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Host-Microbial Interactions | Review

Microbiology of the built environment: harnessing human-associated built environment research to inform the study and design of animal nests and enclosures

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SUMMARY Over the past decade, hundreds of studies have characterized the microbial communities found in human-associated built environments (BEs). These have focused primarily on how the design and use of our built spaces have shaped human-microbe interactions and how the differential selection of certain taxa or genetic traits has influenced health outcomes. It is now known that the more removed humans are from the natural environment, the greater the risk for the development of autoimmune and allergic diseases, and that indoor spaces can be harsh, selective environments that can increase the emergence of antimicrobial-resistant and virulent phenotypes in surface-bound communities. However, despite the abundance of research that now points to the importance of BEs in determining human-microbe interactions, only a fraction of non-human animal structures have been comparatively explored. It is here, in the context of human-associated BE research, that we consider the microbial ecology of animal-built natural nests and burrows, as well as artificial enclosures, and point to areas of primary interest for future research.

KEYWORDS built environment, microbial ecology, animal nests, animal burrows, pathogens, captivity

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INTRODUCTION

Currently, there is a fundamental lack of understanding of the microorganisms that are found in non-human animal (here-forward referred to as "animal") structures. This is particularly important not only in the pursuit of basic scientific knowledge and to inform ecological and evolutionary theory but also in the context of conservation management practices. It is already known, based on robust research efforts from human-associated built environments (BEs), that many microbial interactions are dictated by how human structures are built, used, and where they are located—factors that are also likely to influence both the microbes that are found in animal-associated BEs and individual health outcomes. For example, microbes in nests influence neonate development (1), and a decrease in exposure of captive animals to diverse microbial communities can reduce their ability to ward off pathogens and successfully survive reintroduction (2). Yet, relative to human-focused research efforts, there have been limited studies on the microbiome of natural nests/dens [e.g., references (3–6)] and even less research on how artificial enclosures might alter the composition of bacterial, fungal, viral, and archaeal taxa in ways that could be detrimental to animal health (7–9). Here, we review the literature on how the design and use of human-associated BEs influence the indoor microbiome and examine how these findings can inform the microbial ecology of animal structures. We then pose recommendations for future research, including those related to varied study design and analytical techniques.

HUMAN BUILT ENVIRONMENTS

Human BEs have been defined as "man-made structures, features, and facilities viewed collectively as the places in which people live and work (10)." This includes not only buildings (e.g., homes, offices, daycares, and hospitals) but also cars, public transit, and even outdoor parks and trails that are used for recreation (10, 11). However, for the purpose of this review, we will focus on the buildings themselves, at both the individual and city levels, and the human-microbe interactions that occur within them.

How humans build

Humans are constructing homes and other buildings at an unprecedented rate, such that the indoor biome has become the most rapidly growing biome on Earth (12), and with this, buildings have become increasingly modified and removed from the natural environment. This has resulted in significant shifts in the diversity and types of microbes found in a range of BE types (13–15). Humans tend to maintain a low relative humidity (for comfort and to prevent microbial growth) and maintain our homes and other buildings at temperatures that are not ideal for the proliferation of most microorganisms. Furthermore, through an attempt to seal our built spaces from the outside, we intentionally limit the colonization of indoor spaces by environmental species. This exclusion of outdoor biodiversity and the resulting consequences for human health have been the primary focus of much of the BE research, to date, in which much of this work has been fueled by ecological theory (16) and hinged heavily on the Hygiene and Biodiversity Hypotheses (17, 18).

Another consequence of sealing our BEs, though unintentional, is the accumulation of our own body associates in the spaces where we most frequently spend our time (19–21). This includes not only the innocuous microbes that are commonly found on the skin and in feces (19) but also potential disease-causing organisms (21). Additionally, as body microbes shed, they leave a warm, moist, species-rich environment and often land in a harsh environment that is nutrient and water limited. This difference in habitat characteristics can impose unique selective pressures that increase the rate of horizontal gene transfer events between bacteria and increase the selection for genes associated with antibiotic resistance and survival (e.g., genes associated with drought tolerance) (21). These environmental stressors can ultimately alter survival capability, rates of growth, and evolutionary dynamics (22–24), and the physical and chemical properties of the built materials themselves are an important component to consider.

