# A STUDY OF METHODS FOR ASSESSING SOIL MICROBIAL DIVERSITY

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## ABSTRACT

*Soil microorganisms play a major role in the decomposition of organic matter, regulation of the release of mineral nutrients, and nutrient cycling. The variety of soil microbes has been the focus of a lot of recent study. Understanding the diversity of this complex microbial community in the soil environment is a challenging task. Because of this, it's vital to acquire and comprehend the appropriate methods for studying soil microbial diversity. An overview of the primary research techniques and the idea of soil microbial diversity is given in this publication. The use of molecular and biochemical methods in this domain is then evaluated, including pros and cons. A survey of pertinent recent research is given, along with ideas for further examining soil microbial diversity.*

***Key Words:*** *Ecological Methods, Microbial Communities, Molecular Techniques*

## INTRODUCTION

Soil bacteria comprise a significant fraction of Earth's biodiversity and are vital to biogeochemistry cycles and ecosystem function. Because they are necessary for the decomposition of organic matter, the release of mineral nutrients, and the cycling of nutrients, soil microorganisms have an influence on the amounts of nutrients in the soil, its chemical and physical properties, and ultimately its primary output. The diversity of microorganisms in soil may also be impacted by a broad range of human activities, such as farming, urbanization, pollution, and the use of pesticides. Soil microbial diversity is thus essential for both preserving environmental management and evaluating soil quality. Understanding the enormous diversity of the microbial community in the soil environment has proved to be a challenging endeavor. This is the outcome of methodological limitations as well as a lack of taxonomic expertise. Many studies have lately focused on the diversity of soil microorganisms in an attempt to get a better understanding of the soil black box. Applying and comprehending the appropriate methods for studying soil microbial diversity is so crucial.

## THE SOIL MICROBIAL DIVERSITY CONCEPT

According to Solbrig, the diversity of soil microorganisms is influenced by species variety, genetic diversity, and ecological diversification. Richness, totality, evenness, and dispersion of the species are the constituents of species diversity. However, prokaryotes and asexual organisms struggle to conform to the traditional definition of species, which was created for higher plants and animals. Measures of microbiological diversity have historically included the number of individuals assigned to different taxa and their distribution within them. As a consequence, groupings of microorganisms together have been referred to as functional groups or guilds. Microbial diversity studies have often explored the relative diversities of

communities along a gradient of stress, disturbance, or other biotic or abiotic changes.

## MEASURING SOIL MICROBIAL DIVERSITY

Because of methodological and taxonomic limitations, studying species and genetic diversity has proved to be difficult. The main problem with many traditional physiological and biochemical methods has been their dependence on phenotypic expression analysis and/or microbial culture. Even if a microorganism has shown metabolic activity, many of them are not suitable for cultivation in a laboratory. Moreover, poor gene expression under test conditions has often resulted in negative results when using biochemical test kits. There are presently only two approaches that have been shown to be successful in resolving this problem: signature lipid biomarkers like phospholipid fatty acids and nucleic acid technologies. Most of these methods used principal component analysis or canonical variate analysis to create and analyze multivariate data, or fingerprints. Therefore, methods for measuring microbial diversity in soils have been split into two categories: molecular-based techniques and biochemical-based techniques. These methods are based on the phenotypic and genetic variation of soil microbial communities.

## BIOCHEMICAL-BASED TECHNIQUES

### Plate counts

A common, culture-dependent method that is rapid, inexpensive, and accurate in determining the population's active, heterotrophic component is the plate count. Although 5000 bacterial species have been recorded, only 0.1% to 1% of the soil bacterial community may be cultured with standard laboratory procedures. Limitations include things like temperature, pH, and light for growth. Furthermore, there are an estimated 1.5 million species of fungi worldwide, in contrast to bacteria, although many of these are uncultivable in laboratories using current methods.

### Community level physiological profiles and sole carbon source utilization patterns

The CLPP method is one technique to examine the physiological diversity present in soils. These profiles demonstrate how different carbon substrates may be used by the microbial communities. It is believed that differences in usage patterns correspond to differences in the primary members of the active microbial community. For instance, the metabolic profile of a microbe is generated by the BIOLOG system using 95 different carbon sources. Though the analysis and interpretation of such data are sometimes difficult, the approach has gained favor since it is simple, employs an automated measurement equipment, and offers a plethora of information regarding key functional aspects of microbial communities.

There are further drawbacks. The microbial metabolic profile is not significantly affected by soil fungi or slow-growing bacteria; the BIOLOG systems only assess the metabolic variety of culturable bacteria. Furthermore, the BIOLOG single C-source test plates include significant concentrations of both carbon sources and TTC. The fact that the plates are buffered at a pH that is almost

neutral—apH that is very different from the pH of certain acidic or alkaline soils—may also provide some challenges for some microbes that have effectively adapted to acidic or alkaline soils. While determining the makeup of the soil microbial community, there are disadvantages associated with a number of these characteristics.

