Cell Host & Microbe
Review

# The Landscape Ecology and Microbiota of the Human Nose, Mouth, and Throat

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Landscape ecology examines the relationships between the spatial arrangement of different landforms and the processes that give rise to spatial and temporal patterns in local community structure. The spatial ecology of the microbial communities that inhabit the human body—in particular, those of the nose, mouth, and throat—deserves greater attention. Important questions include what defines the size of a population (i.e., "patch") in a given body site, what defines the boundaries of distinct patches within a single body site, and where and over what spatial scales within a body site are gradients detected. This Review looks at the landscape ecology of the upper respiratory tract and mouth and seeks greater clarity about the physiological factors—whether immunological, chemical, or physical—that govern microbial community composition and function and the ecological traits that underlie health and disease.

## Introduction

In an effort to discern the role of fundamental ecological processes in community assembly, early ecological models assumed organisms to be distributed across a spatially homogeneous environment. Yet, nearly every ecosystem, including the human microbial ecosystem, exhibits distinct patterns of community structure and assembly across gross anatomic sites, suggesting rich habitat differentiation (Costello et al., 2009). One explanation for these distinct patterns is underlying spatial heterogeneity in topographical anatomy (i.e., the "landform"), local chemistry, or both and, in the case of host-associated environments, physiology, as well as tissue type and associated structures, desquamation rates, immune processes, temperature, moisture, and other local conditions. Thus, in treating the human body as a microbial landscape, we must consider the underlying spatial heterogeneity in landforms and environmental features as selective factors that give rise to spatial patterns in microbial community structure and function; these communities, in turn, generate additional spatial heterogeneity.

The theory of landscape ecology, which emerged in the 1960s, sought to explain the spatial patterns and processes operating on a landscape rather than assuming space to be homogeneous (Wiens et al., 1993). When surveying a landscape, the observed types of spatial (and temporal) patterns depend critically on the scale of observation (e.g., a micron, a meter, a kilometer and a second, a day, a year). In turn, the scale of observation informs a variety of parameters—such as patch size, patch density, patch quality, inter-patch distances, and stability. By quantifying these parameters experimentally, ecosystem stability and expected resilience in the face of a variety of disturbances can be modeled using the mathematical underpinnings of landscape ecology. Yet, with few exceptions (Bouslimani et al., 2015; Mark Welch et al., 2016; Swidsinski et al., 2007), most molecular studies of the host-associated microbiota continue to discuss biogeography not as a function of geography per se, but as variation across gross anatomic sites and treat these sites as categorical, discrete entities. For this reason in part, our understanding of the spatial and temporal scale(s) important in the ecology of the microbes and viruses that inhabit the human body is limited.

In this Review, we begin by providing a primer on landscape ecology before discussing some of the processes that give rise to patches while highlighting the importance of spatial scale in commensal microbial communities. Next, we discuss the landscape heterogeneity of the upper respiratory tract and mouth, an excellent anatomic region for studying spatial ecology due to its easy accessibility. Then, we consider the possibility that baseline immune function represents a disturbance regime that is perturbed during acute or chronic infections and is associated with detectable pulse or press perturbations in community structure. We conclude by presenting a few ways in which landscape theory might be applied to the microbiota of the oral and nasal cavities in order to increase our understanding of how pattern and process contribute to the structure and function of host-associated communities.

## Primer on Landscape Ecology

Landscape ecology examines the processes that give rise to spatial patterning in communities across a landscape, "the landforms of a region in the aggregate" (Turner, 1989). The chief proposition of landscape ecology is that the features and spatial arrangement of the landscape dynamically interact with ecosystem function, each shaping the other, such that it is difficult to understand a community without considering the context of its associated landscape (Wiens, 1995a). To facilitate a discussion of this proposition, in this section, we review the basic history, terms, and definitions of landscape ecology while identifying the types of spatial patterns that might be observed in the ecology of the microbiota.

In population biology, a "patch" was historically defined as a spatially clustered population (i.e., a group of interacting

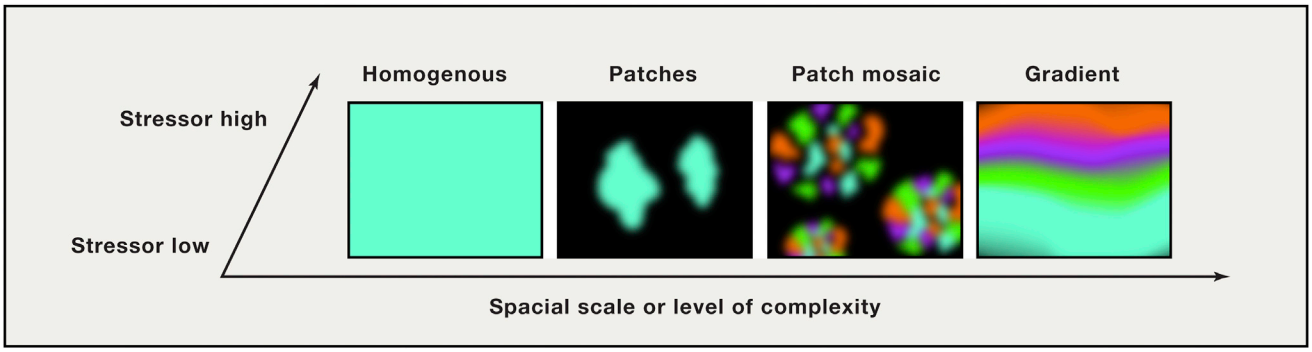


**Figure 1. Spatial Patterns in Landscape Ecology**
A single species that is impervious to an environmental stressor, regardless of intensity (y axis), and that is uniformly distributed across space is said to have a "homogenous" distribution. A "patch" is a spatially clustered population of a single species—in this example, two patches of different sizes can be observed. A "patch mosaic" encompasses a set of patches or patch types in which no discernable pattern can be detected for any patch with respect to the others or to the underlying environmental stressor. Patches in the mosaic may vary with respect to each other: for instance, they may have different sizes, may have sharp or indistinct boundaries, or may be connected or disconnected. A "gradient" describes the spatial pattern in which a patch or patch mosaic varies with respect to some underlying environmental stressor, such as pH, or temperature, for which different species have different optima. Adapted from Wiens (1995b).

organisms of the same species) that could be distinguished from its surroundings (Hutchinson, 1953). The spatial boundaries of a given patch (e.g., city limits) are given by the boundaries of suitable habitat, and each patch is embedded in an inhospitable or neutral matrix (Figure 1). With the development of the theory of island biogeography and meta-population theory, ecologists began considering the interaction of patches (i.e., populations) with each other through migration, dispersal, colonization, and extinction—processes that mediate inter-patch dynamics. In so doing, meta-population biologists solved a fundamental problem in patch biology: that spatially isolated populations have a stochastic, non-zero probability of going extinct and should do so by chance over different timescales (Levins, 1969).

