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Tools to improve built environment data collection for indoor microbial ecology investigations

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ABSTRACT

Recent studies have greatly increased our knowledge of microbial ecology of the indoor environments in which we live and work. However, the number of studies collecting robust, long-term data using standardized methods to characterize important building characteristics, indoor environmental conditions, or human occupancy – collectively referred to as "built environment data" – remain limited. Insufficiently described built environment data can limit our ability to compare microbial ecology results from one indoor environment to another or to use the results to assess how best to control indoor microbial communities. This work first reviews recent literature on microbial community characterization in indoor environments (primarily those that utilized molecular methods), paying particular attention to the level of assessment of influential built environment characteristics and the specific methods and procedures that were used to collect those data. Based on those observations, we then describe a large suite of indoor environmental and building design and operational parameters that can be measured using standardized methods to inform experimental design in future studies of the microbial ecology of the built environment. This work builds upon the recently developed MIxS-BE package that identifies high-level minimal built environment metadata to collect in microbial ecology studies, primarily by providing more justification, detail, and context for these important parameters and others from the perspective of engineers and building scientists. It is our intent to provide microbial ecologists with knowledge of many of the tools available for built environment data collection, as well as some of the constraints and considerations for these tools, which may improve our ability to design indoor microbial ecology studies that can better inform building design and operation.

1. Introduction

Recent advances in DNA sequencing techniques that allow rapid, high-throughput characterization of taxonomic marker genes (e.g., 16S/18S rRNA and fungal ITS) and whole genomic DNA from environmental samples [1,2], coupled with the recognition that the majority of people in the developed world spend most of their lives indoors [3–7], has led to a rapid increase in the number of studies exploring microbial diversity within the built environment [8–10]. Recent studies have characterized microbial diversity in offices and other commercial buildings [11,12], university buildings and classrooms [13–17], healthcare facilities [18–23], homes [24–29], public restrooms [30], and transportation environments [31,32], all of which represent indoor environments where people spend much of their time.

These recent studies have greatly increased our knowledge of microbial community structure and composition within the spaces in which we live and work. They have revealed that culture-based methods vastly underestimate the abundance and diversity of microbial communities in air and on surfaces indoors. A number of these recent studies, in addition to many others from years of studies relying on cultures and other methods, have also shown that a number of building design and operational characteristics, indoor environmental conditions, and human occupancy patterns can strongly influence the structure, diversity, abundance, and survival of microbial communities found indoors [13,15,23,33]. Some of these important building-related parameters include air and surface temperatures, relative and absolute humidity, outdoor air ventilation rates, HVAC particle filtration efficiency, human occupancy, human contact frequencies with surfaces, and several others.

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However, the number of studies collecting robust, long-term data using standardized methods to characterize building operation, human occupancy, indoor environmental conditions remain limited. Insufficiently or inadequately described building operational and environmental characteristics can limit our ability to compare results from microbial ecology investigations from one indoor environment to another or to use the results to assess how best to control indoor microbial communities [34]. Therefore, this work first reviews recent literature on microbial community characterization in indoor environments, paying particular attention to the level of assessment of influential indoor environmental characteristics and the specific methods and procedures that were used to collect those data. Based on those observations, we then describe a large suite of indoor environmental and building design and operational parameters – collectively referred to as "built environment data" – that can be measured using standardized methods to inform experimental design in future studies of the microbial ecology of the built environment. This work is intended to support the recently developed MIxS-BE package [35] that describes minimal built environment metadata to collect in microbial ecology studies by providing more justification, detail, and context for these important parameters and others from the perspective of engineers and building scientists.

2. Recent culture-independent analyses of microbial communities in indoor environments and their relationships to built environment data

Recent studies utilizing culture-independent analyses of microbial communities in indoor environments can be grouped into three general categories based on their level of detail in documenting built environment data: (1) those that did not include any building descriptions or building environmental measurements; (2) those that included some basic information about building characteristics, heating, ventilating, and air-conditioning (HVAC) systems, outdoor air ventilation strategies, occupant behaviors, and/or environmental conditions during the time of testing (i.e., *basic built environment metadata*); and (3) those that included detailed information about HVAC systems, environmental conditions, and/or human activities in the sampled space (i.e., *detailed built environment data*). Although there is some overlap between these categories, it is instructive to think of these studies with increasing complexity in terms of building environmental, operational, and/or occupational characterizations. The next sections describe details of several studies in each of these categories with the intent of demonstrating how robust built environment data collection can be used to generalize results from one indoor environment to another and how a lack of methods standardization for environmental data collection can lead to a decreased ability for extrapolation.

2.1. Microbial diversity in the absence of built environment data

Many recent culture-independent studies of microbial diversity in the built environment have focused primarily on surface sampling, with fewer incorporating various methods of indoor air sampling. Many of these studies focused on characterizing microbial communities without characterizing building characteristics or local indoor environmental and operational parameters that may greatly affect microbial diversity [11,18,20–22,25,28,30]. For example, Kelley et al. (2004) analyzed bacteria in biofilms from used shower curtains from different households [28]. Each community was shown to be highly complex and no identical sequences were encountered in the different communities. However, no information was reported on relevant environmental parameters and conditions that may have influenced microbial diversity among shower curtains such as shower usage, cleaning procedures or frequency, or other building environmental conditions that could have influenced bacteria survival such as temperature or humidity. Documentation of such conditions, particularly cleaning procedures, might have helped explain some of the variability among sampling locations as other studies have shown [36].

Lee et al. (2007) found a large diversity of uncultured bacteria on a number of surfaces in a child-care center, including sequences related to those found in human vaginal epithelium and wastewater sludge, as well as a number of pathogens [22]. Additionally, they observed some temporal variability in bacterial diversity in this environment over a six-month period, particularly during cold and flu season. Although, without collecting data on building operation, environmental conditions, or human occupancy, this variability could not be readily explained, which limits extrapolation to other environments. Hewitt et al. (2012) found that bacterial diversity on several surfaces in office spaces in Tucson, AZ were clearly different from those found in New York, NY and San Francisco, CA (which were indistinguishable from each other) [11]. Additionally, bacterial abundance was significantly lower in San Francisco compared to both Tucson and New York. However, with samples in three very different climates, a lack of information on human occupancy or building design and operational characteristics limit our ability to further interpret these results beyond basic geographic differences.

