June, 2008

Rules of Engagement: Interspecies Interactions that Regulate Microbial Communities

Ainslie Little
Courtney Jaime Robinson, *Howard University*
S Brook Peterson
Kenneth F Raffa
Jo Handelsman

Rules of Engagement: Interspecies Interactions that Regulate Microbial Communities

Ainslie E.F. Little,[1] Courtney J. Robinson,[1] S. Brook Peterson,[1] Kenneth F. Raffa,[3] and Jo Handelsman[1,2]

[1]Department of Bacteriology, [2]Department of Plant Pathology, and [3]Department of Entomology, University of Wisconsin, Madison, Wisconsin, 53706; email: alittle@wisc.edu, cjr@plantpath.wisc.edu, snowbp@u.washington.edu, raffa@entomology.wisc.edu, joh@plantpath.wisc.edu

Annu. Rev. Microbiol. 2008. 62:375–401

First published online as a Review in Advance on June 10, 2008

The *Annual Review of Microbiology* is online at micro.annualreviews.org

This article's doi: 10.1146/annurev.micro.030608.101423

0066-4227/08/1013-0375$20.00

Key Words

microbial ecology, community ecology, community genetics, symbiosis, metagenomics

Abstract

Microbial communities comprise an interwoven matrix of biological diversity modified by physical and chemical variation over space and time. Although these communities are the major drivers of biosphere processes, relatively little is known about their structure and function, and predictive modeling is limited by a dearth of comprehensive ecological principles that describe microbial community processes. Here we discuss working definitions of central ecological terms that have been used in various fashions in microbial ecology, provide a framework by focusing on different types of interactions within communities, review the status of the interface between evolutionary and ecological study, and highlight important similarities and differences between macro- and microbial ecology. We describe current approaches to study microbial ecology and progress toward predictive modeling.

Contents

INTRODUCTION

Biology in the twentieth century was dominated by simplification and order. This was driven by the desire to improve experimental controls and resulted in a landscape of intellectual frameworks unified by reductionism. Study of systems was replaced by study of parts, organism with cells, cells with genes and proteins, and genes and proteins with their atoms. The scientific triumphs were many and the practical outcomes—vaccines, antibiotics, and high-yielding crops—transformed human health and food security. But the cost was a reduction of emphasis, training, and vision in systems-level biology, and with that a reduced ability to address some of the most important current environmental and health challenges.

Community: an assemblage of populations occupying a given area at the same time

Diversity: the richness and evenness of species within a community

As the twentieth century drew to a close, we were confronted by new challenges that rekindled widespread interest and identified the need to understand systems-level biology. Certain human diseases emerged whose origins were understood from landscape-level events that did not fit neatly into a reductionist scheme. Similarly, global climate change, and its underlying human causation, was recognized as a reality, and any realistic solutions required study of interconnecting spheres of society and the biosphere. These events and others like them arrived just as powerful new methods in microbiology emerged to open the way for a renaissance of ecology in general and microbial community ecology in particular. Although the need for systems biology has always been apparent to ecologists, who can offer many examples of ecosystems in which studying a binary interaction led to an erroneous conclusion that was corrected only by introducing more complexity into the model, the change in perspective was a surprise to much of the microbiology community (158).

Over the past century of microbiology, the emphasis on the study of microbes in pure culture has isolated microorganisms from their communities and focused on their behavior in the biologically simple environments of the petri dish and test tube. Although simple model systems have driven an explosion of knowledge in cellular processes and host-microbe interactions over the past two decades, the reality of natural communities demands that we direct attention to complex assemblages as well. Global microbial diversity is enormous, likely representing 10^7 species, of which only 0.01% to 0.1% are known (33, 34, 50, 60, 143). Microbial communities can be complex, with high species richness and unevenness, and their structures are continually influenced by changing biological, chemical, and physical factors. Most microorganisms do not submit readily to growth in the laboratory, leaving microbiologists to either concentrate on the subsets that do perform well under artificial conditions or grope for other methods to describe the species that compose natural communities. Therefore, the

structure of most microbial communities has been difficult to illuminate.

Describing community structure is often a prelude to understanding community function, which has been similarly difficult to elucidate (76, 142, 144). One of the barriers confronting microbial ecologists is the lack of ecological principles that provide the foundation for predictive models. Broadly based, validated principles derived from systems that can be manipulated experimentally would allow for predictions regarding behavior of communities that are less tractable for study. Many of these principles can be borrowed from macroecology, although some need to be reformatted to fit the microbial lifestyle. In this review, we explore the internal processes of microbial communities in an effort to begin to define the principles that underpin ecological and evolutionary patterns of microbial communities. Defining these principles is necessary to enable predictive modeling of ecological dynamics of microbial communities. In addition to their importance to fundamental understanding of the biosphere, predictive models have numerous practical applications. For example, they can provide guidance to strategies for manipulating communities on plant or animal surfaces to suppress pathogens, maintaining community integrity when applying chemicals such as antibiotics or pesticides that could destabilize communities, or successfully introducing a beneficial microorganism such as a biocontrol agent in agriculture or a probiotic in veterinary and human medicine.

ECOLOGICAL PROPERTIES OF MICROBIAL COMMUNITIES

The properties of microbial communities can be divided into two categories: structural and functional. Structural properties describe how the community varies and what it looks like—the types and numbers of members across a range of environments. Functional properties define the community's behavior—how the community processes substrates, interacts with forces in its environment, and responds to perturbations such as invasion.

Structural Properties

Community structure: the composition (membership) of a community and the abundance of individual members

One of the simplest ways to characterize a community is to list its members (composition) and to tabulate the total number of members (richness). To answer such questions as How many different species are there in a given community? and What are they? seems easy and straightforward, but answering them is challenging when they are applied to microbial communities (177). The challenges derive from both biological and statistical issues. Enumeration by culturing limits the description to those members that can be cultured, which constitute the minority (often less than 1%) in most communities. Molecular methods present culture-independent alternatives, which capture far more richness than does culturing (32). Sequence analysis of the 16S rRNA gene is the dominant method of determining identity and phylogenetic relatedness of microorganisms (32), although other genes, such as *rpoB*, may provide greater resolution in phylogenetic associations at the species and subspecies levels (21). To avoid the inherent difficulty in sampling every species in every community, macro- and microbial ecologists often use estimates of richness based on samples of the communities; however, these estimates can vary depending on the estimator chosen and the type of data analyzed (8, 177; see Reference 32 for a review of the difficulties of quantifying properties in microbial communities, the statistics used in the analyses, as well as recent accomplishments in the area). Estimates of richness based on gene sequence relatedness are often calculated using software such as EstimateS and DOTUR (26, 174). DOTUR, for example, has been used for a variety of genes and environments to assign sequences to operational taxonomic units based on phylogenetic distances and to calculate richness estimates at different degrees of phylogenetic resolution (17, 30, 46, 56, 96, 175, 201).

Diversity indices take into account both species richness and evenness of distribution of

Individual: smallest unit within a population; for prokaryotes, it is usually a single cell

Guild: a group of species that occupy a common niche in a given community, characterized by exploitation of environmental resources in the same way

Population: all the individuals of one species in a given area at the same time

Robustness: description of a community's temporal stability and resistance and resilience to perturbations that challenge structure (structural robustness) or function (functional robustness)

species (i.e., abundance of individuals) (8). The diversity of a community is difficult to interpret on its own but can be valuable when used to compare communities. Community structure, like diversity, is an attribute that is most useful when analyzed for comparative purposes. Community structure incorporates both the composition of the community and the abundance of individual members. Diversity and structure measure different aspects of community characteristics, so they can vary independently of each other (78). In soil communities, the presence of a plant community, the introduction of various transgenes into a tree community, and rhizomediation influence composition and structure, but not diversity (40, 111, 214). However, in other situations structural changes influence diversity as well.

In addition to the structure of the entire community, understanding the structure of assemblages within a community can also be important. For example, Perez & Sommaruga (148) monitored the response of the Betaproteobacteria and *Actinobacteria* populations within a lake water community to solar radiation and dissolved organic matter from lake, algal, or soil sources. Functionally defined assemblages, such as guilds, are also of interest because of the activities of community members. In another recent study, the structure of a methanotroph assemblage within a rice field community was monitored using terminal restriction-length polymorphism (T-RFLP) analysis, *pmoA* gene analysis, and stable isotope probing (181). The results indicated that the activity and structure of the methanotroph assemblage (composed of type I and II methanotrophs) fluctuated over time and with CH_4 availability and that type I and type II methanotrophs occupied two different niches within the rice field ecosystem. Other guilds such as ammonium-oxidizers, methanogens, and iron-reducers have been analyzed for structural properties (24, 77, 184). Similarity of community structure can be calculated using a number of computer programs such as S-LIBSHUFF, SONS, TreeClimber, and Unifrac and techniques such as analysis of molecular variance (AMOVA) and homogeneity of molecular variance (HOMOVA) (123, 124, 128, 173, 175, 176, 178). The differences between the hypotheses tested by each of these tools are discussed elsewhere (173).

Robustness

Community robustness is the ability of the community to maintain its functional and structural integrity in the face of potential perturbations (8). This is consistent with other uses of robustness in engineering and statistics that pertain to the heartiness of a system and its ability to function under various, often adverse, conditions (62). Just like complex engineered systems, biological systems, such as cells, tissues, organs, and ecological webs, are composed of diverse and often multifunctional components (100). Systems that maintain their function, characteristic behavior, or some other property despite internal and external perturbations and adapt to their environments are robust (100, 101, 187). Although robustness is a characteristic of all biological systems, it is a relative property that depends on the perturbation and the behavior monitored (187). For example, cancer cells that establish in the human body are particularly robust against the host's defenses, but perturbations against which they are weakly robust offer promising therapies (101).

We use structural robustness, similar to ecosystem stability (although ecosystem stability does not always refer to structure), to describe the constancy in community structure over time (temporal stability), the ability to resist change following a perturbation (resistance), and the return to its native structure following a change to structure (resilience). Components of robustness are often studied individually. Temporal stability, though not always referred to as such, is studied far more often than the other components of robustness. For example, Kikuchi & Graf (99) recently reported that populations in the microbial community of leech crops, comprising *Aeromonas veronii* bv. sobria and a *Rikenella*-like organism, fluctuated within 6 h to 14 days after blood

feeding. The population size of both members initially increased following feeding, but the abundance of *A. veronii* decreased 4 days after feeding while the abundance of the *Rikenella*-like species remained constant over the timescale studied (99). Temporal stability has also been examined in rice field soil, cabbage white butterfly midguts, and a number of aquatic communities (2, 20, 23, 94, 98, 113, 135, 137; C. Robinson, P. Schloss, K. Raffa & J. Handelsman, manuscript submitted).

Functional robustness refers to the ability of a community to maintain a particular activity despite perturbation, which, unlike structural robustness, is not necessarily linked to composition, although links between structure and function have been established many times (1, 22, 24, 28, 61, 181, 184). Saison et al. (171) showed that soil communities exhibited functional and structural resilience to low, but not high, levels of winery compost. In another study, Yannarell et al. (211) found that nitrogen fixation returned to normal levels in a Bahamian microbial mat following a Category Four hurricane, despite a shift in community structure.

At the intersection of functional and structural robustness is the years-old diversity-stability debate. Briefly, the debate questions whether increased community diversity increases or decreases community stability (see References 89 and 131 for reviews of the diversity-stability debate). Macro- and, to a lesser extent, microbial ecology have long sought a common rule that governs the relationship between diversity of a given community and its ecosystem stability, with stability often measured by a specific activity or function. Several years ago, McGrady-Steed et al. (133) found that as diversity increased, decomposition increased, but carbon dioxide uptake decreased in an aquatic microcosm that contained bacteria, protists, and metazoans. They also noted that resistance to invasion increased as the abundance of certain members increased.

