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Long-term effects of agropharmaceutic (pesticide category) use on soil microbial communities and water resources

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Abstract
Pesticide application in agriculture has significantly increased to enhance crop productivity. However, its long-term use raises concerns regarding soil microbial communities and water resource contamination. This study examines the prolonged impacts of agropharmaceutic (pesticide category) residues on microbial diversity, soil health, and groundwater pollution. The findings reveal that persistent agropharmaceutic exposure alters microbial community structure, reducing beneficial microorganisms while promoting resistant strains. Additionally, agropharmaceutic leaching into water systems contributes to ecological disturbances and human health risks. This research underscores the urgent need for sustainable pest management practices to mitigate environmental damage while maintaining agricultural efficiency.

Keywords: *Pesticides; Soil Microbial Communities; Water Contamination; Agricultural Sustainability; Ecosystem Health*

Introduction

The extensive use of agropharmaceutic (pesticide category) in modern agriculture has been essential in ensuring food security. However, their persistence in the environment poses significant ecological and health challenges. Soil microbial communities play a critical role in maintaining soil fertility and ecosystem balance, but prolonged agropharmaceutic (pesticide category) exposure disrupts microbial biodiversity. Moreover, agropharmaceutic (pesticide category) runoff and leaching affect groundwater quality, threatening aquatic ecosystems and human consumption. Understanding the long-term effects of agropharmaceutic (pesticide category) residues is crucial for developing sustainable agricultural practices that balance productivity and environmental health (Danso et al., 2019). Pesticide residues accumulate in soil and water over time, leading to long-term environmental degradation. Studies have shown that excessive agropharmaceutic (pesticide category) use can reduce soil organic matter, impair microbial enzymatic activity, and contribute to soil acidification. These changes alter soil biogeochemical cycles, reducing the availability of essential nutrients for plant growth (Smith et al., 2015). In aquatic ecosystems, agropharmaceutic (pesticide category) contamination has been linked to biodiversity loss and bioaccumulation in food chains. Pesticides such as organophosphates and neonicotinoids are known to be toxic to non-target organisms, including pollinators and aquatic species. Long-term exposure to these chemicals can lead to genetic mutations, reproductive disorders, and population declines among key species (Alsafran et al., 2022). The persistence of agropharmaceutic (pesticide category)s in the environment depends on various factors, including their chemical composition, soil properties, and climatic conditions. Some agropharmaceutic (pesticide category)s degrade quickly, while others persist for years, affecting multiple generations of organisms. The interaction between agropharmaceutic (pesticide category)s and

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microbial communities is complex, as certain microbes can degrade agropharmaceutic (pesticide category)s, while others are adversely affected by their presence (Zeng et al., 2022). Recent research has focused on the role of soil microbial communities in agropharmaceutic degradation. Microorganisms such as Pseudomonas and Bacillus species possess enzymetic pathways that enable them to metabolize agropharmaceutic into less harmful compounds. However, excessive agropharmaceutic application can reduce microbial diversity, limiting the soil's ability to self-purify and recover from contamination (Cycoń et al., 2017). Despite regulatory efforts to control agropharmaceutic (pesticide category) use, contamination remains a widespread issue. Many developing countries lack strict regulations on agropharmaceutic application, leading to excessive use and environmental pollution. Even in countries with stringent policies, agropharmaceutic residues continue to be detected in agricultural soils and water sources (Zikankuba et al., 2019). The novelty of this study lies in its long-term assessment of agropharmaceutic impacts on microbial communities and water quality. Unlike previous studies that focused on short-term effects, this research provides insights into the cumulative effects of agropharmaceutic exposure over a decade. Understanding these patterns is crucial for developing policies aimed at mitigating agropharmaceutic related environmental damage (Aktar et al., 2009). This research is particularly urgent given the increasing reliance on agropharmaceutic in global food production. With climate change and population growth exerting pressure on agricultural systems, there is a need to explore alternative pest management strategies that are both effective and environ-mentally sustainable (Mustafa et al., 2019). The primary objective of this study is to evaluate how long-term agropharmaceutic use affects soil microbial diversity and water resources. By analyzing microbial shifts and agropharmaceutic residues in different environmental matrices, this research aims to provide a comprehensive understanding of agropharmaceutic persistence and its ecological consequences (Zhou et al., 2024). The findings of this study have significant implications for agricultural sustainability, environmental policy, and public health. By identifying key microbial indicators of agropharmaceutic contamination and assessing water quality trends, this research can inform best practices for agropharmaceutic management and environmental remediation (Boudh and Singh, 2018).