Different materials maintain distinctive properties, such as moisture content and potential, surface structure and lability, and the presence of antimicrobial components. Due to this, surface type often explains the vast majority of the variance in microbial communities within a built space (20–22, 25). As an extreme example, soft hydrogels provide high water potential and are often labile, thereby providing an ideal substrate for microbial growth [e.g., reference (26)]. Conversely, copper metals are highly toxic to microbial life, due to the presence of divalent ions that oxidize the bacterial cell wall during vegetative growth, and even have biocidal effects against hardier, sporulating species (27). Studies on the impact of antimicrobial surface materials on microbial growth have demonstrated that alkaline clays select for alkaliphilic bacteria (e.g., *Kocuria rosea*), while acidic mold-resistant paint selects for spore-forming bacteria, such as *Bacillus timonensis* (28). Therefore, designing spaces with the microbiome in mind necessitates the examination of how surface properties influence which taxa can survive and potentially thrive in BEs.

How humans live

Building occupants are a primary determinant of the diversity and types of microorganisms that are found in homes and other buildings. Occupant density, length of stay, their disease state, and the types of occupants (e.g., humans vs dogs) all influence the abundance and compositional variance of indoor-associated microbial signatures. With higher occupant density and longer periods of stay, there is a greater contribution of body-associated microbes into an area (20, 21, 29), and sick individuals inoculate their surrounding spaces with disease-causing organisms. This can not only increase the abundance and potential transmission of pathogens but also the presence of pathogenic microbes can alter the composition of other microbial taxa found on surfaces (30, 31). Moreover, the presence of animals in or around a home increases microbial diversity inside and promotes the colonization of taxa that have the potential to provide protection against the development of autoimmune and allergic diseases in children (32, 33). However, the relative effects of all these factors vary greatly based on how a BE is used. For instance, there would be a lower occupant density and different rates of movement in and out of a single-family home compared to a long-term care facility, both of which would have significantly different occupant demographics than those you might find in a school or on a city bus. In conjunction, the behavioral patterns of those individuals strongly shape the microbial composition. This includes how family roles are differentiated (20), whether a person prefers open or closed windows (34), their proclivity for houseplants (35), and even their propensity/need to use certain medications, such as antibiotics.

The use of antibiotics likely dates back to the origin of our species. Plants with antibiotic properties were used by Neanderthals (36, 37), and thousands of years ago, ancient human civilizations, such as those in Serbia, Egypt, and China, rubbed moldy bread on their wounds to reduce the rate and severity of infection (38, 39). However, it was with the discovery of penicillin by Alexander Fleming in 1928 (40) that our use of antibiotics became both common and widespread. The ubiquity of antimicrobial medications has undoubtedly saved millions of lives, but this rise in usage has not come without consequence. It is now known that the microbiota in human guts is disrupted when antibiotics are ingested, resulting in a decrease in bacterial diversity, the potential for an over-proliferation of noxious organisms, and selection for antimicrobial resistance genes (ARGs) (41–45). These selective properties and the over-usage of antibiotic medications have led to a rise in the abundance of antibiotic-resistant bacteria in BEs—a health concern of such importance that it now ranks in the top 10 global threats listed by the World Health Organization (46). Antibiotic-resistant bacteria can accumulate through direct deposition from fecal matter or indirectly through selection after ingestion and passing of antibiotics into the environment (47, 48). Despite the health risks associated with infection, the full consequences of antibiotic use for personal and environmental health are just beginning to be understood, and even less is known about the underlying selective mechanisms or the dominant modes of exposure to ARGs in the BE. This includes patterns of differential contamination and persistence among locations/surfaces. In one study, daily sampling of the microbiome of hospital rooms identified shifting patterns of antimicrobial resistance in surface-associated communities. However, the genes that were most abundant often did not provide resistance to the antibiotics being administered to the patients (21), suggesting that other factors could influence the emergence of ARGs. Areas of exposure are often thought of in healthcare environments, but this also extends to the BE, more generally. The assemblage of microbial communities unique to homes is a contributing factor to the transfer of ARGs, and homes can be a potential reservoir for the transmission of antibiotic-resistant bacteria to humans (20). Furthermore, we have begun to broadly apply antimicrobials not only to our bodies but also to BE surfaces.

