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Microbial Dynamics in Cotton Soils under Profas Application

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ABSTRACT

Background: Cotton (*Gossypium hirsutum*) is one of the world's most important crops, both economically and agriculturally. While fertilizers and supplements such as Profas are widely used to improve crop productivity, their impact on soil health and microbial communities is not fully understood. This study examined how Profas influences soil microbial diversity and composition in cotton fields. Using V3-V4 amplicon sequencing of the 16S rRNA gene on the Illumina MiSeq platform, microbial communities from soils treated with Profas at 10 ppm and 20 ppm were compared with untreated controls.

Methods: The analysis revealed that Profas application led to marked shifts in microbial populations. Proteobacteria, especially Gammaproteobacteria and members of the Pseudomonadaceae, increased in abundance, whereas overall microbial diversity declined. These patterns were linked to changes in soil nutrient dynamics, pH and the inherent antibacterial properties of Profas. To further explore its impact, nine bacterial strains were isolated and screened for their ability to degrade Profas *in vitro*. Among these, *Prestia flexus* emerged as the most efficient degrader.

Result: The results indicate that Profas not only reshapes soil microbial communities but may also pose ecological risks by reducing biodiversity. At the same time, the identification of microbial strains capable of breaking down Profas highlights a promising approach for the bioremediation of its residues. Taken together, these findings provide new insights into the interactions between synthetic inputs and soil microbes, emphasizing the need for balanced fertilizer practices that support both crop productivity and long-term soil sustainability.

Key words: Bioremediation, Cotton, Metagenomics, Profas, Soil microbes.

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is one of the most important fiber crops worldwide and plays a central role in the agricultural economies of countries such as India, China, Pakistan and the United States. Apart from its value to the textile industry, cotton contributes significantly to rural employment and income generation (Jones *et al*., 2020). With increasing global demand for food, fuel and fiber, cotton production systems have become more intensive, relying heavily on improved varieties, biotechnology and chemical inputs. Herbicide-resistant cotton has been widely adopted to simplify weed management, but continuous herbicide use has led to the development of resistant weed populations (Sathish *et al*., 2022). In some cotton-growing regions, ineffective weed control has resulted in yield losses approaching 90%, demonstrating the urgent need for more sustainable and integrated weed management strategies (Manalil *et al*., 2017).

Insect pests such as bollworms, whiteflies, mites and aphids also pose major threats to cotton productivity. To protect crops from these pests, synthetic pesticides remain the most commonly used tools because of their quick action and reliability (Shipa *et al*., 2021). Global cotton production reached nearly 26 million metric tons in 2018 and much of this yield was maintained through regular pesticide use (Smith *et al*., 2020). However, frequent application of pesticides raises environmental concerns. Studies indicate that repeated pesticide exposure may temporarily or permanently alter soil microorganisms, even when some microbial groups appear to recover after application (Vig *et al*., 2008). Long-term pesticide use can also reduce biodiversity, contaminate soil and water and affect non-target organisms, making it essential to evaluate their wider ecological impacts (Lee *et al*., 2021).

Soil is a biologically active system whose fertility depends on microbial communities responsible for nutrient cycling and organic matter decomposition (Ataikiru *et al*., 2019). Pesticides can disrupt these communities by influencing soil pH, moisture, organic matter content, or directly affecting microbial cells. Such disturbances may reduce microbial diversity and enzyme activity, ultimately affecting soil health. The rhizosphere, the narrow region surrounding plant roots, is especially sensitive to these changes (Rawat *et al*., 2026). Root exudates stimulate diverse microbial populations that help mobilize nutrients, suppress pathogens and promote plant growth (Rathore *et al*., 2018). Beneficial microbes such as *Pseudomonas* and

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Rhizobium enhance nutrient availability and plant resistance. However, heavy pesticide use can shift rhizosphere microbial communities, weaken ecological interactions and reduce soil productivity (Mendes *et al*., 2013).

Emerging contaminants have recently gained global attention, including chemicals whose long-term environmental effects remain uncertain (Mitra *et al*., 2024). Among them, synthetic pesticides remain a major concern because their biological action on pests may unintentionally affect non-target organisms (Sahoo *et al*., 2024). Profas, a widely used pesticide in cotton cultivation, is a good example. It targets the nervous system of pests like bollworms and aphids and is preferred for its high effectiveness and affordability (Martins *et al*., 2022). However, as a broad-spectrum chemical, it can also affect essential soil microorganisms involved in nutrient cycling. Profas persists in soil for several weeks to months depending on environmental conditions and although microbes participate in its degradation, repeated use may suppress sensitive species while encouraging resistant groups, leading to reduced microbial diversity (Bhandari *et al*., 2021). Such changes can disrupt soil processes, affect natural biological control and weaken ecosystem stability (Silva *et al*., 2020).

