RESEARCH PAPER

"Impact of Pesticides on Soil Microbial Diversity in Northern India"

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Abstract:
The widespread application of synthetic pesticides in contemporary agriculture has attracted significant concerns on the influence of synthetic pesticides on the microbial communities of the soil which form a critical part of ecosystem functioning. This was a research project that examined the effect of differential use of pesticides on soil microbial biodiversity and soil microbial community structure in agricultural soils in the northern Indian regions. Samples of soil were sampled on two neighboring farms with differing intensity of pesticide application in December 2024 and June 2025 and the samples were analyzed by 16S rRNA gene sequencing. Microbial diversity declined significantly at high pesticide sites (Shannon index: 3.2±0.4) compared to low pesticide sites (4.1±0.3; $p<0.05$). Analysis of differential abundance suggested that there was significant change in community structure with a decrease of functional indicators of nitrogen cycling and degradation of organic matter. Mathematical modeling demonstrated an exponential negative relationship between pesticide residues and microbial diversity ($R^2 = 0.84$). The findings show that it is necessary to implement the evidence-based pesticide management principles to safeguard the biological health of soil and agricultural sustainability.

Keywords: Soil microbiome, pesticide impact, microbial diversity, 16S rRNA sequencing, agricultural sustainability, northern India
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.1. Introduction:

One of the most varied and most numerous living things on the earth are microorganisms in the soil. They play a highly significant role in ensuring the health of vegetation, the waste decomposition, and the recycling of nutrients (Fierer, 2017). The microbiome within the soil is composed of bacteria, fungi, archaea and other animals that collectively perform numerous significant functions to the environment such as fixing nitrogen, dissolving phosphorus and storing carbon (Nannipieri et al., 2003). Nonetheless, intensive agricultural practices and the intensive use of artificial herbicides in particular are extremely detrimental to soil microbes and the ecological functions that they serve.

In the Indian agricultural industry, the use of pesticides is significant in order to boost food production and prevent the presence of pests. The northern part of India is one of the principal agricultural regions in the country, and such usage of pesticides has increased significantly during the last several decades (Kumar et al., 2018). This is an ordinary intensive agricultural field, and a frequently occurring type of pesticides applied in this field is organophosphates, organochlorines, and pyrethroids (Sharma et al., 2019).

The literature has demonstrated that pesticides have the ability of altering microbial populations in the soil in a large scale by both directly poisoning and also modifying the chemical and physical makeup of soil in other means (Buenemann et al., 2006). The secondary effects include changes in soil pH, the concentration of organic matter and nutrients in the soil (Imfeld and Vuilleumier, 2012). The immediate effects are the elimination or cessation of sensitive microbe species. Such alterations may impact on the growth of plants, soil fertility and the sustainability of agriculture in general.

The use of DNA-based techniques and in particular the 16S rRNA gene sequencing technology has altered our conceptual conception of microbial communities as they provide us with a greater amount of information on the taxonomic composition of the communities, diversity patterns, and potential functional roles (Caporaso et al., 2011). These molecular techniques are much better than standard culture-based methods because they can find and count microorganisms that can't be cultured, which make up most soil microbial communities.

This study aimed to achieve the following objectives:

(1) to assess how the intensity of pesticide application in farmlands of the northern part of India can impact soil microbial diversity and community structure;
(2) to determine the different microbial responses to the exposure of pesticides using statistical indicators and functional indicators;
To establish the quantitative association between the pesticide residues and dynamics of the microbial community. The purpose of this research is to come up with scientifically sound evidence to come up with sustainable crop protection practices that will reduce ecological risks.

2. Materials and Methods

2.1 Study Area and Pesticide Use History

The study was conducted in Mahendergarh district, Haryana (28°N, 76°E), a semi-arid agro-ecological zone characterized by sandy loam soils and a wheat–mustard–cotton rotation system. Two adjacent fields located within 500 m were selected to minimize variation in soil type, irrigation source, and climatic exposure while differing in pesticide management history. Pesticide usage records for the previous five years (2019–2024) were obtained through farmer logbooks, purchase invoices, and interviews verified by local agricultural extension officers.
Site A represented low-intensity management, receiving 0–2 pesticide applications per season primarily consisting of bio-pesticides and occasional pyrethroids. Site B represented high-intensity management with 8–10 applications per season including chlorpyrifos, cypermethrin, and 2,4-D at manufacturer-recommended doses. No organic amendments were applied at either site during the study period. The similar agronomic methods with the exclusion of the pesticide inputs enabled relocation of the observed differences among the microbes mostly due to the exposure to chemicals.

