

PERSPECTIVE

What is microbial community ecology?

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The activities of complex communities of microbes affect biogeochemical transformations in natural, managed and engineered ecosystems. Meaningfully defining what constitutes a community of interacting microbial populations is not trivial, but is important for rigorous progress in the field. Important elements of research in microbial community ecology include the analysis of functional pathways for nutrient resource and energy flows, mechanistic understanding of interactions between microbial populations and their environment, and the emergent properties of the complex community. Some emergent properties mirror those analyzed by community ecologists who study plants and animals: biological diversity, functional redundancy and system stability. However, because microbes possess mechanisms for the horizontal transfer of genetic information, the metagenome may also be considered as a community property.

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Introduction

Analyses of 16S rRNA gene sequences from environmental DNA have shown the extraordinary richness of phylogenetic types found in many microbial habitats (Ley *et al.*, 2006; Walker and Pace, 2007). As microbial ecologists have learned more about ‘who’s there,’ it has heightened interest in understanding ‘what are they doing.’ The concerted activity of interacting microbes was critical to the development of environmental conditions on Earth that led to the evolution of multicellular organisms, and their catalysis of biogeochemical reactions has a central function in sustaining conditions that are compatible with a robust and diverse biosphere. At a time in which human beings are concerned with historically rapid global change, understanding the control mechanisms whereby microbial communities determine ecosystem function is particularly relevant. Current ecosystem simulation models do not include microbial composition, and often neither explicitly consider the effects of environmental conditions on microbial activities nor the interactions between diverse microbial processes (Bardgett *et al.*, 2008). As microbial communities are of primary importance in biogeochemical transformations, a deeper understanding of their dynamics will be critical to refined predictions regarding how the biosphere modulates and responds to future environmental conditions. At a more fundamental level, understanding natural microbial communities

will deepen our understanding of how ecosystems function. In turn, this understanding may elucidate novel interaction mechanisms among multiple species at the single cell level (O’Malley and Dupre, 2007).

There is great interest in applying modern technologies in genome-enabled biology and analytical chemistry to concurrently study both the identity and the function of complex microbial communities (DeLong *et al.*, 2006; Musat *et al.*, 2008). However, effective application requires a clear delineation of what is meant by a ‘microbial community’ and identification of important characteristics specific to community ecology.

What is a microbial community?

The concept of community ecology arose in plant and animal ecology. Communities are defined as multi-species assemblages, in which organisms live together in a contiguous environment and interact with each other. This discipline seeks to analyze how biological assemblages are structured, what are their functional interactions and how community structure changes in space and time. Clements (1916) viewed the community as a ‘supra-organism’, which had a well-defined level of organization with tight interactions among organisms that comprise a causal system and gives rise to emergent properties. The boundaries of the community may have less to do with physical dimensions than the range over which there are strong rather than weak interactions between populations (Levins and Lewontin, 1985). The alternative individualistic concept (Gleason, 1926) is that many species co-occur in a habitat because they tolerate similar physical and chemical

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conditions and do not necessarily interact with each other. The practical delineation of 'community' may then reflect the interests of the ecologist rather than any inherent characteristics.

The problems in rigorously defining community are heightened in the case of microbial ecology. In particular, delineating a 'contiguous environment' and the meaning of 'interact' may be problematic. Microorganisms react to and in turn influence conditions in their microenvironments, which usually have length scales of microns rather than millimeters (Young *et al.*, 2008), except in cases of multicellular structures such as fungal hyphae. However, the consumption of substrates and production of metabolic products in water-saturated sediments and density-stabilized aquatic water columns can generate chemical gradients over meters (Wakeham *et al.*, 2007). The consequence is that these functional groups metabolically 'interact' over many meters. As a result, the strength of interaction among organisms and the defined spatial scale may vary substantially for investigator-defined microbial communities.

