Interactions in the microbiome: communities of organisms and communities of genes

Eva Boon\textsuperscript{1}, Conor J. Meehan\textsuperscript{2,3}, Chris Whidden\textsuperscript{2}, Dennis H.-J. Wong\textsuperscript{2,4}, Morgan G.I. Langille\textsuperscript{2} & Robert G. Beiko\textsuperscript{2}

\textsuperscript{1}Department of Biology, Dalhousie University, Halifax, NS, Canada; \textsuperscript{2}Faculty of Computer Science, Dalhousie University, Halifax, NS, Canada; \textsuperscript{3}Department of Biochemistry and Molecular Biology, Dalhousie University, Halifax, NS, Canada; and \textsuperscript{4}Faculty of Graduate Studies, Dalhousie University, Halifax, NS, Canada

Correspondence: Robert G. Beiko, Faculty of Computer Science, Dalhousie University, 6050 University Avenue, PO Box 15000, Halifax, Nova Scotia, Canada B3H 4R2. Tel.: +1 902 494 8043; fax: +1 902 492 1517; e-mail: beiko@cs.dal.ca

Received 23 April 2013; revised 2 July 2013; accepted 10 July 2013. Final version published online 28 August 2013.

DOI: 10.1111/1574-6976.12035

Editor: Staffan Kjelleberg

Keywords
microbial communities; metagenomics; genome evolution; trait-based ecology; Black Queen Hypothesis; Public Goods Hypothesis.

Abstract
A central challenge in microbial community ecology is the delineation of appropriate units of biodiversity, which can be taxonomic, phylogenetic, or functional in nature. The term ‘community’ is applied ambiguously; in some cases, the term refers simply to a set of observed entities, while in other cases, it requires that these entities interact with one another. Microorganisms can rapidly gain and lose genes, potentially decoupling community roles from taxonomic and phylogenetic groupings. Trait-based approaches offer a useful alternative, but many traits can be defined based on gene functions, metabolic modules, and genomic properties, and the optimal set of traits to choose is often not obvious. An analysis that considers taxon assignment and traits in concert may be ideal, with the strengths of each approach offsetting the weaknesses of the other. Individual genes also merit consideration as entities in an ecological analysis, with characteristics such as diversity, turnover, and interactions modeled using genes rather than organisms as entities. We identify some promising avenues of research that are likely to yield a deeper understanding of microbial communities that shift from observation-based questions of ‘Who is there?’ and ‘What are they doing?’ to the mechanistically driven question of ‘How will they respond?’

Introduction
Microorganisms are everywhere, but they rarely act alone. The best illustration of this fact is the ‘Great Plate Count Anomaly’ (Staley & Konopka, 1985), which claims that $< 1\%$ of all known microorganisms can be successfully cultured on their own. It is now clear that many microorganisms depend on the activity of other microorganisms to successfully grow and reproduce (Schink, 2002; Stolyar \textit{et al.}, 2007; McCutcheon & von Dohlen, 2011; Hug \textit{et al.}, 2012) via mechanisms including acquisition and exchange of metabolites (Stams, 1994; Falony \textit{et al.}, 2006; Carini \textit{et al.}, 2012). The diversity of microbiomes is being explored using surveys that draw on hundreds or thousands of samples (Caporaso \textit{et al.}, 2011; Human Microbiome Project Consortium, 2012; Larsen \textit{et al.}, 2012) and controlled experiments (McNulty \textit{et al.}, 2011; Lawley \textit{et al.}, 2012; Xie \textit{et al.}, 2012; Badri \textit{et al.}, 2013; Smith \textit{et al.}, 2013), with rapid genetic assessment techniques providing much of the evidence for taxonomic and functional diversity. Long-standing questions in microbial ecology such as whether ‘everything is everywhere, but the environment selects’ (Baas Becking, 1934; de Wit & Bouvier, 2006) can now be tested in fine detail by examining the geographic and habitat distributions of microorganisms (Martiny \textit{et al.}, 2006). The web of microbial interactions spans all taxonomic ranks, from strain to superkingdom, and underscores the need for community-centric approaches to understanding microbial diversity (Zarraonaindia \textit{et al.}, 2013).

Microbial ecology has benefited greatly from the adaptation of theories and methods developed initially for multicellular organisms (Prosser \textit{et al.}, 2007). Hypotheses about the distribution of microorganisms can be tested biogeographically by contrasting biotic similarity with habitat distances and geographic distance (Martiny \textit{et al.},
Interactions in the microbiome

2006), using approaches originally developed with macroorganisms in mind. Microbial community ecology has drawn heavily upon classical approaches, in particular the representation of biodiversity in terms of the entities (e.g. species) that are present, often with additional information about the relative abundance of different entities. Although species are commonly thought of as ecological units and thus the most natural entities to count and search for interactions, several reasons motivate the use of other units to quantify biodiversity. Larger taxonomic groups may be of interest because they share one or more important attributes: Class *Amphibia* is seen as a leading indicator of a general decline in biodiversity, in part due to its sensitivity to disturbances in both terrestrial and aquatic habitats (Collins & Crump, 2009), while the balance between *Bacteroidetes* and *Firmicutes* is sometimes treated as a defining feature of the human gut microbiota (Ley et al., 2006; Mariat et al., 2009), even though both bacterial phyla contain a wide range of organisms with distinct ecological roles (Qin et al., 2012). While community ecology considers interactions among entities, the inference of interactions can depend critically on the level at which entities are defined. Models can be used to predict the impact of interactions on expected abundances over time, and web and network structures can capture a complete range of possible pairwise interactions between community members (May, 1973; Menge, 1995; Faust et al., 2012; Larsen et al., 2012). Microbial community ecology has the potential to identify key interactions between microorganisms, with a wide range of important applications in health and the environment (Preidis & Versalovic, 2009; Bakker et al., 2012; Costello et al., 2012).

Success in applying well-developed ecological theories to microorganisms has been achieved in spite of the obvious differences between microorganisms and multicellular organisms. Differences in size, dispersal, dormancy regimes, and growth and reproduction may not prohibit application of the same quantitative techniques to both single-celled and multicellular organisms. However, genome-scale data have given an evolutionary context to the phylogeny function disconnect in microorganisms, particularly bacteria, which has been known for decades (Cowan, 1955; Floodgate, 1962), in the process spawning or reigniting debates about microbial evolution, taxonomy, and the microbial species (Gevers et al., 2005; Konstantinidis & Tiedje, 2005; Bapteste & Boucher, 2008; Doolittle & Zhaxybayeva, 2009). Although a unifying species concept is not needed for ecological analysis, a sound rationale and clear approach (or set of approaches) to define ‘units’ is. The use of uniform taxonomic or phylogenetic thresholds may fail to adequately delineate ecologically cohesive units, especially in microorganisms whose genomes can change rapidly through gene loss, gene duplication, and the acquisition of genes from distant lineages via lateral gene transfer (LGT).

When considering the nature of microbial communities, especially in the inference of interactions that determine community structure, we must assess the potential impact of microbial evolutionary processes on the entities that constitute these communities. In this article, we review several aspects of communities and community interactions, starting with the definition of ‘community’ itself. We then consider different approaches used to define the entities in a potential community, in particular the broad range of trait-based approaches that have recently been developed and applied in different settings. Because traits are ultimately conferred by an organism’s genes, we then examine the evolutionary dynamics of these genes, culminating in two recent hypotheses (McInerney et al., 2011; Morris et al., 2012) that address potential impacts of gene gain and loss on microbial interactions. The dynamic movement of genes through microbial lineages and communities suggests that genes themselves may be treated as valid ecological entities, and we propose a metacommunity framework for the analysis of gene distributions. Finally, we consider the ecological unit definitions that are currently in use, and we highlight how these definitions might be augmented by explicit consideration of interactions and evolutionary models in experimental and analytical techniques.

**Defining and measuring communities and microbiomes**

The term ‘community’ and the related term ‘assemblage’ have long been used in ecology, but their definitions are both fluid and controversial (e.g. Ricklefs, 2008). For example, Fauth et al. (1996) uses assemblage to define ‘phylogenetically related groups within a community’ with a community described as a ‘collection of species occurring in the same place at the same time’. Cornell & Lawton (1992) distinguish ‘interactive’ from ‘noninteractive’ communities based on the presence or absence of biotic interactions. In a similar fashion, Konopka (2009) defines communities as ‘multispecies assemblages, in which organisms live together in a contiguous environment and interact with each other’. We adopt this latter definition of ‘community’ while recognizing that it is neither comprehensive nor universally accepted (e.g. Zarraonaindia et al., 2013). We consider an assemblage to be the set of species (or, more generally, taxa) that are inferred to be in a given place at a given time, based on evidence from morphology or sequence data. Thus, we treat ‘community’ as a refinement of ‘assemblage’, with the additional proviso that taxa interact with one another. These
definitions usefully distinguish observations (assemblages) from testable hypotheses (communities).

The definition of 'microbiome' has a shorter, but equally tortuous history. Although there is consensus that the term was first coined by Joshua Lederberg in 2000 or 2001, confusion arises because the term can be read as 'micro-biome' (the set of resident microorganisms and associated abiotic factors) or 'microbi-ome' (the complete set of genetic information associated with a set of microorganisms). The definition was given by Lederberg & McCray (2001) as ‘...the ecological community of communal, symbiotic, and pathogenic microorganisms that literally share our body space’, and has expanded from its initial application to human-associated microorganisms (Relman, 2002; Turnbaugh et al., 2007) to encompass microorganisms in any setting (Gilbert et al., 2010b). Host-associated microbiota or microbial communities are frequently described as symbionts (Mandel, 2010; Ballal et al., 2011), but this is almost certainly not true for all microorganisms observed in a healthy human. As described above, the existence and nature of interactions among microorganisms and their host represent a hypothesis to be tested. We therefore favor an observation-based definition of the microbiome as the set of microorganisms and their genomes in a particular environment, without any requirement for ecological interactions. Whether one or more communities exist within a given microbiome is a matter for further investigation.

Community interactions

Interactions have been treated as a central feature of communities since the early 1900s, but how these interactions are interpreted has changed many times. Clements (1916) described succession in plant communities, or ‘seres’ as he called them, as a series of associations from pioneer to climax communities. The development of a sere was likened to that of an organism. Gleason (1926) articulated what might be viewed as a first null model of community interactions: ‘Are we not justified in coming to the general conclusion, far removed from the prevailing opinion, that an association is not an organism, scarcely even a vegetational unit, but merely a coincidence?’ Elton (1927), like Clements, also drew the analogy of community and organism when he wrote: ‘animal associations, or better, animal communities, ... are not mere assemblages of species living together, but form closely knit communities or societies comparable to our own’. Many authors viewed interacting organisms in a community as constituting a ‘complex organism’ with emergent properties, as summarized by Phillips (1935). It was only later in the 1950s and the 1960s that the idea of communities as organisms lost its popularity, and so-called ‘species–individualistic’ models gained more popularity (Whittaker, 1967; Ricklefs, 2008). The precise definition of community in any given study is explicitly or implicitly determined by the investigator’s choice of experimental techniques and analytical tools: As Konopka (2009) states, ‘The practical delineation of “community” may then reflect the interests of the ecologist rather than any inherent characteristics’.

A spectrum of degrees of interaction is conceivable (Fig. 1). At one end of this spectrum lies a null interaction model similar to that articulated by Gleason, with distinct organisms found in a particular setting being mutually oblivious or interacting only in trivial ways. In this scenario, the presence of one organism has no effect on the viability of another, which corresponds to the ‘assemblages’ defined previously. At the other end of the spectrum would be coevolved obligate interspecies interactions that are mutually beneficial and highly specific and that bind species so tightly that independent existence, or association with alternative species, is no longer possible. Between these two extremes lie a range of interaction types, from protagonistic (mutualism) to benign (commensalism) to antagonistic (e.g. predation or parasitism), with each interaction type varying from obligate to facultative (Little et al., 2008). Dependencies can be based on metabolic interactions, as in cross-feeding or pathway completion where microorganisms engage in reciprocal or nonreciprocal exchange of metabolites (Heling et al., 1987; Wintermute & Silver, 2010).

Fig. 1. Conceptual representation of communities: (a) no interactions (i.e. a neutral community model), (b) indirect interactions (competition for a resource), (c) direct interactions (cross-feeding and targeted killing). Circles represent individuals, squares indicate a resource, and diamonds indicate a toxic substance.
Extreme examples of tight interactions include the association between the endosymbiotic bacteria *Candidatus ‘Moranella endobia’* and *Candidatus ‘Tremblaya princeps’*, which live inside the cells of the mealybug *Planococcus citri* (McCutcheon & von Dohlen, 2011). In this system, synthesis of several amino acids including phenylalanine, arginine, and isoleucine appears to depend on exchange of pathway intermediates and successive reactions that are carried out by different community members. Less dramatic are systems in which microorganisms depend on pathway end products that must be synthesized by others. Many organisms within the *Dehalococcoides* genus perform reductive dehalogenation, a process of great importance in bioremediation, for example in the commercially successful KB-1 mixed culture (Duhamel et al., 2002; Smidt & de Vos, 2004). However, to be cultured axenically, *Dehalococcoides* requires a specialized reduced medium containing vitamin B12 (Löffler et al., 2012); despite significant efforts, *Dehalococcoides* grows much more slowly and to lower cell density in axenic culture compared with mixed cultures. Metagenomic analysis suggests dependencies on other community members for cofactor precursors and possibly methionine (Hug et al., 2012; see Fig. 2). Dependencies can also be indirect through modification of the surrounding medium, such as the reliance on other organisms to detoxify or sequester harmful compounds in the environment (Morris et al., 2012). In addition to the specific dependencies mentioned above, *Dehalococcoides* strains found in many mixed cultures also depend on other community members for oxygen scavenging (Hug et al., 2012). Negative interactions have been observed between microorganisms at every degree of taxonomic divergence. These can be indirect, based on competition for a particular resource or secretion of a broadly toxic compound. Direct negative interactions involve the targeting of a potential competitor using inhibitory compounds such as antibiotics or bacteriocins, parasitism, or predation (Hibbing et al., 2010).

**Fig. 2.** Interactions supporting the growth and metabolism of the key dehalogenating organisms *Dehalococcoides* and *Geobacter* via metabolite provision (solid arrows) and detoxification via oxygen scavenging (dashed arrows) in the KB-1 mixed culture. Key metabolites and functions provided by other members of the community are underlined. Met, methionine, PCE, perchlorinated ethene.

**Describing the structure of assemblages and communities**

Characterizing the distribution (presence and relative abundance) of an assemblage of microorganisms is a precondition for testing community structure. The assessment of microbial diversity has shifted from primarily culture-based methods to approaches that make use of rapidly improving DNA sequencing technology. Often, a marker gene such as the 16S ribosomal RNA gene (referred to as 16S henceforth) is targeted and sequenced to give an indication of the taxonomic diversity within a given sample (Ward et al., 1990; Amann et al., 1995). There are several drawbacks to such single-gene studies. First, the plasticity of prokaryotic genomes means that the use of 16S as an indicator of diversity often masks many of the differentiating traits between closely related organisms (Dobrindt & Hacker, 2001; Medini et al., 2005). Therefore, the interactions, and the set of distinct entities in a sample, can be difficult to interpret from 16S studies alone. Second, a community is often defined by a stable species composition, but such stability is not always found in microbial settings (Turnbaugh et al., 2009; Booijink et al., 2010; Caporaso et al., 2011; Human Microbiome Project Consortium, 2012). As such, taxonomy-centric definitions may not be sufficient to yield an adequate understanding of microbial ecology (Shade & Handelsman, 2012). An alternative to marker gene studies is environmental whole-genome shotgun (WGS) sequencing as pioneered by such as Venter et al. (2004) and Tyson et al. (2004)’s studies to reveal a metagenome (Handelsman et al., 1998). The resulting set of DNA sequence reads can potentially cover the entire genomes of the sampled microorganisms (given sufficient sequencing effort), not just a given marker gene. Such an approach can reveal the functional complement of a given sample and suggest interactions between members based on such functions. However, assembly and assignment of function and taxonomy to metagenomic sequences is a complicated task that often generates a multitude of low-confidence predictions (Prakash & Taylor, 2012) and ambiguities about which sequences may have originated in the same organism. This leads to difficulty in creating a complete consensus.
of community function and diversity and linking these two aspects to each other.