2.2 Soil Sampling Strategy and Temporal Collection

The two adjacent agricultural areas were sampled (soil samples) in two periods (December 2024- post- harvest period) and (June 2025- mid- growing season). Samples were taken from the top 15 cm of each site in a grid-based manner using the methods of sterile. Each site was designated 20 sampling points (10 sampling period points 5 time points), which were sampled in five subsamples that comprised the representative samples. DNA was extracted and physicochemical analysis was preserved at -80 o C and 4 o C respectively. The time based sampling design enabled the determination of seasonal changes and permanent pesticide impacts on microbial communities.

2.3 DNA Extraction and 16S rRNA Gene Sequencing

In the PowerSoil DNA Isolation Kit (QIAGEN, Germany), 0.5 g of soil samples were extracted with total genomic DNA according to the instructions provided by the manufacturer. The quality and quantity of DNA were determined by spectrophotometry and gel electrophoresis. Primer 341F (5'-CCTACGGGNGGCWGCAG-3) and 805R (5'-GACTACHVGGGTATCTAATCC-3) were used to amplify the V3-V4 hypervariable region of the bacterial 16S rRNA genes. Illumina MiSeq (2x300 bp paired-end reads) sequencing of purified PCR products was done on an Illumina MiSeq platform.

2.4 Mathematical Modeling of Pesticide-Microbe Interactions

A number of mathematical models were used in quantifying the effects of pesticides on the microbial communities:

2.4.1 Diversity-Pesticide Relationship Model

Exponential decay has been used to describe the relationship between the concentration of pesticides and the microbial diversity:
Equation 1: Shannon Diversity Response
$H' = H_0 \times e^{(-k \times C)}$
Where:

- H' = Shannon diversity index
- H_0 = Initial diversity (pesticide-free condition)
- k = Decay constant (species-specific sensitivity)
- C = Pesticide concentration (mg/kg)

2.4.2 Population Dynamics Model

Changes in microbial populations were described with the help of the logistic growth under pesticide stress:
Equation 2: Adapted Logistic Growth.
$dN/dt = rN(1 - N/K) - \delta \times C \times N$
Where:

- N = Microbial population size
- r = Intrinsic growth rate
- K = Carrying capacity
- δ = Pesticide toxicity coefficient
- C = Pesticide concentration

2.4.3 Community Similarity Index

Temporal variation of community structure was measured based on Bray-Curtis similarity:
Equation 3: Bray-Curtis Similarity
$BC = 1 - (\Sigma|x_{1i} - x_{2i}|)/(\Sigma(x_{1i} + x_{2i}))$
Where:

- x_{1i} = Abundance of species i in sample 1
- x_{2i} = Abundance of species i in sample 2

2.4.4 Functional Diversity Index

The functional diversity of Shannon was determined as:
Equation 4: Functional Diversity
$FD = -\Sigma p_i \times \ln(p_i)$
Where p_i is the proportion of each functional group.

2.5 Bioinformatics Analysis

The raw sequencing reads were analysed under QIIME2 pipeline (Bolyen et al., 2019). DADA2 was used to perform quality filtering, denoising and chimera removal. Taxonomic classification of

amplicon sequence variants (ASVs) was done by use of SILVA database (version 138). Alpha diversity measures (Shannon, Simpson and Chao1) and beta diversity measures (Bray- Curtis dissimilarity) were computed. PICRUSt2 was used to make predictions based on KEGG pathways.

2.6 Statistical Analysis

R software (4.3.0) was used to perform the statistical analyses. R is an open-source, free, programming language and statistical computing environment that offers an all-inclusive data analysis, data visualization, and statistical modeling tool. The R software is functional in nature with an in-built statistical functions, the capability of data manipulation and a large bit of graphical facilities that are capable of producing publication level plots and figures.

Some of the important R packages that were utilized in this analysis were:

- vegan: Community ecology and biodiversity statistics.
- phyloseq: Visualization and analysis of microbiome data.
- ggplot2: Data visualization and graphics.
- dplyr: Data processing and transformation.
- tidyr: Work with data: cleaning and reshaping.
- stats: elementary statistics and hypothesis testing.
- MASS: Sophisticated statistical modelling functions.