More recently, Girvan et al. (67) examined the temporal structural stability and the functional resilience of two soil communities of different diversities that had been perturbed with benzene or copper using denaturing gradient gel electrophoresis and monitoring of broadscale (mineralization of ^{14}C-labeled wheat shoot) and narrow-scale (mineralization of ^{14}C-labeled 2,4-dichlorophenol) functions for 9 weeks. Temporal shifts in structure were observed in all soils, although copper-treated and control soils were consistently more similar to each other than either was to the benzene-treated soil (67). This indicates that in some systems the source of the perturbation may be more important than community diversity in structural temporal stability. Benzene perturbation reduced the ability of both communities to perform the narrow niche function; however, the more diverse community reacquired the function by week 9 of the experiment, thereby exhibiting functional resilience (67). Copper treatment increased broadscale function but initially reduced narrow niche function for both communities before they both recovered (67). These findings indicate that diversity and source of perturbation may be important for functional robustness. The results also suggest that soil exhibits functional resilience owing in part to functional redundancy, but that greater diversity in soil communities may also lead to greater resistance and functional stability. The positive association between community diversity and stability is consistent with what has been observed in some macroecological systems such as grassplots (195). More study of microbial community robustness is needed to establish the rules of engagement, i.e., governing principles and predictive models.

INTERACTIONS WITHIN MICROBIAL COMMUNITIES

A first step toward understanding the nature of ecological interactions in natural microbial communities is cataloging mechanisms by which microorganisms interact. Symbiotic interactions can be divided into three overlapping categories, which exist in a continuum from parasitic to mutualistic (**Figure 1**). Parasites (Greek, *para*, "near," and *sitos*, "food") are organisms that live on or in another organism

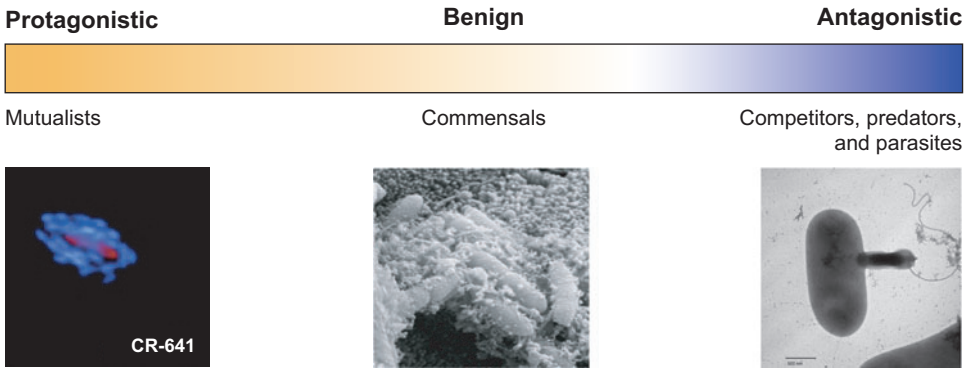

Figure 1

Continuum of interspecific interactions that occur in microbial communities. (*Left*) The beneficial relationships that occur between mutualists such as the phototrophic consortia (shown below) comprising central rod-shaped bacteria in the family *Comamonadaceae* (*red*) and green epibiotic sulfur bacteria (*blue*) (reprinted from Reference 95). Commensal, or benign, relationships are depicted in the middle of the continuum by an electron micrograph of the microbial community of the cabbage white butterfly. (*Right*) Antagonistic relationships are illustrated by an electron micrograph of *Bdellovibrio* attacking *Shewanella oneidsis* (photo credit: Robert Chamberlain, Wayne Rickoll, and Mark Martin).

and obtain all or part of their necessary nutrients at the expense of their host. Commensalism (Latin, *com*, "together," and *mensa*, "table") includes relationships in which one partner derives benefit from the other and the other partner neither is harmed nor benefits from the association. Mutualism (Latin, *mutualis*, "reciprocal") is an association in which both organisms derive benefit from one another. In addition to specific symbiotic interactions, microorganisms can interact antagonistically with other microorganisms via competition for a common resource or via predation of one organism upon another. The mechanisms that dictate interactions among microorganisms are largely responsible for the properties of the community as a whole. Dissecting the binary and tertiary interactions among community members is one essential component to understanding the properties of the community.

Exploitative Competition

As in interactions between macroorganisms, exploitative competition, or competition for nutrients and space, plays an important role in shaping microbial interactions. In eukaryote-associated microbial communities, such as in the human gut, competition for nutrients and space from resident microflora is thought to present one barrier to infection by pathogens, a phenomenon called the barrier effect (74). In cabbage white butterfly larvae, changes in the resident microflora community structure resulting from treatment with antibiotics facilitates invasion by a nonresident, in support of the role of competition from residents in normally preventing invasion (C. Robinson, Y. Ramos, K. Raffa & J. Handelsman, unpublished observations). Probiotic bacteria, such as some *Bifidobacterium* and *Lactobacillus* species, are thought to exert positive effects on host health in part via competitive interactions with pathogenic bacteria for space and nutrients, and also through interference competition by production of toxic compounds (161). In vitro, binding of some probiotic bacteria to cultured epithelial cells can prevent binding of pathogens, in support of the hypothesis that bacteria successfully compete for space (73, 112). Competition for nutrients among bacterial species from functional groups having different nutritional requirements can be important in structuring microbial communities in

nutritionally heterogeneous environments. For example, in microcosms containing picoplanktonic cyanobacteria and heterotrophic bacteria along crossed gradients of glucose and phosphate, the cyanobacteria positively responded to increased phosphate only when glucose was low, presumably because of increased competition from the heterotrophs when organic carbon was supplied (47).

Exploitative and interference competition. The aspect of microbial interactions that has arguably received the most attention is the ability of some microorganisms to produce compounds that, at least in laboratory studies, directly antagonize other microorganisms. It has been assumed that organisms produce these compounds as a means of chemical warfare, providing a competitive edge to the producers by directly inhibiting growth or killing off potential competitors, a form of interference competition. One example in which evidence supports this proposition is in the rhizosphere, where antibiotic production by a number of bacteria contributes to their ability to protect plants from particular pathogens (27, 157). The antibiotics these bacteria produce in vitro have been detected in the rhizosphere (12), and mutants deficient in antibiotic production often exhibit a reduced ability to protect the plant from the pathogen (97, 183, 192) or a reduced fitness in the rhizosphere (129, 153). Additionally, production of the peptide antibiotic trifolitoxin by some strains of *Rhizobium etli* strains contributes to their competitive ability in the rhizosphere, leading to increased occupation of root nodules by producing strains (166, 167). In addition to the rhizosphere systems, evidence from a number of invertebrate-microbe interactions supports the role of antibiotics in antagonistic relationships within communities. For example, antibiotics produced by *Actinomycetes* associated with leafcutter ants protect the ants' fungal gardens from parasitism by another fungus (31, 121). Similarly, larvae of some crustaceans, beewolves, and bark beetles rely on production of antifungal compounds by bacterial symbionts to avoid infection by fungal pathogens (19, 65, 66, 93).

In many cases, however, antibiotic production in vitro has not been demonstrated to result in antagonism in situ, leading to speculation that antibiotics play roles other than as growth inhibitors (39). At subinhibitory concentrations, structurally diverse antibiotics affect transcription of many bacterial genes not necessarily associated with stress responses, suggesting that antibiotics may function as signaling molecules in the environment when produced at low concentrations (39).

Interference competition via signal disruption. Some mechanisms of interference competition between microorganisms are independent of antibiotic production, such as disruption of signaling cascades. Diverse bacteria degrade acyl-homoserine lactone signal molecules (110, 119, 198–200, 210), and the rapid turnover rate of acyl-homoserines in nonsterile soil suggests this is a common bacterial trait at least in that environment (204). Signaling by small peptides can also be disrupted; for example, siamycin, a secondary metabolite produced by a soil *Streptomyces* strain, inhibits signaling by the gelatinase biosynthesis-activating pheromone of *Enterococcus faecalis* (138). The ability to interfere with signaling would provide a competitive advantage if competitive determinants, such as antimicrobial toxin production, were regulated by signaling, as has been suggested (3). However, a clear link between the disruption of signaling and competitive advantage has yet to be established.

Predation

In macroecological systems, predation, or the consumption of one organism by another, is frequently a key stabilizer of community structure (52, 53, 82). The top predator often regulates abundance of other species that in turn regulate other species, providing a cascade of effects that have a sweeping influence. Often, this effect on the community far exceeds the numerical representation of the predator and can transform

entire landscapes, which defines the predator as a keystone species (145, 165).

Predation of bacteria by microbial eukaryotes and bacteriophages provides a key link between microbial and macroorganismal food webs (25, 150) and has global effects on bacterial community structure and composition in many environments. In both freshwater and marine habitats, predation is a leading cause of bacterial mortality (150, 191). In some aquatic environments, top-down control by predation also appears to regulate bacterial population sizes (150). Additionally, predation has been suggested as an influence on bacterial species richness and evenness (150, 213), selectively limiting population sizes of some readily culturable aquatic bacteria that rapidly proliferate when grazing pressure is experimentally reduced (7). In soil, predation by protozoa similarly limits bacterial population sizes and can influence bacterial community composition and structure (25, 137). Difficulties associated with quantification of bacteriophage in soil have hampered efforts to ascertain the role of predation by phage in these ecosystems (5, 207). In engineered microbial communities, predation pressure can influence bacterial population structure by selecting for strains adapted to defend against predator attack (107). Manipulation of microbial communities via phage that prey specifically on lineages of bacteria could be used as a tool to study community dynamics, given the strength of phage selective pressures in some communities (15).

Bacteria or archaea that prey on other bacteria or archaea appear to be relatively rare compared with their eukaryotic counterparts, but examples of each have been described. *Bdellovibrio*-like organisms are obligate bacterial predators, which now appear to be more diverse and widespread than previously recognized (36, 155). Most of these organisms penetrate the outer membrane and wall of their prey and replicate in the cytoplasm, thereby killing the host. However, some, including the *Micavibrio* sp. (Alphaproteobacteria), attach to the outside of prey cells and replicate epibiotically (37). The range of prey organisms targeted by different *Bdellovibrio*-like predators varies but typically includes only a limited number of species (90, 154, 168). A different predatory strategy is employed by a second group of bacterial predators, the myxobacteria (Deltaproteobacteria). Populations of myxobacteria exhibit cooperative, surface-associated motility and collectively subsume prey organisms they encounter by producing secreted and cell-associated degradative enzymes (162). However, unlike *Bdellovibrio*-like organisms, myxobacteria can also obtain nutrients by degrading macromolecules instead of live prey; genome sequencing of *Myxococcus xanthus* suggests that prey bacteria serve as a source of branched-chain amino acids, which the predator does not have the capacity to synthesize (71). In addition to many proteases and cell wall–degrading enzymes, myxobacteria also produce diverse secondary metabolites, which may also play a role in predation or may mediate competition with other species or other myxobacterial strains (55, 71). Finally, a number of gram-negative bacteria release membrane vesicles containing hydrolytic enzymes that can fuse with and lyse other bacteria (91, 117). Membrane-vesicle-mediated lysis may help to extract nutrients from target cells, although this has not been established empirically.

Parasitism

The only prokaryote thought to parasitize another prokaryote is *Nanoarchaeum equitans*, isolated from hydrothermal vents. *N. equitans* is small in physical stature and has a tiny, compact genome, less than 0.5 Mb, predicted to contain a 95% coding sequence (87, 132). This species is completely dependent on its host, the larger archaeon, *Ignicoccus hospitalis* (87, 147). The genome of *N. equitans* lacks many key metabolic functions, including genes encoding glycolysis and trichloroacetic acid cycle enzymes, as well as most amino acid and lipid biosynthesis pathways, indicating that it must obtain many nutrients and metabolites from its host (205). The host species, however, can be found in a free-living state, and association with *N. equitans*

appears to take a toll on its fitness, thus suggesting that the smaller associate is a parasite (87, 147).

Mutualistic and Commensal Interactions

At least as prevalent among microorganisms as the antagonistic interactions described above are interactions in which both partners benefit (mutualism) or in which one partner benefits with no apparent effect on the other (commensalism). Purely commensal relationships may not exist; perhaps we simply have not discovered the benefit to the second partner. More likely, those organisms are either beneficial or harmful to their hosts, depending on the community dynamics in the niche, but researchers have yet to delimit and quantify the costs and benefits exchanged between the host and symbiont. For example, many microbes in the human gut historically termed commensal are now recognized as critical factors in gut and immunity development, nutrient uptake, and homeostasis of the system (85, 86, 159). Furthermore, relationships may be context dependent, that is, an organism could be beneficial under certain conditions and commensal under others.

