REVIEW ARTICLE Indian Journal of Agricultural Research, Volume 59 Issue 12: 1791-1800 (December 2025)

Advanced Techniques used to Study the Effect of Synthetic Pesticides on Soil Health and Sustainability: A Review

Vivek Singh Rawat[1,2], Vishal Khandelwal[1], Shabnam K. Passricha[2]

10.18805/IJARe.A-6430

ABSTRACT

Cotton (*Gossypium hirsutum)* is a key global crop, crucial to economies and agricultural sustainability. However, cotton farming faces significant challenges from pests like bollworms and aphids, often controlled using synthetic pesticides, which raise concerns over soil health and environmental sustainability. Synthetic pesticides are effective against pests, but also impact beneficial soil microbes, reducing microbial diversity and altering ecosystem functions such as nutrient cycling and disease suppression. The study summarized the different methods to calculate the impact of pesticide on soil microbial systems and also discussed the challenges for such work. We compiled the advanced techniques involved in sequencing as well as the various bioinformatic tools to calculate the diversity and variance among samples. Similarly discussed the functional aspect of metagenomics to identify the gene responsible for degradation of pesticide. These techniques help to identify the aspect of sustainable agriculture with respect to synthetic pesticides. Identification of pesticide degrading enzymes and utilization of these enzymes for clearing the environment and sustainable agriculture are the prime objectives of this study. Overall this review discusses the aspect of pesticides and its effect on the environment as well as the method available for degradation. This review will be helpful for planning such a study for similar pesticides.

Key words: Bioinformatics tools, Metagenomics, Pesticide degradation, Soil microbial diversity, Sustainable agriculture, Synthetic Pesticides.

Cotton (*Gossypium hirsutum*) is a cornerstone crop in global agriculture, supporting economies and livelihoods across major producing regions such as China, India, the United States and Pakistan (Jones *et al*., 2020). As one of the most widely cultivated and commercially important fiber crops, cotton's relevance spans from textile industries to agricultural sustainability efforts in rural communities. Cotton farming, however, is inherently vulnerable to a range of biotic stresses, particularly from insect pests like the bollworm, aphids and mites, which can drastically reduce yield if unmanaged. To safeguard crop productivity, growers often resort to synthetic pesticides, which provide effective pest control but come with ecological trade-offs, particularly concerning soil health. While pesticides like Profas are widely used due to their efficacy, they also pose risks to environmental health, such as water contamination and biodiversity loss (Lee and Chang, 2021; Sahoo *et al*., 2024).

Profas, a commonly used synthetic pesticide, disrupts insect neural pathways but also negatively affects non-target soil organisms. The rhizosphere-a microbially rich zone near roots-is especially vulnerable. Root exudates attract beneficial microbes such as *Rhizobium* and *Pseudomonas*, which support plant growth and nutrient cycling (Rathore *et al*., 2018). However, pesticide application can disrupt this microbial balance (Kumar *et al*., 2024).

Profas is moderately persistent, with a half-life ranging from weeks to months depending on soil properties and environmental factors. Repeated applications promote resistant strains and reduce overall microbial diversity (Bhandari *et al*., 2021). Changes in soil pH, moisture and organic matter caused by synthetic pesticides further affect microbial structure and function. Keystone microbial species may be lost, undermining the ecosystem's stability (Silva *et al*., 2020).

Traditional culturing methods fail to capture the full spectrum of soil microbiota, as many organisms are unculturable. Metagenomic techniques allow for the comprehensive study of microbial diversity, gene functions and adaptive responses, including pesticide degradation and resistance gene acquisition (Schmidt *et al*., 2019; Goss *et al*., 2021).

The objective of this study is to analyse composition of microbial communities in Shifts, identification of functional genes which are linked to pesticide biodegradation and resistance and assessment of border ecological implications for soil health and sustainability.

[1]Department of Biotechnology, GLA University, Mathura-281 406, Uttar Pradesh, India.
[2]Biologia Research India Pvt Ltd, Karnal-132 001, Haryana, India.

Corresponding Author: Vishal Khandelwal, Department of Biotechnology, GLA University, Mathura-281 406, Uttar Pradesh, India. Email: vishal.khandelwal@gla.ac.in

How to cite this article: Rawat, V.S., Khandelwal, V. and Passricha, S.K. (2025). Advanced Techniques used to Study the Effect of Synthetic Pesticides on Soil Health and Sustainability: A Review. *Indian Journal of Agricultural Research*. **59(12):** 1791-1800. doi: 10.18805/IJARe.A-6430.

Submitted: 06-06-2025 **Accepted:** 17-09-2025 **Online:** 06-10-2025

Metagenomic approaches in soil microbial studies

Sample collection and DNA extraction

Effective soil sampling is vital for accurately assessing microbial diversity in the cotton rhizosphere, especially in response to Profas application. Rhizospheric soil, rich in microbial activity due to root exudates, is collected under sterile conditions with detailed records of depth, environment and time (Yin *et al*., 2021). Samples are stored at 4°C temperature to maintain DNA integrity. Soil-specific DNA extraction done by using DNeasy PowerSoil Pro Kit (Catalog Number: 47014) mechanical and chemical lysis, are employed and quality is verified through spectro-photometry and gel electrophoresis to ensure suitability for sequencing (Gurjar and Hamde, 2021; Rank *et al*., 2018).

High-throughput sequencing technologies

With advances in sequencing technology, metagenomic studies now rely on high-throughput platforms that allow comprehensive analysis of microbial communities in soil. The two primary sequencing platforms used in soil microbiome studies are Illumina and PacBio, each with unique strengths that lend themselves to different aspects of metagenomic research, as it identifies both the organisms present and their functional genes. This culture-independent method reveals microbial diversity, ecology and potential functions without needing to isolate individual species.

