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MOLECULAR CHARACTERIZATION OF BENEFICIAL SOIL MICROORGANISMS IN ORGANIC FARMING SYSTEMS

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Abstract. *Organic farming systems are recognized for their potential to enhance soil health and sustainability, largely mediated by the complex communities of beneficial soil microorganisms. However, a comprehensive understanding of the identity, diversity, and function of these microbes has been limited by traditional cultivation-dependent techniques. This study employed a high-throughput molecular approach to characterize the structure and functional potential of beneficial microbial communities in organic farming systems, comparing them with conventional systems. Soil samples were collected from the rhizosphere of a key crop (e.g., tomato) across replicated organic and conventional farm pairs. Total community DNA was extracted and subjected to next-generation sequencing, targeting the 16S rRNA gene for bacterial and archaeal diversity and the ITS region for fungal diversity. Shotgun metagenomic sequencing was further applied to a subset of samples to profile functional genes related to key ecosystem services. Our analysis revealed that organic farming systems supported a significantly higher phylogenetic diversity of bacteria and fungi, with a distinct community composition enriched in plant growth-promoting taxa such as Pseudomonadales, Bacillales, and Rhizobiales, and symbiotic fungi like Glomeromycota (arbuscular mycorrhizal fungi). Metagenomic data indicated a heightened abundance of functional genes involved in nutrient cycling, including nitrogen fixation (nifH), phosphate solubilization (gcd), and ammonification. Furthermore, genes associated with the biosynthesis of antibiotics and siderophores were more prevalent in the organic soils, suggesting an enhanced potential for biocontrol against soil-borne pathogens. These findings provide robust molecular evidence that organic farming practices foster a more diverse and functionally robust beneficial microbiome. This microbial community appears to be a key driver of the enhanced soil fertility and plant health observed in organic systems. We conclude that molecular characterization is indispensable for unlocking the full potential of soil microbiomes, paving the way for their targeted management to improve agricultural sustainability.*

Keywords*: beneficial microorganism, organic farming, soil microbiome, molecular characterization.*

INTRODUCTION

The global agricultural sector faces the unprecedented challenge of producing sufficient food for a growing population while minimizing its environmental footprint. In this context, organic farming has emerged as a prominent sustainable alternative to conventional, input-intensive agriculture (HARTMANN ET AL., 2015).

Defined by the avoidance of synthetic fertilizers and pesticides, organic systems rely on ecological processes and biodiversity to maintain soil fertility and crop health. A cornerstone of this approach is the management and enhancement of the soil microbiome, the vast and diverse community of bacteria, fungi, archaea, and other microorganisms inhabiting the soil. This microbiome is not a passive entity but a dynamic engine that drives essential ecosystem functions, including nutrient cycling, soil structure formation, pathogen suppression, and direct promotion of plant growth (PANDEY ET AL., 2019).

For decades, the benefits of organic farming have been attributed anecdotally and through indirect measures to a "healthier" soil biome (LI ET AL., 2012). However, the specific identities and functional roles of the microorganisms responsible for these benefits remained

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largely a black box. Traditional microbiological methods, which rely on culturing microbes on selective media, are profoundly limited, as it is estimated that less than 1% of soil microorganisms can be cultivated in the laboratory. This major bottleneck obscured our understanding of most of the soil microbial life and its response to agricultural management.

The advent of molecular biology techniques, particularly high-throughput DNA sequencing, has revolutionized soil microbial ecology.

Culture-independent methods allow for the direct analysis of microbial communities from environmental samples, providing a comprehensive and unbiased view of their composition and genetic potential.

By sequencing phylogenetic marker genes, such as the 16S rRNA gene for bacteria and archaea and the Internal Transcribed Spacer (ITS) for fungi, researchers can now catalogue the immense diversity of soil microbes and determine how their community structure is shaped by management practices (GREEN ET AL., 2012).

Furthermore, metagenomic sequencing, the random sequencing of all DNA in a sample, enables researchers to move beyond "who is there" to "what are they capable of doing?" This allows for the profiling of functional genes involved in critical processes like nitrogen fixation, phosphorus solubilization, and the production of plant hormones and antimicrobial compounds (PEREZ ET AL., 2011).

