VIEWPOINT

Prokaryotic Diversity—Magnitude, Dynamics, and Controlling Factors

Vigdis Torsvik,[1]* Lise Øvreås, Tron Frede Thingstad

There are probably millions of species in the microorganismal domains Bacteria and Archaea (the prokaryotes), and we are only just beginning to work out the basic principles governing their distribution and abundance in natural environments. One characteristic that has become clear is that prokaryote diversity in aquatic environments is orders of magnitude less than in sediments and soils. Hypotheses and models explaining such differences are under development and are beginning to offer promising insights into the mechanisms governing prokaryote diversity and ecosystem function.

Microscopic prokaryotic organisms are a largely unnoticed part of Earth's biota. They constitute the domains Archaea and Bacteria and consist of possibly millions of different species. Prokaryotic diversity is a product of about 3.8 billion years of evolution—2 billion years longer than that of eukaryotic organisms, and is probably the reason for their extraordinary diversity and habitat range. The prokaryotes are a crucial component of the biosphere because they catalyze processes sustaining all life on Earth and are thus the engines for the biogeochemical cycles. However, only about 4500 species have been characterized, leaving most of the diversity of prokaryotes unexplored.

Traditionally, the unit of diversity is the species, but we do not know whether any naturally occurring entity of prokaryotic species exists, and a variety of definitions for the concept are used for these organisms. First, the "phylophenetic" definition circumscribes the species as a "monophyletic and genomically coherent cluster of individual organisms that show a high degree of overall similarity in many independent characteristics, and is diagnosable by a discriminative phenotypic property" (*1*). Second, a species can be defined as an assemblage of strains sharing 70% or more DNA homology (*2*). Third, in an ecological definition the species and niche concept are linked, and thus a species consists of the organisms occupying the same niche (*3*). Thus, diversity can be defined as the number of prokaryotic species and their relative abundance in a community, or as the amount and distribution of information in a community (*4*).

Magnitude of Prokaryotic Diversity

Diversity estimates for natural bacterial communities have traditionally depended on cultivable species, but results from the use of molecular techniques to measure diversity suggest that reliance on culture has led to a longstanding underestimate of bacterial diversity (Table 1). DNA and RNA analyses imply prokaryotic diversity far greater than was predicted, and are beginning to hint at the role of bacterial and viral diversity in global ecological cycles. For instance, most investigations of prokaryotic diversity relate to surface environments, but recent research suggests that the biota extend deep into Earth's crust, and that the majority of prokaryotic organisms might occur in the oceanic and terrestrial subsurface. The total carbon biomass in subsurface terrestrial microorganisms has been estimated to equal that of all terrestrial and marine plants, and may be the largest constituent of the entire Earth biomass (*5,6*).

The genome size (complexity) of prokaryotic organisms can be calculated from the reassociation rate of denatured (i.e., single-stranded) DNA, which depends on the amount of homogeneous DNA present in a sample. This method can also be used to estimate prokaryotic community genome size (the sum of the sizes of different prokaryotic genomes in a community), which can then be used as a measure of the total genomic diversity in a community (*7*). The DNA reassociation method (*8*) has revealed a high degree of genomic diversity in prokaryotic communities in pristine soil and sediments with high organic content. Here, the DNA diversity seen in 30- to 100-cm^3 samples corresponds to about 3000 to 11,000 different genomes (Table 1). By using the DNA-based species definition, and assuming that strains with $\geq$70% DNA homology belong to the same species, it has been estimated that these samples contain $\sim 10^4$ different prokaryotic species of equivalent abundances.

Aquatic environments appear to support less diversity than soils and sediments. In extreme aquatic environments, such as salt-crystallizing ponds at 22% salinity, the prokaryotic genomic diversity, when estimated by DNA reassociation, appears to correspond to only about seven distinct genomes (Table 1) (*9*). The same technique shows that samples (10-liter) from relatively nutrient-rich freshwater and estuarine waters exhibit a microbial diversity equivalent to $\sim$160 different genomes (*10*). Similarly, Curtis *et al.* (*11*) estimated that 163 different prokaryotic taxa occurred in seawater, based on extrapolation of species-abundance curves. For soil, their corresponding estimate was 6380 different taxa.