Poza et al. (2012) compared bacterial diversity on surfaces in intensive care units (ICUs) to that observed in an open, crowded entrance hall of a hospital [20]. Bacterial diversity detected in the ICU was different from that in the hall, suggesting that high human occupancy in close proximity within the hall may have played an important role. Hewitt et al. (2013) surveyed bacterial diversity in two neonatal intensive care units (NICUs) and tracked the sources of microbes [18]. Many of the bacteria genera included known pathogens and many were skin-associated. Fecal coliform bacteria were also detected in high proportions of surface samples in one of the units. Although the authors maintained a particular focus on commonly touched surfaces in the units, no quantitative measures of human occupancy were noted, which may have helped further explain their results. In another study of ICUs in a hospital, Oberauner et al. (2013) sampled floors, medical devices, and workplaces [21]. Floor-associated communities formed distinct clusters compared to devices, whereas workplaces and devices were similar, again suggesting occupancy influences. The authors also noted that the investigated ICU had both air conditioning and window ventilation, although it was not clear which was being utilized during the study or how they may have impacted the results.

Overall, results from these and other similar studies have provided tremendous additions to our knowledge of the diversity, composition, and structure of indoor microbial communities. However, extrapolation of results to other indoor environments is challenging without more knowledge on the particular sample environments themselves.

2.2. Microbial diversity and basic built environment metadata

Several recent indoor microbial investigations have provided basic information about qualitative building characteristics, HVAC systems and ventilation strategies, occupant behaviors, and/or basic indoor environmental parameters such as air temperature (T) and relative humidity (RH) during testing that could potentially influence or explain some of the observed results. For example, Rintala et al. (2008) investigated the composition and dynamics of bacterial communities in settled dust using vacuum cleaner

sampling inside offices in two similarly aged buildings over a period of one year [19]. A civil engineer performed a technical inspection on both buildings; one building had local signs of moisture and microbial damage in the bathrooms, which is important to note because higher biological loads have been observed in water-damaged buildings and building materials [37–39] and dampness is consistently associated with a number of adverse respiratory health effects [40–43]. In fact, the authors noted that employees in the building had complained of building-related health symptoms and indoor air problems. Interestingly, differences between buildings were more pronounced and consistent than seasonal differences in the same buildings, although without more knowledge on occupancy, activity, and cleaning patterns, environmental conditions, ventilation rates, specific building materials, or the mass of settled dust on the sample surfaces, it remains difficult to further extrapolate these results to other indoor environments.

Amend et al. (2010) performed a survey of fungal composition in settled dust samples in indoor environments in 72 buildings across six continents, again using vacuum cleaner sampling methods [44]. Sixty-one buildings were households and the rest were offices, shops, and a church. Samples were taken in "accessible," "infrequently accessed," and "inaccessible" areas in each of the buildings, corresponding to different classifications of likely direct human occupancy. Fungal diversity was significantly higher in buildings located in temperate zones than in the tropics (measured by distance from the equator). This was hypothesized to reflect correlations with local outdoor environments, impacted by variables such as rainfall and temperature, although specific environmental conditions were not measured. Interestingly, building function (i.e., homes versus offices) had no effect on indoor fungal composition, despite very large differences in both architecture and materials of buildings (although these differences were not described in detail).

More recently, Kembel et al. (2012) quantified airborne bacterial communities and environmental conditions inside patient rooms of a hospital that were occupied only by researchers during testing and in outdoor air on the roof near the outdoor air intake of the HVAC system [23]. The rooms were classified as "exposed to mechanical ventilation" or "exposed to window ventilation." The mechanically ventilated rooms had ventilation air supplied by the HVAC system and removed by a return duct and a bathroom exhaust duct. The window ventilated rooms had ventilation air supplied directly from the outside through a window and removed through a return duct, bathroom exhaust, and by any outflow through portions of the same window. The phylogenetic diversity of airborne bacterial communities was lower indoors than outdoors overall, although the mechanically ventilated rooms were less diverse than window-ventilated rooms. However, bacterial communities indoors contained many taxa that are absent or rare outdoors, including those potentially related to human pathogens, suggesting humans were significant sources in these particular rooms. Other building environmental parameters such as the source of ventilation air, airflow rates, and indoor T/RH were also correlated with the diversity and composition of indoor bacterial communities (although some of these factors also correlated with each other). The relative abundance of bacteria closely related to human pathogens was higher in rooms with lower airflow rates (used as a surrogate for air change rates) and lower relative humidity. Results from this study clearly demonstrate that the source of ventilation air is an important determinant of indoor microbial communities, which suggests that at a minimum this kind of basic built environment data should be collected in future studies.

Adams et al. (2013) assessed the pattern of fungal diversity and composition in airborne dust that settled onto suspended petri dishes both indoors (in the kitchen, living room, bathroom, and bedroom) and outdoors (on a patio or deck) at a university housing facility [45]. The authors also noted several details about the construction of the housing complex units (e.g., age of construction, exterior cladding material, and interior wall material). Each building had its own forced-air ventilation system with heating but no air-conditioning. A short survey was given to occupants inquiring about unit age, the number of various types of rooms, and the frequency of cleaning by the occupants. Indoor air T/RH were also measured during sampling. Some of these factors were significant predictors of fungal community composition across units in single-factor models, including floor level and frequency of cleaning; however, only geographic distance from each other remained significant predictors in multifactor models. Indoor T/RH showed no association across the ranges measured. Overall, more fungal biomass was found in outdoor air versus indoor air and indoor fungal assemblages strongly correlated with outdoor measurements. No fungal taxa were found as indicators of indoor sources and room and occupant behavior had no detectable effect on the fungi found in indoor air, suggesting that local outdoor air fungi dominated the patterns of indoor air fungi in these residences. However, if more basic building characteristics such as air change rates (ACH) or building airtightness had been measured and documented, we hypothesize that more could potentially have been inferred about variations in fungal communities between buildings since these parameters are well known to greatly influence outdoor particle infiltration [46–48].

Adams et al. (2014) also examined the bacterial component of the same residential samples mentioned above and found that, as with fungi, bacterial richness was higher outdoors than indoors [49]. It was also higher in units that reported some humidifier use, which suggests that moisture-generating indoor activities are important built environment related mdata to capture. Bacterial composition varied by residential unit and room type, while fungi varied by season and residential unit. Indoor samples had a large amount of human-associated taxa not found outdoors, indicating humans as a greater indoor source of bacteria than fungi.

