

## THE GENERATION AND MAINTENANCE OF DIVERSITY IN MICROBIAL COMMUNITIES<sup>1</sup>

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Microorganisms play a central role in the regulation of ecosystem processes, and they comprise the vast majority of species on Earth. With recent developments in molecular methods, it has become tractable to quantify the extent of microbial diversity in natural environments. Here we examine this revolution in our understanding of microbial diversity, and we explore the factors that contribute to the seemingly astounding numbers of microbial taxa found within individual environmental samples. We conducted a meta-analysis of bacterial richness estimates from a variety of ecosystems. Nearly all environments contained hundreds to thousands of bacterial taxa, and richness levels increased with the number of individuals in a sample, a pattern consistent with those reported for nonmicrobial taxa. A cursory comparison might suggest that bacterial richness far exceeds the richness levels typically observed for plant and animal taxa. However, the apparent diversity of bacterial communities is influenced by phylogenetic breadth and allometric scaling issues. When these features are taken into consideration, the levels of microbial diversity may appear less astounding. Although the fields of ecology and biogeography have traditionally ignored microorganisms, there are no longer valid excuses for neglecting microorganisms in surveys of biodiversity. Many of the concepts developed to explain plant and animal diversity patterns can also be applied to microorganisms once we reconcile the scale of our analyses to the scale of the organisms being observed. Furthermore, knowledge from microbial systems may provide insight into the mechanisms that generate and maintain species richness in nonmicrobial systems.

**Key words:** bacteria; diversity; microorganisms; molecular surveys; richness.

*“...it is always advisable to perceive clearly our ignorance.”*  
—Charles Darwin

For centuries, ecologists and evolutionary biologists have been busy describing and cataloging the biodiversity on Earth. Despite our best efforts, we still lack robust estimates of species richness for the majority of taxa in most ecosystems. Furthermore, it is often challenging to determine the factors that are most important for determining patterns of species diversity in time and space (May, 1988; Pennisi, 2005). Many basic questions remain unanswered, including why are some habitats more speciose than other habitats, and what are the abiotic, biotic, ecological, and evolutionary forces that determine how many species can be found in a given set of environmental conditions? This is particularly true for many microbial taxa as we often lack even a rudimentary understanding of their diversity patterns.

We do know that the diversity of microorganisms on Earth is truly astounding. In recent years, we have been reminded of this simple fact on a regular basis, both in the scientific literature and in the popular press. For example, just in the past few years, researchers have described high levels of bacterial di-

versity in habitats as diverse as the human skin (Costello et al., 2009), hydrothermal vents at the bottom of the ocean (Huber et al., 2007), polar deserts in Antarctica (Pointing et al., 2009), and cave waters (Chen et al., 2009). Just as astronomers continue to discover new galaxies, microbiologists continue to discover unexpected levels of microbial diversity in unusual environments including showerheads (Feazel et al., 2009), the troposphere (Bowers et al., 2009), and inside rocks (de la Torre et al., 2003). At this point, it is no more noteworthy to report that a given environmental sample harbors high levels of microbial diversity than it would be to report that there are a lot of stars in the sky.

This revolution in our understanding of microbial diversity is a direct result of recent improvements in techniques that allow us to survey the diversity of microbial communities. It is now well recognized that, for many decades, microbiologists had grossly underestimated microbial diversity levels by relying on cultivation-based techniques, which capture only a select few microbial taxa capable of growing rapidly under artificial laboratory conditions (Pace, 1997; Rappé and Giovannoni, 2003). Likewise, with few obvious morphological differences delineating most microbial taxa, direct microscopic analyses of environmental samples are of little use for quantifying microbial diversity. By using high-throughput nucleic-acid-based analyses of microbial communities, researchers have gained new appreciation for the breadth and dynamics of microbial diversity in specific habitats, the spatial and temporal variability in the levels of microbial diversity, and the factors driving this variability (for recent reviews, see Christen, 2008; Hamady and Knight, 2009; Hirsch et al., 2010).

With the widespread use and democratization of sequence-based approaches for surveying microbial diversity, bacterial

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and archaeal sequences have been accumulating in databases at an unflagging rate, as have the number of recognized taxa (Fig. 1). However, we are still very far from a complete catalog of microbial diversity in most environments. Perhaps more importantly, the ecology, physiology, and life history of most microbial taxa—even numerically dominant taxa—remain poorly understood. We have only just begun to understand how diversity is structured within and between habitats and how changes in microbial diversity may influence the stability and functioning of managed and natural ecosystems. Therefore, writing a review on microbial diversity at this time is akin to writing a review on nuclear physics in 1942 at the start of the Manhattan Project. We acknowledge our ignorance, but have chosen not to let our ignorance inhibit our speculations in this review.

Here we will largely restrict our discussion to richness, or alpha diversity patterns, loosely defined as the number of species (or phylotypes or operational taxonomic units, to use terms favored by microbial ecologists) found in a local habitat. It is not our goal to synthesize how specific biotic and abiotic factors affect diversity levels within individual habitats because this topic has been covered elsewhere for many commonly studied ecosystems including soils (Lauber et al., 2009), marine waters (Fuhrman et al., 2008), the human skin surface (Grice et al., 2009), and aquatic sediments (Sapp et al., 2010), to list just a few examples. Although there are interesting ecological questions that can be addressed by examining biogeographic patterns within individual habitat types (e.g., Martiny et al., 2006; Ofiteru et al., 2010), the specific diversity patterns observed are highly dependent on the environment in question. Instead, we focus here on the fundamental processes that are likely to be responsible for the generation and maintenance of microbial diversity regardless of the habitat in question. In other words, we will attempt to answer the simple question: Why do microbial communities harbor such high levels of diversity?

