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Review

Using the microbiota to study connectivity at human–animal interfaces

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Interfaces between humans, livestock, and wildlife, mediated by the environment, are critical points for the transmission and emergence of infectious pathogens and call for leveraging the One Health approach to understanding disease transmission. Current research on pathogen transmission often focuses on single-pathogen systems, providing a limited understanding of the broader microbial interactions occurring at these interfaces. In this review, we make a case for the study of host-associated microbiota for understanding connectivity between host populations at human–animal interfaces. First, we emphasize the need to understand changes in microbiota composition dynamics from interspecies contact. Then, we explore the potential for microbiota monitoring at such interfaces as a predictive tool for infectious disease transmission and as an early-warning system to inform public health interventions. We discuss the methodological challenges and gaps in knowledge in analyzing microbiota composition dynamics, the functional meaning of these changes, and how to establish causality between microbiota changes and health outcomes. We posit that integrating microbiota science with social-ecological systems modeling is essential for advancing our ability to manage health risks and harness opportunities arising from interspecies interactions.

Highlights

Microbiotas are communities of commensal, symbiotic, and pathogenic microorganisms and are very diverse populations which function as their own ecological niche within a host.

The stability of these ecological niches can be disturbed with changed exposures of the host organism, when new and potentially disruptive microbiome components are introduced.

This disruption could be tracked to understand the impacts of new exposures at interfaces, and be used as a proxy to surmise the risks of introduction of emerging pathogens in at-risk populations.

Recent advances in analytical tools may support detailed analysis of the microbiome perturbations and linking these changes to public and ecosystem health.

Form and function of interfaces

A key characteristic of modern societies, driven in particular by human population growth, is the tendency to dominate, manage or exclude wild nature [1]. This often takes the form of physical boundaries – historically, for example, the Romans and several Chinese dynasties built walls between their civilizations and the 'wild' outside [2]. In contemporary societies, planning rules, urban limits, and gazetting of space for particular ecosystem functions also creates boundaries, sometimes delineated by physical structures (fence, wall, trench) and sometimes more open. Inside these spaces, animals and plants live, and their own internal environments, their guts (in the case of animals), are isolated from each other but influenced by the spaces they live in. The within-host environment of different host species therein serve as distinct habitats for microorganisms – some specific to particular hosts (specialist bacteria) and others subject to global dispersal (generalist bacteria) [3,4].

The human desire to dominate nature has resulted in many negative consequences – climate change [5], reductions in biodiversity, and pollution [6], which impact upon the wellbeing of ecosystems at large and humans themselves. The understanding that the health and wellbeing of humans, domestic and wild animals, and the broader environment are closely linked and interdependent is conceptualized by the **One Health approach** [7] (see Glossary) – an important concept in global health. Here, our focus is on '**interfaces**' [8], the boundary between, on the one hand, groups of humans, our domesticated animals, and our altered environment, and on the

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other, 'nature', or a more extensively biodiverse [1] zone. These interfaces are observable at different scales [9]; from satellite views of roads and human infrastructure penetrating the Amazon rainforest, or the contrasting vegetation between a national park and its outskirts, to the edge of an urban agglomeration and a city's agricultural hinterland. Like a landlocked country, one environment may also wholly encapsulate another, which would create a boundary interface around it: a watering point in an otherwise arid zone might create a roughly circular physical interface and be exploited by local human communities and their livestock by day and wildlife by night [10]. Or an urban open air refuse site is an interface between rodents and birds drawn to the available anthropogenic resources and humans engaged in informal sector recycling activities [11,12] (Figures 1 and 2A,B). Whether they live in urban centers, or in intensive or extensive production systems, humans and their domesticated animals are therefore sharing ecosystems with various components of nature, and experience direct and indirect contacts with other species, resulting in a network of interactions at these interfaces [13] (Figure 1). Crucially, these interfaces extend beyond the physical and biological – they exist and are influenced, sometimes managed, by societal interfaces between groups with divergent interests [14]. Socio-political factors play a crucial role in shaping physical interfaces within our environment. As examples, (i) dumpsites may be situated on low-value public land prone to flooding, close to low-income settlements that have historically occupied the same spaces, as they are not under urban development pressure; and (ii) new and intensive farm operations may exist close to wild species' habitats because of historical land tenure decisions that allow physical access and joining together of land parcels. The risks of cross-species contact and disease transmission may thus be heightened [15,16].

