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Microbial Diversity in Soils of Agricultural Landscapes and Its Relation to Ecosystem Function

Thomas M. Schmidt and Clive Waldron

The taxonomic and functional diversity of microbes in soil is stunning. Although they are largely invisible to the naked eye, microbes are pervasive in nature and have a profound impact on Earth's habitability. Like many other terrestrial environments, the top layer of soil at the Kellogg Biological Station Long-Term Ecological Research site (KBS LTER) typically contains 1×10^9 microbes per gram of dry soil, representing between 10,000 and a million species (Gans et al. 2005). Extrapolating the estimated number of microbes in individual soil samples to a global scale yields an estimate of an incredible 26×10^{28} microbes in terrestrial habitats (Whitman et al. 1998). Combined with estimates of microbial abundance in aquatic and subsurface environments, Whitman and colleagues (1998) estimated that microbes contain at least half of the amount of carbon (C) stored in plants and 10 times more nitrogen (N) and phosphorus (P).

The cycling of C, N, P, and other less abundant elements that pass through soil microbial communities influences environments as small as soil aggregates and as far-reaching as global climate. Here, we focus on the composition and function of microbes that drive the cycling of C and N in soils and discuss the ecological significance of their diversity and physiologies within, across, and beyond agricultural landscapes. Compounds containing C and N are major determinants of both crop productivity and climate change, so understanding what controls their transformations will be important for developing agricultural strategies that balance crop yield and ecosystem services against environmental harm.

Our emphasis is on discoveries made in the past two decades at KBS LTER, where experiments have maintained different management practices for >20 years and aspects of C and N cycling are well documented (Robertson and Hamilton

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2015, Paul et al. 2015, Millar and Robertson 2015; Chapters 1, 5, and 9 in this volume). Of particular interest is the partitioning of C and N between bioavailable compounds in the soil and the exchange of greenhouse gases between soils and the atmosphere. These processes are heavily influenced by microbial activity, so it is possible that altered partitioning of C and N could result from changes in the microbial community that are generated by different land uses and management practices. The first step to understanding potential relationships between soil microbes and the production and consumption of greenhouse gases is to determine the structure of microbial communities in different soils.

We begin this chapter with an overview of the composition of microbial communities in soils under different land management practices. We then discuss the relationships between subsets of these communities and the fluxes of methane (CH_4), nitrous oxide (N_2O), and carbon dioxide (CO_2)—the three greenhouse gases that contribute most to climate change (IPCC 2007). Third, we consider the life histories of bacteria in soil and discuss how an ecological perspective has informed new models relating microbial community structure to function. We end the chapter with some thoughts about long-term research to test these models and evaluate the potential for restoring ecological function by manipulating bacterial diversity.

Microbial Diversity in Soil

Molecular Surveys

As in the majority of complex microbial communities in nature, most microbes in soil have yet to be cultured in the laboratory: fewer than 1% of the microbes that can be visualized microscopically grow under traditional cultivation conditions (Staley and Konopka 1985). Cultivated strains provide valuable insights into the metabolism and behavior of microbes from soil (discussed below), but the composition of microbial communities can be determined more rapidly and comprehensively through the characterization of extracted nucleic acids without cultivation. Research at KBS LTER has used some of the most common strategies for these molecular surveys (Fig. 6.1).

The key to comparing these complex microbial communities is obtaining DNA sequences of conserved genes. The nucleotide sequence of a conserved gene is similar in all members because the encoded function of the gene is retained. However, certain nucleotides can be substituted for others without disrupting the gene function. Over time these changes accumulate, so organisms can be grouped according to the location and number of substitutions in their sequences, with the closest relatives having fewest differences. To provide enough material for sequencing, the target gene in an environmental sample must be amplified from each microbe, typically by the process of Polymerase Chain Reaction (PCR). Surveys are often based on comparative analyses of the gene encoding the small subunit ribosomal RNA (SSU rRNA), which has a sedimentation coefficient of 16S in bacteria and Archaea and 18S in eukaryotes.

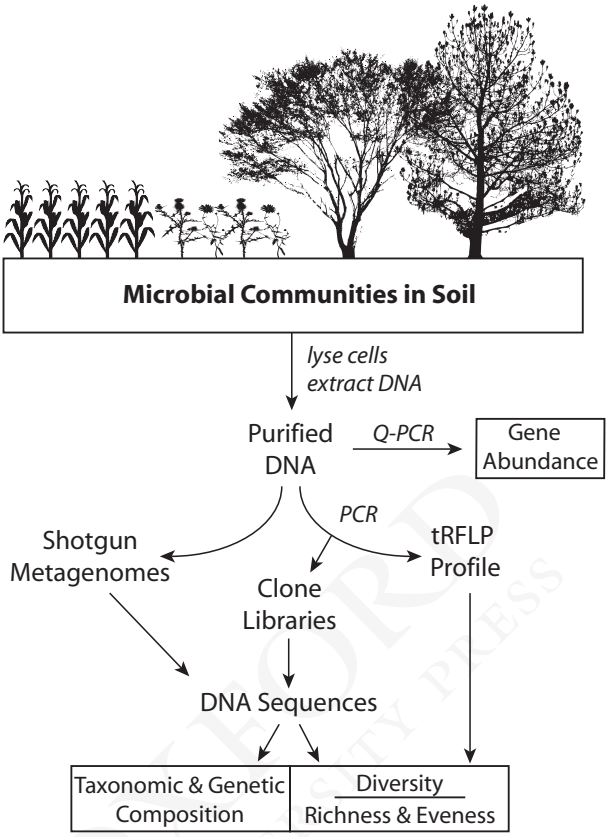

Figure 6.1. An overview of molecular approaches presented in this chapter that have been used to assess the composition and diversity of microbial communities in KBS LTER soils. The polymerase chain reaction (PCR) is used commonly to obtain sufficient quantities of a target gene for subsequent analysis. Quantitative PCR (Q-PCR) is used to estimate gene abundance, while terminal restriction fragment length polymorphism (tRFLP) is a fingerprinting method that provides an overview of gene diversity.

The 16S and 18S rRNA-encoding genes contain regions of strict sequence conservation that are valuable for the design of universal primers to amplify the genes via PCR. These conserved regions are interspersed with regions of sequence variability that are useful for comparative analysis. A major advantage of targeting the SSU rRNA gene for surveys of diverse communities is the opportunity for comparison with the approximately 1.5 million other SSU rRNA gene sequences in curated public databases (Pruesse et al. 2007, Cole et al. 2009). Amplified 16S genes can either be cloned into plasmid vectors and sequenced individually, or sequenced directly with massively parallel "next-generation" sequencing. Because of their high capacity, next-generation technologies offer the potential for considerable in-depth exploration of diversity (Sogin et al. 2006). The 16S gene sequences can be compared at several thresholds of sequence similarity, providing insight into the composition of microbial communities at different taxonomic levels.