## MEASURING MICROBIAL DIVERSITY

Any discussion of microbial diversity must unavoidably start with some background on how we actually measure diversity, including the pitfalls associated with these analyses. Although nonmolecular methods (including phospholipid fatty acid analysis; White and Ringelberg, 1998) have been used in the past to compare microbial diversity levels, techniques based on either DNA or RNA analysis are now widely favored. While we have had a broad array of molecular techniques to assess microbial diversity, including probe-based approaches, DNA/RNA annealing analyses, and fingerprinting approaches that have been reviewed elsewhere (Rondon et al., 1999; Hill et al., 2000; Fuhrman, 2008), they have largely been supplanted by sequence-based analyses of microbial diversity. There are numerous variations for conducting sequence-based assessments of microbial diversity, and new higher-throughput sequencing technologies emerge nearly every week. However, the typical protocol for sequence-based microbial analyses involves extracting DNA (or more rarely, RNA) from the sample in question, PCR amplification of a gene of interest (although non-PCR based techniques can also be used), and sequencing of the amplified gene or gene fragment via either cloning and Sanger sequencing or via direct sequencing of the amplicons by pyrosequencing. Although surveys of other microbial groups often target different genes, in bacterial and archaeal diversity analyses a portion of the gene encoding for the small subunit of ribosomal RNA (i.e., 16S rRNA) is typically sequenced. Compared to other genes, the 16S rRNA gene has a number of clear advantages when it comes to diversity analyses: (1) it has highly conserved regions that permit effective PCR primer design, (2) regions of the gene are sufficiently variable to allow for accurate taxonomic and phylogenetic identification of community members, (3) lateral transfer of this gene between taxa appears to be rare, and (4) since this gene has been widely sequenced in microbial diversity surveys, there is a large amount of accumulated

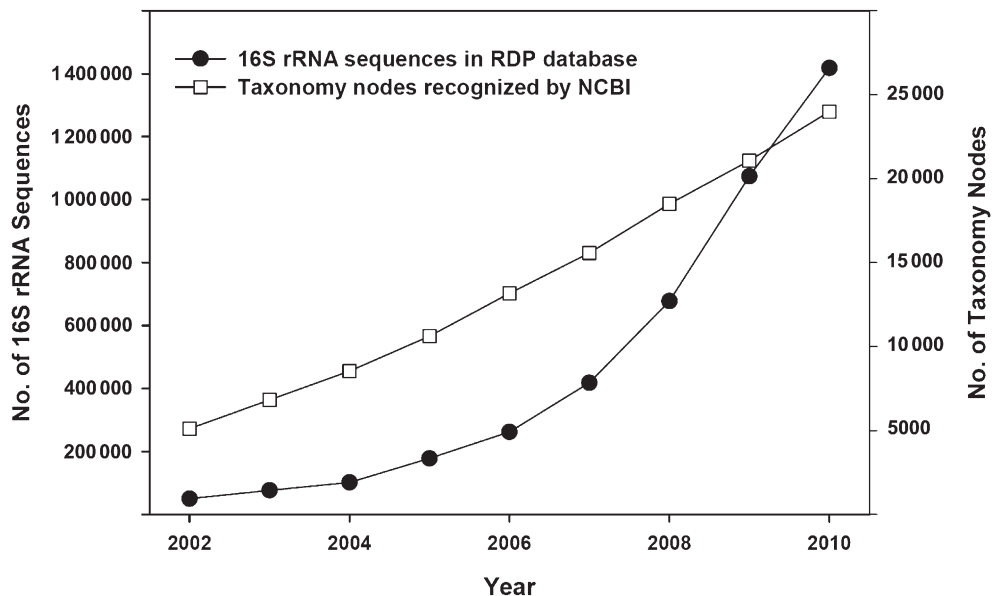


Fig. 1. Growth in the numbers of 16S rRNA sequences (both bacterial and archaeal combined) that have been deposited in a curated database (Ribosomal Database Project; Cole et al., 2009) over the past 8 years (filled circles, left y-axis). Also shown is the increase in the number of bacterial and archaeal taxonomy nodes recognized in the database curated by the National Center for Biotechnology Information (Benson et al., 2009).

16S rRNA sequence data in databases that permit more accurate taxonomic identification and comparisons of community composition across studies (Fig. 1). Once the 16S rRNA gene sequence information has been obtained from a given community, the individual sequences are typically clustered into phylotypes, or operational taxonomic units (OTUs), which are arbitrarily defined groups of sequences that share a certain level of similarity, and these phylotypes are used for taxonomic (e.g., richness, evenness, Shannon index) or phylogenetic (e.g., Faith's phylogenetic diversity) determinations of alpha diversity levels (Faith, 1992; Lozupone and Knight, 2008).

There are some important caveats and limitations that need to be addressed when using sequence-based approaches to estimate microbial richness. Consider, for example, if we were to use a similar approach to survey plant diversity in a forested plot. We would extract DNA from all the plant biomass in the plot, then amplify and sequence a single gene from that pooled DNA sample. We would then use the sequences to cluster the plants into phylotypes and finally estimate alpha diversity and the relative abundance of individual taxa from the sequence data. Individuals that are larger in size (or have more copies of the gene per cell) would appear to be relatively more abundant. Likewise, gene sequences recovered from the plots would not necessarily provide information about a plant's water use efficiency, leaf morphology, shade tolerance, or root architecture.

