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Metagenomic Analysis of Soil Microbial Communities under Regenerative and Conventional Agriculture

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Abstract
Soil microbial communities are essential for maintaining ecosystem stability, nutrient cycling, agricultural productivity, and environmental sustainability. Metagenomic analysis has emerged as a powerful approach for investigating the diversity, structure, and functional potential of soil microorganisms under different agricultural systems. The present review evaluates the microbial dynamics associated with regenerative and conventional agriculture using advanced metagenomic tools including 16S rRNA sequencing and shotgun metagenomics. Findings from recent studies indicate that regenerative agricultural practices such as cover cropping, crop rotation, reduced tillage, and organic amendments significantly enhance microbial diversity, beneficial taxa abundance, nutrient cycling efficiency, and soil ecological resilience. In contrast, conventional agriculture characterized by intensive tillage and excessive chemical inputs is associated with reduced microbial diversity, increased pathogen prevalence, and enrichment of antimicrobial resistance genes. These microbial alterations have important implications for soil health, food safety, environmental quality, and public health within the One Health framework. The review highlights the importance of integrating metagenomic approaches into sustainable agricultural management and public health policy development.

Keywords: *Metagenomics, Soil Microbial Communities, Regenerative Agriculture, Conventional Agriculture, Soil Health, Microbial Diversity, One Health, Sustainable Agriculture.*

Highlights

- Regenerative agriculture significantly improves soil microbial diversity and ecosystem functionality.
- Conventional agricultural practices contribute to microbial imbalance and antimicrobial resistance gene enrichment.
- Metagenomic tools enable comprehensive characterization of soil microbial communities and functional genes.
- Soil microbiome health plays a crucial role in sustainable agriculture, environmental protection, and public health.

Introduction

Soil microbial communities constitute one of the most complex and functionally significant biological systems on Earth, governing essential ecosystem services such as nutrient cycling, organic matter decomposition, soil structure formation, and suppression of plant and human pathogens (1, 2). With advances in molecular biology, particularly metagenomics, it has become possible to comprehensively characterize these microbial communities without the limitations of culture-dependent methods. Metagenomic analysis enables the direct extraction and sequencing of genetic material from environmental samples, providing insights into microbial diversity, taxonomic composition, and functional potential (3, 4). This has significantly enhanced our understanding of how agricultural practices influence soil microbiomes and their downstream impacts on environmental and public health. Agricultural intensification through conventional farming practices—characterized by monoculture systems, excessive tillage, and heavy reliance on synthetic fertilizers and pesticides—has led to substantial alterations in soil microbial diversity and ecological balance (5, 6). Metagenomic studies have consistently reported a decline in microbial richness and evenness in conventionally managed soils, along with a reduction in beneficial microbial taxa involved in nutrient cycling and plant growth promotion (7, 8). Furthermore, these systems often show an increased prevalence of pathogenic microorganisms and antimicrobial resistance genes, raising serious concerns regarding food safety, environmental contamination, and public health risks (9, 10). In contrast, regenerative agriculture has emerged as a sustainable land management approach aimed at restoring soil health and enhancing biodiversity through practices such as reduced tillage, crop rotation, cover cropping, and organic amendments. Metagenomic analyses of regenerative agricultural systems reveal significantly higher microbial diversity, increased abundance of beneficial taxa such as Actinobacteria, Proteobacteria, and mycorrhizal fungi, and enhanced functional gene profiles associated with carbon sequestration, nitrogen fixation, and disease suppression (11, 12). These microbial improvements contribute not only to soil fertility and crop productivity but also to ecosystem resilience and climate change mitigation.Metagenomic approaches, including 16S rRNA gene sequencing and whole-genome shotgun sequencing, have become essential tools for comparing soil microbial communities across different agricultural systems. These techniques allow for high-resolution identification of microbial taxa and functional genes, enabling researchers to detect subtle yet significant shifts in microbial ecology driven by land management practices (13, 14). Additionally, functional metagenomics provides insights into metabolic pathways, resistance gene dissemination, and microbial interactions that are critical for understanding ecosystem health and sustainability. The implications of soil microbial dynamics extend beyond agriculture into the domain of public health. Soil serves as a reservoir for both beneficial microbes and potential pathogens, as well as antimicrobial resistance genes that can be transferred across environmental, animal, and human interfaces (15, 16). Disruptions in soil microbial communities, particularly under conventional agricultural systems, can facilitate the emergence and spread of zoonotic diseases, contamination of food and water resources, and increased exposure to harmful microbial agents. Conversely, regenerative practices that promote microbial diversity and ecological balance can help mitigate these risks and support the principles of the One Health approach, which integrates human, animal, and environmental health. Despite growing evidence supporting the benefits of regenerative agriculture, there remains a need for comprehensive synthesis of metagenomic findings to better understand the extent and mechanisms through which different agricultural practices influence soil microbiomes. This review focuses on the application of metagenomic analysis to evaluate soil microbial communities under regenerative and conventional agriculture, highlighting key differences in microbial diversity, functional potential, and public health implications. By integrating findings from recent studies, this work aims to contribute to the development of sustainable agricultural strategies that promote ecosystem health and safeguard public health.