Obligate associations. In obligate mutualisms each partner depends on the other for survival and reproduction. One particularly elegant obligate microbial association is the phototrophic consortium detected in many freshwater habitats (68). In these assemblages, a central motile, nonphotosynthetic Betaproteobacterium from the family *Comamonadaceae* is surrounded by green sulfur bacteria in an organized structure (59, 95). The epibiotic sulfur bacteria are thought to benefit from the motility provided by the central bacterium, enabling the consortia to chemotax toward sulfide (68). The central *Comamonadaceae* may benefit from carbon secreted by the sulfur bacterium during photoautotrophic growth (68, 69). Additionally, the partners appear to coordinate behaviors via as yet unidentified signal exchange. For example, the consortia chemotax toward sulfide and the organic compound 2-oxoglutarate only in light, which the motile central organism itself cannot detect (69). The consortia also move preferentially to the optimal light wavelength absorbed by the nonmobile photosynthetic bacteria (58, 59).

Several obligate associations between microbial species occur within the context of a eukaryotic host. For example, in the glassy-winged sharpshooter, *Homalodisca coagulata*, metagenomic analysis of its microbial symbionts revealed the presence of complementary amino acid, vitamin, and cofactor biosynthetic pathways in two microbial symbionts, *Baumannia cicadellinicola* and *Sulcia muelleri*. Both symbionts are required to sustain the sharpshooter, which feeds on the amino acid–poor diet of plant xylem (209). Several species from another group of plant sap-feeding insects, the mealybugs, harbor not only multiple symbionts, but one symbiont, a Gammaproteobacterium, is housed inside the second symbiont, a Betaproteobacterium (203). The functions provided to the host by each symbiont have not yet been identified, but the associations appear to be stably maintained and vertically transmitted, as reflected by cospeciation in symbiont phylogenies (190).

Facultative associations. Conditions under which one or both species of a mutualism or commensalism survive and maintain populations in the absence of the other partner are called facultative. Many instances of facultative mutualism in microbe-microbe relationships involve the exchange or sharing of nutritional resources. For example, metabolic cooperation can result from complementary degradative capabilities or from the ability of one organism to make use of byproducts generated by another. In the human oral cavity, metabolic cooperation plays a key role in structuring the complex, multispecies biofilm formed on tooth surfaces. The late successional stage colonizer *Porphymonas gingivalis* benefits the earlier colonizer *Fusobacterium nucleatum* by activating a host protease, plasmin, which *F. nucleatum* subsequently captures and uses to obtain nutrients (35). Another

Coevolution: reciprocal genetic changes in two or more species in response to each other

facultative commensalism is in plant root exudate, where peptidoglycan from the cell wall of *Bacillus cereus* rhizosphere isolates provides carbon to sustain the growth of *Flavobacterium* and *Chryseobacterium* species, which is otherwise carbon limited (151, 152), without impacting the growth of *B. cereus*. In some cases, metabolic cooperation results from the ability of one organism to alleviate the effects of a toxin on another organism. For example, in a model system to evaluate effects of mixed organic waste on organisms important for detoxification, the *p*-cresol-degrading organism *Pseudomonas putida* DMP1 protected the *p*-cresol-sensitive strain *Pseudomonas* sp. strain GJ1, which could then degrade a second common waste compound, 2-chloroethanol (29).

Syntrophy. The hallmark of syntrophic interactions, which can be obligate or facultative, is the coupling of metabolic processes in two organisms, typically by transfer of electrons between the organisms by hydrogen or other carriers, which facilitates metabolisms that would otherwise be thermodynamically unfavorable. Under methanogenic conditions, syntrophy appears to facilitate a number of the intermediate transformations between primary fermentation of complex organic matter and eventual production of methane (172). In methane-rich marine sediments, syntrophy between archaea thought to perform reverse methanogenesis and sulfate-reducing bacteria plays a role in mediating methane oxidation, an important control of the flux of this potent greenhouse gas (10, 75). Degradation of some xenobiotic compounds also relies on syntrophy. For example, interspecies hydrogen transfer from a sulfate-reducing organism facilitates tetrachloroethene dehalorespiration by another organism (48). Similarly, vinyl chloride dechlorination by *Methanosarcina* spp. also requires interspecies hydrogen transfer by a syntrophic partner organism (80).

Coaggregation and multispecies biofilm formation. Many beneficial interactions between microorganisms require the partners to be maintained in close proximity, which is often achieved by the formation of multispecies biofilms or aggregates. In some cases, most notably in the oral cavity of vertebrates, development of complex communities results from specific, receptor-mediated interactions between pairs of organisms, known as coaggregation (109). Early colonizers to tooth surfaces, such as *Streptococcus gordonii* and other viridans streptococci, can bind a variety of host molecules and subsequently facilitate colonization by the second-stage species through coaggregation with specific partners (105). *F. nucleatum*, the most abundant gram-negative bacterium in mouths of healthy people, is thought to serve as a bridge between these early and subsequent late colonizers because of its ability to coaggregate with many species from both classes (104).

EVOLUTION IN MICROBIAL COMMUNITIES

The intersection of ecology and evolution is important to our understanding of communities but has not been sufficiently studied to produce a cohesive framework. Antagonistic and mutualistic behaviors have evolved as adaptations to life in a community. Organisms exploit or compete with each other for resources, leading to the grand diversity of ecological mechanisms in the biological world. Individual species evolve in the context of a community, resulting in coevolution, and the community evolves as a composite of many species. Identifying the selection pressures that favor certain interactions is the key to deriving an evolutionary understanding of microbial communities. And perhaps there is a larger conceptual framework to be developed that will describe microbial community evolution, with the entire community as the unit upon which selection acts.

In many ways, the evolution of prokaryotes and eukaryotes is similar. Natural selection and genetic drift operate on population-level genetic variation caused by mutation and gene flow. Together these processes alter the genetic composition of populations and

directly and indirectly affect the species interactions that dictate community ecology. However, some evolutionary processes play different roles in prokaryotic and eukaryotic populations, and these contrasts are particularly important to consider in the context of microbial community dynamics.

Genetic variation is the target on which selection acts, whereas ecological forces, including biotic factors such as species interactions (competition, parasitism, mutualism), are the agents of selection. Thus, the two fields of study, population genetics and community ecology, seem inevitably coupled through evolution.

Most ecological and evolutionary theory has been developed on the basis of observations made in eukaryotic organisms. Natural selection, developed with plants and animals, and Mendelian genetics, originating from studies of plants, were integrated to form the modern synthesis, which is the basis of current evolutionary theory, but none of the major leaders in the development of the modern synthesis (Fisher, Dobzhansky, Haldane, Wright, Huxley, Mayr, Rensch, Simpson, and Stebbins) focused on prokaryotes. Consequently, models for understanding adaptation, evolution, and speciation in prokaryotic biology were not developed until the early 1980s (114). Advances in molecular biology have propelled the expansion of prokaryotic models for evolution over the past 20 years, from which two major differences between prokaryotic and eukaryotic evolution have emerged: the frequency of recombination and the phylogenetic breadth among which genetic materials can be exchanged. Intragenomic processes such as recombination are likely to have the greatest influence in short-term changes in a community, leading to population adaptation to changing conditions or new metabolic opportunities. Intergenomic processes such as horizontal gene transfer have profound effects on the long-term evolution of communities, possibly leading to the formation of new species (41, 84).

Although a superficial examination of evolutionary processes in prokaryotes and eukaryotes reveals stark differences, deeper examination might unite them. Clonal eukaryotes, for example, may be governed by similar principles as prokaryotes. More significantly, the same forces may regulate hybridization between plant species and interspecies gene transfer in prokaryotes and the resultant afront to the integrity of the species (41, 84).

Intragenomic alterations. Sequential point mutations and gene rearrangements can lead to adjustments in the genotypic and, consequently, phenotypic content of population members. Mutations or alterations that are selected typically improve the fitness of an organism in its current ecological niche, which is necessary to maintain interspecific interactions such as competition, predation, and mutualism. Several hypotheses regarding microbial fitness are readily testable. For example, improvements in fitness over thousands of generations under glucose-limited conditions have been measured (146). An interesting and intensely studied pattern resulting from closely evolving interactions is the coevolutionary process, in which genetically based adaptations in one species invoke reciprocal genetic changes in populations of its partner species (e.g., competitor, parasite) or guilds of species (193, 194).

Horizontal gene transfer. The transfer of genetic information between species is a central mechanism of generating genetic variation in microbial communities. For example, multilocus sequence typing data and proteomic and comparative genomics data indicate that bacterial species in acid mine biofilm communities exchange large (up to hundreds of kilobases) regions of DNA as well as smaller sections that may play a role in resistance to phage (122, 197). Events of horizontal gene transfer can be detected through phylogenetics, by seeking atypical distributions of genes across organisms, or through phylogeny-independent methods that examine genes that appear aberrant in their current genomic context. Complete genome sequencing has arguably been the most important factor in unveiling

instances of horizontal gene transfer, illustrating the impact of horizontal gene transfer on bacterial evolution (6, 10, 13). Perhaps one of the most dramatic impacts of horizontal transfer of genetic information by accessory genetic elements and vectors of genes (e.g., viruses) is ecological. Horizontal gene transfer can enable a microbe to rapidly expand and/or alter its ecological niche, making this genetic process important when considering evolution in microbial communities through deep time (i.e. an evolutionary timescale rather than an ecological timescale). Horizontal gene transfer has also been proposed to contribute to speciation, which is a critical aspect of community function and evolution (41, 84).

Ecological processes, in turn, affect evolution by providing opportunities for interspecies gene transfer and providing selection pressure. The architecture of communities, which dictates proximity of cells of different species, affects the probability of gene exchange across wide phylogenetic distances. The physical and biological features of the community will affect the susceptibility of cells to transformation or transduction, thereby affecting the frequency of gene transfer. The ecological processes and characteristics of the community create the selection pressures that determine the direction of change in frequencies of certain genotypes.

When entire genes or groups of genes are transferred between individuals, especially distantly related individuals, a trait can rapidly sweep through a population under appropriate selection pressure. A contemporary example of this is the rapid spread of antibiotic resistance in bacterial populations. In other instances, the changes can lead to rapid lineage diversification (164). In some cases these changes, especially those that involve the metabolic repertoire, enable the recipient of horizontal gene transfer to invade and adapt in a new ecological niche. Classic examples of niche-altering gene acquisitions include the *lac* operon by *Escherichia coli* and pathogenicity islands by *Salmonella* sp. Changes that enable an organism to invade a new niche(s) have strong implications at the community level: They have the potential to alter interactions between species and the structure, diversity, and robustness of communities. Ultimately, the ecological selection pressures that drive microbial evolution are major contributors to the emergent structure and function of the community.

APPROACHES TO THE STUDY OF MICROBIAL COMMUNITIES

Community ecology, as it pertains to microbes, remains in its infancy. Most studies of microbe-microbe and host-microbe interactions have extracted the organisms from their native community and studied them as binary interactions; indeed, this is simpler to understand and a necessary step toward understanding interspecific interactions in a community context (**Figure 2**). However, the information gained from such studies can be inadequate or misleading, as has been shown repeatedly in macroecological research (18, 120, 182, 185), and as such, results obtained from those studies must be interpreted with caution. To this end, we have parsed microbial ecology studies of communities into four groups on the basis of the questions being asked and presented them chronologically in terms of the order in which questions must be addressed and answered to generate further information on microbial community characteristics and processes. Each question is illustrated with classic experiments and recent technological advances that have enabled their investigation (**Figure 3**).

Who Is Present in the Community?

The first question a community ecologist asks is, Who makes up this community?, and this is indeed a good starting point. But with microorganisms, this is not a trivial question, nor has it been easy to identify the diversity of species found in various communities. Two central techniques are used to identify microbial phylotypes within community samples: One technique relies on culturing, and the other is culture independent.

	 Individual	 Population	 Community
Ecology	**Physiology:** Differential gene expression in response to change	**Demographics:** Birth, death, immigration, emigration	**Community ecology:** Interspecific interactions that shape community structure and function
Genomics	Fine-scale mapping of individual genomes	**Population genomics:** Comparative genomic analyses to assess variation	**Metagenomics:** Genetic potential of collective members of community
Genetics	**Bacterial genetics:** Role of genes under various conditions	**Population genetics:** Allele frequency distribution	**Community genetics:** Interplay between genetic composition of community and ecological community properties

Figure 2

Progression from studies on the individual scale to studies on the community scale.