Community ecology, in turn, extends the domain of meta-population studies from populations of one species to the "community," an assemblage of interacting organisms of two or more species. In spatial ecology, a "patch type" is analogous to a patch but represents a spatially clustered community rather than a population. Patch types are often defined by the dominant organism or land-usage regime (Pickett and Cadenasso, 1995), such as an oak woodland, pine woodland, or coastal sage scrub. As such, the concept of a "community state type" (CST) might be considered as a basic description of a patch type in human microbial ecology, since most researchers use it to denote a community dominated by one organism (e.g., a vaginal community dominated by *Lactobacillus crispatus*) (Ravel et al., 2011). In the human body, dominance by one species is most apparent in the vagina. It is unclear, in general, whether most surveys of the microbiota characterize single patch types or whether they pool multiple patch types since the spatial extent of CSTs is rarely defined. Further, there is some debate in the field of microbial ecology as to how community state types should be defined (Callahan et al., 2016a).

Landscape ecology examines ecosystems across a wide variety of spatial scales surveying the dynamics of spatial patterns known as "patch mosaics" and "gradients." A patch mosaic is a collection of patch types within a spatial territory where patch types exhibit a definite, but seemingly random, spatial distribution with respect to each other (Figure 1). In the mouth, the recently described "cauliflower" arrangement of bacteria is one of the most beautiful visuals of a patch type, consisting of spatially segregated patches of organisms, including *Lautropia*, *Veillonella*, *Haemophilus/Aggregatibacter*, *Capnocytophaga*, and *Streptococcus* spp., among others (Mark Welch et al., 2016). In one representative image, *Lautropia* formed clusters that rarely included other members of the community, while *Streptococcus* was interspersed among clusters of *Haemophilus/Aggregatibacter*, and *Veillonella* occupied peripheral regions where gaps could be found. Large gaps between many patches may be seen, and when larger spatial areas were viewed, different patch types, including "corncob," "cauliflower," and "hedgehog" structures, were found to repeat across space. The repetition of these patch types across space is indicative of a patch mosaic.

Examining the spatial arrangement of patch types and patch mosaics, in turn, leads to some of the fundamental questions in landscape ecology, such as how many distinct patches can be found in a mosaic and why; how large is each patch and why; do patch or patch types have sharp or indistinct boundaries; how connected are the distinct patches; and to what extent do these quantitative features predict the occurrence of spatial arrangements between and across sites. The answers to these questions would enable the prediction of ecosystem stability and resilience (Wiens et al., 1993), allowing the field to move beyond qualitative narratives of spatial patterns toward quantitative descriptions of spatial dynamics and predictions about function.

By examining patch mosaics across the entire landscape, the size, orientation, and arrangement of patches and patch mosaics can be defined (Turner, 1989). These features must be understood to identify gradients—ordered arrangements of patch mosaics (Figure 1). Patch types in a gradient tend to have indistinct boundaries (Wiens et al., 1993) as a result of gradation in underlying environmental factors, such as pH, temperature, and moisture. The recognition and understanding of spatial gradients in microbial community structure across the human body are still

in an early phase. Meanwhile, debate in soil microbiology concerns the question as to whether microbes follow biogeographical patterns that are fundamentally different than those of macroscopic organisms (Fierer et al., 2011; Tripathi et al., 2017).

## Processes that Give Rise to Patchiness

What processes give rise to spatial heterogeneity? This question was first answered by Alan Turing who sought to understand how spatial patterns emerge from a uniform surface (Turing, 1952). Turing studied the unfolding of pattern during morphogenesis by modeling reaction diffusion dynamics. In that seminal work, spatial patterns developed after irregularities were amplified due to system instability in chemical reaction dynamics. These irregularities may have been stochastic, or they may have been emergent features of the system, as is the case with differential gene expression during morphogenesis. In ecosystems, such "Turing irregularities" can give rise to spatial pattern in community structure—in this section, we review some of the factors that may give rise to spatial patterns in ecosystems, including disturbance, abiotic factors, and biotic factors (Hutchinson, 1953). Ecological processes—dispersal, selection, diversification, and drift, as well as priority effects—that give rise to heterogeneity have been reviewed elsewhere (Costello et al., 2012; Fukami, 2015; Martiny et al., 2006).

One of the biggest sources of spatial heterogeneity is "disturbance." Disturbance is defined as an irregularity that perturbs the ecosystem as well as community or population structure. An ecological disturbance is thought to induce spatial heterogeneity by making space available for new colonists and by inducing a temporal irregularity that disrupts the natural course of succession (Levin and Paine, 1974). Disturbances can be one-off events, or they can occur either regularly or stochastically with a specific periodicity and intensity, in which case the periodic oscillations define the disturbance regime (Relman, 2012). One single disturbance event impacting a region may have a different impact on different sites since the "spread of disturbance across a landscape is influenced by spatial heterogeneity" (Turner, 1989). For example, tooth-brushing removes biofilms fairly well from the cheek- and tongue-facing surfaces of teeth, but it tends to be less effective at removing biofilms from the biting surfaces of molars and pre-molars, permitting higher biomass accumulation at those sites. In other words, the intensity of brushing as a disturbance is higher at one site than it is at the other because of spatial heterogeneity in the landscape.

Besides disturbances, a second cause of patchiness is the underlying partitioning of environmental resources or stressors—for example, resident anaerobes in the mouth that prefer a lower redox potential will likely be found where oxygen is limiting in the subgingival crevice, especially in severe cases of periodontitis, or on the dorsal tongue. Other examples include stressors that structure gradients as previously discussed. Biotic interactions—competitive, social, or reproductive—provide a third cause of patchiness. For example, bacteriocins, anti-competitor proteins employed by the microbiota that typically target conspecifics (Zheng et al., 2015), diffuse away from those cells that produce them, creating a concentration gradient and a zone of competitive exclusion, which is observed as the repulsion of the producer and sensitive strains. Similarly, social behaviors typified in bacteria by quorum sensing may generate patchiness by inducing dispersal of surface-associated cells or attachment of planktonic cells, two processes that would naturally lead to different spatial patterns at different timescales. Finally, certain reproductive strategies also give rise to patchiness; an example can be seen in the Cathedrals of the California redwood *Sequoia sempervirens*, in which a circle of clones surrounds the mother tree when that individual reproduces clonally.