Studying transmission at interfaces

The recent increase in infectious disease outbreaks and epidemics, occasionally escalating to pandemics, originating from wild animals, particularly in the context of rapid globally connectivity, has crystallized the fear of **Disease X**, a hypothetical pathogen that could emerge from the other side of these interfaces [17,18]. As a still largely unknown diversity of pathogens is found in wildlife

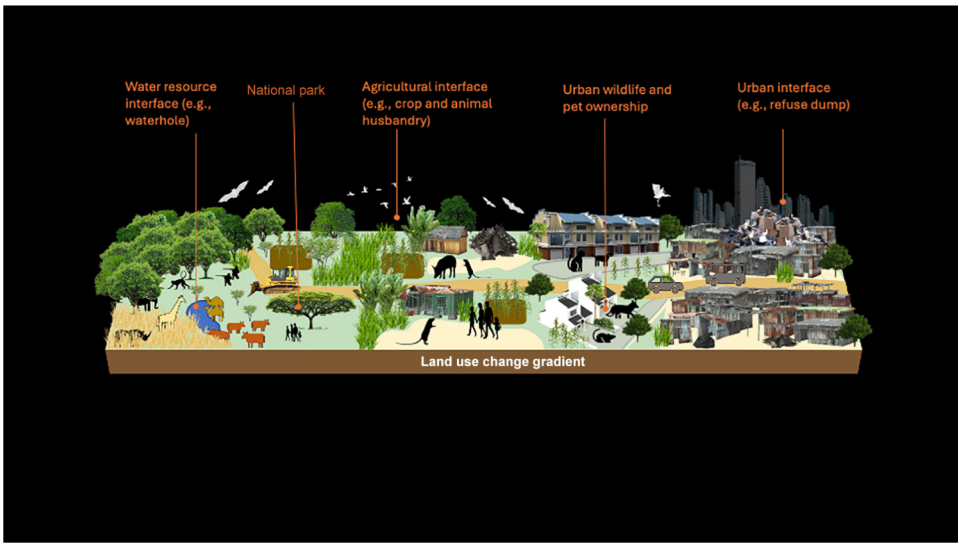

Figure 1. Diagrammatic representation of different types of wildlife/livestock/human interfaces along a hypothetical land-use gradient. At each interface, the likelihood of microbial transfer between animals and humans varies as a function of background microbial diversity and contact intensity; hence, differential shifts in microbiota composition would be expected to occur.

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Figure 2. Examples of interfaces at which humans and animals meet in complex and varied environmental conditions. (A) Urban waste site with scavenging birds near informal settlements. (B) Rural pastoral landscape with livestock at a communal water source. Photo credits: Eric M. Fèvre (A), Dishon M Muloi (B).

hosts, there is a constant threat that this infectious component of biodiversity spills over from wild reservoir communities to other wild or domestic hosts (i.e., **bridge hosts**) ultimately crossing over to humans [19,20]. From there, amplification through human-to-human transmission can lead to epidemics and, eventually, pandemics become more likely – as exemplified by coronavirus disease 2019 (COVID-19) [21].

In disease ecology, hotspots of infectious disease emergence have been identified at wildlife/livestock/human interfaces (WLHI), for example, zones with clear boundaries, like national park buffer zones or fixed spaces where different species interact, such as wet markets [22,23]. WLHI can also be defined temporally by measuring the overlap in time between the use of the same site by different hosts, allowing the identification of the time-window in which a pathogen shed by one host and persisting in the environment may pose a transmission risk to another host occupying the same space. For example, humans sharing space with rodents that have shed *Leptospira* bacteria into the environment can become infected [24,25]. A shared physical space and a shared – or appropriately lagged – time point, creates a pathway for cross-host transmission [11,26]. The drivers that influence these contacts are certainly ecological, but human factors influence ecological processes – such as building design, crop cover choice, or waste storage [27]. WLHI have been mainly studied from a biological and ecological lens and less so from a social or political perspective. WLHI therefore represent complex and constantly evolving **social-ecological systems**, where the nature of interactions and pathogen transmission between non-human hosts and humans is constantly evolving [27]. This dynamicity makes the nature of host contacts at WLHI challenging to predict and leaves us in need of finding new ways to understand interspecies contacts and their consequences. For example, while the epidemiology of avian influenza is well-described – including wild flying and aquatic animals, domestic animals, and humans – the complexity of its transmission cycle allows for **viral reassortment**, making it difficult to predict emergence or human pathogenicity [28].