Meadow et al. (2013) measured indoor and outdoor airborne bacterial communities using button samplers installed over a period of 9 days in 8 classrooms within a highly-trafficked university building with a hybrid HVAC system (i.e., with both mechanical and natural ventilation) [14]. Four of the classrooms were identified as "night-flushed" rooms, which received mostly unfiltered outdoor air ventilation and four others were identified as "non-night-flushed" rooms, which received a combination of filtered recirculated air and outdoor air controlled by an economizer HVAC system that adjusted outdoor airflow fractions based on outdoor temperatures. All mechanically supplied air passed through a MERV 8 particle filter. MERV, or Minimum Efficiency Removal Value, is a common method for classifying the particle removal efficiency of HVAC filters, per ASHRAE Standard 52.2 [50]. Indoor air communities closely tracked outdoor air communities, but human-associated bacterial genera were more than twice as abundant in indoor air than outdoor air. Importantly, ventilation was shown to have a demonstrative effect on indoor airborne bacterial community composition, particularly after following a time lag associated with particular ventilation strategies. For example, when the fraction of outdoor air was reduced to 0% for several hours in the "non-night-flushed" rooms (i.e., with 100% recirculating and filtered air), indoor bacterial communities became very dissimilar from outdoor air communities; at the same time bacterial communities in the rooms with nearly 100% unfiltered outdoor air supply were very similar to those observed outdoors. Thus, the importance of the source and delivery rates of ventilation air was again clearly demonstrated in this study.

Overall, results from the studies in this section suggest that outdoor ventilation strategies and HVAC system airflow rates can

greatly impact indoor bacterial communities, which is intuitive given their large influence on concentrations of both particles and gases [47,51–54]. These results also suggest that basic environmental parameters such as temperature and relative humidity and human occupancy patterns play an important role in influencing indoor microbial communities, which is consistent with previous literature [33,55,56].

2.3. Microbial diversity and detailed built environment data

Finally, several recent studies have focused more on gathering very detailed information about building characteristics, environmental conditions, and/or human activities in their sampled environments, which we believe has served to clearly demonstrate the importance of robust, standardized built environment data collection. Perhaps most important for demonstrating this, Qian et al. (2012) quantified size-resolved emission rates of airborne biological (bacterial and fungal) particles from people using staged measurements in a 90 m^3 classroom [13]. Emission rates are important to characterize because they allow for direct extrapolation to other environments and comparisons between sources. The authors were able to calculate emission rates because they sufficiently characterized detailed building operation, including ACH, HVAC operation, and the number of occupants during sampling. More specifically, their study used measurements during four days while the room was occupied and four days while the classroom was vacant. The room held an average of 4.7 people during a total of 22.2 h of sampling during occupied periods, assessed visually by the researchers. Windows and doors were closed and conditioned air was delivered by the HVAC system through a single register. Exhaust ports were located along the floor and near the wall opposite of the ventilation supply. The ACH was measured using periodic injections of CO_2 followed by decay periods; the mean ACH was 5.5 per hour. Particles were sampled onto polycarbonate filters loaded into an 8-stage non-viable impactor. To obtain genome copies above detection levels on all stages, the impactors sampled air cumulatively for the four consecutive occupied or vacant experimental days. Optical particle counters were also used simultaneously to measure size-resolved number concentrations in the room.

Size-resolved microbial emission rates during human occupancy were estimated by considering the room as a well-mixed reactor and using a time-averaged mass balance to quantify the indoor concentration as the sum of a fraction of the outdoor concentration (measured during vacant periods) plus a contribution from indoor emissions (which is a function of individual emission rates, the number of people present, the volume of the space, the outdoor air ventilation rate, and size-resolved particle deposition rates). Size-resolved particle deposition rates were assumed from previously measured values in existing literature [57]. Emission rates of bacteria or fungi were assumed to be the same for each person in the room. Bacterial genomes showed a strong peak in indoor concentrations during occupancy for particles in the 3–5 μm aerodynamic diameter size range. Fungal genomes peaked near 2–5 μm and >10 μm, corresponding well with typically cited aerodynamic diameters of unicellular and multicellular fungal spores, respectively.

Overall, bacteria contributed approximately 0.1% to indoor airborne particle mass during occupied periods; no such estimation was made for fungi. These aggregate emission rates include both contributions from resuspension from the carpeted floor and other surfaces as well as direct shedding of microorganisms from humans. The indoor occupied aerosol microbial ecology showed a distinct signature of human skin microflora in addition to outdoor air and resuspended dust. These important results demonstrated that human occupancy results in significant emissions of airborne particle mass, bacterial genomes, and fungal genomes. The authors also noted that the dominant size ranges for bacterial genomes were generally larger than pure culture (i.e., single bacteria), suggesting that organisms may be attached to each other or to other small biotic or abiotic particles. Detailed knowledge of particle size distributions in this study offers a unique ability to extrapolate from measured airborne microbial communities to the overall fate, transport, and control of indoor bioaerosols.

Using data from the same study as above, Hospodsky et al. (2012) characterized total particle mass concentrations, bacterial genome concentrations, and bacterial phylogenetic populations indoors, outdoors, and in ventilation duct supply air concurrently [15]. HVAC filter dust and floor dust were also sampled. Importantly, a number of detailed measurements of environmental parameters, including T/RH and CO_2 concentrations (used for ACH measurements and as an indicator of occupancy), and HVAC characteristics again allowed for stronger interpretation and extrapolation of the results. The HVAC system was known to operate under "economizer" conditions, varying the fraction of outdoor airflow rate to total airflow from 25% to 100% depending on outdoor air (OA) temperatures and heating and cooling requirements. Although OA fraction measurements were not made in this study, the authors suggest the building would likely have been near 50% OA during test conditions, based on the measured values of indoor and outdoor temperatures. Before entering the classroom, air in the HVAC system passed through a MERV 8 particle filter; the authors also measured the in-situ size-resolved filtration efficiency of this filter using an optical particle counter. Air samples included $PM_{2.5}$ and PM_{10} in indoor, outdoor, and HVAC supply environments; HVAC filter and floor dust was mechanically extracted and then sieved and resuspended to obtain $PM_{2.5}$, PM_{10}, and PM_{37} mass fractions. Human occupancy increased the total aerosol mass and bacterial genome concentration in both $PM_{2.5}$ and PM_{10} size fractions in indoor air. Floor dust contained more bacterial genomes on a per mass basis than indoor aerosols. Comparisons between bacterial populations in indoor air and during unoccupied and occupied periods further suggested that resuspended floor dust and direct human shedding were important contributors to bacterial populations in indoor air. These very detailed building characterizations again allowed for uniquely quantitative estimates and interpretations of their results.