Taxonomic diversity has historically been expressed in many ways. Assemblages can be considered in terms of the presence or relative abundance of different discretely defined groups, which are circumscribed using either a taxonomic ranking and naming scheme or an assessed degree of genetic relatedness. Measures such as species richness, Shannon diversity, Jaccard dissimilarity, and Bray–Curtis dissimilarity have been applied to microbial communities to assess the impact of different habitat types on biodiversity, by using taxonomy (e.g. the Bacteroidetes/Firmicutes ratio) or by defining operational taxonomic units (OTUs) (Ehrlich & Holm, 1962; Sokal & Sneath, 1963) based on the similarity of marker genes such as 16S (Schloss & Handelsman, 2005; Fig. 3A). Phylogenetic diversity considers the relatedness of different lineages, based on the underlying assumption that phylogenetic relatedness between taxa should correlate with ecological similarity (Martin, 2002). These diversity measures typically quantify the extent to which branches in a rooted phylogenetic tree are unique to one sample or the other, or common to both (Fig. 3B). Weighting by relatedness may give more biologically relevant interpretations of diversity, and phylogenetic diversity measures have gained widespread use in microbial community analysis (Kuczynski et al., 2010; Parks & Beiko, 2013).

Much care is warranted in the choice of the relevant level(s) of diversity. If our focus is the community, it might be reasonable to choose the species boundary as the main delimiter of diversity, because this boundary typically distinguishes the study of populations from that of communities (Prosser et al., 2007). The most widely cited definition of microbial species is that of Dykhuisen & Green (1991), which defines recombination as the key driver of species cohesion and in fact requires the consideration and comparison of multiple gene trees. However, there is still no definition that refutes Cohan & Perry (2007): ‘However, no sequence-based OTU proposed either by systematists or by ecologists appears to correspond to the fundamental units of bacterial ecology’.

**Taxonomy or traits as the basis for communities?**

The focus of microbial ecology on taxonomically and phylogenetically cohesive groups is shared with macroorganism-based ecology and facilitated by the tractability of taxonomic marker genes to sampling and analysis, as well as the availability of large phylogenetic databases such as the Ribosomal Database Project (Maidak et al., 2001) and GreenGenes (DeSantis et al., 2006) for mapping purposes. By contrast, trait-based ecology (Hutchinson, 1957; Green et al., 2008) represents organisms in terms of functional properties that may impact their fitness in a given habitat (i.e. functional traits) and their responses to disturbance. The key to trait-based ecology is the mapping of species information into a functional space that expresses the similarity of morphological, behavioral, or biochemical traits that can influence the ability to occupy particular niches (Hutchinson, 1957; Mouillot et al., 2013); this type of approach recalls microbial classification schemes developed before the advent of DNA sequencing (Sapp, 2005). Because traits mediate the interactions among organisms and between organisms and the environment, many have argued that trait-based approaches are more relevant to community analysis than taxonomic or phylogenetic attributes (McGill et al., 2006; Violle et al., 2007). Although environmental properties will limit the types of organisms that can occupy a particular habitat, the taxa that can potentially occupy that habitat need not be closely related to one another. In some cases, occupancy may be driven by a stochastic ‘lottery’ process (Sale, 1978; Burke et al., 2011a) that need not respect species boundaries or even be constrained to a given clade in a tree.

Taxonomic and phylogenetic approaches to community analysis impose either a discrete or a hierarchical classification of entities (Mishler & Donoghue, 1982; Cohan & Perry, 2007). Phylogenetic approaches require units to be monophyletic, whereas named species would typically be
monophyletic or paraphyletic in practice if not by definition. Trait distributions set aside the expectation of monophyly or paraphyly of units and need not respect clusters or lineages. The majority of an organism’s traits will not respect a species boundary, however defined, and will either be shared with others outside of its species group, vary within its species group, or both. These types of variations motivated Van Valen (1976) to propose the ecological species concept as opposed to the biological (or ‘reproductive’) species concept of Mayr (1942). Convergent evolution can lead distantly related organisms into the same region of ‘trait space’, either via convergent morphological evolution (Kocher et al., 1993; McNab, 2009) or via independent invention of similar systems such as C4 photosynthesis (Sage et al., 2011). A striking example of trait convergence was reported by Fan et al. (2012), who examined the phylogenetic and functional diversity of microorganisms associated with six different types of sponge. The phylogenetic structure was consistent in replicated samples from each sponge and differed markedly between sponge species. However, a range of metabolic and cellular traits including denitrification and cofactor synthesis were consistently enriched relative to seawater samples.

Complementing convergence is the possibility of rapid genotypic and phenotypic divergence, even among organisms that satisfy criteria for membership in the same species. The impact of this divergence has been well documented in many plant species. For instance, genetic variation within Populus angustifolia affects resistance to aphids and influences a wider community of associated macro- and microorganisms (Bailey et al., 2005, 2006; Whitham et al., 2008). While a species-based analysis might capture some aspects of the community in such a case, the key genetic distinctions within P. angustifolia would be completely lost, impeding an understanding of community function.

Traits in microbial ecology

Given the extensively documented genomic and ecological variation in microorganisms, the limitations of taxonomic and phylogenetic approaches will be more acute in these organisms. This motivates the application of trait-based approaches as an alternative (Green et al., 2008). Not surprisingly, the list of traits considered is dominated by those that can be assayed using genomic and related approaches, including sequence dissimilarity, ribosomal copy number, and genome size. Given a metagenome sample that has been functionally annotated using a reference database, it is possible to examine the profile of many or all functional categories of proteins, as has been performed by DeLong et al. (2006) in a depth transect of the ocean, Raes et al. (2011) for a series of sites from the Global Ocean Sampling expedition (Rusch et al., 2007), and others. Because broad functional summaries may miss important differences within groups that drive ecological differences, approaches that target a subset of functions such as nitrogen cycling in soil (Bru et al., 2010), butyrate production in the human gut (Van den Abbeele et al., 2013), or membrane proteins in different ocean habitats (Patel et al., 2010) can be more informative about the relationship between traits and habitat type. Although genomes and metagenomes give a detailed cross section of the functional potential of an organism or a community, it is the phenotypic traits that interact directly with the environment, and these may provide more relevant information in a community analysis (Kim et al., 2009; Gudelj et al., 2010). Phenotypic traits determined by one or a few genes, such as toxin resistance or degradation of a relatively simple carbohydrate, may often be predictable from genotype. However, complex phenotypes such as cell shape, and traits where subtle sequence differences can lead to drastic ecological consequences (such as peptide receptors: Geisinger et al., 2008), will require either more sophisticated modeling or direct experimental characterization of the phenotype of interest (Whitworth, 2008).

The extensive phenotypic diversity within many named species of microorganisms that satisfy the typical criteria for species membership (i.e. 70% DNA–DNA hybridization or 97% identity of the small-subunit ribosomal RNA gene) has been well documented: As Cohan & Perry (2007) state, ‘...the recognized “species” of bacterial systems frequently contain a diversity of populations that are distinct in their biochemistry, physiology, genome content and ecology; classifying an unknown organism to its species thus tells us only vaguely about the organism’s way of life’. This assertion has been shown to be true for oceanic microorganisms such as the remarkably diverse Prochlorococcus marinus (Martiny et al., 2009) and SAR11 (Morris et al., 2002) and for host-associated organisms such as Lactobacillus plantarum (Siezen et al., 2010) and Escherichia coli (Souza et al., 1999; Welch et al., 2002). This diversity highlights the promise of trait-based analysis, but the application of traits in microbial ecology requires a thorough understanding of their genetic underpinnings and the evolutionary processes that generate and sustain them.

Genome evolution and microbial interactions

Processes at the genome level influence the evolution of microbial traits and the emergence of microbial community structure. If we are to consider trait-based
approaches to microbial ecology, then it is essential to understand the evolutionary dynamics of these traits. The following insights gained by comparative analysis of sequenced genomes offer a useful framework to understand why trait-based approaches may be complementary to those based on phylogenetic markers.

Gene loss

At the heart of microbial evolution is a process of genome streamlining that rapidly discards genetic material that is not under selection, a process that appears to carry an advantage to the organism (Lynch, 2006; Koskiniemi et al., 2012). The most striking examples of this process are seen in genomes that are currently in niche transition or have recently undergone such transitions. *Mycobacterium leprae* exemplifies this process: The organism resides in macrophages, but bears residual evidence of a less constrained lifestyle, with more than 1000 pseudogenes providing clear evidence of recent losses of respiration, catabolic, and other pathways (Cole et al., 2001). In such cases, many genes are lost because functions such as host defense or carbohydrate metabolism are no longer needed. Some amount of gene loss can be offset by increasing the density of the interaction network among the proteins that remain (Kelkar & Ochman, 2013), but gene loss may also arise when a resource-intensive function can be performed by one or more other members of the community. The Black Queen Hypothesis (BQH) of Morris et al. (2012) considers the trade-off between the potential cost of losing one or more genes encoding a particular function and the benefit of offloading the resource burden associated with this function onto another member of the community. However, specialization due to loss of function (Fig. 4a) creates a dependency on other community members for that function and therefore requires a certain degree of community stability, potentially in combination with dormancy when key conditions for growth and reproduction—including the presence of essential ecological partners—are not met (Lennon & Jones, 2011).

Lateral gene transfer

Complementing gene loss in microorganisms is LGT, a process of gene gain that can rapidly reshape the ecological capacity of a lineage (Fig. 4b). LGT offers a path by which organisms can recover from the specialization and streamlining that accompany gene loss. While estimates of the extent of LGT vary depending on the microorganisms studied, the analytical methods used, and the way in which transfer events are counted (Beiko et al., 2005; Ge et al., 2005; Kunin et al., 2005; Ragan et al., 2006; Dagan et al., 2008), there is no question that LGT is widespread in microorganisms and appears to play a major role in the generation of functional novelty, at least.
in the short term, than gene duplication (Treangen & Rocha, 2011). LGT clearly violates assumptions of tree-like descent and speciation, introducing connections between distant microbial phyla and even between domains (Puigbó et al., 2009; Dodsworth et al., 2010; Beiko, 2011; Clarke et al., 2013). The mechanisms that enable LGT may not have gene transfer as their primary purpose in microbial cells and may instead serve primarily as agents of resource acquisition and DNA repair (Redfield, 2001) and as mechanisms by which selfish elements propagate themselves. A considerable amount of LGT may conform to the neutral theory of evolution in that many gene acquisitions are deleterious (Skippington & Ragan, 2011) or selectively neutral. Although most genome-scale analyses tend to focus on annotated protein-coding genes, more thorough analysis has shown that many genomes contain the remnants of acquired genes that were presumably neutral and are currently undergoing decay and loss (Hao & Golding, 2006). While LGT events often confer no benefit to the recipient organism, LGT-enabling mechanisms are clearly exploited by organisms in some settings where LGT may be beneficial: For example, loss of DNA repair systems can lead to a ‘LGT mutator’ phenotype (Denamur et al., 2000). Recently, Johnston et al. (2013) demonstrated that some strains of Streptococcus pneumoniae possess methylases that can protect internalized DNA from cleavage by restriction enzymes; the authors argue that this system facilitates the exchange of pathogenicity islands and other material among closely related strains. Further supporting the potentially beneficial role of LGT is the demonstration that acquired genes have been successfully integrated into the host’s regulatory and metabolic networks (Pål et al., 2005; Lercher & Pål, 2008).

What role does LGT have in establishing and maintaining communities? Biofilms are a primary example of a microbial community driven by LGT. Biofilms usually comprise more than one named species (Wolcott et al., 2013) and have been extensively studied in many settings. Recently, the study of medically important biofilms has generated new hypotheses about the role of LGT in communities. Biofilms in chronic infections can persist by subverting host cellular pathways (Kim et al., 2010) to, for example, prevent apoptosis, rather than expressing planktonic virulence factors such as toxins. Microorganisms in biofilms undergo rapid LGT and are often genetically distinct, as exemplified by the fact that 10% of the genes in clinical isolates of Haemophilus influenzae are unique as compared to sequenced laboratory strains (Shen et al., 2006) and the demonstration of in vivo LGT over time within multiple strains of S. pneumoniae infecting a single pediatric patient (Hiller et al., 2010). The distributed genome hypothesis (Ehrlich, 2001; Ehrlich et al., 2010) argues that constituents of some bacterial biofilms collectively possess a community genome that evolves through rapid and focused transfer. This hypothesis suggests that biofilm communities can outcompete host defenses by continuously generating a cloud of novel strains and gene combinations through LGT. Further, this gene acquisition can be regulated through quorum sensing, possibly even between different species (Antonova & Hammer, 2011; Zhu & Li, 2012). A biofilm is a mature example of a community where limited barriers to LGT, distribution of tasks, and close proximity provide incentives to cooperate and maintain the biofilm. The far-reaching level of functional integration has even led some authors to propose that the biofilm itself is the biological individual (Ereshefsky & Pedrosa, 2013) based on both the degree of integration and the similar way in which community members respond to the environment (i.e. a ‘unitary response’ sensu Hull, 1980). Under other definitions of the individual that consider independence of replication, a biofilm is a microbial community with obligate and specific interactions that include even the timed exchange of genetic material.

Outside of biofilms, researchers have tried to understand why obligate associations such as cross-feeding emerge: Pfeiffer & Bonhoeffer (2004) used chemostat simulations to highlight the potential benefits of cross-feeding in ATP production and maintaining low concentrations of enzymes and intermediates, while Zhuang et al. (2011) pointed to membrane space as a potential limiting factor in respiration. The emergence of associations is likely mediated by a number of forces including habitat stability, physiological constraints, and the costs of carrying out reactions. Fan et al. (2012) identified a wide range of different mobile genetic elements (MGEs) in the sponge-associated microorganisms they studied, and suggested that these elements (particularly transposases) might play a role in adaptation of community members to a common host and in disruption of genes that are no longer needed due to the formation of stable associations.

‘Cheating’ microbial strains – microbial strains that have lost important community functions such as quorum sensing, but still acquire resources from other community members (Diggle et al., 2007) – also illuminate the role of LGT in community development. Maintaining functions important to the community on MGEs (e.g. plasmids) or using mechanisms such as quorum sensing to restrict LGT may penalize cheating strains by forcing them to reacquire the lost function (Smith, 2001) or to avoid LGT and be outcompeted (McGinty et al., 2011). Cooperative genes and genes that confer virulence are overrepresented in MGEs (Nogueira et al., 2009), among other traits (Rankin et al., 2011), lending support to this
theory. In a metagenomic study of a contaminated groundwater community, Hemme et al. (2010) found evidence for transfer of genes conferring resistance to many contaminants including mercury and acetone. Harrison & Brockhurst (2012) alternatively suggest that LGT mediated by plasmids is a process of coevolution between chromosomal and plasmid genomes that prevents beneficial genes from simply being absorbed into the chromosomal genome, which would lead to plasmid loss via purifying selection.

LGT and other processes call into the question the utility of phylogenetic cohesion as an exclusive criterion for defining ecological units. If genes can be readily acquired via LGT, then they might be considered a common resource accessible to microorganisms. The Public Goods Hypothesis (PGH) of McInerney et al. (2011) claims that genes are public goods if they satisfy the nonirval and nonexcludable criteria: ‘A good is nonrival if the consumption of the good by one individual does not reduce the availability of that good for another individual. A good is nonexcludable if it is impossible or at least very difficult to exclude the good from being available to everybody’. In treating protein-coding genes, which satisfy these criteria, as the resource, the PGH inverts the BQH to focus on gene acquisition rather than on gene loss as an evolutionary opportunity. Both models offer competitive advantages to organisms that focus their resources on tasks that are not effectively provided by other community members, but the two models are driven by different evolutionary processes (Fig. 4). The interplay of gene acquisition and loss – coupled with other methods of generating novelty such as point mutations and gene duplications – creates an evolutionary and ecological dynamic that may invalidate traditional community models.