Crucial research challenges at these interfaces include predicting transmission pathways between multiple hosts to improve disease spillover management, identifying host and microbial community characteristics that promote or mitigate spillover events, and determining whether sympatric human and animal hosts develop a stable microbiota following prolonged exposure or remain dynamic and susceptible to pathogen spillover. In the context of recent advancements in microbiota research and its implications for host functional processes, here, we review the principles and mechanisms that may drive microbiota exchange at interfaces following interspecies

Glossary

Alpha diversity: a measure of microbiota diversity, defined as the observed richness (number of taxa) or evenness (the relative abundances of those taxa) within a particular sample or community; that is, intra-sample diversity.
Beta diversity: variability in community composition (the identity of taxa observed) between samples or communities; that is, inter-sample diversity.
Bridge hosts: facilitates pathogen transmission from a maintenance (reservoir) population to a target population. To act as a bridge, a host must be capable of either pathogen replication and excretion (without sustaining the infection independently) or mechanical transmission, and must occupy an ecological niche that allows for contact between maintenance and target hosts.
Colonization resistance: mechanisms by which resident intestinal microbiota limit the colonization of pathogens and pathobionts.
Disease X: an unknown diseases caused by pathogen X – an infectious agent currently unknown to cause human disease, but with epidemic or pandemic potential.
Exposome: the cumulative environmental exposures an individual encounters over their lifetime, including microbes, diet, pharmaceuticals, interactions with other hosts (e.g., pets or livestock), pollutants, lifestyle factors (e.g., smoking, physical activity), socioeconomic conditions, and physical or social environment.
Interface (disease): points of interaction where humans overlap in range and time with livestock and wildlife directly, or indirectly (through sharing common environments or consuming animal products); can create pathways for the emergence and cross-species transmission of pathogens.
Microbial signatures: within the context of the microbiome, these are distinct and recognizable patterns in the microbial community, akin to a fingerprint for the microbiome. These signatures may include specific microbial taxa, genetic elements such as AMR genes, or metabolites.
One Health approach: an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals, and

contact. We further explore the potential for monitoring microbiota changes as a means of inferring transmission pathways, which can inform public and ecosystem health strategies and clinical health outcomes. The principles that we will consider and review are: (i) microbiota of hosts with limited or no contact exhibit greater compositional differences than those in frequent contact; (ii) when the microbiota of two hosts come into contact, their compositions influence what is transferred between them, either unidirectionally or bidirectionally; and (iii) microbiota composition, including **alpha** and **beta diversity**, influences resilience to perturbation following interspecies contact. We structure the discussion around these three principles, highlight the knowledge gaps associated with each, and review how microbiota changes may serve as indicators of transmission pathways.

Making the case for studying microbiota at interfaces to infer zoonotic transmission risk

Research into transmission dynamics at interfaces has tended to focus at the scale of single species of primary concern. More recently, transmission dynamics have been inferred through the use of bacterial markers of ecological connectedness such as *Escherichia coli* [29], *Klebsiella pneumoniae* [30], or *Campylobacter* [31] as proxies for transmission between human and animal hosts. While these studies have yielded valuable insights into microbial population structures and identified instances of bacterial transmission between proximate human and animal hosts, they provide a limited view of the transmission dynamics within these structurally and ecologically diverse interfaces. The gut microbiota – described as the community of commensal and pathogenic microorganisms and their mobile genetic elements present in the mammalian gut [32] – has been investigated for bacterial transmission through close social interactions [33–35]. In zoonotic bacteria, there may be a direct functional relevance of this sharing as it often includes genes conferring resistance to antimicrobial drugs. The rapid progress in high-throughput sequencing technologies and analysis of microbiota composition, coupled with a growing appreciation for the microbiota's significance in human health and disease [36,37], have significantly expanded research in this field. These studies have revealed that microbial communities colonizing the human body exert a profound influence on many functional processes of their hosts, including the immune system and host behavior. The diverse symbiotic gut microbiota, which has coevolved with its hosts, plays a crucial role in buffering against pathogen colonization through various mechanisms collectively referred to as **colonization resistance** (as reviewed by Woelfel *et al.* [38] and Caballero-Flores *et al.* [39]. Disruptions in microbiota composition and function increase susceptibility to infections or the overgrowth of deleterious **pathobionts**, ultimately leading to adverse health outcomes. The relative composition of the microbiota, and how it changes (or not) over time, are unique to an individual creating a microbiota 'fingerprint', partly owing to inter-individual environmental exposures (collectively defined as the **exposome** [40]) – the predominant factors shaping microbiota composition such as diet or antibiotic use [41,42]. However, interaction with animals at interfaces contribute to variation in the human microbiota and immune health, depending on the frequency, magnitude, and nature of exposure. For example, in rural settings where much of the world's population resides, proximate animal contacts can facilitate human exposure to animal-associated microbiota [43], potentially disrupting the microbiota ecosystem and, consequently, human health. However, the extent to which exposure to animal microbiota at an interface, particularly wildlife, and whether occasional or continuous at an interface influence the human microbiota's composition, dynamics, and long-term changes remains poorly understood [44]. When humans encounter foreign microbiota, such as through environmental exposure or host contact, colonization occurs only if an available niche exists. This process can result in partial integration of new microbial taxa, displacement of resident communities, or a competition-driven dynamic in which the dominant microbiota prevails through numerical advantage or ecological resilience. Healthy microbiota