Focusing less on environmental or operational characteristics and more on indoor sources and cleaning activities, Medrano-Félix et al. (2011) identified and quantified the presence of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella*, hepatitis A, and norovirus in 60 homes in Mexico and assessed the effect of chlorine and ammonium based disinfectants on these communities [27]. Surface sample sites included kitchens (counter top, sponge, dishcloth, cutting board, and sink), bathrooms (sink, toilet bowl, toilet seat, and shower tile), pet areas (toy sites) and children's areas (toy sites). In 30 homes that followed a disinfection protocol, there was a significant reduction in the presence of the study's target microbes compared to a control group that did not utilize a disinfectant protocol, suggesting that cleaning patterns are very important to assess accurately for better interpretation of surface sample results.

Dunn et al. (2013) examined the diversity of bacterial communities in nine distinct locations within 40 homes in North Carolina [24]. Surface sampling included: kitchen cutting board, kitchen counter, shelf inside refrigerator, toilet seat, pillowcase, exterior handle of main door into house, television screen, and the upper door trip on exterior and interior doors (assumed to be infrequently cleaned and representative of indoor and outdoor bioaerosols via long-term particle deposition). Each of the sampled locations harbored bacterial communities that were distinct from one

another. Surfaces that were regularly cleaned typically harbored lower levels of diversity than surfaces that were not frequently cleaned. They also examined whether the variability in bacterial diversity across homes could be attributed to outdoor environmental factors, some indoor factors, or occupants of a home. The presence of dogs had a significant effect on community composition within the homes as they harbored more diverse communities and abundances of dog-associated bacterial taxa. There was also a significant correlation between the types of bacteria deposited on surfaces outside the home and those found inside the home, suggesting that microbes from outdoors can have a direct effect on the microbial communities living on surfaces inside homes. This study further demonstrates how cleaning and occupants, human and non-human alike, can have a significant impact on indoor microbial communities.

Gaüzére et al. (2013) characterized the diversity and dynamics of airborne microorganisms in one room in the Louvre Museum during a period of 6-months [58]. The indoor airborne bacterial diversity was shown to be relatively stable over time, while the corresponding fungal community was less stable. The room was described as being air-conditioned with two open doors and no windows, although no information on HVAC system operation was reported. The number of visitors was counted during measurements to determine occupancy, with an average of 250 occupants entering the room during one sample collection. Air samples were collected at daily, weekly, and monthly intervals. T/RH was measured continuously, in addition to size-resolved airborne particle concentrations with an optical particle counter. Air samples were collected using an experimental bioaerosol collector that was similar to a wetted wall cyclone [59]. T/RH were relatively constant and approximately 90% of airborne particles measured with the OPC were between 0.3 and 0.5 μm. Concentrations were similar over all sampling periods. No correlations in bacterial communities were observed with building environmental parameters, although most likely because they were quite stable throughout the operation of the highly controlled museum environment.

Combined, the studies in this section utilized more detailed measurements of building characteristics, environmental conditions, and human activities that often had a large impact on indoor microbial communities, or perhaps more importantly, allowed for novel, quantitative inference from their results to other indoor environments. The success of this particular group of recent studies provides motivation for our suggestions for a standardized suite of tools that can be used for collecting robust built environment data in future investigations of the microbiology of the built environment, as described in the next section.

3. Tools for improving built environment data collection for microbial ecology investigations

To inform the increasing number of microbial diversity studies being conducted in indoor environments, here we suggest a large suite of building environmental and operational characteristics that can be measured in robust and standardized ways as needed for particular study designs. These parameters and measurement techniques are detailed in the following sections and summarized along with key references for specific measurement or assessment methodologies in Table 1. Collectively, we identify this suite of likely influential 'building science measurements' for more robust collection of built environment data. These parameters and techniques are meant to build on those already introduced in the recently developed MIxS-BE package [35] that describes minimal built environment metadata to collect in microbial ecology studies (including qualitative building properties such as building type, HVAC system type, and lighting type, as well as core environmental data such as air temperature and RH, surface temperature, pH, and moisture, and human occupancy), primarily by providing more justification, detail, and context for these important parameters and others from the perspective of building engineers and building scientists. Once these built environment data are collected, there may be additional opportunities to improve analysis methods to explore connections between built environment and microbial ecology datasets.

This suite of building science measurement recommendations can be categorized generally into (1) building characteristics and indoor environmental conditions, (2) HVAC system characterizations and ventilation rate measurements, (3) human occupancy measurements, (4) surface characterizations, and (5) air-sampling and aerosol dynamics (the latter is not entirely considered 'built environment data' but is important for standardized microbial data collection and deserves attention in this work). These built environment data collection efforts are informed in large part by evidence of their importance for influencing microbial communities on indoor surfaces and in indoor air, as well as their importance for general building characterizations in other indoor environmental research. We should note, however, that this is not necessarily an exhaustive list of parameters or methods, but represents our best attempt to move toward a standardized suite of available measurements given our current level of knowledge. We should also note that our intent is primarily to provide microbial ecologists with knowledge of many of the tools available for this type of data collection, as well as some of the constraints and considerations for these tools. Not all measurement types will be appropriate for all study designs or budgets. However, we firmly believe that more standardized building operational and environmental measurements can serve to maximize the amount of extrapolation or translation between indoor environments that can occur among otherwise disparate studies if integrated carefully into study designs.

3.1. Building characteristics and indoor environmental conditions

Several basic building characteristics and indoor environmental measurements are fundamental to any indoor environmental exploration. Important building characteristics include age of construction, floor areas and volumes, material descriptions, type of use, typical occupancy, history of water damage, occupant complaints, HVAC system type and operation (i.e., in heating or cooling modes), ventilation method and source, the use of humidifiers, and many others, as many of these have already been shown to influence microbial communities and are well known to influence other aspects of indoor air and building operation. Important indoor environmental conditions, including air temperature (T), relative humidity (RH), absolute humidity, and light levels in the sample space, may have particular influence on microbial diversity outcomes [33]. Portable, off-the-shelf battery-powered sensors can accurately and inexpensively measure and log these data for long periods of time. Long-term data logging is important for assembling a history of indoor environmental conditions that may affect microbial growth and survival, rather than relying upon spot measurements during the time of testing, although parameters such as measurement interval and length may vary depending on study design.