Traditional microbiological methods capture only a small fraction of soil microbial diversity, limiting our understanding of pesticide-induced shifts. Metagenomics has transformed this field by allowing direct sequencing of environmental DNA, helping identify both culturable and unculturable microbes and revealing functional genes and metabolic pathways (Schmidt *et al*., 2019). In pesticide studies, metagenomics helps uncover microbial adaptations, such as resistance genes and biodegradation pathways, which are important for developing sustainable management practices (Goss *et al*., 2021). Considering these challenges, the present study focused on understanding the effects of Profas on soil microbial diversity and community composition in cotton fields in general and to identify its effect on specific microbial taxa as a potential indicator of soil health under pesticide stress and to explore the capacity of soil microbes to degrade Profas for ecological restoration and sustainable pesticide management.

By integrating ecological perspectives with molecular approaches, this study seeks to provide a balanced understanding of Profas as both an agricultural tool and an environmental stressor. The insights gained are expected to guide strategies for sustainable cotton cultivation, where productivity and ecological integrity are not mutually exclusive but mutually reinforcing.

MATERIALS AND METHODS

Collection of soil samples

Soil samples were collected from the rhizosphere of potted cotton plants treated with the insecticide Profas from Shapur Begu village in Sirsa district 29°14′ to 30°0′N latitude and 74°29′ to 75°18′E longitude. Two pesticide concentrations (10 ppm and 20 ppm) were applied, resulting in three treatments: untreated soil (S1), 10 ppm treated soil (S2) and 20 ppm treated soil (S3). Approximately 50 g of soil was collected from the subsurface root zone during the flowering stage using sterile tools to avoid contamination. Samples were sealed in sterile polythene bags and transported immediately to the laboratory at 4°C to preserve microbial and chemical properties. A second round of sampling was carried out after 40 days for detailed physicochemical evaluation.

Physicochemical analysis of soil

Pot experiments were conducted in the departmental net house in 2024-25. After 40 days, soil samples were analyzed in the Department of Agriculture GLA University Mathura-281 406 (U.P.), India, following standard agronomic protocols. Soil pH, organic carbon and available macronutrients were estimated using established procedures, while phosphorus and potassium were quantified using spectrophotometry and flame photometry, respectively. Micronutrients such as Fe, Zn, Mn and Cu were analyzed by atomic absorption spectroscopy following the methods reported by Cota-Ruiz *et al*. (2018); Bandyopadhyay *et al*. (2013); Lindsay and Norvell (1978) and Sivakala *et al*. (2018).

Enrichment and isolation of pesticide-degrading microorganisms

To obtain pesticide-tolerant microbial populations, soil suspensions from treatments were inoculated into minimal salt medium (MSM) containing Profas (10-100 ppm). Cultures were incubated at 30±2°C on a shaker at 150 rpm for one week, followed by three rounds of enrichment. Pure isolates were obtained by repeated streaking on MSM agar. Cultures were preserved on nutrient agar slants at 4°C and in glycerol stocks at -20°C.

Screening and minimum inhibitory concentration (MIC)

Isolates were screened on MSM supplemented with Profas to assess their tolerance levels. MIC was determined by culturing isolates in MSM broth containing increasing pesticide concentrations (10-100 ppm). The highest concentration that supported bacterial growth was recorded as the MIC level.

Antibiotic susceptibility test

Antibiotic sensitivity was evaluated through the disc diffusion assay. A total of 100 µL of overnight-grown culture was spread on nutrient agar plates, followed by placement of commercial antibiotic discs. Plates were incubated at 30°C for 24-48 h and zone diameters were measured to determine susceptibility patterns.

Molecular characterization of isolates

Genomic DNA extraction

Genomic DNA was extracted using a commercial extraction protocol (Biologia Research India Pvt. Ltd.). Bacterial pellets were treated with lysozyme, Proteinase K and RNase A

prior to column-based purification, followed by ethanol washing and elution in preheated buffer.

PCR amplification of 16S rRNA gene

The 16S rRNA gene was amplified using universal primers 27F and 1492R as described by Raghunathan *et al*. (2005). PCR conditions consisted of an initial denaturation at 95°C for 5 min, followed by 32 cycles of denaturation (95°C, 30s), annealing (55°C, 30 s), extension (72°C, 1 min) and a final extension at 72°C for 20 min. Amplicons were examined on 1% agarose gels.

