Microbial diversity and function in soil: from genes to ecosystems

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Soils sustain an immense diversity of microbes, which, to a large extent, remains unexplored. A range of novel methods, most of which are based on rRNA and rDNA analyses, have uncovered part of the soil microbial diversity. The next step in the era of microbial ecology is to extract genomic, evolutionary and functional information from bacterial artificial chromosome libraries of the soil community genomes (the metagenome). Sophisticated analyses that apply molecular phylogenetics, DNA microarrays, functional genomics and *in situ* activity measurements will provide huge amounts of new data, potentially increasing our understanding of the structure and function of soil microbial ecosystems, and the interactions that occur within them. This review summarizes the recent progress in studies of soil microbial communities with focus on novel methods and approaches that provide new insight into the relationship between phylogenetic and functional diversity.

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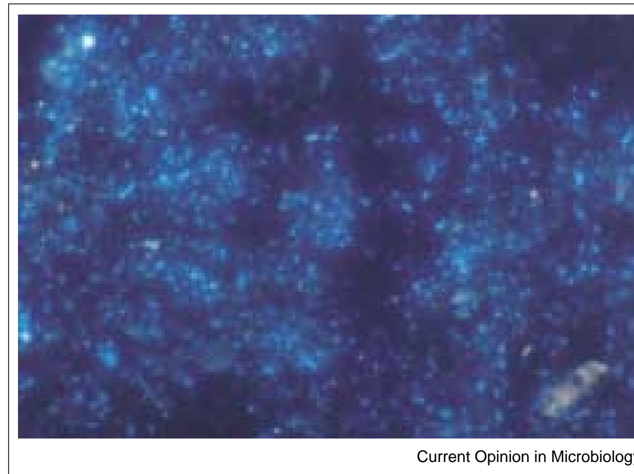
Abbreviations

BAC	bacterial artificial chromosome
BrdU	5-bromo-2′-deoxyuridine
DGGE	denaturant gradient gel electrophoresis
FISH	fluorescent *in situ* hybridization
GC	guanine + cytosine
PCR	polymerase chain reaction
PLFA	phospholipid fatty acid

Introduction

Microbial diversity in soil ecosystems exceeds, by far, that of eukaryotic organisms. One gram of soil may harbor up to 10 billion microorganisms of possibly thousands of different species [1•]. As less than 1% of the microorganisms observed under the microscope (Figure 1) is cultivated and characterized, soil ecosystems are, to a large extent, uncharted. Microbial diversity describes complexity and variability at different levels of biological organization. It encompasses genetic variability within taxons (species), and the number (richness) and relative abundance (evenness) of taxons and functional groups (guilds) in communities. Important aspects of diversity at the ecosystem level are the range of processes, complexity of interactions, and number of trophic levels. Thus, measures of microbial diversity should include multiple methods integrating holistic measures at the total community level and partial approaches targeting structural or functional subsets [2,3]. Diversity may also be considered to be the amount and distribution of information, which is directly applicable to the total genetic diversity or complexity in a community.

Figure 1



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Epifluorescence micrography of soil microorganisms stained with 4′,6-diamidino-2-phenylindole (DAPI). The total bacterial count was 4.2×10^{10} cells gram^{-1} soil (dry weight) by fluorescent microscopy, and 4.2×10^{6} colony-forming units gram^{-1} soil (dry weight) by plating.

The genetic complexity or genome size of microbial community genomes can be assessed by re-association of community DNA. Such broad-scale analysis has revealed [4,5] that the community genome size equals the size of 6000–10 000 *Escherichia coli* genomes in unperturbed organic soils, and 350–1500 genomes in arable or heavy-metal-polluted soils. These values may be conservative, as genomes representing rare and unrecovered microorganisms are probably not included in the analysis. In contrast, the genomic complexity recovered by culturing methods was less than 40 genomes. The total genomic complexity denotes the confines of diversity in terms of genetic information present and provides information about the overall (potential) taxonomic and functional variability at the community level. Because this represents an average diversity value, no detailed information about taxon and functional diversity at lower levels of biological organization is obtained. A number of methods with higher resolving power have been developed for characterization of microbial communities that includes both cultured and uncultured microorganisms. Most of them are based on analyses of ribosomal RNA genes (rDNA). They have uncovered part of the microbial diversity in soil, yielding sequences from many novel phylogenetic lineages. Measures of microbial patterns (fingerprinting) and taxonomic variability have been coupled with analysis of functional genes and activity measurements. Such investigations aim to reveal and understand the relationship