To increase rigor in the meaning of 'microbial community,' it would be valuable for microbial ecologists to explicitly articulate their meaning for each specific research effort. Microbes strongly interacting with each other in a microenvironment comprise a *local community*. However, the distribution of organisms and physicochemical properties within most habitats is patchy; even in apparently well-mixed oligotrophic planktonic habitats, nutrient-rich foci of marine snow may occur (Azam and Malfatti, 2007). The patchwork of local communities has been termed as a *phenomenological community* (Sterelny, 2006); for the purposes of microbial ecology, this would represent a range of macroscale habitats delineated by the investigator, in which the assemblage of microbes persists in spatial association. The phenomenological community could be constrained to a smaller number of populations by defining an *indexical community*—the set of populations that directly interact with a key population or defined biogeochemical process, together with other local populations that affect the directly interacting populations (Sterelny, 2006).

Recent developments in community ecology have begun to recognize that the biological assemblage

cannot be defined without reference to its abiotic environment. An appreciation for the tight interrelationship between microbes and their microscale physical and chemical environments is particularly important for delineation of microbial communities (O'Donnell *et al.*, 2007). In this spirit, it may be instructive to define microbial communities not from a macroscale perspective (for example aquatic vs terrestrial habitats), but rather based on a bottom-up analysis of the physicochemical characteristics of the microenvironment, with upscaling to a spatial domain (the 'contiguous environment') defined by the region over which substantial direct interactions or indirect chemical interactions are occurring. This approach presupposes adequate analysis of the local physicochemical environment, but technical innovations are moving to the microscale level (Young *et al.*, 2008). Konopka (2006) defined four ecosystems derived by considering the environment from the microbe's local perspective (Table 1). Each has particular characteristics that define important selective forces in that habitat, but which also impact the spatial scale over which microbial interactions occur.

Elements of microbial community analysis

Beyond the rigorous definition of a microbial community, there remains the elucidation of its essential characteristics. Levin (1999) proposed that 'the most important challenge for ecologists remains to understand the linkages between what is going on at the level of the physiology and behavior of individual organisms and emergent properties such as the productivity and resiliency of ecosystems.' From this perspective, important elements for analysis of microbial communities comprise fluxes, interactions and properties.

Analysis of community functional pathways

Microbial communities provide ecosystem services (Ducklow, 2007) through the catalysis of biogeochemical reactions. Many reactions in chemoheterotrophs entail conversion of chemical elements from organic forms to inorganic forms that can be used by

Table 1 Microbial-scale ecosystems

<i>Ecosystem type</i>	<i>Examples</i>	<i>Characteristics</i>
Planktonic	Open ocean, lakes	'Oligotroph lifestyle'. High affinity for uptake of multiple nutrients.
Surface-associated, saturated water	Freshwater and ocean sediments, subsurface sediments, microbial mats, biofilms	'Gradient lifestyle'. Hydrodynamic processes and fluid flow determine nutrient fluxes. Biomass density affects gradient steepness.
Surface-associated, unsaturated water	Surface and vadose zone soils	Water availability as limiting factor for activity and dispersal
Macro-organism associated	Gastrointestinal tract, rhizosphere, epiphytes	Patchy nutrient distribution. Dormancy Co-evolution, specific molecular interactions with surface-associated molecules

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primary producers for new biomass synthesis. Others interconvert elements between oxidized and reduced forms, and these coupled reactions can sustain the interacting organisms and have great impact on geological processes (Ehrlich, 1998). Lists of these reactions can be found in the description of biogeochemical cycles (Fenchel *et al.*, 1998).

The analysis of material and energy flow through distributed metabolic networks of microbes could lead to a predictive understanding of ecosystems (Roling *et al.*, 2007). However, it requires a deeper and richer analysis than is presently available. For example, models of terrestrial carbon cycling posit only a few pools of organic C that differ in first-order decay rates (Causarano *et al.*, 2007). Soil organic matter consists of hundreds of different organic substrates, heterogeneously distributed in a three-dimensional matrix. Fifty thousand different microbial taxa may be present in a kilogram of soil (Roesch *et al.*, 2007). Technically, it is possible to measure the rates of specific biogeochemical processes and to census the phylogenetic identities of the resident microbes. It is more difficult to determine which taxon is responsible for a specific biogeochemical process *in situ* (Madsen, 2005). However, increasingly sophisticated analytical methods such as NanoSIMS and Raman spectroscopy can permit the use of stable isotopes to measure process rates, with subsequent analyses of labeled macromolecules to phylogenetically identify the microbial catalyst (Behrens *et al.*, 2008; Huang *et al.*, 2009).