Methodology

Study Design and Literature Collection

The present review was conducted using a comprehensive and systematic evaluation of published scientific literature related to metagenomic analysis of soil microbial communities under regenerative and conventional agricultural systems. Relevant peer-reviewed articles were retrieved from electronic databases including PubMed, Scopus, Web of Science, and Google Scholar using keywords such as "soil metagenomics," "regenerative agriculture microbiome," "conventional farming soil microbes," "16S rRNA sequencing," "shotgun metagenomics," and "soil microbial diversity" (17, 18). Studies published between 2005 and 2025 were preferentially included to capture recent advancements in next-generation sequencing technologies and microbial ecology. Inclusion criteria comprised studies investigating soil microbial diversity, functional gene profiling, microbial abundance, antimicrobial resistance genes, and ecological functions under regenerative and conventional agricultural practices. Studies based solely on culture-dependent techniques were excluded because of their inability to represent the total soil microbial diversity (19, 20).

Soil Sampling and Experimental Design

Most metagenomic studies included in this review employed randomized field sampling from agricultural lands managed under regenerative and conventional systems. Soil samples were generally collected from the rhizosphere and bulk soil at depths ranging from 0–15 cm using sterile augers and stored at 20°C or 80°C prior to DNA extraction to preserve microbial integrity (21, 22).

Regenerative agricultural systems included practices such as:

- Cover cropping
- Crop rotation
- Organic manure application
- Reduced or zero tillage
- Compost amendments
- Agroforestry integration

Conventional systems were characterized by:

- Intensive tillage
- Monoculture cropping
- Synthetic fertilizer application
- Chemical pesticide usage
- Herbicide dependency

Replicated sampling designs with seasonal and spatial variations were considered to ensure reproducibility and ecological representation (23, 24).

DNA Extraction and Metagenomic Sequencing

Microbial DNA was extracted from soil samples using commercially available soil DNA isolation kits such as:

- Qiagen DNeasy PowerSoil Kit
- MoBio PowerSoil DNA Isolation Kit
- NucleoSpin Soil Kit

These kits effectively remove humic acid contaminants that interfere with downstream sequencing reactions (25). After extraction, DNA quantity and purity were assessed using spectrophotometry and fluorometric quantification methods including NanoDrop and Qubit fluorometer analyses (26). High-quality DNA samples were subjected to metagenomic sequencing using next-generation sequencing (NGS) platforms such as:

- Illumina MiSeq
- Illumina HiSeq
- NovaSeq
- Oxford Nanopore
- PacBio SMRT sequencing

Two major sequencing approaches were commonly employed:

1. 16S rRNA Gene Sequencing Used primarily for bacterial and archaeal taxonomic profiling through amplification of hypervariable regions such as V3–V4 and V4–V5 (27).
2. Shotgun Metagenomic Sequencing Used for comprehensive functional and taxonomic analysis by sequencing total microbial DNA from soil samples (28).

Metagenomic Bioinformatics Pipeline

Raw sequencing reads obtained from NGS platforms underwent quality assessment and preprocessing using standard bioinformatics pipelines.