While 16S rRNA genes provide a taxonomic description of microbial communities, sequencing of DNA from microbial communities without initial amplification (also known as "shotgun" sequencing) offers insight into the metabolic potential of communities. The collection of sequences from shotgun libraries constitutes a metagenome. Advances have been made in the application of metagenomic methods in many environments (Dinsdale et al. 2008, Antonopoulos et al. 2009, Dethlefsen and Relman 2011), but microbial communities in soil have received considerably less attention. This is due in part to the remarkable diversity of microbes in soil and in part to the difficulty of defining and sampling microbial communities in such a heterogeneous structural matrix.

Heterogeneity in the physical structure of soil, including aggregates of recalcitrant organic matter, minerals, and microbes, complicates the task of collecting representative samples of the soil environment. Initial characterization of KBS LTER soils suggested that the microbial communities changed dramatically over relatively short distances; major variations in total microbial biomass, respiration, and the number of cultured bacteria were detected over distances as small as 20 cm (Robertson et al. 1997). The importance of spatial heterogeneity to microbial communities at KBS LTER was reinforced through analysis of 16S rRNA genes of *Burkholderia* isolates from the rhizospheres of nearby corn plants in the Biologically Based cropping system of the Main Cropping System Experiment (MCSE; Table 6.1; Robertson and Hamilton 2015, Chapter 1 in this volume). This study revealed dramatic differences in community composition and abundance between bacterial communities on individual plants (Ramette et al. 2005), highlighting the challenge presented by spatial variability in even a single field intensively managed for row-crop production. Variability in bacterial community structure was even detected among soil aggregates in KBS LTER soils. These studies were carried out before high-throughput sequencing became available. Sequence differences among 16S genes were identified indirectly by detecting specific sites for cleavage by restriction enzymes in a process known as Terminal Restriction Fragment Length Polymorphism (tRFLP; Fig. 6.1). As many as half of the differences between tRFLP profiles of 16S rDNA genes from soil could be explained by interaggregate variation, even when particles were less than 2 mm in diameter (Blackwood et al. 2006). This means microbial communities vary significantly from particle to particle—even in the same soil sample. This is a potential source of sampling bias when comparing microbial distributions across field sites or systems, but fortunately, spatial variability in microbial communities can be addressed with sufficient sample size and replication in sampling. For example, RFLP profiles of the genes encoding the final enzymatic step in denitrification (*nosZ*) were identical when triplicate 3-g samples were analyzed from either unmanaged successional communities or agricultural systems at KBS LTER (Stres et al. 2004).

In addition to spatial variability as a potential source of uncertainty, bias can also be introduced in certain steps of processing samples for molecular surveys. Several of these technical challenges have been addressed by research conducted with soils from KBS LTER. Differences in cell lysis and therefore DNA extraction efficiency can distort molecular surveys, revealing only a fraction of the tremendous diversity present in soil communities (Feinstein et al. 2009). One strategy for reducing the

Table 6.1. Description of the KBS LTER Main Cropping System Experiment (MCSE).[a]

Cropping System/Community	Dominant Growth Form	Management
Annual Cropping Systems		
Conventional (T1)	Herbaceous annual	Prevailing norm for tilled corn–soybean–winter wheat (c–s–w) rotation; standard chemical inputs, chisel-plowed, no cover crops, no manure or compost
No-till (T2)	Herbaceous annual	Prevailing norm for no-till c–s–w rotation; standard chemical inputs, permanent no-till, no cover crops, no manure or compost
Reduced Input (T3)	Herbaceous annual	Biologically based c–s–w rotation managed to reduce synthetic chemical inputs; chisel-plowed, winter cover crop of red clover or annual rye, no manure or compost
Biologically Based (T4)	Herbaceous annual	Biologically based c–s–w rotation managed without synthetic chemical inputs; chisel-plowed, mechanical weed control, winter cover crop of red clover or annual rye, no manure or compost; certified organic
Perennial Cropping Systems		
Alfalfa (T6)	Herbaceous perennial	5- to 6-year rotation with winter wheat as a 1-year break crop
Poplar (T5)	Woody perennial	Hybrid poplar trees on a ca. 10-year harvest cycle, either replanted or coppiced after harvest
Coniferous Forest (CF)	Woody perennial	Planted conifers periodically thinned
Successional and Reference Communities		
Early Successional (T7)	Herbaceous perennial	Historically tilled cropland abandoned in 1988; unmanaged but for annual spring burn to control woody species
Mown Grassland (never tilled) (T8)	Herbaceous perennial	Cleared woodlot (late 1950s) never tilled, unmanaged but for annual fall mowing to control woody species
Mid-successional (SF)	Herbaceous annual + woody perennial	Historically tilled cropland abandoned ca. 1955; unmanaged, with regrowth in transition to forest
Deciduous Forest (DF)	Woody perennial	Late successional native forest never cleared (two sites) or logged once ca. 1900 (one site); unmanaged

[a]Site codes that have been used throughout the project's history are given in parentheses. Systems T1–T7 are replicated within the LTER main site; others are replicated in the surrounding landscape. For further details, see Robertson and Hamilton (2015, Chapter 1 in this volume).

complexity to more manageable levels is through fractionation of extracted DNA according to its buoyant density, which is determined by the percentage of base pairs that are guanine:cytosine (G + C content). This strategy increases the capacity to detect bacterial species (defined as >97% identity of the 16S rRNA-encoding gene) in clone libraries (Morales et al. 2009). Using clone libraries from G + C-enrichment, Morales et al. (2009) subsequently designed and tested a collection of primers for PCR. They discovered significant variability in nontarget detection and demonstrated that rigorous empirical validation is necessary before new primers can be used to analyze complex communities using either regular (saturation) or quantitative PCR (Morales and Holben 2009).

While the construction of clone libraries has been a primary tool for assessing genetic landscapes in soil, the recent development of next-generation sequencing techniques offers the opportunity to explore the composition of complex microbial communities in much greater detail. These techniques can document not only the dominant members but also the newly revealed "rare biosphere" (Sogin et al. 2006)—those species in very low numbers that would otherwise go undetected. They can also identify other microbes such as Archaea and Fungi that are not detected in surveys of bacterial 16S genes. Application of next-generation sequencing to DNA extracted from KBS LTER soils has provided one of the first in-depth views of how terrestrial microbial communities differ functionally from complex communities in other ecosystems (Fig. 6.2).