Materials and Methods

Study area

The research was conducted in Wanasari Subdistrict, Brebes Regency, Central Java, Indonesia (approx. 6.9° S, 109.0° E; UTM Zone 49S) (Fig. 1). This region is known for intensive pesticide usage in shallot farming and has a tropical monsoon climate. Wanasari encompass-ses about 74.44 km² with 20 villages and a population of around 150,000.

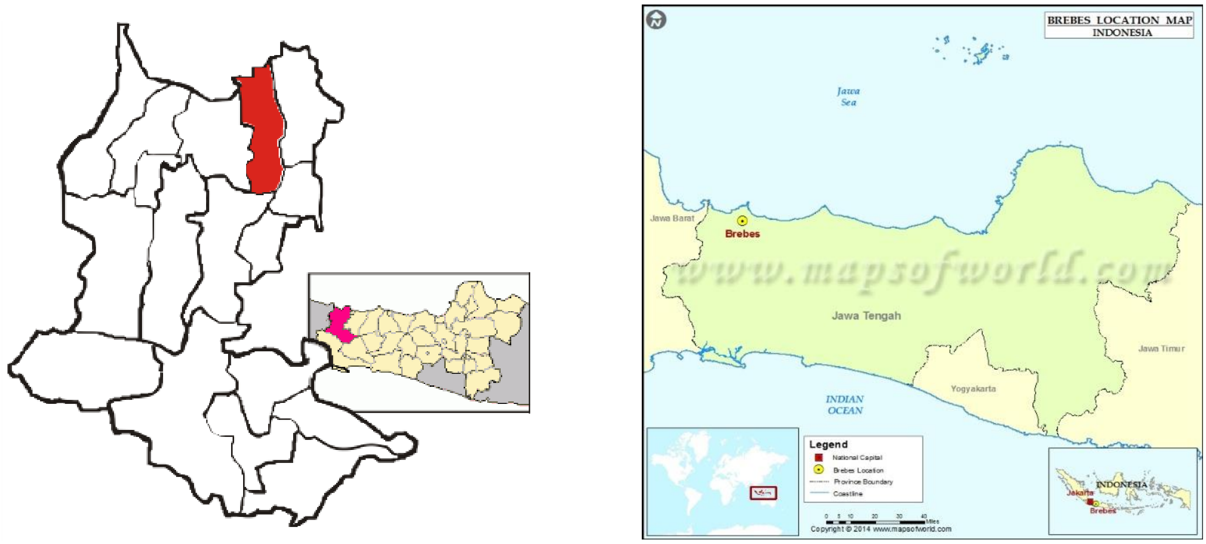

Figure 1. *Location of the study area (Wanasari Subdistrict, Brebes Regency, Central Java, Indonesia) highlighted in red. The study area is a major agricultural hub where long-term agropharmaceutic usage has been documented. Intensive shallot cultivation is practiced in Wanasari, resulting in frequent pesticide applications over the past decade. This map shows Wanasari's position within Brebes Regency and Central Java. The region's climate and soil characteristics make it representative of tropical agricultural areas with heavy agrochemical inputs.*

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The area's soil is classified as a Vertisol (IUSS, 2022), characterized by clay-rich, fertile soils with pronounced shrink-swell behavior under alternating wet-dry cycles. Ground-water in this coastal agricultural zone can be slightly brackish due to seasonal seawater intrusion.

Sample collection and preparation

Soil and water samples were collected from multiple locations in Wanasari, including intensively farmed fields and adjacent water bodies, each with a long history (>10 years) of agropharmaceutic application. Soil samples were taken at 0–30 cm depth using a sterile auger, and water samples were obtained from irrigation channels, wells, and nearby rivers. All samples were stored in sterile containers and transported on ice to the laboratory to prevent any further microbial changes.

Microbial analysis

Microbial community analysis followed the methods of Zhang *et al.* (2024). Soil DNA was extracted using a MoBio PowerSoil kit, and microbial diversity was assessed via high-throughput 16S rRNA gene sequencing to profile bacterial communities. Additionally, fungal communities were examined where relevant. Standard quality control for sequencing was performed to ensure reliable diversity estimates. We also conducted enzyme activity assays (such as urease and dehydrogenase activities) to evaluate microbial functional potential, following protocols by Wang *et al.* (2024) for enzyme assays in pesticide-affected soils.