The removal of potential pathogens is advantageous, but with this, there has also been a loss in many beneficial associations that are important for human health (49). For example, the frequency of vacuum use in Finland has been used as a correlative metric between cleaning in homes and an increase in the prevalence of asthma (i.e., Gammaproteobacteria) (13). Additionally, there is evidence that a shift from traditional to industrialized farming practices in the Hutterite community has led to a markedly higher incidence of asthma among children—nearly four to six times as high as the genetically similar Amish population that still prescribes to traditional farming practices (33). Increased cleaning practices have also been associated with the emergence and persistence of antimicrobial resistance, as seen during the COVID-19 pandemic. The dramatic increase in air and surface cleaning has been correlated with decreased rates of infection from SARS-CoV-2 and the seasonal flu (50, 51), but these altered cleaning regimes have also been implicated in an increase in the abundance of ARGs and healthcare-acquired infections in hospitals (52).

Where humans live

The location of a BE modulates the indoor microbiome. This includes large-scale, biogeographic variation, as well as more localized differences between urban and rural environments or between locations within an individual city. There are global differences in building materials and architectural design, and cultural differences in how that space is occupied (e.g., whether a home is single family or multigenerational). Furthermore, seasonal fluctuations (or a lack thereof) in weather and light availability can influence which microbes are found in buildings.

Globally, temperature and humidity are related to the relative location of the sun and differences in regional habitat complexity/composition. This includes geographical features, such as whether a terrain is mountainous (53) or the percent area of vegetation cover (54), and these regional differences in temperature and humidity are known to influence the indoor microbiome (55). They affect microbial diversity, survivability, and rate of transfer between surfaces and an individual. It has been shown that in BEs with a higher relative humidity, there is an increased rate of microbial sharing (21), which is of particular concern in places like daycares and healthcare facilities. Additionally, regional variations in outdoor fungal communities reflect which species of fungi are found in homes (56), a potentially important consideration when choosing building location and construction materials, as some fungal taxa produce metabolites that can lead to respiratory illness and cognitive impairment (57).

Some of this might also be accounted for by differences in whether a BE is found in an urban or rural setting. Urban spaces promote the colonization and proliferation of some (often pest) taxa, such as the Norway rat (*Rattus norvegicus*) and German cockroach (*Blattella germanica*) (58, 59), but limit exposure to soil- and plant-associated microbes. Haahtela et al. showed that proximity to plant communities, be they from forests or agricultural lands, is associated with the abundance of the types of soil bacteria on the skin that are known to modulate immune development in children (13). Other studies within an individual city have demonstrated that the species richness of bacteria found in the air of homes was positively correlated with the presence of open windows and the proximity of flowering plants to those windows (60). Interestingly, whereas open windows were positively correlated with the load of *Alternaria* fungal allergens in the home, homes with closed windows were dominated by *Aspergillus*-associated allergens (60). This same study also demonstrated that urban, suburban, and rural homes had discrete microbial signatures, that the microbial community composition was negatively correlated to the distance between the homes, and that the presence of dogs increased the geospatial signature variance. This association was linked to the probability that the dogs acted as vectors for transmitting the external microbiome into the home environment (60). The implications of this work are tightly integrated with socioeconomic disparities. Urban homes located in lower socioeconomic areas are often exposed to worse outdoor air pollution, lack green spaces in the immediate environs of the home, and have smaller and less well-ventilated areas, including in locations of high volatile organic compound production (e.g., kitchens) (61)—all of which negatively impact the indoor surface and air microbiomes (62).

