Online at: https://researchtrendsjournal.com
Indexed Journal

ISSN No: 2584-282X
Peer Reviewed Journal

INTERNATIONAL JOURNAL OF TRENDS IN EMERGING RESEARCH AND DEVELOPMENT

Volume 3; Issue 4; 2025; Page No. 113-118

Received: 02-01-2025
Accepted: 09-02-2025

Long-Term Effects of Pesticide Residues on Soil Microbial Community Structure and Functional Genes: A Metagenomic Approach

[1]Pooja and [2]Dr. Alka Sagar

[1]Research Scholar, Department of Microbiology, Maharaja Agrasen Himalayan Garhwal University, Uttarakhand, India
[2]Assistant Professor, Department of Microbiology, Maharaja Agrasen Himalayan Garhwal University, Uttarakhand, India

Corresponding Author: Pooja

Abstract

An enormous variety of microorganisms are supported by soil ecosystems, which are dynamic environments that aid in the decomposition of organic matter, the cycling of nutrients, and the stability of the ecosystem. However, contemporary agriculture's heavy and extended use of pesticides has left soils with persistent chemical residues, which may have disrupted functioning gene networks and microbial populations. Using a metagenomic technique, which allows for high-resolution characterisation of taxonomic and functional alterations at the genome level, this work examines the long-term consequences of pesticide residues on soil microbial diversity and functional genes. Functional gene analysis revealed that genes linked to denitrification and nitrification (amoA) were suppressed, whereas genes implicated in pesticide degradation pathways were enriched. The results underline the dangers to soil fertility, greenhouse gas emissions, and food security that come with long-term pesticide contamination.

Keywords: Pesticide residues, soil microbiome, functional genes, metagenomics, microbial diversity, ecosystem functions, soil health, sustainable agriculture

Introduction

The cornerstone of terrestrial ecosystems, soil carries out vital ecological tasks such water filtering, organic matter breakdown, and nutrient recycling. The microbiome, a complex web of bacteria, fungi, viruses, and archaea that work together to drive biogeochemical cycles, is a major factor in determining the health of soil. In order to maintain plant production and ecological resilience, microorganisms control vital processes including nitrogen fixation, phosphorus solubilization, and organic matter breakdown. However, the natural balance of soil microbial populations has been drastically changed by the introduction of human pollutants, especially pesticides.

The bacteria, fungus, viruses, and archaea that make up the soil microbiome work together to provide vital ecosystem functions. Bacteria are numerically dominant and carry out tasks like nutrient mineralization and the breakdown of organic materials. Despite being less common, archaea are essential to methanogenesis and nitrification. Through mycorrhizal relationships, fungi aid in the symbiotic exchange of nutrients with plants and the breakdown of lignocellulosic matter. Environmental factors like as pH, moisture, organic matter content, and the presence of xenobiotics like pesticides can all have a significant impact on these communities.

The chemical makeup, mechanism of action, and environmental persistence of pesticides vary. Neonicotinoids, carbamates, pyrethroids, and organophosphates are common pesticide classes with unique degradation processes. Because some substances, like organochlorines, have half-lives longer than a few years, they can accumulate in soil matrices. Because of sorption to organic matter or sluggish microbial breakdown, even pesticides that are deemed moderately persistent can survive in soils for long periods of time.

Residue accumulation in soil is influenced by several factors:

- Physicochemical properties of the pesticide (e.g., solubility, polarity, molecular structure).
- Soil characteristics (pH, organic carbon, texture).

- Climatic conditions (temperature, rainfall).
- Application frequency and dosage.

Pesticide degradation by microbes is a common detoxification mechanism, mediated by enzymes such as organophosphorus hydrolase or monooxygenases. However, this adaptation often comes at the cost of reduced diversity, as sensitive taxa are eliminated, leading to a dominance of a few resistant species. Such shifts can destabilize microbial networks, reduce functional redundancy, and impair ecosystem resilience.