Community fingerprinting techniques based on polymerase chain reaction, compared with DNA reassociation methods, indicate that a much lower number of prokaryotic taxa occur in water, usually on the order of 10 species. This discrepancy arises because fingerprinting reveals only the most dominant species, i.e., in water only about 10% of the simultaneously coexisting species are dominant. This number is extremely small compared with the $\approx 10^4$ species estimated to occur in soils and sediments. The three to four order-of-magnitude difference in total bacterial abundance between water and sediments/soils suggests that each dominating taxon in both environments consists of $\approx 10^4$ to 10^5 individuals per gram or milliliter. This finding suggests that there may be mechanisms controlling taxon size that work more or less similarly in all environments, whereas mechanisms controlling the total abundance of the bacterial community work in radically different ways in water compared with soil.

Dynamics and Control of Prokaryotic Diversity

Trophic interactions. Hutchinson (*12*) asked why there are so many phytoplankton species in an apparently homogeneous aquatic environment where all the species present seem to compete for the same mineral nutrient (i.e., there is a "bottom-up" control of diversity operating). Among prokaryotes, competitors can coexist if some mechanism of selective loss is operating (*13–15*) to prevent the most successful competitors from sequestering all the resources. For example, size-selective predation by protozoa will allow bacterial coexistence with phytoplankton of different size classes. Parasitism by host-specific viruses will allow coexistence of different bacterial taxa within the bacterial community (*3*). Such "top-down" control of diversity will in theory work even if all bacterial and phytoplankton taxa are limited by the same substrate (e.g., phosphate).

One consequence of applying a top-down

Department of Microbiology, University of Bergen, Jahnebakken 5, N-5020 Bergen, Norway.

*To whom correspondence should be addressed. E-mail: vigdis.torsvik@im.uib.no

selective-loss mechanism is that large observed differences in total bacterial abundance have to be explained by protozoan predation being severely restricted in soils and sediments. Conversely, the proposed similarity of taxon size in aquatic and soil/sediment environments suggests that rates of viral lysis are similar in both. It is tempting to speculate that the spatially complex matrix of soils and sediments represents more of an obstacle to the movement of protozoa than to the diffusion of small viruses. Indeed, viruses are abundant in sediment pore water (*16*).

In a biogeochemical context, a bottom-up perspective leads to the argument that high microbial diversity is needed for processing all the different types of substrate molecules (resources) produced in the system. A top-down model indicates a fundamentally different concept for coupling diversity and biogeochemical cycles. In such a model, the activities of lytic viruses compensate for the high growth rate of some bacterial species (host groups). If viral lysis is the mechanism that controls diversity, in the sense of allowing competing bacterial species to coexist, then it is bacterial diversity, in the sense of differences in growth rate between coexisting bacteria, that determines the abundance of viruses present (*3*) and thus governs the reflux of particulate organic matter by viral lysis into dissolved organic matter.

Hence, the control of diversity in such models becomes a hierarchical system where the total amount of the limiting resource determines the total amount of biomass that can be produced. Size-selective grazing determines how the biomass is distributed into functional groups (communities), and the host specificity of viruses determines how the functional groups are divided into species.

Evolutionary perspective. The ecological factors and the intrinsic evolutionary mechanisms working at molecular and population levels interact to control prokaryote diversity. One reason for the high genomic diversity observed in prokaryotic communities in soil and sediments is the large populations of organisms and the capacity to accumulate large numbers of mutations. Thus, unlike most eukaryotic populations, every prokaryotic population represents a mixture of genetically diverging clonal cell lines on which natural selection acts. Molecular mechanisms, like lateral DNA transfer and recombination, are also facilitated by high population densities of prokaryotes, and may influence genetic diversity. If lateral transfer occurs within a group of closely related bacteria, it will ensure genetic coherence and slow diversification. By contrast, gene transfer and recombination across species and genus barriers could promote environmental adaptation and the evolution of new traits (e.g., the transfer of antibiotic resistance among different species of bacterial pathogens), thereby increasing diversity. Because high rates of speciation are observed in prokaryotes, one reason for extreme prokaryote diversity might simply be that the speciation rate is faster than the extinction rate (*17*).