between structural and functional diversity in soil microbial ecosystems.

In this review, we summarize the recent progress in studies of soil microbial communities. We present an overview of novel molecular methods for studying all the microorganisms in soil, including those uncultured, and approaches for obtaining information from community genome analysis. The review highlights some recent studies that link phylogenetic groups to function and may contribute to exciting new insight into the relationship between community composition and function. Finally, we discuss the effect of soil physical and chemical conditions on microbial diversity and the importance of functional diversity for soil ecosystem stability and resilience.

Methods to describe diversity

The indications of the vast diversity of uncultured life in soil have stimulated development of methods for culture-independent study of microbial communities. These methods have employed a combination of nucleic acid characterization and microscopy. Over the past two decades, molecular methods, especially 16S rRNA gene sequencing, have become very popular to help identify unknown bacteria [6,7]. In turn, this has led to community analysis using total community DNA extracted from the environment. Broad-scale analysis of community DNA, using techniques such as DNA re-association, provides information about the total genetic diversity of a given bacterial community [4]. A shift in guanine + cytosine (GC) content can be used to detect changes in microbial community structure, but does not tell us anything about the other diversity parameters, which are richness, evenness and composition. Polymerase chain reaction (PCR)-based fingerprinting techniques give a higher resolution and provide information about changes in the whole community structure. These fingerprinting techniques, such as phospholipid fatty acid (PLFA) analysis, denaturant gradient gel electrophoresis (DGGE), amplified rDNA restriction analysis (ARDRA), terminal restriction fragment length polymorphism (T-RFLP) and ribosomal intergenic spacer analysis (RISA), provide information on the species composition, and can be used to compare common species present in samples. However, there are some problems and biases in the PCR amplification step and, therefore, these methods cannot be used as definite indicators of species richness. Despite the PCR problems, a combination of the techniques mentioned above can reveal a great deal about the microbial community diversity. Recently, a method based on integron-targeting PCR assays has been developed to recover genes from environmental DNA without the necessity to know their sequences [8••]. A comprehensive description and discussion of the potential and limitations of the methods are given in the overviews of Kozdrój and van Elsas [2] and Johnsen *et al.* [3].

Single genome versus community genome analysis

Although the complete sequences of more than 60 microbial genomes have provided critical insights into the individual microbial cell, the study of the collective genomes in a community — the 'metagenome' — promises to unlock the secrets of community life. Approximately 60 microbial genomes have been completely sequenced, and hundreds more are in the process of being sequenced. Complete microbial genome sequencing has made it possible to identify and characterize all genes present in a species. This means that we get information about novel metabolic pathways, gene regulatory elements, genes of unknown function, and genes for pathogenesis, virulence and drug resistance [9]. This information also provides insights into the evolution of genes and species. Furthermore, the understanding of species diversity based on comparative genomics has led to a new epoch for biological investigations, using techniques such as microarray analysis [10]. The next step in the era of microbial genomics is to extract functional and evolutionary information from these large datasets and to apply genomics technology to relevant questions in microbial ecology [11••]. Thus, new technology will have an important impact on the understanding of soil microbial ecology. Recently, soil community genomes have been cloned using a bacterial artificial chromosome (BAC) vector. Together, all the genomes of soil microbes can be considered to be one large soil microbial community genome — the metagenome [12••]. Combined comparative analyses of core housekeeping genes like rDNA and functional genes may provide information on both phylogenetic diversity and the potential functional diversity of microbial communities. There are several ways in which environmental microbiology will benefit from microbial genomics. Comparative genomic analysis and microarray technology may be used to determine patterns of gene expression, and to detect novel metabolic pathways. This offers a quick method to access functional information for genes of unknown function. Such information is very useful in functional diversity studies to track highly expressed genes and genes critical in biogeochemical pathways. It is also important to understand how microbial cells are regulated under varying conditions such as carbon supply, energy source and electron acceptor availability, thereby obtaining information on microbial community response to environmental changes. The new tools may also increase our understanding of the process of genome evolution and the factors that regulate diversity.