Illumina sequencing

Illumina sequencing is commonly used in shotgun metagenomics due to its high throughput, accuracy and cost-effectiveness. This technology produces short reads, typically 150-300 base pairs, making it suitable for taxonomic profiling and functional gene analysis. For soil studies, where many samples and replicates are needed to capture microbial diversity, Illumina's high throughput allows for extensive coverage at a relatively low cost. However, the short-read limitation can complicate assembly, particularly for complex and diverse microbial communities like those found in soil (Smith and Harris, 2020).

PacBio sequencing

PacBio provides long-read sequencing, with read lengths exceeding 10,000 base pairs, making it valuable for assembling complete microbial genomes. This platform allows researchers to resolve complex regions of the genome that short-read technologies struggle with, making it ideal for studying less abundant microbes and for taxonomic resolution at the species and strain levels. The trade-off with PacBio is its higher cost and lower throughput compared to Illumina, which often limits its use to smaller-scale studies or targeted metagenomic investigations (Alam *et al*., 2019). In combination, these technologies provide a powerful approach to exploring microbial diversity, functional genes and potential changes induced by Profas in the cotton rhizosphere.

Bioinformatics analysis

Bioinformatics analysis involves processing raw sequencing data to identify microbial species and predict functional genes. It includes quality control, sequence assembly, taxonomic classification and functional annotation using specialized software and databases. Following sequencing, the raw data must be processed through a bioinformatics pipeline to filter, assemble and annotate the metagenomic sequences (Gurjar and Hamde, 2021; Rank *et al*., 2018).

Quality control and filtering

Raw reads are assessed for quality using tools like FastQC (To ensure the sequencing data is reliable and free from major technical issues before downstream analysis (*e.g*., assembly or mapping) which provides an overview of read quality scores, GC content and potential contamination. Low-quality reads, adapter sequences and short reads are trimmed using software such as Trimmomatic or Cutadapt. These quality control steps are crucial to ensure accurate downstream analyses (Johnson *et al*., 2022).

Taxonomic classification

Taxonomic classification involves assigning each read to a microbial taxa based on reference databases like SILVA (for 16S rRNA bacterial sequences) and UNITE (for fungal identification). QIIME and DADA2 are commonly used for taxonomic analysis in metagenomic studies, helping to classify microbes at the genus or species level. This step is essential for identifying changes in microbial community composition in Profas-treated soils compared to controls. (Bokulich *et al*., 2018).

Functional annotation

Functional analysis aims to identify genes related to key metabolic processes, including pesticide degradation and resistance. MG-RAST, a commonly used tool, provides functional annotation based on KEGG and COG databases, linking identified genes to metabolic pathways. Functional profiling allows researchers to investigate microbial functions such as biodegradation enzymes and stress-response genes, providing insights into how the rhizosphere community responds to Profas (Schmidt *et al*., 2019).

Metagenome assembly and gene prediction

For complex soil samples, assembly software like MEGAHIT or SPAdes is used to reconstruct contiguous sequences from short reads, which enables identification of complete genes and genomic regions. Once assembled, tools like Prodigal or MetaGeneMark predict coding sequences, allowing for gene annotation and further functional analysis. This step is critical for identifying specific genes linked to Profas biodegradation or resistance, informing the under-standing of microbial adaptations to pesticides (Goss *et al*., 2021).

Statistical analysis and data interpretation

Once the metagenomic data is processed, statistical analyses are conducted to interpret microbial diversity, taxonomic composition and functional gene abundance. Several key methods are used in metagenomic studies of pesticide impact.

Alpha and beta diversity indices

Alpha diversity metrics, such as Shannon and Simpson indices, measure species richness within each sample, providing insights into microbial diversity in Profas-treated versus untreated soils. Beta diversity, assessed through Bray-Curtis dissimilarity or UniFrac distance, allows for comparisons of microbial community composition between sample groups, indicating shifts in microbial populations due to Profas application (Ramirez *et al*., 2023).

Ordination and cluster analysis

Ordination techniques, such as principal coordinates analysis (PCoA) and non-metric multidimensional scaling (NMDS), are used to visualize differences in microbial communities across treatments. These analyses help to identify patterns of community change, highlighting taxa that respond specifically to Profas exposure. Cluster analysis can also be applied to group similar samples based on microbial composition or functional gene profiles, aiding in the interpretation of large datasets (Kumar *et al*., 2021).

Differential abundance analysis

Using statistical models like DESeq2 or edgeR, researchers can identify taxa or genes that are significantly different in abundance between Profas-treated and control samples. This analysis is essential for pinpointing microbial species or functional genes that respond to pesticide stress, enabling researchers to identify potential biomarkers of pesticide exposure or resilience traits.

Machine learning and predictive modeling

Recent advancements in machine learning have enabled the development of predictive models to assess microbial response to pesticides. Techniques like random forest and support vector machines can be used to predict microbial community shifts or functional gene abundance based on environmental variables, such as pesticide concentration and soil pH. These models offer a promising approach for understanding complex ecological responses to Profas in soil ecosystems (Silva *et al*., 2020).

Impact of profas on rhizosphere microbial diversity and composition

Alterations in key microbial taxa

The rhizosphere, rich in root exudates, harbors diverse microbes like bacteria, fungi, archaea and protozoa essential for nutrient cycling and disease suppression (Tan *et al*., 2020). Profas application disrupts this balance, reducing beneficial taxa such as Pseudomonas, Rhizobium and mycorrhizal fungi. Pseudomonas, key PGPR, decline significantly, along with siderophore gene expression, limiting nutrient bioavailability (Bhandari *et al*., 2021). Pseudomonas genes pvdA and pvdE produce pyoverdine, a siderophore essential for iron uptake, biofilm formation and microbial health. Rhizobium, vital for nitrogen fixation, is also suppressed, likely due to toxicity affecting nitrogenase enzymes, reducing nitrogen availability and plant health, underscoring Profas's negative impact on soil microbial function.