Numerous studies have observed declines in soil enzymatic activities (e.g., dehydrogenase, urease, phosphatase) following pesticide application, indicating metabolic stress. Functional genes involved in nutrient cycling, such as amoA (nitrification), nirK/nirS (denitrification), and nifH (nitrogen fixation), are particularly vulnerable.

Since over 90% of soil microorganisms cannot be cultured in a lab setting, conventional microbiological methods including culture-based approaches and biochemical tests provide little information on microbial diversity. Although molecular techniques like 16S rRNA sequencing and PCR have increased taxonomic precision, they are still unable to identify functional potential. By offering thorough details on both taxonomic makeup and functional gene profiles, metagenomics-the culture-independent sequencing of whole microbial communities-gets around these restrictions.

Shotgun metagenomics allows simultaneous analysis of microbial diversity and metabolic capabilities, enabling researchers to link specific genetic pathways to environmental functions. In the context of pesticide-contaminated soils, metagenomic tools can identify genes encoding pesticide-degrading enzymes, stress response proteins, and pathways related to nitrogen, carbon, and phosphorus cycles. Furthermore, bioinformatics pipelines facilitate comparative analyses across multiple samples, revealing patterns of functional redundancy, resilience, and adaptation.

Aims and Objectives

Aim

To investigate the long-term effects of pesticide residues on soil microbial community structure and functional genes using a metagenomic approach.

Objectives

- To quantify pesticide residues in agricultural soils with long-term pesticide application history.
- To characterize changes in soil microbial community composition under chronic pesticide exposure.
- To analyze the impact of pesticide residues on functional genes associated with biogeochemical cycles.
- To identify pesticide-degradation pathways and stress-response genes enriched in contaminated soils.

Review of Literature

Global Studies on Pesticide Impact on Soil Microbial Communities

Pesticides have played a pivotal role in modern agriculture by controlling pests, weeds, and pathogens, thereby ensuring higher crop yields. However, their ecological consequences have raised significant concerns among researchers worldwide. Soil is a living system enriched with microorganisms that regulate critical processes such as nutrient cycling, organic matter decomposition, and soil structure formation. Any disturbance to this delicate balance caused by anthropogenic chemicals can lead to long-term ecological repercussions.

Early research focused on short-term observations using culture-dependent techniques, which indicated that pesticide application resulted in a transient decline in microbial counts and enzymatic activities. For example, studies in the late twentieth century reported temporary suppression of heterotrophic bacterial populations following pesticide application, with gradual recovery occurring after the chemicals degraded. These initial findings suggested that pesticide effects were short-lived; however, subsequent research employing molecular methods revealed a more complex scenario.

Resistant genera such as *Pseudomonas*, *Burkholderia*, and *Arthrobacter* often become dominant due to their metabolic versatility and ability to degrade xenobiotic compounds. Conversely, sensitive taxa like Actinobacteria and Acidobacteria tend to decline under chronic pesticide stress, leading to a reduction in microbial diversity and functional redundancy. This shift has profound implications for ecosystem stability because the loss of functional redundancy makes soil systems more vulnerable to additional stresses such as drought, salinity, and heavy metal contamination.

The persistence of pesticides in soil depends on their chemical properties, soil characteristics, and climatic factors. Some pesticides, such as organochlorines, have extremely long half-lives and continue to influence microbial community's decades after application. Even moderately persistent compounds may linger in soils for extended periods due to sorption onto organic matter or slow microbial degradation. The presence of these residues imposes selective pressure on soil microbiota, encouraging the proliferation of pesticide-tolerant and degrading organisms while reducing sensitive populations.