Interactions among organisms

Interest in microbial community ecology is a consequence of the postulate that interactions between organisms (microbe–microbe and microbe–metazoan) are of essential importance for understanding ecosystem dynamics and the evolutionary ecology of individual organisms. However, this remains an area in which the cataloging of potential interactions is better developed than mechanistic understanding of specific cases. The field of microbial ecology has correctly been called to task for an insufficient body of theory (Prosser *et al.*, 2007). However, this is one area in which theories on the effects of interactions on community diversity (Reichenbach *et al.*, 2007; Beninca *et al.*, 2008) exceed detailed experimental verification.

Although interspecies competition for nutrient resources is presumed to be widespread, it is difficult to analyze in natural communities; more typically, one detects its outcome as the composition of the microbial community. Laboratory studies of simple, constructed communities and theoretical investigations can provide insights into important molecular mechanisms (Gottschal, 1993; Prado and Kerr, 2008). Interactions can reflect cytoplasmic or extracytoplasmic activities, such as the affinity of membrane-bound transporters for limiting substrates (Robin *et al.*, 2008) or the production of cell-surface-associated or extracellular enzymes for macromolecule hydrolysis (Allison, 2005). However, even at our current state of knowledge, a catalog of microbial interactions (Table 2) goes far beyond simple resource competition (Little *et al.*,

Table 2 Interactions among species—biotic forces that structure community composition

Category	Class	Specific example	Reference
Resource competition	Cell surface: nutrient transporters		Sowell <i>et al.</i> (2009)
	Extracellular enzymes		Allison (2005)
	Siderophores/electron shuttles		Miethke and Marahiel (2007)
Metabolic interactions	Syntrophy		Dolfing (2001)
	Cross-feeding Sequential utilization Chemical modification of environment	Coupled biogeochemical cycles Nitrifiers, xenobiotic catabolism Organic acids alter pH to cause succession	Overmann and van Gernerden (2000) Villarreal <i>et al.</i> (1991)
Allelopathy	Plant chemicals		Bertin <i>et al.</i> (2003)
	Microbial chemicals	Bacteriocins Inhibit quorum sensing Interspecies quorum sensing Root nodule	Gillor <i>et al.</i> (2008), Riley and Wertz (2002) Gonzalez and Keshavan (2006) Ryan and Dow (2008) Cooper (2007)
Signaling	Microbial With multicellular organisms		
Structural	Consortia Biofilms and mats	'Chlorochromatium aggregatum'	Wanner <i>et al.</i> (2008) Des Marais (2003)
Trophic level interactions	Microbial loop		Azam and Malfatti (2007)
Horizontal gene transfer	Parasitism	Viruses	Suttle (2007) Juhás <i>et al.</i> (2009)
	Co-evolution		Moran <i>et al.</i> (2008)

2008). These include not only short-term (physiological) interactions, but also potentially deeper consequences that arise from horizontal gene transfer or co-evolution of traits between different microbes or a microbe and a multicellular organism. Although we have ‘textbook’ examples of these phenomena, the breadth of their ecological significance awaits discovery.

Emergent properties

Emergent properties of a community are characteristics not identifiable by analyzing the component organisms in isolation. Properties of this type can arise when the system is viewed at a high level of organization. An analysis of these properties can lead to an understanding of how the system responds to both external and internal perturbations, and provides the capability to predict system dynamics in response to environmental change.

