74,75]. However, the recent development of novel primers targeting the intergenic spacer region between small- and large-subunit ribosomal RNA genes is being applied to amplify a broader diversity of fungal sequences that could help expand our understanding of BE fungal diversity [75]. Dollive and colleagues have also developed a sorely needed software pipeline for the analysis of eukaryotic 18S rRNA gene sequences [76]. The combination of primers that capture a greater taxonomic diversity and better software tools for analysis should allow greater insight not only into BE fungal diversity but also for a deeper understanding of the interactions between BE fungal and bacterial communities.

One criticism of environmental molecular studies, which is of particular relevance for the BE, is the issue of whether a microbial DNA signature comes from a viable organism. Identification of a microbe through DNA sequencing only verifies that the particular organism was present at some time, but it might no longer be viable, especially considering the extreme conditions present on most surfaces (for example, extremely low humidity, limited nutrients, UV exposure). Understanding which of the microbes in a community persist in a viable state will be especially important for verifying their pathogenic potential. While culturing remains the standard for establishing viability, it is limited in that most organisms are recalcitrant to culture. Therefore, it is essential that we develop methods for both the *in situ* radiolabeling of active organisms and the direct sequencing of rRNA and mRNA from microbial communities. One of the common problems in bacteria transcriptomics is the removal of ribosomal RNA, which comprises 90 to 95% of the total RNA. In eukaryotic cells, the mRNA has a poly-A tail, which can be used to selectively isolate these sequences. Therefore, since 2008, many methods have been developed to remove rRNA and improve the enrichment of bacterial mRNA in community samples (for example, [77,78]). Indeed, the development of rapid rRNA removal and mRNA enrichment tools has led to these methods being applied even in low-biomass environments, such as subsections of human intestinal epithelium tissue, which now put these techniques in reach of researchers working with BE samples [79]. RNA is rapidly degraded in the environment, and its presence suggests the existence of active cells [77]. However, the low biomass on most

Table 2. Comparison of different techniques, including pros and cons, for analysis of microbial communities

Technique	Description	Advantages	Disadvantages
Amplicon sequencing	The amplification and sequencing of a single gene from a broad selection of the microbiome. Traditionally applied to 16S rRNA for bacteria, but now being applied to a wide range of targets	Primarily cost and depth of analysis. Amplicon sequencing is very cheap and enables a rapid and deep characterization of the varying structure of microbial life under changing environmental gradients	This approach provides a narrow field of view, targeting a single gene, be it taxonomic or functionally informative, and only gives information about that one gene. It also can be affected by primer and amplification biases
Genomic sequencing	Sequencing of the genome of representatives of a community, ideally resulting in a single sequence, but more often resulting in 100s of genomic fragments	The genome enables a defined link between potential function and phylogeny, so that one can deduce that species x performs process y. When linked to a cultured cell, it can also be used to define gene function through targeted biochemical tests	Throughput is a problem. Sequencing the genome of isolated organisms has become rudimentary, but few can be isolated. Screening sorted cells from a community, followed by sequencing, is becoming viable, but often results in limited coverage of the genome owing to amplification bias
Metagenomic sequencing	Sequencing of a random sample of the genomic DNA from the cells of a microbial consortium	This technique enables broad observation of the taxonomic and functional genes from an entire community, without the bias associated with amplicon sequencing. With sufficiently deep sequencing, it is also possible to reassemble microbial genomes and other genetic elements	Current sequencing platforms require extensive starting material, although this is changing. Cost can be prohibitive, leading to only shallow characterization of the most dominant microbial taxa. The output only describes potential function, and it is often difficult to link function and phylogeny definitively. Also has the potential to sequence DNA from dead cells
Metatranscriptomic sequencing	Random sequencing of the messenger, small and other RNAs from a microbial community that define the mechanism and response of microbial gene expression	As with metagenomics, this technique enables broad taxonomic and functional characterization, but of expressed genes, which enables deeper analysis of the community and targets the active members of the community. Sequence data can be mapped to known genomes to help identify phylogenetic-specific functional responses	Cost is prohibitive as the steps required to remove the 90-95% of ribosomal RNA to enable deeper characterization of the mRNA are expensive and time consuming, which also limits throughput. RNA is sensitive to degradation, and the half-life of mRNA is very short, which creates biases from sampling the community.
Metaproteomic sequencing	Random sequencing of the amino acid sequences that represent the protein material in a microbial community	The primary advantage is the ability to identify proteins that have not only been expressed as mRNA but have also been folded and have potentially formed active proteins - for example, enzymes - in a cell. When combined with genomics and metatranscriptomics, it is possible to define protein isoforms and map protein function to phylogeny	The primary disadvantage of cost and throughput, which is still higher than metagenomics or metatranscriptomics. By itself, it is complicated to assign taxonomy
Metabolomic sequencing	Random characterization of the metabolic products present in a sample that might have been generated by a microbial community	This is the zenith of microbial activity, and when compared with that of other samples, the relative change in metabolite concentration can explain a lot about the functional consequence of genomic potential, or transcript and protein abundance	As with proteomics, metabolomics is limited by cost and throughput but also by identification of products. It also currently has a limit of detection, with very rare metabolites being hard to detect

surfaces will require innovative strategies to reach the nucleic acid quantities required for most sequencing platforms (that is, 0.5 to 1 µg). The development of novel extraction techniques (for example, MoBio power kits) and sequencing library preparations (for example, Nextera-Illumina, which requires 1 to 50 ng of starting material) means that a solution is potentially imminent.

Concluding remarks

Studies of the unseen majority inside the BE were initiated to determine how bacteria and fungi might affect human health long after it was realized that spending time indoors, away from fresh air, might increase rates of acute and chronic illness. In recent years, it has become apparent that studying the BE might also allow us to

better interrogate the ecological interactions between microorganisms. This means that our artificial environments, in and of themselves, have become a new ecosystem worthy of significant investigation. Research focused on the BE has also allowed us to elucidate how microbes interact in this space, and it is, in turn, enabling us with the help of new technologies to understand better how humans interact with the microbiology of indoor spaces. Recent discoveries in the microbiome of the BE, facilitated by enlightened investment, show us that we share our living spaces with an immense diversity of microbial life, some potentially bad, others good, and most just along for the ride. As the resource and personnel costs of performing high-throughput microbiological studies decrease, it will be possible to perform ever-more-detailed characterization of the rate of transmission of microbes between the BE and ourselves and of the metabolic consequences of sharing our living and working spaces with the invisible biosphere. Without a doubt, this new field of research will lead to discoveries that will change the way that we build, clean and live in our new ecosystem.

Competing interests

The authors declare that they have no competing interests

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