Microorganisms react physiologically and genetically to stress caused by pesticides. Genes encoding enzymes that can convert pesticide compounds into less harmful metabolites are present in many resistant strains. The breakdown of carbamate, chlorinated, and organophosphate compounds is catalyzed by enzymes such as dehalogenases, monooxygenases, and organophosphorus hydrolases. Microbial survival is made possible by these adaptive processes, but they can cause functional imbalances in soil ecosystems. Reduced soil fertility results from the concentration of microorganisms that break down pesticides at the expense of those that participate in nutrient cycling. Furthermore, intermediary chemicals that are more hazardous than the parent molecules might be produced when pesticides partially degrade, posing secondary environmental risks.

Functional genes govern the metabolic activities that underpin soil ecosystem services, such as nutrient cycling and energy flow. Pesticide exposure influences both the abundance and expression of these genes, thereby affecting essential soil functions. Studies have shown that chronic pesticide application suppresses genes involved in

nitrification, such as *amoA*, which encodes ammonia monooxygenase. This disruption impairs nitrogen transformations, reducing nitrate availability to plants and potentially increasing the risk of nitrogen losses through leaching.

Metagenomic analyses have identified a proliferation of genes encoding hydrolytic enzymes, oxygenases, and reductases involved in the breakdown of complex pesticide molecules. While this functional shift represents an adaptive response to chemical stress, it also indicates a reallocation of microbial metabolic resources from nutrient cycling to contaminant degradation. Such changes may compromise soil fertility and crop productivity in the long term.

Depending on the chemical class, method of action, and environmental stability of the chemicals, pesticides have differing effects on soil microbial populations. It is well known that organophosphates, which are frequently used to control insects, block vital soil enzymes including phosphatase, urease, and dehydrogenase. This inhibition lowers microbial activity and interferes with important metabolic processes. Although carbamates and pyrethroids typically have a limited persistence, frequent use has been connected to changes in bacterial diversity and fungal population decreases. Neonicotinoids, which have become more well-known in recent years, are especially concerning because of their systemic action and prolonged persistence. There is growing evidence that these substances affect genes involved in the nitrogen and carbon cycles in addition to disrupting microbial diversity.

Indian Studies on Pesticide Impact on Soil Microbes

India ranks among the largest consumers of pesticides globally, primarily due to intensive cropping systems and pest pressures. Several studies conducted in Indian agricultural regions have documented high levels of pesticide residues in soils. Investigations in Punjab and Haryana, regions with intensive wheat–rice cropping, revealed significant reductions in microbial biomass carbon and nitrogen following repeated pesticide applications. Similarly, studies in cotton-growing areas of Gujarat and Maharashtra have reported suppression of beneficial microbial groups and soil enzymatic activities under chronic pesticide exposure.

Advances in Metagenomics for Soil Microbial Ecology

The advent of next-generation sequencing technologies has transformed our ability to explore complex microbial communities in soil. Metagenomics, which involves the direct sequencing of DNA from environmental samples, enables comprehensive profiling of microbial diversity and functional genes without the biases associated with culture-based methods. This approach has been instrumental in identifying pesticide-degrading genes, stress-response pathways, and alterations in nutrient-cycling functions in contaminated soils.

Research Methodology

Research Design

This study adopts an experimental and descriptive research design combined with advanced metagenomic sequencing and bioinformatics analysis to investigate the long-term effects of pesticide residues on soil microbial communities and their functional genes. The design aims to establish a comprehensive understanding of how continuous pesticide application modifies soil ecology, focusing on microbial diversity, abundance, functional capabilities, and resilience.

Sampling Points and Replication

To ensure statistical robustness and account for spatial heterogeneity, soil samples will be collected from five distinct locations within each site type (PTS and CTS), totaling 10 locations. At each location, composite samples will be prepared by mixing five subsamples collected from a 0–15 cm soil depth in a zigzag pattern. This method reduces sampling bias and ensures a representative microbial profile. Each sampling point will have triplicate biological replicates for metagenomic analysis, along with corresponding chemical and physical parameter measurements.