The relevance of genome streamlining and expansion

The fundamental processes of gene loss and gene gain via LGT impact the evolution of microbial lineages and communities, with global consequences. In the ocean, the ubiquitously distributed and heterotrophic SAR11 group includes an enormous diversity of phylotypes and strains, with wide variation in latitudinal patterns of occurrence and possibly the largest effective population size of any bacterial group (Morris et al., 2002). Genomes of this group are highly streamlined, with extremely short intergenic spacers and a genome size of c. 1.3 Mb (Giovannoni et al., 2005). Large amounts of genome-level variation are present in some regions and in association with particular functions, especially membrane proteins (Wilhelm et al., 2007; Brown et al., 2012). This variation is supported by the observation of very high levels of homologous recombination that disrupt clonal relationships within the group (Vergin et al., 2007), to the point where even ribosomal operons of SAR11 show evidence for homologous recombination (Suzuki et al., 2001). The exchange of genetic material is not limited only to members of the group, as some genes appear to have been acquired from groups such as cyanobacteria (Gilbert et al., 2008; Viklund et al., 2012). A crucial property of this group is the absence of many DNA repair genes such as mutLS that are found in the alphaproteobacterial relatives of SAR11. Because the mutLS complex ordinarily prevents homologous recombination of divergent sequences, its absence from the SAR11 group appears to be responsible for the observed elevated rate of mutation and gene gain and loss (Viklund et al., 2012). Although SAR11 is an especially dramatic example of the opposing forces of genome reduction and gene gain via LGT, it is by no means unique: A primary case study underpinning the BQH is the dependence of the abundant marine photoautotroph P. marinus on other members of its community for peroxide decontamination with many catalase-positive organisms from a wide range of taxonomic groups able to provide this function (Morris et al., 2011). This dependency of P. marinus recalls that of Dehalococciodes described above and in Fig. 2. As microbial communities continue to be explored using laboratory experiments and genetic profiling, many more examples will be discovered.

The ‘use it or lose it’ theme of microbial evolution does not preclude the emergence of relatively large genomes and generalist microorganisms. In contrast with the genome streamlining and ecological partitioning seen in SAR11 and elsewhere, many microorganisms have genomes > 6 megabases in size. These genomes tend to be enriched in genes for regulation, secondary metabolism, and signal transduction (Konstantinidis & Tiedje, 2004; Koonin & Wolf, 2008), with many of these genes acquired via LGT (Cordero & Hogeweg, 2009). The largest prokaryotic genome sequenced to date is that of Sorangium cellulosum strain So ce56 (Schneiker et al., 2007), a standout in the already large myxobacterial group with a genome in excess of 13 megabases of DNA. Its ecology and complement of functional genes are far from being completely elucidated: More than 4400 genes had no assignable function from homology, and 3248 were proper ‘orphans’ with no detectable homologs in any other genome. Among genes with inferred functions, many are associated with secondary metabolite production, cell movement, sophisticated social behaviors including quorum sensing and fruiting body formation, and complex carbohydrate degradation. Evolutionary theory suggests that effective population sizes must be
small, and the role of drift must be substantial, to allow genomes to grow large (Lynch, 2006). Gained genes must be advantageous in the organism’s niche or niches, with only ‘fastidiously growing prokaryotes that inhabit complex, variable environments’ (Koonin & Wolf, 2008) likely to acquire and retain large numbers of new genes. Certainly many (but not all) of the largest microbial genomes are from soil-associated organisms such as the myxobacteria. The genus Pseudomonas contains more than 200 named species of environmental organisms and pathogens, with genomes typically in the range 5–7 MB, many of which can occupy multiple habitats thanks to gene duplication and LGT (Shen et al., 2006; Holloway & Beiko, 2010; Loper et al., 2012). The increased production of secondary metabolites may point to interactions with other microorganisms in a habitat, including negative interactions where the metabolites produced are intended to keep competitors at bay (Borriello, 1990; Cotter et al., 2013).

Mapping genes and molecular systems into a community framework

Having outlined ideas about ecological communities and the evolutionary processes in microorganisms that complicate the relationship between organismal phylogeny and function, we now consider current taxonomic and functional knowledge about microbial communities. These insights will allow us to develop ideas that fuse these aspects of microbial evolution and ecology into potentially new modes of analysis.

The search for a taxonomically defined ‘core’ microbiome

Ecological overlap or equivalence may be at the root of the frequently observed taxonomic differences among samples collected from the same or similar habitats. The most compelling example of this is the absence of a persistent ‘core’ microbiome in many human organs. Huse et al. (2012) examined the distribution of OTUs defined using a 97% identity threshold for different variable regions of the 16S. Oral and stool samples yielded a small number of OTUs that were ubiquitous or nearly so, although these were not necessarily abundant in all samples. Conversely, no OTUs were ubiquitous in many of the vaginal locations sampled, refuting the idea of a ‘core’ vaginal microbiome. Even OTUs that were ubiquitous in oral or stool samples showed differentiation among samples at higher thresholds of sequence identity, suggesting that important differences were being masked at the 97% identity level. Nemergut et al. (2011) examined the distribution of OTUs in different habitats such as soils, lake-water, and saline sediments and found that no OTU was ubiquitous in any habitat even when the sequence identity threshold was set as low as 89%. Deep sequencing of a marine sample (Gibbons et al., 2013) produced significant overlap with OTUs from a range of marine habitats, and the authors suggested that sufficiently deep sequencing at one site would reveal a ‘seed bank’ that encompasses all marine OTUs.

Several causes could contribute to the apparent lack of a ‘core’ in the many habitats examined, beyond the sampling limitations probed by Gibbons et al. (2013). Dispersal limitation and biogeography may play a role (Hanson et al., 2012), with groups such as Pseudomonas (Cho & Tiedje, 2000) and Burkholderia (Pearson et al., 2009), showing strong evidence of spatial structuring. Habitat definitions such as ‘soil’ and ‘gut’ are clearly too broad, as soil microbial diversity is strongly influenced by pH (Fierer & Jackson, 2006) and microhabitat (Carson et al., 2009; Dennis et al., 2009; Reim et al., 2012), and the composition of gut microbiota appears to strongly depend on factors such as diet (Muegge et al., 2011; Wu et al., 2011; Claesson et al., 2012) and the section of the gut that is sampled (Stearns et al., 2011). Although there is no core ‘gut’ microbiome, there may yet be a core ‘healthy transverse colon with high protein and animal fat inputs’ microbiome. Succession may also play a role, as seen for instance in the colonization of dental plaque: The same site can be occupied by ‘early’ or ‘late’ communities that emerge following a disturbance (Human Microbiome Project Consortium, 2012; Teles et al., 2012). Succession was also observed in the multiyear fermentation of American coolship ale, which shows a reproducible pattern in bacteria and yeast species (Bokulich et al., 2012). Finally, the lack of a core may reflect different outcomes of lottery processes as previously described, with observed assemblages reflecting different initial colonization events, where the first established organisms potentially structure the remainder of a community. The existence of positively correlated groups of lineages such as the ‘coabundance groups’ defined by Claesson et al. (2012) and groups of organisms identified in network analysis (Steele et al., 2011; Faust et al., 2012; Friedman & Alm, 2012) does not distinguish between these alternative scenarios. It does, however, suggest that the members of these groups either interact positively with one another and constitute a real community or interact in similar ways with the environment such that all are favored in the same conditions. The observed patterns also support the idea that taxonomic and phylogenetic approaches alone may be insufficient to understand the microbial ecology of a particular habitat (Shade & Handelsman, 2012).
Functional traits in microbial assemblages and communities

If a taxonomic or phylogenetic view fails to resolve a consistent set of community properties, trait-based approaches might yield more coherent results. The ecotype model of Cohan (2002, 2006) retains the requirement that entities constitute clades, but provides a very useful working notion of a set of organisms that are subject to similar evolutionary pressures due to their high relatedness and ecological similarities. However, the evolutionary dynamics of microorganisms allow for rapid change that may bring disparate lineages into conflict, especially if one lineage acquires a particular function of another via LGT. Thus, it becomes more straightforward to focus on ecological similarities, approximated by function defined at one or more levels of organization. How can we integrate functional similarities into a community analysis?

Functional overlap in spite of the apparent lack of an organismal core between samples of the same habitat has already provided convincing arguments in favor of a focus on ecological similarities. A recent example of this has been observed in the microbial communities associated with Ulva australis. Although only six OTUs were present in all sampled habitats (Burke et al., 2011b), and on average, 15% species similarity was seen between samples, and 70% functional similarity was observed across habitats. These functions spanned several categories such as motility, cell adhesion, biofilm formation, interaction with the host, and mechanisms of LGT (Burke et al., 2011a). The proteins involved in these functions in different samples were often phylogenetically distinct, suggesting functional convergence in disparate lineages. Such consistency of function has also been observed with regard to membrane proteins in the ocean. Patel et al. (2010) found correlations between transport proteins and inorganic chemical concentrations, but failed to find a corresponding link with species abundances. These functional profiles also correlated with environmental attributes including pollution, potentially allowing for these gene abundances to be utilized for predictions of such events. Barberán et al. (2012a) report that where 16S fails to differentiate marine microbial communities, genomic traits such as G+C content, genome size, and protein composition dramatically altered beta-diversity patterns and could better discriminate coastal from open-ocean samples and samples from the Atlantic, Pacific, and Indian oceans. Finally, the clinical significance of a shift from taxonomy-based to trait-based community ecology has already been demonstrated through the successful implementation of functional analyses and metagenomic linkage groups to discern microbiomes from type II diabetes patients and healthy individuals (Qin et al., 2012). The above examples all imply that within a given environment certain functional repertoires, defined either by collections of genes or by genomic properties, may be selected for and thus should be the focus of comparisons between habitats.

Although individual genes or ab initio generated combinations of genes may be predictive of phenotype or ecological role (e.g. MacDonald & Beiko, 2010), analyses that treat genes as uncorrelated entities will not always succeed in identifying important functional traits. For instance, Muegge et al. (2011) found that a diverse range of fecal microbial communities from different mammals clustered by diet type when 16S signatures were considered, but not when genes were summarized across all functional categories. Aggregation of genes into pathways and metabolic modules uses known associations between genes and allows for correction of incorrect predictions via gap filling and screening out of unlikely or redundant pathways (Ye & Doak, 2009; Abubucker et al., 2012). At the level of sequenced genomes, pathway- and module-based analyses have identified important functional correlations with periodontal disease (Kastenmüller et al., 2009). It is essential to choose the right trait definition for the question under scrutiny. Conserved traits are often assumed to track genome or organism evolution and thus may be expected to correlate with a wide range of genomic properties and functions (Langille et al., 2013). On the other hand, functional genes, pathways, or modules obtained from WGS confer information about a distinct set of traits that need not correlate with the phylogenetic relationships implied by 16S or other marker genes. To the extent that these different types of information can generate distinct and conflicting patterns, it may be worth combining them in an analysis.

How important are individual genes as mediators of community functions or interactions? Within a single cell, genes and gene products interact in a multitude of ways, for instance by direct chemical interaction, participation in the same biochemical pathway, transcriptional regulation, protein folding and refolding, and subcellular localization. These interactions place constraints on the evolutionary trajectory of genes: For example, the complexity hypothesis (Jain et al., 1999) predicts that genes whose products have many interactions are less likely to undergo LGT, suggesting lower LGT frequencies for ‘informational’ genes that tend to participate in large complexes such as the ribosome as compared with ‘operational’ genes with key metabolic and regulatory roles. This idea was made more explicit by Cohen et al. (2011) who showed that connectivity rather than function was the crucial determinant of gene transferability, which is
consistent with the frequent transfer of aminoacyl-tRNA synthetases that are informational, but have few interaction partners in the cell (Woese et al., 2000; Andam & Gogarten, 2011).

In applying these insights from sequenced genomes to microbial communities, a central question is how these gene product interactions can mediate different types of interaction between community members. Gene loss and gene transfer according to the PGH and BQH along with the processes of duplication and substitution can lead to the formation of new community interactions; several such examples have been outlined above in the ecology of the dechlorinating communities, insect endosymbionts, biofilms, and SAR11. Cross-feeding is an obvious example of a microbial interaction, but some described or implied interactions are more complex and difficult to elucidate. For example, targeted studies of homologous genes from environmental samples have revealed remarkable and seemingly stable sequence diversity (Sableh et al., 2003; Atamna-Ismael et al., 2008; Gabor et al., 2012), suggesting niche specialization (Bielawski et al., 2004) and the potential for rapid changes to nutrient sensitivity and host defense. Given the small amount of variation in these sequences and their presence in closely related strains that may possess identical 16S, the effects of these variations will depend on subtle differences in enzyme specificity or kinetics. Although transcription factors are unlikely to migrate between cells, there have been remarkable demonstrations of the ability of one taxon to induce significant changes in another, with dramatic ecological consequences. An example of this is seen in the lungs of cystic fibrosis patients that are subject to periodic exacerbations of the disease that lead to permanent declines in pulmonary function (Goss & Burns, 2007). With Pseudomonas aeruginosa as a primary pathogen of interest, researchers have identified a class of organisms including the Streptococcus milleri group, collectively termed ‘synergists’ that have neutral to positive impacts on hosts on their own, but increase mortality rates when combined with P. aeruginosa (Sibley & Surette, 2011). The specific interactions that induce the shift in pathogenic status remain to be elucidated, although transcriptional profiling under different association conditions will be highly informative (Duan et al., 2012).

Genes as defining elements of networks and metacommunities

Microbial genomes typically contain many thousands of genes, many of which may mediate community interactions. A challenge in studying the ecological role of genes is the possibility that different genes may have opposing effects on organismal interactions. Furthermore, opposing selection processes at the gene vs. organism level would obscure the link between gene and community. A gene-centric view of communities will liberate microbial ecology from exclusively marker gene-driven approaches, but untangling the effects of different genes may require models that can accommodate distributional, phylogenetic, and selective information about those genes. Networks that incorporate these types of information would thus better reflect the dynamics of a microbial community, which may allow for variable taxonomic membership while retaining functional parameters. We turn our attention now to promising ecological frameworks based on gene exchange that may suit this purpose.

Gene exchange communities

Up to now, we have considered microbial communities as defined by Konopka (2009) and others based on physical proximity of a set of organisms and the requirement that a set of organisms interact. However, LGT enables a different view of communities, where interactions between organisms are defined strictly on the basis of gene exchange (Jain et al., 2003; Skippington & Ragan, 2011). These gene exchange communities (GECs) are often represented by a graph or network structure with nodes signifying organisms or taxonomic groups and edges between nodes indicating evidence of LGT between a pair of groups. Additionally, edges can be directed if the identity of the donor and recipient can be reliably inferred and can also be weighted to reflect the extent of gene flow along a particular edge. DNA is sufficiently stable in the environment that GECs need not respect community or habitat boundaries and can span organisms that live in multiple habitats (Hooper et al., 2009; Holloway & Beiko, 2010; Smillie et al., 2011). Different types of DNA molecules including mobile phages and plasmids as well as chromosomes (Lima-Mendez et al., 2008; Halary et al., 2010) can also be considered separately in GECs. Such a vehicle-centric approach (Skippington & Ragan, 2011) can highlight the role of extrachromosomal elements in mediating LGT interactions between organisms.

Metacommunities of genes

Taking the idea of GECs one step further, the idea that genes mediate interactions suggests that analyses could consider communities of genes (once again in the ecological sense of ‘community’) in place of communities of organisms. An important recent development in microbial ecology is the application of metacommunity theory to microbial systems (Fig. 5a). A metacommunity was defined by Leibold et al. (2004) as ‘a set of local
communities that are linked by dispersal of multiple potentially interacting species’. The definition does not require that species interact with one another and therefore encompasses all assemblages whether or not they satisfy our definition of communities. Modeling linkages allows the simultaneous consideration of dispersal, competition, and other processes and can be used to test hypotheses about the dynamics of assemblages and communities. In this framework, for instance, a person or an individual organ can be viewed as a ‘patch’ occupied by a microbial community, with assembly of that community mediated by the metacommunity in situations such as recovery from disturbance and invasive species (Costello et al., 2012). Declerck et al. (2013) applied metacommunity principles in the investigation of plankton community similarity, testing the effects of variable nutrient availability and dispersal rate in an outdoor mesocosm experiment. Varying nutrient availability did not affect the similarity between communities, although there was some evidence that nutrient addition did have a significant impact on community structure. However, even tiny amounts of dispersal between communities (corresponding to 0.009% of total volume) were sufficient to make these communities more similar to one another.