Taxonomic and Functional Diversity

Greater taxonomic diversity within soil microbial communities has been revealed as the resolving power of analytical methods has improved—from culture collections to biochemical profiling (fatty acid methyl esters) to DNA sequences (clone libraries, then metagenomes). The remarkable diversity in soil results in large part from a wide variety of microbes present in low numbers. For example, analysis of a 5000-member clone library from the MCSE Conventional system (Table 6.1) led to an estimate of 3500 bacterial species (defined by 97% sequence identity of 16S rRNA genes) (Morales et al. 2009). Yet 80% of the 1700 species actually identified were encountered three times or less, so the projection that the soil contained about 3500 species is almost certainly an underestimate. Similarly, a study of 409 clones of 18S rDNA from basidiomycete fungi yielded a surprising variety of 241 species of basidiomycetes (Lynch and Thorn 2006). While there is not yet a complete description of the microbial diversity in any soil environment, statistically rigorous comparisons of the more abundant members of the community are now possible.

An in-depth assessment of the effect of row-crop agriculture on taxonomic diversity in microbial communities is currently under way across the KBS LTER landscape. Initial results, based on approximately 15,000 sequences of 16S rRNA encoding genes for replicated plots in the MCSE Deciduous Forest and Conventional systems of the MCSE (Table 6.1), revealed approximately 10,000 species in each treatment (Schmidt et al. unpublished). Surprisingly, despite the dramatic differences in these ecosystems, there is not an obvious difference in the phylum level composition of communities. As with other studies of bacterial diversity in soil (Janssen

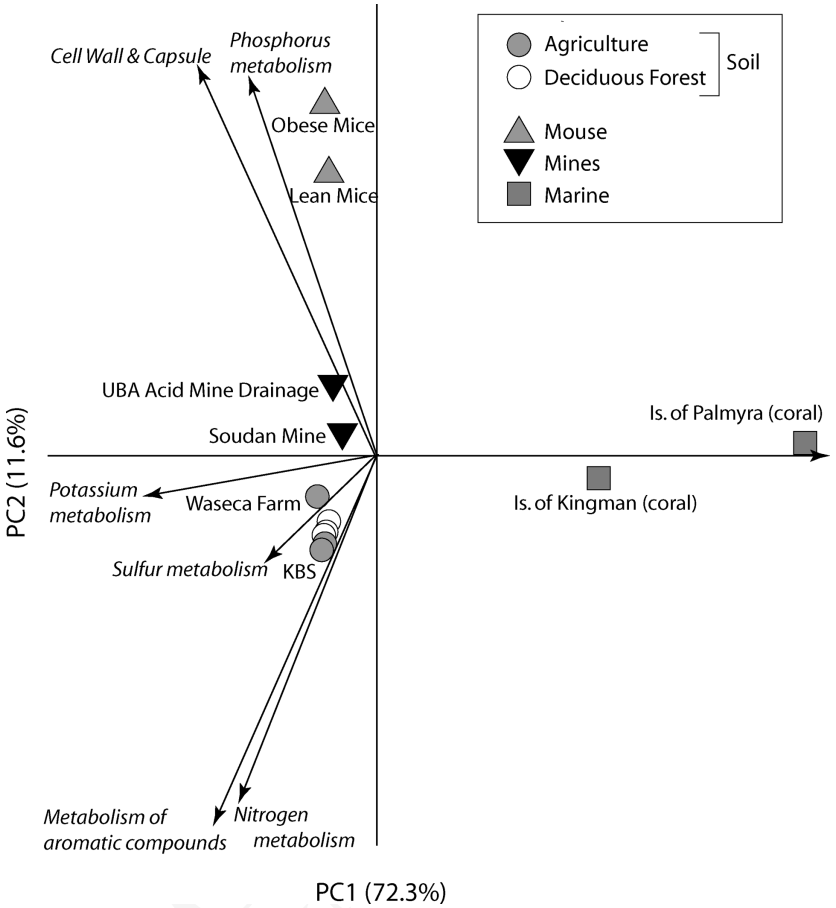

Figure 6.2. Principal components analysis of annotated shotgun metagenomes of microbial communities from soils (KBS LTER and Waseca Farm in Wisconsin), subterranean mines, marine coral reefs, and mouse guts. Axes depict the first two principal components (PCs) and indicate the % of variation explained by each. The functional diversity in soils is clearly distinguished from other biomes, driven in part by genes involved in the nitrogen cycle and the degradation of aromatic compounds. The length of each vector is proportional to the impact of the metabolic pathways in discriminating shotgun metagenomes.

2006), the phyla Proteobacteria and Acidobacteria are most abundant, constituting approximately 75% of the bacteria in soils at KBS LTER. The Proteobacteria are of particular interest because they include the majority of species that catalyze the consumption of CH_4 and production of N_2O—two important greenhouse gases whose emission to the atmosphere is influenced by land management (Gelfand and Robertson 2015, Chapter 12 in this volume). Given the reproducible differences in the fluxes of these gases among MCSE systems (Robertson et al. 2000), the composition of the Proteobacteria community may have an especially important influence on greenhouse gas production in managed landscapes, as discussed in the next section.

The most common bacterial phyla identified by 16S gene sequences (Proteobacteria, Actinobacteria, Cytophagales, Planctomycetes, Verrucomicrobia, and the Acidobacteria) were also investigated by the characterization of RNA extracted from KBS LTER soils. Because the concentration of rRNA in a cell is positively correlated with growth rate, direct probing of RNA provides an estimate of changes in the overall metabolic status of microbes. Changes in rRNA abundance reveal that soil microbial communities are dynamic and capable of responding to seasonal events. The relative abundance of microbial groups is also affected by local environments, so recognizable patterns of community structure can be related to land management (Buckley and Schmidt 2003). It is also worth noting that a low ratio of rRNA to rRNA-encoding genes suggests low overall metabolic activity, leading Jones and Lennon (2010) to propose that dormancy contributes to the maintenance of microbial diversity in lakes. Given the extensive diversity and low growth rates in soil microbes, it is worth considering dormancy as a major mechanism for the preservation of diversity in terrestrial habitats as well (Lennon and Jones 2011).

While detailed taxonomic characterization of communities can be derived by targeting the 16S rRNA encoding genes, analysis of shotgun metagenomes is currently the best approach for identifying the metabolic potential of a community. This provides a comprehensive catalog of DNA sequences in the soil and can indicate, through similarity to known genes, the relative abundance of metabolic functions and pathways that are encoded in that soil. Such data from KBS LTER revealed a previously unknown and systematic artifact in metagenomes (Gomez-Alvarez et al. 2009) that can be identified and removed with an online tool (Teal and Schmidt 2010)—a critical step in making quantitative metagenome comparisons. With this artifact removed, an initial assessment of the functional diversity in KBS LTER soils was made from replicate plots of the MCSE Deciduous Forest and Conventional corn–soybean–wheat systems. The metagenomes were annotated using the MG-RAST tool developed at Argonne National Laboratories (http://metagenomics.nmpdr.org) and compared to metagenomes from other biomes. Based on a principal components analysis of the annotated metagenomes, the functional diversity in soils was clearly distinguished from other biomes (Fig. 6.2). Nitrogen metabolism was one of the major features driving the distinction between the microbial communities in soils from those in other environments.