## Spatial Scale in the Landscape Ecology of the Microbiota

One critical question in microbiome research is how to couple our analytical techniques with the spatial or temporal scale(s) required to identify patterns and underlying mechanisms important in the ecology of the microbes and viruses that inhabit the human body. The grain of observation profoundly influences our ability to observe patterns. If the grain is too coarse, such as what is gained by using nasal lavage or oral rinses to sample the nasal or oral cavities, then the community that is observed is a statistical sample of a heterogenous landscape. If the grain of observation is too fine, a single patch or patch type might be misinterpreted as representative of the entire landscape.

This raises the question of what spatial scales are relevant to the genesis of spatial patterns and processes in the communities of the human body. Our ability to detect spatial patterns depends on the spatial scale of organisms in a given ecosystem. For plants, patches are typically observed at small spatial scales (1 m), patch mosaics are observed at larger spatial scales (50 km), and gradients are observed at the most expansive spatial scales (200 km) (Ehrenfeld et al., 1997). Microbial ecology, on the other hand, must focus on variation between entities separated in space on a scale that is appropriate to their body size. Viruses range in size from 20 to 450 nm, while bacteria vary in size from 0.3 μm for *Mycoplasma* (diameter) to the average cell, *Escherichia coli*, which is 1.1 to 1.5 μm wide and 2 to 6 μm long. The spatial extent of a population or community of micron-sized organisms will depend on the scale of the processes that give rise to their spatial arrangement across space, as already discussed.

Imaging studies provide initial insights into the scales important in the spatial organization of the microbiota. Microbial colonization of the nasal turbinates may be sparse and patchy with single bacterial cells seeding the surface of epithelia at seemingly random locations (Swidsinski et al., 2007). On the other hand, inflamed adenoids are punctuated by focal patches and highly confluent polymicrobial biofilms that sometimes disrupt epithelial surface integrity. A similar survey revealed that bacterial biofilms collected from a single tooth surface in the absence of disease range up to hundreds of microns in radius (Mark Welch et al., 2016). Patch sizes on epithelial surfaces are known to be limited compared to those on non-shedding surfaces of teeth. Similarly, desquamation rates, which differ between tissues, may restrict patch size even on epithelial surfaces. Taken together, these observations suggest that additional in vivo or ex vivo work (e.g., on whole teeth rather than tooth swabs) is needed to characterize the size(s) of individual biofilms in each of the major body site habitats.

In order to refine our scale of observation, it may be useful to collect samples along annotated georeferenced transects,

obtaining sample site coordinates and topographical data with imaging as is done in macroecology rather than simply describing anatomic regions. In the nasal cavity, for instance, geographic variation in nasal flow velocity is thought to create focal hotspots of high sheer stress at specific points throughout the mucosa (Doorly et al., 2008). A hypothesis that might be tested is that these hotspots influence microbial colonization of epithelia in the nasal cavity. If communities vary according to nasal flow velocity or other environmental factors, geographic location may be as important as the type of epithelial surface on which a community is found.

Another way to identify the appropriate scale is to determine the amount of "detail that can be ignored without producing results that contradict specific sets of observations" (Levin, 1992). In this light, interpersonal variation can sometimes be viewed as a confounding variable, as it is too expansive a spatial scale across which to make inferences about the spatial organization of nasal or oral microbial communities. The extent to which interpersonal variation overshadows inter-site variation has sparked debate about whether communities inhabiting the anterior nares (ANs) and the nasal mucosa differ (Yan et al., 2013; Ramakrishnan et al., 2017; Kaspar et al., 2016; Wos-Oxley et al., 2016). The degree to which interpersonal variation obscures finer-scale spatial or temporal patterns is evident in studies that present separate ordination plots for each subject (Hauser et al., 2016; Kaspar et al., 2016; Ramakrishnan et al., 2017) or that present a different ordination for each site, coloring samples by subjects (Sato et al., 2015; Wos-Oxley et al., 2016). In recognizing that interpersonal variation is a confounding variable in these analyses, rather than a comparator, it becomes possible to manage its effects using numerical ecology. As an example, separate ordinations could be performed, as above, but rather than presenting individual ordinations for each subject, a multiple-tables analysis could be performed to examine the consistency of spatial patterns in community structure across subjects. Another approach where geographic coordinates have been obtained, through imaging or modeling, would be to perform a trend surface analysis, which would enable identification of large scale spatial structures.

## Landscape Ecology of the Human Nasal Cavity

The spatial heterogeneity of the nasal cavity likely shapes the distribution of organisms across its surfaces. Microbes inhabiting the anterior nares confront a wide variety of host features that are not present elsewhere in the nasal canal, including stiff, coarse hairs known as vibrissae that litter a keratinized stratified squamous epithelium, specialized tissue with known microbial associations (Figure 2). At ambient temperature, this region tends to be cooler than other nasal sites and sites throughout it are subject to upwelling from sweat and sebaceous glands, which percolate through the region. These micro-environmental differences may give rise to spatial patterns in community structure. For example, sebum secreted from the sebaceous glands appears to be an important determinant of the preferential colonization of *Propionibacterium* spp. (Mukherjee et al., 2016).

The nasal mucosa, which lies just 2 cm beyond the anterior nares, differs from the nares in several ways that may considerably influence microbial community structure. Yan et al. (2013) found that communities at three nasal sites, anterior naris, middle meatus, and sphenoethmoidal recess (Figure 2) differed according to epithelium type. A variety of anatomic and physiological features may explain this finding. While microbes in the nares are subject to ambient temperature, temperatures in the turbinates increase by ~4.5°C, reaching ~33°C by the time air reaches the nasopharynx (NP; Keck et al., 2000). This temperature gradient, which induces the formation of a moisture gradient, may differentially influence gene expression in both pathogens and commensals at different locations along the nasal passages. In addition, some bacteria colonize the crypts of the pseudostratified columnar ciliated epithelium of the mucosa (Swidsinski et al., 2007), a niche that is inaccessible to inhabitants of the nares. Likewise, drainage from sinuses occurs at specific locations throughout the mucosa, e.g., maxillary sinus into the middle meatus and ethmoidal sinuses into the sphenoethmoidal recess, which presumably creates local patches reflective of the sinus source pool. These features are also lacking in the nares.