Novel methods linking phylogenetic groups to their activities and function

Perhaps the greatest challenge facing microbiology today is the problem of linking phylogeny and function. The methods based on 16S rRNA analysis provide extensive information about the taxa present in an environment, although they provide little insight into the functional role of each phylogenetic group. Metagenomic analysis provides some functional information through genomic sequence and expression of traits, but other methods are required to link specific functions with the group responsible for them. The concomitant quantitative and comparative analyses of expressed rRNA genes and genes for key enzymes in

relation to environmental factors can be used to obtain information about the phylogeny and ecology of functional bacterial groups responsible for processes like denitrification, nitrification and methane oxidation.

Environmental functional gene arrays could be constructed using oligonucleotid probes to target gene expression or genes coding for key enzymes in all biogeochemical cycles. These can be used for specific detection of gene expression in the environment. Investigations in which attempts have been made to associate specific microorganism or microbial groups with their ecological function have been performed on functional groups of bacteria, using genes for key enzymes such as nitrate reductase [13], ammonia monooxygenase [14] and methane monooxygenase [15]. Another strategy to look for specific functional groups is to use microsensor measurements of chemical profiles in relation to the distribution of different bacterial taxa to identify environmental conditions favored by particular bacteria [16,17•]. It remains, however, a major challenge in soil microbial ecology to ascribe microbial processes to specific microorganisms. Before the microorganisms are cultivated, their ecological functions must be elucidated through culture-independent characterization that links phylogenetic and functional genetic analyses to metabolic activities in soil.

Microradioautography, using the uptake of specific radiolabeled substrates by individual cells, can be used to detect and quantify the active populations utilizing this substrate. In order to link the uptake of a specific substrate with the phylogenetic identity of a specific bacterial cell, microautoradiography has been used in combination with fluorescent *in situ* hybridization (FISH) of microbial cells using fluorescent, group-specific phylogenetic probes (targeting rRNA) and fluorescence microscopy. Studies in which microautoradiography and FISH are combined in natural and engineered environments are rapidly increasing [14,16,18,19]. The application of stable isotope probing (SIP) and phospholipid fatty acid (PLFA) labeling to determine functionally active components of microbial communities is also becoming increasingly used in microbial ecology studies [20,21••]. In SIP, ^{13}C-DNA produced during the growth of metabolically distinct microbial groups on a ^{13}C-enriched carbon source can be resolved from ^{12}C-DNA by density gradient centrifugation. The DNA can then be fractionated, and each fraction can be further analyzed taxonomically and functionally by gene probing and functional analysis. This method, therefore, offers a powerful technique for identifying microorganisms that are actively involved in specific metabolic processes. Another method in which activity can be linked to phylogenetic information is the incorporation of 5-bromo-2′-deoxyuridine (BrdU) into DNA to detect metabolically active community members in response to substrate or other stimuli. BrdU can be added to a culture of microbial cells, and metabolically active members of a community will then incorporate BrdU into their DNA [22,23]. Yin *et al.* [24•] recently used this technique to determine the extent of functional redundancy along a soil reclamation gradient in a highly contaminated mine spoil. Different carbon amendments were used and significant differences were detected in the microbial populations that had incorporated BrdU in their DNA, indicating a bacterial functional redundancy within this microbial community. A problem using this technique is that selective stimulation of bacteria that are not actually active before substrate amendment may occur.