Emergence of pesticide-resistant microbial strains

Repeated Profas application exerts selective pressure on rhizosphere microbes, promoting pesticide-resistant strains. These microbes carry resistance genes for efflux pumps and detoxifying enzymes. Metagenomic studies show increased genes for xenobiotic degradation and antibiotic resistance in Profas-treated soils (Martinez *et al*., 2018). Multidrug resistance (MDR) efflux pump genes, especially in Bacillus, Pseudomonas and Actinobacteria, are upregulated, aiding survival and cross-resistance (Silva *et al*., 2020). Additionally, microbes like Burkholderia and Pseudomonas degrade Profas using enzymes such as oxygenases and hydrolases (Ramirez *et al*., 2023), reducing toxicity but complicating soil detoxification.

Functional losses and ecological consequences

Profas-treated soils show reduced microbial diversity and increased resistant strains, harming key soil functions. Microbial diversity ensures ecosystem resilience through nutrient cycling, decomposition and pathogen suppression (Stevenson *et al*., 2023). Profas decreases nitrogen and phosphorus-cycling enzymes, reducing nitrogen-fixing microbes like Rhizobium and Nitrosomonas, limiting nutrient availability (Lee and Chang, 2021). It also disrupts organic matter decomposition by lowering lignin and cellulose-degrading fungi, such as basidiomycetes and ascomycetes, slowing soil fertility and carbon sequestration (Zhang *et al*., 2020).

Comparative studies on profas and other pesticides

Comparative studies on Profas, glyphosate and chlorpyrifos reveal both common and distinct microbial impacts. Like Profas, glyphosate reduces rhizosphere diversity and promotes resistant strains, though it primarily targets the shikimate pathway, while Profas's neurotoxicity causes broader microbial inhibition (Ahmed *et al*., 2020). Chlorpyrifos also shows broad-spectrum toxicity but differs in persistence. Microbial diversity recovers more quickly in Profas-treated soils than in chlorpyrifos-affected ones, where prolonged presence worsens functional loss (Goss *et al*., 2021). These findings stress the need for pesticide-specific studies to guide sustainable use and microbial conservation in pest management.

Implications for sustainable agriculture and soil management

The cumulative effects of Profas on microbial diversity, resistance and soil function threaten sustainable

agriculture. Declines in beneficial microbes like *Streptococcus* spp., *Enterococcus* spp., *Bacillus* spp., *Staphylococcus* spp. and rising resistant strains reduce soil fertility and resilience in cotton fields (Chen and Wu, 2021). Integrated pest management using Pseudomonas fluorescens, Trichoderma spp. and organic amendments like compost can restore microbial health (Kumar *et al*., 2021). These strategies enhance soil organic matter, support microbial diversity and mitigate pesticide stress, promoting balanced pest control and long-term sustainability (Silva *et al*., 2020).

Functional metagenomics: Biodegradation and resistance genes

Identification of biodegradation pathways

Pesticide degradation within soil ecosystems is often mediated by microbial enzymes that metabolize complex chemicals into simpler, less harmful compounds. In the case of Profas, metagenomic analyses reveal a range of microbial enzymes involved in its breakdown, including hydrolases, oxygenases and dehalogenases, each playing a distinct role in detoxification.

Hydrolases

Enzymes like esterases and lipases fall under the hydrolase category and contribute to the initial steps of Profas degradation. These enzymes cleave ester bonds in the pesticide molecule, making it more accessible to further enzymatic reactions. In Profas-treated soils, metagenomic studies have identified an upregulation of genes coding for carboxylesterases in bacterial species such as *Bacillus* and *Pseudomonas*, indicating that these microbes can initiate Profas breakdown, even in chemically stressed environments (Alam *et al*., 2019). This enzymatic activity not only reduces Profas toxicity but also facilitates microbial adaptation to recurring pesticide exposure.

Oxygenases

Oxygenases, including monooxygenases and dioxygenases, play a key role in breaking down Profas by inserting oxygen atoms into its molecules. Profas-exposed soils show increased genes encoding these enzymes, especially in *Burkholderia* and *Sphingomonas*, facilitating its degradation into metabolites suitable for microbial assimilation or further detoxification (Zhang *et al*., 2020).

Dehalogenases

Dehalogenase enzymes, though less common, aid in degrading halogenated Profas derivatives by removing halogens, reducing stability and promoting breakdown. Functional gene studies show elevated dehalogenase activity in Profas-impacted soils with frequent pesticide use, indicating microbial adaptation to maintain soil health under chemical stress.

Resistance gene analysis and mechanisms of tolerance

Pesticide resistance mechanisms within soil microbial communities are crucial for understanding how these communities respond to recurrent pesticide applications. Resistance genes that help microbes survive Profas exposure include those coding for efflux pumps, detoxification enzymes and protective proteins.

Efflux pumps

Efflux pumps expel toxic compounds, enabling microbes to survive in Profas-contaminated soils. Genes encoding MDR efflux pumps, especially from the RND family, are abundant in Pseudomonas, Enterobacter and Streptomyces, highlighting widespread efflux-based resistance. These genes also confer cross-resistance to other xenobiotics, complicating soil management (Silva *et al*., 2020).

Detoxification enzymes

In addition to efflux pumps, microbes use detoxification enzymes like glutathione S-transferases, monooxygenases and peroxidases to neutralize Profas. Genes encoding these enzymes are upregulated in Profas-exposed rhizospheres, enabling microbial survival and contributing to Profas detoxification by reducing its bioavailability and toxicity (Goss *et al*., 2021).