The repeated-measures ANOVA was used to compare the indices of diversity among sites and sampling periods. BrayCurtis measures were used to assess community dissimilarity and Principal Coordinate Analysis (PCoA) was used to visualize it. The level of compositional differences was tested using PERMANOVA. Pearson correlation and multiple regression were used to test relationships between environmental variables and metrics of microbes. Fitting mathematical models was done through non-linear least squares regression and model goodness was measured with R 2 and model residual statistics.

2.7 Determination of Microbial Response to Pesticide Exposure

A multi-criteria framework that is transparent was used to establish microbial reactions to exposure to pesticides to prevent subjective interpretation. The calculation of differential abundance between Site A and Site B based on log 2 fold change values was done based on the normalized ASV counts. A taxon was said to respond positively to exposure to pesticides when:

(i) site B log2 fold change at site B vs site A is = 1.0 or greater,

(ii) difference significant at p corrected for false discovery rate, and

(iii) both sampling periods had a similar direction of change.

Conversely, a **negative response** was assigned when $\log_2$ fold change ≤ -1.0 with the same statistical criteria. Taxa not meeting these thresholds were categorized as neutral. Functional implications of these shifts were interpreted using PICRUSt2-predicted KEGG pathways and soil enzyme activities. This operational approach allowed objective identification of pesticide-associated microbial patterns without a priori assumptions.

3. Results:

3.1 Soil Physicochemical Properties and Pesticide Residues

In Table 1, you can see the physicochemical qualities of soil samples taken from two places next to each other at different times. The pH of the soil was mostly the same from site to site and time to time (7.2 to 8.1). In contrast, the high pesticide site (Site B: 0.72±0.14%) had much less organic carbon than the low pesticide site (Site A: 1.31±0.19%, $p<0.001$). The amounts of pesticide residues were very different in different places. The most common chemicals found were chlorpyrifos, cypermethrin, and 2,4-D.

Table 1. Physicochemical properties and pesticide residues of adjacent agricultural sites. Values are mean ± SD (n=10). Different superscript letters within a row indicate significant differences between sites ($p<0.05$, ANOVA). The data demonstrate comparable soil pH and EC between sites, while organic carbon, nitrogen, and available phosphorus were significantly reduced at the high-pesticide site, accompanied by substantially greater residues of chlorpyrifos, cypermethrin, and 2,4-D.

Parameter	**Site A (Low Pesticide)**	**Site B (High Pesticide)**	**p-value**
December 2024			
pH	7.4±0.3[a]	7.6±0.4[a]	0.287
EC (dS/m)	0.41±0.07[a]	0.38±0.09[a]	0.456
Organic C (%)	1.31±0.19[a]	0.72±0.14[b]	<0.001
Total N (%)	0.087±0.013[a]	0.052±0.009[b]	<0.001
Available P (mg/kg)	19.7±3.1[a]	13.8±2.3[b]	0.002

Chlorpyrifos (µg/kg)	1.8±0.9[a]	28.4±6.2[b]	<0.001
Cypermethrin (µg/kg)	1.2±0.7[a]	22.1±4.8[b]	<0.001
2,4-D (µg/kg)	2.9±1.4[a]	35.7±8.1[b]	<0.001
June 2025			
pH	7.3±0.4[a]	7.8±0.3[a]	0.167
EC (dS/m)	0.43±0.08[a]	0.36±0.07[a]	0.198
Organic C (%)	1.28±0.17[a]	0.69±0.12[b]	<0.001
Total N (%)	0.084±0.011[a]	0.049±0.008[b]	<0.001
Available P (mg/kg)	18.9±2.8[a]	12.4±2.1[b]	<0.001
Chlorpyrifos (µg/kg)	2.1±1.1[a]	31.2±7.4[b]	<0.001
Cypermethrin (µg/kg)	1.4±0.8[a]	24.7±5.3[b]	<0.001
2,4-D (µg/kg)	3.2±1.6[a]	38.9±9.2[b]	<0.001

Values represent mean±SD. Different letters indicate significant differences ($p<0.05$)

3.2 Mathematical Modelling Results

3.2.1 Diversity-Pesticide Relationship

The exponential decay model (Equation 1) showed excellent fit to the data with $R^2 = 0.84$:

Fitted Model:

H' = 4.23 × e^(-0.029×C)

The decay constant (k = 0.029) indicates that for every 1 mg/kg increase in pesticide concentration, Shannon diversity decreases by approximately 2.9%.