Phylogenetic analysis

Sequenced amplicons were analyzed using NCBI BLAST (Altschul *et al*., 1990). Multiple sequence alignment was carried out using ClustalW and phylogenetic trees were constructed using the neighbour-joining method in MEGA 6.0 (Saitou and Nei, 1987; Tamura *et al*., 2013).

Growth kinetics and biodegradation analysis

Growth curve analysis was performed by inoculating nutrient broth with 24-hour-old cultures and measuring OD600 at regular intervals. Biodegradation studies were conducted in MSM containing 100 ppm Profas, with samples collected every 6 h for up to 36 h. Pesticide extraction followed the QuEChERS method (Anastassiades *et al*., 2003) and residual Profas was quantified using GC-MS following Kumar and Philip (2006).

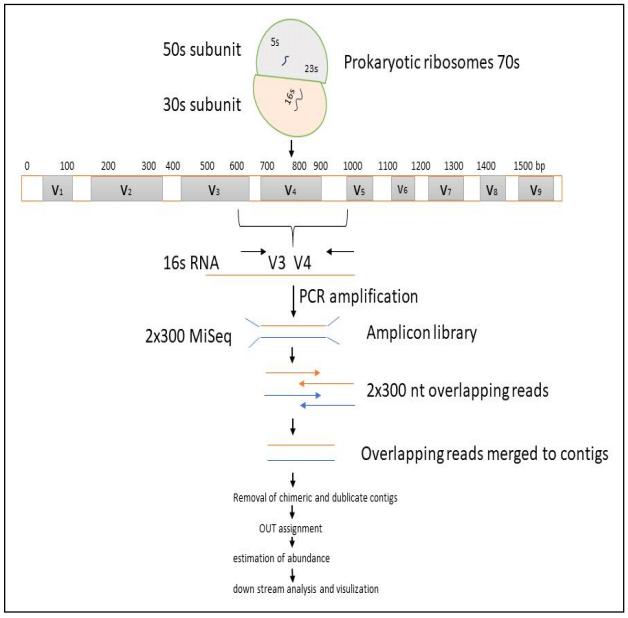


Fig 1: The flowchart representing the 16S rRNA metagenomic workflow.

Metagenomic library preparation and sequencing

Soil community DNA was extracted using commercial kits (Qiagen; Zymo Research). DNA quality was assessed by Nanodrop and agarose gel electrophoresis. The V3-V4 region of the 16S rRNA gene was amplified using modified primers. The sequencing workflow is illustrated in Fig 1. Amplified fragments were purified, indexed and sequenced on an Illumina MiSeq platform (2 × 300 bp).

GC-MS analysis

GC-MS quantification was carried out using an oven temperature of 100°C, injector temperature of 260°C and nitrogen as the carrier gas at 1.2 mL/min. A retention window of 30 min was used and detection was carried out with a ^63Ni electron capture detector.

RESULTS AND DISCUSSION

Sequencing output and read statistics

Metagenomic DNA extracted from the control (S1), 10 ppm pesticide-treated (S2) and 20 ppm pesticide-treated (S3) soil samples yielded 383,567, 381,196 and 224,782 high-quality reads, respectively (Table 1). The QIIME pipeline processed these reads, providing a comprehensive overview of microbial diversity, community structure and potential functional attributes. These datasets formed the basis for analyzing how Profas, as a soil amendment, influenced the microbial communities under varying treatment concentrations.

Microbial diversity at the phylum and class levels

Bacterial taxa predominated across all samples. In the control soil (S1), the dominant phyla included Proteobacteria, Firmicutes, Bacteroidetes, Acidobacteria and Nitrospirae. Pesticide exposure caused marked alterations to this structure. At 10 ppm (S2), Proteobacteria increased significantly, while Actinobacteria declined, along with moderate reductions in Firmicutes and Bacteroidetes; Acidobacteria remained relatively stable. Under higher concentration (S3, 20 ppm), overall microbial diversity decreased notably, with significant reductions in most phyla (Fig 2). Actinobacteria was the most prevalent class in control soils, but treated soils exhibited a shift favoring Alpha-,Beta- and Gammaproteobacteria. The latter increased substantially in S3, suggesting their adaptive advantage to chemical stress environments (Fig 3). This compositional change aligns with the broader observation that chemical inputs can exert selective pressures on soil microbes, reducing sensitive lineages while enriching tolerant populations (Ni *et al*., 2025; Peprah *et al*., 2025).

Table 1: Preliminary reads data.