Quality Control and Filtering

Low-quality reads, sequencing adapters, and chimeric sequences were removed using software such as:

- FastQC
- Trimmomatic
- Cutadapt
- DADA2 (29, 30)

Taxonomic and Functional Analysis

Operational Taxonomic Unit (OTU) Clustering

For 16S rRNA sequencing data, operational taxonomic units (OTUs) or amplicon sequence variants (ASVs) were generated using:

- QIIME2
- Mothur

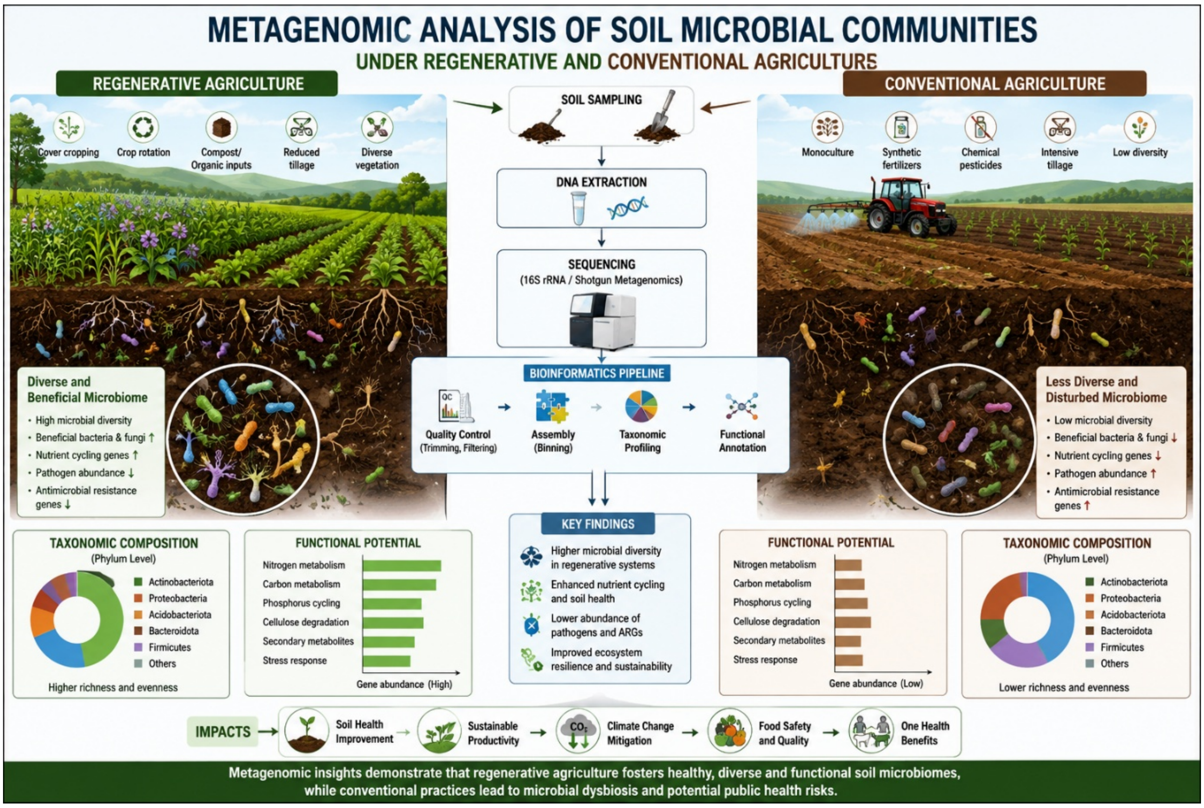

Figure 1. Metagenomic analysis of soil microbial communities

- USEARCH (31)

Taxonomic assignment was performed against reference databases including:

- SILVA
- Greengenes
- Ribosomal Database Project (RDP) (32)

Shotgun Metagenomic Functional Annotation

Functional gene annotation and pathway analyses were conducted using tools such as:

- MG-RAST
- HUMAnN
- MetaPhlAn
- MEGAN
- Kraken2 (33, 34)

Functional genes related to:

- Nitrogen cycling
- Carbon metabolism
- Phosphorus solubilization
- Antibiotic resistance
- Pathogen virulence

Microbial Diversity Analysis

Alpha diversity indices including:

- Shannon diversity index
- Simpson diversity index
- Chao1 richness estimator

were calculated to evaluate microbial richness and evenness within soil samples (35). Beta diversity analyses based on Bray–Curtis dissimilarity and Principal Coordinate Analysis (PCoA) were employed to compare microbial community composition across agricultural systems (36).