In addition to these obvious limitations, there are other key quirks associated with sequence-based microbial surveys that need to be carefully considered when reporting and interpreting microbial diversity patterns. First, few studies are likely to capture the full extent of microbial diversity within a given sample. One reason for this is that PCR primers rarely amplify all members of a given taxonomic group without introducing biases (Wintzingerode et al., 1997; Baker et al., 2003; Fuhrman, 2008), so the amplification step will often lead to some groups being missed, or at least underrepresented, in surveys. Second, it is logistically and financially difficult to survey the full extent of diversity for broad taxonomic groups of microorganisms such as bacteria and fungi (Schloss and Handelsman, 2004; Curtis and Sloan, 2005; Fierer et al., 2007). Even with the most advanced high-throughput technology, ecologists are challenged by undersampling, owing to the tremendous diversity and abundance of microorganisms in nearly all ecosystems. Just as plant ecologists are often unable to survey every plant in a given plot, microbiologists will invariably miss rarer taxa in community surveys. Last, there are often errors introduced during the PCR step (e.g., chimeras, Qiu et al., 2001), the sequencing itself (Quince et al., 2009), and OTU-picking algorithms (Huse et al., 2010) that, singly or in combination, lead to the overestimation of microbial diversity. Although it may be true that "microbial diversity is beyond practical calculation" (Wilson, 1999), we can compare relative changes in bacterial richness levels across individual samples even if sampling is incomplete, as long as analyses are conducted in a consistent manner and data are rarefied to a set sampling depth (Lozupone and Knight, 2008; Shaw et al., 2008; Costello et al., 2009).

#### COMPARING BACTERIAL RICHNESS ACROSS HABITATS

The term *microbe* is so vague as to be meaningless, as meaningless as if biologists were to begin using the term *macrobe* to refer to all plants and animals (the main difference being that

the human ego is sensitive to the idea of being lumped together with mosses and jellyfish). Although archaea, viruses, microeukaryotes, and fungi all harbor high levels of diversity and have fascinating diversity patterns, we focus here on bacteria because they have been surveyed across the widest array of habitats using modern molecular techniques. Also, despite some noteworthy exceptions (e.g., decomposing plant litter, deep subsurface environments, some fermented foods and drinks), bacteria likely dominate the pool of microbial biomass in most environments. We will not attempt an exhaustive review of the many hundreds of papers that have described bacterial diversity levels from environmental samples because differences in methodologies make direct comparisons tenuous or impossible. Instead, we restricted our analyses to those studies that met a number of criteria. First, we needed to be able to approximate the number of cells that were likely to be found in the individual sample or samples that were analyzed in a given study. Second, we only included those studies that conducted relatively comprehensive sequence-based surveys of the 16S rRNA gene (in most cases, more than a thousand sequences per sample) that were obtained via direct PCR amplification of community DNA. Furthermore, we only compared richness levels at a single level of taxonomic resolution, the species level, recognizing that there is no consensus definition of a microbial species and that the species definitions commonly applied to plants and animals are usually unrelated (and perhaps irrelevant) to microbial species definitions (Rossello-Mora and Amann, 2001; Rossello-Mora and López-López, 2008). Here we define species as those sequences that share  $\geq 97\%$  similarity in their 16S rRNA gene sequences, recognizing that this cutoff is somewhat arbitrary but widely applied. Although microbiologists more commonly use the terms operational taxonomic units (OTUs) or phylotypes when discussing taxonomic diversity, we use the term *species* here to avoid unnecessary jargon, acknowledging that even for plants and animals what constitutes a species is often undetermined and subject to debate.

In most environments, the accurate determination of the full extent of bacterial richness is a Sisyphaean task, and a comprehensive examination of richness levels across a broad range of environments remains to be conducted (though see Lozupone and Knight, 2007). However, we can still compare relative richness levels across selected environments (Fig. 2) and the shape of species-accumulation curves (Fig. 3). Not surprisingly, the richness of bacterial communities is highly variable across environments. Some environments, including the atmosphere (Bowers et al., 2009), glacial ice (Simon et al., 2009), and highly acidic stream waters (Tyson et al., 2004) harbor relatively low numbers of bacterial species, while other environments, including soils (Fierer et al., 2007; Roesch et al., 2007), microbial mats (Ley et al., 2006), and marine water (Sogin et al., 2006) are likely to have thousands of bacterial species in a given sample (Fig. 2) with the total number of species yet to be determined (evident from the lack of asymptotes in the curves shown in Fig. 3). Also, we know that, in most environments, the vast majority of bacterial species are rare (Pedrós-Alfó 2006; Sogin et al., 2006). For example, across all of the samples included in Fig. 3, the most dominant species represented, at most, only 2–5% of the sequences obtained from a given sample.