Spatial heterogeneity. The structural complexity of soil and sediments is important for population-level diversification because it allows resources to be partitioned and creates new niches, thereby enhancing prokaryote specialization and division into distinct ecological species. The potential for spatial isolation provided by the soil matrix provides a mechanism for controlling diversity in soil that differs markedly from the "top-down" control of diversity that is more likely to operate in aqueous environments. Soils and sediments are chemically complex too, and steep gradients of substrate concentrations, redox potential, and pH also contribute to the formation of large numbers of microhabitats.

Temporal heterogeneity. Most terrestrial communities intermittently suffer disturbances, such as starvation, desiccation, freezing/thawing, or human activity. Altered environmental conditions and resource availability create opportunities for new species to become established, and disturbances will ensure that communities include a mixture of different stages of succession (*18*). However, strong and frequent disturbances will cause the disintegration of the microhabitats and disruption of the boundaries between populations, allowing local resources to become available to a larger proportion of the entire microbial biomass. Consequently, microorganisms with a potentially high growth rate (r-strategists) will become numerically dominant and reduce the evenness of the species distribution. A competitive diversity pattern like this is seen in an arable soil (*19*) (Table 1) where major and frequent disturbances decrease diversity compared with soils from a nearby pasture.

Other environmental factors, such as eutrophication, may also lead to bell-shaped responses in diversity where at low eutrophication levels, an increase in nutrients allows an increase in the complexity of the food web, whereas at high levels of eutrophication, more nutrients may be channeled to a few dominating species, further decreasing the evenness of species distribution. In extreme cases, an accumulation of toxic metabolites or other detrimental effects can occur, and are likely to reduce diversity even more. The dramatic environmental effects of high levels of eutrophication are evident from studies of sediments beneath fish farms, where prokaryotic diversity may only be 50 genome equivalents compared with a diversity of ~11,000 genome equivalents in pristine sediments (Table 1). Fish-farm sediments are subjected to heavy organic input from fish-feed pellets, which ultimately reduces resource heterogeneity. This leads to a decrease, not necessarily in the number of species present, but in the evenness of species distribution of the community.

Matters of Scale

A full understanding of the differences in prokaryote diversity patterns in soil and water requires investigation at different environmental scales. The relative scale between what constitutes the size of a habitat required for a prokaryote, and the size of samples taken for observation, is an important consideration.

Predictions from simple models of homogeneous habitats can provide valuable information about factors like speciation, dispersal, and biological interactions controlling local diversity (α diversity) (*20*) in aquatic environments and probably in soil microhabitats. However, diversity in soil cannot be explained completely by simple models. At the scales used for most quantitative estimates of prokaryotic diversity in soil, knowledge of habitat structure and spatial and temporal variability is essential. Hence, the striking differences in prokaryotic diversity observed in soil and water not only relate to spatial diversity (β diversity), but also to the size of the organisms involved. Because of their small size, pro-

Table 1. Prokaryotic abundance as determined by fluorescence microscopy and total genomic diversity in prokaryotic communities calculated from the reassociation rate of DNA isolated from the community (*9*). Community genome complexity is described as numbers of base pairs (bp). Genome equivalents are given relative to the *Escherichia coli* genome (4.1×10^6 bp).

DNA source	Abundance (cells cm^{-3})	Community genome complexity (bp)	Genome equivalents	Ref.
Forest soil	4.8×10^9	2.5×10^{10}	6000	(*8*)
Forest soil, cultivated prokaryotes	1.4×10^7	1.4×10^8	35	(*8*)
Pasture soil	1.8×10^{10}	(1.5×10^{10})–(3.5×10^{10})	3500–8800	(*22*)
Arable soil	2.1×10^{10}	(5.7×10^8)–(1.4×10^9)	140–350	(*22*)
Pristine marine sediment	3.1×10^9	4.8×10^{10}	11,400	(*8*)
Marine fish-farm sediment	7.7×10^9	2.0×10^8	50	(*8*)
Salt-crystallizing pond, 22% salinity	6.0×10^7	2.9×10^7	7	(*9*)

karyotic diversity in a 100-cm^3 soil sample can be compared to the regional diversity of macroorganisms (γ diversity) (*20*).

Despite a growing knowledge of the magnitude of prokaryote diversity, most of the prokaryotes seen in natural environments are uncultivated, and their functional roles and diversity are unknown. The realization that genes for harvesting of light energy occur widely in marine prokaryotic genomes (*21*) is a striking demonstration of the need to know more about prokaryotic diversity in order to understand how they contribute to the ecological and biogeochemical functioning of our ecosystems.

