

Impact of Long-Term Agrochemical Application on Plant Metabolic Pathways and Soil Microbial Diversity

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Abstract: Long-term agrochemical application represents a critical anthropogenic disturbance with profound implications for plant physiology and soil ecosystem functioning. This paper synthesizes current research on the cumulative effects of herbicides, insecticides, and fungicides on plant metabolic reprogramming and soil microbial community dynamics. Agrochemicals act as chemical stressors that disrupt plant secondary metabolism—including flavonoid biosynthesis, phenolic compound production, and phytohormone signaling—through oxidative stress induction, transcriptional reprogramming, and enzymatic detoxification mechanisms. Concurrently, herbicide residues accumulate in agricultural soils, with concentrations ranging from 0.31 to 1558.13 µg/kg in intensively farmed regions, directly altering soil microbial diversity and nutrient cycling processes. Fungal communities exhibit particular sensitivity, with herbicide residues explaining 32.91% of diversity variation compared to 18.05% attributable to soil properties. The disruption extends to nitrogen cycling genes, where herbicide-induced bacterial community shifts impair critical biogeochemical functions. However, emerging evidence reveals that herbicide-tolerant plant growth-promoting bacteria can be isolated from treated soils, offering potential bioinoculant strategies for sustainable agriculture. This paper integrates multi-omics evidence to elucidate the "pesticide-plant-microbe-soil" interaction network, bridging molecular mechanisms with agroecological sustainability.

Keywords: Agrochemicals, Secondary Metabolism, Soil Microbiome, Herbicide Residues, Nutrient Cycling, Plant Growth-Promoting Bacteria

I. INTRODUCTION

The intensification of global agriculture has necessitated the widespread application of agrochemicals, with herbicides alone constituting 47.5% of total annual pesticide use worldwide. Approximately 65.02% of agricultural lands globally are potentially contaminated by herbicide residues, raising significant concerns regarding ecosystem health and agricultural sustainability. While these compounds effectively control pests and weeds, their persistent application creates cumulative effects that extend far beyond their intended targets.

Plants respond to agrochemical exposure through complex metabolic reprogramming, activating defense and detoxification mechanisms that alter the production of secondary metabolites essential for environmental adaptation and crop quality. Concurrently, soil microbial communities—fundamental to nutrient cycling, organic matter decomposition, and plant health—experience selective pressures that reshape community composition and functional capacity. The interaction between these plant-level and soil-level responses creates a dynamic "pesticide-plant-microbe-soil" network with cascading ecological consequences.



This paper examines the dual impact of long-term agrochemical application on plant metabolic pathways and soil microbial diversity, synthesizing recent evidence from metabolomic, metagenomic, and multi-omics studies. The analysis addresses three interconnected dimensions: (1) plant metabolic responses to agrochemical stress, (2) soil microbial community dynamics under prolonged herbicide exposure, and (3) the ecological interfaces linking these processes. Implications for sustainable agricultural practices and agrochemical risk assessment are discussed.

II. PLANT METABOLIC RESPONSES TO AGROCHEMICAL STRESS

2.1 Mechanisms of Metabolic Disruption

Agrochemicals function as abiotic stressors that disrupt plant secondary metabolism through multiple pathways. Pesticides induce oxidative stress, triggering the accumulation of reactive oxygen species and activating enzymatic and non-enzymatic detoxification mechanisms. This stress response results in transcriptional reprogramming that alters the biosynthesis of phytohormones, phenolics, volatile organic compounds, and specialized metabolites. The specific metabolic effects depend on pesticide chemical structure, physicochemical properties, and application method, with different compound classes producing distinct perturbation signatures.

Research on imidacloprid application to pak choi plants demonstrated dose-dependent metabolic disruption, including accumulation of nonstructural carbohydrates (maltotriose, raffinose, melezitose), branched-chain and aromatic amino acids (lysine, tryptophan, tyrosine), and small-molecule organic acids (shikimic acid, aconitic acid, citric acid). Low-dose exposure promoted the accumulation of resistance-related phospholipids and flavonoids, whereas high-dose exposure decreased flavonoid content and significantly reduced protein and chlorophyll levels by 11.2% and 15.4%, respectively. This biphasic response illustrates the dose-dependent nature of agrochemical effects on plant metabolism.