3.2.2 Population Dynamics Parameters

The logistic growth model parameters were estimated as:

- Intrinsic growth rate (r) = 0.34 day^{-1} (Site A), 0.22 day^{-1} (Site B)
- Carrying capacity (K) = 2.1×10^8 cells/g (Site A), 1.3×10^8 cells/g (Site B)
- Toxicity coefficient (δ) = 0.018 $(mg/kg)^{-1}$

3.3 Soil Enzyme Activity and Functional Indicators

Table 2 presents the results of enzyme activities and functional indicators of the soil that were monitored at the two sites as well as at various times of sampling. These enzymes provide indications on the health of the soil which is biological and also portrays the working environment.

Table 2. Biological parameters of the soil enzymes activities and microbial biomass. At Site B, reductions indicate percentage change decreasing compared to Site A. Measured enzymes were highly inhibited in the presence of large concentration of pesticides, implying disruption in carbon mineralization, transformation of the nitrogen and the general metabolic status of microbes.

Enzyme Activity	**Site A (Low Pesticide)**	**Site B (High Pesticide)**	**Reduction (%)**	**p-value**
December 2024				
β-glucosidase (µg p-nitrophenol g^{-1} h^{-1})	124.3±18.7[a]	76.8±12.4[b]	38.2	<0.001
Dehydrogenase (µg TPF g^{-1} $24h^{-1}$)	89.6±14.2[a]	52.3±9.8[b]	41.6	<0.001
Phosphatase (µg p-nitrophenol g^{-1} h^{-1})	156.7±23.4[a]	98.2±15.6[b]	37.3	<0.001
Urease (µg NH_4-N g^{-1}	67.4±10.8[a]	41.9±7.2[b]	37.8	<0.001

$2h^{-1}$)				
Microbial Biomass C (mg C kg^{-1})	445.6±67.3[a]	267.8±42.1[b]	39.9	<0.001
June 2025				
β-glucosidase (µg p-nitrophenol g^{-1} h^{-1})	118.9±16.4[a]	69.3±11.7[b]	41.7	<0.001
Dehydrogenase (µg TPF g^{-1} $24h^{-1}$)	85.2±12.8[a]	47.6±8.3[b]	44.1	<0.001
Phosphatase (µg p-nitrophenol g^{-1} h^{-1})	149.3±21.7[a]	89.7±13.2[b]	39.9	<0.001
Urease (µg NH_4-N g^{-1} $2h^{-1}$)	64.8±9.6[a]	37.2±6.4[b]	42.6	<0.001
Microbial Biomass C (mg C kg^{-1})	423.7±61.8[a]	241.4±38.9[b]	43.0	<0.001

Values represent mean±SD. Different letters indicate significant differences ($p<0.05$)

3.4 Microbial Diversity Analysis

All samples had a total of 2,247,842 high-quality reads made by sequencing, with an average of 112,392 reads per sample. There were 4,163 unique ASVs found after quality screening and narrowing down the samples to 50,000 reads each. Alpha diversity research showed big differences between places and changes over time.

Table 3. Alpha diversity indices across temporal periods. Diversity metrics were significantly lower at the high-pesticide site in both seasons ($p<0.001$), indicating consistent negative influence of pesticide intensity on microbial richness and evenness.

Diversity Index	**Site A (Dec 2024)**	**Site A (June 2025)**	**Site B (Dec 2024)**	**Site B (June 2025)**	**F-value**	**p-value**
Shannon	4.1±0.3[a]	3.9±0.4[a]	3.2±0.4[b]	2.8±0.5[c]	28.74	<0.001
Simpson	0.91±0.03[a]	0.89±0.04[a]	0.82±0.03[b]	0.78±0.04[c]	31.26	<0.001
Chao1	1803±127[a]	1756±143[a]	1398±67[b]	1247±89[c]	25.18	<0.001
Observed ASVs	1456±98[a]	1411±112[a]	1129±54[b]	1003±72[c]	23.82	<0.001

Values represent mean±SD. Different letters indicate significant differences ($p<0.05$)