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VIEWPOINT

Life and the Evolution of Earth's Atmosphere

James F. Kasting[1]* and Janet L. Siefert[2]

Harvesting light to produce energy and oxygen (photosynthesis) is the signature of all land plants. This ability was co-opted from a precocious and ancient form of life known as cyanobacteria. Today these bacteria, as well as microscopic algae, supply oxygen to the atmosphere and churn out fixed nitrogen in Earth's vast oceans. Microorganisms may also have played a major role in atmosphere evolution before the rise of oxygen. Under the more dim light of a young sun cooler than today's, certain groups of anaerobic bacteria may have been pumping out large amounts of methane, thereby keeping the early climate warm and inviting. The evolution of Earth's atmosphere is linked tightly to the evolution of its biota.

Microorganisms are important for many reasons, not the least of which is their responsibility, direct or indirect, for the production of nearly all of the oxygen we breathe. Oxygen is produced during photosynthesis by a reaction that can be written as $CO_2 + H_2O \rightarrow CH_2O + O_2$. Here, "$CH_2O$" is a geochemist's shorthand for more complex forms of organic matter. Most photosynthesis on land is carried out by higher plants, not microorganisms; but terrestrial photosynthesis has little effect on atmospheric O_2 because it is nearly balanced by the reverse processes of respiration and decay. By contrast, marine photosynthesis is a net source of O_2 because a small fraction (~0.1%) of the organic matter synthesized in the oceans is buried in sediments. This small leak in the marine organic carbon cycle is responsible for most of our atmospheric O_2.

Although higher plants (e.g., kelp) are found in the oceans, most marine photosynthesis is performed by single-celled organisms. The most abundant of these are eukaryotic algae, such as diatoms and coccolithophorids (Fig. 1). Roughly 99% of primary production can be attributed to such organisms (*1*). Prokaryotic bacteria are also important for another reason. Though they make up only ~1% of marine biomass, cyanobacteria (or blue-green algae) are the main organisms responsible for fixing nitrogen (*1*). This capability is quite remarkable because the enzyme responsible for reducing N_2, nitrogenase, is poisoned by O_2. Thus, cyanobacteria have had to evolve complex mechanisms for protecting their nitrogenase. Some, such as the filamentous *Anabaena* spp., do so by fixing nitrogen only in specialized cells called heterocysts. Other cyanobacteria fix nitrogen at night and photosynthesize by day. Still others, such as *Trichodesmium* spp. (very abundant in tropical waters), fix nitrogen in the morning and photosynthesize in the afternoon (*2*). Such specificity shows that these are highly evolved pieces of biological machinery.

In some sense, when it comes to producing oxygen, cyanobacteria are the entire story. Because cyanobacteria can live anaerobically and aerobically, they are universally believed to have been responsible for the initial rise of atmospheric O_2 around 2.3 billion years ago (Ga) (*3*, *4*). Comparison of ribosomal RNA from cyanobacteria with portions of the DNA inside chloroplasts implies that all eukaryotes, including algae and higher plants, derived their photosynthetic capabilities from cyanobacteria by way of endosymbiosis (*5*). The *Prochlorococcus* spp., an important component of today's marine ecosystem, may be the living ancestor of the cyanobacterium involved in this event (*6*). It appears that oxygenic photosynthesis—an extremely complex biochemical process—was "invented" only once, and a primitive cyanobacterium was the organism responsible.

Though the production of O_2 is the most notable effect of organisms on the atmosphere, it is by no means their only one. Our modern atmosphere contains numerous trace gases (e.g., CH_4, N_2O, CH_3Cl, COS, dimethyl sulfide) whose sources are almost entirely biological. Some of these gases influence climate today by contributing to the atmospheric greenhouse effect. Concentrations of CH_4 (methane) and N_2O (nitrous oxide) have been increasing in recent

[1]Department of Geosciences, 443 Deike, The Pennsylvania State University, University Park, PA 16802, USA. [2]Department of Statistics, Rice University, Houston, TX 77251–1892, USA.

*To whom correspondence should be addressed. E-mail: kasting@essc.psu.edu