

# A Brief Note on Measurement and Interactions of Microbial Diversity

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## Editorial

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## ABOUT THE STUDY

For many years, soil ecosystem modellers assumed that the amount of soil microbial biomass was the primary driver of decomposition and nutrient cycles, with substrate availability, temperature, and moisture serving as the primary governing variables. Microbiologists began assessing approaches capable of detecting linkages between changes in community composition and ecosystem processes after discovering the great diversity and dynamic nature of the soil microbiome. It is impossible to include all available microbiological methods in a single chapter due to the tremendous proliferation of microbiological methods, particularly nucleic acid-based assays, over the last few decades. Instead, here are brief descriptions of a few of these technologies that can be used to investigate the soil microbiome.

### Soil health

In terms of physical and chemical qualities, soils are quite diverse. Soil scientists have been attempting to identify soil types in terms that represent their ability to function in both natural and managed environments for a long time. In this context, the phrase "soil quality" was coined, but more lately, the term "soil health" has gained popularity. The ability of a soil to work within its surroundings, support plant and animal productivity, and maintains or improves water and air quality is referred to as soil quality. Soil health expands on this concept by recognising that, like humans, soils work best when they are healthy.

Soil microbial communities are extremely important for soil health; they must be morphologically and phenotypically diverse in order to successfully occupy the multiple micro-niches found in soil, as well as process and divide the wide range of substrate inputs that occur throughout the year. Organic matter content, aggregate stability, water infiltration, crusting, erosion, and acidity are all important indicators of soil health. Microbial activity has a significant impact on these parameters, both directly and indirectly.

Changes in these soil qualities inevitably alter the niches filled by soil microorganisms, resulting in changes in the microbial community's content, composition, and activity. Soil microbiologists have devised methodologies to acquire insight into community composition and dynamics at both the taxonomic and functional levels as part of the endeavour to define soil health. One major goal is to determine which agricultural methods promote and maintain soil health.

### Enzymes

Microorganisms create intracellular and extracellular enzymes that are ultimately responsible for metabolism. For processes including denitrification, nitrification, cellulose breakdown, and a variety of other C, N, and nutrient transformations, assays have been devised to quantify potential enzyme activity. A soil sample is added to slurry containing a buffered solution with supplementary C and  $\text{NO}_3$  in the case of denitrification.

The headspace is depleted of oxygen, and acetylene and  $\text{N}_2$  are introduced. Because acetylene prevents the final step of denitrification, a buildup of  $\text{N}_2\text{O}$  occurs. The denitrification rate is then calculated using the accumulated  $\text{N}_2\text{O}$ . Colorimetric or fluorometric assays, such as the phosphatase assay described, are commonly used to measure the activity of enzymes involved in the breakdown of C molecules. A colour or light signal is created when the targeted substrate is broken down in these circumstances. The aggregation of those signals can be converted into the substrate's response rate and conversion potential [1-5].

### Cellular fatty acids

Lipids are necessary components of all living things. Phospholipids, glycolipids, lipopolysaccharides, and lipoproteins are all types of lipids found in microbes. Microbial lipids contain a wide range of fatty acids, which are utilised to identify bacteria. In general, soil microbiologists investigate the fatty acid profiles of soils using two rather simple techniques. Fatty acids are freed from soil lipids using one method, which involves heating a soil sample at  $100^\circ\text{C}$  in a solution of NaOH and methanol. The fatty acids are heated under hot acidic conditions to form their methyl ester derivatives (FAME), which are then extracted into an organic solvent such as hexane and evaluated using gas chromatography [6,7].

Lipids can also be extracted from soil using a mixture of water, chloroform, and methanol, and then separated into several classes (neutral, glycolipids, and phospholipids). The Phospholipid Fraction (PLFA) is usually the one that gets the most attention, and it's studied following hydrolysis and methylation in alkaline circumstances. The PLFAs are extracted and evaluated using an organic solvent and gas chromatography. In most cases, 20-50 fatty acids may be recognised and distinguished. Because various PLFA markers (and other fatty acids) are diagnostic for specific microbial groups, these data can be used to discriminate between microbial communities in different soils or in the same soil under different management settings [8-10].

This examination can also be done quantitatively and utilised to determine the amount of microbial biomass present. Because both bacteria and fungus have ester-linked phospholipids, the relative abundance of certain fungal and bacterial PLFAs can be utilised to calculate the fungal: bacterial ratio. Common whole-soil lipid-based analyses have two drawbacks: they can be used to identify broad groupings like Gram-positive bacteria and

mycorrhiza, but they have limited taxonomic resolution and cannot distinguish between species, and Archaea have ether linkages and are not detected using the same extraction protocol.

### DNA sequencing

Many branches of biology have been transformed by new molecular technology and computing resources, perhaps none more so than microbial ecology. Increased capacity to sequence millions of lengths of DNA simultaneously has essentially overcome the limits of culture and microscopy in capturing the diversity of the soil microbial community. For example, multiple studies have used PCR to target portions of the 16S rRNA genes, then used bioinformatics to match those sequences against publically available databases for sequence identification and diversity metric calculation.

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