ANIMAL BUILT ENVIRONMENTS

Animal BEs have been less well-defined than their human-associated counterparts, and they are more variable in structure design. Depending upon the taxa, the form and function of animal nests can vary significantly from open-air, nightly sleeping spots that are used by a single animal (e.g., chimp nests) to elaborate nests that are more enclosed, persist for generations, and house multiple individuals (e.g., ant nests and prairie dog burrows). Yet, despite this variation, animals use their spaces in similar ways to humans, including to find comfort and protection from the environment (63), manage interactions with other animals (e.g., avoid predation) (64), rear their young (65–67), and store resources (68). Additionally, just as humans have evolved in the context of their interactions with other species, so too have animals, and the microbial ecology of nests can provide insights into these evolutionary relationships, including how these associations have shaped everything from immune development to behavior. For example, fungus-farming ants have co-evolved with certain fungi—their primary food source— where the ants exhibit specific behaviors that promote these beneficial species' interactions. The ants select leaves from plant species that are preferred by the fungus to promote fungal growth, and further, they secrete molecules with antimicrobial properties to reduce antagonistic interactions inside their nests by preventing bacterial overgrowth (69–71). Within this symbiosis and with most ecological systems, the ecology of animals in their nest environments is highly complex, and therefore, we will be unable to include all animal-microbe interactions that have been described in the literature. We will, however, attempt to highlight those studies that we find to be representative of the broader scope of nest ecology. Here, we focus on the nests and burrows built by terrestrial animals and artificial structures, including supplemental nests that are located in the natural habitat (72) or enclosures that are completely removed from the environment, such as those found in zoos.

How animals build

There have been a number of studies on the shape and size of animal nests and burrows, such as those built by great apes (73–77) and rodents (78), but a characterization of the nest size, function, and use patterns have seldom been paired with microbial data. This limits our ability to fully assess the intricacies of nest ecology for most wild animals (7, 79). However, there is evidence that nest design modulates the microbial assemblage by promoting the influx and persistence of specific taxa and by limiting colonization from environmental sources (4). Two design-related factors that are likely to play a role in microbial community composition are temperature and humidity, which are strong determinants of microbial survival and transmission in human BEs (21, 55). Animals design their nests in ways that stabilize these environmental variables (80, 81), and there is evidence that temperature and humidity variation influences nest microbiota

(82, 83). Parsing out the relative effects of how sealed a nest is from the outside (i.e., limiting environmental colonization) and temperature/humidity regulation for maintaining positive microbial interactions is essential, particularly when considering the optimal design of artificial nests and enclosures.

It is also worth noting that some species use nests built by other animals. These opportunistic animals can be either specialists or generalists. Whereas specialists, like the Brown-headed Cowbird (*Molothrus ater*) (84), use only the nests of other birds, generalists use nests built by many different species and/or nests that are located across a range of habitat types. Raccoons (*Procyon lotor*), for instance, use Muskrat (*Ondatra zibethica*) dens and Wood Duck (*Aix sponsa*) boxes, as well as arboreal sleeping sites in trees (85). Due to a generalist's use of various nest types and sites, the microbes they are likely to encounter are modulated by the species-specific characteristics and individual-specific behaviors of the nest builders. Though there are no studies on this topic, it can be assumed that a generalist's exposures are likely to be more varied over time than species that are closely tied to the nests they build, and it is unclear how nest microbiomes are generally affected by the occupation of a passing "invader." The answer to these questions is likely to depend on the factors associated with occupant demographics (as discussed more in the next section), including the number and size of individuals, length of stay, their disease state, and the relative rate of microbial shedding by each individual.

Another potential factor that could influence nest-associated microbial assemblage is the type of building material used in construction. In both human and animal BEs, material type selects for particular microbial communities (22–24, 86). Evidence from bird nests shows that some building substrates, here being feathers and specific plant species, have greater antimicrobial properties than others. This selection of materials is non-random, and it has been shown to increase nesting success (3, 87, 88). This non-random selection and curation of materials for nest construction are not limited to birds, and there is evidence that other animals construct their nests with the microbiome in mind, particularly as it relates to a reduction in the accumulation of pathogens (4, 89). In addition, material substrate will vary based on whether a nest is an above-ground structure or a subterranean burrow. Whereas above-ground nests might contain more plant-associated microbes and/or be more openly exposed to the environment, burrows are likely to host a greater abundance of soil-associated microbes and be more protected from environmental variation.

