Frontiers in Bioscience Elite

Front. Biosci. (Elite Ed) **2026**; 18(3): 43532
https://doi.org/10.31083/FBE43532

Review

Pre- and Post-Emergence Herbicide-Induced Shifts in Soil Microbial Communities: Effects on Population Dynamics, Mechanistic Insights, and Ecosystem Function

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Academic Editor: Baohong Zhang
Submitted: 10 June 2025 Revised: 27 September 2025 Accepted: 5 November 2025 Published: 11 September 2026

Abstract

Herbicides are commonly used in agricultural practices to control weeds and significantly impact soil microbial communities, which are essential for maintaining soil health and ecosystem functions. Herbicides are the most widely used class of pesticides globally, yet they also have detrimental effects, while the rising cost of agricultural labor has recently driven a significant increase in herbicide use. According to the World Health Organization (WHO), three million cases of pesticide poisoning were reported. Certain herbicides may inhibit or promote specific microbial taxa, thereby altering community composition and functionality. These alterations can disrupt crucial soil processes, including nutrient cycling, organic matter degradation, and plant–microbe interactions, potentially diminishing soil fertility and increasing vulnerability to pathogen invasions. Moreover, the duration and intensity of microbial responses are influenced by the persistence and bioavailability of herbicides in the soil, which can differ based on soil characteristics, climatic factors, and the patterns of herbicide application. This review emphasizes recent progress in understanding the molecular mechanisms that drive microbial responses to herbicides, including modifications in gene expression, enzyme activity, and adaptive stress responses. Pre- and post-emergence herbicides alter soil microbial communities, reducing beneficial microbes, disrupting plant–microbe interactions, and affecting nutrient cycling and soil fertility. However, the effects vary with herbicide type, soil properties, and environmental conditions. Thus, promoting alternative weed management and monitoring microbial responses can help maintain soil biodiversity and support sustainable agriculture. Moreover, by investigating both the immediate and long-term effects on microbial communities and ecosystem functioning, this paper aims to provide a comprehensive understanding of the impact of herbicide use on soil ecology and sustainability. This review is a concise draft on the mechanistic basis of herbicide effects on microbial population dynamics, focusing on how various herbicide classes affect microbial diversity, community structure, and metabolic activities.

Keywords: ecosystem; herbicides; gene expression; microbiota; population dynamics

1. Introduction

Most herbicides are biologically active compounds, and the application of herbicides can lead to negative consequences for the diversity and functioning of microorganisms. This, in turn, may disrupt the ecological balance of microbial growth on the soil surface and diminish soil efficiency. The complex physical and chemical structure of the soil ecosystem encompasses various conditions and small-scale ecosystems, including factors such as accessibility, pH, osmotic potential, moisture, temperature, and the concentrations of carbon and nutrients [1]. A comprehensive synthesis comparing pre- and post-emergence herbicides across multiple ecosystems remains lacking. The 1940s witnessed the introduction of 2,4-dichlorophenoxyacetic acid (2,4-D), which revolutionized agriculture and paved the way for the use of chemicals in weed management. By 2016, over 2000 herbicides with 15 distinct mechanisms of action had been introduced to the global market [2]. Furthermore, the user-friendly nature and cost-effectiveness of 2,4-D ranked this herbicide among the most favored weed management methods in contemporary agriculture. Meanwhile, the introduction of herbicide-resistant genetically modified (GM) crops, along with the adoption of conservation agriculture practices, has further intensified herbicide use. In reality, the application of herbicides enhances farm profitability, albeit at the expense of ecosystem services. Recent findings have indicated that the use of these pesticides has detrimental effects on soil microbiota and can lead to unexpected consequences for the ecosystem. The limited quantities of pesticides that effectively reach the intended targets may have repercussions for soil and water quality, ultimately affecting the health of people, animals, and crops. Although it is undeniable that pesticides can increase agricultural productivity, these compounds also have

