Characterizing microbial communities through space and time

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Abstract

Until recently, the study of microbial diversity has mainly been limited to descriptive approaches, rather than predictive model-based analyses. The development of advanced analytical tools and decreasing cost of high-throughput multi-omics technologies has made the later approach more feasible. However, consensus is lacking as to which spatial and temporal scales best facilitate understanding of the role of microbial diversity in determining both public and environmental health. Here, we review the potential for combining these new technologies with both traditional and nascent spatio-temporal analysis methods. The fusion of proper spatio-temporal sampling, combined with modern multi-omics and computational tools, will provide insight into the tracking, development and manipulation of microbial communities.

Introduction

Microorganisms are ultimately responsible for the health of all other organisms. Communities of microbes decompose materials, recycle nutrients, break down pollutants, and aid in the digestion of food in "higher" animals -- they are the "ubiquitous janitors of the Earth" (1). Microbes can also cause disease, destroy our food sources, and degrade our structures (as reviewed in (1)). Early studies characterized a limited snapshot of microbial diversity. However, to predict or manipulate microbially mediated processes, we must understand spatial and temporal patterns of diversity at multiple levels, Figure 1.

Because of the astounding diversity of microbial communities(2), the ability to characterize their fine-scale temporal and spatial variation has only become achievable within the past 5 years. Next-generation "-omics" technologies such as high-throughput amplicon,

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metagenome, and metatranscriptome sequencing (3, 4) allow collection of thousands to millions of sequences at prices the average researcher can now afford. Recent research using next-generation technologies has focused on temporal and spatial associations(5-8) of microbial communities, concentrating on clustering (e.g. microbial communities from different body habitats cluster separately(9)) or correlations (e.g. soil bacterial diversity is correlated with pH(10)) However, unifying theories linking overall spatial and temporal variation in microbial community structure to system functional processes have remained elusive. Under similar environmental conditions, microbial communities can have strikingly different composition and function(11, 12); conversely, different community configurations can function similarly(13, 14).

Here, we discuss how community statistics from ecology can increase understanding of microbial community structure-function relationships. This review covers recent advances in molecular, culture-independent studies, and highlights advances in statistical methods for detecting and explaining spatially and/or temporally explicit patterns in microbial community composition. We conclude that combined spatial and temporal studies that exploit inexpensive DNA sequencing, together with improved bioinformatics tools and large-scale automated sampling techniques, will aid in understanding and predicting both the temporal and spatial patterning of microbial communities, and the processes that underlie these patterns.

Foundations of Modern Microbial Community Ecology

Advances in sequencing technology and analytic tools to quantify differences between communities have enabled the recent boom in microbial diversity studies. Limitations in sequencing cost and coverage have largely been overcome with next generation sequencing technologies that directly read single-nucleotide additions to DNA strands (454 and Illumina)., Analytic obstacles due to issues of species identification were conceptually harder to overcome. For example, the traditional definition of "species-level" Operational Taxonomic Units (OTUs) at 97%, although useful for an overview of diversity, is problematic because it assumes that rates of evolution and inherent genetic variation are equivalent across species, and excludes phylogenetic information contained in sequence data(15-17). Phylogenetic community distance metrics such as UniFrac(18) reduce the impact of arbitrary OTU thresholds and often provide better community clustering than taxon-based methods, because even an imperfect phylogenetic tree gives a far better picture of relationships among microbes than does the "phylogeny" where all taxa are equally related implicitly assumed by taxon-based approaches(19).

Spatial studies of microbial diversity

Understanding how microbial communities vary at different spatial scales is important because diversity hotspots and deserts can be identified, correlations with environmental factors can be detected, and hypotheses about dispersal limitation or stochasticity of community assembly can be tested. These issues of spatial scaling can be critical in downstream applications; for example, it is essential to know the scale at which microbial diversity varies when designing a bioprospecting sampling campaign to maximize the diversity of organisms or genes surveyed(20-22).