How animals live

For humans, there are estimates of the rate of bacterial shedding from our bodies (29), but as far as we are aware, these estimations do not exist for other animals. Furthermore (with the exception of ants and other arthropods), most studies that characterize microbes from wild animals focus primarily on the fecal microbiome rather than microbes from other body sites, such as the skin/fur. The majority of microbial biomass is found in the gut, making fecal samples useful for tracking microbial dynamics [e.g., reference (90)]. However, this focus has resulted in a paucity of studies exploring body-environment interactions and microbial exchange. Therefore, it is hard to quantify the relative contribution of body-associated microbes from animals into nests or from nests to the animals' bodies. Though the magnitude and direction of this microbial exchange have yet to be measured, it is clear that there is a sharing of microbes (7, 91–94), indicating the importance of the nest microbiome itself and that of the individuals that use those spaces. Therefore, occupant demographics are important to consider when assessing the diversity and types of microbiota found in animal nests.

Occupant demographics have the potential to influence the nest microbiome in a variety of ways. Both humans and other animals intentionally decrease the abundance of pathogens with various hygiene practices and the use of antimicrobials. This includes either the direct cleaning of their bodies and waste removal (95–98) or through the curation of antimicrobial-producing taxa (7, 99). It is unknown, however, whether the presence of antimicrobial-producing taxa in nests increases selection for ARGs on interior surfaces, but it can be posited that any selection for resistance is less frequent in natural nests than in artificial enclosures. Due to the properties commonly associated with artificial nests (e.g., lower nutrient availability), it is probable that there is less microbial diversity, a greater accumulation of pathogens, and environmental stressors that can lead to an increase in horizontal gene transfer events and the abundances of ARGs (as seen with human BEs) (21). Furthermore, zoos and other captive facilities administer topical, oral, and intravenous medications, and of particular concern is the use of antimicrobials *en masse* in animal production facilities, where ARG selection is rife (100, 101).

Where animals live

Animals are found on every continent and in every defined habitat type on Earth, and their home ranges can extend from small, discreet areas to large tracts of land. Animals that are more widely distributed are exposed to a broader range of climatic conditions, such as greater variability in temperature and humidity, and they are likely to have more diverse environmental exposures in the form of other animals, plants, microbes, and metabolites. For instance, a species of spider (*Stegodyphus dumicola*) that is widely distributed throughout Southern and Central Africa has a variable nest microbiome that is dictated by the local environment (5). Also, differences in nest microbiomes might vary significantly based on time of year, as microbes found in the air are influenced by seasonal fluctuations (102). How nest location influences the microbiome for a range of nest types will not only be an important area of research as the climate changes and species ranges shift but also in the context of habitat alteration and increased urbanization.

The differences in urban and rural home microbiomes have been well-established in humans (60, 103–106), but there is only one study of which we are aware that quantifies differences in nest microbiomes between these two environments (97). In this study, the authors examined bacteria collected from the feathers and nests of the Mountain Chickadee (*Poecile gambeli*) and found that though nests in urban locations hosted a similar richness of bacterial taxa, there was a greater abundance of potential pathogens. Additionally, there was more variability in community composition in urban nests, and they found evidence that there was some sharing of microbes between nests and individuals, suggesting that urban environments alter bacterial community structure and promote pathogen accumulation in ways that could be detrimental to animal health (97). This is, perhaps, not surprising as there have been a number of studies correlating urbanization and an increase in rates of infectious disease in wildlife (107), and that environmental microbes (e.g., those associated with soil, water, and plants) are different between urban areas and more rural areas (108–110). As urbanization continues to spread and become the dominant way of living, globally, humans must assess how changes in land development and use influence the health of the animals with which we share our altered habitats. However, importantly, it is not only the animals that live outdoors that are affected by human preferences and behaviors. In conservation sciences, as well as in places that are used for entertainment purposes (e.g., some sanctuaries, wildlife parks, and zoos), there has been the construction of novel habitats altogether.