Overlaying the cilia is a shifting blanket of mucus, which is comprised of two distinct layers, an aqueous fluid with which cilia interact (Figure 2) and an overlaying mucus layer comprised of more than 100 proteins, including mucins which agglomerate in "rafts," antimicrobial proteins like lactoferrin, lysozyme, and peroxidase, as well as secretory IgA (sIgA), albumin, fibrinogen, and lipid-binding proteins (Casado et al., 2005). Myriad mechanisms underlying interactions between microbes and mucins have been identified—the ability or inability of an organism to interact with mucus likely influences whether it persists on a mucosal surface (Zanin et al., 2016). The blanket of mucus is transported through the nasal cavity before being drained into the nasopharynx (Figure 2), where microbes may gain access to the soft and hard palates of the oral cavity.

During the first year of life, the density of the nasal microbiota increases with age while alpha diversity decreases (Mika et al., 2015). Nasopharyngeal communities of the infant tend to be dominated by taxa associated with the skin, including *Staphylococcus* and *Corynebacterium* spp. (Teo et al., 2015). These taxa are later succeeded in the nasopharynx by *Moraxella* or *Alloiococcus* spp., which when dominant in a community tend to be fairly stable (Biesbroek et al., 2014). When either *Haemophilus* or *Streptococcus* spp. colonize the nasal cavity and ascend to dominance in a community, they tend to be displaced quickly, at least in infancy. Early patterns of community succession in the nasal cavity, in turn, determine the likelihood a given patch type will transition to another since different invading colonists seem to have different probabilities of displacing dominant strains. For example, in adults, the ability of *Staphylococcus aureus* to invade patch types dominated by *Streptococcus pneumoniae* is limited (Bogaert et al., 2004; Chien et al., 2013; Cremers et al., 2014), though other colonists can and do displace *S. pneumoniae* as the dominant member.

The mechanisms governing CST transitions in nasal communities are now being defined. One mechanism is the elaboration of proteins or small molecules by one organism to antagonize or inhibit the growth of another (Abreu et al., 2012; Zipperer et al., 2016). Similar antagonism can be achieved as a side effect of metabolism as demonstrated by an elegant study showing that the commensal *Corynebacterium accolens* impairs *S. pneumoniae* colonization by metabolizing host lipids to oleic acid, a potentially mutualistic action that may limit the density

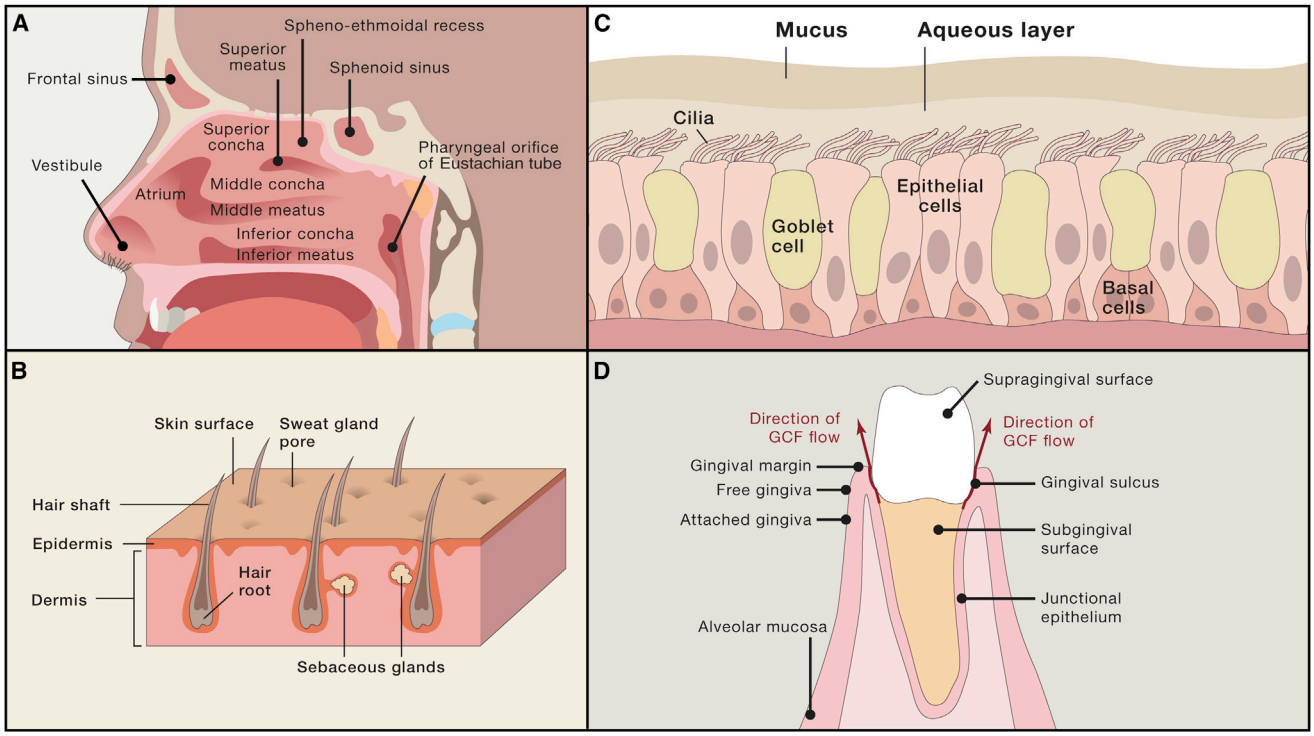


**Figure 2. Spatial Organization of the Nasal Cavity and Some Microenvironments of the Nose and Mouth**

(A) Organization of the nasal cavity. Schematic illustrating the spatial arrangement of the nasal vestibule, the nasal conchae, nasal meatuses, and the frontal and sphenoid sinuses.

(B) Surface and subsurface structures in the nasal vestibule. Host features in the nasal vestibule that may create distinct microbial habitats include the sweat glands, sebaceous glands, and nasal hair, all of which are absent in the nasal mucosa.

(C) Topography of the nasal mucosa. Microbes interact with host features in the nasal mucosa that are absent in the vestibule, including cilia that move a blanket of mucus across the pseudostratified columnar epithelium.