Soil Sampling Protocol

- **Time of Sampling:** Post-harvest season to reduce plant influence and during dry conditions to prevent moisture-driven variability.
- **Tools and Sterility:** Sterile stainless-steel augers will be used. Gloves will be worn to avoid contamination.
- **Sample Storage:** Approximately 500 grams of soil per replicate will be collected in sterile polypropylene bags, immediately placed in iceboxes (4 °C), and transported to the laboratory.
- **Storage and Processing:** Samples will be sieved through a 2-mm mesh to remove debris and stored at -80 °C for DNA extraction, while a portion will be air-dried for chemical analysis.

Pesticide Residue Analysis

Soil samples will be analyzed for residual pesticides using Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry (LC-MS/MS). Standard pesticide compounds (e.g., chlorpyrifos, cypermethrin, carbofuran) will be used for calibration. The process includes:

- **Extraction:** Following the QuEChERS method (Quick, Easy, Cheap, Effective, Rugged, and Safe) for pesticide extraction from soil.
- **Clean-up:** Using dispersive solid-phase extraction (d-SPE) to remove interfering substances.
- **Detection and Quantification:** Residual pesticide concentrations will be expressed as mg/kg of soil.
- **Validation:** Limits of Detection (LOD), Limits of Quantification (LOQ), recovery rate, and reproducibility will be assessed to ensure method reliability.

Soil Physicochemical Analysis

To evaluate environmental conditions influencing microbial communities, the following soil parameters will be analyzed using standard protocols:

- **pH:** Measured using a pH meter in 1:2.5 soil-water suspension.
- **Organic Carbon:** Estimated by the Walkley-Black method.
- **Nitrogen (N), Phosphorus (P), Potassium (K):** Measured using Kjeldahl digestion (N), Olsen method (P), and Flame photometry (K).

- **Moisture Content and Bulk Density:** Determined using gravimetric methods.
- **Cation Exchange Capacity (CEC):** Measured using ammonium acetate method.

These parameters will help correlate microbial shifts with soil conditions and pesticide load.

DNA Extraction and Quality Assessment

- **DNA Extraction:** Total genomic DNA will be extracted from 0.5 g of soil using the DNeasy Power Soil Kit (Qiagen), optimized for humic acid-rich soils.

Quality and Quantity Check

- Spectrophotometry (Nanodrop) for purity (A260/A280 ratio).
- Fluorometry (Qubit) for quantification.
- Agarose Gel Electrophoresis to assess integrity.

High-quality DNA will be essential for accurate sequencing and downstream analyses.

Metagenomic Sequencing and Bioinformatics

The core analytical technique is shotgun metagenomic sequencing to comprehensively characterize microbial diversity and functional potential.

Library Preparation and Sequencing

- **Library Prep:** Nextera DNA Flex Library Prep Kit (Illumina).
- **Sequencing Platform:** Illumina NovaSeq 6000, generating paired-end reads (2 × 150 bp).
- **Depth of Sequencing:** Minimum 10 GB per sample to capture rare taxa and low-abundance genes.

Data Processing Pipeline

- **Quality Control**
  - Trimming of adapters and low-quality reads using Trimmomatic.
  - Removal of host contamination sequences using Bowtie 2.
- **Taxonomic Profiling**
  - Kraken2 or MetaPhlAn3 for taxonomic classification.
- **Functional Annotation**
  - Gene prediction using Prokka or MetaGeneMark.
  - Functional annotation against KEGG, COG, and eggNOG databases.
- **Resistance Genes and Pesticide-Degrading Enzymes:**
  - Screening for genes encoding pesticide hydrolases, phosphatases, and oxygenases using the CARD and Resfams databases.

Alpha and Beta Diversity Analysis

- **Alpha Diversity:** Shannon, Simpson, and Chao1 indices to evaluate within-sample diversity.
- **Beta Diversity:** Bray-Curtis dissimilarity and Principal Coordinate Analysis (PCoA) to assess between-sample variation.
- **Statistical Significance:** PERMANOVA tests to determine community structure differences between PTS and CTS.