Figure 2 also shows that there is a significant positive correlation between the species richness of a sample and the number of individuals in a sample (Fig. 2,  $r^2 = 0.40$ ,  $P < 0.01$ ). However, a note of caution: the estimates contained in Fig. 2 should only be treated as best guesses because many important environments

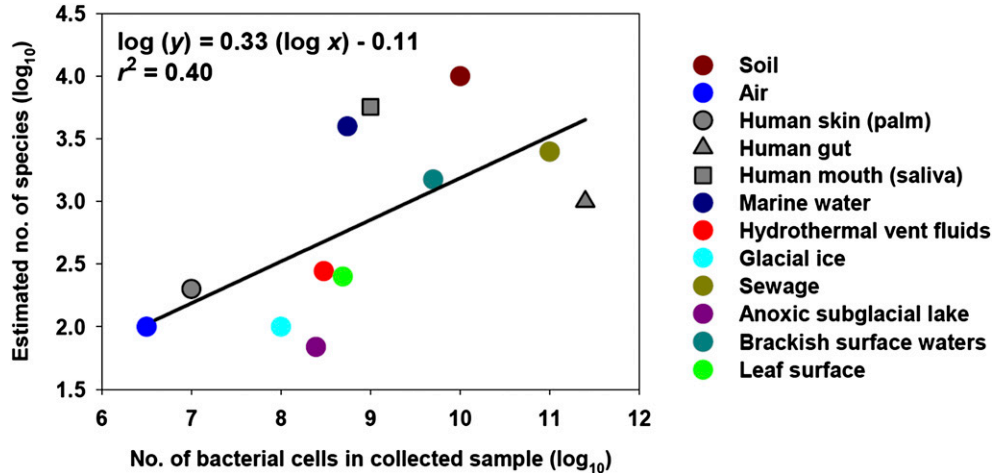


Fig. 2. Estimated number of bacterial species (based on 16S rRNA similarity) vs. the estimated number of cells in the collected sample. See Table 1 for additional details on these data and their sources. Note that these values represent only coarse estimates of population sizes and richness levels based on the most comprehensive data sets currently available. Likewise, we are assuming that these individual samples are representative of the broader habitat in question, but this may not necessarily be the case.

were excluded from our analyses due to a paucity of relevant data, differences in methodologies and inconsistent processing of sequences across studies. However, these results do suggest that samples with highly diverse bacterial communities are often those that contain large numbers of bacterial cells. There are various explanations for the observed relationship between bacterial richness and number of individuals in a sample (Rosenzweig,

1995). First, more species may be recovered from samples with more individuals because of sampling effects. In other words, if more individuals had been recovered from the atmosphere, we may have found that this sample was just as diverse as the soil sample, which contained approximately four orders of magnitude more cells (Table 1). Although one could partially account for this sampling effect by applying rarefaction

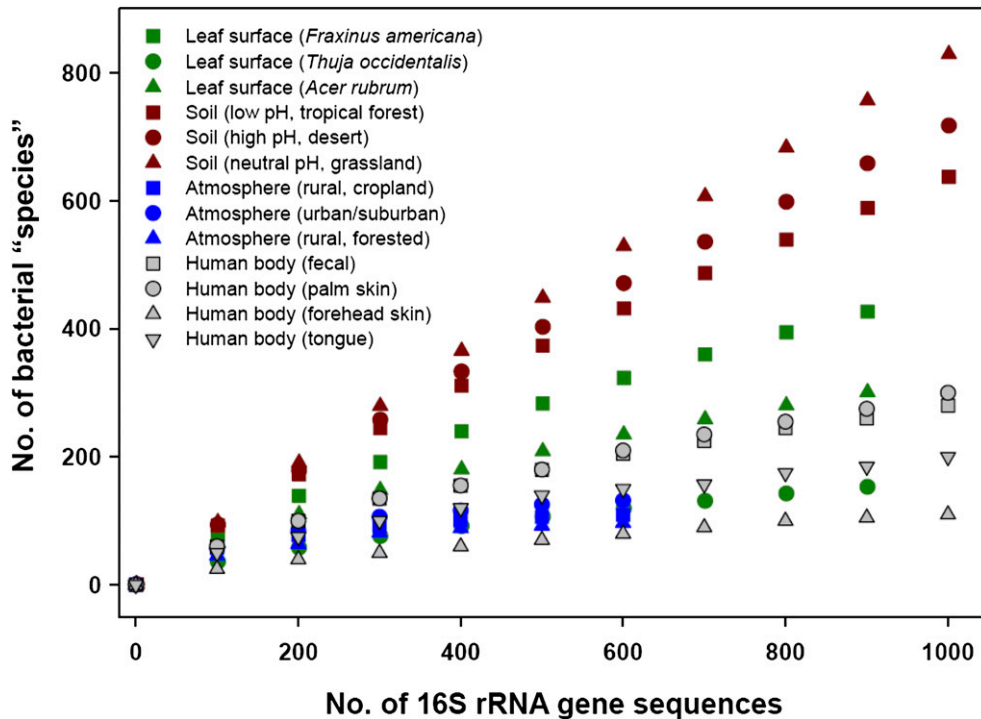


Fig. 3. Rarefaction curves from individual samples collected from selected environments. The x-axis shows the number of gene sequences obtained per sample with the y-axis showing the number of bacterial "species" observed per sample, with a "species" defined at the 97% sequence similarity level (see Comparing bacterial richness across habitats section). All sequencing and sequence analyses were conducted in an identical manner for all samples shown here. However, note that there were only 600 sequences per sample for the atmosphere samples. Leaf surface data are from Redford et al. (2010), soil data from Lauber et al. (2009), atmosphere data from Bowers et al. (2010), and data on the human body from Costello et al. (2009).