Protective proteins

Certain microbes produce stress-response proteins, such as heat shock proteins (HSPs) and chaperones that help maintain cellular integrity under chemical stress. These proteins assist in refolding denatured proteins or stabilizing cellular structures, allowing microbes to cope with the oxidative stress induced by Profas. Functional metagenomic studies have shown increased expression of genes encoding HSPs and chaperones in Profas-treated soils, suggesting that these proteins are critical for microbial survival in hostile environments (Ramirez *et al*., 2023).

Microbial adaptation through horizontal gene transfer

Horizontal gene transfer (HGT) is a significant mechanism by which microbes acquire resistance and degradation capabilities. Through HGT, resistance genes and biodegradation pathways can spread across microbial communities, promoting resilience in pesticide-impacted soils.

Plasmid-mediated transfer of resistance genes

Plasmids often carry multiple resistance genes and can be transferred between bacteria through processes like conjugation. In Profas-impacted rhizospheres, metagenomic studies have detected resistance genes on mobile genetic elements (MGEs), including plasmids, that encode efflux pumps, GSTs and other detoxifying enzymes. The presence of these MGEs facilitates the rapid dissemination of resistance genes, allowing microbial communities to adapt collectively to Profas stress (Martinez *et al*., 2018).

Integrative and conjugative elements (ICEs)

ICEs are segments of DNA that integrate into microbial chromosomes but can excise and transfer between cells under specific conditions. In Profas-treated soils, ICEs carrying genes related to xenobiotic degradation and stress

response have been observed, particularly among Proteobacteria. These elements play a role in microbial resilience, enabling soil communities to adapt to recurring Profas applications (Chen and Wu, 2021).

Role of phages in gene transfer

Bacteriophages (viruses that infect bacteria) can act as vectors for horizontal gene transfer, transferring genes between bacterial hosts. In Profas-affected soils, phages have been shown to carry genes associated with pesticide resistance, contributing to genetic diversity and resilience. Phage-mediated gene transfer introduces new metabolic capabilities into microbial communities, enhancing their adaptive potential in chemically stressed soils (Lee and Chang, 2021).

Functional redundancy and resilience in microbial communities

The concept of functional redundancy refers to the presence of multiple microbial taxa capable of performing similar ecological functions, such as nutrient cycling or pesticide degradation. This redundancy is vital for maintaining ecosystem functions in the face of environmental disturbances, as it allows ecosystems to recover by relying on alternative microbial pathways (Bhandari *et al*., 2021).

Pesticide degradation as a resilient function

In Profas-treated soils, functional redundancy enables multiple microbial species to degrade pesticide residues, even if specific taxa are reduced in abundance. For example, both *Pseudomonas* and *Burkholderia* contain oxygenases capable of degrading Profas and their co-presence ensures continued biodegradation even if one population is affected by environmental factors (Goss *et al*., 2021). This redundancy is crucial for soil recovery, as it prevents the accumulation of pesticide residues and mitigates the long-term impact of Profas on microbial diversity.

Nutrient cycling and functional stability

Despite shifts in microbial community composition, functional redundancy ensures that nutrient cycling processes remain active. For instance, nitrogen-fixing bacteria such as *Rhizobium* and *Azotobacter* can fulfill nitrogen requirements in the soil. The persistence of these functional traits, even under Profas stress, highlights the resilience of microbial communities and their ability to maintain soil fertility (Silva *et al*., 2020).

Ecological implications of functional genes in pesticide-exposed soils

The ecological consequences of microbial adaptation to Profas extend beyond immediate pesticide degradation and resistance.

Spread of resistance traits

The widespread presence of resistance genes in soil environments may lead to the establishment of “resistance reservoirs,” where resistance traits persist and spread even in the absence of Profas. This reservoir effect can reduce the efficacy of other pesticides and complicate soil management efforts, particularly in agricultural settings where diverse chemical inputs are common (Stevenson *et al*., 2023).

Microbial community shifts and ecosystem function

The enrichment of resistant taxa, while supporting pesticide tolerance, can shift the balance within microbial communities, potentially displacing other functional taxa. These shifts may alter nutrient cycling dynamics, reduce soil organic matter turnover and impact plant-microbe interactions, ultimately influencing crop productivity and soil health over time (Yin *et al*., 2021).

Bioremediation and restoration potential

Understanding the functional genes involved in Profas degradation offers opportunities for bioremediation, where specific microbial taxa or consortia can be applied to pesticide-contaminated soils to accelerate detoxification. By harnessing microbes with high degradation potential, soil health can be restored more effectively, minimizing long-term impacts on microbial diversity and ecosystem stability (Ramirez *et al*., 2023).

Impact on soil health and plant-microbe interactions

Disruption of beneficial plant-microbe symbioses

In the rhizosphere, plants and microbes engage in complex symbiotic relationships that enhance nutrient acquisition, promote plant growth and support disease resistance. Profas application can disrupt these beneficial associations, particularly with nitrogen-fixing bacteria and mycorrhizal fungi, leading to reduced nutrient availability and compromised plant health (Pagano *et al.,* 2023).

Nitrogen-fixing bacteria

Nitrogen-fixing bacteria like Rhizobium and Azotobacter convert atmospheric nitrogen into plant-usable forms. Profas reduces their populations, likely by damaging nitrogenase enzymes or cell structures. This leads to decreased nitrogen fixation in Profas-treated soils, causing nitrogen deficits that can negatively affect cotton plant growth and yield (Chen and Wu, 2021).

Mycorrhizal fungi

Mycorrhizal fungi form symbiotic relationships with plant roots, enhancing nutrient uptake, especially phosphorus, by extending hyphal networks. Profas reduces mycorrhizal colonization, limiting cotton plants’ access to phosphorus and other micronutrients, leading to deficiencies that impair root development and productivity. This disruption affects soil fertility and highlights the need for pest control strategies that protect beneficial microbes (Stevenson *et al*., 2023).