Sample	Reads1	%GC	Read length	Reads2	%GC	Read length
S1	383567	53	251	383567	55	251
S2	381196	53	251	381196	54	251
S3	224782	54	251	224782	55	251

Taxonomic and functional shifts at family levels

At the order level, Actinomycetales, Burkholderiales and Rhizobiales dominated in control soils but declined considerably under Profas exposure (Fig 4). Pseudomonadales, however, became prominent under pesticide treatment, particularly at 20 ppm, highlighting potential adaptive qualities for coping with chemical stress. A similar trend was observed at the family level: Actinomycetales, Bradyrhizobiaceae and Comamonadaceae were dominant in control soils, whereas Pseudomonadaceae became predominant in S2 and even more abundant in S3 (Fig 5). Genus-level analysis demonstrated high relative abundance of Actinomycetes and Pseudomonas. Pseudomonas spp. increased notably in S2 and S3, supporting prior evidence of their pesticide-degrading capability (Steiner *et al*., 2024). Genera such as *Bacillus*, *Prevotella* and *Rhodoplanes* along with taxa affiliated with *Actinomycetales* were abundant in the control but declined under higher Profas doses. These observations indicate that elevated concentrations of the amendment reduce

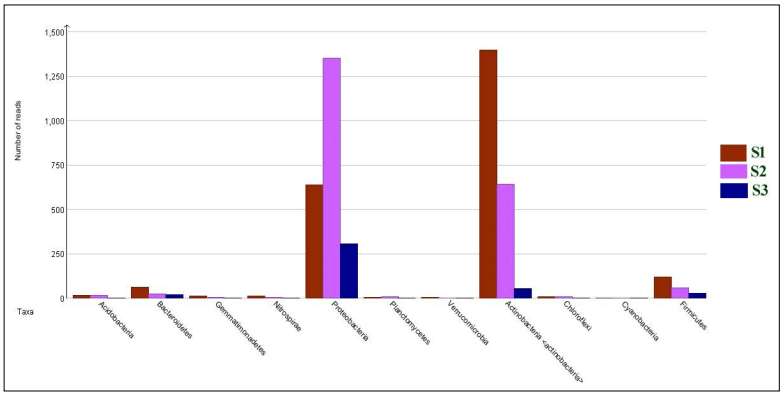


Fig 2: Phylum-level distribution of microbial communities in samples S1, S2 and S3 based on sequencing read abundance.

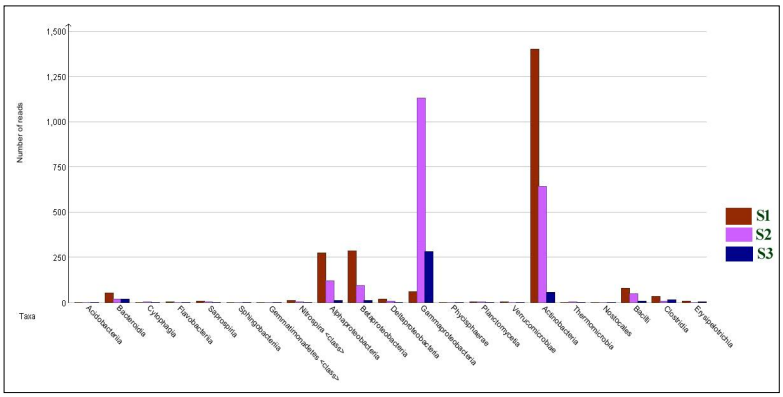


Fig 3: Class-level distribution of microbial communities in samples S1, S2 and S3 based on sequencing read abundance.

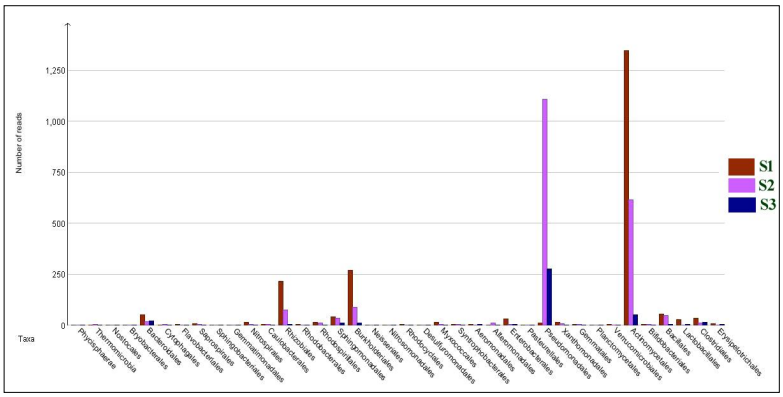


Fig 4: Order-level composition of microbial communities in samples S1, S2 and S3 based on sequencing read abundance.

microbial evenness and favor specialized taxa with enhanced stress resistance.