Environmental Drivers of Diversity

Chemical and physical factors that affect the distribution of microbes in soil are poorly understood. However, the application of molecular techniques is providing the capacity to identify environmental factors that influence microbial distributions in nature. Culture-independent approaches (Pace 2009) are particularly useful for exploring the biology of bacteria from phyla that are poorly represented in culture collections. These include one of the most abundant phyla in soil, the Acidobacteria. In two recent studies (Eichorst et al. 2007, 2011), the distributions of Acidobacteria in relation to physical and chemical characteristics of soil were determined across the MCSE using partial sequencing of cloned 16S rRNA genes. The percentage of subdivision 1 Acidobacteria was correlated with soil pH, being highest in the most

acidic soils (Eichorst et al. 2007, 2011). To determine if this relationship was significant for other terrestrial environments, previously published sequences from a variety of soil environments were similarly analyzed. The combined datasets revealed a significant correlation ($p < 0.004$) between pH and the percentage of Acidobacteria in subdivision 1 (Fig. 6.3A). The potential for plant polymers to influence the distribution of Acidobacteria was assessed in a molecular survey using clone libraries

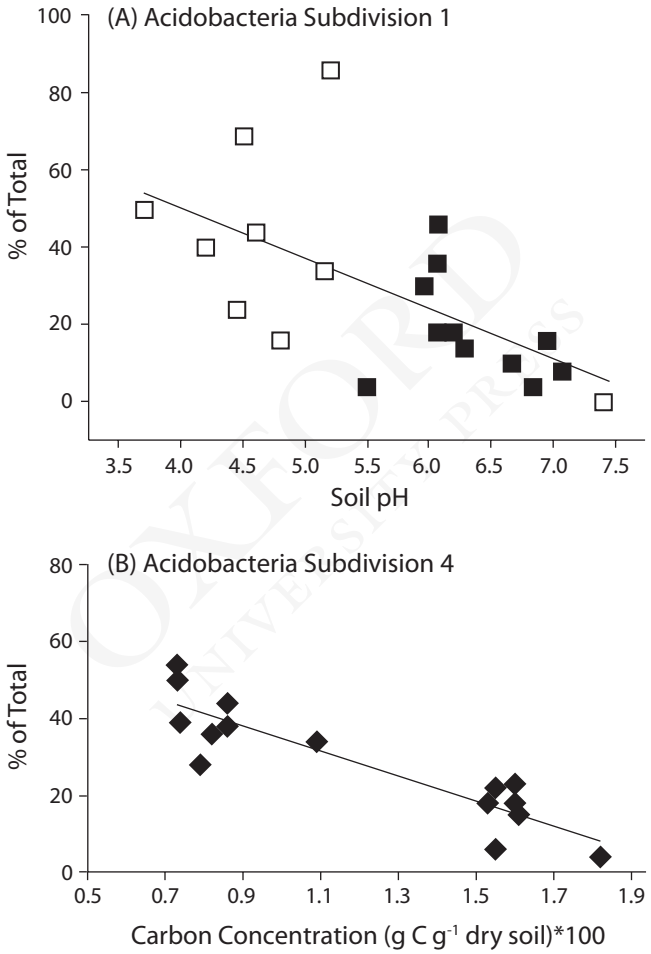

Figure 6.3. The relationship between environmental characteristics and Acidobacteria, one of the most abundant phyla in soils. (A) The proportion of Acidobacteria belonging to Subdivision 1 is related to the pH of the soil environment both at KBS LTER (solid symbols) and in soils worldwide (open symbols). Adapted from Eichorst et al. (2007). (B) The proportion of Acidobacteria in Subdivision 4 correlates with soil carbon in the agricultural and managed grassland systems at the KBS LTER. Adapted from Eichorst et al. (2011). Both panels demonstrate how data from molecular surveys are laying a foundation for understanding factors that influence the distribution of microbes in soil.

from native and agricultural soils at KBS LTER (Eichorst et al. 2011). The distribution of Acidobacteria varied with the C content of soil. In particular, subdivision 4 of Acidobacteria was most abundant in agricultural soils (Fig. 6.3B), which contain less organic C than forested soils (Paul et al. 2015, Chapter 5 in this volume).

Given that plants are the major source of organic C for soil microbes, it is reasonable to expect a coupling between the composition of plant and microbial communities in terrestrial ecosystems. Both physiological phospholipid fatty acid (PLFA) profiles and metabolic Biolog assays of rhizosphere soils obtained from different plant species indicate that plant species composition impacts microbial activity and functional diversity within soil microbial communities (Broughton and Gross 2000). Further, tRFLP fingerprints of DNA extracted from various soil fractions indicate that the highest diversity of microorganisms is associated with rapidly cycling soil C from freshly deposited plant residues (Paul et al. 2015, Chapter 5 in this volume). These studies also reveal that heterogeneity in soil microbial communities results from small-scale spatial heterogeneity in the availability and composition of plant residues, which is consistent with plant biomass as a driver of microbial community structure. Molecular surveys of microbial communities in soil provide a means to identify environmental factors that influence community structure and help set the stage for studies that relate the structure of microbial communities with their functions.

Relating Structure and Function of Microbial Communities

Relationships between Biological Diversity and Function

Ongoing studies at the Cedar Creek LTER (e.g., Tilman et al. 2001) and elsewhere (e.g., Suding et al. 2005) are exploring relationships between plant species diversity and ecosystem function. These studies have demonstrated, for instance, that plant diversity is positively correlated with net primary productivity (Tilman et al. 2001) and C sequestration in soil (Adair et al. 2009). Although the underlying explanation for such diversity–function relationships is vigorously debated, complementary contributions from different plant species appear to be of fundamental importance (Fargione et al. 2007). Given the positive relationship between the diversity of plant species and the magnitude of ecosystem processes, we asked if there might be similar relationships between bacterial communities and the processes they catalyze in KBS LTER ecosystems. Evidence from elsewhere suggests that such a relationship is unlikely for those microbially catalyzed processes, such as biomass decomposition, involving a wide variety of organisms (Schimel 1995, Groffman and Bohlen 1999). However, the number of species in a bacterial community (species richness) may be important for those processes catalyzed by fewer, more specialized species (Cavigelli and Robertson 2000). Given that microbes are responsible for the exchange of greenhouse gases with the atmosphere, including the consumption of atmospheric CH_4 and production of N_2O, the relationships among bacterial diversity and these processes seem especially important to explore.