TABLE 1. Estimates of cell concentrations (conc.) and numbers of bacterial species from a wide range of environments and the approximate volume of sample used to obtain the estimates. Bacterial species are defined as taxa that share  $\geq 97\%$  similarity in their 16S rRNA gene sequences. We only report average cell concentrations for those studies in which multiple samples were analyzed from a given environment. Note that, in many cases, estimated cell concentrations and species numbers were not obtained from the same study, and the numbers provided represent only rough approximations based on the available information. Only those studies that conducted relatively deep surveys of the bacterial communities via high-throughput sequencing were used for the richness estimates reported here.

Habitat	Cell conc. (Source)	Mean no. per sample (Source)	Total volume of sample
Soil	$10^{10}$ cells/cm <sup>3</sup> (1)	10000 (2)	1 cm <sup>3</sup>
Air	$10^6$ cells/m <sup>3</sup> (3)	100 (3)	2.5–5.4 m <sup>3</sup>
Human skin	$10^7$ cells/cm <sup>2</sup> (4)	200 (5)	70 cm <sup>2</sup>
Human gut	$10^{12}$ cells/g feces (6)	1000 (7)	0.2 g
Human mouth (saliva)	$10^8$ cells/mL saliva (8)	5700 (9)	10 mL
Marine water	$10^5$ to $10^6$ cells/mL sea water (10)	4000 (10)	1000 mL
Marine hydrothermal vent fluids	$10^5$ cells/mL (11)	260 (11)	3 L
Glacial ice	$10^6$ cell/mL (12)	100 (13)	100 mL
Sewage	$10^9$ cells/mL (14)	2500 (14)	100 mL
Anoxic subglacial lake	$10^6$ cells/mL (15)	70 (15)	250 mL
Baltic Sea (brackish surface water)	$10^6$ cells/mL (16)	1500 (16)	5000 mL
Leaf surfaces	$10^5$ cells/cm <sup>2</sup> (17)	250 (17)	5000 cm <sup>2</sup>

Notes: Sources for estimates: (1) Torsvik et al., 2002; (2) Fierer et al., 2007; Roesch et al., 2007; (3) Bowers et al., 2009; (4) Fredricks, 2001; (5) Fierer et al., 2008; (6) Vaahntovu et al., 2005; (7) Dethlefsen et al., 2007; Turnbaugh et al., 2009; (8) Lauber et al., unpublished manuscript; (9) Keijsers et al., 2008; (10) Sogin et al., 2006 and references therein; (11) Huber et al., 2007; (12) Sharp et al., 1999; (13) Simon et al., 2009; (14) McLellan et al., 2010; (15) Gaidos et al., 2009; (16) Andersson et al., 2010; (17) Redford et al., 2010.

techniques, doing so leads to a loss of information, and in some instances, richness would likely remain correlated with the number of individuals (Rosenzweig, 1995). If we assume that the microbial surveys from different ecosystems in Fig. 2 were sampled with comparable effort, then cross-ecosystem patterns of bacterial richness would not simply be a result of sampling differences but instead a likely product of various ecological and evolutionary processes, which we examine in more detail in the following section. Perhaps most importantly, the results in Fig. 2 illustrate the gaps in our knowledge of bacterial diversity. Despite the exponential increase in the volume of microbial sequences (Fig. 1), microbiologists do not adhere to a set of “best practices” when sampling, processing samples, and reporting their data. Moreover, sequence data are not always accompanied by metadata (e.g., cell number, soil type, temperature), which are critical for addressing questions using comparative approaches. As high-throughput sequencing becomes more commonplace and projects such as the Earth Microbiome Project get underway (see <http://www.earthmicrobiome.org/>), our estimates of bacterial richness patterns will undoubtedly improve, particularly if data are collected in a consistent manner to permit direct comparisons of richness levels across environments.

#### FACTORS INFLUENCING THE RICHNESS OF MICROBIAL COMMUNITIES

Using a combination of theoretical, comparative, and experimental approaches, microbiologists have, over the past few decades, gained substantial insight into the ecological and evolutionary processes that influence patterns of microbial diversity. Next, we briefly highlight a subset of the more important mechanisms that are likely to contribute to the high levels of microbial species richness that are typically observed in a variety of ecosystems.

**Metabolic diversity**—The taxonomic diversity of a microbial community is, at least in part, a reflection of its underlying

metabolic diversity. This point becomes apparent when one contrasts the metabolic diversity of macroscopic and microscopic organisms. In general, plants generate energy through photosynthesis, while animals ingest and aerobically oxidize organic matter. Thousands of microbial species make a living using the same metabolic pathways. For example, chlorophyll-based photosynthesis is found in six different bacterial phyla (Bryant et al., 2007). Arguably, the mechanisms of phototrophy are far more diverse among microorganisms than in plants. For example, some bacteria (e.g., *Rhodobacter*) are capable of using reduced sulfur compounds as a source of electrons instead of H<sub>2</sub>O. In addition, metagenomic studies over the past decade have revealed a new type of phototrophy where rhodopsin genes function as a light-driven proton pump in heterotrophic bacteria throughout the oceans (Béjà et al., 2001). In at least a few cases, these different forms of phototrophy have demonstrable effects on the maintenance of biodiversity. For example, some cyanobacteria have evolved distinct accessory pigments (e.g., phycobiliproteins) that allow species to coexist via niche partitioning of the light spectrum (Stomp et al., 2004). There are likely to be just as many (if not more) microbial species that generate ATP using a combination of inorganic chemicals (e.g., H<sub>2</sub>S, Fe<sub>2</sub><sup>+</sup>, NH<sub>3</sub><sup>+</sup>) and “alternate” electron acceptors (e.g., NO<sub>3</sub><sup>-</sup>, Fe<sub>3</sub><sup>+</sup>, Mn<sub>4</sub><sup>+</sup>, SO<sub>4</sub><sup>3-</sup>, or CO<sub>2</sub> instead of O<sub>2</sub>) (Megonigal et al., 2004). Some of these microbial taxa are capable of switching between different types of metabolism (e.g., mixotrophs and facultative anaerobes), a strategy that is thought to be adaptive in fluctuating environments (Crane and Grover, 2010). In addition, microbial biodiversity may be maintained through complex trophic interactions involving multiple species or ecotypes. For example, it has been shown in laboratory experiments that the coexistence of *E. coli* variants is achieved through “cross feeding”, whereby one strain degrades a single limiting resource (i.e., glucose) and excretes intermediates (acetate and glycerol) that support the growth of other strains (Rosenzweig et al., 1994; Pfeiffer and Bonhoeffer, 2004). In addition, tightly coevolved mutualistic interactions involving microbes are commonly found in nature (e.g., Schink, 1997; Currie et al., 2003), and it seems likely that