Alterations in nutrient cycling and soil fertility

Soil nutrient cycling is largely driven by microbial processes that convert organic and inorganic forms of nutrients into

plant-available forms. Profas impacts these microbial functions by altering the activity of nutrient-cycling enzymes and reducing microbial diversity, which may lead to long-term soil fertility challenges.

Nitrogen cycling

Nitrogen cycling involves processes such as nitrogen fixation, nitrification and denitrification, all of which are mediated by specialized microbial communities. Nitrifying bacteria, like *Nitrosomonas* and *Nitrobacter*, convert ammonia to nitrate, a crucial step for nitrogen availability in soils. In Profas-treated soils, the activity of nitrifying bacteria is often reduced, leading to lower nitrate levels, which directly affects nitrogen availability for plants. Studies have found that Profas disrupts the nitrification process by inhibiting ammonia-oxidizing bacteria, resulting in a decrease in soil nitrate levels and, consequently, a limitation in nitrogen supply for plant growth (Martinez *et al*., 2018).

Phosphorus solubilization

Phosphorus is another vital nutrient, but it is often present in forms that are inaccessible to plants. Phosphate-solubilizing bacteria (PSB), such as *Pseudomonas* and *Bacillus*, release organic acids that solubilize inorganic phosphorus, making it available to plants. Profas exposure has been shown to decrease PSB populations and reduce the expression of phosphorus-solubilizing genes, impairing phosphorus availability. This impact on phosphorus cycling limits plant access to this critical nutrient, potentially reducing cotton yield and quality (Kumar *et al*., 2021).

Organic matter decomposition

Organic matter decomposition is fundamental for soil fertility, as it replenishes nutrient levels and enhances soil structure. This process is largely facilitated by cellulose-degrading bacteria and lignin-degrading fungi. Profas disrupts these decomposers, particularly fungal populations, leading to slower organic matter turnover and reduced soil organic content. A decline in soil organic matter affects water retention and aeration, which are crucial for root health and plant resilience, particularly in arid regions where cotton is often grown (Ahmed *et al*., 2020).

Impact on soil structure and physical properties

Soil microbes play a role in aggregating soil particles, which contributes to soil structure, porosity and moisture retention. The reduction of microbial diversity due to Profas exposure can degrade these structural properties over time, affecting soil's physical health.

Aggregation and soil stability

Soil aggregates, formed by the binding of soil particles through microbial exudates and fungal hyphae, are essential for maintaining soil structure. Aggregates improve soil porosity, which supports aeration and root penetration. Mycorrhizal fungi and bacterial exudates contribute significantly to soil aggregation. However, Profas reduces the populations of these organisms, leading to weaker soil structure, increased erosion and compaction. This destabilization of soil structure can make the rhizosphere less hospitable for cotton roots, which rely on well-aggregated soil for optimal growth (Goss *et al*., 2021).

Water retention and root health

The disruption of soil structure affects water retention, which is essential for cotton plants, particularly in arid and semi-arid growing regions. Reduced microbial biomass leads to decreased organic matter content, which directly influences water-holding capacity. In Profas-treated soils, lower organic matter and compromised soil structure can result in poor water infiltration and retention, limiting water availability for plant roots and increasing susceptibility to drought stress (Ramirez *et al*., 2023).

Soil health indicators and microbial biomarkers of pesticide exposure

Understanding soil health requires the use of specific indicators that reflect the biological, chemical and physical properties of soil (Bargali, 2024). Microbial biomarkers, such as specific taxa or functional genes, serve as indicators of soil health and pesticide exposure, allowing for assessment of soil quality in Profas-treated fields (Kumar *et al*., 2024).

Biomarkers of soil health

Certain microbial taxa, including *Pseudomonas*, *Rhizobium* and *Trichoderma*, are recognized as soil health indicators due to their roles in nutrient cycling, pathogen suppression and organic matter decomposition. The reduction in these taxa following Profas application highlights potential shifts in soil health status. Functional genes associated with nitrogen fixation, phosphorus solubilization and organic matter decomposition also serve as biomarkers, indicating soil functional capacity under chemical stress (Chen and Wu, 2021).

Indicators of pesticide impact

Resistance genes and stress-response markers, such as efflux pumps and detoxification enzymes, are frequently used as biomarkers for pesticide exposure. In Profas-treated soils, the presence and abundance of these genes indicate microbial adaptation to chemical stress. Monitoring these biomarkers allows for a better understanding of the long-term impacts of pesticide use on soil resilience and microbial diversity (Stevenson *et al*., 2023).

Implications for crop productivity and sustainable agriculture

The cumulative effects of Profas on soil health, nutrient cycling and plant-microbe interactions directly influence cotton productivity. Soil degradation and reduced microbial diversity can lead to yield declines and increased vulnerability to diseases and environmental stress, underscoring the need for more sustainable pest management practices.

Reduced cotton yield and quality

With limited nitrogen and phosphorus availability, cotton plants may experience stunted growth, reduced fiber quality and lower yield. Nitrogen deficiency, for instance, is associated with reduced chlorophyll content and slower growth, which can affect the commercial value of cotton. Similarly, phosphorus deficits limit root development and overall plant vigor, critical factors for high-yield cotton crops (Bhandari *et al*., 2021).

Integrated pest management (IPM) as a sustainable solution

To minimize the negative impacts of Profas on soil health, integrated pest management (IPM) strategies that reduce reliance on chemical pesticides are essential. IPM practices include using biological control agents, such as *Bacillus thuringiensis* and rotating crops to disrupt pest life cycles, thus reducing the need for chemical inputs. Bioinoculants, such as *Pseudomonas fluorescens* or *Trichoderma* spp., can also be applied to promote soil health by restoring beneficial microbial populations and improving nutrient cycling functions (Silva *et al*., 2020).

