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# Biochemistry and Metagenomic Techniques in Restored Soils with Organic Amendments

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**Abstract**

The practice of open-pit mining produces unfavorable conditions in the ecosystem. The damage soil and vegetable due to mining activities produce growing erosion problems and soil fertility. The use of different organic amendments and the study of soil microbial consortia could favor the soil restoration. In this review, we will examine the development of methodologies from traditional tools based on biochemical properties and enzymatic activities to last generation methodologies based on metagenomics for the study of microbial communities in restored soils with organic amendments.

**Keywords:** Biochemistry techniques; Metagenomic techniques; Restored soils; Organic amendments

**Abbrevations:** USW: Urban Solid Waste; PCR: Polymerase Chain Reaction; DGGE: Denaturant Gradient Gel Electrophoresis; PLFAs: Profile of Acid Grades Such as Phospholipids; PGP: Plant Growth Promoting Bacteria

## Introduction

Opencast mining causes serious environmental problems. This practice leads to the loss of the entire soil and plant cover, considerably increasing erosion problems and loss of soil fertility [1]. All of this, the limitations in physical-chemical soil properties accompanied by low microbial activity hinder plant regeneration activity [2]. Therefore, a solution to this problem could be the use of organic amendments, such as sewage sludge, compost of plant remains and urban solid waste (USW) or manure to restore degraded soils [3,4], burned forest soils [3] and technosols [5].

Some authors have suggested the need to use organic amendments for soil restoration [6], as they optimize the price [1], provide organic matter and restore absent microbiota that is vital for soil structural formation and contribute to plant establishment and the transformation of organic matter [7,8]. Many authors confirm the benefit of using sewage sludge to improve soil properties such as the water retention capacity, organic matter content and nutrients such as nitrogen and phosphorus [2,9] which stimulate microbial activity [10], making it a high value amendment [11]. Likewise, compost from plants debris contains a large amount of organic carbon and nutrients such as nitrogen, calcium, magnesium and potassium [10,12], as well pruning debris act as a mulch layer favoring water retention and reducing evapotranspiration [13]. In addition, this type of compost is free of pollutants, so it is catalogued as a high-quality amendment [14]. Shi et al. [15] observed that manure promotes microbial activity. Edaphic microorganisms play a crucial role in the biogeochemical cycles of the soil [16], contributing through their enzymatic activity to the recycling of nutrients, necessary to make them accessible to plants and other microbes [17].

Soil microorganisms are a key factor in soil quality and evolution [18]. Organic amendments favor the development of soil microorganisms and their activity [1], and therefore, the performance of restoration [19]. Hence, it is necessary to have a deep knowledge and a better understanding of the dynamics of soil microbial communities in restoration, in order to ensure the success of organic amendments [20].

This review will analyze the evolution of methodologies for the study of microbial communities in soils restored by application of organic amendments from traditional tools based on biochemical properties and enzymatic activities to last generation methodologies based on metagenomics.

## Biochemistry Techniques

The traditional techniques of soil microbial activity analysis applied in restored soils are based on studying the general metabolic activity. They group different biochemical properties such as soil basal respiration, used to determine global microbial activity or soil enzymatic activity involved in the carbon, nitrogen and phosphorus cycles [4,21,22]. The profile of acid grades such as phospholipids (PLFAs) [21] used to evaluate changes

in community structure is also studied. In order to know the microbial components, bacteria and fungi, molecular tools supported by the Polymerase Chain Reaction (PCR) combined with denaturant gradient gel electrophoresis (DGGE), as well as cloning and sequencing of rRNA 16S [4,23,24]. These molecular techniques have been used for the study of microbial communities in restored soils with sludge and USW compost from limestone quarries [1].

The study of the soil enzymatic activity is very important, given that they report about the activity of microbial communities and respond faster than the physico-chemical soil properties to changes in the environment [25,26] such as the application of organic amendments. Several authors have observed increases in the activities involved in the biochemical processes of nutrient cycles [16,27], such as the carbon cycle (dehydrogenase, cellulase, α-glucosidase, β-glucosidase, invertase, ...) [17,26,28-30], the nitrogen cycle (catalase, urease, protease-casein, protease-BAA, ...) [25,29-32] and phosphorus cycle (phosphatase, phosphomonoesterase, phosphosdiesterase ) [15,17,26,33]. For example, catalase activity is related to soil respiration, microbial activity and organic matter content [31]. The enzyme urease is a potential factor to determining the nitrogen content [32]. Soil phosphatase catalyses the hydrolysis of ester-phosphate bonds [15], producing the release of assimilable phosphate by plants and microorganisms [33], while cellulase activity contributes to the formation and release of humus, improving soil fertility and accelerating biomineralization processes [28]. Nitrification potential is another biochemical technique used to analyze the soil nitrogen cycle. Topac Sagban [34] studied the potential of nitrification in soils treated with sewage sludge and observed that urease activity, arginine ammonification and heterotrophic soil bacteria influenced by this parameter. Other traditional techniques were based on the isolation of microorganisms from serial dilutions [35] with specific protocols such as Beringer [36] to isolate nitrogen-fixing strains. However, in these biochemical techniques, it is not known qualitatively and quantitatively the involvement of the organisms involved, as well as the specific communities that develop in the restored soils.