Such metacommunity analyses can yield significant insights into the dominant forces that influence microbial community structure. A promising extension of metacommunity theory is the explicit consideration of phylogenetic relatedness of the taxa in a study, which can highlight cases where the distribution of a taxon is restricted for historical reasons (Urban & Skelly, 2006; Cavender-Bares et al., 2009; Leibold et al., 2010). Malcom (2011) additionally considered the role of gene networks in shaping the phenotypes that allow organisms to compete in patches. Given the possibility of shifting the focus of a community investigation from species to genes (or functions, however defined), it may be worth investigating whether metacommunity models that were originally developed with taxonomic units in mind can be equally well applied to sets of genes (Fig. 5b). In this setting, one could view a microorganism (or a population of organisms) as a patch that is colonized by a set of genes, with an analogy between the historical biogeographic constraints of, for example, Leibold et al. (2010) and the phylogenetic histories (vertical or otherwise) of individual functional genes. The complete collection of genes in a population (roughly equivalent to the pan-genome of a species: Tettelin et al., 2005, 2008) could then be modeled as a metacommunity. ‘Selfish’ elements such as transposons and restriction-modification systems are good candidates for ecological analysis due to their high mobility. For example, Venner et al. (2009) reviewed ‘genome ecology’ approaches that treat transposable elements as interacting elements with host eukaryotic genomes as the niche, while Hooper et al. (2009) identified transposases, shared via LGT, which bridged multiple habitats. Restriction/modification systems are highly mobile, can impact on the fitness of their host in many ways, and interact with one another in ways that are often lethal to the cell (Kobayashi, 2001), and would likely map well into a metacommunity framework.

At the whole-genome level, an intriguing example of the application of community genetic ideas to microbial ecology is the exploration by Reno et al. (2009), who examined the biogeography of seven Sulfolobus islandicus genomes distributed across three locations in the United States and Russia. In this case, strong evidence of allopatric speciation was observed, with no gene flow between

---

**Fig. 5.** Metacommunity approaches in microbial community analysis. (a) Metacommunity of organisms, with locations as encompassing units, lines indicating migration pathways and different taxa indicated with color. (b) Metacommunity of genes, with organisms as units. Gray clouds represent the core genome, while colored circles indicate the presence or absence of different genes of different functional classes in the pan-genome. Lines indicate sharing of genes; gray lines connecting taxon 6 with other taxa represent reduced levels of LGT due to decreased efficiency of homologous recombination. The phylogenetic tree indicates the relationships between taxa based on a marker gene such as 16S.
Defining and redefining the units of analysis

Having considered different ways of thinking about microbial communities in light of the evolutionary processes that shape the genomes of their constituents, we now consider the different definitions of ecological units that can be subjected to diversity analysis. Any ecological analysis of microorganisms will critically rest on the definition of the units or taxa to be counted, compared, and contrasted. Even before the advent of rapid DNA sequencing, a range of unit definitions emerged to balance taxonomy, phylogeny, and traits.

Taxonomic approaches have made use of OTUs, the application of which can be agnostic to the existence of taxonomic labels, thus allowing measurements of diversity and dissimilarity in the absence of a satisfactory taxonomic scheme. Because an OTU can be based upon any of a multitude of evolutionarily cohesive characters, including subregions of the 16S or other phylogenetic markers such as cpn60 (Case et al., 2007; Links et al., 2012), it allows for a range of markers to be utilized as the basis for diversity within a given sample or community (Hugenholtz et al., 1998; Schellenberg et al., 2011). A limitation of the OTU approach is that inferred groups for a fixed identity threshold will be different based on the choice of marker due to LGT or rate variation, potentially leading to different conclusions (Brousseau et al., 2001; Schellenberg et al., 2009). There is also sensitivity of the choice of method used to generate OTUs: Assignments can vary drastically depending on whether a 97% OTU is defined to require that a given sequence match all other sequences at this threshold or better (the ‘furthest neighbor’ approach) or whether it is sufficient that a given sequence matches any other sequence in the OTU (the ‘nearest neighbor’ approach). Phylogenetic approaches to diversity such as genome-based classification and inference among microorganisms (Klenk & Göker, 2010; Chan et al., 2012) address some of the limitations of OTU analysis (Lozupone et al., 2007). Still, they present a single picture of diversity that is dependent on a canonical hierarchical relationship.

A refinement of single marker diversity measures that still relies on phylogenetic cohesion is the concept of ecotypes (Cohan, 2002, 2006). This concept gives weight to common taxonomic properties between members of the same species, but differentiates based upon small changes in gene content or expression, which may allow for greater fitness within an ecological niche. Konstantinidis et al. (2006) defined ecotypes, based upon average nucleotide identity, to be members of a species that have accumulated a few small extra genetic elements or mutations for environmental adaptation, but otherwise preserve the overall genetic signature of the species. These approaches integrate the notion of OTU relatedness within a tight cluster, but allow for lineage segregation based upon small changes to the overall genetic background. Another departure from phylogenetically defined units is the concept of genovars, groups of strains that share distinct genetic content profiles (Porwollik et al., 2004) and form homologous recombination pools (Ahmed et al., 2012). These distinguishable groups that are below the level of species, but above the level of strain would likely form clusters within a taxonomy-free OTU study and would also group with other genovars in a species classification. Therefore, groupings such as genovars require a combination of functional and taxonomic diversity measures to understand their potential for pathogenicity or other functional features.

Pathogenicity and other phenotypic traits can also be shared collectively by members of a microbial assemblage or community and constitute a basis for classification. For example, pathogroups are polymicrobial biofilm communities that are integral to infections where the entire compilation of microorganisms contributes to a generalized pathogenic phenotype (Dowd et al., 2008). Such polymicrobial biofilms demonstrate diverse community properties, which allow for invasion of host tissue and subsequent cell adherence in tandem with passive antibiotic resistance and metabolic handovers (Wolcott et al., 2013). These biofilms are composed of highly integrated yet diverse parts with active mechanisms of recruitment to ensure such variety is achieved.
Coordinated yet diverse communities have also been observed in non-pathogenic settings such as dental plaques (McBride & Van der Hoeven, 1981; Kuramitsu et al., 2007). These biofilms are clear examples of where the diversity of the community matters and interplay between such diverse members, be they of differing species, strains, or levels in between, form the basis of a community and directed associated interactions with the habitat. Studies based upon single marker genes in an organism-by-organism setting will not elucidate such patterns, because a coupling of functional trait-based approaches and taxonomic contexts is required to observe fine-grained diversity and related community functionality. This coupling can inform experimental procedures for studying such communities and their interaction networks.

Another approach that can link taxonomically disparate organisms is the use of phylogenetic networks (Hilario & Gogarten, 1993; Beiko et al., 2005; Kunin et al., 2005; Beiko, 2011; Dagan, 2011; Parks & Beiko, 2012), rather than phylogenetic trees, as the basis for phylogenetic beta-diversity. Such networks can represent the uncertainty in a phylogenetic tree (e.g. Parks & Beiko, 2012) or show conflicting similarity relationships as derived from a sampling of many genes rather than a single phylogenetic marker. The latter type of network could modify diversity values by downweighting the ecological differences among organisms that participate in the same gene exchange community, for instance by tracing the shortest path between a pair of taxa instead of the canonical relationship derived from a marker gene.

Testing the community hypothesis

Because our working definition presents microbial communities as a hypothesis rather than a mere set of observations, experimental and computational approaches need to be designed with communities in mind. In Box 1, we outline protocols that assess the growth response of microorganisms, target the metabolites they produce, and enable a genomic view of organismal interactions. Given the central importance of sequence data in microbiomics, the remainder of this section is focused on emerging methods that can target the question of microbial communities.

Computational approaches for marker gene and metagenomic data

Bioinformatics, central to the analysis of microbial communities, will benefit from the development of new descriptive standards such as MIMARKS to describe marker genes (Yilmaz et al., 2011) and the emergence of reference databases and formats that aim to adhere to these standards (Gilbert et al., 2010b; Ivanova et al., 2010; McDonald et al., 2012). An important first question is how the current taxonomic and phylogenetic strategies for inferring community structure can be augmented with additional information about function and distribution (Fig. 6). Martiny et al. (2009) demonstrated the value of using different OTU thresholds to discover different environmental correlates in samples of P. marinus. In using supervised learning approaches to classify microbiome samples based on OTU abundance, Knights et al. (2011) found similar performance across a wide range of OTU thresholds and suggested that ‘hybrid models using several levels of phylogenetic binning will outperform those constrained to any one bin size, and this is certainly an area that requires further research’. Large reference databases such as the Earth Microbiome Project associate marker gene distributions with a wide range of habitat and temporal information; these resources will provide a rich reference set against which new data sets can be compared.

While phylogenetic proximity is the most commonly used surrogate for ecological similarity, as evidenced by the proliferation of studies using OTU-based and phylogenetic beta-diversity measures to infer community similarity, these new sources of information suggest new approaches based on habitat similarity and/or co-occurrence. Recent work suggests that molecular functions are in many cases predictable from marker gene information, if phylogenetically close reference genome sequences are available (Langille et al., 2013; Martiny et al., 2013). Although the genome evolution processes described above can lead to different degrees of predictability for different types of molecular function and for different lineages, these observations suggest that OTU analyses can be enriched with functional information to produce better definitions of ecological units and predictions of ecological roles (Fuhrman, 2012). Given the value and predictive power of phylogenetic marker genes, especially at the genus and species level, we anticipate that the implicit functional information conveyed by marker genes, along with explicit information from functional genes (e.g. functional gene networks: Bittner et al., 2010) and habitat distribution (e.g. co-occurrence networks: Chaffron et al., 2010; Barberán et al., 2012b; Faust et al., 2012), will prove a powerful combination in the ecological analysis of microorganisms (Fig. 6). For example, Layeghifard et al. (2012) made the connection between phylogenetic networks and species dispersal, using the network approach of Boc et al. (2010) for inferring networks from genetic data, to reconstruct migration networks from geographic and biodiversity information.
Interactions in the microbiome

Box 1. Experimental approaches to investigating assemblages and communities

Although sequencing of environmental DNA currently dominates the study of microbial assemblages and communities, a variety of allied technologies are required to determine what is transcribed (metatranscriptomics: Poretsky et al., 2005; Leininger et al., 2006; Moran et al., 2013), the proteins present in a sample (metaproteomics: Rodríguez-Valera, 2004; Wilmes & Bond, 2004; Ram et al., 2005), and what metabolites are produced (metabolomics: Weckwerth, 2003). Metaproteomics (Wilmes & Bond, 2004) can reveal new functional genes and metabolic pathways in a sample. Ram et al. (2005) used ‘shotgun’ mass spectrometry approaches to identify correlations between organismal abundance and level of protein expression and highlight the apparent importance of hypothetical proteins as well as proteins involved in refolding (e.g. chaperones) and oxidative stress. Metaproteomics has also been used to identify strain-level variation in Candidatus ‘Accumulibacter phosphatis’ protein expression in enhanced biological phosphorus removal (EBPR) communities (Wilmes et al., 2008a) and directly link these proteins to EBPR metabolic processes (Wilmes et al., 2008b). These approaches have been combined to further characterize microbial communities (e.g. Gilbert et al., 2010a; Teeling et al., 2012; Yu & Zhang, 2012). While none of these approaches can necessarily identify the precise nature of an interaction, they can be used to gauge the impact of shifts in environment or assemblage on the function of an organism.

Single-cell isolation and sorting techniques can be used to subdivide communities and facilitate genomics, proteomics, and transcriptomics of a select group of cells to gain insight about community ecology (Müller et al., 2012). For example, Jehmlich et al. (2010) used cell-sorting techniques to separate E. coli K-12 from Pseudomonas putida KT2440 in a mixed culture of 5 × 10⁶ cells and applied proteomics to identify proteins that were expressed in each subpopulation. The sequenced genomes of five cells of the Verrucomicrobia obtained through single-cell isolation from bacterioplankton communities revealed that these organisms are capable of hydrolysis of a wide variety of polysaccharides, which is important in bacterioplankton communities (Martinez-Garcia et al., 2012).

Potential interactions in a set of microorganisms can be assessed by measuring the impact they have on microbial growth rates in culture. Experimental systems such as microcosms, chemostats, and mixed cultures have produced a large body of knowledge about the evolution, ecology, and physiology of organisms and communities in a wide variety of natural and artificial habitats. Tresesica-Mlynarz & Ward (1995) discovered the importance of mixed cultures in degradation of polycyclic aromatic hydrocarbons: Mixed cultures of P. putida, an unknown flavobacterium, and P. aeruginosa degraded a wider range of polycyclic aromatic hydrocarbons, relative to pure cultures of each bacterium. Kuenen (1983) examined the role of competition between specialists and a generalist in a mixed culture: Specialists Thiobacillus neapolitanus and Spirillum G7 with generalist Thiobacillus A2 were placed in various growth media; Thiobacillus A2 outcompeted the other strains on mixed media, while the specialists were more successful on specialized media. Sher et al. (2011) discovered that co-cultures including one of two closely related strains of the photoautotroph P. marinus and one of 344 strains of various heterotrophic bacteria enhanced growth curves in a manner dependent on the relatedness of the P. marinus strains. As with transcriptomic and other types of data described above, it may not be possible to deduce the exact nature of an interaction from co-culture experiments alone.

Historical limitations to characterization of community metabolism are being remedied with new laboratory techniques. Available techniques include stable isotope probing (SIP) for culture-independent tracking of molecules through microbial communities and their members (Kreuzer-Martin, 2007) and imaging mass spectrometry (Watrous et al., 2011) for collecting direct evidence of chemical interaction between community members. DNA stable isotope probing (DNA-SIP) combines stable isotope tagging to molecules to allow the identification and function of organisms that metabolize the tagged molecules (Chen & Murrell, 2010). Schloss & Handelsman (2003) suggested the use of DNA-SIP to subdivide microbial communities for metagenomic sequencing, while a proof of concept was used to isolate large DNA fragments from uncultured soil bacteria (Dumont et al., 2006). Other work used DNA-SIP and metagenomics to dramatically increase the chance of finding specific functional genes from metagenomes (Knietsch et al., 2003; Sul et al., 2009).

Fine-scale understanding about the interaction between any two organisms can also be obtained through insertional mutagenesis and depletion (iMAD), which combines bacterial mutagenesis and RNA interference. Using iMAD, the dynamics of interaction between Legionella pneumophila and its host was resolved, revealing the network of proteins that are required for intracelluar growth of L. pneumophila (O’Connor et al., 2012). Desorption electrospray ionization (DESI) has verified already known metabolic interactions between competing Bacillus subtilis and Streptomyces coelicolor (Watrous et al., 2010), and nano-DESI has been used to examine the molecular networks of living colonies, including the possible identities of unknown metabolites through time (Watrous et al., 2012). Nano-DESI could be potentially useful in reconstructing the metabolism of a community of organisms (i.e. a multispecies metabolic network), establishing alternative organismal physiologies, and when combined with sequencing, help in the verification of gene function.
Another promising approach is to construct more explicit models of microbial and community function to address the partitioning of functions across taxa in a sample. Our understanding of metagenomic samples is constrained by the large number of hypothetical proteins for which reliable functions are not available (Galperin & Koonin, 2004; Ellrott et al., 2010) and the high degree of misannotation of some functional families of proteins (Schnoes et al., 2009; Radivojac et al., 2013). However, educated guesses about protein function can be made based on cues such as phylogeny, genetic linkage, subcellular localization, and metabolic pathway cohesion (Yu et al., 2010; Engelhardt et al., 2011; Yelton et al., 2011), and these predictions can be improved through computational means (Chen et al., 2013) and tested experimentally (Mirete et al., 2007; Yamada et al., 2012). Predicted genes in metagenomes can be subjected to both functional and taxonomic assignment, to divide the functional profile of the microbiome by organism or lineage. Although taxonomic assignment is imperfect (McHardy & Rigoutsos, 2007; MacDonald et al., 2012) especially when reference taxa are lacking or multiple strains are present, this information can nonetheless be used to determine which organisms are providing which crucial functions in a community (Hug et al., 2012). Ideally, such analyses can reveal metabolic pathway discontinuities or ‘handoff points’ that correspond to syntrophic or other types of association (Fig. 7).