Central to the investigation of microbial spatial patterns is one of the most-contested hypothesis in microbial ecology: "everything is everywhere, but the environment selects" (23); i.e. that local environmental conditions, or 'filters', select for community assemblages that can best exploit or survive. Although several important environmental filters have been well-studied (e.g. (18, 24-27), historical filters (i.e. traces of past events or community states) are also important in structuring communities (28, 29). Thus, a primary goal of

spatial analysis techniques should be to measure the separate contribution of history (e.g. community assembly and patterns of microbial dormancy) and environment as a function of the spatial configuration of the dataset. Ultimately, spatial pattern analysis improves our ability to predict microbial diversity hotspots, and where microbes and their associated functions are abundant in a given habitat.

Indeed, there are some strongly predictive patterns in spatial microbial diversity. For example, taxa-area analysis (regression of taxa observed vs. sampling area) has been adopted from island biogeography(30). As with macroorganisms, larger areas harbor more microbial lineages, and the taxa-area relationship has been referred to as one of the only 'laws' of ecology(31). In contrast, other spatial patterning of microbial alpha diversity deviates from patterns observed for macroorganisms. For example, the well-established pattern in macroorganisms that diversity decreases with latitude seems to apply to marine (32)but not soil microbes (10), and significant relationships between elevation and microbial diversity have not been observed (33).

As noted above, phylogenetic beta diversity has been widely applied: it has been shown to vary systematically at scales ranging from between continents(17, 27, 34) to within a single hand or computer keyboard(35). Whether communities of similar composition cluster together in space can be detected using variograms, which graphically represent the relationship between two distance matrices(7, 17); the statistical significance of these associations can explored with the Mantel test(36). These representations also allow researchers to fit models that explicitly determine the range of spatial autocorrelation (37) and produce input for spatially explicit prediction algorithms, such as kriging or spatial-partial regression(38), which explain the overall patterns of variation and faithfully interpolate community structure at sites not directly observed.

Although community-level measurements and associations provide useful tools for microbial biogeography, studies of individual taxa that are key players in an ecosystem are also critical: for example, knowing that cyanobacteria are early colonizers because they fix nitrogen and act as primary producers provides a level of insight that cannot be obtained purely from patterns of similarity and difference at the whole community level. Recently employed methods that refine overall spatial patterns in microbial communities include subdividing analyses by taxon (ranging from species to phylum-level), which can be used as inputs for niche modeling (7, 39), and using ordination techniques to identify indicator taxa (these include SAMOVA, DFA, and SIMPER), Table 1. These methods allow the user to identify an individual clade or consortium, and, when applied to metagenomic data, specific genes, that affect function (e.g. the ability of gut Bacteroidetes in Japanese people to degrade polysaccharides found in seaweed, presumably horizontally transferred from marine Bacteroidetes consumed along with sushi(40)). These newer methods share similar goals and often-statistical methodologies, but detailed comparisons on the same data have not yet been performed and there is not yet consensus about their strengths and weaknesses (41).

Temporal studies of microbial diversity

Like spatial studies, temporal studies can identify taxa shared at different times, correlations with environmental conditions that affect the communities, and the relative contribution of different processes, including stochastic processes and priority effects, to community structure (14, 42). However, unlike spatial studies, temporal studies have provided greater insight into processes. Recent studies in different environments have shown that some communities exhibit cyclical patterns (26), others exhibit a monotonic trajectory (e.g. the development of the gut microbial community from the newborn to the adult (43)), and others

remain relatively stable over time (e.g. the human mouth community (44), or the hypolimnion of stratified bog lakes(45)).