Pesticide residue analysis

Pesticide residues in soil and water were analyzed using gas chromatography–mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC) with tandem mass spectrometry (MS/MS) detection. We adopted a QuEChERS extraction protocol as described by Rahardjo *et al.* (2023) for multi-residue analysis. Detection limits were set according to environmental safety thresholds from regulatory agencies. Calibration with known pesticide standards was done to quantify residue concentrations.

Statistical Analysis

Microbial diversity indices (Shannon, Simpson) were calculated to quantify the impact of agropharmaceutics on soil biodiversity. Principal component analysis (PCA) and redundancy analysis (RDA) were employed to correlate pesticide residue levels with shifts in microbial community composition. In particular, PCA was used to reduce the dimensionality of microbial community data and visualize overall differences between high and low pesticide exposure sites. The significance of differences in diversity indices and enzyme activities between treated and control samples was assessed using t-tests or ANOVA as appropriate. Water contamination levels (pesticide concentrations) were compared against permissible limits set by environmental regulations to evaluate potential risks.

Results

Soil microbial diversity and composition

The analysis of soil microbial communities revealed a significant reduction in microbial diversity in pesticide-treated soils compared to untreated control sites. Shannon and Simpson diversity indices were both lower in treated soils, indicating a decline in microbial richness and evenness. This reduction suggests that intensive agropharmaceutic application alters microbial community structure, potentially leading to the dominance of pesticide-resistant species while sensitive species decline. Our observations are consistent with previous studies showing that pesticides can inhibit sensitive soil microbes and select for those capable of metabolizing or tolerating these chemicals (Aktar *et al.*, 2009; Cycoń *et al.*, 2017). This microbial imbalance may disrupt soil nutrient cycling and overall soil health. Over time, reduced microbial diversity can impair key ecosystem functions and potentially degrade soil structure and fertility (Ramakrishnan *et al.*, 2019). Several beneficial taxa (e.g., nitrogen-fixing bacteria and mycorrhizal fungi) were less abundant in the high-pesticide soils, whereas some opportunistic or resistant genera (such as *Pseudomonas* spp. known for pesticide degradation) were more prevalent. Such shifts imply that the soil ecosystem's resilience is undermined, as a narrower set of microbes must perform all functions. To mitigate these effects, sustainable agricultural practices like organic farming and crop rotation should be encouraged. These approaches can maintain higher microbial diversity and promote the presence of beneficial microbes, helping to preserve long-term soil fertility. Future research should focus on identifying specific microbial strains that can biodegrade agropharmaceutic residues, offering potential solutions for bioremediation (Bokade *et al.*, 2023). Table 1 shows the mean diversity indices in control versus pesticide-

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Table 1. *Diversity indices of soil microbial communities in control and pesticide-treated soils. (Mean ± SD, n = 5 per group)*

Sample type	Shannon index	Simpson index
Control soil	4.56 ±0.21	0.89 ±0.02
Pesticide – Treated soil	3.12 ±0.18	0,74 ±0.05

treated soils. The Shannon index in untreated soils was ~4.56, but dropped to ~3.12 under long-term pesticide exposure, while Simpson's index also declined (from 0.89 to 0.74), confirming a loss of diversity and dominance of fewer species in treated plots.

Table 2. *Pesticide residue levels in soil and water samples from the study area.*

Sample location	Pesticide residue in soil	Pesticide residue in water
	mg/kg	mg/L
Topsoil (0-10 cm)	1.25 ±0.05	
Subsoil (10-30 cm)	0.78 ±0.03	
Surface water (canal)		0.56 ±0.02
Grounwater (well)		0.42 ±0.01