Systems biology approaches have been developed to model the flux of metabolites within and between community members. Existing models can struggle with the number of steps and the complexity of interactions involved, but simplified approaches that focus on particular functions of interest have yielded testable predictions (Stolyar et al., 2007; Salimi et al., 2010; Zhuang et al., 2012; Zomorrodi & Maranas, 2012). Although interactions predicted from metabolic networks and models still need to be tested through experimentation, they will quickly be able to highlight potential positive and negative interactions between microorganisms based on metagenomic data. Enumerating many different types of communities in this fashion will reveal which types of dependencies (for instance, cofactor synthesis, carbohydrate degradation, and dependence on others for oxygen scavenging) have emerged most often.

![Fig. 6. Computing diversity (expressed here as the dissimilarity between samples A and B) with multiple types of data. (a) A phylogeny of marker genes, which serves as the basis for most studies of microbial beta-diversity. (b) The distribution (i.e., phylogenetic profile) of different classes of genes can highlight associations that do not necessarily coincide with the phylogeny in (a), suggesting evolutionary and possibly functional connections between more distant taxa. (c) Co-occurrence networks display positively and negatively correlated sets of taxa, highlighting possible species sorting effects and functionally equivalent or similar taxa. Such taxa could contribute relatively little to overall beta-diversity. (d) Like the distributions in (b), phylogenies of nonmarker genes can recapitulate the dispersal of genes across a set of taxa in a nonvertical manner and identify taxa that are more functionally similar than their marker gene phylogeny would suggest.

![Fig. 7. Identifying handoff points in metagenome samples. Steps in a directed, branching metabolic pathway are shown, with colored squares indicating the presence of a given reaction in different members of a microbial community. Some organisms such as the blue individual in both communities encode only the first few steps of the pathway and do not require the products of later steps. However, handoff points (indicated with ‘*’) are steps where an organism depends on others members of the community for synthesis of a particular metabolite. The handoff point locations differ for the orange taxon in (a) and the pink taxon in (b), possibly due to different combinations of LGT and gene loss in the impacted organisms.

© 2013 Federation of European Microbiological Societies. Published by John Wiley & Sons Ltd. All rights reserved.
An evolutionary context for microbial communities

The evolutionary trajectory of genomes can inform us about processes of community formation and specialization of microorganisms. The interactions between organisms in a putative community can be probed using the methods described above, but comparisons against completely sequenced reference genomes highlight differences that may reflect recent community evolution, including evidence of ‘public goods’ sharing or gene losses according to the BQH. The application of metacommunity theory to genes in a set of metagenomes in concert with closely related sequenced genomes would produce novel ecological views of the metagenome in relation to habitat and the partitioning of genes among organisms. This approach extends ideas already developed in the examination of shared gene pools in environmental organisms such as *S. islandicus* (Reno et al., 2009) and lineages such as *Listeria* that include both pathogenic and environmental isolates (Dunn et al., 2009). Johnson & Winquist (2011) proposed that changes in hygiene have impacted on the biogeography of gut bacteria, effectively altering the metacommunity structure by increasing the fragmentation of communities. Such shifts would undoubtedly impact LGT regimes as well.

Evolutionary pressures manifested at the gene level will also shed light on the role of different genes in a community. In *M. leprae*, the ratio of nonsynonymous to synonymous mutations (dN/dS) can be used to identify genes under reduced selection pressure, indicating possible losses of function that will ultimately lead to deletion from the genome. Calculated dN/dS ratios significantly > 1.0 constitute evidence for positive selection and may indicate rapid adaptation to a new or changing habitat (Ohta, 1992). Increased rates of nonsynonymous substitution have also been observed in reduced endosymbionts (Kuo et al., 2009) and have been interpreted to reflect reduced effective population sizes in pathogens (Warnecke & Rocha, 2011). In the human microbiome, Schloissnig et al. (2013) found no genes with strong evidence of positive selection, but identified antimicrobial genes and bile salt hydrolase as the best candidates for undergoing localized and rapid nonsynonymous change. Conversely, an examination of iron uptake genes from the Global Ocean Sampling database identified a number of iron uptake and metabolism genes that showed evidence of positive selection, suggesting adaptations to nutrient limitation that echo the quantitative study of Patel et al. (2010). Complementing the study of substitution rates is the search for evidence of genetic recombination within genomes. Recombination and LGT have been shown to replace parts of genes as well as entire genes (Schouls et al., 2003; Chan et al., 2009, 2011), and recombination within some types of genes such as those encoding surface proteins could be interpreted as a modifying force akin to positive selection (Hollingshead et al., 2000; Baldo et al., 2010).

Conclusion

The preeminent questions in microbial ecology today can be traced back to the original diversity surveys of van Leeuwenhoek and ecological studies of succession in the late 17th century. The intervening 300 years have provided evidence that microorganisms are alive and abundant in every setting on Earth, can cause disease, possess genetic material, are central to nutrient cycling, and can evolve quickly to adapt to new challenges and opportunities. With every significant new technique developed to study microorganisms, the predominant thinking of earlier periods has been overturned, reshaping the debate about their fundamental nature and the roles they play (Sapp, 2005). Affordable and fast DNA sequencing drives the latest revolution, and the rate at which genomic (including metagenomic) data are accumulating easily outstrips our capacity to thoroughly analyze and reason about microbial communities. Even where informed analysis is possible, important conclusions can be entirely dependent on the statistical and computational tools that are applied and the choice of approach used to categorize diversity and function. A striking example of this dependency is the claim that sets of microorganisms in the human gut can be classified into ‘enterotypes’ that differ qualitatively in their composition and function. Arumugam et al. (2011) claimed the existence of enterotypes based on the observation that the taxonomic profile associated with any gut sample could be assigned to one of three enterotypes. However, other evidence suggests that enterotypes are a product of the analytical methods used to analyze the data (MacDonald et al., 2012; Yatsunenko et al., 2012; Koren et al., 2013) and that diversity is best represented as a gradient rather than a finite and small number of discrete states (Jeffery et al., 2012; Yatsunenko et al., 2012). Although more data (larger samples, more individuals, time series) are essential to resolving this question, microbial community theory will have a central role to play as well, because the degree of interactions will influence the tendency of communities to behave like discrete entities or gradients.

Even microbial communities with low apparent diversity present a multitude of challenges. The extreme acid mine drainage biofilm environment has been probed extensively using sequence-based and complementary approaches (Tyson et al., 2004; Belnap et al., 2010; Jiao et al., 2011). While the community is dominated by a narrow range of lineages including *Leptospirillum* and...
Ferroplasma (Tyson et al., 2004), population-level analysis has revealed extensive sequence diversity and evidence of LGT among closely related strains (Allen et al., 2007; Eppley et al., 2007), rare lineages of tiny Archaea (Baker et al., 2006), multiple lineages of acidophilic eukaryotes, some of which act as hosts to bacterial endosymbionts (Baker et al., 2003, 2009), and complex bacteriophage interactions with rapid turnover of CRISPR sequences (Andersson & Banfield, 2008). Modeling the evolutionary and ecological interactions within even this ‘simple’ community is a daunting task; a challenge that is only amplified in less extreme environments that have more niches, higher richness and diversity, and greater disturbances including increased competition through dispersal. Clearly, lineage-based analyses alone are insufficient to the task of modeling communities.

A question that is highly pertinent to microbial ecology was posed by Webb et al. (2010): ‘Can ecological performance generally be predicted by a single or just a few traits or are many traits required?’ Currently, our knowledge of many microbiomes is based on a single trait, the 16S, which is present in all prokaryotes, but conveys no direct evidence of ecological differences. Using genomes and metagenomes shifts the balance from too few to too many traits, and analyzing all genes indiscriminately will lose important functional differences in an ocean of largely uninformative functional information. For this reason, we see a great deal of promise in approaches that fuse phylogenetic information (which can serve as a proxy for many traits that are conserved at low-to-medium taxonomic ranks) with specific functions of interest and information about habitat distribution (with species sorting as another imperfect proxy for function). In concert with these approaches to diversity, we believe that treating genes as ecological agents will yield vital new insights into the question of whether shifts in taxonomic composition necessarily imply shifts in community function. If different organisms in different communities are fulfilling the same roles, it is more likely that the communities differ in their taxonomic composition due to stochastic processes such as founder effects or density-dependent effects such as phage predation. Conversely, functional differences – even in only a few critical pathways – could reflect subtle, but important habitat differences and dramatically altered ecosystem services. This focus on functional attributes, and the corresponding view of genes as agents that confer selective advantages, echoes the ‘selfish gene’ hypothesis of Dawkins (1976), but allows genes even more freedom to follow distinct evolutionary trajectories thanks to LGT. However, identifying crucial functional differences is a daunting challenge given the confounding effects of hypothetical proteins and incorrect functional annotations. Identified pathways may also be irrelevant if an organism either is incapable of expressing that pathway or is merely a ‘tourist’ that is isolated from interactions with the host or other observed microorganisms. Replicated experiments and the use of complementary approaches such as transcriptomics will provide partial solutions to these problems (Knight et al., 2012).

Microbiomics is already being applied in a wide range of settings. The preeminent example in human health is the use of fecal transplants containing a defined mixture of nonpathogenic organisms to cure Clostridium difficile infection (Lawley et al., 2012; Petrof et al., 2013). Community construction, enrichment, and amendment are being used to optimize many microbial bioremediation processes (Duhamel et al., 2004; Brune & Bayer, 2012; Mikesková et al., 2012; Patel et al., 2012), while environmental monitoring has revealed startling shifts in the Arctic Ocean microbiota, a potential harbinger of further shifts as the state of the region changes (Comeau et al., 2011). A deeper understanding of the relationships and dependencies between microorganisms in a community will recast central questions in microbiology from ‘Who is there?’ and ‘What are they doing?’ to ‘How will they respond?’ New techniques for community analysis and modeling will influence the design of experiments and suggest interventions to produce desirable changes in microbial community function. With this will come the realization of the full potential of microbiomics.

Acknowledgements

We are grateful to W. Ford Doolittle, Elizabeth Edwards, Donovan Parks, and Joseph Bielawski for feedback on earlier versions of the manuscript. We also thank Jonathan Eisen for his discussion on the definition of ‘microbiome’ (http://phylogenomics.blogspot.ca/2009/03/human-microbiome-term-being-used-in.html). E.B., C.J.M. and M.G.I.L. are supported by the Canadian Institutes for Health Research. C.W. is supported by the Natural Sciences and Engineering Research Council of Canada and the Killam Trusts. D.H.J.W. is supported by the Dalhousie Centre for Comparative Genomics and Evolutionary Bioinformatics. R.G.B. acknowledges the support of Genome Atlantic and the Canada Research Chairs program.

Authors’ contribution

E.B. and C.J.M. are co-first authors.

References

Abubucker S, Segata N, Goll J et al. (2012) Metabolic reconstruction for metagenomic data and its application to the human microbiome. PLoS Comput Biol 8: e1002358.
Interactions in the microbiome

Ahmed A, Earl J, Retchless A et al. (2012) Comparative genomic analyses of 17 clinical isolates of Gardnerella vaginalis provide evidence of multiple genetically isolated clades consistent with speciation into genovars. J Bacteriol 194: 3922–3937.

Allen EE, Tyson GW, Whitaker RJ, Detter JC, Richardson PM & Banfield JF (2007) Genome dynamics in a natural microbial strain population. P Natl Acad Sci USA 104: 1883–1888.

Amann RI, Ludwig W & Schleifer KH (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev 59: 143–169.

Andam CP & Gogarten JP (2011) Biased gene transfer in microbial evolution. Nat Rev Microbiol 9: 543–555.

Andersson AF & Banfield JF (2008) Virus population dynamics and acquired virus resistance in natural microbial communities. Science 320: 1047–1050.

Antonova E & Hammer B (2011) Quorum-sensing autoinducer molecules produced by members of a multispecies biofilm promote horizontal gene transfer to Vibrio cholerae. FEMS Microbiol Lett 322: 68–76.

Arumugam M, Raes J, Pelletier E et al. (2011) Enterotypes of the human gut microbiome. Nature 473: 174–180.

Atamna-Ismaeel N, Sabehi G, Sharon I, Witzel KP, Labrenz M, Andersson AF & Hammer B (2011) Quorum-sensing gene transfer challenges principles of microbial systematics. Trends Microbiol 16: 200–207.

Badri DV, Zolla G, Barkker MG, Manter DK & Vivanco JM

Bailey JK, Deckert R, Schweitzer JA, Rehill BJ, Lindroth RL, Gehring C & Whitham TG (2005) Potential impact of soil microbiomes on the leaf metabolome and on herbivore feeding behavior. New Phytol 169: 264–273.

Bailey JK, Weerasinghe S, Welker S, Rittman BE, Brodie EL, Detter JC et al. (2011) The major human gut bacterial phylum Planctomycetes harbors an abundant and novel gene for proteorhodopsin. ISME J 5: 758–766.

Barberán A, Bates ST, Casamayor EO & Fierer N (2012a) Using network analysis to explore co-occurrence patterns in soil microbial communities. ISME J 6: 343–351.

Barberán A, Fernández-Guerra A, Bohannan BJ & Casamayor EO (2012b) Exploration of community traits as ecological markers in microbial metagenomes. Mol Ecol 21: 1909–1917.

Beiko RG (2011) Telling the whole story in a 10,000-genome world. Biol Direct 5: 6.

Beiko RG, Harlow TJ & Ragan MA (2005) Highways of gene sharing in prokaryotes. P Natl Acad Sci USA 102: 14332–14337.

Belnap CP, Pan C, VerBerkmoes NC, Power ME, Samatova NF, Carver RL, Hettich RL & Banfield JF (2010) Cultivation and quantitative proteomic analyses of acidophilic microbial communities. ISME J 4: 520–530.

Bielawski JP, Dunn KA, Sabehi G & Béja O (2004) Darwinian adaptation of proteorhodopsin to different light intensities in the marine environment. P Natl Acad Sci USA 101: 14824–14829.

Bittner L, Halary S, Payri C, Cruaude C, de Reviers B, Lopez P & Baptiste E (2010) Some considerations for analyzing biodiversity using integrative metagenomics and gene networks. Biol Direct 5: 47.

Boc A, Philippe H & Makarenkov V (2010) Inferring and validating horizontal gene transfer events using bipartition dissimilarity. Syst Biol 59: 195–211.

Bokulich NA, Bamforth CW & Mills DA (2012) Brewhouse-resident microbiota are responsible for multi-stage fermentation of American coolship ale. PLoS ONE 7: e35507.

Bouillet MB, El-Aidy S, Rajilic-Stojanovic M, Heilig HG, Troost FL, Smidt H, Kleerebezem M, De Vos WM & Zoetendal EG (2010) High temporal and inter-individual variation detected in the human ileal microbiota. Environ Microbiol 12: 3213–3227.

Borriello SP (1990) The influence of the normal flora on Clostridium difficile colonisation of the gut. Ann Med 22: 61–67.

Brousseau R, Hill JE, Prefontaine G, Goh SH, Harel J & Hemmingsen SM (2001) Streptococcus suis serotypes characterized by analysis of cpn60 gene sequences. Appl Environ Microbiol 67: 4828–4833.
Brown MV, Lauro FM, DeMaere MZ et al. (2012) Global biogeography of SAR11 marine bacteria. Mol Syst Biol 8: 595.

Brune KD & Bayer TS (2012) Engineering microbial consortia to enhance biomining and bioremediation. Front Microbiol 3: 203.

Burke C, Steinberg P, Rusch D, Kjelleberg S & Thomas T (2011a) Bacterial community assembly based on functional genes rather than species. P Natl Acad Sci USA 108: 14288–14293.

Burke C, Thomas T, Lewis M, Steinberg P & Kjelleberg S (2011b) Composition, uniqueness and variability of the epiphytic bacterial community of the green alga Ulva australis. ISME J 5: 590–600.

Caporaso JG, Lauber CL, Costello EK et al. (2011) Moving pictures of the human microbiome. Genome Biol 12: R50.