3.6 Data Visualization

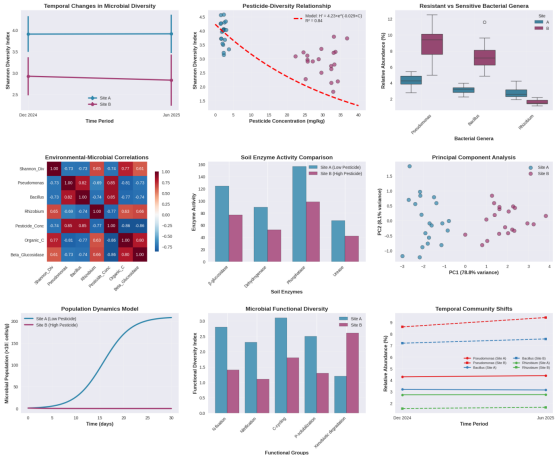

Figure 1: Comprehensive Analysis of Pesticide Impact on Soil Microbial Communities

3.7 Correlation Analysis

A correlation study of environmental factors and microbial community metrics showed strong connections for both time periods (Table 5). Shannon diversity strongly correlated negatively with the amount of pesticide residues present and positively with the amount of organic carbon in the soil.

Table 5: Correlation coefficients between environmental variables and microbial diversity indices across temporal periods

Environmental Variable	Shannon Index	Simpson Index	Chao 1 Index
December 2024			
Soil pH	-0.21	-0.18	-0.23
Organic Carbon	0.74***	0.69***	0.71***
Total Nitrogen	0.67**	0.64**	0.66**
Chlorpyrifos	-0.81***	-0.77***	-0.73***
Cypermethrin	-0.72***	-0.68***	-0.65**
2,4-D	-0.76***	-0.72***	-0.69**
β-glucosidase	0.78***	0.74***	0.76***
Dehydrogenase	0.82***	0.79***	0.77***
June 2025			
Soil pH	-0.19	-0.16	-0.21
Organic Carbon	0.78***	0.73***	0.75***
Total Nitrogen	0.71***	0.68**	0.70***
Chlorpyrifos	-0.84***	-0.79***	-0.76***
Cypermethrin	-0.75***	-0.71***	-0.68**
2,4-D	-0.79***	-0.74***	-0.71***
β-glucosidase	0.81***	0.76***	0.78***
Dehydrogenase	0.85***	0.81***	0.79***

***, *, * *indicate significance at p<0.001, p<0.01, and p<0.05, respectively*

4. Result & Discussion:

4.1 Impact of Pesticides on Microbial Diversity

The current study offers some quantitative data that there are significant changes in the microbial diversity of soil as related to the intensive use of pesticides. The exponential decay relationship (R^2 = 0.84) indicates a systematic decline in Shannon diversity with increasing pesticide residues, supporting the hypothesis that chemical stress acts as a major ecological filter. Since the study locations were close to each other, and similar in edaphic features, the observed variation can be explained by exposure to pesticides primarily and not due to the inherent heterogeneity of the soils.

The large decrease in the Shannon diversity (4.1 to 2.8) among the proximate sites indicates that pesticides may be having a more significant influence than the local environmental conditions such as the soil type and temperature. Time revealed that the phenomenon of loss of diversity is not only constant, but can be even aggravated by time. Indicatively, between December 2024 and June 2025, diversity continued to reduce in Site B.

4.2 Insights from Mathematical Modelling

The exponential decline model (H' = 4.23 × e^(-0.029×C)) gives us a way to figure out how much microbial variety will be lost based on the concentration of pesticides. The decay constant (k = 0.029) indicates that microbes in the soil are extremely sensitive to pesticides with some 3 percent of their species count being lost with every one mg/kg increment of pesticide.

The population dynamics models revealed that exposure to pesticides reduces the growth rates as

well as the population of microbes that can survive in an area. The toxicity number (= 0.018) demonstrates that pesticides have moderate and high impact on population dynamics that can affect the ability of communities to recover and restore wellbeing.

4.3 Activity of Enzymes and Loss of Function

The decrease in the soil enzyme activities by 37 to 44 percent in pesticide-heavy areas indicates that the soil organic processes were seriously impaired, and it also means that there was reduced cycling of carbon, the entire metabolic activity, the supply of phosphorus, and the reduction of nitrogen.

Positive significant correlations between enzyme activities and diversity measures (r = 0.780.85) indicate that ecosystem health is related to microbial diversity. According to this connection, it appears that the loss of variation has a direct result of a soil ecosystem that does not work effectively.