(D) Tooth microenvironments. Microbes may colonize the supragingival or subgingival surfaces of teeth or the mucosa surrounding each tooth. After teeth erupt, gingival crevicular fluid (GCF) begins to leak into the oral cavity and may influence community composition above the gums. Also note the location of the junctional epithelium, a potential site of baseline immune activity in the gums.

of this "pathobiont" on our epithelial surfaces (Bomar et al., 2016). Members of the commensal microbiota may also induce a CST transition by manipulating the immune system. For example, in the presence of *S. pneumoniae*, *Haemophilus influenzae* appears to upregulate the expression of chemokines that lead to the complement-mediated phagocytic removal of *S. pneumoniae* from the neighborhood (Lysenko et al., 2005).

## Landscape Ecology of the Human Oral Cavity

The human oral cavity also provides a unique opportunity to study the spatial ecology of microbial communities due to its accessibility and wealth of unique microbial habitats. The clearest topographical feature that distinguishes microbial communities is whether the superficial tissue layer of a given site is shedding (oral mucosa) or non-shedding (dental enamel) (Human Microbiome Project Consortium, 2012). In this section, we discuss the features of the oral landscape, which may give rise to spatial patterning in oral microbial communities.

Keratinization of the oral mucosa creates spatial heterogeneity. The stratified squamous epithelia of the oral mucosa can be subdivided into several functional types—the masticatory, lining, and specialized mucosa—each distinguished by functional histologic features. The superficial layer of the masticatory mucosa that lines the hard palate, the dorsal tongue surface, and keratinized gingiva proximal to supragingival tooth surfaces consists of a cornified envelope of orthokeratinized (i.e., the superficial cell layer lacks nuclei) or parakeratinized (i.e., the superficial cell layer is pyknotic) cells. By contrast, the lining mucosa of flexible tissues—like the soft palate, ventral tongue surface, floor of the mouth, and labial mucosa—lacks keratinization. An example of the specialized mucosa is found in the region of the papillae on the dorsal surface of the tongue, which give it a bumpy appearance.

Importantly, spatial heterogeneity in keratinization and the spatial arrangement of papillae at sites across the dorsal tongue have been shown by microscopy to be associated with spatial patterning in microbial colonization of that surface (Aufdemorte and Cameron, 1981). More recent work has demonstrated that community structure differs between the dorsal tongue and the lateral or ventral tongue surfaces (Aas et al., 2005; Mager et al., 2003). These patterns likely arise as a consequence of the surface topography of sites, which determines the intimacy with which different sites come into contact with each other.

In addition to these differences in topography, surfaces vary with respect to their proximity to the nearest salivary gland, a major source of environmental disturbance in the mouth. The minor

salivary glands form a dense and expansive network that punctuates the labial, palatal, and buccal mucosa, releasing viscous, highly proteinaceous secretions with poor buffering capacity (Dawes and Wood, 1973). These secretions bathe the surfaces from which they emanate, as well as opposing surfaces, creating heterogeneity that likely explains, in concert with other factors, the observation that communities found on cheek-facing aspects of individual teeth differ from those on tongue-facing aspects (Sato et al., 2015; Simón-Soro et al., 2013).

The three major salivary glands differ in their secretory rates and salivary composition (Schneyer and Levin, 1955), giving rise to gradients in salivary film velocity, oral clearance, and intra-plaque pH across the teeth (Dawes et al., 1989; Kleinberg and Jenkins, 1964; Wolff and Kleinberg, 1998). Moreover, the salivary glands also give rise to spatial variation in patterns of wetness and dryness across different geographic regions of the mucosa, suggesting that microbial communities inhabiting soft tissues may vary along a moisture or pH gradient (Wolff and Kleinberg, 1998), although, to our knowledge, this has not been tested.

Despite the existence of multiple known compartments in the mouth, most surveys of oral communities provide limited insight into the spatial patterning of supragingival communities across expansive spatial scales. This is because most extant studies, including our own (Bik et al., 2010), have reported findings of biofilms pooled from multiple tooth surfaces, used saliva as a sample of supragingival surfaces, or used rinsing samples instead. The grain of resolution afforded by such techniques does not permit interrogation of the fine-scale spatial variation of communities across sites. Of the studies that have analyzed independent samples of each tooth surface, most treat the unit of spatial variation—the physical location of a tooth in the mouth—as a categorical variable, such as tooth number, tooth class, or tooth aspect (Haffajee et al., 2009; Mager et al., 2003). We have recently shown in a pilot experiment that microbial communities inhabiting the exposed tooth surfaces of healthy humans vary not only based on tooth aspect and tooth class, but as a function of the physical distance separating sites in a manner that is consistent with a spatial gradient (Callahan et al., 2016a).

The oral cavity is a dynamic ecosystem that varies over time in ways that influence spatial patterns of microbial community assembly. The eruption of our dentition can be compared to the uplift of mountains, as both processes describe the dynamics of landform development and the emergence of new habitat into an existing ecosystem. Infants enter the world toothless and remain that way for ~6 months when teeth begin erupting. The deciduous teeth erupt over the first 2 years of life and are gradually shed and replaced by the permanent dentition between the ages of 6 and 12. Importantly, different tooth classes (e.g., molars, incisors) erupt in a stereotypic sequence at different developmental ages, making some of our teeth older than others within an individual, yet comparable in morphology and tooth age between individuals. Once teeth break through the gumline (Figure 2), gingival crevicular fluid, complement, phagocytes, and other components from the bloodstream begin, in minute measure, leaking into the mouth, providing novel growth substrates for some organisms at the same time as adding additional mechanisms of immune control. In infants, community assembly in the oral cavity reflects this extended process of geomorphogenesis; within a day of birth, *Streptococcus salivarius* and *Streptococcus mitis* colonize the oral mucosa, while *Streptococcus sanguinis*, which preferentially colonizes dental enamel, is not seen until after the teeth erupt (Carlsson et al., 1975).

## Dispersal across Anatomic Sites

Different gross anatomic sites are connected to each other. Some of these sites may serve as sources of colonists for other sites, which serve as "sinks." The nasal and oral cavities, for example, both drain to the pharynx, which ultimately connects through the trachea to the lungs or through the esophagus to the stomach, which is connected to the gut (Figure 3). In this section, we highlight several studies of dispersal between body sites and identify the obstacles researchers face in characterizing these dynamics.