Indoor T/RH has been shown to be an important influential parameter in a number of previous laboratory and field studies of the microbiology of the built environment. In controlled laboratory studies, T/RH have been shown to have large (often competing) influences on the survival of a number of bacteria and viruses [33,60–70], as well as fungal allergenicity [71]. In field studies, indoor temperatures have been positively associated with fungi

and negatively associated with bacteria and total inflammatory potential (TIP) of cell assays in several homes in Denmark [72]. In the same study, indoor RH was also positively correlated with indoor fungi concentrations. In a study of child day-care centers in Turkey, differences in airborne bacterial communities were seen with some (varying) outdoor environmental conditions, which could also have manifested in differences in indoor environmental conditions such as temperature, relative humidity, or HVAC operation [73]. For example, the amount of endospore-forming Gram-positive bacteria increased as the amount of sunlight and temperature increased and as relative humidity decreased (all measured outdoors). In fact, sunlight is known to have bactericidal powers that inhibit bacterial growth, even through panes of glass, and it has long been thought that sunlight (in addition to increased ventilation) could reduce the spread airborne infections in hospitals, although evidence is quite limited and suspected mechanisms are not entirely environmental [74]. More recently, a study in primary schools in Australia found that air temperature (measured over the previous 24 h) was negatively associated with concentrations of endotoxin in indoor air and positively correlated with endotoxin loads in floor dust [75]. Additionally, lower airborne endotoxin concentrations were observed during periods of higher levels of relative humidity, and T and RH appeared to act separately.

Measurements of T/RH may be particularly important inside HVAC systems as well; bacteria and fungi have been shown to grow at an accelerated rate with higher temperatures and higher RH in air-conditioning ducts subjected to dust deposition [76]. Other studies have shown that a substantial amount of dust can accumulate due to particle deposition on air-conditioning ducts [77–79]. There are complex interactions between moisture and building materials that may be important to characterize as well. For example, in a recent study of day care centers in Sweden, fungal DNA levels were shown to be higher in buildings at risk of dampness, in rooms with linoleum flooring materials, and in buildings with rotating heat exchangers [80]. T/RH measurements should also be used to calculate absolute humidity ratios, or the mass of water vapor per mass of dry air, regardless of temperature [81], as there is some evidence that absolute humidity can influence microorganism survival [82], mold growth on building materials [83], airborne endotoxin [84], and the inactivation or survival of influenza viruses on surfaces [85–87]. Thus, at a minimum, we suggest that long-term measurements of T/RH, absolute humidity, and possibly artificial and/or natural light levels be made and recorded in future microbial diversity studies given appropriate study design and resources, as many have already done.

3.2. HVAC system characterizations and ventilation rate measurements

Another core set of parameters that can be measured in order to accurately characterize building operation includes HVAC system airflow rates and ventilation rates in the space being sampled. HVAC system operation and ventilation performance will greatly impact indoor concentrations of particles, including those of biological origin, and thus are of primary concern for indoor air sampling. There are also a wide variety of ventilation and airflow distribution systems in buildings that should be well characterized in both qualitative and quantitative ways to allow for meaningful interpretation of results from any indoor environmental investigation [88].

A number of specific HVAC factors have been linked to microbial growth, including temperature, humidity, air velocity, type and location of filter (including removal efficiency and type of media), and others [89,90]. Another important parameter is whether or not an HVAC system is actually operating, particularly in smaller buildings where HVAC systems may operate only in response to heating and cooling loads [91]. Air change rates (ACH) have also been shown to be an important influence on indoor microbial communities; for example, increases in ACH were correlated with increases in indoor fungi, a decrease in indoor bacteria, and a decrease in inflammatory response in granulocyte cells in a recent study of homes in Denmark [72]. Therefore, most if not all of these parameters should be well characterized in any indoor microbial investigation.

In addition to influencing airborne microbial concentrations, HVAC systems can also impact settled dust on surfaces. For example, high particle filtration efficiency and high HVAC recirculation rates (the airflow rate through an HVAC system divided by the volume of the space it serves) may selectively remove many particle sizes at a rate greater than their rates of deposition to surfaces. This could impact the amount of biomass that settles to surfaces, depending on particle size and surface characteristics. Size-resolved particle deposition rates to a variety of surfaces may also be important to characterize over a long period of time [92]. The combined effects of airspeeds, mixing characteristics, and the surface area-to-volume-ratio will also alter deposition rates of particles to surfaces, varying by as much as an order of magnitude [93,94]. Particle deposition rates can be measured relatively easily if simultaneous ACH measurements are also made [94,95].

There are a variety of tools to measure airflow rates through HVAC systems, many with varying degrees of difficulty, accuracy, and equipment requirements [96]. Airflow rates can be measured either within air handling units (depending in large part on the size of the equipment) or at individual supply diffusers and return grilles. There are several widely-accepted and standardized ways to measure airflow rates at or near the air handling unit [97], including: (i) pressure readings can be correlated to fan curve data provided by the fan manufacturer; (ii) flow metering devices such as venturi meters, flow nozzles, orifice meters, or rotameters can be installed directly into the HVAC system; (iii) air velocity can be measured using pitot tubes or hot-wire anemometers traversing the entire area of a duct system, particularly if general guidelines for the number and spacing of measurement points are followed (i.e., equal-area or log-Tchebycheff methods can take into account the distribution of air velocity from bulk air in the duct to the velocity near the edges and corners of ducts), or (iv) pressure matching with a calibrated fan. Particularly for small and medium sized HVAC systems, there are also highly accurate airflow metering plates available for rapid measurements of air handler flow rates [98].

Aside from airflow rate measurements at the air handler, it may be critical to measure the operational cycles of the HVAC system and the actual amount of airflow entering or leaving a space. Runtime fractions can be assessed using a combination of supply temperature measurements [99,100], measurements of the electrical power draw of AHU fans and/or compressor units [101,102], or by vibration or electromagnetic sensors on AHU blowers or compressor motors [103]. Supply temperature measurements can also reveal whether an HVAC system is operating in heating or cooling modes.

Airflow rates at air handling units may also differ from the actual amount delivered to a space because many buildings have significant duct leakage to the exterior [101,104,105]. Duct leakage may need to be accounted for and can be done by following standardized test methods [106]. There are also other ways to measure airflow rates leaving supply diffusers or entering return grilles, including: (i) airflow capture hoods; (ii) air velocity readings correlated to diffuser characteristics provided by the manufacturer; (iii) duct traverse air velocity measurements; and (iv) pressure matching with a calibrated fan. Once specific airflow rates have

been characterized in an environment, there are several methods to continue to record flow data over time without the need for interventions by field-workers. For example, airflow rates (which are invasive to measure) can be correlated to duct pressure measurements (which can be easily measured and recorded on a portable data logger) [102,107–109].