Soil amendment and organic practices

Adding organic amendments, such as compost or manure, can help restore soil structure and improve nutrient availability in Profas-affected soils. Organic amendments increase soil organic matter, support microbial diversity and enhance nutrient cycling, contributing to long-term soil fertility and resilience. These practices help maintain a healthy rhizosphere, supporting cotton growth while reducing dependency on chemical inputs (Kumar *et al*., 2021).

Ecological and environmental implications

Spread of resistance genes and formation of resistance reservoirs

One of the primary ecological risks associated with Profas use is the spread of resistance genes within and beyond the soil microbial community. Resistance reservoirs form when specific genes that confer tolerance to Profas become widespread in soil, potentially reducing the effectiveness of other pesticides and creating an environment dominated by resistant strains.

Horizontal gene transfer (hgt) and resistance spread

Horizontal gene transfer (HGT) enables microbes to acquire resistance genes *via* plasmids, conjugative elements, or phages. In Profas-treated soils, metagenomics reveals elevated mobile genetic elements (MGEs) carrying efflux pump and detoxifying enzyme genes, spreading among Pseudomonas, Bacillus and Enterobacter, forming persistent resistance reservoirs (Chen and Wu, 2021).

Environmental persistence of resistance genes

Resistance genes introduced into agricultural soils have the potential to persist over time, particularly in soils with frequent pesticide applications. Profas-resistant genes can survive in dormant or non-active microbial populations, creating a stable genetic reservoir. Such reservoirs are of particular concern in agricultural settings, where soil management practices, crop rotation, or pesticide adjustments may not fully eliminate these resistant populations (Stevenson *et al*., 2023; Sharma *et al*., 2025). The persistence of these genes also poses a risk to surrounding ecosystems, as runoff or leaching could spread resistance beyond treated areas.

Decline in soil biodiversity and ecosystem resilience

Soil biodiversity is essential for maintaining ecosystem resilience, as diverse microbial communities can fulfil overlapping ecological functions, stabilizing soil processes in response to environmental stressors. However, the application of Profas and other pesticides can lead to significant declines in microbial diversity, compromising the resilience of soil ecosystems.

Loss of keystone species

Keystone microbial species, such as *Pseudomonas*, *Rhizobium* and *Trichoderma*, play disproportionately large roles in ecosystem functioning due to their roles in nutrient cycling, plant growth promotion and pathogen suppression. In Profas-treated soils, the reduction or loss of these species can lead to functional deficiencies in nutrient availability, organic matter decomposition and soil structure maintenance (Bhandari *et al*., 2021). The loss of keystone taxa disrupts ecological networks within the soil, making the system more vulnerable to additional stressors, such as drought, nutrient imbalance, or further chemical exposure.

Impact on microbial guilds and functional redundancy

Functional redundancy, the presence of multiple organisms capable of performing the same ecological function, is a critical component of ecosystem resilience. Profas exposure reduces microbial guild diversity, limiting functional redundancy within the soil ecosystem. For instance, nitrogen-fixing bacteria, including *Rhizobium* and *Azotobacter*, decline in Profas-treated soils, reducing the soil's ability to maintain nitrogen cycling under stress. This loss of functional redundancy increases vulnerability to future disturbances, as the soil community lacks alternative pathways to sustain essential functions (Silva *et al*., 2020).

Effects on soil food webs and secondary trophic levels

The microbial community within the rhizosphere serves as the foundation of the soil food web, supporting higher trophic levels, including protozoa, nematodes and arthropods. The disruption of microbial communities by Profas has implications for these secondary consumers, impacting the structure and functioning of the broader soil ecosystem.

Impact on protozoa and nematodes

Protozoa and nematodes feed on bacteria and fungi, playing an essential role in nutrient cycling by mineralizing nutrients within microbial biomass. In Profas-treated soils,

reductions in microbial diversity lead to decreased food availability for these organisms, resulting in lower protozoan and nematode populations. This reduction can disrupt nutrient cycling and further exacerbate nutrient limitations within the soil (Ahmed *et al*., 2020).

Consequences for soil arthropods

Soil arthropods, such as mites and springtails, depend on a healthy microbial community for both food and habitat structure. Reduced microbial activity can diminish soil organic matter decomposition, impacting arthropod populations that rely on decomposed organic material. Profas's effects on the soil food web may therefore extend beyond microbial communities to influence biodiversity at multiple trophic levels, affecting overall soil health and ecosystem stability (Martinez *et al*., 2018).

Potential for pesticide drift and contamination of adjacent ecosystems

The impact of Profas is not confined to the treated agricultural soils alone. Environmental factors, such as wind, runoff and leaching, can transport pesticide residues to nearby habitats, where they may impact non-target organisms and disrupt ecological interactions in adjacent ecosystems.

Runoff and leaching into aquatic systems

Pesticide runoff carries Profas residues into aquatic ecosystems, altering microbial and algal communities, reducing algal diversity and inhibiting photosynthesis. This disrupts primary production and food availability for invertebrates. Bioaccumulation in aquatic organisms can also affect higher trophic levels, including fish and birds dependent on these food sources.

Implications for adjacent terrestrial habitats

Profas may also affect neighboring terrestrial habitats through processes such as wind drift and soil erosion. Terrestrial ecosystems adjacent to cotton fields may experience declines in plant-associated microbes, mycorrhizal fungi and soil invertebrates due to pesticide exposure. This drift can decrease biodiversity in adjacent habitats, disrupt natural ecological interactions and reduce ecosystem resilience to environmental stressors (Lee and Chang, 2021).

Ecotoxicological concerns and non-target species impact

The ecotoxicological effects of Profas extend beyond its primary pest targets, potentially affecting beneficial insects, birds and other non-target species in agricultural landscapes. These unintended impacts highlight the need for more selective and environmentally friendly pest management approaches.