Just as many humans, especially those in Westernized societies, live primarily in environments that are strikingly different from those in which we evolved, removing an animal from its natural environment greatly alters their species interactions in ways that can be detrimental to health and survival. For captive animals, their body microbes are less diverse than those of wild individuals (2, 8, 9, 111–113), and this loss in diversity can make the animal more susceptible to disease and less able to survive upon reintroduction (2). Yet, there is a lack of environmental microbiological data from different types of enclosures. Captive environments are often built and designed based on caretaker needs, visibility to the public, and/or to confine and isolate an individual, and they can include everything from large preserves to small enclosures. Certainly, as seen in human BEs, the more removed an animal is from its natural habitat (e.g., less density and diversity of local plants and loss of interaction with other animals), the greater the potential for differences in rates of microbial exposures that occur within those spaces. However, there are currently no paired studies on the microbes found in natural animal nests to those found in artificial enclosures, even though structure-body interactions are of high relevance to animal health and of particular importance for conservation sciences.

Studies of body-associated microbes from laboratory mice, when compared to wild mice, create a particularly stark contrast which might give us some insight into these natural/captive dynamics. Mice kept specific-pathogen free, under controlled laboratory conditions with significant inbreeding, have led to models with very different immune responses, especially compared to humans (114). Recent studies have demonstrated that rewilding lab mouse intestines with fecal pellets from wild mice can lead to a significant alteration in the immune response (115). This lends credence to the idea that impaired immune responses resulting from reduced microbial exposure can be rescued through an introduction to diverse microbes.

Finally, one place where animals are frequently found outside of the natural environment is in homes. Home microbiomes and the relative influences of many of the features or activities that alter the composition of the taxa found within have been well-characterized, and a few studies have demonstrated a strong contribution of animal microbes inside (33, 60). However, the animals themselves have been less examined, despite evidence that urban lifestyles increase the incidence of allergies in dogs compared to those that live in rural areas (116). Furthermore, the same study demonstrated that dogs with allergies were more likely to have owners with allergies, suggesting a similar relationship in both humans and dogs between environmental exposures and the development of disease.

RESEARCH RECOMMENDATIONS

There is a great need for diverse animal-focused BE studies, including those that encompass a broad range of taxa, robust sampling strategies, and multi-layered sample collection and analytical techniques. Though human-associated BE research can be used to infer the microbial ecology of animal nests/enclosures, there are both obvious and nuanced differences between these BE types. Quantifying the differences in structural characteristics and complexities is needed to advance our broader knowledge of ecology and evolution and to identify which factors are consequential for the promotion of animal health. This is of high importance as our climate changes, displacing animals from their native ranges, and a greater number of animals are brought into enclosures for pleasure, rehabilitation, or conservation purposes. Here, we provide recommendations for future studies in this field.

Inclusion of a broader range of animal taxa, geographic locations, and habitat types

To date, much of the nest-associated microbiome research has focused on a limited diversity of taxa and biogeographic regions, with studies coming primarily from arthropods (4, 5, 117–121) and birds (3, 88, 122, 123). Moreover, from the studies on birds, much of the research is focused on how the microbiome influences nesting success rather than providing a more general characterization of nest microbial ecology and how that varies over time, space, and between species. Even at the scale of nest shape, size, and function, there have been no studies for most animal taxa. As a greater number of wild animal populations become the focus of study, we suggest the inclusion of nest ecology, not only as it relates to the animal itself but also in the context of biogeographic, regional, and local environmental characteristics. With observational studies that address the natural structure, function, and microbiome of nests from diverse animal species, the relative importance of taxonomic and environmental variation would be more easily discernable. This integrated framework could then be used to better predict the ways in which animals maintain health-promoting microbial interactions.

Inclusion of more natural and artificial nest/enclosure types

There is a pressing need for paired studies that include both natural nests and artificial enclosures. Captivity has been shown to alter the body microbiome in a broad range of species (2, 111–113), resulting in an increase in infection risk and reduced survivability upon reintroduction, and there is evidence from both human (20, 21) and animal (7, 9) studies that microbial sharing occurs between individuals and their environment. Yet, there is almost nothing known about the microbiome of the enclosures themselves. Additionally, discrete areas within nests/enclosures harbor different microbes (4), and nest location influences the composition of those communities (124), so studies on how site selection and use patterns influence microbial colonization should be included. This would allow for the incorporation of spatial differences into the design of artificial nests (be they supplemental or captive enclosures). This work would be particularly relevant in the field of endangered species conservation. To prevent further population declines, it is imperative to understand how architectural design, differential use patterns, and habitat structure modulate the microbiome in both natural and artificial BE types.