Culture-based methods. Koch's discovery that bacteria could be isolated and grown in pure culture on solid artificial medium enabled the discipline bacteriology to develop. Growing bacteria in pure culture provided morphological and physiological data, which together provided the basis to identify bacterial species. More advanced culture techniques incorporated various nutrients and abiotic conditions that closely mimicked the environments from which the samples were isolated. However, recent estimates indicate that less than 1% of the membership of many communities is culturable, making it necessary to assess the identity of the as yet uncultured organisms to generate a complete list of community members.

Culture-independent methods. One molecular approach that provides a powerful complement to culture-dependent techniques is the amplification of 16S rRNA gene sequences directly from environmental samples, such as soil, using PCR and universal or domain-specific primers, which is usually followed by clone library construction. Clones are then screened to analyze sequence differences (11), or restriction fragment length polymorphisms (136), which are then used to identify species. To date, 16S rRNA gene amplification and identification remain the most reliable tool to describe prokaryotic species. Because the gene is universal and can therefore be used as an identifier for any bacterial or archaeal species, it accounts for both culturable and nonculturable prokaryotic organisms, and it has phylogenetic meaning. There are, however, limitations to the 16S rRNA gene approach to phylogeny. Interspecies gene transfer muddies phylogenetic assessments derived from 16S rRNA. If organisms are hybrids with fragments of DNA of different organismal origins, then what is a species? Should they be defined by the 16S rRNA gene affiliation or by a census of functional genes? Prokaryote phylogeny is an emergent field and the species concept will be one to grapple with in the future.

The universal primers used to assess entire communities may not detect all species. Recent work suggests significant differences in the groups whose 16S rRNA genes are amplified when the universal primers are replaced by miniprimers with very broad specificity (88). All methods impose bias and it is likely that

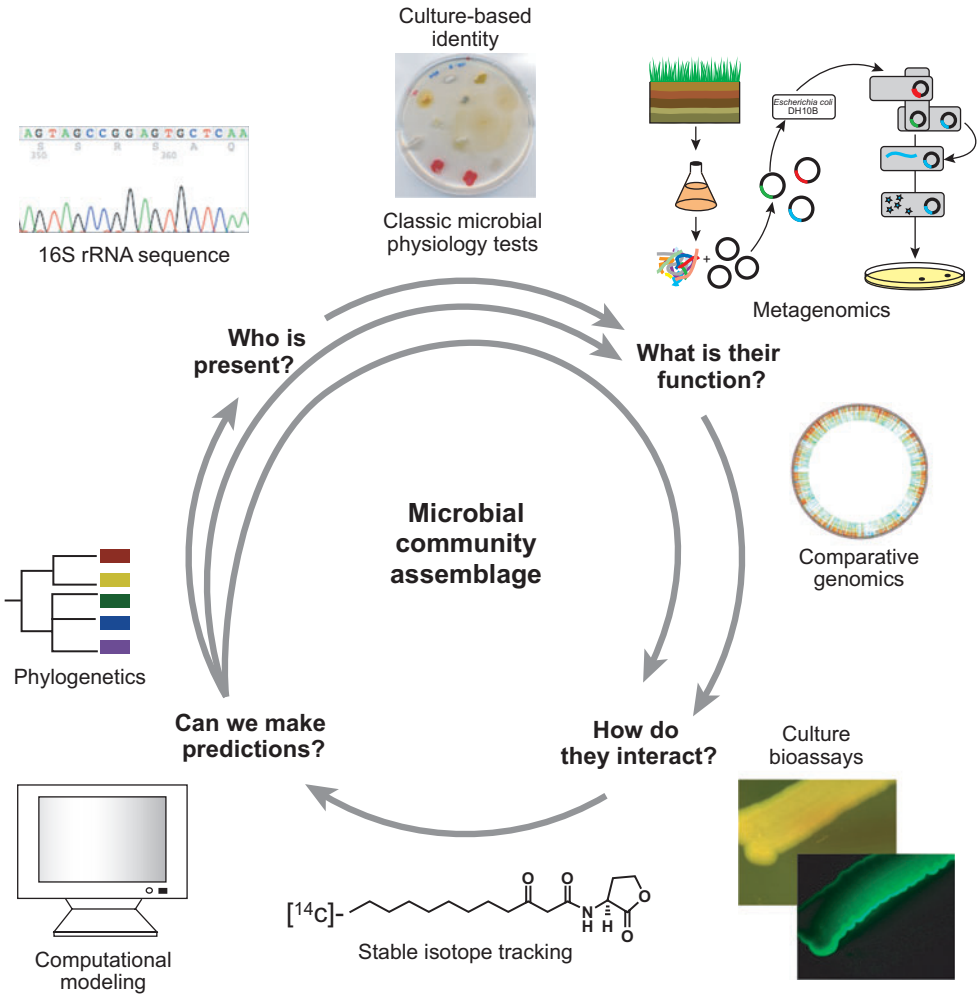

Figure 3

Four groups of questions in microbial ecology and some techniques used to address them.

further development of phylogenetic tools will reveal greater diversity and perhaps groups of organisms that are not suspected from current surveys.

What Are the Functions of Individual Organisms?

After identifying which organism(s) is present within a community, the next challenge for microbial ecologists is to identify which organism demonstrates each of the various metabolic processes in its native community.

Classic in vitro microbial physiology. Up until the last 15 years of the twentieth century, pure-culture experimental setup, as described above, was the central method of associating organisms with metabolic processes. In an effort to understand metabolic processes within the context of a microbe's native community, it was typical to inoculate enrichment cultures with samples from the natural environment of interest, determine which bacteria grow, and then make inferences on the basis of substrate use about the microbe's activity in its native community.

Metagenomics. The diversity of the as yet unculturable members of microbial communities is vast compared with that of the culturable members (9, 13, 14, 108, 118, 130, 156). To capture and study the functional diversity of these organisms, a new field designated metagenomics has sprung to life. Metagenomics is the culture-independent analysis of genomes from an assemblage of microorganisms. A metagenomic analysis entails extracting DNA directly from soil, cloning it into a culturable host bacterium, and analyzing it (169, 186). This method has recently been used for massive capture and sequencing of DNA from the Sargasso Sea (202), acid mine drainage (197), a Minnesota soil sample (196), and a global ocean survey (170). DeLong et al. (42) applied metagenomics to planktonic microbial communities in the North Pacific Subtropical Gyre, in which they identified stratified microbial communities through comparative genomics delineated by taxonomic zonation and functional and metabolic potential. Through detailed analysis of the genes in each stratum, they inferred the photosynthetic activity at various depths in the ocean. They also found a surprisingly high frequency of cyanophage-infected cells (up to 12%), which likely structure the planktonic community via predation.

Functional metagenomics. Entire phyla in soil are known only by their 16S rRNA gene signatures, with nothing known about their physiology, genetics, or role in the soil community. Most work in metagenomics is driven by sequence analysis, but this work is limited by the ability to recognize gene function on the basis of sequence alone. Because many of the genes isolated from the environment have no significant similarity to genes of known function, an alternative approach is to search for genes of a particular function by functional metagenomics. In functional metagenomics, genes are sought that confer a function of interest on a host bacterium. This method requires that the genes be expressed, but it does not require that their functions be recognizable by sequence (79, 83, 102, 103, 126, 188, 208).

Stable isotope probing. Stable isotope probing (SIP) involves introducing a stable isotope-labeled substrate into the community and tracking the movement of the substrate by extracting diagnostic molecules (e.g., lipids and nucleic acids) to determine which molecules have incorporated the substrate. Stable isotope ratios have been used by ecologists to follow resource use through trophic levels for decades (43, 44, 179). More recently, stable isotopes have become a tool used by microbial ecologists to track the movement of substrates through microbial communities and identify which community members utilize which substrates. The main advantages of SIP are that it does not rely on culturability, and it allows direct observation of substrate movement with minimal disruption of the natural environment and community. SIP in microbial communities has been recently reviewed elsewhere (106).

Single-cell analyses. Fully comprehending community function necessitates understanding the function and activities at all levels, including that of individual members. Individuals within a population vary in levels of expression of certain genes and growth rates (92). For example, using flow cells and laser scanning, Strovas et al. (189) revealed that individuals within *Methylobacterium extorquens* grown continuously vary in cell size at division, division time, and growth rate, and they respond differently to a substrate shift. In addition to explaining more about individual members of a community, single-cell analyses should also prove useful for studying rare and uncultured organisms. Although used to amplify low-quantity DNA for metagenomic studies, multiple displacement amplification, a technique that involves random primers and ϕ29 DNA polymerase), can also be used to amplify whole genomes of single cells (72, 81, 139). Multiple displacement amplification combined with technologies for capturing individual cells, such as microfluids, may lead to situations in which the genomes of rare members could be analyzed to determine the potential function of the member within its community.

Metagenomics: culture-independent analysis of DNA extracted directly from communities in environmental samples

How Do Organisms Interact?

Interactions are the fulcrum of communities. Studying them is essential to understanding community function, but that study is challenging. Lessons from study of cultured organisms can guide methodological choices. Culturing has been, by far, the most impactful method introduced into microbiology since the microscope. The study of organisms in pure culture has produced the staggering depth and breadth of current knowledge about cellular microbiology. Likewise, coculture can be used to study interactions in a controlled environment in which variables can be manipulated. Bacterial genetics has been similarly influential, providing experimental precision and rigor that elevate associations to causal relationships. The adaptation of cellular genetics to study interactions among organisms is likely to yield surprises and provide a foundation for community ecology principles.

Coculture experiments. Although the trend in the twentieth century was toward reduction, which meant studying microbes in isolation or sometimes pairs, studying organismal interactions in their native communities, or in assemblages that more closely resemble native communities, is not a recent idea. In 1895, Winogradsky isolated *Clostridium pasteurianum*, an organism that fixes free nitrogen from the air (163, 197). But through a series of pure-culture and coculture experiments, it became clear to Winogradsky that *C. pasteurianum*, a strict anaerobe, could only fix nitrogen under aerobic conditions. If *C. pasteurianum* was grown in close association with an extreme aerobe that essentially creates an anaerobic environment for *C. pasteurianum*, nitrogen fixation was restored. Studying microbial assemblages in culture remains an important method to investigate organismal interactions. In 1993 Gilbert and colleagues (64) reported that the application of *Bacillus cereus* UW85, an antibiotic-producing strain used for biological control, to the soybean rhizosphere increased the abundance of bacteria from the *Cytophaga-Flavobacterium* (CF) group. More recently, Peterson et al. (152) used coculturing to determine that the commensal relationship between *B. cereus* UW85 and the bacteria from the CF group is mediated by a *B. cereus* peptidoglycan. Although coculture experiments are invaluable to our understanding of interspecies interactions, a main restriction is that they are limited to a small number of species interacting together in liquid or on a plate.

Bacterial genetics. Just as bacterial genetics has provided profound insight into the function of organisms in pure culture, it can lead to an understanding of species interactions in communities. The foundation of genetics is construction of random mutations followed by mutant hunts. The randomness coupled with assessment of phenotype produces a minimally biased approach in which the genes required for a certain function or process are identified. In this age of gene arrays and genomics, mutant hunts have been replaced by more-directed methods, but broad searches remain critical to expanding our knowledge beyond the human imagination. Targeted approaches typically require the researcher to make educated predictions about the nature or type of genes involved in a process. Broad mutant hunts enable the bacteria to answer that question. Historically, the answers have been surprising, and many of the genes identified would not have been predicted to be involved in the process on the basis of sequence alone.

The community context presents new challenges for bacterial genetics. Identifying genes involved in community function requires complex screens that will be difficult to apply to large collections of mutants, but the search will be worthwhile. In addition, studies of targeted mutants in communities can be revealing. For example, the study of genes involved in quorum sensing in pure culture and in a community provides very different insights. The ubiquitous presence of genes involved in quorum-sensing indicates that density-dependent cell-cell communication is a common mode of bacterial communication (6, 127, 134). Demonstration of signal exchange between strains in a

community in a caterpillar gut provided surprising evidence of stability of quorum-sensing signal in a high pH environment (B. Borlee, G. Geske, C. Robinson, H. Blackwell & J. Handelsman, manuscript in revision). Genes that code for antibacterial compounds are found in several environmental isolates (16, 45, 51, 116). The role of antimicrobials in nature is controversial (16, 38, 45, 51, 116, 212). Some studies, however, have shown that producing these compounds yields a competitive advantage for bacteria (57, 63). An antibacterial protein produced by a marine bacterium is important for its competition with other marine bacteria for the formation of biofilms as well as dispersal of cells from biofilms (125, 160).