ecosystems. It recognizes the health of humans, domestic and wild animals, plants, and the wider environment (including ecosystems) are closely linked and interdependent.

Pathobionts: microorganisms typically existing as harmless symbionts that, under specific circumstances, could lead to disease outcomes.

Social-ecological systems: ecological systems (composed of interacting biological units) intricately linked with and affected by one or more social systems. It recognizes that humans are not separate from nature, but rather an integral part of it.

Viral reassortment: a genetic exchange process in which two or more parental viruses co-infect a single host cell and exchange genome segments, producing hybrid viral progeny with genome segments from multiple parental strains.

exhibit intrinsic resistance to colonization, as each microbial member occupies a defined niche (nutrient–niche hypothesis [45]), granting more diverse microbiota resilience against disease (diversity–stability hypothesis [46,47]).

While many studies have demonstrated strain-level microbiota transmission between hosts (reviewed elsewhere [48]), a broader perspective suggests that each contact point holds a wealth of **microbial signatures** – both commensal and pathogenic. Simply, when a connection occurs (and this connection may be indirect), some of these signatures are transmitted. The question then becomes: can we observe and quantify this transmission highway? Is it simply an observable movement that indicates contact? Microbes exist along a spectrum of colonization and persistence, with some microbes capable of establishing long-term colonization within a host or population, while others are transient. Understanding the dynamics of microbial colonization and persistence is crucial for human health, particularly as transient bacteria can be reintroduced through external contacts, whereas persistent taxa, often vertically transmitted, may be irreplaceable if lost [49]. For instance, antibiotic exposure – either directly in humans or indirectly through agricultural settings – can lead to the permanent loss of beneficial, highly persistent microbes [49]. Comparative microbiota analysis of Hadza hunter-gatherers in Tanzania and industrialized populations in Nepal and California identified the loss of 124 gut-resident taxa in the latter, underscoring the impact of industrialization and lifestyle shifts on gut microbiome diversity [50]. In hospital neonatal units, colonization by pathogenic, antimicrobial resistant (AMR) bacteria often occurs within 48 h, yet outbreaks of invasive disease remain infrequent [51,52], suggesting that neonatal and environmental microbiota are highly interconnected [53] through microbial transmission and dispersal, with only some exchanges leading to significant health outcomes. Although immediate health outcomes may not always be evident, the long-term impact of these microbial exchanges – potentially manifesting downstream in time – remains unknown. We need to shift our conceptualization of microbiota composition to one that encompasses the broader implications of microbial exchange and considers persistence at an ecosystem scale (as reviewed by Robinson *et al.* [54]). Several factors have been hypothesized to influence gut microbiota colonization, including transmission routes and mechanisms, intrinsic microbial traits (such as aerotolerance and spore formation) [55], and host social interactions. However, the extent to which these factors facilitate or impede microbial dispersion and colonization – thereby modulating transmission dynamics – remains poorly understood [56]. For instance, the precise route of gut microbiota colonization has yet to be fully elucidated, although prevailing hypotheses suggest that the fecal–oral route is the primary mode of gut microbiota transmission [55].