Characterization of microbial sharing between bodies and nests

Animals share microbes with their environment (7, 92–94). However, the direction and magnitude of this interaction and its consequences for animal health are unknown. For instance, the fur of Key Largo woodrats (*Neotoma floridana smalli*) harbors similar bacteria to those found inside their nests (7), but it is not known if the woodrats are primarily sloughing off body microbes (as is seen in human BEs) or if they are being readily colonized by the microbes in their nests. If these rodents are frequently colonized by nest-associated bacteria, selection of building materials that promote positive species interactions is of high priority, particularly if an animal is moved from the natural habitat into a captive environment. To address this, more longitudinal, quantitative studies are required to track the movement of microbial taxa and biomass. In addition, these studies should involve concomitant analysis of animal immune responses to identify how different exposure scenarios influence immunological dynamics.

More diverse studies on animal-associated pathogens

There are a number of studies, especially from the bodies of animals, that assess zoonotic pathogen risk [e.g., reference (125)]. However, outside of animal production and with some obvious exceptions, such as monitoring the fungus implicated in the spread of white-nose syndrome among bats (126) and the search for pathogens that might be related to colony collapse in bees (127), there is little research on animal-associated pathogens (i.e., what is making them sick, rather than what has the potential to make humans sick). This is incredibly important not only for individual species conservation efforts but also when considering species interactions and ecosystem ecology, health, and stability over time. Nest microbiomes offer a unique look into which microbes have pathogenic potential for the animals that live there. Just as exposure to pathogens has increased in human BEs with our transition into more modified and sealed spaces (21, 128), there is a risk for a potential increase in selection for and accumulation of animal-associated pathogens as they are moved into modified captive environments. And, further, though it is known that captivity alters which microbes live on and around an animal, habitat alterations (such as the spread of urban spaces) have the potential to alter pathogen abundance and exposure potential for wildlife, and therefore, should be included in future studies.

Addition of a variety of sample collection types and analytical techniques

Many studies on animal nests have been limited to culturing and 16S rRNA and/or ITS gene analyses (3, 4, 7, 79, 88, 122). Though researchers can gain significant insight into the types and composition of organisms that are present using these approaches, the resolution of these analyses is limited. This field would, therefore, benefit from the inclusion of other characterization techniques, such as shotgun metagenomics. Metagenomics would allow for the assessment of the genetic and functional potential of the microbial community, as well as allow for the identification of individual microbial strains (129, 130). Additionally, metagenomic methods would provide a whole community approach with the inclusion of not only bacteria and fungi but also viruses and archaea. Identification of which taxa might be driving species interactive effects within that environment could be better predicted, and metagenomic sequencing would be useful in identifying taxa of interest (e.g., those that could potentially produce novel antibiotic therapies) and taxa of concern (e.g., pathogenic microorganisms). This could aid in the discovery of novel antibiotics, which are a global health need (131). Also, as human-animal interactions become more frequent (132), the ability to identify taxa and track locations with high potential for zoonotic disease reservoirs is becoming increasingly important. Finally, the characterization of metabolite profiles would allow for a deeper understanding of species' interactive effects (130) and provide insight into how they differ between nest/enclosure types and in response to use patterns and environmental variation.

CONCLUSIONS

The primary focus of built environment microbiome research, to date, has been on the places where humans live, work, and produce food. This has practical utility, as it has provided us with evidence to inform policy and management decisions that promote human health. However, as we have discussed in this review, there is great utility in studying the microbiome of wild and captive animal structures. From identifying the emergence of zoonoses in animal populations to improving animal welfare, understanding how microbes colonize and influence animal health in nesting environments is of high importance. We, therefore, call for a more systematic effort to characterize the evolved context of animal-microbe interactions in the natural world, so that we may better understand nest microbial ecology and replicate health-promoting interactions in captivity.

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