Carini P, Steindler L, Beszteri S & Giovannoni SJ (2012) Nutrient requirements for growth of the extreme oligotroph 'Candidatus Pelagibacter ubique' HTCC1062 on a defined medium. ISME J 7: 592–602.

Carson JK, Campbell L, Rooney D, Clipson N & Gleeson DB (2009) Minerals in soil select distinct bacterial communities in their microhabitats. FEMS Microbiol Ecol 67: 381–388.

Case RJ, Boucher Y, Dahllof I, Holmström C, Doolittle WF & Kjelleberg S (2007) Use of 16S rRNA and rpoB genes as molecular markers for microbial ecology studies. Appl Environ Microbiol 73: 278–288.

Cavender-Bares J, Kozak KH, Fine PV & Kembel SW (2009) The merging of community ecology and phylogenetic biology. Ecol Lett 12: 693–715.

Chaffron S, Rehrauer H, Perlthaler J & von Mering C (2010) A global network of coexisting microbes from environmental and whole-genome sequence data. Genome Res 20: 947–959.

Chan CX, Beiko RG, Darling AE & Ragan MA (2009) Lateral transfer of genes and gene fragments in prokaryotes. Genome Biol Evol 1: 429–438.

Chan CX, Beiko RG & Ragan MA (2011) Lateral transfer of genes and gene fragments in Staphylococcus extends beyond mobile elements. J Bacteriol 193: 3964–3977.

Chan JZ, Halachev MR, Loman NJ, Constantinidou C & Pallen MJ (2012) Defining bacterial species in the genomic era: insights from the genus Acinetobacter. BMC Microbiol 12: 302.

Chen Y & Murrell JC (2010) When metagenomics meets stable-isotope probing: progress and perspectives. Trends Microbiol 18: 157–163.

Chen I-MA, Markowitz VM, Chu K et al. (2013) Improving microbial genome annotations in an integrated database context. PLoS ONE 8: e54859.

Cho JC & Tiedje JM (2000) Biogeography and degree of endemicity of fluorescent Pseudomonas strains in soil. Appl Environ Microbiol 66: 5448–5456.

Claesson MJ, Jeffery IB, Conde S et al. (2012) Gut microbiota composition correlates with diet and health in the elderly. Nature 488: 178–184.

Clarke M, Lohan AJ, Liu B et al. (2013) Genome of Acanthamoeba castellani highlights extensive lateral gene transfer and early evolution of tyrosine kinase signaling. Genome Biol 14: R11.

Clements FE (1916) Plant Succession; An Analysis of the Development of Vegetation. Carnegie Institution of Washington, Washington DC.

Cohan FM (2002) What are bacterial species? Annu Rev Microbiol 56: 457–487.

Cohan FM (2006) Towards a conceptual and operational union of bacterial systematics, ecology, and evolution. Philos Trans R Soc Lond B Biol Sci 361: 1985–1996.

Cohan FM & Perry EB (2007) A systems for discovering the fundamental units of bacterial diversity. Curr Biol 17: R373–R386.

Cohen O, Gophna U & Popko T (2011) The complexity hypothesis revisited: connectivity rather than function constitutes a barrier to horizontal gene transfer. Mol Biol Evol 28: 1481–1489.

Cole ST, Ewigmeier K, Parkhill J et al. (2001) Massive gene decay in the leprosy bacillus. Nature 409: 1007–1011.

Collins JP & Crump ML (2009) Extinction in Our Times. Global Amphibian Decline. Oxford University Press, Oxford, UK.

Comeau AM, Li WK, Tremblay JE, Carmack EC & Lovejoy C (2011) Arctic Ocean microbial community structure before and after the 2007 record sea ice minimum. PLoS ONE 6: e27492.

Cordero OX & Hogeweg P (2009) The impact of long-distance horizontal gene transfer on prokaryotic genome size. P Natl Acad Sci USA 106: 21748–21753.

Cornell HV & Lawton JH (1992) Species interactions, local and regional processes, and limits to the richness of ecological communities: a theoretical perspective. J Anim Ecol 61: 1–12.

Costello EK, Stagaman K, Dethlefsen L, Bohannan BJ & Relman DA (2012) The application of ecological theory toward an understanding of the human microbiome. Science 336: 1255–1262.

Cotter PD, Ross RP & Hill C (2013) Bacteriocins – a viable alternative to antibiotics? Nat Rev Microbiol 11: 95–105.

Cowen ST (1955) Symposium. The principles of microbial classification. Introduction. The philosophy of classification. J Gen Microbiol 12: 314–321.

Dagan T (2011) Phylogenomic networks. Trends Microbiol 19: 483–491.

Dagan T, Artzy-Randrup Y & Martin W (2008) Modular networks and cumulative impact of lateral transfer in prokaryote genome evolution. P Natl Acad Sci USA 105: 10039–10044.

Dawkins R (1976) The Selfish Gene. Oxford University Press, New York City.
Interactions in the microbiome

lineages of Listeria under an “extended mosaic genome concept”. Mol Biol Evol 26: 2605–2615.

Dykhuizen DE & Green L (1991) Recombination in Escherichia coli and the definition of biological species. J Bacteriol 173: 7257–7268.

Ehrlich GD (2001) The biofilm and distributed genome paradigms provide a new theoretical structure for understanding chronic bacterial infections. 41st Interscience Conference on Antimicrobial Agents and Chemotherapy; December 16–19, 2001; Chicago, Illinois.

Ehrlich PR & Holm RW (1962) Patterns and populations: basic problems of population biology transcend artificial disciplinary boundaries. Science 137: 652–657.

Ehrlich GD, Ahmed A, Earl J, Hiller NL, Costerton JW, Stoodley P, Post JC, DeMee P & Hu FZ (2010) The distributed genome hypothesis as a rubric for understanding evolution in situ during chronic bacterial biofilm infectious processes. FEMS Immunol Med Microbiol 59: 269–279.

Ellrott K, Jaroszewski L, Li W, Wooley JC & Godzik A (2010) Expansion of the protein repertoire in newly explored environments: human gut microbiome specific protein families. PLoS Comput Biol 6: e1000798.

Elton CS (1927) Animal Ecology. Sidgwick and Jackson, London.

Engelhardt BE, Jordan MI, Srouji JR & Brenner SE (2011) Genome-scale phylogenetic function annotation of large and diverse protein families. Genome Res 21: 1969–1980.

Eppley JM, Tyson GW, Getz WM & Banfield JF (2007) Genetic exchange across a species boundary in the archaeal genus Ferroplasma. Genetics 177: 407–416.

Ereshefsky M & Pedros M (2013) Biological individuality: the case of biofilms. Biol Philos 28: 331–349.

Falony G, Vlachou A, Verbrugghe K & De Vuyst L (2006) Cross-feeding between Bifidobacterium longum BB536 and acetate-converting, butyrate-producing colon bacteria during growth on oligofructose. Appl Environ Microbiol 72: 7835–7841.

Fan L, Reynolds D, Liu M, Stark M, Kjelleberg S, Webster NS & Thomas T (2012) Functional equivalence and evolutionary convergence in complex communities of microbial sponge symbionts. P Natl Acad Sci USA 109: E1878–E1887.

Faust K, Sathirapongsasuti JF, Izard J, Segata N, Gevers D, Raes J & Huttenhower C (2012) Microbial co-occurrence relationships in the human microbiome. PLoS Comput Biol 8: e1002606.

Fauth JE, Bernardo J, Camara M, Resetarits WJ Jr, Van Buskirk J & McCollum SA (1996) Simplifying the jargon of community ecology: a conceptual approach. Am Nat 147: 282–286.

Fierer N & Jackson RB (2006) The diversity and biogeography of soil bacterial communities. P Natl Acad Sci USA 103: 626–631.

Floodgate GD (1962) Some remarks on the theoretical aspects of bacterial taxonomy. Bacteriol Rev 26: 277–291.

Friedman J & Alm EJ (2012) Inferring correlation networks from genomic survey data. PLoS Comput Biol 8: e1002687.
Fuhrman JA (2012) Metagenomics and its connection to microbial community organization. F1000 Biol Rep 4: 15.
Gabor E, Niehaus F, Ahle W & Eck J (2012) Zooming in on metagenomics: molecular microdiversity of Subtilisin Carlsberg in soil. J Mol Biol 418: 16–20.
Galperin MY & Koonin EV (2004) 'Conserved hypothetical' proteins: prioritization of targets for experimental study. Nucleic Acids Res 32: 5452–5463.
Ge F, Wang LS & Kim J (2005) The cobweb of life revealed by genome-scale estimates of horizontal gene transfer. PLoS Biol 3: e316.
Geisinger E, George EA, Chen J, Muir TW & Novick RP (2008) Identification of ligand specificity determinants in AgrC, the Staphylococcus aureus quorum-sensing receptor. J Biol Chem 283: 8930–8938.
Gevers D, Cohen FM, Lawrence JG et al. (2005) Opinion: re-evaluating prokaryotic species. Nat Rev Microbiol 3: 733–739.
Gibbons SM, Caporaso JG, Purrung M, Field D, Knight R & Gilbert JA (2013) Evidence for a persistent microbial seed bank throughout the global ocean. P Natl Acad Sci USA 110: 4651–4655.
Gilbert JA, Mühling M & Joint I (2008) A rare SAR11 fosmid clone confirming genetic variability in the ‘Candidatus Pelagibacter ubique’ genome. ISME J 2: 790–793.
Gilbert JA, Meyer F, Jansson J et al. (2010a) The Earth Microbiome Project: meeting report of the “1 EMP meeting on sample selection and acquisition” at Argonne National Laboratory October 6 2010. Stand Genomic Sci 3: 249–253.
Gilbert JA, Field D, Swift P et al. (2010b) The taxonomic and functional diversity of microbes at a temperate coastal site: a “multi-omic” study of seasonal and diel temporal variation. PLoS ONE 5: e15545.
Giovannoni SJ, Tripp HJ, Givan S et al. (2005) Genome streaming in a cosmopolitan oceanic bacterium. Science 309: 1242–1245.
Gleave HA (1926) The individualistic concept of the plant association. Bull Torrey Bot Club 1: 7–26.
Goss CH & Burns JL (2007) Exacerbations in cystic fibrosis. 1: Handelsman J, Rondon MR, Brady SF, Clardy J & Goodman RM (1998) Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. Chem Biol 5: R245–R249.
Hanson CA, Fuhrman JA, Horner-Divine MC & Martiny JB (2012) Beyond biogeographic patterns: processes shaping the microbial landscape. Nat Rev Microbiol 10: 497–506.
Hao W & Golding GB (2006) The fate of laterally transferred genes: life in the fast lane to adaptation or death. Genome Res 16: 636–643.
Harrison E & Brockhurst MA (2012) Plasmid-mediated horizontal gene transfer is a coevolutionary process. Trends Microbiol 20: 262–267.
Helling RB, Vargas CN & Adams J (1987) Evolution of Escherichia coli during growth in a constant environment. Genetics 116: 349–358.
Hemme CL, Deng Y, Gentry TJ et al. (2010) Metagenomic insights into evolution of a heavy metal-contaminated groundwater microbial community. ISME J 4: 660–672.
Hibbing ME, Fuqua C, Parsek MR & Peterson SB (2010) Bacterial competition: surviving and thriving in the microbial jungle. Nat Rev Microbiol 8: 15–25.
Hilario E & Gogarten JP (1993) Horizontal transfer of ATPase genes—the tree of life becomes a net of life. Biosystems 31: 111–119.
Hiller NL, Ahmed A, Powell E et al. (2010) Generation of genic diversity among Strepctococcus pneumoniae strains via horizontal gene transfer during a chronic polyclonal pediatric infection. PLoS Pathog 6: e1001108.
Hollingshead SK, Becker R & Briles DE (2000) Diversity of PapA: mosaic genes and evidence for past recombination in Streptococcus pneumoniae. Infect Immun 68: 5889–5900.
Holloway C & Beiko RG (2010) Assembling networks of microbial genomes using linear programming. BMC Evol Biol 10: 360.
Hooper SD, Movromatis K & Kyrpides NC (2009) Microbial co-habitation and lateral gene transfer: what transposases can tell us. Genome Biol 10: R45.
Hug LA, Beiko RG, Rowe AR, Richardson RE & Edwards EA (2012) Comparative metagenomics of three Dehalococcoides-containing enrichment cultures: the role of the non-dechlorinating community. BMC Genomics 13: 327.
Hugenholtz P, Goebel BM & Pace NR (1998) Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. J Bacteriol 170: 4765–4774.
Hull D (1980) Individuality and selection. Annu Rev Ecol Syst 11: 311–332.
Hus H, Reiters VA, Kyrpides NC et al. (2007) Metagenomic project classification of metagenome projects. ISME J 1: 51–57.
Jain R, Rivera MC, Moore JE & Lake JA (2003) Horizontal gene transfer accelerates genome innovation and evolution. *Mol Biol Evol* 20: 1598–1602.

Jeffery IB, Claesson MJ, O’Toole PW & Shanahan F (2012) Categorization of the gut microbiota: enterotypes or gradients? *Nat Rev Microbiol* 10: 591–592.

Jehnlich N, Hübßchmann T, Gesell Salazar M, Völker U, Benndorf D, Müller S, von Bergen M & Schmidt F (2010) Advanced tool for characterization of microbial cultures by combining cytomics and proteomics. *Appl Microbiol Biotechnol* 88: 575–584.

Jiao Y, D’haeseleer P, Dill BD, Shah M, Verberkmoes NC, Hettich RL, Banfield JF & Thelen MP (2011) Identification of biofilm matrix-associated proteins from an acid mine drainage microbial community. *Appl Environ Microbiol* 77: 5230–5237.

Johnson MA & Winquist RJ (2011) Island biogeography effects on microbial evolution may contribute to Crohn’s disease. *Biochem Pharmacol* 82: 1801–1806.

Johnston C, Martin B, Granadel C, Polard P & Claverys J-P (2013) Programmed protection of foreign DNA from restriction allows pathogenicity island exchange during pneumococcal transformation. *PLoS Pathog* 9: e1003178.

Kastenmüller G, Schenk ME, Gasteiger J & Mewes HW (2009) Uncovering metabolic pathways relevant to phenotypic traits of microbial genomes. *Genome Biol* 10: R28.

Kelkar YD & Ochman H (2013) Genome reduction promotes increase in protein functional complexity in bacteria. *Genetics* 193: 303–307.

Kim HD, Shay T, O’Shea EK & Regev A (2009) Transcriptional regulatory circuits: predicting numbers from alphabets. *Science* 325: 429–432.

Kim M, Ashida H, Ogawa M, Yoshikawa Y, Mimuro H & Sasakawa C (2010) Bacterial interactions with the host epithelium. *Cell Host Microbe* 8: 20–35.

Klenk HP & Göker M (2010) En route to a genome-based classification of Archaea and Bacteria? *Syst Appl Microbiol* 33: 175–182.

Knietzsch A, Bowien S, White D, Gottschalk G & Daniel R (2003) Identification and characterization of coenzyme B12-dependent glycerol dehydratase- and diol dehydratase-encoding genes from metagenomic DNA libraries derived from enrichment cultures. *Appl Environ Microbiol* 69: 3048–3060.

Knight R, Jansson J, Field D et al. (2012) Unlocking the potential of metagenomics through replicated experimental design. *Nat Biotechnol* 30: 513–520.

Knights D, Costello EK & Knight R (2011) Supervised classification of human microbiota. *FEMS Microbiol Rev* 35: 343–359.

Kobayashi I (2001) Behavior of restriction-modification systems as selfish mobile elements and their impact on genome evolution. *Nucleic Acids Res* 29: 3742–3756.

Kocher TD, Conroy JA, McKay KR & Stauffer JR (1993) Similar morphologies of cichlid fish in Lakes Tanganyika and Malawi are due to convergence. *Mol Phylogenet Evol* 2: 158–165.

Konopka A (2009) What is microbial community ecology? *ISME J* 3: 1223–1230.

Konstantinidis KT & Tiedje JM (2004) Trends between gene content and genome size in prokaryotic species with larger genomes. *P Natl Acad Sci USA* 101: 3160–3165.

Konstantinidis KT & Tiedje JM (2005) Towards a genome-based taxonomy for prokaryotes. *J Bacteriol* 187: 6258–6264.