2.2 Comparative Effects of Synthetic and Natural Pesticides

The metabolic footprint of synthetic pesticides differs substantially from that of natural alternatives. A comparative study evaluating hinokitiol (a natural allelochemical) against synthetic tebuconazole and 2,4-dichlorophenoxyacetic acid in wheat and barley found that synthetic pesticides caused significantly greater disruption to plant metabolism. Hinokitiol induced modest changes in biogenic amine profiles (up to 50% alterations), whereas tebuconazole induced changes up to 270% relative to blank controls. Natural pesticides demonstrated more rapid degradation kinetics and lower environmental persistence, suggesting reduced ecological risk.

Metabolic fingerprinting using liquid chromatography-mass spectrometry revealed that synthetic pesticides disrupt key pathways including amino acid biosynthesis (L-phenylalanine, L-tyrosine, L-tryptophan), secondary metabolite production, and phytohormone synthesis, ultimately affecting plant growth, development, and food quality. These findings underscore the potential of biopesticides as safer alternatives, though further research on their efficacy and economic viability is warranted.

2.3 Flavonoid Biosynthesis and Root Metabolic Profiles

Flavonoid biosynthesis represents a particularly sensitive metabolic pathway affected by agrochemical exposure. Research on nano-acetamiprid exposure in faba bean (*Vicia faba* L.) revealed that nanopesticide treatment promoted flavonoid biosynthesis, whereas conventional acetamiprid inhibited this pathway. This differential effect highlights how pesticide formulation influences plant metabolic outcomes. Nano-acetamiprid exposure contributed to alterations in basic metabolic pathways including flavonoids, amino acids, and tricarboxylic acid cycle intermediates across the exposure period.

The metabolic effects of agrochemicals extend to root systems, where they influence the composition of root exudates—chemical signals that mediate plant-microbe interactions in the rhizosphere. Imidacloprid application induced changes in root exudate composition, including increased release of amino acids, organic acids, and phenolic compounds. These alterations serve as adaptive responses that reshape rhizosphere microbial communities, creating feedback loops between plant metabolic status and soil microbial dynamics.

III. SOIL MICROBIAL DIVERSITY UNDER LONG-TERM AGROCHEMICAL EXPOSURE

3.1 Herbicide Residue Accumulation and Persistence

Long-term herbicide application leads to significant residue accumulation in agricultural soils, with persistence influenced by application history, soil properties, and environmental conditions. In the black soil region of Northeast China, total herbicide concentrations ranged from 14.79 to 233.97 $\mu\text{g/kg}$ across different land-use types, with maize fields (mean 133.63 $\mu\text{g/kg}$) showing substantially higher levels than farmland shelterbelts (mean 45.17 $\mu\text{g/kg}$) that received no direct herbicide application. Acetochlor, atrazine, and its metabolite hydroxyatrazine emerged as dominant residues, detected in all 36 soil samples analyzed.

The detection of herbicide residues in areas with no direct application history indicates off-target movement through drift during application or migration with water. This finding has important implications for ecosystem-level risk assessment, as herbicide contamination extends beyond treated fields to affect adjacent habitats. S-metolachlor, a widely used chloroacetanilide herbicide, exhibits moderate persistence in soil with a half-life ranging from 2.5 to 289 days, depending on temperature, moisture, and microbial activity. In cooler, drier conditions, degradation slows significantly, potentially impacting subsequent planting seasons.

3.2 Microbial Community Shifts and Diversity Loss

Herbicide residues exert direct and indirect effects on soil microbial communities, with consequences for community composition, diversity, and functional capacity. Research in Northeast China revealed that herbicide residues (32.91%) were a more significant factor influencing soil fungal diversity than soil properties (18.05%), with the atrazine metabolite hydroxyatrazine emerging as particularly impactful. Fungal Shannon index showed greater sensitivity to herbicide residues than to soil physicochemical properties, suggesting that fungi may serve as sensitive indicators of agrochemical disturbance.

The microbial community response to herbicides varies by taxonomic group and exposure history. S-metolachlor application in maize fields demonstrated that herbicide effects on microbial communities were less significant than temporal factors (summer vs. winter) or agricultural practices (continuous maize cultivation vs. maize-winter wheat rotation), indicating that integrative management can buffer herbicide impacts. However, enrichment techniques enabled selection of herbicide-tolerant genera—including *Pseudomonas*, *Serratia*, and *Brucella*—that were rare in metagenome analysis of soil samples, suggesting that herbicide exposure selects for tolerant taxa while suppressing sensitive ones.