**Fatty acid methyl ester (FAME) and phospholipid fatty acid (PLFA) analyses**

The fatty acid methyl ester method provides information on the makeup of the microbial community based on fatty acid groupings. The major taxonomic groups within a community may be identified based on the unique fatty acid content that constitutes a rather stable portion of the cell biomass. Therefore, changes to the fatty acid profile would also have an impact on the makeup of the community and the amount of microbial biomass.

According to Zelles, the PLFA method has been used to elucidate the many strategies that microorganisms employ to adapt to changed environmental circumstances across a wide range of soil kinds, management approaches, climatic causes, and disturbances. In order to streamline evaluation procedures and improve the assessment of soil microbial communities, Zelles recommended classifying PLFAs into a number of chemically unique subgroups. This way, only subgroups thought to be engaged in key activities would be investigated.

**MOLECULAR-BASED TECHNIQUES**

Microbial ecologists have been using molecular methods more and more in the last several years to look into the distribution and activity of microorganisms in the environment. Many methods have been developed to identify bacteria in soils, such as DNA cloning and sequencing, fluorescent in situ hybridization, polymer chain reaction based technologies, and nucleic acid hybridization, among others.

**Nucleic acid hybridization and fluorescent in situ hybridization (FISH)**

In molecular bacterial ecology, nucleic acid hybridization with specific probes is a crucial qualitative method. These hybridization techniques may be used to in situ hybridization inside cells or to extract DNA and RNA. Using the FISH method, the spatial distribution of bacteria in biofilms has also been successfully investigated. However, the traditional FISH method has a number of sensitivity limitations that prevent it from identifying cells with low ribosome concentration. Because low ribosome concentration per cell was often linked with poor physiological activity, slow-growing or starving cells might go unnoticed. To overcome this limitation, FISH created a tyramime signal amplification method that allowed for the investigation of slow-growing microorganisms. Nucleic acid hybridization, or FISH, also has the disadvantage of being insensitive in the absence of high copy number sequences.

**Guanine plus cytosine (G + C) content**

Diversity in soil bacteria may be studied by examining changes in the DNA's guanine plus cytosine makeup. This procedure is based on the knowledge that microorganisms differ in their G + C content and that taxonomically related species differ by only 3% to 5%. This method provides a coarse degree of resolution since different taxonomic groups could have the same mol

percentage range of G + C. Melting curves provide microbial community profiles that are indicative of the overall genetic diversity. Although this method is considered low resolution, it may nevertheless be utilized to demonstrate broad changes in the structure of microbial communities, especially when diversity is low. One advantage of this approach is that it does not suffer from PCR bias when it comes to DNA extraction, quantification, or identifying rare members of the microbial communities. This makes it possible to identify and examine some of the less prevalent bacteria in the community that fractionation would have prevented PCR from picking up on. But it requires a large amount of DNA.

**PCR- based techniques**

In diversification research, polymer chain reaction-based molecular techniques have been used to overcome the limitations of culture-based procedures. This detection method has several uses in ecological and environmental research. Environmental DNA that has been directly collected may be used as a PCR template. Prokaryote identification and study have made extensive use of PCR, which targets the 16s rDNA. However, the use of internal transcribed spacer regions and 18s rDNA in the investigation of fungal populations is growing. Target DNA is amplified using specific or universal primers, and the resulting products are sorted using different techniques.

After that, specific community information may be extracted from the amplified PCR product via primer hybridization. Both temperature gradient agents and denaturing agents may be used in gradient gel electrophoresis. DGGE and TGGE are two similar methods for studying microbial diversity. In theory, DGGE may be used to segregate DNA that varies by just one base pair. TGGE uses the same concept as DGGE, except temperature is employed as the gradient rather than chemical denaturants. These techniques were first developed to identify point mutations in DNA sequences. Muyzer lists the following advantages of DGGE and TGGE: they can monitor changes in microbial populations; they are rapid, precise, reproducible, and fairly priced; and they can analyze several samples at once.

**Random amplified polymorphic DNA (RAPD)**

Randomly amplified polymorphic DNA polymerase chain reaction, or RAPD-PCR, is a rapid and simple technique to identify pertinent genetic markers and evaluate organismal genetic diversity at various taxonomic levels. This technique includes staining the amplified products with ethidium bromide via electrophoresis, amplification of the PCR product using random primers, and examination of the gel images utilizing imaging instruments. RAPD bands are then graded as binary presence or absence characteristics in order to generate a matrix of RAPD phenotypes. One way to quantify genetic diversity is to look at the polymorphic band percentage. Compared to other molecular markers, this method has the advantages of being quick, simple to apply, and rich in polymorphic DNA. However, because to the short primers, there isn't much repetition.