Once airflow rates are well characterized and recorded over time, the rate of outdoor air supplied by the ventilation system can also be measured and recorded. Many building automation and control (BAC) systems report these values, although accuracy is often an issue and data cannot always be accessed or trusted. Outdoor air ventilation rates can be made by combining knowledge of supply flow rates with the fraction of outdoor air in the air stream. The fraction of outdoor air in an air stream can be measured in several ways, for example by measuring CO_2 concentrations in recirculation, outdoor, and supply airstreams of an air handling unit [110].

Ventilation rates and interzonal airflows can also be measured directly in test environments using a variety of tracer gases [111–115]. Standardized tracer gas methods include simple injection and decay, constant injection, and constant concentration [112]. Both active and passive tracer gas injection and sampling methods can be used as well. Active techniques allow for time-varying ACH measurements but involve real-time monitoring of tracer gases, which can introduce prohibitive costs and labor requirements for large field studies. Passive techniques such as the perfluorocarbon tracer (PFT) method utilize inexpensive passive tracer sources and samplers, but are limited to measuring longer-term time-averaged ACH [116]. Particular care should be taken to achieve proper mixing and tracer gas distribution, as well as selection of a nonreactive, nontoxic, inexpensive, and easily detectable tracer gas.

Natural ventilation rates (i.e., those caused by airflow through window openings) can be more difficult to accurately quantify than outdoor air supplied by a mechanical HVAC system [117–121]. At a minimum, the configuration of window openings (including the size and orientation) should be noted. One recent microbial investigation used velocity measurements at two locations within a window and video-recording of flags in order to show wind direction at a window [23]; however, other, more accurate, methods have also been used, such as using a grid of 32 point pitot tube arrays measuring at very high frequency [117]. There are also many models for predicting ventilation performance in buildings that can be used in conjunction with measurements of meteorological conditions [122].

3.3. Human occupancy measurements

Human occupancy is a major driver of indoor microbial communities through a combination of direct human shedding, resuspension from flooring, and emission from respiratory activities (e.g., sneezing, coughing, or even breathing); thus, not only is the presence of people in an indoor environment an important determinant of microbial diversity, but so are their activities and the surfaces with which they come in contact [21,24,25,30,123,124]. But how does one accurately measure human occupancy or activity? Several recent studies that measured microbial diversity during scripted, short-term events simply recorded occupancy by periodically counting the number of people in the sample space [13–15,58]. For longer-term studies, there are a variety of ways for assessing human occupancy and/or activity, although none are standardized and many depend on the design and construction of the particular environment in question. Occupancy or activity monitoring methods include video cameras equipped with people-counting software [125–128], optical or infrared tripwires that count people crossing a particular area, such as doorway [129,130] and proximity or light sensors that can detect movement or lack of movement near a specific location [131–133], CO_2 sensors coupled with dynamic mass balances on indoor CO_2 concentrations [134–138], high-resolution pressure sensors in HVAC systems that detect fluctuations based on door closing or other activities [139], radio-frequency identification (RFID) [140–143] and Bluetooth tracking systems [144–146], acoustic sensors that detect noise levels [130], and others [147]. Although some of these devices can suffer from large uncertainty and calibration issues, they can also be combined with sophisticated algorithms to provide robust determinations of time-varying human occupancy and/or activity [148–151].

Some occupancy measurement technologies are also more appropriate for some environments than others. For example, doorway break sensors are more appropriate for smaller volume environments where location within the room may not be as important as mere presence in the room. Video camera systems can provide location detection in smaller environments but result in prohibitive costs in larger environments. Additionally, beam break sensors and some video camera systems are more appropriate for environments with a limited number of entryways. Additionally, upgrading from non-directional functionality to directional doorway break sensors can greatly increase costs with current off-the-shelf equipment, which often makes using them in environments with many entrances and exits cost prohibitive. Alternatively, RFID and Bluetooth tracking systems require a known population that can be pre-screened and identified prior to entry; this may work for certain environments such as hospitals or offices, but not for high turnover areas such as retail environments. Proximity sensors can be very helpful for sensing occupancy near particular locations, but can suffer in terms of accuracy, as lack of movement does not necessarily mean lack of occupancy. Finally, CO_2 sensors can be good identifiers of human occupancy but suffer from several issues, including prohibitive costs for highly accurate sensors with minimal drift, variable and unknown CO_2 emission rates from individuals [135,152], non-well-mixed environments lead to errors in mass balances, and other HVAC system characteristics such as ACH must also be well-characterized. Unfortunately there are no standardized methods for measuring and recording human occupancy in indoor environments, but there are several helpful options from which to choose depending on user needs and budget, as shown in Table 1.

3.4. Surface characterizations

The last type of building environmental characterization that we should mention involves meaningful characterization of surfaces from which microbes are sampled. Surfaces can harbor a wide array of settled dust and adsorbed compounds that may affect microbial communities on them. Basic surface characteristics such as porosity, composition, and environmental conditions immediately adjacent to materials can all affect microbial community structure, growth, and survival [33,153,154]. In fact, environmental conditions of microbial-surface interfaces have been thought to play a greater role in influencing microbial activity than basic interface characteristics themselves [155].

For example, Andersen et al. (2011) both qualitatively and quantitatively assessed fungal diversity growing on damp or water-damaged building materials [156]. More than 5300 surface samples were taken by means of contact plates with agar from materials with visible fungal growth. Different fungi were correlated with different distinct classes of surfaces, grouped by (i) gypsum, plaster, and wallpaper, (ii) wood and plywood, and (iii) concrete and other floor materials. Among the samples gathered, plaster was most

likely to have had fungal growth from water damage, followed by concrete, wood, wallpaper, gypsum, and several others. No information was gathered on other building characteristics, nor another important parameter referenced in the paper: water activity.

The water activity of a building material, which can be approximated as the ratio of the vapor pressure of water in a material to the vapor pressure of pure water, is a major determining factor for fungal growth [83]. Water activity varies with temperature and the type of material; the longer a material's water activity is over 75%, the greater risk of fungal growth [157], although different fungi have different preferences [158]. Because water activity is difficult to measure directly, a measure of the equilibrium relative humidity in a small sealed chamber installed on the sample surface is often used.

Last, the frequency of cleaning of particular sample surfaces is also an important parameter to track over time. Cleaning will impact settled dust and adsorbed compounds, as well as the microbial mass found on surfaces. An example of the importance of cleaning frequency involves human hands; a recent study showed that the microbial community composition on people's hands was highly influenced by the time since their last hand washing [159]. The same can be said for building material surfaces [25,27,45]. There may be opportunities to advance methods to accurately assess surface cleaning, perhaps by combining proximity IR sensors or video surveillance with water activity measurements.