these taxa contribute appreciably to estimates of microbial richness. New molecular tools, including genomics and transcriptomics, are providing unprecedented insight into the metabolic capacity, not only of individual strains (e.g., Yoder-Himes et al., 2009), but entire assemblages of species (e.g., Mou et al., 2008). Such information will almost certainly improve our understanding about the metabolic factors that control microbial species richness. Nevertheless, it remains unclear whether metabolic specialization alone can explain the coexistence of thousands of species in a single sample. Even though the metabolic niche breadth of microorganisms is impressive, there are still a finite number of resources that can be used for generating energy. Similar concerns have been expressed over the years by plant ecologists (Grubb, 1977) and limnologists (Hutchinson, 1959). Thus, in the following sections, we briefly highlight additional factors that are known to affect patterns of microbial diversity.

**Microbial evolution**—As with other taxa, evolutionary processes are critical for understanding patterns of microbial biodiversity. However, microorganisms have a number of unique attributes that affect their rates and modes of evolution. First, many species achieve large population sizes, have relatively fast generation times, and undergo rapid rates of mutation (Kassen and Rainey, 2004). Together, these features should promote the evolution of novel phenotypes or ecotypes, which can ultimately give rise to new species (Elena and Lenski, 2003). Although the majority of microorganisms reproduce asexually, recombination is achieved through a suite of processes that fall under the umbrella of lateral gene transfer. Lateral gene transfer can occur via transformation, the uptake of extracellular DNA; conjugation, the swapping of plasmids between cells; and transduction, the virus-mediated movement of genes among cells. Such lateral transfer has the potential to introduce novel traits into distantly related taxa, which has implications for species interactions and ecosystem processes (Boucher et al., 2003). For example, recent studies have shown that the swapping of phosphorous acquisition genes is prevalent for some of the dominant bacterial taxa in the nutrient-limited open ocean (Coleman and Chisholm, 2010). Extinction is another evolutionary process that affects patterns of biodiversity. Owing to their large population sizes and ability to resist harsh conditions, it has been hypothesized that high levels of microbial diversity may be attributed in part to low rates of extinction (Dykhuizen, 1998; Horner-Devine et al., 2003, but see Weinbauer and Rassoulzadegan, 2007). Unfortunately, there are very few, if any, good estimates of microbial extinction rates.

**Microbial biogeography**—The spatial patterns of species richness provide insight into the processes that regulate biodiversity (MacArthur and Wilson, 1967). However, a long-held belief in microbiology is that “everything is everywhere, but the environment selects” (Baas-Becking, 1934). This idea led to the commonly held notion that microorganisms, owing primarily to their small size, exhibit cosmopolitan distributions. In other words, microbiologists thought that biogeographic distributions were not influenced by dispersal limitation. Recent studies in microbial ecology have challenged this dogma using a variety of comparative and experimental approaches (Bell et al., 2005; Fierer and Jackson, 2006). Through the construction of distance–decay relationships and species area curves, it has been shown that microbial species are not ran-

domly distributed across the landscape (Horner-Devine et al., 2004). In some cases, the distribution of microbial taxa is correlated with spatial heterogeneity of the habitat suggesting that species may be sorted via environmental filtering (Horner-Devine et al., 2004; Fierer and Jackson, 2006). In fact, it has been shown that soil bacteria can be locally adapted to edaphic characteristics at the scale of only a few meters (Belotte et al., 2003). Taken together, recent studies in microbial biogeography suggest that high dispersal rates do not trump local processes via mass effects (Logue and Lindstrom, 2010) and that niche-based processes and historical contingencies are likely to play an important role in determining patterns of microbial species richness (Martiny et al., 2006).

**Microbial dormancy**—Dormancy refers to an individual’s ability to enter a reversible state of reduced metabolic activity. Dormancy is a bet-hedging strategy that generates a seed bank, which allows populations to persist through unfavorable environmental conditions. In plant and animal systems, ecologists have demonstrated that dormancy can allow for the coexistence of competing species (Caceres and Tessier, 2003) and thus maintain biodiversity (Chesson and Warner, 1981). Although microbiologists have known about dormancy in bacteria for more than a century, it has only recently been shown that dormancy can have implications for the diversity of microbial communities (Jones and Lennon, 2010). Many microbial species can survive for prolonged periods in a dormant state. For example, viable bacteria have been retrieved from 500 000-yr-old permafrost samples (Johnson et al., 2007). Although there are costs associated with dormancy, it appears to be a prevalent life history strategy that is used by a wide range of microbial taxa in a variety of habitats. A recent compilation of literature data estimated that, on average, 90% of the bacteria in soils are metabolically inactive (Lennon and Jones, 2011). In such systems, dormancy should have important implications for a variety of ecological processes that influence microbial diversity, including succession and recovery from disturbance events (Lennon and Jones, 2011).