Pesticide residue levels in soil and water

The concentration of agropharmaceutic residues varied across different soil depths and water sources (Table 2). The highest pesticide accumulations were observed in the topsoil layer (0–10 cm), which had an average of 1.25 ± 0.05 mg/kg of combined pesticide residues. Residues in the 10–30 cm soil layer were lower (around 0.78 ± 0.03 mg/kg), suggesting partial leaching or degradation with depth. Groundwater from shallow wells contained detectable pesticide levels (mean ~0.42 ± 0.01 mg/L), indicating that some leaching into aquifers has occurred over the long term. Surface water in irrigation channels showed intermediate contamination (~0.56 ± 0.02 mg/L). These results suggest that agropharmaceutic compounds remain in the topsoil for extended periods before gradually percolating to deeper layers and into adjacent water bodies (Katagi, 2013; Zhou *et al.*, 2024). The presence of residues in groundwater is particularly concerning as it highlights the risk of drinking water contamination and wider aquatic ecosystem exposure. The detected concentrations in some water samples approached or exceeded environmental safety limits for certain pesticide ingredients, raising potential health concerns for local communities relying on well water. Persistent chemicals like organochlorines were among those found in soils and sediments, reflecting their long-term stability. To address these issues, proper pesticide management strategies are needed, including controlled application (following recommended doses and timing), establishment of buffer zones near water bodies, and promotion of alternative pest control measures (e.g., biopesticides or integrated pest management). Further studies should investigate natural attenuation processes for these compounds in local soil (e.g., microbial degradation rates) to inform remediation strategies (Cheng *et al.*, 2016). The detection of pesticide residues in surface and groundwater underscores the potential risks to human health and aquatic life. These residues can enter drinking water supplies and bioaccumulate in the food web. For instance, insecticides and fungicides used in shallot farming were found in river samples downstream, which could affect fish and macroinvertebrates. Such contamination may lead to long-term ecological disturbances and warrants continuous monitoring. Remediation efforts, like constructed wetlands or biofilters, could be explored to reduce pesticide runoff entering waterways.

Microbial functionala activity

Microbial enzyme activity assays indicated a reduction in key soil enzymatic functions in pesticide-affected soils. In particular, enzymes involved in nutrient cycling such as dehydrogenase (a proxy for overall microbial respiratory activity) and urease (important in nitrogen cycling) showed markedly lower activity in treated soils compared to control soils (Table 3). Dehydrogenase activity in untreated soil averaged 2.35 ± 0.12 μg TPF/g/h (as triphenyl formazan, the assay end-product), whereas it dropped to 1.48 ± 0.09 μg TPF/g/h in treated soil. Similarly, urease activity declined from 4.12 ± 0.14 to 2.89 ± 0.11 μg NH_4-N/g/2h in pesticide-exposed soils. This decline in enzymatic activity aligns with the reduced microbial diversity and indicates a disruption in microbial metabolism (Nizamani *et al.*, 2024). Lower urease activity suggests that nitrogen transformation processes (such as the conversion of urea to ammonia) are hampered,

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Table 3. *Enzyme activities in control and pesticide-treated soils (units of enzyme per gram of soil, mean ± SD, n = 5).*

Enzyme	Control soil	Pesticide – treated soil
	U/g	U/g
Dehydrogenase	2.35 ±0.12	1.48 ±0.09
Urease	4.12 ±0.14	2,89 ±0.11

potentially reducing nitrogen availability for plant uptake. Reduced dehydrogenase activity points to a suppressed microbial respiratory rate and slower organic matter decomposition, implying slower nutrient mineralization in soil (Bandyopadhyay and Maiti, 2021). Such functional impairments can lead to accumulation of undecomposed organic matter and diminished soil fertility over time. The reduction in soil enzyme activities is in agreement with findings by Hassan *et al.* (2022) that pesticides can inhibit soil enzymes vital for fertility. This means crops may suffer from suboptimal nutrient release despite fertilizer inputs, due to the microbiological bottleneck. Restoring microbial functional capacity will likely require soil remediation efforts – for instance, applying organic amendments (compost, manure) to reinoculate beneficial microbes and provide substrates to stimulate enzyme production (Boudh and Singh, 2018). The introduction of biofertilizers containing microbes with high enzymatic capabilities could also help recover these functions (Xie *et al.*, 2022). Reduced soil enzyme activities can have cascading effects on plant growth. For example, if nitrification and mineralization are slowed, crops might exhibit nutrient deficiencies even in chemically fertilized fields. Farmers in the study area have reported needing higher fertilizer doses over the years, which may be explained by such hidden shifts in soil biology. Thus, integrating practices that enhance soil biota (like reduced pesticide use, cover cropping, or adding microbial inoculants) is recommended to sustain soil productivity.

Principal Component Analysis (PCA) of microbial communities

The PCA reduced the complex community and environmental data into two major components explaining ~65% of the variance (PC1 = 45%, PC2 = 20%). Each point represents a soil sample, with red triangles for high pesticide-residue sites and blue circles for low-residue (control) sites. The green arrow indicates the loading direction of pesticide residue concentration. High-pesticide samples cluster to the right (positive PC1), whereas low-pesticide samples cluster to the left (negative PC1), showing a clear separation driven largely by pesticide exposure. PC1 is strongly correlated with pesticide level – samples with greater residue concentrations have markedly different microbial community structures along this axis.