Human behavior plays a critical role in shaping exposure pathways [33,35,57,58], with the evolution of human culture and practices (such as animal husbandry or antibiotic use [59]) facilitating microbial exchange and dissemination [33,60]. For example, adults in Honduras with closer social relationships exhibited higher rates of microbiota strain sharing [35]. More sociable wild macaques had higher rates of acquiring beneficial commensal *Faecalibacterium* genera, whereas less social individuals were more likely to acquire pathogenic *Streptococcus* genera [61]. Furthermore, a study in Peru and El Salvador demonstrated extensive networks of resistome similarity among sympatric human, animal, and environmental microbiota [62]. These findings highlight the significance of examining microbiota transmission within the framework of social networks [60] also present at interfaces, which fundamentally shape inter- and intra-host contact patterns and exposure dynamics [63]. Key questions remain whether specific taxa, entire microbiota, or a combination of both are socially transmissible, and whether beneficial or mutualistic bacteria are preferentially transmitted over pathogenic or harmful ones.

Microbiota perturbations and health outcomes

An individual's microbiota is shaped by its genetic composition [64] and cumulative exposures (exposome) to environments and microorganisms over time, forming a relatively stable 'microbial equilibrium'. At an interface, disruptions to this equilibrium, whether from novel ecological interactions or new exposures, can be viewed as transient shifts toward the original or an alternative stable state, transitions between distinct equilibrium states, or an oscillatory pattern of stability influenced by complex environmental forces (Figure 3) [65–67]. This demonstrates 'microbial fluidity' – the dynamic reshaping of microbial communities – providing a framework for understanding host–environment interactions and tracing both contemporary and historical microbial exchanges. A host's capacity to restore microbial equilibrium in the aftermath of one of these perturbations depends on a combination of intrinsic factors such as current taxa membership and immune function, and extrinsic factors, such as the frequency and nature of exposure [65,68]. Longitudinal analyses of microbiota perturbation patterns reveal that regular exposures establish a 'microbiota baseline' within individuals, with significant deviations from this baseline manifesting as: increased beta diversity dispersion, reduced alpha diversity, decreases in symbionts and increases in pathobionts, or elevated AMR resistance and virulence traits [69]. Tracking these microbial shifts could provide valuable public health indicators for assessing the consequential impact of interactions at WLHI interfaces. However, the magnitude, direction, and implications of these changes are likely to vary depending on interface type; for example, along land-use gradients where the type of wildlife contact differs along the gradient. Empirical studies investigating these patterns specifically at WLHI are few and far between, but we draw parallels from human and wildlife health research, where extensive evidence demonstrates the relationship between microbiota diversity, health outcomes, and colonization resistance. For example, rats in anthropogenically altered habitats exhibit lower gut microbial alpha diversity and a more dispersed community structure [56], while American white ibises in urbanizing areas experience microbiota shifts characterized by reduced diversity and increased shedding of pathogenic *Salmonella* [57]. This

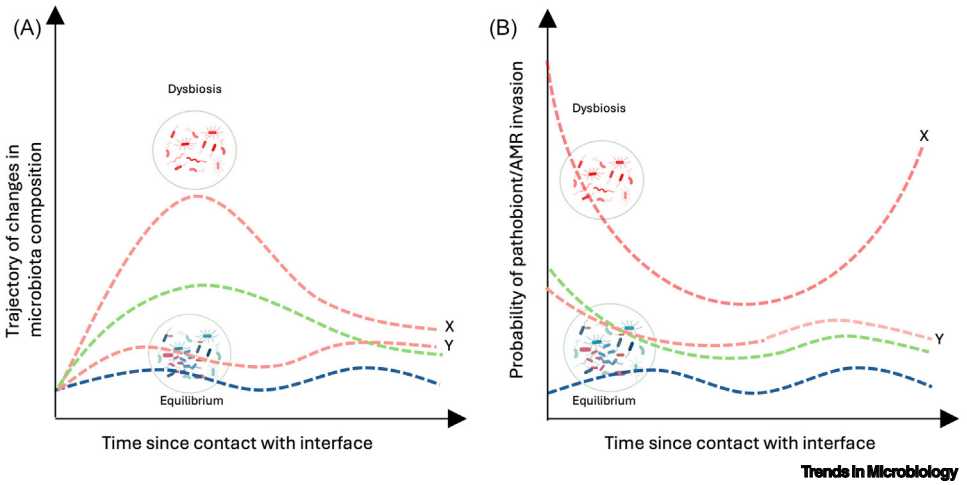

Figure 3. Conceptual graphical representation of hypothesized trajectories of gut microbiota compositional change over time in hosts following initial and ongoing exposure to a human–animal interface. (A) Trajectories of microbiota compositional change (e.g., alpha or beta diversity); (B) trajectories of the probability of pathobiont or antimicrobial resistance (AMR) invasion. In both panels, the blue line represents a host with regular, long-term historical contact with the interface; the green line represents a host with repeated but irregular historical contact; and the red lines represent hosts with no prior exposure. Hosts with no prior exposure to the interface are subdivided into X, a host with a less functionally diverse microbiome (which we hypothesize is therefore more susceptible to dysbiosis), and Y, a host with a more functionally diverse microbiome (which we hypothesize is therefore less susceptible to dysbiosis).