**Neutral processes**—Despite obvious distinctions in the niches occupied by many microbial taxa, recent studies suggest that neutral processes may be important for understanding patterns of microbial diversity. Neutral theory assumes that species are functionally equivalent and that the relative abundance of species is influenced by stochastic events and random dispersal from a regional pool of taxa. Originally developed to explore diversity patterns of forest communities, neutral theory can explain a number of common phenomena in ecology, including species area relationships and species abundance distributions (Hubbell, 2001). Since then, neutral theory has been applied to microbial systems. When invasion dynamics were examined, the dynamics of two-thirds of the *Pseudomonas* isolates from a culture collection were neutral or near-neutral under laboratory conditions (Zhang et al., 2009). Neutral patterns have also been observed in more complex microbial communities (Sloan et al., 2006, 2007). For example, dominance patterns of ammonia-oxidizing bacteria in wastewater treatment communities were generally consistent with predictions from neutral theory (Ofiteru et al., 2010). However, fits of the observed and predicted data were improved when models included environmental information, suggesting that both niche and neutral processes contribute to patterns of microbial biodiversity.

IS MICROBIAL DIVERSITY REALLY FAR GREATER THAN “MACROBIAL” DIVERSITY?

It is now almost a cliché to claim that the diversity of microorganisms in a given environment is high. Nevertheless, it boggles the mind to think of an environmental sample harboring thousands of bacterial species (Figs. 2, 3). Often, microbiologists find these diversity estimates surprising or unexpected, which is understandable when we consider that, for many decades, microbiologists had grossly underestimated microbial diversity on Earth (Pace, 1997). With new data in hand, there is a temptation to compare the diversity of microbial and “macrobial” (plant and animal) communities. The conclusions from such comparisons almost always reveal that microbial diversity in individual samples is far greater than the local plant and animal diversity. After all, some of the most species-rich plant, insect, and vertebrate communities appear to pale in comparison to the many thousands of bacterial species found in a single gram of soil or a few liters of ocean water (Fig. 2). However, any direct comparison between macrobial diversity and microbial diversity is tenuous at best. Below, we discuss two important features that may help reconcile the apparent differences between microbial and macrobial diversity.

**Phylogenetic breadth**—When we quantify bacterial diversity, we are effectively measuring diversity across an entire domain of life. In contrast, when we quantify plant or animal diversity, we are only measuring diversity within a relatively

small portion of the eukaryotic domain (Fig. 4). For this reason, any qualitative comparison between macrobial and microbial diversity must account for differences in phylogenetic breadth. If we were to assess total eukaryotic diversity (sum of plant, animal, fungal, and all unicellular eukaryotic taxa) within a given plot, it may actually be very comparable to bacterial diversity, especially considering the enormous diversity of fungi and unicellular eukaryotic lineages (Fig. 4). The greater phylogenetic breadth covered in bacterial diversity estimates compared to plant or animal estimates is somewhat tempered by the commonly used bacterial species definition (97% sequence similarity in the small subunit rRNA gene, the same definition used in Figs. 2 and 3). If we were to apply this same species definition to plant and animal taxa, it would effectively lump together many taxa that are currently recognized as unique species (e.g., many apes). Nevertheless, the enormous amount of bacterial diversity contained within environments is less impressive once we consider that bacteria have been diversifying for at least 3.7 billion years and that the phylogenetic breadth captured in bacterial diversity surveys is far larger than in most macrobial diversity surveys.

**The problem of scale**—Ecologists sample microorganisms and macroorganisms with different effort, which can bias diversity estimates. The discrepancy in effort arises when we use similar approaches for sampling organisms with logarithmic-scale differences in body size. For example, hectare-size plots are typically used to survey tree diversity, yielding sample