Taxonomic diversity. The central property in community ecology is diversity. An inventory of diversity within a discrete sample has two important components—taxon richness and the relative abundance of different taxa in the community. The analysis of 16S rRNA gene sequences directly from environmental samples (Tringe and Hugenholtz, 2008) has greatly enriched our understanding of global microbial diversity. However, the financial costs of sequencing in the past meant that habitats were usually grossly undersampled relative to their richness. However, new sequencing technologies can now remove that restriction, and even very rare taxa can be detected (Sogin *et al.*, 2006). One benefit of deeper sampling is a more precise determination of the relative abundance of different taxa in a habitat. Analysis of relative abundance is conceptually very important to the other properties of communities discussed below, but a consequence of undersampling is that detection and quantitation of taxa present at low relative abundance is imprecise. New DNA sequencing technologies (pyrosequencing) can provide enough data (although more limited phylogenetic resolution), and the application of theoretical constructs to community assembly (Curtis *et al.*, 2006) can suggest methods of analysis that provide informative rank-abundance distributions for microbial communities.

The term ‘diversity’ can be applied at different ecological scales, and it is important to be clear about the intended meaning. Whittaker (summarized in Magurran, 2004) described a matrix of diversity indices that can be applied to microbial systems at a wide range of spatial scales. Complementary information is provided by inventory diversity (taxonomic diversity within spatially defined habitats) and differentiation diversity indices (the variation in an inventory diversity index). Microbial ecologists generally fail to accurately articulate which diversity index they have

measured. It is important to understand whether those analyses represent differentiation of pattern diversity (as would be true of a large number of subsamples from local environments) or measures of between-habitat diversity.

Functional diversity and functional redundancy. The analysis of taxonomic diversity across the microbial world is important on its own, as it illuminates the richness of evolutionary history over the past 3 billion years. However, for the analysis of microbial community ecology and the functioning of ecosystems, the determination of functional diversity is of greatest importance. Higher levels of redundancy for ecological functions should be related to the reliability with which an ecosystem will continue to deliver services in the face of environmental changes (Naeem *et al.*, 1998) (Figure 1). The central question is how to assay and quantify functional diversity. Phylogeny is informative to an adequate level in a small number of cases (for example methane-oxidizing bacteria (McDonald *et al.*, 2008)). However, it is more common to find that specific ecological functions are distributed across a wide breadth of taxa (nitrogen fixation and denitrification provide two examples).

In some cases, there are conserved regions of functional genes (such as sulfite reductase *dsrA* for sulfate reduction (Miletto *et al.*, 2007) and nitrite or nitrous oxide reductases for nitrate reduction (Mills *et al.*, 2008)) that can be used to assess the abundance of these genes in natural samples. Analysis of sequence differences among amplicons by fingerprinting techniques (Wertz *et al.*, 2006) will permit an analysis of functional diversity, at least for abundant community members. On the other hand, there are other important functions such as extracellular electron transfer, in which key functional

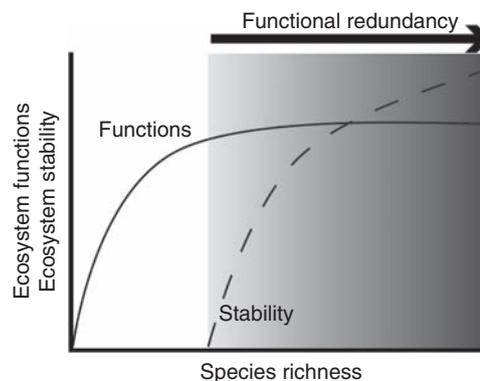


Figure 1 Conceptual model of relationship between functional redundancy and ecosystem stability (its resistance and resiliency to environmental perturbations). As new taxa are added to a pristine ecosystem, the number of ecological functions that are present increases. However, at some point, additional taxa do not add novel capacities, but do add redundancy to specific functions. Highly redundant systems are more likely to retain a function after an environmental stress because the probability of a resident taxon that is stress resistant is greater.

proteins, extracytoplasmic multiheme cytochromes, found in metal-reducing bacteria such as *Anaeromyxobacter*, *Geobacter* and *Shewanella* species, are poorly conserved. The other limitation to this molecular approach for analysis of functional diversity is that novel genes and enzymes will not be recognized.