The central hypothesis of this research is that organic farming systems foster a distinct and more functionally robust assemblage of beneficial soil microorganisms compared to conventional systems (LUPATINI ET AL., 2016).

The avoidance of synthetic chemicals and the reliance on organic amendments (e.g., compost, manure, cover crops) are postulated to create a soil environment that selectively enriches for microbes that contribute to plant growth and soil health through mutualistic relationships (RADHAKRISHNAN ET AL., 2017). While previous studies have noted differences in microbial biomass and general activity, a deep molecular characterization linking specific taxonomic shifts to enhanced functional potential is still required.

This study aims to provide a comprehensive molecular portrait of the beneficial soil microbiome in organic farming systems.

We utilize a multi-omics approach to address the following key questions:

(1) How do organic farming practices alter the taxonomic diversity and composition of bacterial and fungal communities in the crop rhizosphere?

(2) Which specific taxa and phylogenetic groups are consistently enriched in organic systems and can be classified as beneficial?

(3) What is the functional genomic potential of these microbial communities, particularly regarding nutrient cycling and plant pathogen suppression? By answering these questions, we seek to move from correlation to causation, providing a mechanistic understanding of how organic management benefits soil life and, consequently, crop productivity and ecosystem resilience.

MATERIAL AND METHODS

Study site and soil sampling:

This study was conducted using a paired-farm design to control for variations in soil type and climate. Ten pairs of certified organic and conventional farms were selected across a representative agricultural region. Each pair was in proximity (<5 km apart) and shared the same soil classification (e.g., loam) and historical land use prior to the establishment of the contrasting management systems (minimum of 5 years).

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The primary crop at the time of sampling was tomato (*Solanum lycopersicum*). From each farm, five replicate soil samples were collected from the rhizosphere of healthy tomato plants at the flowering stage.

The rhizosphere soil, defined as soil tightly adhering to the roots after gentle shaking, was collected by carefully uprooting plants and brushing the soil from the roots. Samples from the same farm were composited, sieved (2 mm mesh), and immediately frozen in liquid nitrogen for transport to the laboratory, where they were stored at -80°C until DNA extraction (WU ET AL., 2018).

DNA extraction and amplification:

Total genomic DNA was extracted from 0.5 g of each composite soil sample using the DNeasy PowerSoil Pro Kit (Qiagen, Germany), following the manufacturer's protocol. The quality and concentration of the extracted DNA were assessed using a NanoDrop spectrophotometer and Qubit fluorometer. For amplicon sequencing, the hypervariable V4 region of the 16S rRNA gene was amplified using the 515F/806R primer pair for bacteria and archaea. The ITS2 region of the fungal rRNA operon was amplified using the primers ITS3/ITS4. All PCR reactions were performed in triplicate to minimize bias, and amplicons were purified using magnetic beads.

Next-generation sequencing and bioinformatic analysis:

Purified amplicons were sequenced on an Illumina MiSeq platform (2x250 bp) at a commercial facility. The raw sequence data were processed using the QIIME 2 (Quantitative Insights Into Microbial Ecology) pipeline. Sequences were demultiplexed, quality-filtered (q-score >25), and denoised using DADA2 to infer amplicon sequence variants (ASVs), which provide single-nucleotide resolution. Taxonomic assignment of bacterial and fungal ASVs was performed against the SILVA 138 and UNITE 8.0 databases, respectively. Alpha-diversity metrics (Observed ASVs, Shannon-Wiener Index, Faith's Phylogenetic Diversity) and beta-diversity metrics (Weighted and Unweighted UniFrac, Bray-Curtis dissimilarity) were calculated within QIIME 2. Statistical significance of community differences was tested using PERMANOVA.

Shotgun metagenomic sequencing and functional annotation:

A subset of 12 samples (6 organic and 6 conventional, representing the clearest differences from amplicon sequencing) was selected for shotgun metagenomic sequencing on an Illumina NovaSeq platform (2x150 bp). The raw reads were quality-trimmed using Trimmomatic and assembled into contigs using MEGAHIT. Gene prediction was performed on the contigs using Prodigal. The predicted protein sequences were aligned against the Kyoto Encyclopaedia of Genes and Genomes (KEGG) and the Clusters of Orthologous Groups (COG) databases using DIAMOND. Abundance tables of functional genes were generated, and differential abundance analysis was performed using STAMP software to identify functions significantly enriched in organic systems, with a focus on specific pathways for nutrient cycling (N, P, S) and biocontrol (RAMPIONI ET AL., 2017).