Can We Make Predictions at the Community Scale?

Perhaps the most important implication of information on ecological community principles and dynamics from both an application and a theoretical standpoint is the potential to build predictive models. By defining fundamental principles of microbial community ecology derived from phylogenetically and ecologically disparate systems amenable to experimental manipulation, we can generate models to predict information about other communities that are less tractable for study. Much of the baseline research needed to generate such models falls into the categories described above, namely, who is present in a community, what is their function, and how do they interact with one another. Advances in molecular biology, and computational biology in particular, have allowed development in predictive ecological modeling that is poised for application to microbial communities.

Integrative modeling. In parallel with the recurring theme of moving from reductionist science to systems-based biology, many current approaches toward understanding properties of microbial communities include integrating biochemistry, thermodynamics, metabolite transport and utilization, metagenomic sequencing, regulatory and metabolic network analysis, and comparative and evolutionary genomics. On the mechanistic scale, several groups aim to predict microbially mediated metabolic activities in specific environments using microarrays and to predict a microbe's behavior and lifestyle directly from its genomic sequence (4). On the ecological scale, ecological niche modeling packages can output predictions of geographic ranges for species on the basis of current species records and layers of environmental data (e.g., GARP, Genetic Algorithm for Rule-set Production).

Community genetics. An integrative field of study becoming increasingly important in generating predictions about how microbial community members interact is community genetics, which involves coupling changes in genetic distributions with species interactions and community structure (49, 206). A few studies of macroecological systems have generated predictions. For example, Whitham and colleagues found that genetic variation in plants affects diverse communities of insect herbivores, birds, and fungi (206). Other studies have shown that population dynamics and trophic interactions affect the rate at which pests evolve resistance to genetically engineered crops. Experimental results from a predator-prey system (rotifers that consume algae) provided the basis for building models for ecological and evolutionary hypotheses about the predator-prey cycles. These studies in situations involving rapid evolution model successfully predicted ecological consequences (180).

There has been little application of community genetics to make predictions of ecological phenomena in predominantly microbial communities, but it will be a fruitful avenue of research. Community genetics in microbial communities has the special power of using constructed, defined mutants that can be introduced into a community. The behavior of the community in the presence of the mutant and wild type can be compared, providing insight into the role of a single gene in a population of a species and in the community

Community genetics: studies of the interplay between community genetic composition and community structure and function

context. Just as bacterial genetics brought power and precision to the dissection of bacterial cell processes in the twentieth century, the same approaches will transform our understanding of community processes in the twenty-first century.

SUMMARY POINTS

1. An important feature of microbial communities is robustness, which has structural and functional components.
2. Understanding microbial community ecology necessitates study of evolutionary mechanisms that underlie community structure.
3. Recent advances in molecular biology provide a means to address questions about microbial communities, including both culturable and as yet uncultured members.
4. Microbial communities offer a unique opportunity to apply community genetics in a manner that is difficult in most macroorganism communities: Introduction of defined mutants into communities will advance our understanding of the interplay between community genetic composition and community structure and function under various selection pressures.
5. The approaches described here will contribute to the inputs needed to build and test predictive models that will elucidate principles that govern community interactions, providing a set of rules of engagement among community members that dictate community structure and function.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We are grateful for support from an Interdisciplinary Hatch Project from the University of Wisconsin College of Agricultural and Life Sciences and the Howard Hughes Medical Institute. We thank Karen Cloud-Hansen for helpful discussions of an earlier draft of the manuscript.

LITERATURE CITED

1. Adamczyk J, Hesselsoe M, Iversen N, Horn M, Lehner A, et al. 2003. The isotope array, a new tool that employs substrate-mediated labeling of rRNA for determination of microbial community structure and function. *Appl. Environ. Microbiol.* 69:6875–87
2. Alonso-Saez L, Balague V, Sa EL, Sanchez O, Gonzalez JM, et al. 2007. Seasonality in bacterial diversity in north-west Mediterranean coastal waters: assessment through clone libraries, fingerprinting and FISH. *FEMS Microbiol. Ecol.* 60:98–112
3. An D, Danhorn T, Fuqua C, Parsek MR. 2006. Quorum sensing and motility mediate interactions between *Pseudomonas aeruginosa* and *Agrobacterium tumefaciens* in biofilm cocultures. *Proc. Natl. Acad. Sci. USA* 103:3828–33
4. Antonovics J. 1992. Towards community genetics. In *Plant Resistance to Herbivores and Pathogens: Ecology, Evolution, and Genetics*, ed. RS Fritz, EL Simms, pp. 426–49. Chicago: Univ. Chicago Press

5. Ashelford KE, Day MJ, Fry JC. 2003. Elevated abundance of bacteriophage infecting bacteria in soil. *Appl. Environ. Microbiol.* 69:285–89
6. Bassler BL. 1999. How bacteria talk to each other: regulation of gene expression by quorum sensing. *Curr. Opin. Microbiol.* 2:582–87
7. Beardsley C, Pernthaler J, Wosniok W, Amann R. 2003. Are readily culturable bacteria in coastal North Sea waters suppressed by selective grazing mortality? *Appl. Environ. Microbiol.* 69:2624–30
8. Begon M, Harper J, Townsend C. 1990. *Ecology: Individuals, Populations, and Communities*. Cambridge, MA: Blackwell. 945 pp.
9. Bintrim SB, Donohue TJ, Handelsman J, Roberts GP, Goodman RM. 1997. Molecular phylogeny of Archaea from soil. *Proc. Natl. Acad. Sci. USA* 94:277–82
10. Boetius A, Ravenschlag K, Schubert CJ, Rickert D, Widdel F, et al. 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature* 407:623–26
11. Bond PL, Hugenholtz P, Keller J, Blackall LL. 1995. Bacterial community structures of phosphate-removing and nonphosphate-removing activated sludges from sequencing batch reactors. *Appl. Environ. Microbiol.* 61:1910–16
12. Bonsall RF, Weller DM, Thomashow LS. 1997. Quantification of 2,4-diacetylphloroglucinol produced by fluorescent *Pseudomonas* spp. in vitro and in the rhizosphere of wheat. *Appl. Environ. Microbiol.* 63:951–55
13. Borneman J, Skroch PW, O'Sullivan KM, Palus JA, Rumjanek NG, et al. 1996. Molecular microbial diversity of an agricultural soil in Wisconsin. *Appl. Environ. Microbiol.* 62:1935–43
14. Borneman J, Triplett EW. 1997. Molecular microbial diversity in soils from eastern Amazonia: evidence for unusual microorganisms and microbial population shifts associated with deforestation. *Appl. Environ. Microbiol.* 63:2647–53
15. Breitbart M, Wegley L, Leeds S, Schoenfeld T, Rohwer F. 2004. Phage community dynamics in hot springs. *Appl. Environ. Microbiol.* 70:1633–40
16. Brinkhoff T, Bach G, Heidorn T, Liang L, Schlingloff A, Simon M. 2004. Antibiotic production by a Roseobacter clade-affiliated species from the German Wadden Sea and its antagonistic effects on indigenous isolates. *Appl. Environ. Microbiol.* 70:2560–65
17. Brodie EL, DeSantis TZ, Parker JPM, Zubietta IX, Piceno YM, Andersen GL. 2007. Urban aerosols harbor diverse and dynamic bacterial populations. *Proc. Natl. Acad. Sci. USA* 104:299–304
18. Bronstein J, Barbosa P. 2002. Multi-trophic/multi-species interactions: the role of nonmutualists in shaping and mediating mutualisms. In *Multitrophic Interactions*, ed. T Tscharntke, BA Hawkins, pp. 44–66. Cambridge, UK: Cambridge Univ. Press
19. Cardoza YJ, Klepzig KD, Raffa KF. 2006. Bacteria in oral secretions of an endophytic insect inhibit antagonistic fungi. *Ecol. Entomol.* 31:636–45
20. Carrino-Kyker SR, Swanson AK. 2008. Temporal and spatial patterns of eukaryotic and bacterial communities found in vernal pools. *Appl. Environ. Microbiol.* 74:2554–57
21. Case RJ, Boucher Y, Dahllof I, Holmstrom C, Doolittle WF, Kjelleberg S. 2007. Use of 16S rRNA and *rpoB* genes as molecular markers for microbial ecology studies. *Appl. Environ. Microbiol.* 73:278–88
22. Chandler DP, Jarrell AE, Roden ER, Golova J, Chernov B, et al. 2006. Suspension array analysis of 16S rRNA from Fe- and SO42-reducing bacteria in uranium-contaminated sediments undergoing bioremediation. *Appl. Environ. Microbiol.* 72:4672–87
23. Chenier MR, Beaumier D, Fortin N, Roy R, Driscoll BT, et al. 2006. Influence of nutrient inputs, hexadecane, and temporal variations on denitrification and community composition of river biofilms. *Appl. Environ. Microbiol.* 72:575–84
24. Chu H, Fujii T, Morimoto S, Lin X, Yagi K, et al. 2007. Community structure of ammonia-oxidizing bacteria under long-term application of mineral fertilizer and organic manure in a sandy loam soil. *Appl. Environ. Microbiol.* 73:485–91
25. Clarholm M. 2002. Bacteria and protozoa as integral components of the forest ecosystem—their role in creating a naturally varied soil fertility. *Antonie van Leeuwenhoek* 81:309–18
26. Colwell RK. 2005. EstimateS Ver. 7.5: statistical estimation of species richness and shared species from samples. (Software and User's Guide) **http://viceroy.eeb.uconn.edu/EstimateSPages/EstimateSSupport.htm**