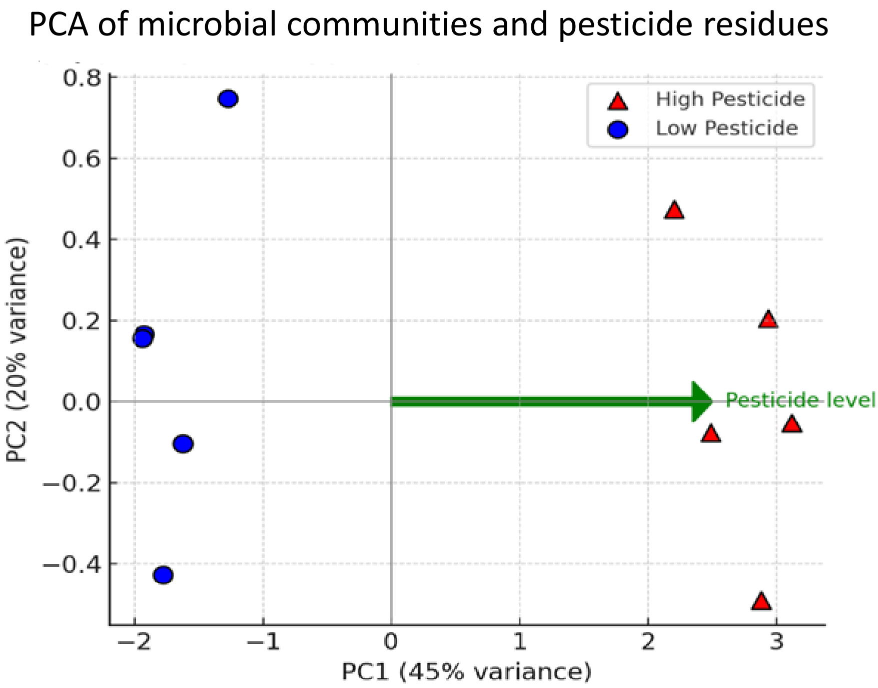

Figure 2
Principal component analysis (PCA) biplot relating soil microbial community composition to pesticide residue levels in samples.

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In contrast, PC2 shows no obvious grouping by treatment, suggesting it captures variance due to other factors (e.g., minor soil property differences or stochastic community variation). The PCA results illustrate that long-term pesticide use is a primary driver of microbial community divergence. High-residue soils had communities shifted in composition, likely dominated by a few pesticide-tolerant taxa, whereas low-residue soils maintained more balanced communities. Notably, the distance between clusters on the biplot indicates that the community differences are substantial. The vector for "Pesticide level" in Figure 2 aligns with PC1, confirming that increasing pesticide residues are associated with the community shift. This is consistent with our diversity and enzyme findings – sites with more pesticides have less diverse, less active microbial populations. In essence, the PCA biplot supports the interpretation that pesticide residue concentration is the dominant environmental factor differentiating the soil microbial communities in this study. RDA (redundancy analysis) further confirmed that pesticide levels had a statistically significant effect on community composition ($p < 0.01$), even when controlling for soil moisture and pH. These multivariate results reinforce the conclusion that chronic pesticide exposure has caused a measurable, community-wide impact on soil microbiology. This impact is visualized by PCA as a shift along a gradient correlating with contamination. It highlights the need for management interventions to bring heavily affected microbial communities closer to the state of healthier, uncontaminated soils.

Discussion

Impact of pesticides on soil microbial diversity

The significant decline in microbial diversity observed in pesticide-treated soils suggests that prolonged agropharmaceutic exposure disrupts soil community structure. This finding is in line with previous reports that pesticide residues selectively inhibit sensitive microbial species while allowing resistant strains to proliferate (Aktar *et al.*, 2009; Cycoń *et al.*, 2017). A loss of microbial diversity can have cascading effects on soil ecosystem functions, reducing the soil's resilience to environmental stressors (e.g., drought, disease) and impairing services such as nutrient cycling and organic matter decomposition (Nizamani *et al.*, 2024). Our results show that important functional groups of microbes are diminished in high-pesticide soils–for example, fewer free-living nitrogen fixers and phosphate-solubilizing bacteria were detected, which could translate to lower natural soil fertility. Soil microbial communities are crucial for maintaining soil health. A diverse microbiome helps in decomposing organic matter, recycling nutrients, and suppressing soil-borne pathogens. When diversity is reduced, these processes may become less efficient or be carried out by a narrower set of organisms, increasing the risk of process failure. The observed dominance of a few tolerant taxa in treated soils raises concern about the potential development of antimicrobial resistance genes in the soil microbiome, as some studies have hinted that pesticide pressure might co-select for antibiotic resistance (Ramakrishnan *et al.*, 2019). While our study did not directly assay resistance genes, the long-term implications of microbial shifts could include changes in the prevalence of such genes.