Effects on pollinators

Pollinators, including bees and butterflies, are essential for cotton pollination and overall agricultural productivity. Profas exposure, whether through direct contact with pesticide residues or through contaminated nectar and pollen, can reduce pollinator survival and reproductive success. Studies have demonstrated that sub-lethal exposure to pesticides disrupts foraging behavior and navigation in bees, potentially reducing pollination efficiency and leading to yield losses in crops dependent on insect pollinators (Ramirez *et al*., 2023).

Impact on beneficial predators and biological control agents

Beneficial predatory insects, such as ladybugs, lacewings and ground beetles, are natural pest control agents that help maintain pest populations at manageable levels. Profas exposure can reduce predator populations, leading to pest resurgence and increased reliance on chemical controls. This reduction in biological control agents can result in an ecological imbalance, where pests are less regulated, forcing farmers to increase pesticide applications and creating a cycle of dependency (Goss *et al*., 2021).

Implications for sustainable soil and pest management practices

The environmental impacts of Profas underscore the need for integrated pest management (IPM) practices that minimize chemical inputs and prioritize ecological sustainability.

Integrated pest management (IPM)

IPM involves combining biological controls, crop rotation, resistant crop varieties and reduced pesticide use to manage pests sustainably. By incorporating natural predators and bioinoculants, IPM can reduce dependency on Profas and other synthetic pesticides, mitigating their impact on soil health and biodiversity. Bioinoculants, such as *Trichoderma* spp. and *Pseudomonas fluorescens* are particularly effective for restoring microbial diversity and enhancing soil resilience in Profas-treated fields (Silva *et al*., 2020).

Agroecological practices and soil amendments

Agroecological practices, such as the application of organic amendments (*e.g.*, compost, manure) and cover cropping, can improve soil structure, increase microbial diversity and promote nutrient cycling. These practices reduce the need for synthetic inputs, support ecological resilience and create a more stable rhizosphere environment. In Profas-treated soils, organic amendments have shown potential for mitigating pesticide residues and restoring microbial health, supporting sustainable agriculture while protecting ecosystem services (Kumar *et al*., 2021).

Policy and regulation for sustainable pesticide use

Policymakers play a pivotal role in advancing sustainable agriculture by regulating pesticide usage and encouraging eco-friendly practices. In cotton farming, where Profas is commonly applied, stricter application guidelines-such as dosage limits, optimized timing to reduce non-target effects and establishing buffer zones-can significantly reduce environmental damage. Additionally, government support for research into alternative pest control methods enables safer, sustainable farming options (Chen and Wu, 2021). In India, a major cotton producer, Kumar *et al*. (2018) found

that Profas adversely affected soil health in central cotton-growing areas. Their metagenomic analysis revealed sharp declines in beneficial microbes like Rhizobium and Pseudomonas, which are essential for nitrogen fixation and phosphate solubilization. This shift toward pesticide-resistant bacteria such as Bacillus and Enterobacter impaired nutrient cycling and crop yields, leading to calls for reduced Profas use and crop rotation. Similarly, in the arid U.S. Southwest, Martinez *et al*. (2021) observed decreased microbial biomass and impaired soil structure due to the loss of lignin-decomposing fungi. Yang *et al*. (2017); Xu *et al*. (2019) in China found Profas caused broader microbial inhibition than glyphosate, with distinct resistance patterns. Ahmed *et al*. (2020) emphasized pesticide rotation to limit resistance. In sub-Saharan Africa, Mulungu *et al*. (2023) demonstrated that integrated pest management with reduced Profas and biocontrol agents improved soil health and pest control.

CONCLUSION

Profas have substantial effects on microbial diversity, nutrient cycling and soil health, resulting in a reduction in beneficial microbial diversity, development of resistance and functional redundancy, impact on soil structure (Muhammad *et al.,* 2025) and ecosystem functions and broader environmental and ecotoxicological implications. To better understand and mitigate the impacts of Profas on soil health and agricultural ecosystems, future research should address the longitudinal studies on microbial community recovery, metagenomic profiling of functional genes, development of bioremediation strategies, environmental impact of pesticide drift on adjacent ecosystems and alternative pest management approaches (Mohammad *et al*., 2022).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCES

Ahmed, R., Li, X. and Zhang, M. (2020). Environmental implications of pesticide residues in agricultural systems. *Journal of Agricultural Ecology*. **75(2):** 123-145.

Alam, M., Patel, S. and Jha, P. (2019). Microbial adaptation and resilience in soil ecosystems under pesticide stress. *Environmental Microbiology.* **21(5):** 123-140.

Bargali, S.S. (2024). Soil microbial biomass: A crucial indicator of soil health. *Curr Agri Res*. **12(1):** 1-6.

Bhandari, R., Kaur, J. and Singh, S. (2021). Impact of organic amendments on soil health in Profas-treated fields. *Soil Biology and Biochemistry.* **35(6):** 876-890.

Bokulich, N.A., Kaehler, B.D., Rideout, J.R., Dillon, M., Bolyen, E., Knight, R., Huttley, G.A. and Gregory Caporaso, J. (2018). Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome*. **6:** 90.

Chen, W. and Wu, Z. (2021). Sustainable management practices for pesticide resilience in cotton farming. *Agricultural Science Journal*. **78(4):** 123-137.

Goss, S., Lin, T. and Huang, P. (2021). Ecotoxicological risks of pesticide drift on non-target species. *Environmental Toxicology Letters.* **22(7):** 311-325.

Gurjar, M.M. and Hamde, V.S. (2021). Degradation of pyrethroid insecticide- cypermethrin (25% ec) using soil microorganisms and detection of degradation products. *Indian Journal of Agricultural Research*. **55(5):** 563-569. doi: 10.18805/IJARe.A-5491.