The effect of soil structure and environmental conditions on microbial diversity

Soil is a very complex system that comprises a variety of microhabitats with different physicochemical gradients and discontinuous environmental conditions. Microorganisms adapt to microhabitats and live together in consortia with more or less sharp boundaries, interacting with each other and with other parts of the soil biota. A number of investigations emphasize the impact of soil structure and spatial isolation on microbial diversity and community structure [11••,25,26]. Analysis of the spatial distribution of bacteria at microhabitat levels showed that, in soils subjected to different fertilization treatments, more than 80% of the bacteria were located in micropores of stable soil micro-aggregates (2–20 μm) [25]. Such microhabitats offer the most favorable conditions for microbial growth with respect to water and substrate availability, gas diffusion and protection against predation. Particle size had a higher impact on microbial diversity and community structure than did factors like bulk pH and the type and amount of organic compound input [26]. Results showed that the microbial diversity in fractions with small soil particles was higher than that in fractions with large soil particles, and that most of the soil microbial community was particle-specific. A high diversity of bacteria belonging to the *Holophaga*/*Acidobacterium* division and *Prosthecobacter* were present in small particles (silt and clay). Large particles (sand) harbored few members of the *Holophaga*/*Acidobacterium* division, and were dominated by bacteria belonging to the α-proteobacteria. Other investigations indicate that the type and amount of available organic substrates strongly influence the abundance of microbial groups and their functional diversity in soil ecosystems [27,28]. Smit *et al.* [29•] used their own data and data from recent literature on the distribution of 16S rDNA sequences among five main bacterial divisions to search for relationships between the abundance of microbial groups and soil nutritional status. Their results suggested that soil with a high content of readily available nutrients showed positive selection for α- and γ-proteobacteria, this being indicative of r-selection, which is selection for bacteria with potentially high growth rates. In low-nutrient soil or soil with a high content of recalcitrant substrates, the percentage of *Acidobacterium* increased, this being indicative of k-selection, which is selection for bacteria with lower growth potential but higher capability to compete for substrates. The ratio between the number of proteobacteria and *Acidobacterium* was suggested to be indicative of the nutritional status of

soils. The ratio was low in oligotrophic soil, medium in agricultural soil with low organic input, and high in agricultural soil with high organic input [29•,30].

Competitive interactions are thought to be a key factor controlling microbial community structure and diversity [11••]. Soil structure and water regime influence competitive interactions by causing spatial isolation within communities. Soil with high spatial isolation showed high microbial diversity, whereas soil with lower spatial isolation showed much lower diversity and was dominated by a few microorganisms. The high diversity in soil with high spatial isolation may also have been caused by a higher heterogeneity of carbon resources in this soil and, consequently, a higher niche variation. Soil bacteria are subjected to considerable seasonal fluctuations in environmental conditions such as temperature, water content and nutrient availability. An important issue to elucidate is how environmental changes and seasonal variations influence qualitative variation in community composition. Smit *et al.* [29•] found that bacterial biomass did not change significantly during the seasons, but that both culturing and molecular fingerprinting techniques demonstrate significant variations in community composition. Culturing techniques show that the proportion of fast-growing bacteria was lowest in winter and highest in summer, and that the highest species richness was found in spring and autumn. This seemed to correlate with enhanced microbial activities and nutrient input from fertilization (spring) and plant debris after harvest (autumn). Molecular fingerprinting indicated that the community, to a large extent, consisted of stable dominant populations, but that less-abundant populations, as revealed by low-intensity bands on a DGGE gel, showed distinct seasonal differences. Culture-dependent methods and molecular methods reveal strikingly different microbial populations in soil. It has been demonstrated that Gram-positive bacteria with high GC content [29•] and Gram-positive bacteria with low GC content [31] were abundant among isolates, but had very low abundance or were nearly absent among clones.