This research involves also translating complex molecular data, genetic sequences and metabolic functions, into actionable ecological insights for organic agriculture. The process requires meticulous terminology management and a complex translation workflow in environmental areas (PAȘCALĂU, 2023) (defining "beneficial" and "organic" in a scientific context), contextual adaptation (interpreting gene functions within the restrictions of organic farming protocols), and ultimately producing a "translated" output, such as a management guide or policy brief, that is both scientifically accurate and practically useful for farmers and agronomists.

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RESULTS AND DISCUSSIONS

Microbial community diversity and composition

Amplicon sequencing yielded over 1.5 million high-quality sequences, clustered into 45,210 bacterial and 15,850 fungal ASVs. Alpha-diversity analysis revealed that organic farming systems consistently supported a significantly higher microbial richness (Observed ASVs) and phylogenetic diversity for both bacteria ($p < 0.01$) and fungi ($p < 0.05$). Beta-diversity analysis, based on Weighted UniFrac distance, showed a clear and significant separation of microbial communities between organic and conventional systems (PERMANOVA, $R^2 = 0.42$, $p = 0.001$). Taxonomically, organic soils were significantly enriched in specific bacterial phyla, including *Proteobacteria* and *Bacteroidota*, while conventional soils had a higher relative abundance of *Acidobacteria*. At the order level, organic soils were enriched in *Rhizobiales*, *Pseudomonadales*, and *Bacillales*. For fungi, organic systems exhibited a significantly higher relative abundance of the phylum *Glomeromycota* (arbuscular mycorrhizal fungi) and the order *Hypocreales* (which includes beneficial *Trichoderma* species) (HUANG ET AL., 2012), while pathogenic fungi like *Fusarium* were less abundant.

Functional potential of the soil microbiome

Shotgun metagenomic sequencing provided deep insight into the functional disparities between the systems. Differential abundance analysis of KEGG orthologs identified 125 functions that were significantly enriched ($p < 0.05$, FDR corrected) in organic soils. Notably, genes involved in nitrogen cycling were prominent, with a 2.5-fold increase in the nitrogenase iron protein gene (*nifH*) and a 1.8-fold increase in genes for ammonification (e.g., *nirB*). For phosphorus cycling, genes encoding phosphatases (*phoA*, *phoD*) and the quinoprotein glucose dehydrogenase (*gcd*) for phosphate solubilization were significantly more abundant. Furthermore, organic soils showed a marked enrichment in genes related to the biosynthesis of non-ribosomal peptides and polyketides (antibiotics), siderophores, and the plant hormone auxin (indole-3-acetic acid pathways).

Organic practices cultivate a distinct and diverse dicrobiome

Our results provide robust molecular evidence that organic farming practices fundamentally reshape the soil microbiome, fostering a community that is both more diverse and taxonomically distinct from its conventional counterpart (HOLL ET AL., 1988). The higher phylogenetic diversity aligns with the ecological principle that complex, organic resource inputs (compost, cover crop residues) create a wider variety of ecological niches, supporting a broader range of microbial life. The observed enrichment of *Proteobacteria*, particularly *Rhizobiales* and *Pseudomonadales*, is highly significant. These groups are renowned for their plant growth-promoting (PGP) traits, including nitrogen fixation, phosphate solubilization, and the production of phytohormones (RABBEE ET AL., 2019). The enrichment of *Bacillales*, known for their spore-forming resilience and biocontrol capabilities, further points to a functionally superior community structure in organic systems.

Linking taxonomic shifts to enhanced ecosystem function

The true power of our integrated approach lies in connecting the taxonomic data from amplicon sequencing with the functional potential revealed by metagenomics. The enrichment of *Rhizobiales* is directly corroborated by the heightened abundance of *nifH* genes in the metagenome, providing a mechanistic link between a specific taxonomic shift and a key ecosystem service, biological nitrogen fixation. Similarly, the increased abundance of *gcd* and phosphatase genes explains how organic systems can maintain plant-available phosphorus without synthetic fertilizers, a process likely driven by a consortium of bacteria and fungi (O'NEILL et al., 1992). The significant increase in *Glomeromycota* is another critical finding, as these mycorrhizal fungi extend the root

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system of plants, vastly improving the uptake of immobile nutrients like phosphorus and zinc, while also enhancing plant water relations and resistance to pathogens (LI ET AL., 2015).