27. Compant S, Duffy B, Nowak J, Clement C, Barka EA. 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. *Appl. Environ. Microbiol.* 71:4951–59
28. Cottrell MT, Kirchman DL. 2000. Natural assemblages of marine Proteobacteria and members of the Cytophaga-Flavobacter cluster consuming low- and high-molecular-weight dissolved organic matter. *Appl. Environ. Microbiol.* 66:1692–97
29. Cowan SE, Gilbert E, Liepmann D, Keasling JD. 2000. Commensal interactions in a dual-species biofilm exposed to mixed organic compounds. *Appl. Environ. Microbiol.* 66:4481–85
30. Cox CR, Gilmore MS. 2007. Native microbial colonization of *Drosophila melanogaster* and its use as a model of *Enterococcus faecalis* pathogenesis. *Infect. Immun.* 75:1565–76
31. Currie CR, Poulsen M, Mendenhall J, Boomsma JJ, Billen J. 2006. Coevolved crypts and exocrine glands support mutualistic bacteria in fungus-growing ants. *Science* 311:81–83
32. Curtis TP, Head IM, Lunn M, Woodcock S, Schloss PD, Sloan WT. 2006. What is the extent of prokaryotic diversity? *Philos. Trans. R. Soc. London Ser. B* 361:2023–37
33. Curtis TP, Sloan WT. 2004. Prokaryotic diversity and its limits: microbial community structure in nature and implications for microbial ecology. *Curr. Opin. Microbiol.* 7:221–26
34. Curtis TP, Sloan WT, Scannell JW. 2002. Estimating prokaryotic diversity and its limits. *Proc. Natl. Acad. Sci. USA* 99:10494–99
35. Darenfed H, Grenier D, Mayrand D. 1999. Acquisition of plasmin activity by *Fusobacterium nucleatum* subsp. *nucleatum* and potential contribution to tissue destruction during periodontitis. *Infect. Immun.* 67:6439–44
36. Davidov Y, Friedjung A, Jurkevitch E. 2006. Structure analysis of a soil community of predatory bacteria using culture-dependent and culture-independent methods reveals a hitherto undetected diversity of *Bdellovibrio*-and-like organisms. *Environ. Microbiol.* 8:1667–73
37. Davidov Y, Huchon D, Koval SF, Jurkevitch E. 2006. A new alpha-proteobacterial clade of *Bdellovibrio*-like predators: implications for the mitochondrial endosymbiotic theory. *Environ. Microbiol.* 8:2179–88
38. Davies J. 2006. Are antibiotics naturally antibiotics? *J. Ind. Microbiol. Biotechnol.* 33:496–99
39. Davies J, Spiegelman GB, Yim G. 2006. The world of subinhibitory antibiotic concentrations. *Antimicrobials/Genomics* 9:445–53
40. de Carcer DA, Martin M, Karlson U, Rivilla R. 2007. Changes in bacterial populations and in biphenyl dioxygenase gene diversity in a polychlorinated biphenyl-polluted soil after introduction of willow trees for rhizoremediation. *Appl. Environ. Microbiol.* 73:6224–32
41. de la Cruz F, Davies J. 2000. Horizontal gene transfer and the origin of species: lessons from bacteria. *Trends Microbiol.* 8:128–33
42. DeLong EF, Preston CM, Mincer T, Rich V, Hallam SJ, et al. 2006. Community genomics among stratified microbial assemblages in the ocean's interior. *Science* 311:496–503
43. Deniro MJ, Epstein S. 1978. Influence of diet on distribution of carbon isotopes in animals. *Geochim. Cosmochim. Acta* 42:495–506
44. Deniro MJ, Epstein S. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. *Geochim. Cosmochim. Acta* 45:341–51
45. Derzelle S, Duchaud E, Kunst F, Danchin A, Bertin P. 2002. Identification, characterization, and regulation of a cluster of genes involved in carbapenem biosynthesis in *Photorhabdus luminescens*. *Appl. Environ. Microbiol.* 68:3780–89
46. Diaz PI, Chalmers NI, Rickard AH, Kong C, Milburn CL, et al. 2006. Molecular characterization of subject-specific oral microflora during initial colonization of enamel. *Appl. Environ. Microbiol.* 72:2837–48
47. Drakare S. 2002. Competition between picoplanktonic cyanobacteria and heterotrophic bacteria along crossed gradients of glucose and phosphate. *Microb. Ecol.* 44:327–35
48. Drzyzga O, Gottschal JC. 2002. Tetrachloroethene dehalorespiration and growth of *Desulfitobacterium frappieri* TCE1 in strict dependence on the activity of *Desulfovibrio fructosivorans*. *Appl. Environ. Microbiol.* 68:642–49
49. Dungey HS, Potts BM, Whitham TG, Li HF. 2000. Plant genetics affects arthropod community richness and composition: evidence from a synthetic eucalypt hybrid population. *Evol. Int. J. Org. Evol.* 54:1938–46

50. Dykhuizen DE. 1998. Santa Rosalia revisited: Why are there so many species of bacteria? *Antonie van Leeuwenhoek* 73:25–33
51. Emmert EAB, Klimowicz AK, Thomas MG, Handelsman J. 2004. Genetics of zwittermicin A production by *Bacillus cereus*. *Appl. Environ. Microbiol.* 70:104–13
52. Estes JA. 1996. Predators and ecosystem management. *Wildl. Soc. Bull.* 24:390–96
53. Estes JA, Crooks K, Holt R. 2001. Predators, ecological role of. In *Encyclopedia of Biodiversity*, ed. SA Levin, 4:857–78. San Diego, CA: Academic Press
54. Feil EJ, Holmes EC, Bessen DE, Chan MS, Day NP, et al. 2001. Recombination within natural populations of pathogenic bacteria: short-term empirical estimates and long-term phylogenetic consequences. *Proc. Natl. Acad. Sci. USA* 98:182–87
55. Fiegna F, Velicer GJ. 2005. Exploitative and hierarchical antagonism in a cooperative bacterium. *PLoS Biol.* 3:e370
56. Francis CA, Roberts KJ, Beman JM, Santoro AE, Oakley BB. 2005. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *Proc. Natl. Acad. Sci. USA* 102:14683–88
57. Franks A, Egan S, Holmstrom C, James S, Lappin-Scott H, Kjelleberg S. 2006. Inhibition of fungal colonization by *Pseudoalteromonas tunicata* provides a competitive advantage during surface colonization. *Appl. Environ. Microbiol.* 72:6079–87
58. Frostl JM, Overmann J. 1998. Physiology and tactic response of the phototrophic consortium "Chlorochromatium aggregatum." *Arch. Microbiol.* 169:129–35
59. Frostl JM, Overmann J. 2000. Phylogenetic affiliation of the bacteria that constitute phototrophic consortia. *Arch. Microbiol.* 174:50–58
60. Gans J, Wolinsky M, Dunbar J. 2005. Computational improvements reveal great bacterial diversity and high metal toxicity in soil. *Science* 309:1387–90
61. Gentile ME, Lynn Nyman J, Criddle CS. 2007. Correlation of patterns of denitrification instability in replicated bioreactor communities with shifts in the relative abundance and the denitrification patterns of specific populations. *ISME J.* 1:714–28
62. Geraci A. 1991. *IEEE Standard Computer Dictionary: Compilation of IEEE Standard Computer Glossaries*. New York: Inst. Elect. Electron. Eng. Inc.
63. Giddens SR, Houliston GJ, Mahanty HK. 2003. The influence of antibiotic production and pre-emptive colonization on the population dynamics of *Pantoea agglomerans* (*Erwinia herbicola*) Eh1087 and *Erwinia amylovora* in planta. *Environ. Microbiol.* 5:1016–21
64. Gilbert G, Parke JL, Clayton MK, Handelsman J. 1993. Effects of an introduced bacterium on bacterial communities on roots. *Ecology* 74:840–54
65. Gil-Turnes MS, Fenical W. 1992. Embryos of *Homarus americanus* are protected by epibiotic bacteria. *Biol. Bull.* 182:105–8
66. Gil-Turnes MS, Hay ME, Fenical W. 1989. Symbiotic marine bacteria chemically defend crustacean embryos from a pathogenic fungus. *Science* 246:116–18
67. Girvan MS, Campbell CD, Killham K, Prosser JI, Glover LA. 2005. Bacterial diversity promotes community stability and functional resilience after perturbation. *Environ. Microbiol.* 7:301–13
68. Glaeser J, Overmann J. 2003. Characterization and in situ carbon metabolism of phototrophic consortia. *Appl. Environ. Microbiol.* 69:3739–50
69. Glaeser J, Overmann J. 2003. The significance of organic carbon compounds for in situ metabolism and chemotaxis of phototrophic consortia. *Environ. Microbiol.* 5:1053–63
70. Gogarten JP, Doolittle WF, Lawrence JG. 2002. Prokaryotic evolution in light of gene transfer. *Mol. Biol. Evol.* 19:2226–38
71. Goldman BS, Nierman WC, Kaiser D, Slater SC, Durkin AS, et al. 2006. Evolution of sensory complexity recorded in a myxobacterial genome. *Proc. Natl. Acad. Sci. USA* 103:15200–5
72. Gonzalez JM, Portillo MC, Saiz-Jimenez C. 2005. Multiple displacement amplification as a prepolymerase chain reaction (pre-PCR) to process difficult to amplify samples and low copy number sequences from natural environments. *Environ. Microbiol.* 7:1024–28
73. Gopal PK, Prasad J, Smart J, Gill HS. 2001. In vitro adherence properties of *Lactobacillus rhamnosus* DR20 and *Bifidobacterium lactis* DR10 strains and their antagonistic activity against an enterotoxigenic *Escherichia coli*. *Int. J. Food Microbiol.* 67:207–16

74. Guarner F, Malagelada JR. 2003. Gut flora in health and disease. *Lancet* 361:512–19
75. Hallam SJ, Putnam N, Preston CM, Detter JC, Rokhsar D, et al. 2004. Reverse methanogenesis: testing the hypothesis with environmental genomics. *Science* 305:1457–62
76. Handelsman J. 2004. Metagenomics: application of genomics to uncultured microorganisms. *Microbiol. Mol. Biol. Rev.* 68:669–85
77. Hansel CM, Fendorf S, Jardine PM, Francis CA. 2008. Changes in bacterial and archaeal community structure and functional diversity along a geochemically variable soil profile. *Appl. Environ. Microbiol.* 74:1620–33
78. Hartmann M, Widmer F. 2006. Community structure analyses are more sensitive to differences in soil bacterial communities than anonymous diversity indices. *Appl. Environ. Microbiol.* 72:7804–12
79. Healy FG, Ray RM, Aldrich HC, Wilkie AC, Ingram LO, Shanmugam KT. 1995. Direct isolation of functional genes encoding cellulases from the microbial consortia in a thermophilic, anaerobic digester maintained on lignocellulose. *Appl. Microbiol. Biotechnol.* 43:667–74
80. Heimann AC, Batstone DJ, Jakobsen R. 2006. *Methanosarcina* spp. drive vinyl chloride dechlorination via interspecies hydrogen transfer. *Appl. Environ. Microbiol.* 72:2942–49
81. Hellani A, Coskun S, Sakati N, Benkhalifa M, Al-Odaib A, Ozand P. 2004. Multiple displacement amplification on single cell and possible preimplantation genetic diagnosis applications. *Fertil. Steril.* 82:S28
82. Henke SE, Bryant FC. 1999. Effects of coyote removal on the faunal community in western Texas. *J. Wildl. Manag.* 63:1066–81
83. Henne ADR, Schmitz RA, Gottschalk G. 1999. Construction of environmental DNA libraries in *Escherichia coli* and screening for the presence of genes conferring utilization of 4-hydroxybutyrate. *Appl. Environ. Microbiol.* 65:3901–7
84. Hoffmeister M, Martin W. 2003. Interspecific evolution: microbial symbiosis, endosymbiosis and gene transfer. *Environ. Microbiol.* 5:641–49
85. Hooper LV. 2004. Bacterial contributions to mammalian gut development. *Trends Microbiol.* 12:129–34
86. Hooper LV, Midtvedt T, Gordon JI. 2002. How host-microbial interactions shape the nutrient environment of the mammalian intestine. *Annu. Rev. Nutr.* 22:283–307
87. Huber H, Hohn MJ, Rachel R, Fuchs T, Wimmer VC, Stetter KO. 2002. A new phylum of Archaea represented by a nanosized hyperthermophilic symbiont. *Nature* 417:63–67
88. Isenbarger TA, Finney M, Ríos-Velázquez C, Handelsman J, Ruvkun G. 2008. Miniprimer PCR, a new lens for viewing the microbial world. *Appl. Environ. Microbiol.* 74:840–49
89. Ives AR, Carpenter SR. 2007. Stability and diversity of ecosystems. *Science* 317:58–62
90. Jurkevitch E, Minz D, Ramati B, Barel G. 2000. Prey range characterization, ribotyping, and diversity of soil and rhizosphere *Bdellovibrio* spp. isolated on phytopathogenic bacteria. *Appl. Environ. Microbiol.* 66:2365–71
91. Kadurugamuwa JL, Beveridge TJ. 1996. Bacteriolytic effect of membrane vesicles from *Pseudomonas aeruginosa* on other bacteria including pathogens: conceptually new antibiotics. *J. Bacteriol.* 178:2767–74
92. Kaern M, Elston TC, Blake WJ, Collins JJ. 2005. Stochasticity in gene expression: from theories to phenotypes. *Nat. Rev. Genet.* 6:451–64
93. Kaltenpoth M, Gottler W, Herzner G, Strohm E. 2005. Symbiotic bacteria protect wasp larvae from fungal infestation. *Curr. Biol.* 15:475–79
94. Kan J, Suzuki MT, Wang K, Evans SE, Chen F. 2007. High temporal but low spatial heterogeneity of bacterioplankton in the Chesapeake Bay. *Appl. Environ. Microbiol.* 73:6776–89
95. Kanzler BEM, Pfannes KR, Vogl K, Overmann J. 2005. Molecular characterization of the nonphotosynthetic partner bacterium in the consortium "Chlorochromatium aggregatum." *Appl. Environ. Microbiol.* 71:7434–41
96. Katayama T, Tanaka M, Moriizumi J, Nakamura T, Brouchkov A, et al. 2007. Phylogenetic analysis of bacteria preserved in a permafrost ice wedge for 25,000 years. *Appl. Environ. Microbiol.* 73:2360–63
97. Keel C, Schnider U, Maurhofer M, Voisard C, Laville J, et al. 1992. Suppression of root diseases by *Pseudomonas fluorescens* CHA 0: importance of the bacterial secondary metabolite 2,4-diacetylphloroglucinol. *Mol. Plant-Microbe Interact.* 5:4–13