A weakness of most current applications of community metagenomics or metaproteomics to complex communities is that gene sequences and proteins are disassociated from the intact organism that possessed them. As a result, the analyses cannot place a specific function in the context of the other functional properties of the organism. A measure of functional redundancy could be derived from cataloging genes or proteins with annotated functions and correlated to ecosystem resiliency to experimentally imposed perturbations (such as Hg stress). However, a mechanistic understanding will require analysis of the co-occurrence of those functional genes or gene products with stress resistance determinants in discrete organisms, either by bioinformatic analysis of single genomes or physiological analyses of cultivated organisms. For complex microbial communities, we may have to wait for the advent of technologies, whereby several hundred bacterial cells could be individually plucked from a habitat, and their genomes amplified (if necessary) and sequenced in a high-throughput and inexpensive manner. In this way, the community metagenome would comprise a list of functional genes in the complete context of the other genetic determinants within the organism.

Stability/resistance/resilience. Each of these terms has been applied to a description of ecosystem response to environmental perturbations. Resistance is related to the degree that a system does not exhibit an acute loss of function after imposition of a stress, whereas resilience connotes the rate at which functionality returns after an acute stress effect (Pimm, 1984). 'Stability' could then represent either the resistance of a system to acute, toxic effects or resilience, taking into account a longer-term perspective, in which stress imposes selection for resistant taxa present at low relative abundance. The stability concept refers to the functional properties of the community (Tilman, 1999), rather than to the population sizes of the constituents, as the latter are very dynamic in response to environmental changes.

Theoretical and empirical work in ecology has investigated the idea that higher diversity within a community will lead to increased ecosystem stability (Loreau, 2000; Tilman *et al.*, 2006). Several mechanisms have been put forward for this; these mechanisms are not mutually exclusive: (i) individual ecotypes may differ in their effects on ecosystem processes; thus, particular combinations of ecotypes may result in distinctive ecosystem properties. However, layered on top of this are empirical

observations of (ii) a richness effect, in which a mixture of ecotypes performs differently than the average performance of the individuals in isolation. There are many possible mechanisms, and microbial ecology represents an experimentally tractable discipline, in which it may be possible to identify the molecular mechanisms. Richness effects can arise from complementary properties of taxa within the communities such as niche partitioning or facilitation (mutualistic or commensal interactions). Alternatively, some taxa may have dominant effects and there is a higher probability that one of these dominant forms is present in a highly diverse community (the sampling effect).

Community genetic potential. The community properties articulated above have all been developed within plant and animal ecology and are applicable to microbial community ecology. However, it is useful to consider whether microbial communities possess a unique community property—the community metagenome. Microbes (particularly Bacteria and Archaea) have mechanisms for gene transfer across broad phylogenetic barriers that do not occur at ecologically significant rates in other organisms. Thus, the resilience of microbial communities over a period of a few years to environmental perturbations may rest in part on the frequency of horizontal gene transfer within microbial communities. Two examples of responsiveness under strong selection pressure are the dissemination of antibiotic resistance determinants (Hughes and Datta, 1983) and the development of metabolic pathways for the catabolism of novel xenobiotic organic compounds (Juhas *et al.*, 2009).

This suggests a role for the cataloging of genes (metagenomes) or protein sequences (metaproteomes) from microbial communities. The technology exists to conduct these surveys. However, substantial advances in informatics are still required to make them useful in an ecological sense. The specificity of annotation that is satisfactory for comparative genomics is inadequate to define ecological functionality. That is, analysis of microbial community functionality requires not only the identification of a protein family, but also an understanding of the range of substrate usage. A larger problem is the substantial fraction (approximately 30%) of genes in sequenced bacterial genomes, for which no function is assignable at all. Perhaps their functions are unknown because they are unimportant under laboratory culture, which may mean that they are very significant for some ecological function in nature, but this would require significant experimentation to uncover.