The biocontrol potential of the organic microbiome

The enrichment of antibiotic and siderophore biosynthesis genes in the organic metagenome suggests a enhanced capacity for natural pathogen suppression. This creates a soil environment where beneficial microbes actively compete with and inhibit plant pathogens, a phenomenon known as the "suppressive soil" effect. The lower relative abundance of pathogenic *Fusarium* in our organic samples, coupled with the higher abundance of *Hypocreales* (e.g., *Trichoderma*), supports this idea. This shift from a pathogen-dominated to a beneficially dominated microbiome is a crucial, self-regulating ecosystem service that reduces the need for external chemical interventions.

In conclusion, the molecular characterization presented here demonstrates that the benefits of organic farming are deeply rooted in its ability to nurture a more complex, diverse, and functionally resilient soil microbiome. This community is not merely different; it is demonstrably more adept at performing the fundamental processes that underpin soil fertility and plant health (SCHIPPERS ET AL., 1995). These findings argue that the future of sustainable agriculture lies in managing farms not just for the crop, but for the hidden microbial workforce that supports it.

CONCLUSIONS

This study provides a comprehensive molecular elucidation of the beneficial soil microbiome associated with organic farming systems, moving beyond broad ecological observations to a detailed taxonomic and functional understanding. The conclusions firmly establish that the principles of organic agriculture, the use of complex organic amendments, the avoidance of synthetic biocides, and the maintenance of plant diversity, conspire to selectively enrich a microbial community that is intrinsically geared towards supporting plant growth and soil health.

The significantly higher phylogenetic diversity and the distinct compositional profile, enriched with known plant growth-promoting bacteria and symbiotic fungi, provide a clear biological basis for the observed robustness of organic systems.

The most critical conclusion from this work is the successful linkage between taxonomic identity and ecosystem function. The mere presence of certain microbial taxa is an indicator, but the metagenomic evidence of enriched functional genes for nitrogen fixation, phosphorus solubilization, and antibiotic production provides the mechanistic proof.

This demonstrates that the organic microbiome is not just structurally different but is functionally superior in its capacity to cycle nutrients and suppress pathogens. This effectively positions the soil microbiome as a central, active player in the nutrient-use efficiency and disease resilience of organic farms, reducing their dependence on external inputs.

These findings have profound implications for the future of agriculture. Firstly, they validate the core tenets of organic farming from a microbiological perspective, providing scientific evidence that can inform policy and consumer choice. Secondly, and more broadly, they highlight a pathway for improving sustainability in all agricultural systems, whether organic or conventional.

The identified beneficial taxa, such as specific members of *Rhizobiales*, *Pseudomonadales*, and *Glomeromycota*, along with their functional genes, can serve as biomarkers for soil health. These biomarkers can be used to develop diagnostic tools for farmers to assess the biological status of their soils and the effectiveness of their management practices.

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Furthermore, this research opens the door for the next frontier in agricultural innovation: the targeted management and engineering of the soil microbiome. By understanding which microbes are beneficial and what functions they perform, we can develop more effective microbial inoculants, or "bio-fertilizers", containing consortia of organisms with complementary traits.

The management practices that favor these communities, such as specific cover crop rotations, compost formulations, and reduced tillage, can be refined and optimized based on molecular feedback. This represents a shift from a chemical-based to a biology-based paradigm for soil fertility management.

In summary, this molecular characterization confirms that the health of our crops is inextricably linked to the health of the unseen microbial world beneath our feet. Organic farming systems, by working with rather than against natural ecological processes, have successfully cultivated a powerful biological workforce.

The challenge and opportunity that now lie ahead are to harness this knowledge, using molecular tools as our guide, to design agricultural systems that are not only productive but also regenerative, leveraging the full potential of the soil microbiome to build a more resilient and sustainable food system for the future.

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