The microbial diversity of soil and the interactions between different trophic levels were elucidated in a simple ecosystem model in which primary producers (plants) and decomposers (microbes) were linked through cycling of a limiting nutrient factor [32] for the primary producers. The model shows that the efficiency of nutrient recycling from organic compounds to decomposers is a key parameter that controls ecosystem processes (productivity and biomass of the functional groups). The model predicts that microbial diversity has a positive effect on nutrient cycling efficiency, and contributes to increased ecosystem processes. One major effect that microbial diversity can have on ecosystem processes is to ensure that all organic compounds are recycled. Organic compound diversity may have a negative or neutral effect on a stable ecosystem. Most soils are exposed to fluctuating environmental conditions and, in fluctuating ecosystems and a long-term perspective, the high diversity of organic substrate is likely to have a positive effect on the function. The relationship between microbial diversity and soil processes may not be linear because many processes are carried out by a consortium of microorganisms. In interacting consortia, small linear changes in microbial diversity may result in non-linear changes in process.

Functional diversity of soil microorganisms

Functional diversity is an aspect of the overall microbial diversity in soil, and encompasses a range of activities. The relationship between microbial diversity and function in soil is largely unknown, but biodiversity has been assumed to influence ecosystem stability, productivity and resilience towards stress and disturbance. The catabolic response profile (CRP) [33], which is a measure of short-term substrate-induced respiration, has been used to calculate the diversity (range and evenness) of catabolic functions expressed *in situ*. Catabolic diversity has been used to investigate the effect of stress and disturbance on the diversity and resilience of soil microbial communities. When matching soils from different long-term-managed environments (crop and pasture) were subjected to stress and disturbance, it was demonstrated that microbial communities with low catabolic evenness (crop fields) were less resistant to stress and disturbance than were microbial communities with high catabolic evenness (pasture). Other soil properties might also have contributed to the resistance. After a major disturbance (such as landslips, volcanic eruptions or retreat of glaciers), marked changes in functional diversity (catabolic evenness) in developing soil ecosystems have been demonstrated [34]. The functional diversity was initially low in non-vegetated (underdeveloped) sites, but as vegetation was established, the diversity rapidly increased and, in older successions, the catabolic evenness declined. These diversity patterns broadly paralleled patterns in plant and fungal diversity. The relationships between microbial community composition and physiological capacity [35] were elucidated using PLFA profiles and two functional measures, namely substrate utilization capacities (BIOLOG) and enzyme activities. Broad-scale community composition (PFLA profiles) correlated well with specific enzyme activities, especially enzymes responsible for initial degradation of macromolecules such as lignocellulose. The lack of correlation between BIOLOG and PLFA assays may be due to the fact that BIOLOG selects for a minor part of the microbial community utilizing readily available simple substrates, whereas PLFA includes the total community. Macromolecule degradation and demineralization enzyme activities may measure functions that immediately respond to litter input and are more clearly related to changes in the active part of microbial communities.

Conclusions and future directions

Recent advances in soil community analysis using molecular methods agree with the earlier data on total genetic diversity by indicating an enormous microbial diversity in soil. Soil diversity exceeds that of aquatic environments,

and is a great resource for biotechnological exploration of novel organisms, products and processes. Novel methods and approaches enable us to explore this vast diversity. Studies of sequence information from organisms in soil microhabitats and their gene expression under different conditions will provide guidelines for designing new and improved culturing methods that resemble their natural niches. New tools in bioinformatics and statistical analysis enable us to handle the huge amount of data obtained through multidimensional studies that combine growth-independent molecular analyses with analyses of microbial growth, activity and physiology, and integrate measures of environmental parameters. Such polyphasic studies integrate different aspects of microbial diversity and provide a more complete picture of microbial diversity and a deeper understanding of the interactions in soil microbial ecosystems. Studies of microbial sequences, comparative genomics and microarray technology will improve our understanding of the structure/function relationships and the effects of abiotic and biotic factors on soil microbial communities. It is conceivable that the research field dealing with the interaction of genomes with the environment will be an important topic in the future.

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