raises critical questions on the extent to which anthropogenic impacts shaping wildlife microbiota contribute to zoonotic pathogen risks for humans and livestock.

Furthermore, microbiota perturbations resulting from inter-host interactions have been shown to facilitate the dispersal of AMR strains and their resistance determinants. Humans exposed to antibiotic-treated livestock [70–72] or wildlife that interact closely with tourists [73] exhibit remodeling of their microbiota and enhanced acquisition of AMR bacteria and resistance genes. Once acquired, AMR genes can persist within the gut microbiota – particularly in the abundant *Bacteroidales* symbionts [74] – serving as reservoirs for horizontal transfer to opportunistic pathogens [75]; thus, this sharing amplifies the risk of AMR emergence and persistence with consequent public health implications.

While most research has focused on the negative consequences of inter-host microbiota transmission, there may also be potential benefits. For instance, children aged 4 months to 6 years in rural Nicaragua exposed to environments contaminated with cattle and dog feces exhibited higher gut microbiota diversity [76], mirroring findings in children under 5 years in rural Kenya who engage in livestock feeding chores [77]. Similarly, a recent study on Wisconsin dairy farms identified bacterial co-occurrence between cattle and farmers, including enrichment of taxa associated with protection from diarrheal disease [72], reinforcing hypotheses on the adaptive advantages of microbiota exchange [78]. Additionally, multiple studies (reviewed in [79]), indicate that people exposed to dogs exhibit greater gut microbiota diversity and composition. These findings, along with broader microbiota research linking farm exposure to lower allergy and asthma prevalence, suggest that increased gut microbiota diversity may enhance microbial resilience and functional capacity, contributing to improved immune regulation and disease resistance – echoing the hygiene hypothesis [80]. In summary, there is a tight relationship between microbiota dynamics and host immunity, which we argue could be invaluable for public health, allowing the identification of high-risk connections before pathogen transmission becomes consequential.

In human health, changes in alpha and beta diversity of the gut microbiota have been proposed as promising indicators for various neurological diseases [81], metabolic disorders [82–85], and even longevity [86]. Similarly, in the context of ecosystem health, microbiota surveillance has been used as a noninvasive approach to monitor wildlife population health [87–89]. We posit that if used as a form of bio-indicator, these observations could be extended to support predictions of infectious disease at interfaces. More broadly, at the population level, they could function as a reliable early-warning system for public health events of concern, as microbiota changes often precede clinical manifestations of disease. Despite this apparent potential, the application of microbiota monitoring in infectious disease epidemiology – particularly in response to environmental exposures at animal interfaces – remains underexplored. To comprehensively assess the functional consequences of microbiota changes, several key research questions must be addressed. First, defining the relevant temporal and spatial scales of microbiota monitoring is critical, distinguishing between local and broader geographic scales [90] as well as short-term (e.g., seasonal) and long-term trends [91]. Establishing these parameters is essential for accurately interpreting microbiota shifts and devising realistic sampling intervals.

Second, a single metric is unlikely to capture the full functional impact of microbiota shifts, as these depend on the required scale of resolution used to define transmission. One approach is to analyze broad ecological correlations between diversity or composition, which may be relatively straightforward but could prioritize dominant taxa while overlooking ecologically or functionally significant low-abundance taxa. By contrast, targeted analyses of specific microbial determinants – such as the correlation between bile salt hydrolases and the inhibition of

Clostridioides difficile – using quantitative PCR, or deep metagenomic sequencing could provide higher resolution. Explicitly defining the appropriate metric is essential for testing hypotheses about microbiota perturbations, their functional roles, and the underlying ecological processes driving these shifts.

Third, it is necessary to establish biologically and ecologically meaningful thresholds for microbiota perturbations – considering both magnitude and frequency – to distinguish significant shifts from background variation driven by broader ecological and social dynamics, especially if we are proposing that there be an inflection point at which action might be taken.