**Amplified fragment length polymorphism (AFLP)**

To rapidly check for genetic variation, AFLPs, PCR-based markers, are used. One of the main characteristics of AFLP-PCR is its capacity to examine many unique DNA regions spread randomly across the genome at the same time. In essence, AFLP methods may be used in PCR amplification to detect polymorphisms of genomic restriction regions. Vos et al. state that AFLP markers have shown potential for assessing genetic variability among individuals, groups, and independently emerging lineages, such as species. The incapacity of AFLP-PCR to identify homologous markers, or alleles, is its main flaw. Because of this, this method is less useful for studies like heterozygosity studies that need precise allelic state assignment. But since AFLPs can be used to swiftly and efficiently make precise, high-resolution markings, they are emerging as a powerful tool for ecologists and evolutionary biologists.

The terms restriction fragment length polymorphism (RFLP) and terminal restriction fragment length polymorphism (T-RFLP) are interchangeable. RFLP is a culture-independent technique for assessing the diversity of the microbial population using a DNA sequencer.

To acquire relevant data, it is essential to ensure both the reproducibility of the RFLP banding pattern and the completion of digestion. rDNA that has been amplified by PCR is usually digested using base pairs cutting restriction enzyme. When it comes to communities analysis, agarose or non-denaturing polyacrylamide gel electrophoresis are used to find various lengths. The fluorescence tag on one PCR primer is the sole distinction between T-RFLP and RFLP. T-RFLP fingerprints are widely used to track shifts in microbial diversity across time and place. There are two automated techniques: ribosomal intergenic spacer analysis and intergenic spacer analysis. Automated ribosomal intergenic spacer analysis is a well-liked DNA-based community fingerprinting method that is highly reliable and high-resolution for finding differences across large fungal communities. Based on its length polymorphism, the ribosomal intergenic spacer region—which is located between the 16s and 23s rRNA genes—provides the foundation for RISA. The technique has been effectively used to fingerprint mixed populations and simple communities as well as to describe, categorize, and type strains. Even among closely related strains, there are differences in the size and nucleotide sequence of the non-coding ribosomal internal spacer region.

Ribosomal intergenic spacer analysis uses differences in the length of internal transcribed spacer portions of rRNA genes to rapidly classify samples into functional taxonomic groupings. It is possible for individuals from different species to have the same ITS fragment size. Even though ARISA uses a different taxonomic resolution than species level, it nonetheless offers a trustworthy representation of community composition. Changes in the species composition are therefore correlated with changes in two OTU assemblages.

**Single-strand conformational polymorphism (SSCP)**

Separating PCR-amplified rRNA and rDNA molecules has allowed SSCP to examine the structure and dynamics of

microbial communities with effectiveness. The method relies on changes in DNA sequence to achieve differential intramolecular folding of single-stranded DNA. Resolution is made possible by a shift in the electrophoresis mobility of single-stranded PCR amplifications caused by DNA secondary structure. Pure cultures of soil microorganisms and community fingerprints of wild rhizospheric microbial communities from different plants may be recognized by SSCP. SSCP analysis ought to be easier than DGGE or TGGE as it doesn't need primers when using GC-clamp or gradient gel equipment. A single bacterial species may provide several bands in addition to PCR bias because of multiple operons or single-stranded PCR conformations. An further method of identifying community members is to enhance the growth of microorganisms using certain enrichments. Investigations involving guilds or functional groups benefit from this approach.

**FUTURE PERSPECTIVES**

Research on soil microbiological diversity is hampered by taxonomic and methodological limitations. Thus, efforts have been made by soil microbiologists to enhance molecular approaches. Research on soil microbial diversity may benefit from the use of recently developed bioinformatics and microarray technologies in the biological sciences (McLachlan et al., 2004; Mount, 2004). Although they have limitations, molecular methods may provide information on species that are not cultivable. The optimal strategy for investigating soil microbial diversity is hard to determine.

To get the most information, soil microbial diversity should be investigated utilizing a variety of assays with different endpoints and resolutions. Measuring total microbial diversity is difficult in modern microbial ecology, particularly for species that are hard to cultivate, erratic, or in low abundance. Little is known about the temporal and geographical variability of soil microorganisms because of their intrinsic diversity and spatial dispersion. A more representative sampling regime might be provided by minimizing sampling variability via the use of power analyses to establish sample size and geostatistical analysis to describe the geographical distribution of subsurface microorganisms.

It is unclear how plant microorganisms and soil interact, how structural diversity and function of below- and above-ground ecosystems work, and how microbial diversity and function in soil interact. To fully understand the complexity of biological, chemical, and physical aspects, ecologists, pedologists, microbiologists, and botanists should work together (Dobrovol'skaya et al., 2001).

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