Metagenomic Workflow of Soil Microbial Community Analysis

Table 1. Comparative Microbial Characteristics under Agricultural Systems

Parameter	Regenerative Agriculture	Conventional Agriculture
Microbial Diversity	High	Reduced
Beneficial Bacteria	Increased	Decreased
Soil Organic Carbon	Enhanced	Declined
Antibiotic Resistance Genes	Lower	Higher
Pathogen Abundance	Reduced	Increased
Nutrient Cycling Efficiency	Improved	Impaired

Table 2. Major Methodological Findings from Previous Studies

Authors	Year	Major Findings
Caporaso et al.	2012	Developed QIIME platform for high-throughput microbial community analysis
Knight et al.	2018	Advanced microbiome analytical standards for metagenomics
Venter et al.	2016	Regenerative systems showed higher microbial richness
Banerjee et al.	2019	Soil microbiomes influence ecosystem multifunctionality
Sun et al.	2015	Conventional farming increased antibiotic resistance genes
Schmidt et al.	2019	Cover cropping improved beneficial microbial abundance

Figure 2. General Workflow of Soil Metagenomic Analysis

Soil Sampling

↓

DNA Extraction

↓

DNA Quality Assessment

↓

NGS Sequencing
(16S rRNA / Shotgun)

↓

Quality Filtering

↓

Bioinformatics Analysis

↓

Taxonomic Classification

↓

Functional Gene Annotation

↓

Statistical & Ecological Analysis

↓

Interpretation of Soil Microbial Dynamics

Figure 3. Major Bioinformatics Tools Used in Soil Metagenomics

Sequencing Data

↓

FastQC → Quality Assessment

↓

Trimmomatic → Read Filtering

↓

QIIME2 / DADA2 → OTU/ASV Analysis

↓

Kraken2 / MetaPhlAn → Taxonomic Profiling

↓

MG-RAST / HUMAnN → Functional Annotation

↓

R software → Statistical Visualization

Discussion

Metagenomic analysis has significantly transformed the understanding of soil microbial ecology by enabling comprehensive characterization of microbial communities and their functional capacities under different agricultural systems. The findings reviewed in the present study demonstrate substantial differences in microbial diversity, abundance, and ecological functions between regenerative and conventional agricultural practices. Regenerative agricultural systems consistently exhibited greater microbial richness, higher taxonomic diversity, and improved functional gene abundance compared to conventional systems (38, 39). These observations indicate that regenerative management practices support healthier and more resilient soil ecosystems. One of the major findings from metagenomic studies is the increased abundance of beneficial microbial taxa under regenerative agriculture. Soil microorganisms such as Actinobacteria, Proteobacteria, Firmicutes, and arbuscular mycorrhizal fungi play essential roles in nutrient cycling, nitrogen fixation, phosphorus solubilization, and suppression of soil-borne pathogens (40, 41). Regenerative practices including cover cropping, crop rotation, compost application, and reduced tillage create favorable environmental conditions for microbial proliferation by increasing soil organic matter and reducing physical disturbance (42). Enhanced microbial diversity improves soil aggregation, water retention, and nutrient availability, thereby promoting sustainable crop production and ecological stability. Conversely, conventional agricultural systems were associated with significant reductions in microbial diversity and functional stability. Intensive tillage disrupts soil structure and microbial habitats, while excessive use of synthetic fertilizers and pesticides negatively affects beneficial microbial populations (43, 44). Metagenomic analyses have shown that prolonged chemical exposure leads to the dominance of stress-tolerant and opportunistic microbial taxa while suppressing ecologically beneficial organisms (45). Such alterations can impair nutrient cycling efficiency, reduce soil fertility, and increase susceptibility to plant diseases. Another important observation is the increased prevalence of antimicrobial resistance genes (ARGs) and pathogenic microorganisms in conventionally managed soils. Studies have reported that excessive application of agrochemicals and animal manure containing antibiotic residues contributes to the enrichment and dissemination of ARGs within soil microbial communities (46, 47). Soil acts as a major environmental reservoir for resistance genes, which may subsequently transfer to human and animal pathogens through horizontal gene transfer mechanisms. This poses serious public health concerns due to the increasing global burden of antimicrobial resistance. Metagenomic investigations also revealed that regenerative agriculture contributes to enhanced ecosystem multifunctionality