In summary, molecular censuses of microbes in nature have uncovered a breathtaking genetic diversity; in addition, new biogeochemical pathways and catalysts continue to be discovered by cultivation, albeit at a slower pace. In the past few years, technologies that sequence DNA, mRNA or proteins

have been applied to natural communities as 'discovery-based' science. This is appropriate at an early phase to search for patterns (now at the level of genes and proteins rather than taxa) that can lead to hypotheses regarding the causes of those patterns. However, one route to produce functional connections between microbial diversity and ecosystem properties is the rigorous application of community ecology concepts to microbial ecosystems, in ways that are testable and lead to a predictive synthesis. Some of the technical opportunities and challenges for future research were mentioned above: the increased sensitivity of stable isotope analyses through new mass spectrometry techniques that can be coupled to phylogenetic analyses of labeled macromolecules to identify the important microbial catalysts and the major challenge of extracting precise functional information from the annotation of nucleic acid or protein sequences.

Additional directions for future research that would aid in going beyond a descriptive to a predictive science of microbial community ecology might include several other experimental, computational and technical approaches:

- (1) *Expansion of experimental approaches beyond those most commonly used in microbial ecology*: a common experimental regime is manipulation of environmental factors in the field or in confined samples to analyze effects of abiotic factors on ecosystem processes. However, it is difficult to assess how microbial community structure impacts ecosystem functioning from these experiments. Application of experimental approaches such as common garden experiments and reciprocal transplants can provide community-level insights (Reed and Martiny, 2007).
- (2) *Predictive models at the community scale*: this is a daunting task, and current models of aquatic or terrestrial ecosystems (Izaurrealde *et al.*, 2006) neither attempt to include microbiological diversity nor spatial heterogeneities. A path forward would include scaling at the fundamental microbial ecosystem level (Table 1). For example, in terrestrial ecosystems, individual soil aggregates could represent an appropriate scale of community structure; one can collect data on the physical structure of the matrix, and modeling techniques (such as individual-based approaches) can be applied to incorporate biological diversity and spatial heterogeneity (O'Donnell *et al.*, 2007). This individual-based approach has been applied to multi-species biofilms (Picioreanu *et al.*, 2004). If these modeling approaches were successful, other computational techniques would be necessary to upscale predictions to larger spatial scales.
- (3) *Microbial model systems to test ecological theory*: microcosm experiments have been criticized as unrepresentative surrogates of natural ecosystems (see Jessup *et al.*, 2004). However,

the perceived constraints of these experiments also provide the means to rigorously test sophisticated ecological theories—the experimentalist can simplify a system to its fundamentals, control the scale and effects of environmental factors and reduce spatial heterogeneity. A recent example (Wittebolle *et al.*, 2009) illustrated how >1000 microbial microcosms were deployed to test the effects of evenness in relative abundance on functional stability of ecosystems. The positive effects of evenness on the constructed microbial communities' responses to environmental stressors have implications for larger-scale ecosystems in which invasive species or anthropogenic activities have altered community structure (Naeem, 2009).

- (4) *Explicitly directing research efforts to the three specific elements of microbial community analysis identified above*: the analysis of community functional pathways will benefit from the increasing sophistication of stable isotope analyses mentioned above; a limitation is that instrumentation is currently rare because of its expense. There are also new, mass spectrometric-based methods to resolve the complex suite of organic substrates present in soil or water, although the determination of molecular formulas from these data is in its infancy (Reemtsma, 2009). Among the emergent properties of microbial communities, functional redundancy is one that is of substantial theoretical interest, and which might be tractable to measure, at least for some well-understood biogeochemical functions (examples include nitrogen fixation and aromatic dioxygenases). This can be accomplished through data mining of metagenomes or targeted approaches such as PCR amplification of known functional genes or development of activity-based proteomic approaches (Cravatt *et al.*, 2008).

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