Konstantinidis KT, Ramette A & Tiedje JM (2006) The bacterial species definition in the genomic era. *Philos Trans R Soc Lond B Biol Sci* 361: 1929–1940.

Koonin EV & Wolf YI (2008) Genomics of bacteria and archaea: the emerging dynamic view of the prokaryotic world. *Nucleic Acids Res* 36: 6688–6719.

Koren O, Knights D, Gonzalez A, Waldron L, Segata N, Knight R, Huttenhower C & Ley RE (2013) A guide to enterotypes across the human body: meta-analysis of microbial community structures in human microbiome datasets. *PLoS Comput Biol* 9: e1002863.

Koskiniemi M, Sun S, Berg OG & Andersson DI (2012) Selection-driven gene loss in bacteria. *PLoS Genet* 8: e1002787.

Kreuzer-Martin HW (2007) Stable isotope probing: linking functional activity to specific members of microbial communities. *Soil Sci Soc Am J* 71: 611.

Kuczynski J, Liu Z, Lozupone C, McDonald D, Fierer N & Knight R (2010) Microbial community resemblance methods differ in their ability to detect biologically relevant patterns. *Nat Methods* 7: 813–819.

Kuenen JG (1983) The role of specialists and generalists in microbial population interactions. *Foundations of Biochemical Engineering: Kinetics and Thermodynamic in Biological Systems, Vol. 207*, chapter 10 (Blanch HW, Papoutsakis ET & Stephanopoulos G, eds), pp. 229–251. ACS Symposium Series.

Kunin V, Goldovsky L, Darzentes N & Ouzounis CA (2005) The net of life: reconstructing the microbial phylogenetic network. *Genome Res* 15: 954–959.

Kuo CH, Moran NA & Ochman H (2009) The consequences of genetic drift for bacterial genome complexity. *Genome Res* 19: 1450–1454.

Kuramitsu HK, He X, Lux R, Anderson MH & Wenyuan S (2007) Interspecies interactions within oral microbial communities. *Microbiol Mol Biol Rev* 71: 4653–4670.

Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Knight R, Beiko RG & Huttenhower C (2013) Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat Biotechnol* (in press).

Larsen PE, Field D & Gilbert JA (2012) Predicting bacterial community assemblages using an artificial neural network approach. *Nat Methods* 9: 621–625.

Lawley TD, Clare S, Walker AW et al. (2012) Targeted restoration of the intestinal microbiota with a simple, defined bacteriotherapy resolves relapsing...
Clostridium difficile disease in mice. PLoS Pathog 8: e1002995.

Layeghifard M, Peres-Neto PR & Makarenkov V (2012) Using directed phylogenetic networks to retrace species dispersal history. Mol Phylogenet Evol 64: 190–197.

Lederberg J & McCray AT (2001) ‘Ome Sweet ‘Omics—a genealogical treasury of words. Scientist 15: 8.

Leibold MA, Holyoak M, Mouquet N et al. (2004) The metacommunity concept: a framework for multi-scale community ecology. Ecol Lett 7: 601–613.

Leibold MA, Economou EP & Peres-Neto P (2010) Metacommunity phylogenetics: separating the roles of environmental filters and historical biogeography. Ecol Lett 13: 1290–1299.

Leininger S, Urih T, Schloter M, Schwark L, Qi J, Nicol GW, Prosser JI, Schuster SC & Schleper C (2006) Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442: 806–809.

Lennon JT & Jones SE (2011) Microbial seed banks: the ecological and evolutionary implications of dormancy. Nat Rev Microbiol 9: 119–130.

Lercher MJ & Pål C (2008) Integration of horizontally transferred genes into regulatory interaction networks takes many million years. Mol Biol Evol 25: 559–567.

Ley RE, Turnbaugh P, Klein S & Gordon JI (2006) Microbial ecology: human gut microbes associated with obesity. Nat Rev Microbiol 4: 110–120.

Lima-Mendez G, Van Helden J, Toussaint A & Leplae R (2006) Rules of engagement: interspecies interactions that enable de novo assembly of metagenomic sequence data. PLoS ONE 7: e9755.

Little AEF, Robinson CJ, Peterson SB, Raffa KF & Handelsman J (2008) Rules of engagement: interspecies interactions that regulate microbial communities. Annu Rev Microbiol 62: 375–401.

Loeffler FE, Yan J, Ritalahti KM, Adrian L, Edwards EA, Konstantinidis KT, Müller JA, Fullerton H, Zinder SH & Spormann AM (2012) Dehalococcoides mccartyi gen. nov., sp. nov., obligately organohalide-respiring anaerobic bacteria relevant to halogen cycling and bioremediation, belong to a novel bacterial class, Dehalococcoidia classis nov., order Dehalococcoidales ord. nov. and family Dehalococcoidaceae fam. nov., within the phylum Chloroflexi. Int J Syst Evol Microbiol 63: 625–635.

Loper JE, Hassan KA, Mavrodi DV et al. (2012) Comparative genomics of plant-associated Pseudomonas spp.: insights into diversity and inheritance of traits involved in multilocus interactions. PLoS Genet 8: e1002784.

Lozupone CA, Hamady M, Kelley ST & Knight R (2007) Quantitative and qualitative β diversity measures lead to different insights into factors that structure microbial communities. Appl Environ Microbiol 73: 1576–1585.

Lynch M (2006) Streamlining and simplification of microbial genome architecture. Annu Rev Microbiol 60: 327–349.

MacDonald NJ & Beiko RG (2010) Efficient learning of microbial genotype-phenotype association rules. Bioinformatics 26: 1834–1840.

MacDonald NJ, Parks DH & Beiko RG (2012) Rapid identification of high-confidence taxonomic assignments for metagenomic data. Nucleic Acids Res 40: e111.

Maidak BL, Cole JR, Lilburn TG, Parker CT Jr, Saxman PR, Farris RJ, Garrity GM, Olsen GJ, Schmidt TM & Tiedje JM (2001) The RDP-II (Ribosomal Database Project). Nucleic Acids Res 29: 173–174.

Malcom JW (2011) Gene networks and metacommunities: dispersal differences can override adaptive advantage. PLoS ONE 6: e21541.

Mandel MJ (2010) Models and approaches to dissect host-symbiont specificity. Trends Microbiol 18: 504–511.

Mariat D, Firmesse O, Levenez F, Guimaraes V, Sokol H, Doré J, Corthier G & Furet JP (2009) The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. BMC Microbiol 9: 123.

Martin AP (2002) Phylogenetic approaches for describing and comparing the diversity of microbial communities. Appl Environ Microbiol 68: 3673–3682.

Martinez-Garcia M, Brazel DM, Swan BK et al. (2012) Capturing single cell genomes of active polysaccharide degraders: an unexpected contribution of Verrucomicrobia. PLoS ONE 7: e53514.

Martiny JB, Bohannan BJ, Brown JH et al. (2006) Microbial biogeography: putting microorganisms on the map. Nat Rev Microbiol 4: 102–112.

Martiny AC, Tai AP, Veneziano D, Princeau F & Chisholm SW (2009) Taxonomic resolution, ecotypes and the biogeography of Prochlorococcus. Environ Microbiol 11: 823–832.

Martiny AC, Treseder K & Pusch G (2013) Phylogenetic conservatism of functional traits in microorganisms. ISME J 7: 830–838.

May RM (1973) Stability and Complexity in Model Ecosystems. Princeton Univ, Press.

Mayr E (1942) Systematics and the Origin of Species. Columbia Univ, Press, New York.

McBride BC & Van der Hoeven JS (1981) Role of interbacterial adherence in colonization of the oral cavities of gnotobiotic rats infected with Streptococcus mutans and Veillonella alcalescens. Infect Immun 33: 467–472.

McCUTCHEON JP & von Dohlen CD (2011) An interdependent genome architecture. Curr Biol 21: 1366–1372.

McDonald D, Clemente JC, Kuczynski J et al. (2012) The Biological Observation Matrix (BIOM) format or: how I learned to stop worrying and love the one-ome. Gigascience 1: 7.

McGill BJ, Enquist BJ, Weiher E & Westoby M (2006) Rebuilding community ecology from functional traits. Trends Ecol Evol 21: 178–185.
McGinty SE, Rankin DJ & Brown SP (2011) Horizontal gene transfer and the evolution of bacterial cooperation. Evolution 65: 21–32.

McHardy AC & Rigoutsos I (2007) What’s in the mix: phylogenetic classification of metagenome sequence samples. Curr Opin Microbiol 10: 499–503.

McInerney JO, Pisani D, Bapteste E & O’Connell MJ (2011) The Public Goods Hypothesis for the evolution of life on Earth. Biol Direct 6: 41.

McNulty NP, Yatsunenko T, Hsiao A et al. (2011) The impact of a consortium of fermented milk strains on the gut microbiome of gnotobiotic mice and monozygotic twins. Sci Transl Med 3: 106ra106.

Medini D, Donati C, Tettelin H, Masiagnani V & Rappuoli R (2005) The microbial pan-genome. Curr Opin Genet Dev 15: 589–594.

Meehan CJ & Beiko RG (2012) Lateral gene transfer of an ABC transporter complex between major constituents of the human gut microbiome. BMC Microbiol 12: 248.

Menge BA (1995) Indirect effects in marine rocky intertidal interaction webs: patterns and importance. Ecol Monogr 65: 21–74.

Mikesková H, Novotný C & Svobodová K (2012) Interspecific interactions in mixed microbial cultures in a biodegradation perspective. Appl Microbiol Biotechnol 95: 861–870.

Mirete S, de Figueras CG & González-Pastor JE (2007) Novel nickel resistance genes from the rhizosphere metagenome of plants adapted to acid mine drainage. Appl Environ Microbiol 73: 6001–6011.

Mishler BD & Donoghue MJ (1982) Species concepts: a case for pluralism. Syst Zool 31: 491–503.

Moran MA, Satsinsky B, Gifford SM et al. (2013) Sizing up metatranscriptomics. ISME J 7: 237–243.

Morris RM, Rappe MS, Connon SA, Vergin KL, Siebold WA, Carlson CA & Giovannoni SJ (2002) SAR11 clade dominates ocean surface bacterioplankton communities. Nature 420: 806–810.

Morris JJ, Johnson ZI, Szul MJ, Keller M & Zinser ER (2011) Dependence of the cyanobacterium Prochlorococcus on hydrogen peroxide scavenging microbes for growth at the ocean’s surface. PLoS ONE 6: e16805.

Morris JJ, Lenski RE & Zinser ER (2012) The Black Queen Hypothesis: evolution of dependencies through adaptive gene loss. MBio 3: e00396–13.

Mouillot D, Graham NA, Villéger S, Mason NW & Bellwood DR (2013) A functional approach reveals community responses to disturbances. Trends Ecol Evol 28: 167–177.

Muege BD, Kuczynski J, Knights D, Clemente JC, González A, Fontana L, Henriassat B, Knight R & Gordon JJ (2011) Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. Science 332: 970–974.

Müller S, Hübschmann T, Kleinstuber S & Vogt C (2012) High resolution single cell analytics to follow microbial community dynamics in anaerobic ecosystems. Methods 57: 338–349.

Nemergut DR, Costello EK, Hamady M et al. (2011) Global patterns in the biogeography of bacterial taxa. Environ Microbiol 13: 135–144.

Nogueira T, Rankin DJ, Touchon M, Taddei F, Brown SP & Rocha EPC (2009) Horizontal gene transfer of the secretome drives the evolution of bacterial cooperation and virulence. Curr Biol 19: 1683–1691.

O’Connor TJ, Boyd D, Dorer MS & Isberg RR (2012) Aggravating genetic interactions allow a solution to redundancy in a bacterial pathogen. Science 338: 1440–1444.

Ohta T (1992) The nearly neutral theory of molecular evolution. Annu Rev Ecol Syst 23: 263–286.

Pál C, Papp B & Lercher MJ (2005) Adaptive evolution of bacterial metabolic networks by horizontal gene transfer. Nat Genet 37: 1372–1375.

Parks DH & Beiko RG (2012) Measuring community similarity with phylogenetic networks. Mol Biol Evol 29: 3947–3958.

Parks DH & Beiko RG (2013) Measures of phylogenetic differentiation provide robust and complementary insights into microbial communities. ISME J 7: 173–183.

Patel PV, Gianoulis TA, Bjørnson RD, Yip KY, Engelmann DM & Gerstein MB (2010) Analysis of membrane proteins in metagenomics: networks of correlated environmental features and protein families. Genome Res 20: 960–971.

Patel V, Jain S & Madamwar D (2012) Naphthalene degradation by bacterial consortium (DV-AL) developed from Alang-Sosiya ship breaking yard, Gujarat, India. Bioresour Technol 107: 122–130.

Pearson T, Giffard P, Beckstrom-Sternberg S et al. (2009) Phylogeographic reconstruction of a bacterial species with high levels of lateral gene transfer. BMC Biol 7: 78.

Petrof EO, Gloor GB, Vanner SJ, Weese SJ, Carter D, Daigneault MC, Brown EM, Schroeter K & Allen-Vercoe E (2013) Stool substitute transplant therapy for the eradication of Clostridium difficile infection: ‘RePOOPulating’ the gut. Microbiome 1: 3.

Pfeiffer T & Bonhoeffer S (2004) Evolution of cross-feeding in microbial populations. Am Nat 163: E126–E135.

Phillips J (1935) Succession, development, the climax and the ocean’s surface. PLoS ONE 6: e16805.

Poretzky RS, Bano N, Buchan A, LeCleir G, Kleikemper J, Pickering M, Pate WM, Moran MA & Hollibaugh JT (2005) Analysis of microbial gene transcripts in environmental samples. Appl Environ Microbiol 71: 4121–4126.

Porwollik S, Boyd EF, Choy C, Cheng P, Florea L, Proctor E & Parks DH & Beiko RG (2014) Characterization of Salmonella enterica Subspecies I genovars by use of microarrays. J Bacterial 186: 5883–5898.

Prakash T & Taylor TD (2012) Functional assignment of metagenomic data: challenges and applications. Brief Bioinform 13: 711–727.
Preidis GA & Versalovic J (2009) Targeting the human microbiome with antibiotics, probiotics, and prebiotics: gastroenterology enters the metagenomics era. *Gastroenterology* 136: 2013–2031.

Prosser JL, Bohannan BJ, Curtis TP et al. (2007) The role of ecological theory in microbial ecology. *Nat Rev Microbiol* 5: 384–392.

Puigbó P, Wolf YI & Koonin EV (2009) Search for a ‘Tree of Life’ in the thicket of the phylogenetic forest. *J Biol* 8: 59.

Qin J, Li Y, Cai Z et al. (2012) A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* 490: 55–60.

Radivojac P, Clark WT, Oron TR et al. (2013) A large-scale evaluation of computational protein function prediction. *Nat Methods* 10: 221–227.

Raes J, Letunic I, Yamada T, Jensen LJ & Bork P (2011) Toward molecular trait-based ecology through integration of biogeographical, geographical and metagenomic data. *Mol Syst Biol* 7: 473.

Ragan MA, Harlow TJ & Beiko RG (2006) Do different surrogate methods detect lateral genetic transfer events of different relative ages? *Trends Microbiol* 14: 4–8.

Ram RJ, Verberkmoes NC, Thelen MP, Tyson GW, Baker BJ, Blake RC 2nd, Shah M, Hettrich RL & Banfield JF (2005) Community proteomics of a natural microbial biofilm. *Science* 308: 1915–1920.

Rankin DJ, Rocha EPC & Brown SP (2011) What traits are carried on mobile genetic elements, and why? *Hereditas* 106: 1–10.

Redfield RJ (2001) Do bacteria have sex? *Nat Rev Genet* 2: 634–639.

Reim A, Lüke C, Krause S, Pratscher J & Frenzel P (2012) One millimetre makes the difference: high-resolution analysis of methanoxidizing bacteria and their specific activity at theoxic-anoxic interface in a flooded paddy soil. *ISME J* 6: 2128–2139.

Relman DA (2002) New technologies, human-microbe interactions, and the search for previously unrecognized pathogens. *J Infect Dis* 186: S254–S258.