## Metagenomics Study

The existence of beneficial rhizospheric bacteria for vegetation, known as Plant Growth Promoting Bacteria (PGP), are necessary for successful restoration, since they are capable of fixing nitrogen, solubilizing phosphates, producing plant growth stimulants, acting as biocontrol agents or inducing resistance against pathogens [37]. Knowing whether such microbial communities proliferate in restored soils with organic amendments could be an excellent indicator of the evolution of restoration treatments and thus contribute to optimizing these treatments. Recently, new next-generation methodologies based on mass sequencing have emerged to amplify rRNA 16S. These techniques guarantee very detailed molecular information, both taxonomic and genomic (metagenomic) of soil microbial communities and relative abundance [18]. At present, we find two technologies:

a. The sequencing of amplicons obtained from the PCR of genes, such as ribosomal RNA, which is used as a phylogenetic marker.

b. The "shot-gun" with which we can sequence DNA or RNA (metatranscriptomic) directly, allowing knowledge of the genes expressed by prokaryotes, including those of rRNA.

These methodologies based on metagenomics arose in the 90's, allowing to know the DNA of organisms present in a microbial community, for this reason, it is the tool used to identify genes of interest involved in biological processes of the soil [38].

Restoring soils with organic amendments produces a change in existing microbial communities, as new communities inhabiting these amendments are introduced [39,40]. The application of metagenomic studies could favor the study of the dynamics and evolution of microbial communities in restored soils, being able to combine with "shot-gun" technique allowing at the same time to study what functions they present and how these microbial communities are modified.

Schmalenberger et al. [24] observed 12 years after restoring a soil with compost and gypsum that the microbiota was similar to semi-natural soils, abounding microorganisms *Acidobacteriaceae, Nitrosomonadaceae, Caulobacteraceae* and *Anaplasmataceae*. In contrast, communities of *Chitinophagaceae, Beijerinckiaceae, Xanthomonadaceae* and *Acetobacteraceae* proliferated in untreated soils related to environments with low organic matter content, high salinity and pH [41-43]. Demonstrating that untreated soils did not go towards a development similar to natural soils in the medium to long term. Bastida et al. [44] studied, 25 years after of restoration, the changes produced in microbial communities between restored soils with organic amendments and unrestored soils. The restored plots exhibited microorganisms more specialized in the degradation of plants remains. Bacteroidetes, Planctomycetes and Alpha-proteobacteria being the most abundant, and the Ascomycota division the most represented fungi. The presence of labile plant substrates increasing the presence of labile plant substrates that influence the structure of the edaphic microbial community. In this sense, combining these metagenomic techniques with "shot-gun" sequencing would allow to make it possible to know the microorganisms beneficial to plants, which favor the cycling of nutrients from the soil and the growth of plant cover.

Other authors have been interested in the taxonomy-function relationship. Shikata et al. [45] temporarily sampled with metagenomics in restored soils with bovine compost. They observed that initially *Herbivorax saccincola* and bacteria belonging to the *Pelotomaculum genus* were more abundant, while at the end of the study the *Tepidanaerobacter* and *Tepidimicrobium genera* increased, concluding that there was a progressive change in the dynamics of soil microbial communities. In early stages, the microbial community exposed a preference for sugars and over time other communities showing a preference for other organic acids and alcohols, demonstrating that non-cellulolytic strains


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helped accelerate the efficient degradation of lignocellulose of *H. saccincola*. Similarly, Guo et al. [46] conducted a study using the 16S rRNA gene sequence and shot-gun metagenomic sequencing to compare the taxonomic and functional communities of the soil microbiome. In this study, a natural revegetation was carried out and changes in the microbial community of the soils and how it affected the vegetation were studied chronologically for 30 years. The authors concluded that these communities favored nutrient cycling and soil fertility, influencing plant taxonomic diversity and cover, thus demonstrating significant changes in taxonomic diversity and edaphic microbial function in arid and semi-arid ecosystems.

In other studies, conducted on soils of restored mines with household waste from the mines, the proliferation of prokaryotes involved in the main soils, functions favoring fertility was studied, using a marker gene and sequencing the metagenome by "shot-gun" [47].

The combination of metagenomic techniques can also be a successful way to study contaminated soils, both terrestrial and marine. Cabral et al. [48] characterized the microbial potential and the degradative activity of aromatic compounds in mangrove sediment samples using metagenomic and metatranscriptomic approaches. They found functions involved in the degradation of aromatic compounds.

It should be noted that not only can an individual omic be used, but that, for example, Malla et al. [49] undertook a review in which they addressed the integrating role of multi-omic approaches. They discuss how these orientations help to understand and explore the structural and functional aspects of soil microbial consortia. In short, the knowledge of soil microorganisms and their functions could help to select the most optimal microorganisms to increase the fertility and guarantee the success in restoration. Therefore, in the future, inoculum of selected microorganisms could be used in order to accelerate the process of development and edaphic evolution.

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