Temporal studies are now moving beyond observing temporal patterns using ordination or other visualizations of community associations. A growing toolbox of descriptive, non-parametric statistics for temporal microbial dynamics exists. These techniques include correlation networks and analysis of community rate of change over time, and can be used for exploring temporal patterns, then relating these patterns to specific biotic or abiotic drivers. For example, temporal associations between commonly occurring OTUs in aquatic microbial time series have be uncovered using correlation-based networks analyses, such as local similarity analysis (46-48). Specifically, different ecosystems sharing a regional or climactic driver were shown to exhibit similar changes in their microbial communities (e.g. (46, 49)). In addition, aquatic bacterial communities correlate far better with phytoplankton communities than with physical and chemical properties of the system (50). There are also non-parametric methods that specifically test for temporal structure such as cycles, trajectories, and serial objects (e.g. (36, 51)). Finally, measuring rates of change in the whole microbial community or in specific taxa over time allows comparisons of these rates across ecosystems or experimental treatments(52).

An extensive legacy of aquatic timeseries observations exists due to routine limnological and marine sampling efforts, and timeseries are now becoming available in other systems such as wastewater treatment (e.g. (53, 54)), host-associated systems(44), and air (e.g. (55)). The accumulation of more of these rich time series datasets for microbial communities across different ecosystems will likely provide essential the first baseline for the expected nature and scale of variability in microorganisms. An added benefit will be improved detection of responses to disturbance events in microbial communities outside of the expected variation, such as those observed after episodic typhoon events in a sub-tropical lake (56)or in the human gut after antibiotic treatment(57). Such studies of response to perturbation are critical for developing an understanding of factors that lead to resilience in different communities, and for predicting microbial responses to a changing planet.

Combining spatial and temporal studies of microbial communities towards predictive models

Combining spatial or temporal series can reveal key features of a system. For example Caporaso et al. (44) combined both spatial and temporal components in a microbial study to assess the variation of microbial communities of the human microbiome. The addition of extensive temporal sampling to a previous study(9) led to a novel perspective: although each spatially explicit location on the human body retains a compositional difference from other locations, the communities within each location shift over time. That is, each location retains only a small 'temporal core' of species-level phylotypes within a community over time.

Analysis tools developed for spatial studies can sometimes be applied to temporal studies, or vice versa. For example, in(58), variograms were used to identify the temporal scales at which *E. coli* concentrations increased within a watershed. Similarly, wavelet analysis, a method to find the dominant periodic phenomena in a time series by decomposing those signals on a local timescale, was used in an *in vitro* gut microbial community analysis which revealed the strongest population cycles in Bacteroidetes and Firmicutes(59). Creative applications of techniques developed for one type of study to others will likely yield additional benefits in future, as will merging the two types of studies. For example, samples taken along a spatial gradient that vary due to time, known as chronosequences, e.g. ecosystem succession (60), glacial recession(61), spoil heap development (62) inherently have both spatial and temporal components. However, spatial and temporal patterning are

conflated in such studies; techniques such as niche modeling could potentially assist in resolving these issues. Similarly, studies that track timeseries at multiple sites or in multiple subjects will be essential for understanding factors that affect community dynamics as well as structure. Describing different resolutions of microbial community spatial variability, from microns to continents, and temporal variability, from hours to decades, will inform prediction of dynamics and responses to novel events.

Conclusion

Our review has described issues concerning the description of microbial communities and their relevant impact on the systems in which they reside. We have also described the use of existing and nascent analytical tools. In the past, studies of spatial and temporal dynamics of microbial communities have been limited to descriptions, rather than predictive models (7, 22). The advent of high-throughput multi-omics tools and the decreasing cost of automated sampling equipment enables adoption by the scientific community. Eventually, model-based approaches will be a key goal of future studies of microbial communities, and will allow us to test and appropriately apply what we think we have learned.

Acknowledgments

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Highlights

Microbial community studies with spatial or temporal axes have become more common due to the democratization of sequencing technologies

Current analysis tools allow us to statistically test our hypothesis but give few insights into causal relationships or the development of predictive models

There are multiple scales of temporal and spatial dynamics in microbial communities that have yet to be described in many systems, but will inform prediction.