Microbial diversity indices and compositional structure

Alpha diversity indices Chao1, Shannon and Simpson reflected clear differences among treatments. The control (S1) displayed the highest species richness and evenness, while S2 and S3 showed reductions in both attributes. Importantly, S3, despite having the highest pesticide exposure, retained greater estimated species richness than S2 based on rarefaction patterns, suggesting the adaptive survival of certain resistant groups. These dynamics imply that low and high concentrations of Profas affect microbial populations differently, reducing moderate diversity at low doses but encouraging selective adaptation at higher levels. Krona plots revealed hierarchical changes in community structure. *Micrococcaceae* accounted for approximately 40% of the diversity in S1, followed by Actinomycetes and genera like *Oxydanes* and *Arthrobacter*. In contrast, Pseudomonas dominated S2 and S3, contributing 54% and 43% of the diversity, respectively (Fig 6 and 7). The predominance of Pseudomonadaceae supports earlier findings that nutrient enrichment and fertilizer residue favor fast-growing, metabolically versatile bacterial groups (Romero *et al*., 2025; Ni *et al*., 2025). Alpha diversity visualizations further supported these shifts. Rarefaction curves for S1 reached higher plateaus compared with treated soils, indicating greater overall richness. S2 showed early increases but plateaued quickly, while S3 exhibited a more varied OTU abundance pattern. Shannon index trends mirrored these findings, showing reduced evenness in higher Profas concentrations.

Phylogenetic diversity (PD) estimates revealed that the S2 sample had a sharp initial rise in PD due to the presence of diverse lineages, while S3 displayed lower PD values, signifying stronger dominance by specific groups. Beta diversity analysis (PCoA) illustrated distinct separations between control and treated soils, with PC1 and PC2 explaining 33.59-66.41% of community variation. S1 and S2 clustered closely, whereas S3 was clearly separated, demonstrating dose-dependent shifts in microbial composition. These transitions reaffirm that Profas

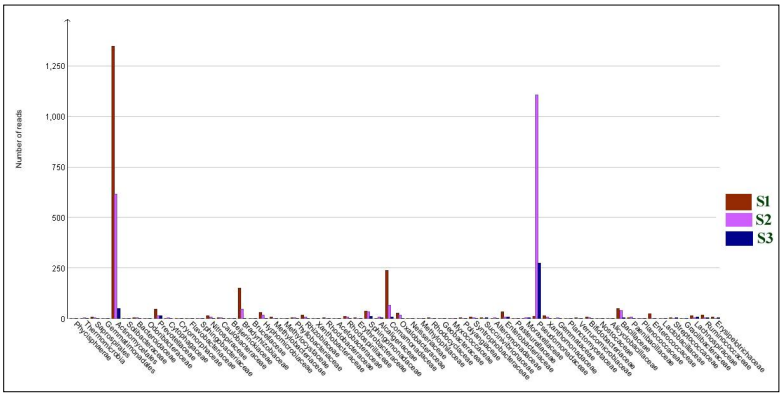


Fig 5: Family-level composition of microbial communities in samples S1, S2 and S3 based on sequencing read abundance.

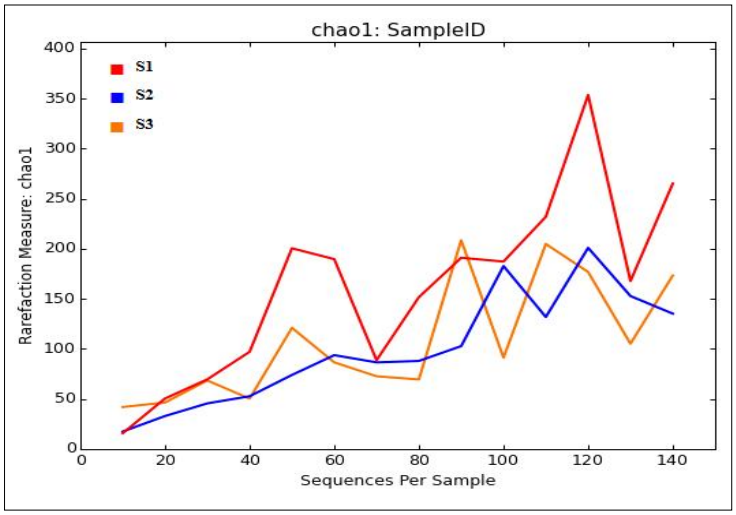


Fig 6: Chao1-based alpha diversity rarefaction curves for samples S1, S2 and S3.

dramatically influences soil microbial communities, particularly at higher concentrations.