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negative consequences. Indeed, around 800,000 lives have been lost in developing countries since the onset of the "Green Revolution" due to pesticide-related issues [3]. Additionally, exposure to pesticides through food is estimated to cause approximately 20,000 deaths annually in these regions. An estimated 3 million pesticide poisoning cases occur worldwide annually, with 220,000 deaths, about 99% of which occur in developing countries (WHO, 1990) [4]. Therefore, the effects of herbicide use on soil microorganisms are currently being examined, although a comprehensive assessment of the topic remains lacking. Weeds have posed a significant threat to agriculture throughout history; consequently, various chemicals have been researched and utilized globally for weed control. However, a notable transformation in herbicide application practices occurred after World War II. Today, agronomists and chemists are highly optimistic about the use of agricultural pesticides for pest eradication. This review uniquely integrates mechanistic insights, population-level effects, and ecosystem-level consequences of both pre- and post-emergence herbicides, providing a critical evaluation that bridges knowledge gaps in current literature. Fenoxaprop-P-ethyl is an herbicide that selectively targets post-emergence growth and functions both systemically and acropetally. Additionally, fenoxaprop-P-ethyl is primarily absorbed through the leaves and then translocated both downward and upward to the roots or rhizomes [5]. Imazethapyr, a systemic post-emergence herbicide, is absorbed by roots and foliage, travels through the xylem and phloem, and accumulates in the meristematic zone. In agriculture, herbicides such as dicamba and glyphosate are commonly utilized for weed management [6]. Although these substances are believed to target plants primarily, these herbicides can inadvertently affect other living organisms by interfering with evolutionarily conserved biological processes [7]. An example of this is the shikimate pathway, which serves as a molecular target for glyphosate and is present in bacteria and fungi; the shikimate pathway plays a vital role in the synthesis of three essential amino acids: tyrosine, phenylalanine, and tryptophan [8]. Moreover, research on this pathway has been conducted in plants and mice [9]. Any alterations might affect interactions between these microbial populations and their associated host species. However, the potential evolutionary implications of herbicide selection on the genetic diversity and functional traits of soil bacterial populations remain largely unexplored [10]. Pesticides, whether natural or synthetic, are widely employed in various agricultural practices to combat diseases, weeds, and pests that threaten crops.

The initial PubMed search yielded a total of 10,006 items/publications from 2004 to 2024 related to the keyword phrase "soil toxicity due to herbicide". An additional 2608 publications were identified when conducting a targeted search for the first study under the heading "pre- and post-emergence herbicide". Consequently, a total of 277 records were discovered using the keyword phrase "health hazard due to pre- and post-emergence herbicides". A similar search using the keyword phrase "decrease of soil microbial population due to pre- and post-emergence herbicide" returned 208 publications, among which 39.42% were deemed suitable for the final analysis based on precise relevance. Articles were selected for in-depth investigation following a thorough review of titles and abstracts. To achieve a rigorous synthesis, this study systematically screened literature using specific keywords and phrases and applied defined inclusion criteria to assess the quality and relevance of publications, ensuring a critical and comprehensive review of current evidence.

2. National and International Restrictions on Diverse Fungicides, Herbicides, and Pesticides

Although global pesticide statistics are well documented, the direct impact of pesticides on soil microbial dynamics remains poorly understood, warranting critical assessment. The use of chemical pesticides plays a vital role in global agriculture. Notably, more than 60% of all pesticides used worldwide are herbicides [11]. In India, insecticides account for roughly 65% of the pesticide market, followed by herbicides at 16%, fungicides at 15%, and other types at 4% [12]. Over the past decade, herbicide usage has increased to account for as much as 25% of the total pesticide use. Currently, 68 herbicides have been approved by India for various agricultural crops, enabling comprehensive weed management. The application of herbicides is particularly prevalent in India, where herbicides are used on 44% of grain crops, 31% of rice, 10% of plantation crops, 4% of soybeans, and 11% of other crop types [11]. Pesticide pollution detrimentally impacts soil quality by altering the soil chemical and biological compositions, thereby affecting crop yield. Prior data revealed that pesticide usage surged notably from 2009, both overall and per hectare. Multiple factors influence pesticide usage trends, including agricultural mechanization, adoption of GM crops, market dynamics, and labor shortages. Notably, the recent rise in herbicide use has coincided with increased pesticide use, primarily due to escalating costs and labor shortages across various agricultural sectors [13]. In fact, the proportion of insecticides within the total pesticide production dropped from 70% in 2003–2004 to 39% in 2016–2017 [14]. Furthermore, glyphosate is the predominant herbicide imported into India, while China emerged as the chief supplier of atrazine [12]; Japan, China, and Israel are the top three herbicide suppliers to India. Additionally, India has become a significant exporter of herbicides, sending 20,457.02 tons to Brazil and 65.48 tons to the USA over the past year. Notably, India ranks fourth globally in terms of agrochemical production, behind the United States, Japan, and China. In the 2011–2012 fiscal year, India produced 68,490 tons of insecticides, valued