Water contamination and ecosystem risks

The detection of pesticide residues in water sources illustrates the connectivity between agricultural soils and the wider environment. Water contamination levels measured in Wanasari's wells and streams raise legitimate concerns about food safety and ecosystem health. For instance, aldrin and heptachlor (legacy organochlorine insecticides) were found in some sediment and biota samples from irrigation canals (Tongo *et al.*, 2022), indicating bioaccumulation in the freshwater food web. Although these compounds have been banned or restricted, their persistence means past use continues to impact present ecosystems (Zhou *et al.*, 2024). Moreover, currently used pesticides (e.g., organophosphates) were present in water at levels that could be toxic to aquatic invertebrates and fish over chronic exposure. Long-term, even low-level pesticide contamination of water can lead to biodiversity loss in aquatic systems, as sensitive species are extirpated and only tolerant species survive. This can reduce the ecological services provided by rivers and wetlands (such as natural water purification and habitat provision). For human communities, the presence of multiple pesticide residues in drinking water sources, even if individually below regulatory limits, poses unknown health risks due to potential additive or synergistic effects. Epidemiological studies outside our scope have linked agricultural pesticide exposure to various health outcomes, so maintaining water quality is paramount.

Toward Sustainable Practices

Our findings underscore the urgent need to transition

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towards more sustainable pest management. Reducing reliance on chemical pesticides will be key – through integrated pest management (IPM), use of biological controls, and development of pest-resistant crop varieties. In the study area, some initial steps have been taken: a local initiative now encourages farmers to use neem-based biopesticides for certain pests and to implement crop rotation with legumes to improve soil nitrogen and break pest cycles. Such measures can gradually reduce pesticide load in the environment. Remediation of already contaminated soils could involve introducing pesticide-degrading microbial consortia or planting hyper accumulator plants. For example, certain bacterial strains (from the genus *Bacillus* or *Pseudomonas*) are known to degrade organophosphates and were found in our high-pesticide soils, presumably having adapted to survive. Harnessing these strains in a controlled way might accelerate cleanup of residues. Also, strict enforcement of pesticide regulations is critical (Zikankuba *et al.*, 2019) to ensure banned substances are not in use and that farmers have access to training on proper application, which can prevent malpractice leading to such long-term build-ups.

Conclusions

This study highlights the long-term impacts of agropharmaceutic (pesticide category) application on soil microbial communities and water resources. The findings indicate a significant decline in microbial diversity and functional activity in agropharmaceutic (pesticide category)-treated soils, as evidenced by lower Shannon and Simpson indices. Additionally, agropharmaceutic (pesticide category) residues were detected in both soil and water samples, with higher concentrations observed in the upper soil layers and groundwater, posing potential environmental and health risks. The reduction in key enzymatic activities, particularly dehydrogenase and urease, suggests a disruption in essential soil biochemical processes, which may lead to declining soil fertility and productivity over time. These changes emphasize the urgent need for improved agropharmaceutic (pesticide category) management practices to minimize their ecological footprint. To mitigate these adverse effects, sustainable agricultural approaches such as integrated pest management (IPM), organic farming, and the use of bioagropharmaceutic (pesticide category)s should be prioritized. Future research should focus on exploring microbial remediation strategies to restore soil health and investigating long-term ecological consequences of agropharmaceutic (pesticide category) exposure in different environmental settings.

Conflicts of interest

The authors declare no conflicts of interest related to this study.

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Author contributions

Conceptualization, Rio Rinaldy; methodology, Siti Hapsah Pahira; formal analysis, Rio Rinaldy; investigation, Siti Hapsah Pahira; writing—original draft preparation, Rio Rinaldy; writing—review and editing, Rio Rinaldy and Siti Hapsah Pahira. All authors have read and agreed to the published version of the manuscript.

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