4.4 Evidence-Based Interpretation of Microbial Shifts

The statistical model used in this experiment made it possible to objectively identify microbial groups with a consistent response to the intensity of pesticides. Instead of classifying on the basis of previous assumptions, fold-change thresholds and temporal stability were used to classify. Some of the taxa were highly enriched during the high-pesticide location in both seasons indicating ecological selection in the context of chemical stress. On the other hand, those groups that were related to nutrient cycling showed serious losses, which conformed to the decreases in enzyme activities.

Combination of community information and functional indicators increases the biological value of such patterns. Taxa-specific decreases that were correlated with nitrogen conversion were accompanied by reduced urease and dehydrogenase activities, suggesting that compositional changes were reflected in quantifiable functional deficiency. The same associations have been noted in agro-ecosystems under the chronic exposure to pesticides where community simplification is accompanied by metabolic versatility loss.

The study however identifies that response categories are associated with field-level tests and not direct toxicity tests. Potential causes of observed patterns may also include soil properties, past history, and the indirect trophic interactions. Consequently, only the ecological correlations based on the statistical evidence and functioning measurements can have interpretations provided.

4.5 Effects on Function and the Environment

There were indicators of functional suppression of important soil enzymes suggesting that exposure to pesticides in the environment was associated with significant suppression of the enzymes. Reduced β-glucosidase and dehydrogenase activity are indications of decreased carbon turnover and total microbial respiration, and reduced phosphatase and urease activity are indications of limitations on phosphorus mobilization and nitrogen mineralization.

- Nitrogen Cycling Problems: Rhizobium, Bradyrhizobium, and Nitrospira are lost, which reduce the effectiveness of the processes of fixation of nitrogen and nitrification.
- Carbon Cycling Problems: Reduced activity of 2-glucosididase and decreased Carbon-cycling bacteria influence the digestion of organic wastes.
- Availability of phosphorus: A reduced phosphatase activity results in increased difficulty in having phosphorus absorbed by plants.
- Soil Health Degradation: The general decline in the numbers and activity of the microorganisms and enzymes indicate that the biological health of the soil has been undermined.

4.6 Changes in Time and the Potential for Recovery

The time series analysis revealed that there was not much space to recover high pesticide spots. The microbe communities at Site A remained generally similar during successive sample periods whereas those at Site B deteriorated with time. This leaves the implication that the high usage of pesticides can alter the soil ecosystems in permanent fashion that cannot be reversed.

According to the mathematical models, the process of healing would require large drops in the quantity of pesticides applied and increased time to heal the community.

4.7 Implications for Management

Long-term pesticides management plans would come as a good solution to the quantitative associations in the study:

- Determining the limit: The exponential decay model can be used to determine such limits/thresholds of the use of pesticides, which will have minimal environmental impact.
- Monitoring programmes: Microbial diversity indices and enzyme activities as identified in this study can be used as quantitative measures of regular evaluation of agricultural soil biological quality in the presence of various pesticides.
- Recovery Planning: The population dynamic models may be used to prepare plans and schedules of repair.
- Deep Agriculture: Mathematical relationships are used to have selective application of pesticides depending on the biological capacity of the soil.

Although the adjacent-site design limited the problem of edaphic variation, the observational study design is not sufficient to determine causality; therefore, the conclusion will be made through the context of statistically significant relationships.

5. Conclusions

This study offers field-based molecular findings that are largely linked to degradation of soil

microbial biodiversity and functionality in agro-ecosystems across the north of India, which is heavily dependent on intense use of pesticides. The major conclusions are:

1. Quantitative relationship: Microbial diversity exhibited an exponential decline with increasing pesticide residues (R^2 = 0.84), demonstrating a predictable ecological response to chemical intensity.
2. Functional impairment: The key enzyme activities reduced in high-pesticide soils (37-44 percent) and the biomass of microorganisms was lower, which means that the soils were not able to mineralize carbon, transform nitrogen and mobilize phosphorus.
3. Reorganization of the community: The analysis of the distribution of abundance indicated that the changes in composition were similar across seasons and were confirmed by the use of PERMANOVA and the correlation with the pesticide residues.
4. Methodological contribution: The research presented a clear statistical platform of determining the reactions of microbes to pesticides, combining sequencing data with the soil biochemical indicators.
5. Management implications: The findings have highlighted the importance of sound scheduling of pesticides, encouraging the use of bio-pesticides, and frequent observation of soil biological parameters to ensure the sustainability of soil fertility in the long run.

The cause and effect of the future research that involves controlled microcosm experiments plus field measurements will help better understand cause and effect and recovery possibilities of impacted microbial communities.

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