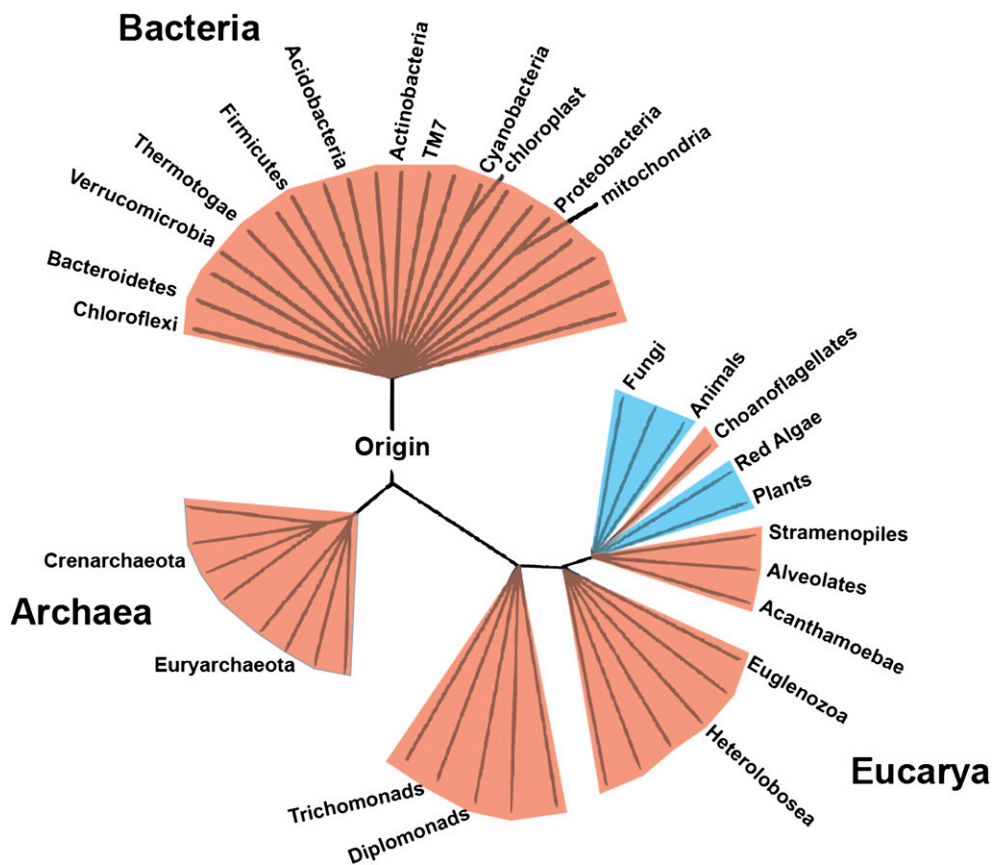


Fig. 4. A version of the “universal” tree of life based on rRNA sequence analysis, adapted from Pace (2009), with multicellular lineages indicated in blue and those lineages that are primarily unicellular highlighted in red.

areas that are approximately 50 000 times bigger than an individual tree (assuming that a tree "footprint" is 0.2 m<sup>2</sup>). In contrast, a 0.5 g sample of soil or fecal material has a volume that is approximately one-trillion times larger than a typical bacterium of 1 μm<sup>3</sup>. Based on biogeographic scaling relationships (MacArthur and Wilson, 1967), we can expect that these differences in sampling effort will influence conclusions about the levels of diversity in microbial and macrobial communities. More specifically, this disparity in scale may lead to the misleading assumption that bacterial diversity is somehow far higher than typically observed for "macro"-organisms when in reality we are just sampling microbial and macrobial communities at very different scales. In other words, the astounding levels of microbial diversity could simply be explained by the statement from Werner Heisenberg (1962, p. 58): "What we observe is not nature itself, but nature exposed to our method of questioning."

If we were to sample microbial and macrobial communities on comparable spatial scales, would microbial richness be more in line with richness estimated from typical surveys of plant and animal diversity? To address this question, we begin by making some simple assumptions about the allometric relationship between individuals (microbe vs. plant) relative to the dimensions of a standard sized plot (1 m<sup>2</sup>). We assume that an average microbe is 1 μm<sup>2</sup>, while the area of a grassland plant stem is 1 cm<sup>2</sup>. Given a uniform nonoverlapping distribution, bacteria can achieve a maximum density of 10<sup>12</sup> individuals/m<sup>2</sup>, while plants can achieve a maximum density of 10<sup>4</sup> individuals/m<sup>2</sup>. Based on the differences in population densities alone, it is clear that plants will likely be undersampled relative to microbes. To sample plants with the same effort that we sample microbes, ecologists would need to increase their plot sizes by four orders of magnitude to 10 km<sup>2</sup>. Conversely, if microbial ecologists wanted to sample bacteria at the same scale that ecologists sample grassland plant communities, they would need to obtain 100-μm<sup>2</sup> soil collections, which is equivalent to the size of a fine sand particle. These simple calculations reveal that there are obvious discrepancies when it comes to sampling microbial and macrobial communities. Differences in sampling effort may not only bias our comparisons of microbial and macrobial diversity, but also have implications for how we interpret species interactions at the microbial scale. For example, in an average soil core, we are effectively lumping multiple habitats and environmental gradients, which on human scales, would be equivalent to the heterogeneity encountered in ~1000 km<sup>2</sup>.

**Conclusions**—"From the elephant to butyric acid bacterium—it is all the same!" —Albert Jan Kluyver (1926)

By definition, any unifying concepts in the study of biological diversity must consider microorganisms because they represent the bulk of the phylogenetic diversity and a major portion of the living biomass on Earth. However, many of our dominant paradigms in the fields of ecology and biogeography, including those developed from studies of latitudinal or elevational gradients in diversity, succession patterns, and island biogeography, have largely been derived from work on plants and animals. One reason for this myopia has been methodological; until recently, microbiologists could not survey microbial diversity as easily as ecologists could conduct field surveys of plant and animal diversity. However, that argument is now moot. With recent methodological advances, it is now feasible to explore the evolutionary and ecological explanations for the seemingly

high levels of microbial diversity observed in many environments. In all likelihood, many of the concepts developed to explain and predict plant and animal diversity patterns can be applied to microorganisms once we reconcile the scale of our analyses to the scale of the individual organisms being observed. However, a number of concepts may simply not apply; the factors controlling plant and animal diversity may fundamentally differ from those factors controlling microbial diversity. If true, this suggests that ample opportunities exist for microbiologists and "macrobial" ecologists to work together to broaden the search for universal patterns and mechanisms governing the distribution of biological diversity on Earth.

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