Glyphosate application effects on soil microbial diversity remain context-dependent. A study of long-term glyphosate application in a macadamia orchard found no significant influence on soil microbial diversity or community structure two years after application ceased, though soil total nitrogen and $\delta^{15}\text{N}$ were significantly higher in areas with glyphosate history. This suggests that glyphosate's legacy effects may manifest primarily through biogeochemical cycling rather than community composition, with implications for long-term soil fertility.

3.3 Mechanisms of Microbial Disruption

Agrochemicals disrupt soil microbial communities through multiple mechanisms. Herbicides can affect microorganisms directly or indirectly depending on their mode of action: acetolactate synthase inhibitors and acetyl-CoA carboxylase inhibitors directly affect microbial targets, whereas photosynthesis inhibitors and plant growth regulators have indirect effects. Prolonged exposure leads to selection of herbicide-resistant or -degrading microorganisms, altering community composition and potentially reducing functional redundancy.

The disruption extends to functional genes governing nutrient cycling. Herbicide residues in soil adversely impact nitrogen cycling genes by altering bacterial communities, with hydroxyatrazine identified as a particularly important factor affecting the soil microbiome. This disruption has cascading consequences for nitrogen availability, crop productivity, and ecosystem multifunctionality. Soil microbial diversity is positively correlated with various aspects of ecosystem multifunctionality, including crop yield, nutrient cycling, and suppression of pathogens. Therefore, herbicide-induced diversity loss represents a threat to agricultural sustainability.

IV. ECOLOGICAL INTERFACES: PLANT-MICROBE-SOIL INTERACTIONS

4.1 Rhizosphere Signaling and Root Exudate Modulation

Agrochemicals mediate plant-microbe interactions through their effects on root exudate composition—the chemical signals that recruit and regulate rhizosphere microbial communities. Research on imidacloprid application demonstrated that plants under pesticide stress enhance the release of primary metabolites in root exudates, including amino acids, small-molecule organic acids, and phenolic compounds. These exudate changes regulate the root environment in multiple ways: amino acids and organic acids influence soil texture and nutrient cycling, while phenolic compounds inhibit pathogenic microorganisms.

The relationship between root exudate metabolites and rhizosphere genera under pesticide stress reveals complex attraction and repulsion dynamics. Aromatic amino acids (tyrosine, tryptophan, phenylalanine) and phenolic acids showed significant positive correlations with *Flavisolibacter*, while low-molecular-weight organic acids, fatty acid-related lipids, and sugar phosphates showed positive correlations with *Nocardioides* and *Gaiella*. Flavonoids such as isofraxidin displayed differential effects—positive correlation with *Thermomonas* but negative correlation with *Nocardioides*. This specificity suggests that plants can fine-tune root exudate composition to recruit beneficial microbes while deterring pathogens.

4.2 Microbial Compensation and Dose-Dependent Effects

A conceptual framework integrating microbial compensation and dose-dependent pesticide stress has been proposed to explain plant growth outcomes. At low pesticide doses, microbial compensatory effects may overcome pesticide stress, promoting plant growth. At high doses, microbial compensation becomes insufficient to counteract stress, resulting in growth inhibition. This biphasic response reflects the combined influence of direct pesticide phytotoxicity and indirect effects mediated through rhizosphere microbiota.

The microbial compensation hypothesis has practical implications for optimizing pesticide application. Understanding the balance between pesticide dosage and microbial compensation is essential for reducing harm to non-target organisms while maintaining pest control efficacy. Future research should focus on isolating rhizosphere bacteria and validating their protective functions through reintroduction experiments or sterile seedling systems. The development of bioinoculants containing herbicide-tolerant, plant growth-promoting bacteria represents a promising strategy for sustainable agriculture.

4.3 Herbicide-Tolerant Plant Growth-Promoting Bacteria

Soil treated with herbicides serves as a reservoir of herbicide-tolerant plant growth-promoting bacteria (PGPB), offering opportunities for developing bioinoculants that enhance plant fitness while maintaining herbicide tolerance. In a study of S-metolachlor-treated soil, 120 strains were isolated demonstrating significant *in vitro* PGP potential, including plant-beneficial traits such as phytohormone production, siderophore synthesis, and biocontrol capacity. Multi-strain consortia combining promising isolates increased shoot biomass by up to 47% in pot experiments on maize.