Researchers have examined whether the middle ear and/or the adenoids serve as a reservoir for the bacterial agents of otitis media with effusion (OME) not only because the nasal canal is connected to the middle ear via the Eustachian tube (ET) (Figure 2), but also because the nasopharynx is often colonized by organisms implicated in OME, including *S. pneumoniae*, *H. influenzae*, *Moraxella catarrhalis*, and *Alloiococcus otitidis*. In one study, community composition of the middle ear closely resembled that of the external auditory canal (EAC) both in abundance and in community similarity, leading the authors to consider the EAC to be a likely source for OME (Chan et al., 2017). A competing theory with supporting evidence from microscopy is that the middle ear is seeded by the adenoids (Torretta et al., 2013). Patch types comprised of *S. aureus*, *M. catarrhalis*, and *S. pneumoniae* were found on the adenoid adjacent to the ostia of the ET more frequently than in the region of the nasopharyngeal dome. Interestingly, for unknown reasons, microbial clusters near the ET were more often polymicrobial than were the clusters on the nasopharyngeal dome. And interestingly, isolates derived from the ET region were more likely to form biofilms in vitro than were the isolates from the region of the NP. Taken together, these data led the authors to conclude that the adenoids are a more likely source for OME. By contrast, Chan et al. (2016) tested the adenoid theory using 16S rRNA data, concluding that the adenoids were an unlikely source of colonists to the middle ear since middle ear effusions (MEF) were dissimilar in community structure to adenoids. Dissimilarity appeared to be driven by the differential abundance of *Alloiococcus* found in high and low abundance in the MEF and adenoids, respectively. Implicit in this conclusion is the hypothesis that the size of different *Alloiococcus* populations in this ecosystem drives dispersal dynamics between these sites.

The sinuses experience flooding during respiratory colds as well as during physiological reflexes like coughing or sneezing. As a result of transient spikes in intranasal pressure, nose blowing pushes as much as 1 mL of nasal mucus into the ostiomeatal complex as well as the ethmoid and sphenoid sinuses (Gwaltney et al., 2000). The periodic flooding of the sinuses with mucins, a nutrient source, as well as microbes trapped in the mucus, suggests this habitat is functionally similar to a floodplain. The nasal mucus may transport colonists to the paranasal sites, and the nutrient influx may cause blooms in the sinus microbiota, a community found even in healthy humans (Abreu et al., 2012; Aurora et al., 2013). To determine the source pool

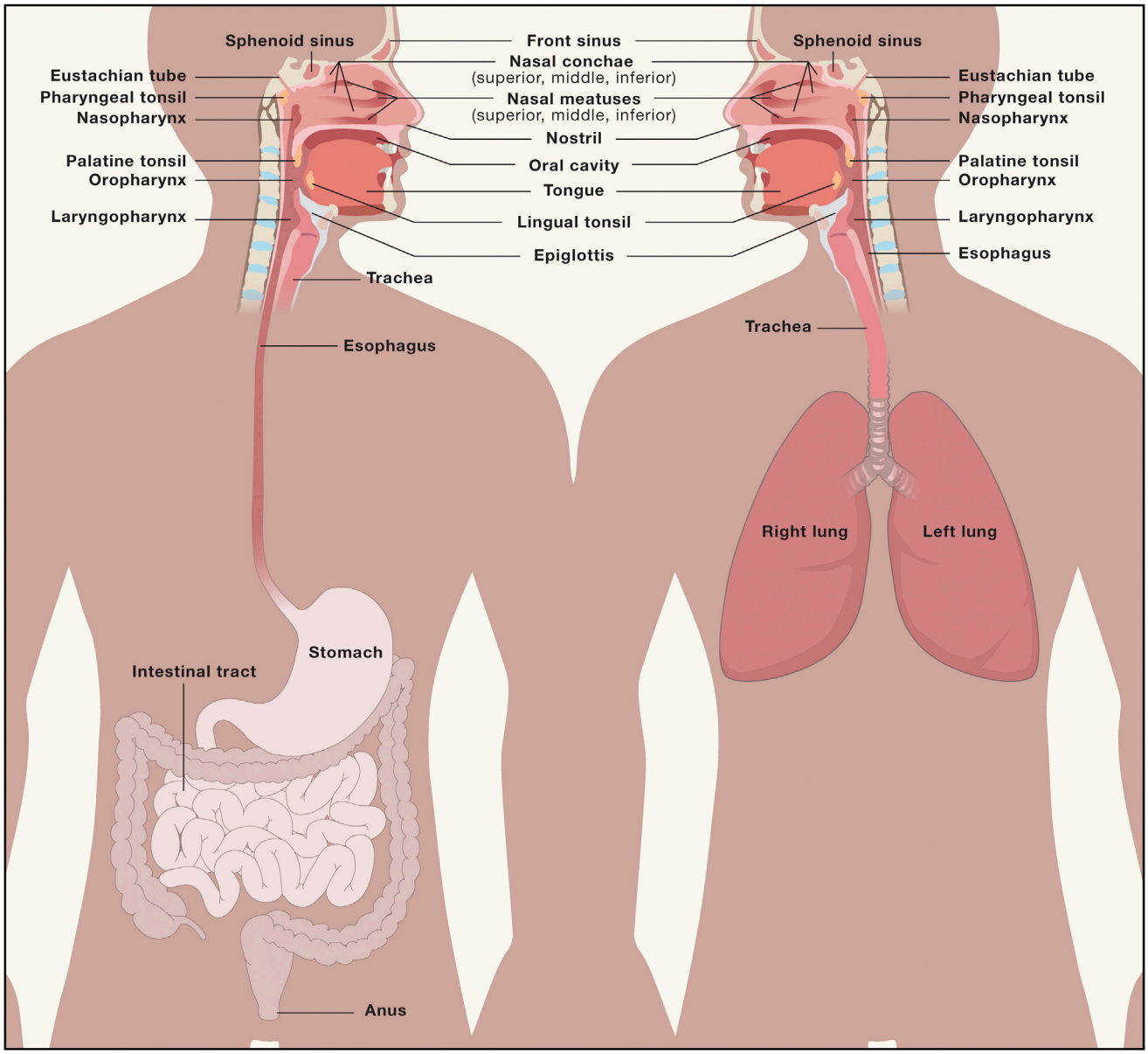


**Figure 3. Understanding Microbial Dispersal across the Human Body**
A schematic illustrating some of the distal body sites to which microbes may disperse from the nose, mouth, or throat, as well as some interconnections among sites, which are discussed in the main text. For example, the pharyngeal tonsils, also known as the adenoids, may serve as a reservoir for middle ear infections as a result of dispersal via the Eustachian tube.