Addressing these questions demands appropriate study designs and the sampling of representative populations alongside genomic and computational analyses. For example, longitudinal surveillance of human and animal cohorts sharing environmental resources (e.g., shared water resource), coupled with appropriately matched control populations, and well curated metadata, could help establish links between microbiota changes, individual disease risks, and broader population-wide trends associated with the interface contact. Undertaking empirical studies to understand these complex relationships should be a research priority.

Tools for studying microbiota function

A key objective of microbiota research is to identify factors that meaningfully perturb the microbiota in a way that has functional implications of these perturbations. We must move beyond the simple description or observation of compositional changes at isolated time points. Increasingly, studies are adopting longitudinal designs and time-series analyses [35,92,93], utilizing tools to estimate broad temporal variations in microbiota composition – particularly beta diversity and community dispersion. However, these approaches often fall short in explaining variability in microbiota responses to specific perturbations [94]. Part of the challenge in analyzing microbiota data temporally lies in its compositional nature, where changes in the relative abundance of one taxon are negatively correlated with changes in others [95,96]. Study designs are further limited by sparse temporal sampling, statistical complexities such as autocorrelation, and the nonlinear, nonadditive relationships between compositional shifts and functional outcomes [97]. Owing to the increasing emphasis on mechanistic investigation, various computational and statistical methods have been developed. Generalized additive models and linear mixed-effect models are commonly applied to analyze longitudinal trends, identifying significant temporal changes and their drivers [98]. Dynamic Bayesian models complement these approaches by capturing time-dependent ecological interactions and characterizing the factors influencing microbiota dynamics, providing a robust framework for investigating temporal patterns and their implications [99,100]. The predictive power of microbiota monitoring depends on distinguishing critical perturbations from reversible or unimportant deviations. Emerging machine learning tools, such as large language models [101], offer potential for deciphering complex systems with hidden structures and relationships. However, these models require extensive training datasets, which are currently lacking. A central question in the microbiota field is the directionality of causality; specifically, does microbiota composition (and associated changes therein) predict health outcomes, or do health outcome shape the composition? This revisits Koch's postulates and Bradford Hill's criteria for establishing causation. Tools such as labeled directed acyclic graphs help characterize causal structures for microbiota –mediated outcomes, disentangling complex relationships and subsequently improving inference about the direction of causality [102]. Improved understanding of these causal pathways will facilitate the development of targeted microbiota-related interventions. Higher resolution methodologies for analyzing strain-level microbiota sharing [103] will undoubtedly improve our understanding of microbial transmission at WLHIs. These tools provide fine-grained insights into microbial exchange and the potential functional impacts of strain-level variation.

Concluding remarks

Anthropogenic activities continue to shape the form and function of interfaces, influencing patterns of connectivity between humans and animals, and, in turn, the transmission of microorganisms. Where these microorganisms are pathogenic, such transmission events become critical points in the emergence of disease. Microbiota science offers a high-resolution lens through which to study the ecology of putative transmission occurring at WLHI. It not only reveals the composition and function of microbial communities but also serves as a potential bioindicator, providing valuable insights into the broader ecological and health impacts of interspecies interactions. Developing a comprehensive eco-epidemiological understanding of these complex, multi-host systems is crucial and must evolve alongside the rapidly advancing field of microbiota science. This requires shifting beyond a human-centric perspective toward a One Health approach, which better informs interdisciplinary strategies that mitigate risks associated with WLHIs while harnessing the potential co-benefits for public and ecosystem health. To this end, future microbiome research should transition from exploratory studies to hypothesis-driven investigations, integrating innovative analytical and experimental methodologies. Key priorities include elucidating the functional consequences of microbiota variation following interspecies contact and quantifying the relative contributions of different interfaces to microbial transmission (see Outstanding questions).

Outstanding questions

How do temporal and spatial variations in interspecies host contact at interfaces influence human gut microbiota structure, and how does this vary among different interface types?

How frequently should microbiota changes be monitored to effectively inform public health strategies?

How do social networks and behavioral patterns – their nature, frequency, and magnitude – shape microbial transmission at WLHIs?

Do compositional changes mean that there are functional dynamics? And how many time points are necessary to reveal meaningful microbiota change dynamics?

What tools and methodologies are most effective for studying microbiota perturbations, their functional implications, and causal structures?

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Declaration of interests

The authors declare no competing interests.

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