Microbial interactions and community assembly at microscales
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In most environments, microbial interactions take place within microscale cell aggregates. At the scale of these aggregates (~100 μm), interactions are likely to be the dominant driver of population structure and dynamics. In particular, organisms that exploit interspecific interactions to increase ecological performance often co-aggregate. Conversely, organisms that antagonize each other will tend to spatially segregate, creating distinct micro-communities and increased diversity at larger length scales. We argue that, in order to understand the role that biological interactions play in microbial community function, it is necessary to study microscale spatial organization with enough throughput to measure statistical associations between taxa and possible alternative community states. We conclude by proposing strategies to tackle this challenge.

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Introduction
One of the most fundamental properties of complex biological systems is their multi-scale structure. Multicellular organisms are formed by the hierarchical organization of tissues, fibers, proteins, and amino acid motifs, all the way down to DNA [1]. Likewise, ecosystems have a hierarchical arrangement: from meta-communities to communities, populations, individuals, pathways, and genes (Figure 1). This hierarchical structure is more than just a convenient way to organize a textbook. It is, in fact, essential to the perceived macroscopic properties of the whole. For instance, the mechanical properties of bone tissue cannot be explained only from the properties of the collagen fibers that compose it. Instead they depend on the mesoscopic organization, for example, the packing of fibrils and the density of cross-links [1]. Similarly, in ecology, there is significant evidence that local, microscopic interactions between and within populations affect properties such as resistance to perturbations, efficiency of resource utilization, rates and yields of biomass production, etc. [2–4]. All these are macroscopic properties that depend on how the microscopic building blocks (genes, genotypes, and cells) are assembled.

Yet, the classical mantra of microbial ecology (‘who is there and what are they doing’) suggests that functions of microbial ecosystems can be reduced to the functions of their building blocks — genotypes and their genes — without knowledge of how these building blocks interact. Over almost two decades, microbial ecology has experienced a revolution driven by -omic technologies, which has allowed researchers to enumerate the building blocks. Simultaneously, a number of efforts have been made to infer associations between these building blocks from -omics data [5–12]. However, these statistical associations are often inferred from coarse-grained samples, which are collected at the scales of ecosystems, and not at the scales of local communities or populations (Figure 1).

In many natural environments (Table 1), cells of diverse taxonomic origins aggregate in patches of high local cell density, either attached to surfaces or to each other in multicellular flocs. Within those local patches, which are often on the order of 100 μm (Table 1), cell–cell distances are short enough for diffusible metabolites to reach neighboring cells. At these scales, local ecological interactions, which can inhibit or promote growth of microbial populations, directly influence community structure and dynamics. But, these physical associations between microorganisms may be short-lived. Microscale communities frequently assemble and disassemble by migration, attachment, and detachment from surfaces and cells. Thus, ecological interaction networks are highly dynamic and depend on the interplay between behavior (chemotaxis, attachment, etc.) and cell–cell interactions.

How these discrete microscale communities assemble may dictate the structure-function mapping of microbial ecosystems. This is not only because ‘who sits next to whom’ can determine what type of metabolic conversions are realized and with what efficiency, but also because the combined effects of ecological interactions and spatial structure can influence community diversity and stability.
Multi-scale nature of microbial ecosystems. At the scale of meters or kilometers, microbial communities are driven by coarse-grained environmental parameters and may appear stable due to the averaging of multiple variable meso-environments and micro-environments. On the other end of the spectrum, at scales of 1–10 μm, we can measure the behavior and function of single cells. However, in between, there are multiple nested layers of ecological structure. At the scale of ~cm-m, depending on the rate of mixing in the system, we are likely to sample meta-communities, most likely in the form of ensembles of microscale aggregates connected by dispersal. Community properties at these scales are likely driven by differences in dispersal and small-scale abiotic gradients. In environments like the ocean, dental plaques, sediments, and others, the cell aggregates that comprise local communities are found at the scale of ~10–1000 μm, but often at the lower end of this range. It is at the scale of these cell aggregates (~100 μm) that biological interactions between organisms are most likely to have a measurable effect on population dynamics and composition. However, current -omic techniques disrupt this structure and can only provide us with raw repertoires of taxa and genes, while imaging techniques are limited in throughput.

Here we argue that studying microbial communities in high-replication at the local patch scale is necessary, both to reconstruct ecological interaction networks and to understand the functional impact of microbial interactions (Box 1).