for the sinus microbiota, one group assessed the similarity of communities in the anterior nares, nasopharynx, and ethmoid sinus (ES) before and at 2 and 6 weeks after sinus surgery (Hauser et al., 2016). Communities of the ES 6 weeks after surgery were most similar to those of the AN and ES pre-surgery, leading the authors to conclude that the AN may serve as the source of post-disturbance colonists. Communities of the NP, on the other hand, were ecologically dissimilar to those of the ES, leading the authors to conclude that the NP is an unlikely source of sinus colonists. Of interest, communities inhabiting the NP were as dissimilar to the ES as the ES was to itself before and 2 weeks after the disturbance, raising the question as to whether the observed level of dissimilarity that excluded the NP must also exclude the ES as a likely source of its own repopulation.

In attempting to understand the relationship between the bacteria found in the upper and lower respiratory tracts, researchers discovered a biomass gradient that distinguishes between the two anatomical sites (Charlson et al., 2011). The adapted island model was subsequently proposed to explain decreasing community richness at lung sites as a function of increasing distance to the supraglottis, the proposed source (Dickson et al., 2015). Later work led the authors to conclude that the oral cavity may seed both the lungs and the stomach since these sites are more similar to each other

than they are to nasal communities (Bassis et al., 2015). Prior work from our group has also shown a large overlap in the composition of the microbiota of the stomach, mouth, and esophagus (Bik et al., 2006). An operational taxonomic unit (OTU) level analysis of Human Microbiome Project (HMP) 16S rRNA amplicon data revealed high levels of similarity between communities of the distal colon and oral cavity, but not of the colon and nasal or skin communities (Ding and Schloss, 2014), leading these workers to postulate that the oral cavity may seed the gastrointestinal tract, an attractive but as yet unproven proposition.

The most convincing examples of bacterial dispersal across the human landscape come from culture-based studies demonstrating that the *S. aureus* strains found in the anterior nares of an individual are the strains found in *S. aureus* bacteremia of the same individual (von Eiff et al., 2001). Yet, since *S. aureus* inhabits multiple regions in the nasal cavity, including the anterior nares, the middle meatus, and other turbinates (Yan et al., 2013), it is hard to say that the nares serves as the source for *S. aureus* bacteremia, as has often been claimed, and the processes giving rise to dispersal from the true source(s) remain insufficiently characterized. Similarly, carriage of *S. aureus* in the anterior nares predisposes individuals to soft tissue and skin infections, such as cutaneous abscesses, at sites far removed from the nose—what gives rise to this phenomenon and whether the anterior nares per se is the source remain unclear (Johnson et al., 2015).

Valuable insights into the obstacles researchers face when examining dispersal across anatomic sites have come into focus from these early studies. First, community-based similarities (or dissimilarities) and population abundances do not provide direct evidence that a site is (or is not) the source of colonists for another site. Analyzing exact sequences and finding the same ribosomal sequence variations (Callahan et al., 2016b) at each site would provide more compelling evidence but may not yet meet the gold standard of culture-based tests and full-genome sequencing due to amplicon bias, potential sequencing errors, and the limited resolution of the highly conserved 16S rRNA gene.

Future work using strain-resolved metagenomics (Donati et al., 2016) may improve our understanding of how often and how far microbes travel across the human body. Likewise, culture-based and imaging work should supplement 16S rRNA-based surveys to achieve the same effect. Second, our understanding of dispersal across the human body is limited by our incomplete characterization of the spatial patterns and scales important in the ecology of these sites. This is an important point since dispersal-colonization dynamics are influenced by the orientation of patch types relative to each other and to the environment, the distance between patches, the number and quality of patches, and the dispersal capability of the organism(s) in question (Gadgil, 1971).

## The Immune System as a Source of Landscape Transformation

Interactions between commensals and the immune system appear to be important in shaping the topography of the human landscape. The magnitude of the immune response appears to vary depending on whether a surface is colonized by a commensal or a pathogen. In mice, nasal colonization by the commensal *Lactobacillus murinus* induced Th1 immune responses of the nasal cavity to a measurable but lesser degree than *Streptococcus pyogenes* colonization (Costalonga et al., 2009). Furthermore, germ-free mice have reduced epithelial and mucosal thickness, more collagen, fewer goblet cells, and smaller nasal-associated lymphoid tissue compared to pathogen-free mice, indicating that commensals shape the landscape (Jain et al., 2016).

A baseline level of cell-mediated immune function dramatically shapes the landscape of the oral cavity. Diminished alveolar bone in the oral cavities of germ-free mice and rats was thought to be paradoxical (Baer and Fitzgerald, 1966) given that bone loss was presumed to follow chronic inflammation triggered by an aberrant subgingival community, as in generalized periodontitis. In an effort to address this paradox, other work identified hallmarks of inflammation—mast cells and basophils—in the junctional epithelia (Figure 2) of germ-free animals (Wolf et al., 1973), and recent molecular studies showed that the junctional epithelia constitutively express the pro-inflammatory cytokines IL-1$\beta$ and TNF-$\alpha$ at comparable levels in germ-free and conventional animals (Tsukamoto et al., 2012). Collectively, these studies imply that the gingiva is subject to low-grade inflammation in the absence of microbial exposure. Moreover, mice reared under conventional conditions were found to upregulate the chemokines KC/CXCL1 and MIP-2 as compared to germ-free animals (Tsukamoto et al., 2012), suggesting that the commensal microbiota fine-tunes landscape form and function via the immune system.

## Pathogens as Disturbance: Mechanisms of Interplay between Host and Microbe

Acute infections may be viewed as a ''pulse disturbance'' when a pathogen directly or indirectly modifies community composition or structure. Viral infections, for example, have been shown to modulate the structure of the nasopharyngeal microbiota. In healthy children, a single upper respiratory infection (URI) can reduce the phylogenetic diversity of nasopharyngeal communities (Santee et al., 2016). Moreover, children who experience a large number of URIs tend to have lower nasopharyngeal community richness and diversity than children who experience fewer URIs, suggesting a high frequency of disturbance can have persistent effects. Different patterns can be expected to arise as a consequence of different infections; human rhinovirus (HRV) depresses community richness less than does respiratory syncytial virus (RSV) (Rosas-Salazar et al., 2016).