98. Kent AD, Jones SE, Lauster GH, Graham JM, Newton RJ, McMahon KD. 2006. Experimental manipulations of microbial food web interactions in a humic lake: shifting biological drivers of bacterial community structure. *Environ. Microbiol.* 8:1448–59
99. Kikuchi Y, Graf J. 2007. Spatial and temporal population dynamics of a naturally occurring two-species microbial community inside the digestive tract of the medicinal leech. *Appl. Environ. Microbiol.* 73:1984–91
100. Kitano H. 2002. Computational systems biology. *Nature* 420:206–10
101. Kitano H. 2003. Cancer robustness: tumour tactics. *Nature* 426:125
102. Knietsch A, Bowien S, Whited G, Gottschalk G, Daniel R. 2003. Identification and characterization of coenzyme B-12-dependent glycerol dehydratase- and diol dehydratase-encoding genes from metagenomic DNA libraries derived from enrichment cultures. *Appl. Environ. Microbiol.* 69:3048–60
103. Knietsch A, Waschkowitz T, Bowien S, Henne A, Daniel R. 2003. Metagenomes of complex microbial consortia derived from different soils as sources for novel genes conferring formation of carbonyls from short-chain polyols on *Escherichia coli*. *J. Mol. Microbiol. Biotechnol.* 5:46–56
104. Kolenbrander PE, Andersen RN, Blehert DS, Egland PG, Foster JS, Palmer RJ Jr. 2002. Communication among oral bacteria. *Microbiol. Mol. Biol. Rev.* 66:486–505
105. Kolenbrander PE, Andersen RN, Moore LV. 1990. Intrageneric coaggregation among strains of human oral bacteria: potential role in primary colonization of the tooth surface. *Appl. Environ. Microbiol.* 56:3890–94
106. Kreuzer-Martin HW. 2007. Stable isotope probing: linking functional activity to specific members of microbial communities. *Soil Sci. Soc. Am. J.* 71:611–19
107. Kunin V, He S, Warnecke F, Peterson SB, Garcia Martin H, et al. 2008. A bacterial metapopulation adapts locally to phage predation despite global dispersal. *Genome Res.* 18:293–97
108. Kuske CR, Ticknor LO, Miller ME, Dunbar JM, Davis JA, et al. 2002. Comparison of soil bacterial communities in rhizospheres of three plant species and the interspaces in an arid grassland. *Appl. Environ. Microbiol.* 68:1854–63
109. Lamont RJ, El-Sabaeny A, Park Y, Cook GS, Costerton JW, Demuth DR. 2002. Role of the *Streptococcus gordonii* SspB protein in the development of *Porphyromonas gingivalis* biofilms on streptococcal substrates. *Microbiology* 148:1627–36
110. Leadbetter JR, Greenberg EP. 2000. Metabolism of acyl-homoserine lactone quorum-sensing signals by *Variovorax paradoxus*. *J. Bacteriol.* 182:6921–26
111. LeBlanc PM, Hamelin RC, Filion M. 2007. Alteration of soil rhizosphere communities following genetic transformation of white spruce. *Appl. Environ. Microbiol.* 73:4128–34
112. Lee YK, Puong KY. 2002. Competition for adhesion between probiotics and human gastrointestinal pathogens in the presence of carbohydrate. *Br. J. Nutr.* 88(Suppl. 1):S101–8
113. Lepere C, Boucher D, Jardillier L, Domaizon I, Debroas D. 2006. Succession and regulation factors of small eukaryote community composition in a lacustrine ecosystem (Lake Pavin). *Appl. Environ. Microbiol.* 72:2971–81
114. Levin BR. 1981. Periodic selection, infectious gene exchange, and the genetic structure of *E. coli* populations. *Genetics* 99:1–23
115. Levin BR, Bergstrom CT. 2000. Bacteria are different: observations, interpretations, speculations, and opinions about the mechanisms of adaptive evolution in prokaryotes. *Proc. Natl. Acad. Sci. USA* 97:6981–85
116. Li J, Beatty PK, Shah S, Jensen SE. 2007. Use of PCR-targeted mutagenesis to disrupt production of fusaricidin-type antifungal antibiotics in *Paenibacillus polymyxa*. *Appl. Environ. Microbiol.* 73:3480–89
117. Li Z, Clarke AJ, Beveridge TJ. 1998. Gram-negative bacteria produce membrane vesicles which are capable of killing other bacteria. *J. Bacteriol.* 180:5478–83
118. Liles MR, Manske BF, Bintrim SB, Handelsman J, Goodman RM. 2003. A census of rRNA genes and linked genomic sequences within a soil metagenomic library. *Appl. Environ. Microbiol.* 69:2684–91
119. Lin Y-H, Xu J-L, Hu J, Wang L-H, Ong SL, et al. 2003. Acyl-homoserine lactone acylase from *Ralstonia* strain XJ12B represents a novel and potent class of quorum-quenching enzymes. *Mol. Microbiol.* 47:849–60

120. Little AEF, Currie CR. 2008. Black yeast symbionts compromise the efficiency of antibiotic defenses in fungus-growing ants. *Ecology* 89:1216–22
121. Little AEF, Murakami T, Mueller UG, Currie CR. 2006. Defending against parasites: Fungus-growing ants combine specialized behaviours and microbial symbionts to protect their fungus gardens. *Biol. Lett.* 2:12–16
122. Lo I, Denef VJ, Verberkmoes NC, Shah MB, Goltsman D, et al. 2007. Strain-resolved community proteomics reveals recombining genomes of acidophilic bacteria. *Nature* 446:537–41
123. Lozupone C, Knight R. 2005. UniFrac: a new phylogenetic method for comparing microbial communities. *Appl. Environ. Microbiol.* 71:8228–35
124. Lozupone CA, Hamady M, Kelley ST, Knight R. 2007. Quantitative and qualitative beta diversity measures lead to different insights into factors that structure microbial communities. *Appl. Environ. Microbiol.* 73:1576–85
125. Mai-Prochnow A, Evans F, Dalisay-Saludes D, Stelzer S, Egan S, et al. 2004. Biofilm development and cell death in the marine bacterium *Pseudoalteromonas tunicata*. *Appl. Environ. Microbiol.* 70:3232–38
126. Majernik A, Gottschalk G, Daniel R. 2001. Screening of environmental DNA libraries for the presence of genes conferring Na(+)(Li(+))/H(+) antiporter activity on *Escherichia coli*: characterization of the recovered genes and the corresponding gene products. *J. Bacteriol.* 183:6645–53
127. Manefield M, Turner SL. 2002. Quorum sensing in context: out of molecular biology and into microbial ecology. *Microbiol.* 148:3762–64
128. Martin AP. 2002. Phylogenetic approaches for describing and comparing the diversity of microbial communities. *Appl. Environ. Microbiol.* 68:3673–82
129. Mazzola M, Cook RJ, Thomashow LS, Weller DM, Pierson LS 3rd. 1992. Contribution of phenazine antibiotic biosynthesis to the ecological competence of fluorescent pseudomonads in soil habitats. *Appl. Environ. Microbiol.* 58:2616–24
130. McCaig AE, Grayston SJ, Prosser JI, Glover LA. 2001. Impact of cultivation on characterisation of species composition of soil bacterial communities. *FEMS Microbiol. Ecol.* 35:37–48
131. McCann KS. 2000. The diversity-stability debate. *Nature* 405:228–33
132. McCliment EA, Voglesonger KM, O'Day PA, Dunn EE, Holloway JR, Cary SC. 2006. Colonization of nascent, deep-sea hydrothermal vents by a novel archaeal and nanoarchaeal assemblage. *Environ. Microbiol.* 8:114–25
133. McGrady-Steed J, Harris PM, Morin PJ. 1997. Biodiversity regulates ecosystem predictability. *Nature* 390:162–65
134. Miller MB, Bassler BL. 2001. Quorum sensing in bacteria. *Annu. Rev. Microbiol.* 55:165–99
135. Moss JA, Nocker A, Lepo JE, Snyder RA. 2006. Stability and change in estuarine biofilm bacterial community diversity. *Appl. Environ. Microbiol.* 72:5679–88
136. Moyer CL, Dobbs FC, Karl DM. 1994. Estimation of diversity and community structure through restriction fragment length polymorphism distribution analysis of bacterial 16S rRNA genes from a microbial mat at an active, hydrothermal vent system, Loihi Seamount, Hawaii. *Appl. Environ. Microbiol.* 60:871–79
137. Murase J, Noll M, Frenzel P. 2006. Impact of protists on the activity and structure of the bacterial community in a rice field soil. *Appl. Environ. Microbiol.* 72:5436–44
138. Nakayama J, Tanaka E, Kariyama R, Nagata K, Nishiguchi K, et al. 2007. Siamycin attenuates fsr quorum sensing mediated by a gelatinase biosynthesis-activating pheromone in *Enterococcus faecalis*. *J. Bacteriol.* 189:1358–65
139. Neufeld JD, Chen Y, Dumont MG, Murrell JC. 2008. Marine methylotrophs revealed by stable-isotope probing, multiple displacement amplification and metagenomics. *Environ. Microbiol.* 10:1526–35
140. Neuhauser C, Andow DA, Heimpel GE, May G, Shaw RG, Wagenius S. 2003. Community genetics: expanding the synthesis of ecology and genetics. *Ecology* 84:545–58
141. Ochman H, Lawrence JG, Groisman EA. 2000. Lateral gene transfer and the nature of bacterial innovation. *Nature* 405:299–304
142. Pace NR. 1995. Opening the door onto the natural microbial world: molecular microbial ecology. *Harvey Lect.* 91:59–78