Methane and Methanotrophs

The decrease in CH_4 consumption that accompanies conversion of forest or grassland to row-crop agriculture is well documented (Smith et al. 2000, Robertson et al. 2000), but had not been tied to the diversity of CH_4-oxidizing microbes (methanotrophs) in these soils. Levine et al. (2011) compared molecular surveys of bacteria in KBS LTER soils to *in-situ* measurements of CH_4 fluxes. These surveys were based on *pmoA*—a gene that codes for one of the subunits of CH_4 monooxygenase, the first enzyme in the pathway of CH_4 oxidation. Across MCSE systems, CH_4 consumption varied by ~7-fold and was greater in soils with a higher number of methanotroph species (Fig. 6.4A). Additionally, the temporal stability of CH_4 oxidation throughout the year increased with methanotroph richness: in different MCSE systems, CH_4 oxidation was less variable (there was less variance among system replicates) in treatments harboring the highest methanotroph richness (Fig. 6.4B). Levine et al. (2011) attributed increased stability to a greater capacity for diverse methanotroph communities to oxidize CH_4 under a broader set of environmental conditions.

The MCSE also provides an opportunity to examine the recovery of CH_4 oxidation and methanotroph diversity following abandonment from agriculture. The rate of CH_4 consumption and the number of methanotroph species both increase following the cessation of agricultural activities. Extrapolating from the current rate at which methanotroph richness and CH_4 consumption are being reestablished, Levine et al. (2011) estimate that approximately 80 years from the time of abandonment will be needed for CH_4 oxidation to return to the levels of native undisturbed soils. This relationship also suggests that managing lands to conserve or restore methanotroph richness (see Gelfand and Robertson 2015, Chapter 12 in this volume) could help mitigate increasing atmospheric concentrations of this potent greenhouse gas.

Bacteria and Nitrous Oxide Production

Nitrous oxide is another potent greenhouse gas of biological origin. Approximately half of contemporary anthropogenic N_2O emitted to the atmosphere is from agricultural soils (IPCC 2007) and its emission is accelerated by N fertilizer use (Millar and Robertson 2015, Chapter 9 in this volume). Nitrous oxide can be produced by both nitrifying and denitrifying bacteria (Robertson and Groffman 2015), but stable isotope tracing indicates that in agricultural soils at KBS LTER, it is made primarily by denitrifiers (Ostrom et al. 2010). During denitrification, microbes use nitrate (NO_3^-) in place of oxygen (O_2) as a terminal electron acceptor for respiratory metabolism. A key enzyme in denitrification is nitrite reductase (*nir*) encoded by either *nirK* or *nirS* genes (Fig. 6.5). Huizinga (2006) found that denitrifiers in KBS LTER soils primarily carry *nirK*, but her molecular surveys did not find any patterns in the distribution of denitrifiers with *nirK* that could be linked to N_2O flux.

However, there may be a pattern in the distribution of denitrifiers that carry a gene that codes for another enzyme involved in N_2O fluxes, N_2O reductase (*nos*). The net production of N_2O from denitrification is dependent not only on the activities of the enzymes nitrite reductase (nir) and nitric oxide reductase

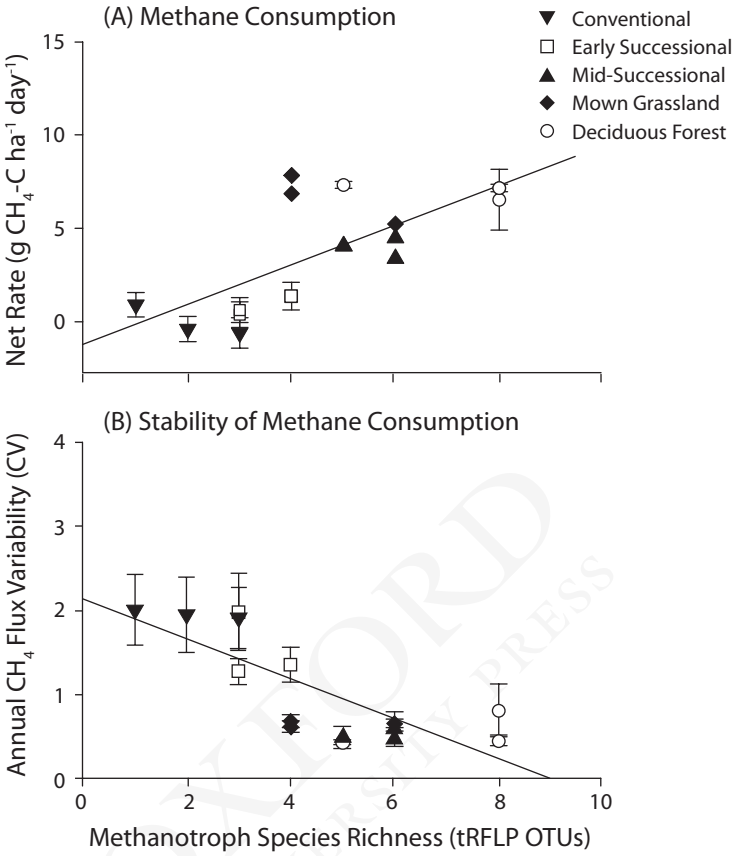

Figure 6.4. The number of bacterial species in soil that oxidize methane (methanotrophs) is directly related to both the rate (A) and stability of methane consumption (B) at KBS LTER. Methanotroph species richness is based on the number of Operational Taxonomic Units (OTUs) defined by tRFLP analysis of methane (CH_4) monooxygenase, the first enzyme in the CH_4 oxidation pathway. Methane fluxes were estimated *in situ* using chambers placed over the soil. The temporal stability of CH_4 consumption rates is expressed as the coefficient of variation (CV) over the annual period of measurements. Redrawn from Levine et al. (2011).

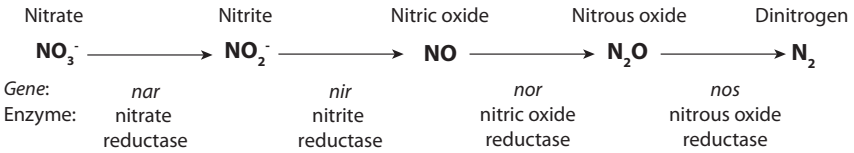

Figure 6.5. The bacterial denitrification pathway, including metabolic intermediates, essential genes and enzymes. A key enzyme in denitrification, producing the first gaseous metabolite, is nitrite reductase (*nir*) encoded by the *nirK* or *nirS* genes. Both genes have been used to survey the diversity of denitrifying bacteria.