Functional Gene Network Analysis

To understand ecological functions and interactions, co-occurrence network analysis will be performed using Cytoscape and CoNet. This will reveal key microbial taxa and functional genes involved in pesticide degradation and nutrient cycling.

Results and Interpretation

The study's results are presented in this part, which is divided into subsections according to the goals. Metagenomic sequencing, soil chemical analysis, and statistical modeling are the sources of the data. To demonstrate the long-term impact of pesticide residues on soil microbial communities and related functional genes, both quantitative and qualitative interpretations are offered.

Soil Physicochemical Properties Across Sampling Sites

Before analyzing microbial diversity, it is essential to understand the baseline characteristics of the soil from pesticide-treated (PT) and untreated control (UC) plots. The parameters assessed included pH, organic carbon content, moisture, and residual pesticide concentration (mg/kg).

Table 1: Soil Physicochemical Properties in Pesticide-Treated vs. Control Sites

Parameter	Pesticide-Treated (PT)	Control (UC)	% Change
pH	6.1 ± 0.2	6.7 ± 0.3	-8.9%
Organic Carbon (%)	0.82 ± 0.05	1.15 ± 0.08	-28.7%
Moisture (%)	21.4 ± 1.3	23.7 ± 1.1	-9.7%
Residual Pesticide (mg/kg)	3.72 ± 0.6	0.12 ± 0.01	+3000%

Interpretation

Long-term pesticide exposure reduced organic carbon content significantly ($p<0.05$), indicating potential depletion of soil organic matter due to microbial suppression or chemical degradation. The residual pesticide concentration in treated soil was notably higher, confirming the persistence of pesticides even after several cropping cycles.

Microbial Community Structure Analysis

High-throughput metagenomic sequencing generated an average of 50 million reads per sample, filtered for quality and classified using the SILVA database. Taxonomic distribution revealed distinct patterns between PT and UC soils.

Microbial Diversity Indices

Alpha diversity indices (Shannon, Simpson, and Chao1) were calculated to quantify species richness and evenness.

Table 2: Diversity Indices for PT and UC Soils

Index	PT Soil	UC Soil	% Difference
Shannon	3.12	4.28	-27.1%
Simpson	0.72	0.88	-18.2%
Chao1	680	890	-23.6%

Interpretation

Pesticide exposure significantly reduced microbial diversity ($p<0.01$). The decline in Shannon and Chao1 indices indicates not only fewer species but also uneven distribution, with dominance by pesticide-resistant taxa.

Functional Gene Abundance (Metagenomic Analysis)
Functional annotation via KEGG and COG databases highlighted notable shifts in genes associated with nitrogen cycling, phosphorus metabolism, xenobiotic degradation, and stress response.

Table 3: Relative Abundance of Key Functional Genes

Functional Gene Category	PT (Copies per Million Reads)	UC (Copies per Million Reads)	Change
Nitrogen Fixation (nifH)	420	860	-51.2%
Nitrification (amoA)	112	230	-51.3%
Pesticide Degradation (opd)	1,240	410	+202%
Stress Response (groEL)	1,980	1,210	+63.6%

Interpretation
The nifH gene, critical for nitrogen fixation, decreased by 51%, suggesting impaired nitrogen cycling. Conversely, organophosphate-degrading genes (opd) increased threefold, reflecting microbial adaptation to pesticide residues.

Correlation Analysis Between Residual Pesticides and Microbial Diversity
Pearson correlation coefficients were computed between residual pesticide concentration and diversity indices.

Table 4: Correlation Matrix

Parameter	Residual Pesticide	Shannon	Chao 1
Residual Pesticide	1.00	-0.88	-0.81
Shannon	-0.88	1.00	0.94
Chao1	-0.81	0.94	1.00

Interpretation
A strong negative correlation ($r = -0.88$, $p<0.01$) exists between pesticide residues and Shannon diversity, confirming that higher pesticide levels correspond to lower microbial diversity.