Future spatial or temporal analysis must use both spatial and temporal components with model-based algorithms to properly assess and predict microbial community divergence between health states
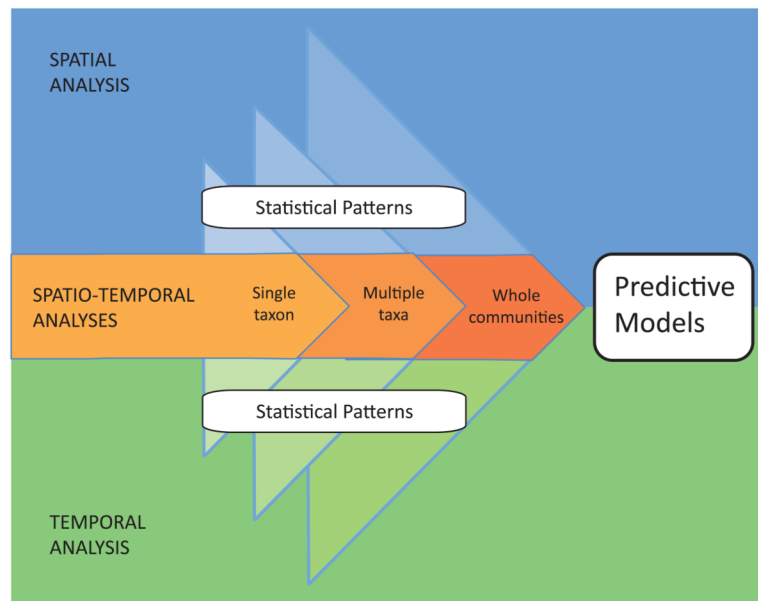
Figure 1. Improving our understanding of the variability of microbial communities
Spatial and temporal studies ranging in scale from that of individual taxa to communities have allowed us to detect patterns of distribution. However, in order to be able to fully understand the nature and ranges of variability in microbial systems, research focusing on improving our ability to *predict* whole communities across heterogenous space and time is much needed. Building upon existing studies and tools of analysis will help us move from simply describing statistical patterns to developing better predictive models. The accumulation of spatio-temporal studies for microbial communities across different ecosystems will provide essential information about the expected scales of variability, allowing for better biological and ecological interpretations of deviations from normal ranges of variability.

Table 1
Different types of spatial and temporal analysis, and their applications

This table highlights the deep connections between spatial and temporal studies, and the frequent applicability of techniques developed for one type of analysis to the other. Further spatial and temporal sampling in projects such as the Earth Microbiome Project will likely highlight additional deep connections.

Original use (developed for)	Analysis type	Analytical method / tool	Information provided when applied in spatial studies	Information provided when applied in temporal studies
Spatial studies	Correlative	Variograms	Indication of spatial autocorrelation	Identification of the appropriate time scale for sampling
Spatial studies	Correlative	Taxa-accumulation plots	An indication of how many more taxa are detected by sampling larger areas (i.e. taxa-area curves)	An indication of how many more taxa are detected by sampling additional time points
Spatial studies	Predictive	Kriging	Interpolation of data at locations missing direct observation (e.g. continuous chloropleth maps)	Interpolation of data at time points missing direct observation
Temporal studies	Correlative	Frequency transformations	NA (or not yet determined?)	Identification of cyclical or periodic fluctuations in community structure/composition over time
Both	Correlative	Ordination methods	Geographic structuring of taxa/ communities based on a metric of similarity	Temporal structuring of taxa/ communities based on a metric of similarity
Both	Correlative	Network analyses	Association or co-occurrence patterns of taxa and/or communities over space	Association or co-occurrence patterns of taxa and/or communities over time
Both	Predictive	Niche modeling	Prediction of expected taxon/ community distributions in space, indication of important environmental filters	Forecasting of taxon/community distributions with changing conditions over time, assessment of niche conservation