**Local interactions and ecological function**

One implication of ‘who sits next to whom’ is that populations in close physical proximity may have complementary metabolic repertoires that improve their functional productivity. Why combinations of taxa can perform certain functions better than taxa in isolation is an exciting and relatively open question in microbial evolution. In the most well-studied cases, populations consume the metabolic waste products of others, often as electron donor or acceptors in anaerobic environments [13]. However, in many cases, these synergisms emerge through direct complementation of eroded genetic repertoires [14]. For instance, many different lineages across the microbial tree of life have lost the ability to synthesize their own amino acids and rely on those produced by a host or neighboring microbes [15,16].
Genomic studies suggest that metabolic complementation plays a crucial role in natural microbial communities. For example, Zelezniaik et al. [17**] showed computationally that there is a general trend for locally co-occurring populations to be enriched in metabolic complementarities, suggesting that interactions among micro-organisms are common and likely to emerge from pairing of incomplete or complementary metabolic pathways. In a separate experimental study in methanogenic communities, Embree et al. [18**] showed that amino acid auxotrophies create interdependencies between populations that control energy flux and contribute to community
Box 1 A case study: community ecology on particulate organic matter.

In oceans, lakes, and other aquatic environments, organic particles — ranging from decaying crustaceans to fish fecal pellets to polysaccharide gels — serve as nutrient-rich scaffolds for microbial communities (Table 1) [51]. Microbes from the surrounding water flock to these particles and assemble into dense multi-species consortia that consume and recycle particle resources before they sink out of zones of high productivity in the ocean. The assembly of local communities on particles is shaped by the interplay between cell behavior and ecological interactions. Traits such as swimming speed, chemotaxis, and surface attachment [52] control the order of arrival of organisms to a particle, as well as their residence time. At the same time, ecological interactions such as quorum sensing [53,54], chemical antagonism [55] and exploitation of public goods [56] inhibit or facilitate growth. By modulating the abundance of particle-degrading bacteria and their exposure to particle surfaces, local interactions on POM can thus control the rates of particle degradation and biomass production, and consequently, the rates of carbon remineralization in the water column. Similar processes are likely to control the assembly of microscale biofilms in many other environments, including those in the oral microbiome or in granular sludge in waste water treatment reactors [50].

Robustness. Altogether, interactions through metabolic complementarities are common in nature and can have a large impact on community function.

Interactions and (in)stability

Besides direct functional consequences like metabolic complementation, ecological interactions and spatial assembly can also affect the stability of the community (i.e., the ability to buffer environmental change) [19,20]. A robust result of population dynamics models is that strong mutual antagonism or interference competition — where two species compete with each other more than with themselves — makes coexistence between species globally unstable [21]. Therefore, in a well-mixed environment, one species wins over the other.

However, in the presence of spatial structure, the instabilities created by mutually antagonistic interactions manifest as spatial patterns and coexistence at large scales. For instance, mutually antagonistic colonizers of nutrient patches will exclude each other on any individual patch, depending on order of arrival, but will coexist globally. Thus, differences in early stages of colonization can drive each individual micro-patch to alternative states characterized by different patterns of species abundance (Figure 2). Consistent with this hypothesis, strains of *Bacillus* sampled from the same 4 cm² area chemically inhibit each other less frequently than bacteria from distant (30 m) locations [22*]. Thus, community assembly and interference competition may contribute to spatial segregation of antagonistic genotypes in soil.

Alternative states on local patches can increase global diversity and consequently, the functional stability of ecosystems [23–25]. Ecosystems with more redundant groups of species can function under a larger number of environmental conditions, an idea known as the spatial insurance hypothesis [23]. This is because redundancy ‘insures’ ecosystems against fluctuations in the abundance of any individual species, thereby allowing diversity to improve the global health of ecosystems. However, testing this hypothesis requires community structure information in high-replication at the single patch level, which is currently difficult to obtain.

Inverting the problem: from patterns to interactions.

To infer interaction networks from -omics data, various network reconstruction algorithms have been proposed. These include model-independent methods (e.g., species correlations across samples), and model-dependent methods (e.g., fitting data to a Lotka–Volterra model) [7,8,10,26–28]. Correlation-based methods assume that positive interactions increase the likelihood that interacting species mix or fluctuate in a correlated fashion, whereas negative interactions increase the likelihood that species segregate spatially or fluctuate in an anti-correlated fashion. Thus, these methods infer the sign and strength of ecological associations by exploiting the patterns of spatial segregation and mixing described earlier.

However, these inference methods are limited by the fact that interaction-derived spatial patterns typically have characteristic length scales that are much smaller than traditionally sampling scales. In particular, standard coarse-grained taxonomic surveys collect community data at ‘bucket’ scales, averaging over thousands of locally assembled communities. No matter how sophisticated the inference algorithm may be, the quality of the predictions can only be as good as the input data. For this reason, taxon-taxon interactions inferred from coarse-grained samples most likely capture similar responses to abiotic parameters (e.g., temperature or pH), rather than biotic interactions. This problem is likely pervasive over a wide range of ecosystems. However, solving the problem is not easy. It requires (i) sampling at the local patch scale with high replication and (ii) differentiating interaction-driven community structure patterns from those created by abiotic physiochemical factors.