(nor) that make N_2O, but also on the activity of N_2O reductase (nos) that reduces N_2O to the innocuous gas N_2 (Fig. 6.5). A tRFLP analysis of cloned *nosZ* genes (Stres et al. 2004) revealed greater diversity in the denitrifying community in the MCSE Conventional system than in the Mown Grassland (never tilled) treatment. Furthermore, even though nirS was less abundant than nirK, quantitative PCR revealed that this agricultural community had a higher ratio of *nirS* genes to *nosZ* genes and so had greater potential to produce N_2O (rather than N_2) from the same amount of NO_3^- (Morales et al. 2010). This represents the intriguing possibility that agricultural land use has selected for a subset of denitrifiers that may accelerate N_2O production.

Another possible functional difference between denitrifying bacteria from agricultural and successional soils at KBS LTER was suggested by a study of N_2O reductases from cultured representatives. Even though these cultured species represent only a small fraction of the denitrifying bacteria, the average O_2-sensitivity of their N_2O reductases varied significantly between the two soils (Cavigelli and Robertson 2001). Next-generation sequencing technologies will enable analysis of all the bacterial *nosZ* genes that code for N_2O reductase in agricultural and successional communities.

Microbial Respiration and Carbon Dioxide Flux

Increasing worldwide demand for agriculture to produce food, fuel, and fiber affects reservoirs of soil organic matter (SOM), which are highly responsive to both changing land use and shifts in climate (Paul et al. 2015, Chapter 5 in this volume; Robertson et al. 2015, Chapter 2 in this volume). Microorganisms play a crucial role in determining the turnover of SOM: they rapidly assimilate and respire labile fractions to CO_2 or transform organic matter to more recalcitrant compounds that are critical to C sequestration and long-term soil productivity. Approximately half of the C lost as CO_2 from soils is due to the metabolism of heterotrophic microbes, with the remainder ascribed primarily to plant root respiration (Hanson et al. 2000). Predicting the fate of microbially processed C (i.e., assimilation into biomass vs. respiratory loss) is thus critical to the development of robust models that accurately predict terrestrial C transformations.

Carbon dioxide emission from soils varies across MCSE systems (Paul et al. 1999), but unlike the specialized functions of CH_4 consumption or N_2O production, no direct relationship exists between CO_2 emission and bacterial richness in KBS LTER soils (Levine et al. 2011). Although the number of bacterial species does not vary dramatically with land management, the composition of the microbial heterotroph communities does. And because C cycling involves a broad diversity of microbes, it may be in the changing composition of heterotrophic microbes that we find explanations for the variation in respiratory CO_2 production. Most biogeochemical models of C cycling assume that microbial communities assimilate C at a fixed rate. For example, there are a number of transformations of C in the widely used CENTURY model (Fig. 6.6; Parton et al. 1987), and it is assumed that in each transformation, 55% of the C consumed by microbes is oxidized to CO_2, with the remainder incorporated into

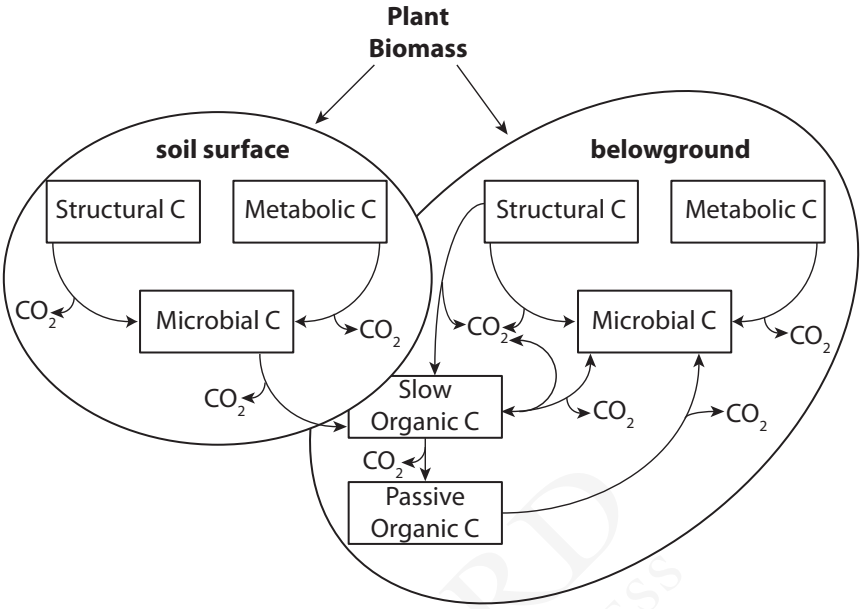

Figure 6.6. The soil subsystem of the Century Model (Parton et al. 1987) includes microbial transformations of plant biomass where assimilated carbon is apportioned between carbon dioxide (CO_2) and microbial biomass both at the soil surface and belowground. In both cases, the model assumes that 55% of the carbon assimilated by microbes is oxidized to CO_2 with the remainder incorporated into cell biomass, which is equivalent to a bacterial growth efficiency of 0.45.

cell biomass. This assumption has profound consequences for the predicted fate of C, including its retention in soil and the potential for global-scale feedbacks.

While the assumption that 55% of the C metabolized by microbes is released as CO_2 may be reasonable for pure cultures provided with substrates and nutrients in optimal proportions, the proportion of carbon respired to CO_2 changes under substrate or nutrient limitation (del Giorgio and Cole 1998). The efficiency by which microbial communities use resources to produce more biomass is not determined by a single enzyme or pathway, as the assumption implies, but rather by their ability to coordinate cellular activity with environmental signals. The net result of this integrated cellular system is a balance between growth and respiration that is characteristic of each microbe. To understand the rate and controls of microbial transformations of C in soil, we need to advance our knowledge of the processes that influence C metabolism in different members of the community. In other words, it is critical that we better understand the fundamental physiological and ecological mechanisms that control the assimilation and fate of C processed by soil microbes. Studies of microbial populations in KBS LTER soils are contributing to this understanding and are the subject of the next section.

Ecology of Soil Microbes

Cultivation and Characterization of Ecologically Relevant Microbes

While molecular surveys are helping to delineate the distribution and temporal dynamics of microbes in the environment, understanding the ecology of microbes requires studying the functions of the entire organism, not just its DNA sequences. Culture-based approaches—in which individual strains or consortia of microbes are isolated and grown—provide the most information about life history traits of microbes that thrive in the soil environment. Life history traits are characteristics that influence the growth, reproduction, or survival of an organism. Few studies of microbes embrace the notion of life histories, perhaps because they do not have obvious counterparts to plant or animal life history traits such as clutch size or parental care. Since natural selection favors individuals who are best able to survive and leave viable progeny regardless of whether they are single- or multicellular, the study of life history traits in microbes should be as useful as it has been in plants and animals and can provide a common currency to compare ecological strategies.