Molecular identification and functional potential of bacterial isolates

Profas treatment significantly influenced microbial functional profiles. It altered soil pH, nutrient availability and organic content, leading to changes in microbial biomass and activity. Enhanced levels of nitrogen, phosphorus and potassium likely promoted nitrogen-fixing bacteria and other nutrient-cycling groups. In contrast, potential antimicrobial effects of some Profas components suppressed certain microbial taxa. Within these communities, Proteobacteria, notably Gammaproteobacteria, exhibited resilience and even increased in abundance, reflecting their adaptability to nutrient-rich and chemically variable environments.

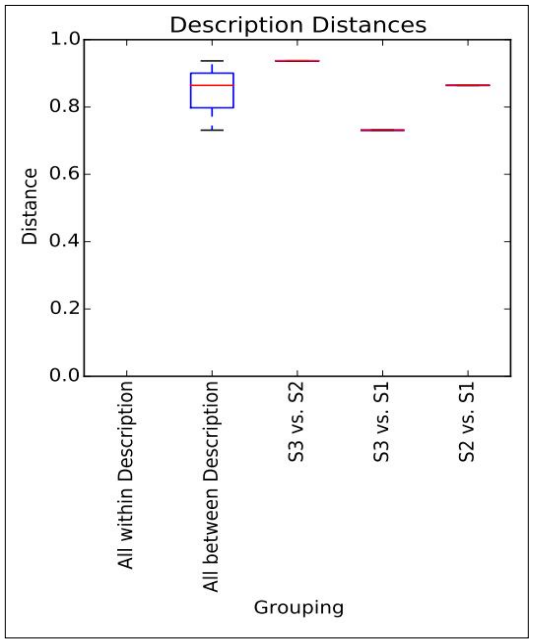


Fig 7: Distance-based (beta) diversity showing within- and between-sample dissimilarities among microbial communities.

Nine bacterial isolates were obtained using enrichment culturing and assessed for pesticide degradation by GC-MS. GC-MS analysis of the sample revealed distinct and well-resolved chromatographic peaks between approximately 4.5 and 5.6 min, corresponding to cypermethrin, the active ingredient of the pesticide Profas. The presence of multiple closely eluting peaks reflects the separation of cypermethrin stereoisomers, a characteristic feature of pyrethroid insecticides. The high intensity of these peaks and a stable baseline indicate reliable detection and minimal interference, confirming the persistence of cypermethrin residues in the analyzed sample. This chemical confirmation is directly relevant to Profas exposure, as it validates that increasing Profas doses result in measurable cypermethrin residues. Consequently, the GC-MS results provide a mechanistic basis for the observed dose-dependent alterations in microbial community structure under higher Profas treatments, linking chemical residue presence with biological effects (Fig 8). GC-MS analysis showed a single, sharp peak at approximately 7.8-8.0 min, confirming the presence of profenofos, an organophosphate insecticide. The well-resolved peak and low background noise indicate reliable identification based on characteristic retention time and mass spectral matching. This finding is directly relevant to Profas, as profenofos is an active ingredient in the formulation, confirming actual chemical exposure in the system. The detection of profenofos supports the observed dose-dependent biological effects under Profas treatment, linking residue presence with ecological or microbial responses (Fig 9). Among them, Prestia flexus achieved the highest degradation rate, indicating its strong bioremediation capability (Table 2). The genomic sequences of all strains were submitted to the NCBI database for accession numbers (Table 3). Phylogenetic analysis based on the Neighbor-joining method and maximum composite likelihood distances revealed clear evolutionary relationships with established bacterial lineages (Fig 10). This confirms that Profas exerts selective pressure favoring taxa with higher metabolic and ecological fitness, particularly Proteobacteria and Pseudomonadaceae.

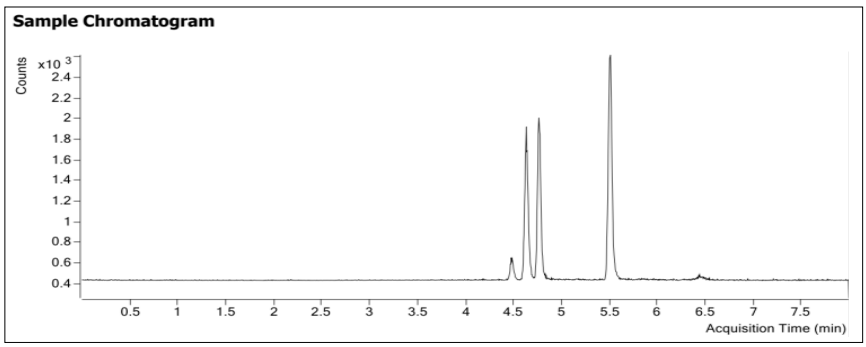


Fig 8: GC-MS chromatogram showing characteristic cypermethrin peaks (4.5-5.6 min), confirming the presence of cypermethrin, the active ingredient of Profas, in the sample.