143. Pace NR. 1997. A molecular view of microbial diversity and the biosphere. *Science* 276:734–40
144. Pace NR, Stahl DA, Lane DJ, Olsen GJ. 1985. Analyzing natural microbial populations by rRNA sequences. *ASM News* 51:4–12
145. Paine RT. 1969. A note on trophic complexity and community stability. *Am. Nat.* 103:91–93
146. Papadopoulos D, Schneider D, Meier-Eiss J, Arber W, Lenski RE, Blot M. 1999. Genomic evolution during a 10,000-generation experiment with bacteria. *Proc. Natl. Acad. Sci. USA* 96:3807–12
147. Paper W, Jahn U, Hohn MJ, Kronner M, Nather DJ, et al. 2007. *Ignicoccus hospitalis* sp. nov., the host of 'Nanoarchaeum equitans.' *Int. J. Syst. Evol. Microbiol.* 57:803–8
148. Perez MT, Sommaruga R. 2007. Interactive effects of solar radiation and dissolved organic matter on bacterial activity and community structure. *Environ. Microbiol.* 9:2200–10
149. Perna NT, Plunkett G 3rd, Burland V, Mau B, Glasner JD, et al. 2001. Genome sequence of enterohaemorrhagic *Escherichia coli* O157:H7. *Nature* 409:529–33
150. Pernthaler J. 2005. Predation on prokaryotes in the water column and its ecological implications. *Nat. Rev. Microbiol.* 3:537–46
151. Peterson SB. 2008. *Interactions between rhizosphere bacteria*. PhD thesis. Univ. Wis.-Madison
152. Peterson SB, Dunn AK, Klimowicz AK, Handelsman J. 2006. Peptidoglycan from *Bacillus cereus* mediates commensalism with rhizosphere bacteria from the Cytophaga-Flavobacterium group. *Appl. Environ. Microbiol.* 72:5421–27
153. Pierson LS, Pierson EA. 1996. Phenazine antibiotic production in *Pseudomonas aureofaciens*: role in rhizosphere ecology and pathogen suppression. *FEMS Microbiol. Lett.* 136:101–8
154. Pineiro SA, Sahaniuk GE, Romberg E, Williams HN. 2004. Predation pattern and phylogenetic analysis of *Bdellovibrionaceae* from the Great Salt Lake, Utah. *Curr. Microbiol.* 48:113–17
155. Pineiro SA, Stine OC, Chauhan A, Steyert SR, Smith R, Williams HN. 2007. Global survey of diversity among environmental saltwater *Bacteriovoracaceae*. *Environ. Microbiol.* 9:2441–50
156. Quaiser A, Ochsenreiter T, Klenk HP, Kletzin A, Treusch AH, et al. 2002. First insight into the genome of an uncultivated crenarchaeote from soil. *Environ. Microbiol.* 4:603–11
157. Raaijmakers JM, Vlami M, de Souza JT. 2002. Antibiotic production by bacterial biocontrol agents. *Antonie van Leeuwenhoek* 81:537–47
158. Raffa KF. 2004. Transgenic resistance in short-rotation plantation trees. In *The Bioengineered Forest: Challenges for Science and Society*, ed. SH Strauss, HD Bradshaw, pp. 208–27. Washington, DC: RFF Press
159. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. 2004. Recognition of commensal microflora by Toll-like receptors is required for intestinal homeostasis. *Cell* 118:229–41
160. Rao D, Webb JS, Kjelleberg S. 2005. Competitive interactions in mixed-species biofilms containing the marine bacterium *Pseudoalteromonas tunicata*. *Appl. Environ. Microbiol.* 71:1729–36
161. Rastall RA, Gibson GR, Gill HS, Guarner F, Klaenhammer TR, et al. 2005. Modulation of the microbial ecology of the human colon by probiotics, prebiotics and synbiotics to enhance human health: an overview of enabling science and potential applications. *FEMS Microbiol. Ecol.* 52:145–52
162. Reichenbach H. 1999. The ecology of the myxobacteria. *Environ. Microbiol.* 1:15–21
163. Rhee SK, Liu XD, Wu LY, Chong SC, Wan XF, Zhou JZ. 2004. Detection of genes involved in biodegradation and biotransformation in microbial communities by using 50-mer oligonucleotide microarrays. *Appl. Environ. Microbiol.* 70:4303–17
164. Riley MS, Cooper VS, Lenski RE, Forney LJ, Marsh TL. 2001. Rapid phenotypic change and diversification of a soil bacterium during 1000 generations of experimental evolution. *Microbiology* 147:995–1006
165. Ripple WJ, Beschtab RL. 2003. Wolf reintroduction, predation risk, and cottonwood recovery in Yellowstone National Park. *For. Ecol. Man.* 184:299–313
166. Robleto EA, Borneman J, Triplett EW. 1998. Effects of bacterial antibiotic production on rhizosphere microbial communities from a culture-independent perspective. *Appl. Environ. Microbiol.* 64:5020–22
167. Robleto EA, Scupham AJ, Triplett EW. 1997. Trifolitoxin production in *Rhizobium etli* strain CE3 increases competitiveness for rhizosphere. *Mol. Plant-Microbe Interact.* 10:228–33
168. Rogosky AM, Moak PL, Emmert EA. 2006. Differential predation by *Bdellovibrio bacteriovorus* 109J. *Curr. Microbiol.* 52:81–85

169. Rondon MR, August PR, Bettermann AD, Brady SF, Grossman TH, et al. 2000. Cloning the soil metagenome: a strategy for accessing the genetic and functional diversity of uncultured microorganisms. *Appl. Environ. Microbiol.* 66:2541–47
170. Rusch DB, Halpern AL, Sutton G, Heidelberg KB, Williamson S, et al. 2007. The Sorcerer II global ocean sampling expedition: northwest Atlantic through eastern tropical Pacific. *PLoS Biol.* 5:e77
171. Saison C, Degrange V, Oliver R, Millard P, Commeaux C, et al. 2006. Alteration and resilience of the soil microbial community following compost amendment: effects of compost level and compost-borne microbial community. *Environ. Microbiol.* 8:247–57
172. Schink B. 2002. Synergistic interactions in the microbial world. *Antonie van Leeuwenhoek* 81:257–61
173. Schloss PD. 2008. Evaluating different approaches that test whether microbial communities have the same structure. *ISME J.* 2:265–75
174. Schloss PD, Handelsman J. 2005. Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. *Appl. Environ. Microbiol.* 71:1501–6
175. Schloss PD, Handelsman J. 2006. Introducing SONS, a tool for operational taxonomic unit-based comparisons of microbial community memberships and structures. *Appl. Environ. Microbiol.* 72:6773–79
176. Schloss PD, Handelsman J. 2006. Introducing TreeClimber, a test to compare microbial community structures. *Appl. Environ. Microbiol.* 72:2379–84
177. Schloss PD, Handelsman J. 2007. The last word: books as a statistical metaphor for microbial communities. *Annu. Rev. Microbiol.* 61:23–34
178. Schloss PD, Larget BR, Handelsman J. 2004. Integration of microbial ecology and statistics: a test to compare gene libraries. *Appl. Environ. Microbiol.* 70:5485–92
179. Schoeninger MJ, Deniro MJ, Tauber H. 1983. Stable nitrogen isotope ratios of bone collagen reflect marine and terrestrial components of prehistoric human diet. *Science* 220:1381–83
180. Shertzer KW, Ellner SP, Fussmann GF, Hairston NG. 2002. Predator-prey cycles in an aquatic microcosm: testing hypotheses of mechanism. *J. Anim. Ecol.* 71:802–15
181. Shrestha M, Abraham W-R, Shrestha PM, Noll M, Conrad R. 2008. Activity and composition of methanotrophic bacterial communities in planted rice soil studied by flux measurements, analyses of pmoA gene and stable isotope probing of phospholipid fatty acids. *Environ. Microbiol.* 10:400–12
182. Sih A, Crowley P, McPeek M, Petranka J, Strohmeier K. 1985. Predation, competition, and prey communities—a review of field experiments. *Annu. Rev. Ecol. Syst.* 16:269–311
183. Silo-Suh LA, Lethbridge BJ, Raffel SJ, He H, Clardy J, Handelsman J. 1994. Biological activities of two fungistatic antibiotics produced by *Bacillus cereus* UW85. *Appl. Environ. Microbiol.* 60:2023–30
184. Smith JM, Castro H, Ogram A. 2007. Structure and function of methanogens along a short-term restoration chronosequence in the Florida Everglades. *Appl. Environ. Microbiol.* 73:4135–41
185. Stanton ML. 2003. Interacting guilds: moving beyond the pairwise perspective on mutualisms. *Am. Nat.* 162:S10–23
186. Stein JL, Marsh TL, Wu KY, Shizuya H, DeLong EF. 1996. Characterization of uncultivated prokaryotes: isolation and analysis of a 40-kilobase-pair genome fragment from a planktonic marine archaeon. *J. Bacteriol.* 178:591–99
187. Stelling J, Sauer U, Szallasi Z, Doyle FJ III, Doyle J. 2004. Robustness of cellular functions. *Cell* 118:675–85
188. Streit WR, Daniel R, Jaeger K-E. 2004. Prospecting for biocatalysts and drugs in the genomes of noncultured microorganisms. *Curr. Opin. Biotechnol.* 15:285–90
189. Strovas TJ, Sauter LM, Guo X, Lidstrom ME. 2007. Cell-to-cell heterogeneity in growth rate and gene expression in *Methylobacterium extorquens* AM1. *J. Bacteriol.* 189:7127–33
190. Thao ML, Gullan PJ, Baumann P. 2002. Secondary (gamma-Proteobacteria) endosymbionts infect the primary (beta-Proteobacteria) endosymbionts of mealybugs multiple times and coevolve with their hosts. *Appl. Environ. Microbiol.* 68:3190–97
191. Thingstad TF. 2000. Elements of a theory for the mechanisms controlling abundance, diversity, and biogeochemical role of lytic bacterial viruses in aquatic systems. *Limnol. Oceanogr.* 45:1320–28
192. Thomashow LS, Weller DM. 1988. Role of a phenazine antibiotic from Pseudomonas fluorescens in biological control of Gaeumannomyces graminis var. tritici. *J. Bacteriol.* 170:3499–508

193. Thompson JN. 1994. *The Coevolutionary Process*. Chicago: Univ. Chicago Press. 376 pp.
194. Thompson JN. 2005. Coevolution: the geographic mosaic of coevolutionary arms races. *Curr. Biol.* 15:R992–94
195. Tilman D, Wedin D, Knops J. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature* 379:718–20
196. Tringe SG, von Mering C, Kobayashi A, Salamov AA, Chen K, et al. 2005. Comparative metagenomics of microbial communities. *Science* 308:554–57
197. Tyson GW, Chapman J, Hugenholtz P, Allen EE, Ram RJ, et al. 2004. Community structure and metabolism through reconstruction of microbial genomes from the environment. *Nature* 428:37–43
198. Uroz S, Chhabra SR, Camara M, Williams P, Oger P, Dessaux Y. 2005. *N*-acylhomoserine lactone quorum-sensing molecules are modified and degraded by *Rhodococcus erythropolis* W2 by both amidolytic and novel oxidoreductase activities. *Microbiology* 151:3313–22
199. Uroz S, D'Angelo-Picard C, Carlier A, Elasri M, Sicot C, et al. 2003. Novel bacteria degrading *N*-acylhomoserine lactones and their use as quenchers of quorum-sensing-regulated functions of plant-pathogenic bacteria. *Microbiology* 149:1981–99
200. Uroz S, Oger P, Chhabra SR, Camara M, Williams P, Dessaux Y. 2007. *N*-acylhomoserine lactones are degraded via an amidolytic activity in *Comamonas* sp. strain D1. *Arch. Microbiol.* 187:249–56
201. Vasanthakumar A, Delalibera I, Handelsman J, Klepzig KD, Schloss PD, Raffa KF. 2006. Characterization of gut-associated bacteria in larvae and adults of the southern pine beetle, *Dendroctonus frontalis* Zimmermann. *Environ. Entomol.* 35:1710–17
202. Venter JC, Remington K, Heidelberg JF, Halpern AL, Rusch D, et al. 2004. Environmental genome shotgun sequencing of the Sargasso Sea. *Science* 304:66–74
203. von Dohlen CD, Kohler S, Alsop ST, McManus WR. 2001. Mealybug [beta]-proteobacterial endosymbionts contain [gamma]-proteobacterial symbionts. *Nature* 412:433–36
204. Wang Y-J, Leadbetter JR. 2005. Rapid acyl-homoserine lactone quorum signal biodegradation in diverse soils. *Appl. Environ. Microbiol.* 71:1291–99
205. Waters E, Hohn MJ, Ahel I, Graham DE, Adams MD, et al. 2003. The genome of *Nanoarchaeum equitans*: insights into early archaeal evolution and derived parasitism. *Proc. Natl. Acad. Sci. USA* 100:12984–88
206. Whitham TG, Young WP, Martinsen GD, Gehring CA, Schweitzer JA, et al. 2003. Community and ecosystem genetics: a consequence of the extended phenotype. *Ecology* 84:559–73
207. Williamson KE, Radosevich M, Wommack KE. 2005. Abundance and diversity of viruses in six Delaware soils. *Appl. Environ. Microbiol.* 71:3119–25
208. Winogradsky S. 1895. Researches sur l'assimilation de l'azote libre de l'atmosphere par les microbes. *Arch. Sci. Biol.* 3:297–352
209. Wu D, Daugherty SC, Van Aken SE, Pai GH, Watkins KL, et al. 2006. Metabolic complementarity and genomics of the dual bacterial symbiosis of sharpshooters. *PLoS Biol.* 4:e188
210. Yang F, Wang L-H, Wang J, Dong Y-H, Hu JY, Zhang L-H. 2005. Quorum quenching enzyme activity is widely conserved in the sera of mammalian species. *FEBS Lett.* 579:3713–17
211. Yannarell AC, Steppe TF, Paerl HW. 2007. Disturbance and recovery of microbial community structure and function following Hurricane Frances. *Environ. Microbiol.* 9:576–83
212. Yim G, Wang HH, Davies J. 2007. Antibiotics as signalling molecules. *Philos. Trans. R. Soc. London Ser. B* 362:1195–200
213. Zhang R, Weinbauer MG, Qian P-Y. 2007. Viruses and flagellates sustain apparent richness and reduce biomass accumulation of bacterioplankton in coastal marine waters. *Environ. Microbiol.* 9:3008–18
214. Zul D, Denzel S, Kotz A, Overmann J. 2007. Effects of plant biomass, plant diversity, and water content on bacterial communities in soil lysimeters: implications for the determinants of bacterial diversity. *Appl. Environ. Microbiol.* 73:6916–29

Annual Review of Microbiology
Volume 62, 2008

Contents