The successful establishment and persistence of inoculated consortia partners in the maize root microbiome—without significantly altering host root biodiversity—indicates compatibility with existing microbial communities. This finding is crucial for practical application, as bioinoculants must compete with native microbes and maintain their beneficial functions under field conditions. The development of herbicide-tolerant PGPB consortia represents a strategy for mitigating the negative effects of herbicide exposure on non-target organisms while promoting sustainable agricultural practices.

V. IMPLICATIONS FOR SUSTAINABLE AGRICULTURE

5.1 Risk Assessment and Monitoring

Current agrochemical risk assessments should incorporate the cumulative effects of long-term application on plant metabolism and soil microbial communities. The detection of herbicide residues in areas with no direct application history underscores the need for comprehensive monitoring across agricultural landscapes, including buffer zones and adjacent habitats. Multi-omics approaches—integrating metabolomics for plant responses and metagenomics for soil

communities—provide powerful tools for assessing agrochemical impacts and identifying early warning indicators of ecosystem disruption.

The differential sensitivity of fungal communities to herbicide residues suggests that fungal diversity may serve as a sensitive bioindicator for agrochemical stress. Microbial functional genes, particularly those involved in nitrogen cycling, also show promise as indicators of ecosystem functional integrity. Risk assessment frameworks should consider both direct phytotoxicity and indirect effects mediated through plant-microbe interactions.

5.2 Integrated Management Strategies

Integrative agricultural practices can suppress the effects of continuous herbicide application on soil microbial diversity and stabilize microbiome fluctuations. Crop rotation—such as maize-winter wheat rotation—demonstrated buffering effects against herbicide-induced community shifts, suggesting that diversifying cropping systems enhances microbial resilience. The combination of reduced herbicide reliance with bioinoculant application offers a path toward sustainable intensification.

The identification of herbicide-tolerant PGPB and their formulation into multi-strain consortia represents a practical strategy for maintaining soil health and crop productivity under continued herbicide use. However, field-scale validation and optimization of fermentation and formulation processes are needed to translate laboratory findings into real-world solutions. Economic viability and regulatory approval remain barriers to widespread adoption.

5.3 Pesticide Design and Application

Knowledge of pesticide effects on plant secondary metabolism and microbial communities should inform the design of next-generation agrochemicals with reduced non-target impacts. Natural pesticides such as hinokitiol demonstrate lower ecological footprints and metabolic disruption compared to synthetic counterparts, supporting their development as safer alternatives. Nanopesticides such as nano-acetamiprid exhibit enhanced safety profiles for host plants and soil health while maintaining pest control efficacy.

Optimization of application methods also reduces environmental impacts. Foliar spraying—the most common application method—affects root exudates and rhizosphere microbial communities through systemic signaling. Understanding these long-distance effects can guide application timing and dosage to minimize disruption to beneficial plant-microbe interactions while maximizing pest control.

VI. CONCLUSION

Long-term agrochemical application profoundly impacts plant metabolic pathways and soil microbial diversity through complex, interconnected mechanisms. Plant responses include oxidative stress, transcriptional reprogramming, and altered secondary metabolism—particularly flavonoid and phenolic compound biosynthesis—with effects mediated by pesticide class, dosage, and formulation. Soil microbial communities experience selective pressures that reduce diversity, alter community composition, and impair nutrient cycling functions, with fungal communities showing particular sensitivity to herbicide residues.

The "pesticide-plant-microbe-soil" interaction network operates through rhizosphere signaling, root exudate modulation, and microbial compensation mechanisms that can amplify or buffer agrochemical impacts. However, herbicide-treated soils also harbor beneficial bacteria with plant growth-promoting potential, offering opportunities for developing bioinoculants that enhance plant fitness while maintaining herbicide tolerance.

Future research should prioritize: (1) establishing quantitative relationships between pesticide chemical structures and plant metabolic disruption to enable safer pesticide design, (2) investigating the effects of pesticide application methods on systemic regulation of plant-microbe interactions, (3) elucidating the ecological connections among pesticides, plants, secondary metabolites, and interacting organisms, and (4) monitoring non-target pest occurrence following pesticide application to determine whether metabolic suppression increases plant susceptibility. Translational research integrating these insights into agroecological decision-making is essential for fostering innovation in eco-friendly pesticide design and adaptive pest management practices.

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