and environmental sustainability. Functional gene profiling demonstrated higher abundance of genes associated with carbon sequestration, methane oxidation, nitrogen metabolism, and biodegradation pathways in regenerative systems (48, 49). These microbial functions are critically important for mitigating greenhouse gas emissions and improving climate resilience. Increased microbial-mediated carbon storage under regenerative agriculture further supports global efforts toward climate change mitigation and sustainable land management. From the perspective of public health and One Health, soil microbial diversity is closely linked with food safety, environmental quality, and disease prevention. Healthy soil microbiomes suppress pathogenic organisms through microbial competition and production of antimicrobial compounds (50). Reduced microbial diversity under conventional agriculture may facilitate pathogen emergence, contamination of agricultural produce, and increased transmission of zoonotic diseases. Regenerative systems, by maintaining ecological balance and microbial resilience, may therefore contribute to improved food quality and reduced health risks. The application of advanced metagenomic tools such as shotgun sequencing, metatranscriptomics, and functional genomics has further enhanced understanding of microbial interactions and ecosystem processes. Bioinformatics platforms including QIIME2, MG-RAST, HUMAnN, and Kraken2 have enabled large-scale comparative analyses of microbial communities across diverse agroecosystems (51, 52). These technologies allow precise identification of microbial biomarkers associated with soil health and agricultural sustainability. Despite significant advancements, several challenges remain in soil metagenomic research. Soil microbial communities are highly heterogeneous and influenced by climatic conditions, soil type, crop species, and management intensity (53). Variations in sampling methods, sequencing depth, and bioinformatics pipelines may also affect reproducibility and interpretation of results. Furthermore, many soil microorganisms remain unculturable and functionally uncharacterized, limiting the understanding of their ecological roles. Future research integrating multi-omics approaches, long-term field experiments, and machine learning-based analyses may provide deeper insights into soil microbiome dynamics and their implications for sustainable agriculture and public health. Overall, the reviewed evidence strongly supports the hypothesis that regenerative agricultural practices enhance soil microbial diversity, improve ecological functioning, and reduce environmental and public health risks compared to conventional farming systems. Metagenomic analysis serves as a powerful scientific tool for monitoring soil health and guiding evidence-based agricultural policies aimed at achieving sustainability and One Health goals.

Conclusion

Metagenomic analysis has emerged as an advanced and highly effective approach for understanding the structure, diversity, and functional potential of soil microbial communities under different agricultural systems. The findings reviewed in this study clearly demonstrate that regenerative agriculture promotes greater microbial diversity, improved functional gene abundance, enhanced nutrient cycling, and increased ecological resilience compared to conventional agricultural practices. In contrast, conventional farming systems characterized by intensive tillage and excessive chemical inputs contribute to microbial imbalance, reduced soil fertility, enrichment of pathogenic microorganisms, and increased dissemination of antimicrobial resistance genes. The application of next-generation sequencing technologies and bioinformatics tools has provided valuable insights into the complex interactions between soil microbes, agricultural management, environmental sustainability, and public health. Regenerative agricultural practices not only improve soil quality and crop productivity but also support climate change mitigation, ecosystem stability, and food safety within the broader One Health framework. Therefore, integrating metagenomic monitoring into agricultural management and public health policies can play a critical role in promoting sustainable food systems and environmental protection. Future research focusing on long-term multi-omics studies and standardized metagenomic methodologies will further strengthen the understanding of soil microbiomes and facilitate the development of resilient agricultural ecosystems for global health sustainability.

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