Microbiologists have traditionally used media that contain abundant organic compounds and other nutrients to grow and study microbes. These "rich" media formulations select for copiotrophic microbes—organisms that capitalize on the availability of abundant resources and grow quickly. Cultivating oligotrophs—organisms that thrive in resource-poor environments—requires a different strategy. Key culturing elements are growth media with limited nutrients, atmospheres with less than ambient O_2 concentrations, and sufficient time for slow-growing colonies to form (e.g., Janssen et al. 2002, Stevenson et al. 2004). This approach has enabled many never or rarely cultured bacteria to be grown in the lab, including representatives of the abundant but poorly characterized phyla Acidobacteria and Verrucomicrobia.

Acidobacteria are present in soils worldwide (Janssen 2006) and abundant in many KBS LTER soils, as noted earlier, yet few cultivated representatives of this cosmopolitan phylogenetic group exist. Presumably, they are unable to compete with the fast-growing bacteria that prosper on the rich media commonly used in microbiology laboratories. To learn more about the metabolic properties and potential ecological roles of members of this phylum, Eichorst et al. (2007) isolated Acidobacteria strains from KBS LTER soils using incubation conditions and media designed to mimic their natural environment. Cultivation conditions included low concentrations of nutrients, plant polymers as sole C and energy sources, and extended (3 to 4-week) incubation periods. Altered incubation atmospheres with decreased concentrations of O_2 and elevated levels of CO_2 resulted in a slightly acidified medium with a pH similar to *in-situ* measurements of soil pH at KBS LTER.

When plant polymers were used as a C and energy source, the diversity of Acidobacteria growing in culture increased relative to those cultured on simple sugars (Eichorst et al. 2011). All the cultivated strains of Acidobacteria contained either one or two copies of the 16S ribosomal RNA-encoding gene that, along with

a relatively slow doubling time (10–15 hours at ~23ºC), suggests an oligotrophic lifestyle. Several of these strains produce a carotenoid pigment that is thought to protect against oxidative stress when exposed to ambient O_2 concentrations. The need for its production in ambient atmospheres suggests a sensitivity to O_2 that would explain optimal growth rates under reduced O_2 atmospheres. The optimal growth of these Acidobacteria under slightly acidic pH and low O_2 concentrations, conditions commonly observed in many soils, is consistent with their widespread distribution and abundance in soils.

The strains in one collection from KBS LTER are sufficiently similar, but distinct enough from previously named Acidobacteria, to warrant creation of a new genus, *Terriglobus. T. roseus*—the pigment-producing strain—has been defined as the type species of the genus (Eichorst et al. 2007). Studies are under way to explore the role of the extensive extracellular polysaccharide produced by *Terriglobus* strains (Fig. 6.7) in the formation of soil aggregates and to document the capacity of the strains to degrade complex plant polymers.

The characterization of microbes in culture is a time-intensive endeavor that has faded in popularity as access to molecular approaches has widened. As a result, half of the 70+ known bacterial phyla have been identified solely from rRNA gene sequences (Pace 2009), making it difficult to draw inferences about their genetics, physiology, or ecology. The study of single cells (including deriving their complete genome sequences) offers an intriguing new possibility for advancing our knowledge of microbial physiology and the lifestyle trade-offs that underlie the distribution and activities of bacteria in nature.

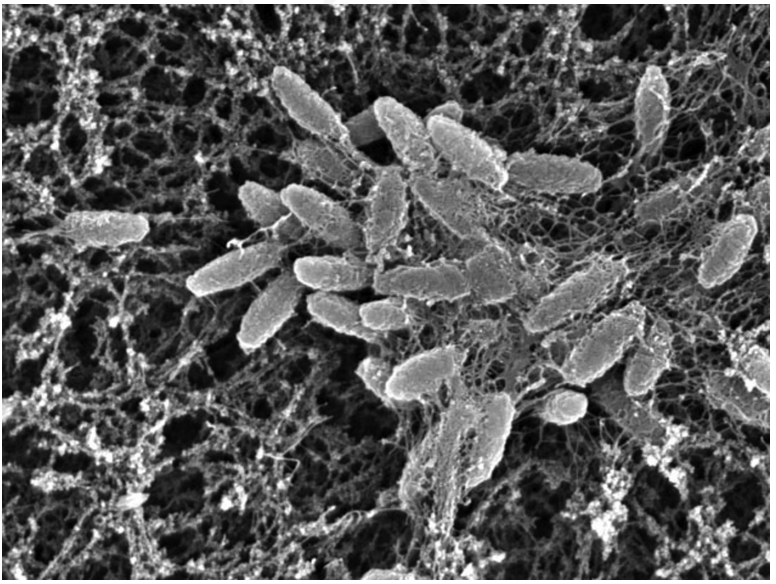

Figure 6.7. A scanning electron micrograph of an isolate of the phylum Acidobacterium revealing an extensive extracellular polysaccharide matrix that may be important in the formation of soil aggregates. From Eichorst et al. (2007).

Trade-Offs between Power and Efficiency

Little is known about the selective pressures that shape microbial communities in soil and, in particular, about fluctuations in environmental conditions that might trigger the growth of distinct microbes. Ecological strategies of microbes are commonly described on a spectrum of alternative responses to nutrient supply. At one extreme are oligotrophs that are most competitive when organic resources are scarce. At the other end are copiotrophs that thrive when nutrients are suddenly abundant. Klappenbach et al. (2000) isolated a collection of bacteria from KBS LTER soils to develop and test a model positing that these opposing strategies involve a trade-off between "power" in the rapid response to an influx of resources and "efficiency" in the use of scarce resources. A copiotroph's capacity for rapid growth in response to an influx of resources may be the selective pressure driving the maintenance of multiple copies of rRNA operons in its genome. The cost of maintaining this capacity for rapid response and growth would be detrimental to efficiency in nutrient-poor environments, so oligotrophs may be under selective pressure to minimize the number of their rRNA-encoding genes.

Considerable evidence has accumulated from KBS LTER isolates to support a conceptual model in which the number of rRNA operons encoded by a bacterium, which ranges from 1 to 15 (Lee et al. 2009), is indicative of where an organism lies on a spectrum of ecological strategies between oligotrophy (few rRNA operons) and copiotrophy (many rRNA operons; Fig. 6.8) (Klappenbach et al. 2000,