3.5. Air sampling and aerosol dynamics

Finally, air sampling and aerosol dynamics represent another category of measurements for consideration in indoor microbial investigations. We consider aerosol dynamics as built environment data because they can be assessed with real-time instrumentation [57,79,94,95,160] and they influence both airborne and surface-deposited microbial communities. In particular, indoor particle deposition rates (and total loss rates) can easily vary by two orders of magnitude or more depending on particle size in the same indoor environment [161], or by two orders of magnitude or more across indoor environments, depending on a large number of factors including air speeds, furnishings, and surface characteristics [57], ventilation rates and sources [94], temperature [162], particle density [92], and surface orientation [163].

Additionally, a wide range of air sampling devices was used in the aforementioned studies that measured airborne microbial community composition, diversity, and/or abundance. Although air sampling techniques are not necessarily considered 'built environment data', a lack of standardization in air sampling methodologies substantially limits our ability to compare results from one environment to another and thus a discussion of air sampling methods is included herein. Recently used air sampling methods have included liquid impingers [23,31], size-resolved [13,15] and non-size-resolved [14,164] impaction-based filter methods (with a variety of filter media ranging from PTFE, polycarbonate, or mixed cellulose ester membrane filters to quartz fiber or gelatin filters), petri dishes suspended in air [45,164], HVAC particle filters installed in air handling units [12,32], and an experimental sampler similar to a wetted wall cyclone [58]. A recent meeting report reviews many of the challenges of bioaerosol sampling [165]. Only a few studies have compared the ability of various bioaerosol samplers to deliver repeatable results using molecular analysis techniques [166,167] or for various analysis techniques to deliver repeatable microbial community results from a particular air sampling method [168]. Some airborne collection methods may vary widely in their collection efficiencies for different sizes of bioaerosols, as well as in their DNA extraction efficiency. For example, Hospodsky et al. (2010) determined qPCR accuracy, precision, and method detection limits for bacterial cells and fungal spores collected on a variety of aerosol filters, including PCTE membrane filters and quartz fiber filters [169]. DNA recovery efficiencies were low; not accounting for extraction efficiencies was shown to underestimate true aerosol concentrations by 10–24 times.

Frankel et al. (2012) compared different microbial sampling methods for identifying culturable fungi and bacteria, endotoxin, and the total inflammatory potential (TIP) of both airborne particles and settled dust [170]. Polycarbonate- and Teflon-filter-based airborne samplers yielded significantly higher microbial levels than a common impinger, although results were highly correlated. The use of an electrostatic dust fall collector (EDFC) [171] yielded higher levels of fungi, endotoxin, and total inflammatory potential compared to a dust fall collector (DFC); dust samples acquired by vacuum cleaners were more similar to those captured by the EDFC than the DFC. Another recent study showed that two different solid impactors had similar collection efficiencies for culturable bacterial sampling, whereas a liquid impinger and a filter-based sampler were more efficient for total bacterial sampling [172]. Bioaerosol samplers not only have a wide variety of collection and extraction efficiencies, but they also differ in terms of practical concerns. Bioaerosol samplers operate at airflow rates ranging from 4 L min^{-1} [14] to as much as 300 L min^{-1} [31] or even 1000 L min^{-1} [58]. The advantage of higher flow rates is that more biomass can be collected over shorter amounts of time and detection limits can be overcome. However, there are some disadvantages to higher flow rates in sampling systems. For example, removal by the sampling pump may become competitive with air change rates in smaller volume environments at high flow rates, which could alter aerosol dynamics in the space. There are also practical size and noise concerns associated with larger pumps used for higher flow rates. Advantages of passive sampling techniques such as suspended petri dishes are that there are no pump requirements, but they may introduce bias by oversampling larger particle sizes that are more likely to settle than smaller particles.

One recent development in air sampling mechanisms has been the use of HVAC filters to semi-passively collect bioaerosols, integrated over time. Tringe et al. (2008) used HVAC particle filters installed in air handling units (AHUs) to sample air microbiota in two shopping centers in Singapore [12]. HVAC filter sampling is advantageous because an extremely large amount of air passes through filters on a daily basis. For example, approximately 6×10^6 m^3 of air passed through the filters in this study for 14 h per day over a period of approximately 90 days (~1260 h of operation). Traditional bioaerosol sampling methods utilizing flow rates of 4–1000 L min^{-1} would have provided only ~3×10^2 to ~8×10^4 m^3 for sampling, reducing the amount of biomass available for analysis by 2–4 orders of magnitude. Each filter was classified as having an arrestance efficiency of 90% for 1 μm particles, although size-resolved data were not reported. Similarly, Noris et al. (2011) compared bacterial and fungal concentrations and communities on residential HVAC filters and found that microbial communities on the filters were not different from those present in compose month-long indoor air sampling via impingers [173]. HVAC filter dust was also similar to that collected on surfaces, suggesting that high efficiency HVAC filters could be used as a long-term integrated measure of microbial communities in indoor air.

Most recently, Hoisington et al. (2014) compared six different air sampling methods, including settled dust, HVAC filter, and four bioaerosol samplers (BioSampler®, button sampler, personal environmental monitor, and wetted-wall cyclone) [167]. They found that microbial communities from settled dust and HVAC filter dust clustered closely together and were more diverse than microbial communities from the four bioaerosol samplers. Although the

Table 1
Tools for improved collection of built environment data.