Characterizing spatial structure in natural microbial communities in high throughput with micron-scale resolution

Several methods exist that allow us to visualize ‘who sits next to whom’ in complex natural communities. The most well known among these is fluorescence *in situ* hybridization (FISH), in which fluorescent probes bind to specific microbial sequences and are visualized via microscopy. Combining FISH-based techniques with many modes of microscopy, researchers have characterized the microscale spatial structure of microbial communities in a diverse range of ecosystems, including the oral...
Microbial interactions can create bistability, where one population outcompetes the other depending on initial conditions. This is shown in the upper panel, which depicts the phase space of a Lotka-Volterra (LK) model with interference competition. However, in stochastic cellular automata simulations where initial populations are randomly initialized at 50:50 ratios (lower panel) the same type of interactions lead to the emergence of large segregated patches dominated by one species or the other. (b) Mutualistic interactions lead to stable coexistence. Upper panel shows the result of a Lotka-Volterra model with mutualistic interactions. In stochastic cellular automata (lower panel) mutualisms manifest as strong mixing between species. (c) Extending these ideas to larger networks of potential interactions, antagonisms can create patterns of exclusion that segregate locally assembled communities across patches, provided that the scale of the patch is comparable to the length scale over which antagonistic effects manifest themselves. Thus, positive interactions like metabolic complementation should be more frequent within a patch than expected from null models without spatial structure.

Increasing replication with synthetic systems

Using these imaging techniques, a natural next step is to characterize the statistical properties of individual microscale communities in high-throughput, including robust patterns of taxon co-occurrence and divergence to alternative states. Such an increase in throughput may be technologically feasible, particularly with spectral FISH methods. However, to interpret results in light of underlying ecological interactions, we need to control for variability in patch composition and historical contingencies explicitly. In the ideal case, each patch would be identical in physiochemical composition and life history, but this type of controlled, highly replicated patch structure does not exist in most natural ecosystems. Therefore, synthetic or semi-synthetic laboratory systems, in which patch properties are tightly controlled, can complement studies of naturally occurring microbial communities.
Colonizing. Furthermore, the colonization process can be visualized in real-time by coupling microfluidic devices with microscopy [35–38]. For example, a recent study used a coral-on-a-chip system to visualize the dynamical process by which a coral polyp is infected by a known coral pathogen (*Vibrio corallilyticus*) with a level of spatiotemporal resolution that could not be achieved in a natural marine ecosystem [38]. Altogether, microfluidic devices are a powerful addition to the microbial ecology toolbox, but to date, have not been extended to microbial communities as diverse as those found in nature.

In our lab, we have developed a complementary approach, which allows us to study community assembly on defined nutrient patches, starting from a complex microbial milieu. We use nutrient-rich synthetic particles as spatially isolated, chemically defined scaffolds for microbial communities [39**]. We immerse these particles in diverse, naturally occurring microbial assemblages (coastal seawater, sediments, soil samples, etc.), and over several days, microbial communities self-assemble on these particle scaffolds (Figure 3). By controlling the patch size and composition, as well as the pool of potential colonizers, this approach allows us to analyze many individual communities as discrete entities, each of which is a self-organized replicate from the same pool of colonizers. This model system offers a new way to broach the question of ‘who tends to co-occur with whom’ at scales of 10–100 μm, a first step towards reconstructing interactions between taxa in a complex community. By comparing the microbial communities associated with many individual particles, we hope to identify robust statistical associations between taxa across many replicate communities, to probe the space of possible communities, and potentially identify alternative stable states.

**Conclusions**

Here, we have highlighted the interplay between microbial interactions and microscale spatial community assembly. Studying the two phenomena in conjunction will enable us to understand the potential for functional complementation, as well as to improve inferences of microbial interaction networks. Ultimately, however, the challenge will be to understand how local interactions influence ecosystem processes — for example, the rates of biomass production and substrate turnover. This is particularly challenging for non-trophic interactions such as public good exploitation. Addressing this problem will require a combination of biophysical modeling and controlled experiments in the lab.

The study of locally assembled communities as discrete entities with high replication has the potential to enable the discovery of alternative community states driven by ecological interactions and not by abiotic factors. Ultimately, we hope to assess the robustness of microscale community assembly, including if shifts in individual taxa can move communities towards alternative states. In the future, this knowledge could be used to control and even engineer microbial communities.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

● of special interest
●● of outstanding interest


A systematic survey of potential for metabolic exchange in over 800 communities shows that metabolic complementarities are common in natural communities and can provide an advantage in nutrient-poor conditions.


Demonstration that amino acid auxotrophies arise in methanogenic consortia, creating a layer of non-trophic interactions on top of the energy flow network.


Demonstrates that chemical antagonism is less common among Bacillus strains sampled from the same cubic centimeter of soil than among those sampled from distant locations.


Detailed analysis of the complex spatial structure in human dental plaque using spectral fluorescence in situ hybridization. This technique offers tremendous opportunities to systematically measure microbial spatial statistics in microbial communities.


36. Kim HJ, Li H, Collins JJ, Ingber DE: Contributions of microbiome and mechanical deformation to intestinal bacterial


Study with synthetic community scaffolds analogous to marine particles shows that particle colonization dynamics in the ocean follows primary succession controlled by public good production and motility.