Ren ML, Held NL, Fields CJ, Burke PV & Whitaker RJ (2009) Biogeography of the *Sulfolobus islandicus* pan-genome. *P Natl Acad Sci USA* 106: 8605–8610.

Ricklefs RE (2008) Disintegration of the ecological community. *Am Nat* 172: 741–750.

Rodriguez-Valera F (2004) Environmental genomics, the big picture? *FEMS Microb Lett* 231: 153–158.

Rusch DB, Halpern AL, Sutton G et al. (2007) The Sorcerer II Global Ocean Sampling expedition: northwest Atlantic through eastern tropical Pacific. *PLoS Biol* 5: e77.

Sabehi G, Massana R, Bielawski JP, Rosenberg M, Delong EF & Béja O (2003) Novel Proteorhodopsin variants from the Mediterranean and Red Seas. *Environ Microbiol* 5: 842–849.

Sage RF, Christin PA & Edwards EJ (2011) The C(4) plant lineages of planet Earth. *J Exp Bot* 62: 3155–3169.

Sale PF (1978) Coexistence of coral reef fishes — a lottery for living space. *Environ Biol Fish* 3: 85–102.

Salimi F, Zhuang K & Mahadevan R (2010) Genome-scale metabolic modeling of a clostridial co-culture for consolidated bioprocessing. *Biotechnol J* 5: 726–738.

Salyers AA, Gupta A & Wang Y (2004) Human intestinal bacteria as reservoirs for antibiotic resistance genes. *Trends Microbiol* 12: 412–416.

Sapp J (2005) *The Bacterium's Place in Nature. Microbial Phylogeny and Evolution*, pp. 1–52. Oxford University Press, New York, NY.

Schellenberg J, Links MG, Hill JE, Dumonceaux TJ, Peters GA, Tyler S, Ball TB, Severini A & Plummer FA (2009) Pyrosequencing of the chaperonin-60 universal target as a tool for determining microbial community composition. *Appl Environ Microbiol* 75: 2889–2898.

Schellenberg J, Links MG, Hill JE, Hemmingsen SM & Dumonceaux TJ (2011) Pyrosequencing of chaperonin-60 (cpn60) amplicons as a means of determining microbial community composition. *Methods Mol Biol* 733: 143–158.

Schink B (2002) Synergistic interactions in the microbial world. *Antonie Van Leeuwenhoek* 81: 257–261.

Schloissnig S, Arumugam M, Sunagawa S et al. (2013) Genomic variation landscape of the human gut microbiome. *Nature* 493: 45–50.

Schloß PD & Handelsman J (2003) Biotechnological prospects from metagenomics. *Curr Opin Biotechnol* 14: 303–310.

Schloß PD & Handelsman J (2005) Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. *Appl Environ Microbiol* 71: 1501–1506.

Schneiker S, Perlova O, Kaiser O et al. (2007) Complete genome sequence of the myxobacterium *Sorangium cellulosum*. *Nat Biotechnol* 25: 1281–1289.

Schneiders AM, Brown SD, Dodewski I & Babcock PC (2009) Annotation error in public databases: misannotation of molecular function in enzyme superfamilies. *PLoS Comput Biol* 5: e1000605.

Schous AM, Schot CS & Jacobs JA (2003) Horizontal transfer of segments of the 16S rRNA genes between species of the *Streptococcus anginosus* group. *J Bacteriol* 185: 7241–7246.

Shade A & Handelsman J (2012) Beyond the Venn diagram: the hunt for a core microbiome. *Environ Microbiol* 14: 4–12.

Shen K, Sayeed S, Antalis P et al. (2006) Extensive genomic plasticity in *Pseudomonas aeruginosa* revealed by identification and distribution studies of novel genes among clinical isolates. *Infect Immun* 74: 5272–5283.

Sher D, Thompson JW, Kashtan N, Croal L & Chisholm SW (2011) Response of *Prochlorococcus* ecotypes to co-culture with diverse marine bacteria. *ISME J* 5: 1125–1132.

Sibley CD & Surette MG (2011) The polymicrobial nature of airway infections in cystic fibrosis: Cangene Gold Medal Lecture. *Can J Microbiol* 57: 69–77.

Siezen RJ, Tzenova VA, Castioni A, Weh M, Phan HTK, Rademaker JLW, Starenburg MJ, Kleerebezem M, Molenaar D & Van Hylckama Vlieg JET (2010) Phenotypic
and genomic diversity of Lactobacillus plantarum strains isolated from various environmental niches. Environ Microbiol 12: 758–773.

Skippington E & Ragan MA (2011) Lateral genetic transfer and the construction of genetic exchange communities. FEMS Microbiol Rev 35: 707–735.

Smith H & de Vos WM (2004) Anaerobic microbial dehalogenation. Annu Rev Microbiol 58: 43–73.

Smillie CS, Smith MB, Friedman J, Cordero OX, David LA & Alm EJ (2011) Ecology drives a global network of gene exchange connecting the human microbiome. Nature 480: 241–244.

Smith J (2001) The social evolution of bacterial pathogenesis. Proc Biol Sci 268: 61–69.

Smith MI, Yatsunenko T, Manary MJ et al. (2013) Gut microbiomes of Malawian twin pairs discordant for kwashiorkor. Science 339: 548–554.

Sokal RR & Sneath PHA (1963) Principles of numerical taxonomy. Freeman, San Francisco, WH.

Souza V, Rocha M, Valera A & Eguiarte LE (1999) Genetic structure of natural populations of Escherichia coli in wild hosts on different continents. Appl Environ Microbiol 65: 3373–3385.

Staley JT & Konopka A (1985) Measurement of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. Annu Rev Microbiol 39: 321–346.

Stams AJ (1994) Metabolic interactions between anaerobic bacteria in methanogenic environments. Antonie Van Leeuwenhoek 66: 271–294.

Stearns JC, Lynch MD, Senadheera DB, Tenenbaum HC, Goldberg MB, Cvitkovitch DG, Croitoru K, Moreno-Hagelsieb G & Neufeld JD (2011) Bacterial biogeography of the human digestive tract. Sci Rep 1: 170.

Steele JA, Countway PD, Xia L et al. (2011) Marine bacterial, archaeal and protistan association networks reveal ecological linkages. ISME J 5: 1414–1425.

Stoljar S, Van Dien S, Hillesland KL, Pinel N, Lie TJ, Leigh JA & Stahl DA (2007) Metabolic modeling of a mutualistic microbial community. Mol Syst Biol 3: 92.

Sul WJ, Park J, Quensen JF, Rodrigues JL, Seliger L, Tsoi TV, Zylstra GJ & Tiedje JM (2009) DNA-stable isotope probing integrated with metagenomics for retrieval of biphenyl dioxygenase genes from polychlorinated biphenyl-contaminated river sediment. Appl Environ Microbiol 75: 5501–5506.

Suzuki MT, Béja O, Taylor LT & Delong EF (2001) Phylogenetic analysis of ribosomal RNA operons from uncultivated coastal marine bacterioplankton. Environ Microbiol 3: 323–331.

Teeling H, Fuchs BM, Becher D et al. (2012) Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. Science 336: 608–611.

Teles FR, Teles RP, Sachdeo A, Uzel NG, Song XQ, Torresyap G, Singh M, Papas A, Haffajee AD & Socransky SS (2012) Comparison of microbial changes in early redeveloping biofilms on natural teeth and dentures. J Periodontol 83: 1139–1148.

Tettelin H, Maignani V, Cieslewicz MJ et al. (2005) Genome analysis of multiple pathogenic isolates of Streptococcus agalactiae: implications for the microbial “pan-genome”. P Natl Acad Sci USA 102: 13950–13955.

Tettelin H, Riley D, Cattuto C & Medini D (2008) Comparative genomics: the bacterial pan-genome. Curr Opin Microbiol 11: 472–477.

Treangen TJ & Rocha EP (2011) Horizontal transfer, not duplication, drives the expansion of protein families in prokaryotes. PLoS Genet 7: e1001284.

Trzeszicka-Mlynarz D & Ward OP (1995) Degradation of polycyclic aromatic hydrocarbons (PAHs) by a mixed culture and its component pure cultures, obtained from PAH-contaminated soil. Can J Microbiol 41: 470–476.

Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R & Gordon JI (2007) The human microbiome project. Nature 449: 804–810.

Turnbaugh PJ, Hamady M, Yatsunenko T et al. (2009) A core gut microbiome in obese and lean twins. Nature 457: 480–484.

Tyson GW, Chapman J, Hugenholtz P, Allen EE, Ram RJ, Richardson PM, Solovyev VV, Rubin EM, Rokhsar DS & Banfield JF (2004) Community structure and metabolism through reconstruction of microbial genomes from the environment. Nature 428: 37–43.

Urban MC & Skelly DK (2006) Evolving metacommunities: toward an evolutionary perspective on metacommunities. Ecology 87: 1616–1626.

Van den Abbeele P, Belzer C, Goossens M, Kleebezem M, De Vos WM, Thas O, De Weirtd R, Kerckhof EM & Van de Wiele T (2013) Butyrate-producing Clostridium cluster XIVa species specifically colonize mucins in an in vitro gut model. ISME J 7: 949–961.

Van Valen L (1976) Ecological species, multispecies, and oaks. Taxon 25: 233–239.

Venners S, Feschotte C & Biémont C (2009) Dynamics of transposable elements: towards a community ecology of the genome. Trends Genet 25: 317–323.

Venter JC, Remington KJ, Robertson PD, et al. (2004) Environmental genome shotgun sequencing of the Sargasso Sea. Science 304: 66–74.

Vergin KL, Tripp HJ, Wilhelm LJ, Denver DR, Rappé MS & Giovannoni SJ (2007) High intraspecific recombination rate in a native population of Candidatus pelagibacter ubique (SAR11). Environ Microbiol 9: 2430–2440.

Vilkuna J, Ettema TJ & Andersson SG (2012) Independent genome reduction and phylogenetic reclassification of the oceanic SAR11 clade. Mol Biol Evol 29: 599–615.

Viole C, Navas ML, Vile D, Kazakou E, Fortunel C, Hummel I & Garnier E (2007) Let the concept of trait be functional!. Oikos 116: 882–892.

Ward DM, Weller R & Bateson MM (1990) 16S rRNA sequences reveal numerous uncultured microorganisms in a natural community. Nature 345: 63–65.
Warnecke T & Rocha EP (2011) Function-specific accelerations in rates of sequence evolution suggest predictable epistatic responses to reduced effective population size. *Mol Biol Evol* **28**: 2339–2349.

Watrous J, Hendricks N, Meehan M & Dorrestein PC (2010) Capturing bacterial metabolic exchange using thin film desorption electrospray ionization-imaging mass spectrometry. *Anal Chem* **82**: 1598–1600.

Watrous JD, Alexandrov T & Dorrestein PC (2011) The evolving field of imaging mass spectrometry and its impact on future biological research. *J Mass Spectrom* **46**: 209–222. doi:10.1002/jms.1876.

Watrous J, Roach P, Alexandrov T et al. (2012) Mass spectral molecular networking of living microbial colonies. *PNAS* **109**: E1743–E1752.

Webb CT, Hoeting JA, Ames GM, Pyne MI & LeRoy Poff N (2010) A structured and dynamic framework to advance traits-based theory and prediction in ecology. *Ecol Lett* **13**: 267–283.

Weckwerth W (2003) Metabolomics in systems biology. *Annu Rev Plant Biol* **54**: 669–689.

Welch RA, Burland V, Plunkett G 3rd et al. (2002) Extensive mosaic structure revealed by the complete genome sequence of uropathogenic *Escherichia coli*. *PNAS* **99**: 17020–17024.

Whitham TG, DiFazio SP, Schweitzer JA, Shuster SM, Allan GJ, Bailey JK & Woolbright SA (2008) Extending genomics to natural communities and ecosystems. *Science* **320**: 492–495.

Whittaker RH (1967) Gradient analysis of vegetation. *Biol Rev* **49**: 207–264.

Whitworth DE (2008) Genomes and knowledge – a questionable relationship? *Trends Microbiol* **16**: 512–519.

Wilhelm LJ, Tripp HJ, Givan SA, Smith DP & Giovanoni SJ (2007) Natural variation in SAR11 marine bacterioplankton genomes inferred from metagenomic data. *Biol Direct* **2**: 27.

Wilmes P & Bond PL (2004) The application of two-dimensional polycrylamide gel electrophoresis and downstream analyses to a mixed community of prokaryotic microorganisms. *Environ Microbiol* **6**: 911–920.

Wilmes P, Andersson AF, Lefsrud MG, Wexler M, Shah M, Zhang B, Hettich RL, Bond PL, VerBerkmoes NC & Banfield JF (2008a) Community proteogenomics highlights microbial strain-variant protein expression within activated sludge performing enhanced biological phosphorus removal. *ISME J* **2**: 853–864.

Wilmes P, Wexler M & Bond PL (2008b) Metaproteomics provides functional insight into activated sludge wastewater treatment. *PLOS ONE* **3**: e1778.

Wintermute EH & Silver PA (2010) Dynamics in the mixed microbial concourse. *Genes Dev* **24**: 2603–2614.

Woese CR, Olsen GJ, Ibba M & Soll D (2000) Aminoacyl-tRNA synthetases, the genetic code, and the evolutionary process. *Microbiol Mol Biol Rev* **64**: 202–236.

Wolcott R, Costerton JW, Raoul D & Cutler SJ (2013) The polymicrobial nature of biofilm infection. *Clin Microbiol Infect* **19**: 107–112.

Wu GD, Chen J, Hoffmann C et al. (2011) Linking long-term dietary patterns with gut microbial enterotypes. *Science* **334**: 105–108.

Xie Y, Luo Z, Li Z, Deng M, Liu H, Zhu B, Ruan B & Li L (2012) Structural shifts of fecal microbial communities in rats with acute rejection after liver transplantation. *Microbiol Ecol* **64**: 546–554.

Yamada T, Waller AS, Raes J, Zeleznik A, Perchat N, Perret A, Salanoubat M, Patil KR, Weissenbach J & Bork P (2012) Prediction and identification of sequences coding for orphan enzymes using genomic and metagenomic neighbours. *Mol Syst Biol* **8**: 581.

Yatsunenko T, Rey FE, Manary MJ et al. (2012) Human gut microbiome viewed across age and geography. *Nature* **486**: 222–227.

Ye Y & Doak TG (2009) A parsimony approach to biological pathway reconstruction/inference for genomes and metagenomes. *PLOS Comput Biol* **5**: e1000465.

Yelton AP, Thomas BC, Simmons SL, Wilmes P, Zemla A, Thelen MP, Justice N & Banfield JF (2011) A semi-quantitative, synteny-based method to improve functional predictions for hypothetical and poorly annotated bacterial and archaeal genes. *PLOS Comput Biol* **7**: e1002230.

Yilmaz P, Kottmann R, Field D et al. (2011) Minimum information about a marker gene sequence (MIMARKS) and minimum information about any (x) sequence (MiXxS) specifications. *Nat Biotechnol* **29**: 415–420.

Yu K & Zhang T (2012) Metagenomic and metatranscriptomic analysis of microbial community structure and gene expression of activated sludge. *PLOS ONE* **7**: e38183.

Yu NY, Wagner JR, Laird MR et al. (2010) PSORTb 3.0: improved protein subcellular localization prediction with refined localization subcategories and predictive capabilities for all prokaryotes. *Bioinformatics* **26**: 1608–1615.

Zarraonaindia I, Smith DP & Gilbert JA (2013) Beyond the genome: community-level analysis of the microbial world. *Biol Philos* **28**: 261–282.

Zhu P & Li M (2012) Recent progresses on AI-2 bacterial quorum sensing inhibitors. *Curr Med Chem* **19**: 174–186.

Zhuang K, Vemuri GN & Mahadevan R (2011) Economics of membrane occupancy and respiro-fermentation. *Mol Syst Biol* **7**: 500.

Zhuang K, Ma E, Lovley DR & Mahadevan R (2012) The design of long-term effective uranium bioremediation strategy using a community metabolic model. *Biotechnol Bioeng* **109**: 2475–2483.

Zomorrod AR & Maranas CD (2012) OptCom: a multi-level optimization framework for the metabolic modeling and analysis of microbial communities. *PLOS Comput Biol* **8**: e1002363.