Ecological and agricultural interpretations

The findings highlight how Profas application reshapes soil microbial ecology, leading to both beneficial and adverse outcomes. The decline in microbial diversity, especially under higher dosages, could weaken ecosystem resilience and hinder essential processes like nutrient cycling, disease suppression and organic matter decomposition (Ni *et al*., 2025). However, enrichment of functionally versatile taxa such as Pseudomonas may mitigate some adverse effects by maintaining key biochemical pathways.

From an agricultural perspective, these structural changes may influence rhizosphere interactions, nutrient use efficiency and plant growth. Fertilizer-induced microbial shifts could affect nitrification, phosphorus solubilization and beneficial symbioses. Therefore, optimal dosage management is crucial to preserve both microbial biodiversity and soil productivity. The isolation of efficient Profas-degrading strains such as Prestia flexus further underscores the potential of leveraging native soil microbiota for bioremediation and sustainable agricultural practices. Collectively, these results show that while Profas enhances nutrient cycling in the short term, improper dosing or prolonged exposure may lead to long-lasting microbial imbalance.

Table 2: The analysis of the nine bacterial strains using GC-MS demonstrates their potential to degrade profas.

Sample	Bacterial isolates	Profas
S2-1	*Streptococcus parauberis*	92.36
S2-4	*Enterococcus faecium*	90.03
S2-8	*Bacillus pumilus*	89.14
S2-11	*Staphylococcus aureus*	90.36
S2-11b	*Bacillus cereus*	88.16
S3-7	*Bacillus tequilensis*	75.55
S3-9	*Prestia flexus*	73.47
S3-3	*Lysinibacillus macrolides*	74.39
S3-12	*Enterococcus faecalis*	74.10

Table 3: NCBI accession numbers of the nine isolated bacterial strains.

Sample	Bacterial isolates	Accession no.
S2-1	*Streptococcus parauberis*	PQ432457
S2-4	*Enterococcus faecium*	PQ432456
S2-8	*Bacillus pumilus*	PQ432517
S2-11	*Staphylococcus aureus*	PQ432458
S2-11b	*Bacillus cereus*	PQ432518
S3-7	*Bacillus tequilensis*	PQ432516
S3-9	*Prestia flexus*	PQ432519
S3-3	*Lysinibacillus macrolides*	PQ432459
S3-12	*Enterococcus faecalis*	PQ432455

Limitations and future directions

While this study provides valuable insights, several limitations should be acknowledged. Experiments were conducted under controlled pot conditions, which cannot fully represent field realities such as soil heterogeneity, seasonal variability and interactions with diverse soil biota. The work also focused mainly on bacterial communities, leaving fungal, archaeal and viral groups unexplored. Future research should incorporate multi-omics approaches to obtain a more comprehensive understanding of soil microbiome responses to chemical amendments. Long-term field trials are needed to determine the persistence of microbial shifts and the cumulative impacts of repeated Profas applications. Assessing functional genes linked to nutrient cycling, stress tolerance and xenobiotic degradation would further clarify how microbes adapt to chemical stress and contribute to soil health. Integrating microbial data with soil physicochemical properties may support fertilizer strategies that balance productivity with microbial diversity (Peprah *et al*., 2025; Ewere *et al*., 2024). Profas application significantly influences soil microbial diversity, community structure and functional potential. Although alpha diversity decreased at higher Profas concentrations, taxa such as Proteobacteria and Pseudomonadaceae were enriched, indicating resilience. The isolation of Profas-degrading strains, particularly *Prestia flexus*, highlights microbial adaptability and bioremediation potential, underscoring the need for balanced, sustainable fertilization practices.