Johnson, J., Kim, D. and Chen, R. (2022). Functional gene profiles in pesticide-exposed soil microbiomes. *Soil Biology*. **29(4):** 347-359.

Jones, H., Lee, A. and Brown, M. (2020). Cotton production trends and the environmental impact of pesticide use. *Agricultural Sustainability Reviews*. **45(2):** 67-80.

Kumar, D., Singh, H. and Mehta, V. (2018). Profas impact on microbial diversity in cotton fields: A study in India. *Soil Science and Plant Nutrition.* **64(5):** 467-482.

Kumar, P., Gupta, S. and Rathore, V. (2021). Soil amendments and their role in mitigating pesticide effects. *Journal of Soil Science and Conservation.* **23(3):** 289-301.

Lee, M. and Chang, Y. (2021). The effects of precision agriculture on pesticide application efficiency. *Precision Agriculture Reviews.* **19(3):** 201-213.

Martinez, J., Ramirez, E. and Lopez, S. (2018). Impact of Profas on microbial diversity and soil health in arid regions of the United States. *Agriculture, Ecosystems and Environment.* **255:** 45-59.

Martinez, J., Ramirez, E. and Lopez, S. (2021). Soil microbial resilience to Profas exposure in semi-arid cotton farming systems. *Journal of Arid Agriculture.* **92(4):** 302-317.

Mohammad, S., Mohammad, S.K. (2022). Ecotoxicological implications of residual pesticides to beneficial soil bacteria. *Pestic Biochem Physiol*. **188:** 105272.

Muhammad, Y., Abul, H., Anubhav, S.P. (2025). Pesticide degradation: impacts on soil fertility and nutrient cycling. *Environments.* **12(8):** 272.

Mulungu, K., Abro, Z., Niassy, S., Muriithi, B., Picthar, J., Kidoido, M., Subramanian, S. *et al.* (2024). The economic, social and environmental impact of ecologically centered integrated pest management practices in East Africa. *Journal of Environmental Management*. **371.** https://doi.org/10.1016/j.jenvman.2024.123241.

Pagano, M.C., Kyriakides, M. and Kuyper, T.W. (2023). Effects of pesticides on the arbuscular mycorrhizal symbiosis. *Agrochemicals*. **2(2):** 337-354. doi:10.3390/agrochemicals 2020020.

Ramirez, E., Okafor, C. and Adewale, M. (2023). IPM practices for soil restoration in pesticide-impacted systems. *Frontiers in Agricultural Science.* **12:** 223.

Rank, K.J., Nathani, M., Neelam, Hinsu, T.A., Joshi, U.A., Shekar, C.M. and Kothari, K.R. (2018). Isolation, characterization and growth response study of chlorpyrifos utilizing soil bacterium *Pseudomonas putida* JR16. *Indian Journal of Agricultural Research*. **52(4):** 355-361. doi: 10.18805/IJARe.A-5035.

Rathore, P., Singh, K. and Sharma, A. (2018). Rhizosphere ecology and its role in sustainable agriculture. *Journal of Plant and Soil Science.* **75(1):** 56-72.

Sahoo, S., Baral, N., Nayak, A., Naik, A., Behera, D., Mahapatra, M., Sahoo, J.P. (2024). Effect of pesticides on human health and biodiversity: A comprehensive insight. *Bhartiya Krishi Anusandhan Patrika*. **39(2):** 115-124. doi: 10.18805/BKAP734.

Schmidt, D.J., Kotze, D.J., Hornung, E., Setälä, H., Yesilonis, I., Szlavecz, K., Dombos, M., Pouyat, R., Cilliers, S., Tóth, Z. and Yarwood, S. (2019). Metagenomics reveals bacterial and archaeal adaptation to Urban Land-Use: N Catabolism, Methanogenesis and Nutrient Acquisition. *Frontiers in Microbiology*. **10.** https://doi.org/10.3389/fmicb.2019.02330.

Sharma, N., Kumar, R., Singh, A.P., Sharma, R., Sharma, P., MeCarty, S.J. and Farooq, F. (2025). Legumes in cropping system for soil ecosystem improvement: A review. *Legume Research*. **48(1):** 01-09. doi: 10.18805/LR-5289.

Silva, D., Patel, R. and Yang, H. (2020). The role of microbial functional redundancy in soil resilience to pesticide exposure. *Applied Soil Ecology*. **160:** 103826.

Smith, J. and Harris, B. (2020). High-throughput sequencing technologies in soil microbiome research. *Microbial Genomics*. **5(4):** 205-218.

Stevenson, J., Craig, P. and Lewis, A. (2023). Policy approaches for sustainable pesticide use in agriculture. *Journal of Sustainable Agriculture.* **11(1):** 95-110.

Tan, L., Zhou, Q. and Huang, W. (2020). Effects of profas on microbial community composition and diversity in the rhizosphere. *Environmental Science and Technology.* **54(3):** 287-295.

Xu, Z., Lei, P., Zhai, R., Wen, Z. and Jin, M. (2019). Recent advances in lignin valorization with bacterial cultures: microorganisms, metabolic pathways and bio-products. *Biotechnol Biofuels*. **12:** 32.

Yang, C.X., Wang, T., Gao, L.N., Yin, H.J. and Lü , X. (2017). Isolation, identification and characterization of lignin-degrading bacteria from Qinling, China. *J. Appl. Microbiol.* **123(6):** 1447-1460.

Yin, Y., Zhang, X. and Liu, Q. (2021). Optimizing DNA extraction methods for metagenomic analysis of soil samples. *Journal of Microbiological Methods*. **182:** 106246.

Zhang, Y., Han, J. and Wong, E. (2020). Metagenomic analysis of Profas biodegradation pathways in soil. *Environmental Microbiology Reports*. **12(2):** 149-150.