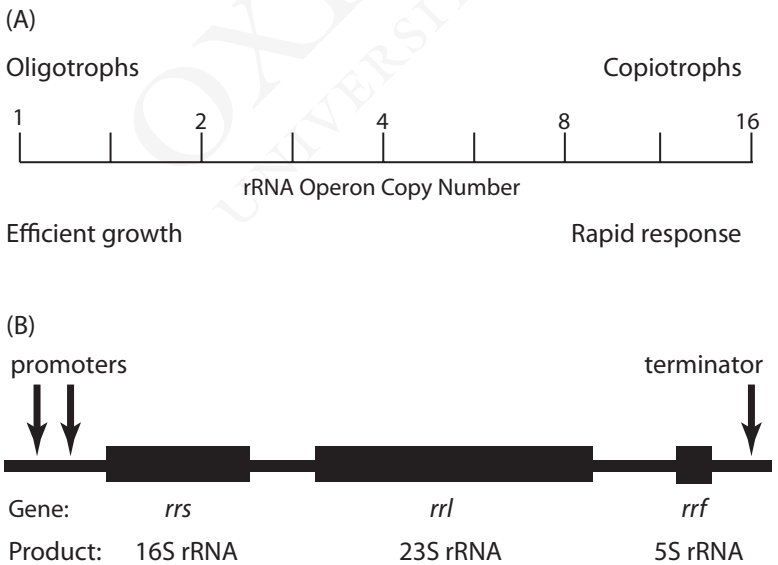

Figure 6.8. A) The number of rRNA operons encoded by a bacterium ranges from one to 15 and is proposed to be indicative of where an organism lies on a spectrum of ecological strategies between oligotrophy (few rRNA operons) and copiotrophy (many rRNA operons). B) The typical structure of an rRNA operon in bacteria.

Stevenson 2000, Dethlefsen and Schmidt 2007). For instance, bacteria that form visible colonies rapidly (<48 hours) upon exposure to nutritionally complex media contain an average of 5.5 copies of the SSU rRNA encoding gene, whereas bacteria that respond slowly contained an average of 1.4 copies (Klappenbach et al. 2000). These findings reveal phenotypic effects associated with the number of rRNA operons that underlie the distribution and abundance of bacterial populations in soil.

In addition to determining the number of rRNA operons, we have examined the efficiency with which the protein-synthesizing machinery operates in microbes. This machinery typically makes up more than half of a microbe's dry weight and consumes a majority of the cell's energy during rapid growth. The translation of mRNA into protein has been studied extensively in model organisms; nevertheless, while the translational apparatus is qualitatively similar in structure and function across all known life, little is known about variations between organisms in translational performance. The macromolecular composition of phylogenetically diverse oligotrophic and copiotrophic soil bacteria suggests that differences in translational power (normalized rate of protein synthesis) and associated maintenance costs help explain the fundamental trade-offs between the rapid growth in copiotrophs vs. the efficient use of resources in oligotrophs (Dethlefsen and Schmidt 2007). Analysis of bacterial genomes, in particular the patterns of codon usage in protein-coding genes, supports this model (Dethlefsen and Schmidt 2005).

When this model is applied to denitrifiers, the positive relationship between the number of rRNA-coding genes and maximum growth rate becomes apparent (Fig. 6.9). It suggests that the influence of land management on the composition of denitrifiers results from disturbance favoring fast-responding (copiotrophic) denitrifiers.

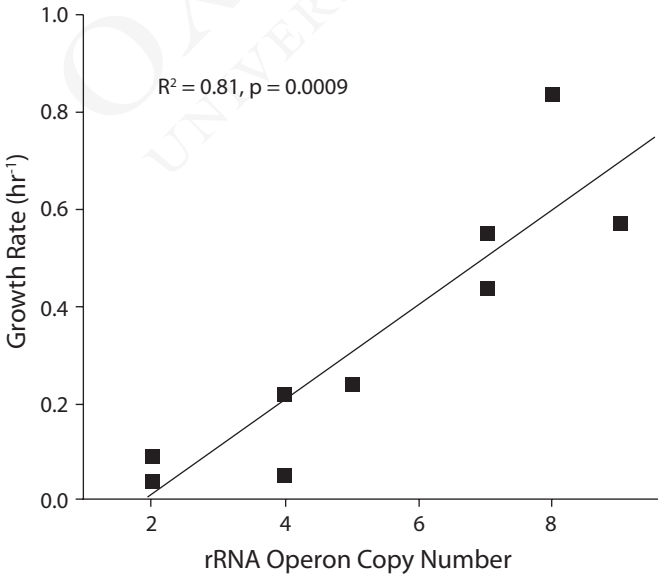

Figure 6.9. The relationship between growth rate and the number of rRNA operon copies in sequenced genomes of cultured denitrifying bacteria.

Based on this body of research, one might expect that bacteria that make efficient use of resources are favored in habitats where the concentration of resources is perpetually low. A minimum concentration of resources is required to provide maintenance energy, which is the sum of energy expenditures that are not directed toward growth, for example, maintaining a charged membrane or motility. At very low resource concentrations, the majority of assimilated C is therefore respired for maintenance purposes. Under these conditions, most C resources will be oxidized to CO_2 to provide energy rather than biomass. Consequently, bacterial growth efficiency should be low in resource-limited environments. When C and nutrient resources are more abundant, a greater proportion of assimilated C can be allocated to biomass production, making growth efficiency higher. Studies to measure the efficiency of C utilization in different MCSE systems are under way and will be a reasonable test of this model's ability to accurately predict the composition of bacterial communities in soil.

Summary

Microbial communities in soil are critical to the productivity and health of the biosphere and, in particular, to the cycling of C and N that underpin agricultural productivity and climate change. However, we are only just beginning to understand the composition and function of these complex soil microbial assemblages. The replicated array of managed and unmanaged ecosystems at KBS LTER has been an invaluable resource for studying the effects of land use on soil microbial communities and their impact on the biosphere. Molecular surveys reveal a tremendous taxonomic and functional diversity of microbes in KBS LTER soils—a diversity so large that even state-of-the-art, large-scale DNA sequencing methods have yet to reveal its full extent. Despite the challenges associated with measuring this enormous diversity, there are clear patterns in the distribution of microbes across the KBS LTER landscape, and the relationships between the structure of microbial communities and ecosystem-level processes such as C and N cycling are being revealed. One of the most striking relationships uncovered to date is the positive correlation between methanotroph diversity and CH_4 consumption.

As we continue gathering fundamental information about the structure of different bacterial communities, we must ask more questions about the connections between their varying compositions and the major ecological functions that are altered by agronomic management. It is particularly important that we begin to address cause and effect: Is a function modified directly by a change in the bacterial community, or are they independently influenced by land use?

The answers to these questions will affect future microbial research at KBS LTER in two key ways. The first will be to accelerate evolution of the current, largely observational approach to a more experimental strategy that manipulates variables *in-situ* to test hypotheses about the role of the bacterial community. These experiments will then lead to the second phase of research: developing and evaluating practices to maximize agricultural productivity while reducing environmental impact. If these practical applications involve direct manipulation of bacterial

communities, we will have some major new challenges to overcome. Perhaps the most pressing, in light of discoveries that microbial communities may take decades to recover their diversity, will be to find ways to accelerate changes in the composition—and hence the function—of microbial communities in agricultural soils. The availability of large-scale replicated experimental systems reflecting different land management practices will ensure that the KBS LTER continues to play a pivotal role in both fundamental and applied aspects of microbial ecology research.

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