Interestingly, the commensal microbiota affects the likelihood that either a URI or a lower respiratory infection (LRI) will occur. Children with NP communities dominated by Moraxella, Haemophilus, or Streptococcus were more likely to experience LRIs when infected with either HRV or RSV as compared to those with other community types (Teo et al., 2015). Moraxella, Haemophilus, and Streptococcus, moreover, were independent predictors of acute respiratory symptoms, including fever, suggesting that the invading viral pathogen is not the only organism that modulates immune function and inflammation during acute respiratory infections. *S. pneumoniae* appears to take advantage of inflammation in viral-induced asymptomatic URI (Glennie et al., 2016). In the presence of virus, *S. pneumoniae* increased

mucosal factor H (FH), but not SLP1 or β-defensin-2 or lactoferrin; high levels of FH, in turn, induced inflammation allowing *S. pneumoniae* population size to bloom to a much higher density as compared to individuals with low FH levels. Mechanistically, FH appeared to facilitate the adherence and subsequent internalization of *S. pneumoniae* in nasopharyngeal epithelial cells, where population growth is not restricted by complement-mediated opsonophagocytosis. *S. pneumoniae* often colonizes the upper respiratory tract in healthy individuals, though this organism does, of course, cause disease, a transition that may be strongly influenced by acute viral infection. Collectively, these data suggest that acute viral infections modulate microbial community structure and function.

Chronic inflammation may be viewed as a "press disturbance" if it causes long-standing and persistent changes in the composition or structure of microbial communities. One such example is chronic rhinosinusitis (CRS), characterized by prolonged inflammation of the sinuses and a shift from a Th1 to a Th2 response (Aurora et al., 2013). Microbial communities in the paranasal sinuses of CRS patients are less rich and less diverse than commensal communities in healthy individuals (Wagner Mackenzie et al., 2017). Linear discriminant analysis identified the genus *Corynebacterium* as a potential biomarker that was over-represented in CRS. Abreu et al. (2012) have beautifully shown that mice challenged with *Corynebacterium tuberculostearicum* after antibiotic-mediated depletion of the commensal community developed goblet cell hyperplasia and mucin hypersecretion, two hallmarks of CRS. In that work, the commensal community protected against this immunopathology since depletion of the microbiota was required to see the emergence of sinonasal pathology. In stark contrast, another group found that nasal lavage samples of the microbiota collected from patients with CRS, but not healthy controls, stimulated the induction of Il-5 in peripheral leukocytes isolated from healthy controls as well as from the same host (Aurora et al., 2013). This work suggests that chronic inflammatory conditions, such as CRS, represent an altered ecological landscape, one that is both enforced by aberrant immune cells and responses and reinforced by a dysfunctional microbiota.

In the oral cavity, the prolonged absence of salivary flow induces a press disturbance. The movement of saliva through the mouth underlies the variable exchange rates between whole saliva and plaques on different dental surfaces as well as oral clearance from larger compartments (Dawes, 1989). Not only do such heterogeneities make certain dental surfaces more or less susceptible to demineralization, but they also provide a primary basis (e.g., pH) for structuring the biogeography of the oral microbiota. In healthy individuals, dental caries usually takes years to develop, but caries can manifest on the timescale of months in individuals with chronic low salivary flow (i.e., hyposalivation) (Sreebny and Valdini, 1988). Moreover, individuals with hyposalivation have more decayed, filled, and missing teeth compared to controls, even in patient populations that practice ultra-fastidious oral hygiene (Abraham et al., 1998), and, the pattern of caries attack in these individuals shifts from the biting surfaces of teeth in the posterior toward the smooth and root surfaces of teeth in the anterior compartment, sites that are infrequently attacked in otherwise healthy individuals (Dreizen et al., 1977).

A rich history surveying this phenomenon extends back to the 1950s. There is general consensus that hyposalivation selects for caries-associated bacteria, such as *Lactobacillus* spp., *Candida albicans*, and *Streptococcus mutans* (Almståhl et al., 2003), indicating that the loss of salivary flow represents a sustained ecological disturbance that alters ecosystem function. Given that the pattern of dental caries shifts in a site-specific manner in these individuals relative to healthy controls, a natural question is whether or not the loss of salivary flow exerts site-specific effects on oral microbial communities. All extant studies examining the impact of hyposalivation on the microbiota have relied on pooled plaque samples from multiple tooth surfaces or rinsing samples or sampling of just a handful of sites to survey supragingival community structure, thereby obscuring the extent to which shifts in community composition and structure occur, if any, at different biogeographic sites across the dentition.

## Perspectives and Future Directions

An important unanswered question in the field of microbiome research is what spatial (and temporal) scales are relevant to the bacteria that inhabit the human body. To define scales for the microbiota that are analogous to those defined for macroecology, researchers need to undertake sufficiently powered observational studies with the goal of ascertaining the average size of a single microbial population or community, the spatial extent of patch mosaics, and the scales along which gradients occur on the human body. Community function will be as important to measure as community structure. Once the spatial scales for a given habitat have been determined, it will be possible to describe the types, sizes, and extent of spatial patterns observed in the microbiota. Coupled with perturbation experiments, the underlying processes—stochastic, biotic, and disturbance—driving spatial patterns can be elucidated. The nose, mouth, and throat are particularly amenable to such lines of inquiry because these habitats are more easily accessible to sample collection as compared to other body sites.

The application of landscape ecology to the field of microbiome research also requires a shift from describing sample sites as categorical variables (e.g., anterior nares, middle meatus) toward thinking of them as georeferenced ones. Obtaining geographic coordinates, as well as a model of the topography of anatomic site, through imaging, would allow investigators to estimate critical ecological parameters, including dispersal distance, defined here as the geometric distance between two patch types. Moreover, having geographic coordinates would enable researchers to test variation in community features as a function of the physical distance separating sites or separating a site with respect to some environmental stressor. Another important and unknown parameter in the ecology of the microbiota is the frequency at which different patches (and patch types) go extinct, as well as the frequency with which they repopulate within and between different gross anatomic sites. Knowledge of the dispersal, colonization, and extinction parameters would enable modeling of community dynamics, for example, in the wake of antibiotic disturbance. With these and other studies of landscape ecology in the nose, mouth, and throat, a more comprehensive understanding will be acquired of the environmental parameters in health, setting the stage for more mechanistically informed and predictive interventions in disease.

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