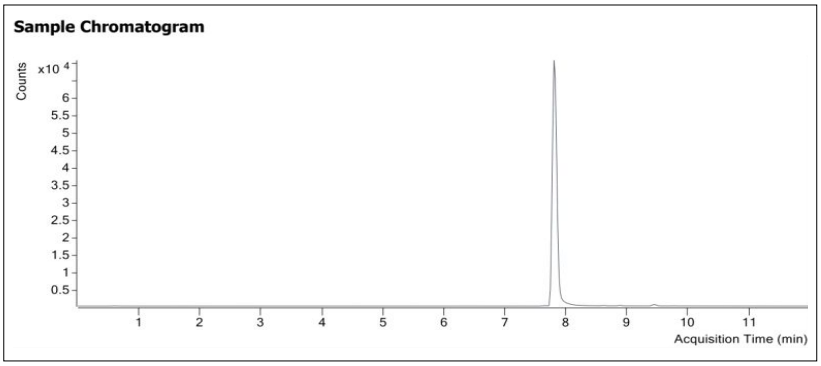


Fig 9: GC-MS chromatogram showing a distinct profenofos peak at ~7.8 min, confirming the presence of profenofos, an active ingredient of Profas, in the sample.

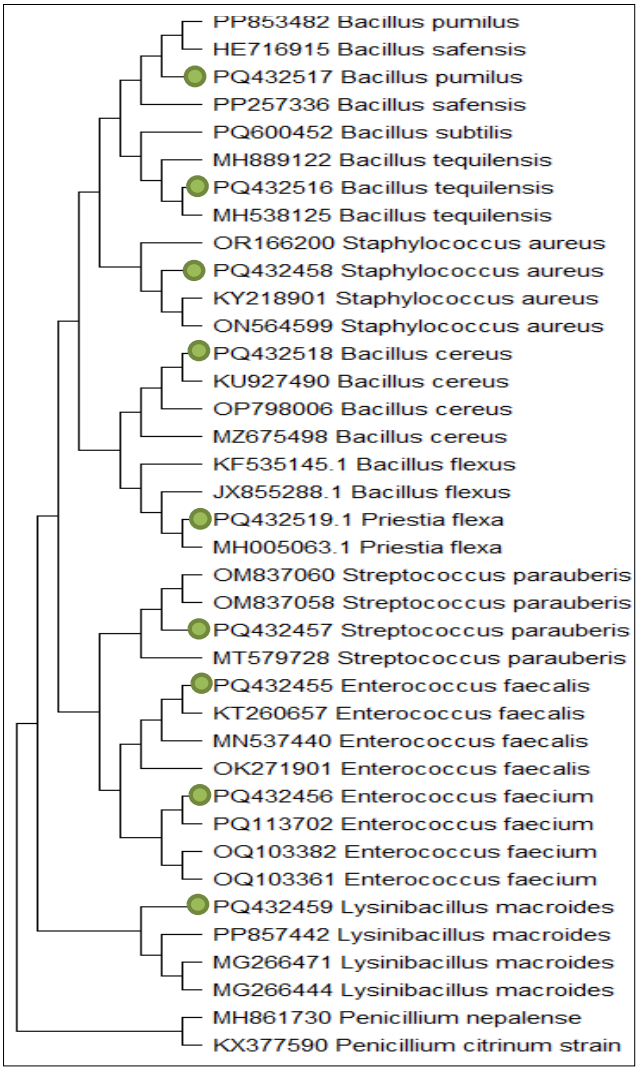


Fig 10: Taxonomic clades of bacterial samples spanning from profas treated soil of cotton plant pots, showing the root clade.

CONCLUSION

This study shows that Profas significantly influenced soil microbial diversity, community composition and functional potential. Metagenomic analysis revealed that untreated soil contained a highly diverse microbiome dominated by Actinobacteria and Proteobacteria, whereas Profas-treated soils showed selective enrichment of Gammaproteobacteria and Pseudomonadaceae at higher concentrations. Alpha and beta diversity analyses indicated a decline in microbial richness and evenness with increasing Profas levels, demonstrating that chemical amendments can disrupt soil microbial balance. Molecular identification of isolates highlighted the resilience of certain taxa, with *Prestia flexus* showing the strongest Profas-degrading ability, underscoring the adaptive capacity of soil bacteria and their potential role in bioremediation.

These findings suggest that while chemical pesticide may reduce overall diversity, they can also promote metabolically versatile and ecologically important groups that support nutrient cycling and organic matter decomposition. Understanding these shifts is essential for sustainable agriculture, as optimized fertilizer application can help maintain microbial diversity while encouraging beneficial taxa and minimizing ecological disruption.

Future work should include long-term field studies to evaluate the persistence of microbial changes and cumulative effects of repeated Profas use. Integrating multi-omics tools and expanding analyses to fungal, archaeal and viral communities will provide a more complete understanding of soil ecosystem responses. Overall, Profas shows a dual impact reduced diversity at higher doses and selective enrichment of key functional microbes informing strategies for sustainable soil management and microbe-assisted bioremediation.

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Disclaimers

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Informed consent

Not applicable.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish or preparation of the manuscript.

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