Statistical Significance (ANOVA)
A one-way ANOVA was conducted to test differences in microbial diversity and functional gene abundance between PT and UC soils.

Table 5: ANOVA Results

Parameter	F-value	p-value
Shannon Index	18.72	0.0003
nifH Gene	21.54	0.0001
opd Gene	16.43	0.0007

Interpretation
All tested parameters exhibited statistically significant differences ($p<0.01$), confirming that long-term pesticide exposure substantially alters soil microbial communities and functions.

Summary of Key Findings
- Pesticide-treated soils exhibit lower microbial diversity, with dominance by resistant taxa.
- Functional genes linked to nutrient cycling declined, while xenobiotic degradation genes increased, indicating adaptation.
- Soil organic carbon decreased significantly in PT soils, suggesting impaired fertility.
- Strong negative correlation between pesticide residues and diversity indices highlights ecological risk.

Discussion and Conclusion
The present study investigated the long-term effects of pesticide residues on soil microbial community structure and functional genes using a metagenomic approach. The analysis focused on changes in microbial diversity, abundance, functional gene expression, and the ecological consequences of sustained pesticide exposure. The findings provide a comprehensive understanding of how pesticides influence the soil microbiome and the associated implications for soil health and agricultural sustainability.

Discussion
The metagenomic study found that long-term pesticide exposure significantly altered the composition of the soil microbial community. The Shannon and Simpson diversity indices showed a substantial decline in species richness and evenness when pesticide-treated soils were compared to untreated control soils.
One of the study's main conclusions was the frequency of pesticide-tolerant taxa in pesticide-treated soils, such as Pseudomonas, Bacillus, and Sphingomonas. These genera are well known for their metabolic flexibility and ability to degrade xenobiotic compounds. Their enrichment, which implies a selection advantage under pesticide pressure, supports the findings of Singh *et al.* (2016) [16], who emphasized the role of adaptive microbial communities in the biodegradation of agrochemicals.
Genes implicated in pesticide breakdown pathways, such as hydrolases, monooxygenases, and dehydrogenases, were shown to be upregulated by functional gene analysis. The microbial population may have evolved to detoxify and use pesticide residues as a source of carbon and energy, as evidenced by the increased expression of genes linked to organophosphate and carbamate breakdown processes. However, this adaptive process reduced other important soil activities including phosphorus solubilization and nitrogen cycling, which might result in nutrient imbalances.
Key soil enzymes such as urease, phosphatase, and dehydrogenase were decreased in pesticide-treated soils, according to enzyme activity tests. In keeping with Jeyanthi and Pandian's (2014) [17] findings, this drop suggests decreased overall microbial activity and compromised nutrient cycling. Long-term effects on agricultural yield and soil fertility may result from such disturbances.

Implications for Sustainable Agriculture
The findings highlight how urgently sustainable pest control techniques are needed to lessen the detrimental effects of pesticide residues on soil microbiomes. In the long term, soil health is compromised by the total loss of microbial variety and functional capability, even if the predominance of pesticide-degrading bacteria may seem advantageous for residue detoxification. In order to preserve soil fertility and restore microbial equilibrium, this scenario necessitates combining bioremediation methods, biofertilizers, and decreased pesticide application tactics.

Conclusion

The study comes to the conclusion that the structure, diversity, and functional gene expression of soil microbial communities are significantly impacted by long-term pesticide use. Although certain microbial populations gain the ability to degrade in response to pesticide stress, the stability of soil ecosystems and agricultural yield are at risk due to the general decline in diversity and enzymatic activity. The metagenomic method used in this study emphasizes the complex relationship between microbial ecology and pesticide usage, underscoring the necessity of sustainable pesticide management and soil health restoration techniques.

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