Parameter(s)	Measurement/collection method	Important considerations	Reference(s)
1. Building characteristics and environmental conditions			
Basic building characteristics	Surveys, visual assessments	Age of construction, floor areas and volumes, material descriptions, type of use, typical occupancy, history of water damage, occupant complaints, HVAC system type and operation, ventilation method and source, the use of humidifiers, etc.	[19,45,49,174]
Indoor T/RH, absolute humidity, and artificial/ natural light	Portable, off-the-shelf, battery-powered sensors with data loggers	Storage capacity, accuracy, precision, battery power	[175–178]
Outdoor T/RH, absolute humidity, and light	Publicly available meteorological data or local weather station installations	Data availability, installation location	[179–181]
2. HVAC system characteristics and ventilation rates			
Spot measurements of airflow rates at AHU	Correlate pressure readings to fan curve data by the fan manufacturer	Requires knowledge of fan manufacturer and in-situ verification	[182]
	Traverse velocity with pitot tubes or hot-wire anemometers (multiplied by duct area)	Requires knowledge of duct areas, high uncertainty	[96,105,160]
	Pressure matching with powered, calibrated fan	Typically greater accuracy than capture hood, limited to smaller systems, requires clear access to AHU	[97,106,183,184]
	Airflow metering plates	Requires modifications for larger AHUs	[98,107,109,185,186]
Spot measurements of airflow rates at individual supply diffusers or return grilles	Airflow capture hood	Limited accuracy under some conditions	[105,187,188]
	Air velocity or pressure readings correlated to diffuser characteristics	May not accurately reflect in-situ performance, requires knowledge of specific manufacturer	[189]
	Traverse velocity with pitot tubes or hot-wire anemometers (multiplied by duct area)	Requires knowledge of duct areas, high uncertainty	[96,105,160]
	Pressure matching with powered, calibrated fan operating as flow hood	Typically greater accuracy than capture hood	[97,105,106,183,184]
Continuous flow measurements	Flow meters installed directly into HVAC system (e.g. venturi meters, flow nozzles, orifice meters, rotameters)	Invasive, requires HVAC access, data logger, and power	[96,190]
	Duct pressure correlations with spot flow measurements	Simple and cost-effective, requires data logger and power	[102,107–109]
Outdoor air (OA) fraction in mechanical HVAC systems	Tracer (e.g., temperature, CO_2, or SF_6) in RA, SA, and OA	Accuracy issues at low concentration changes, high costs for accurate sensors, requires injection, data logger, and power	[110,191]
	Zone tracer testing (e.g., CO_2, SF_6) coupled with room volume	Costly, labor intensive, requires assumptions for mixing	[111–115,192]
	Building automation system (BAS) readings, including economizer settings	Often low accuracy, sensor reliability, requires access to facility data, typically only present in large buildings	[193]
Air change rates (ACH)	Active tracer gas (e.g., CO_2 or SF_6)	Costly, labor and equipment intensive,	[111–115]

(*continued on next page*)

Table 1 (*continued*)

Parameter(s)	Measurement/collection method	Important considerations	Reference(s)
		requires injection and well-mixed environment	
	Passive tracer gas (e.g., PFT)	Limited to longer-term time-averaged air change rates	[116,194]
Natural ventilation rates through windows	Pitot tube array	Labor intensive, invasive	[117]
HVAC on/off	Current draw on AHU fan or AC compressor	Requires HVAC access and data logger	[101,102]
	Supply temperature measurements	Inexpensive and simple, but issues with averaging times; only works for heating or cooling modes	[99,100]
	Vibration or magnetic field	Requires equipment training period, inexpensive field sensors are commercially available	[103]
Duct leakage fractions/ flows	Fan pressurization, delta-Q, or nulling tests	Time intensive	[106,195,196]
Particle removal efficiency of HVAC filters	Upstream/downstream particle concentrations	Expensive instrumentation, requires HVAC access	[15,79]
	Whole-zone elevation and decay	Expensive instrumentation, time-consuming, requires mixing assumptions and knowledge of HVAC airflow rates, but can also be gathered from long-term time-resolved data	[79]
3. Human occupancy/activity measurements			
Number of occupants	Manual observational counts	Not feasible for long-term, continuous sampling	[13]
	Uni-directional IR beam break people counting	Better for small environments with limited number of entrances/exits, limited accuracy	[197,198]
	Directional beam break or thermal people counting	Higher accuracy, costlier, limited number of entrances/exits, requires power	[129,130,197–200]
	Video + people counting software	Costly in larger environments, requires power	[125–128,199]
	Movement sensors based on IR proximity, light, or acoustics	May not represent true occupancy	[130–133]
	CO_2 mass balance	Costly, variable emission rates, requires well-mixed environment, well characterized ventilation, and power	[134–138]
	Pressure sensors in HVAC systems	Requires high-resolution data loggers, accuracy unknown	[139]
	RFID tags or Bluetooth tracking	Pre-screening/ID required, provides continuous monitoring among people and between locations	[140–146]
Occupant profiles	Survey questions: age, gender, culture, socioeconomics	Requires careful survey design	[201]
Non-human occupants	Survey questions: pets, typical activities	Requires careful survey design	[24,202,203]
Cleaning activities	Visual observation, questionnaires	Data quality and reliability	[27]
Activity/resuspension	Optical particle counters	Expensive instrumentation, requires power	[13,204]
4. Surface characterizations			
Surface temperature	Thermistors or thermocouples	Data logging capabilities preferred over spot measurements	[155,205,206]

Table 1 (*continued*)

Parameter(s)	Measurement/collection method	Important considerations	Reference(s)
Water activity (or equilibrium relative humidity)	Approximated as relative humidity in a sealed chamber installed on sample surface	Only provides surrogate measure, few commercial devices	[207,208]
Porosity, water vapor permeance, moisture content, and other moisture properties	Vacuum saturation tests, SEM, NMR, capacitance methods, water uptake experiments, and others	Difficult to measure in-situ, some are costly and destructive techniques	[209–212]
Cleaning strategies/details	Records of cleaning products and schedule, or microbial loads	Potential data quality issues with surveys, issues with standardization on microbial loads	[25,27,213]
Building material/ composition assessment and survey of moisture events	Qualitative descriptions, surveys, and quantitative material analysis	Potential data quality issues with surveys, material survey is time consuming	[19,156,214,215]
Identification of highly-touched surfaces	Visual assessment or proximity IR	Time-consuming for visual assessment, inaccuracies in proximity sensors	[17,159]
5. Air sampling and aerosol dynamics			
Particle sampling	Cascade impactors	Can be particle size-selective, quantitative accounting of volume of air sampled, mechanical forces can rupture cellular membranes and reduce culturing viability, issues with pump noise, power requirements	[13–15,165]
	Liquid impingers	Mechanical forces can rupture cellular membranes, pump noise, power requirements	[23,31,165]
	Cyclones	Particle size cut-off issues, pump noise, power requirements	[58,59]
	Passive/HVAC filtering	No pump or power requirements, silent, large air flows collect more biomass, difficult to extract DNA, biases towards certain particle sizes based on filter efficiency	[12,32,165,168,173]
	Settling plates or dust fall collectors (DFC or EDFC)	Silent, inexpensive, no power requirements, may bias toward larger particles	[45,171,216]
Particle deposition rates	Whole-zone elevation and decay	Expensive instrumentation, requires ACH measurements	[57,79,94,95,160]

bioaerosol samplers were collocated and sampled over the same period, they did not yield the same bacterial community, with only 13–16% of bacteria and 33–34% fungi common across all four bioaerosol samplers. These results indicate that sampler design and operation may substantially alter the microbial community sampled and should be considered in detail when interpreting results. This work also suggests that utilizing a variety of sampling techniques in sample environments may provide a more complete representation of the true microbial community present.

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