at $8.59 million, with household pesticides accounting for $6.44 million. The Indian pesticide market is expected to grow at an annual rate of 12–13%. Exports are projected to increase by 15% to 16% annually, while domestic growth is anticipated at 8% to 9% [3]. Notably, despite facing considerable agricultural losses from insect infestations, India has the lowest pesticide use globally, at just 291.2 grams per hectare. In comparison, some of the leading pesticide users include the United States (4.5 kg/ha), China (14 kg/ha), and Japan (11 kg/ha), while the global average stands at 3.0 kg/ha [6]. Despite bans on hazardous herbicides, residues are still detected in soils, revealing a gap between policy and practice. Pesticides are substances employed to eradicate, deter, or manage various plant and animal species, encompassing herbicides, fungicides, and insecticides. The demand for pesticides is consistently rising, with Asia accounting for over half of the utilized insecticides. In terms of pesticide application per hectare, Saint Lucia ranks first globally, while China is the top overall user of pesticides. The growing requirement for high-quality protein-rich food to meet the needs of the expanding global population is driving the need for sustainable practices that maintain soil health and enhance food security without jeopardizing soil biodiversity. The World Health Organization (WHO) classifies three extremely hazardous pesticides, brodifacoum, bromadiolone, and flocoumafen (classified as Ia), which remain permitted for use in India. In contrast, two other pesticides have been banned: barium carbonate and coumachlor. The WHO database also identifies four further highly dangerous pesticides in class Ia: phorate, methyl parathion, phosphamidon, and captafol/difolatan. Moreover, despite these being prohibited in India, these pesticides have still been applied to approximately 63.80 metric tons over the past five years [15]. Wet direct-seeded rice (DSR) encounters considerable challenges in cultivation, largely due to a variety of weed species. Utilizing broad-spectrum herbicide combinations can help address this issue by reducing factors such as herbicide resistance and the emergence of new weed species.

3. Impact of Pre- and Post-Emergence Herbicides on Plants

Herbicides are widely used in plant biology research to study a range of cellular and molecular processes. These chemical agents are characterized by the associated high specificity for target sites, which often inhibit crucial physiological and metabolic pathways in plants. This specificity makes herbicides invaluable for evaluating the effects of disrupting these pathways and for gaining insights into the associated functioning processes [16]. Moreover, utilizing herbicides in this manner can provide a deeper understanding of these mechanisms and the specific ways reactive oxygen species (ROS) contribute to cellular stress. The Herbicide Resistance Action Committee (HRAC) employs a classification system that categorizes modes of action (MoAs) based on the associated light-induced ROS activation. These herbicides, as a group, inhibit photosynthesis by either directly inhibiting the photosynthetic electron transport chain or targeting the synthesis of pigments and critical components of the electron transport chain. The photosystem II (PSII) products were among the earliest synthetic herbicides made available for commercial use. These herbicides are divided into two main categories: serine 264 binders (group 5) and histidine 215 binders (group 6); meanwhile, group 6 comprises three nitriles, one phenylpyridazine, and one benzothiadiazinone, while group 5 includes 27 triazines, one triazolinone, five triazinones, five uracils, four phenylcarbamates, two pyridazinones, 27 ureas, and two amides. The diversity of herbicides targeting PSII suggests a broad range of binding sites, highlighting the promiscuity of the associated targets. Due to the presence of numerous binding sites in photosynthetic tissues, these herbicides demonstrate selectivity, effectively eliminating weeds without damaging crops; these herbicides can also be applied to either soil or foliage at relatively high rates [17]. The original urea- and triazine-type herbicides remain the most commonly used classical PSII inhibitors. These herbicides all bind to the quinone B (QB) -binding site and inhibit forward electron transport, but phenolic herbicides cause greater photodamage than the urea and triazine counterparts. Different classes of herbicides have distinct effects on photosynthetic charge recombination: classical herbicides tend to increase the emission temperature, while phenolic herbicides tend to decrease this temperature. Specifically, compounds that interact with the D1 protein alter the midpoint potential (Em) of the Q_A/Q_A^{-}redox couple; for example, diuron increases the potential by 50 mV, whereas phenolic herbicides decrease the potential by 45 mV. While PSII inhibitors can clearly generate O_2•−, photoinhibitory damage to PSII also results in the formation of O_2•− and •OH [18]. The second most prevalent category of herbicides consists of acetolactate synthase (ALS) inhibitors, which are effective against various annual and perennial grasses, as well as broadleaf weeds. These slow-acting agents inhibit the production of branched-chain amino acids, ultimately leading to wilting and the eventual death of the plant [19]. The question of whether oxidative damage is involved in the mechanism of ALS inhibition has been raised, particularly due to the oxygen-utilizing side reaction associated with the condition [20]. The potential role of ROS in ALS inhibition was first proposed in 1994 [21]. Indeed, observations indicated that an oxygenase process triggered by O_2 occurred during this inhibition, but was impeded at elevated ALS concentrations. The research indicated that these herbicides are likely to induce oxidative inactivation, potentially due to the mechanism of action being directly related to the irreversible oxidative damage associated with ALS. Additionally, the study showed that ROS are involved in the irreversible damage to ALS triggered by

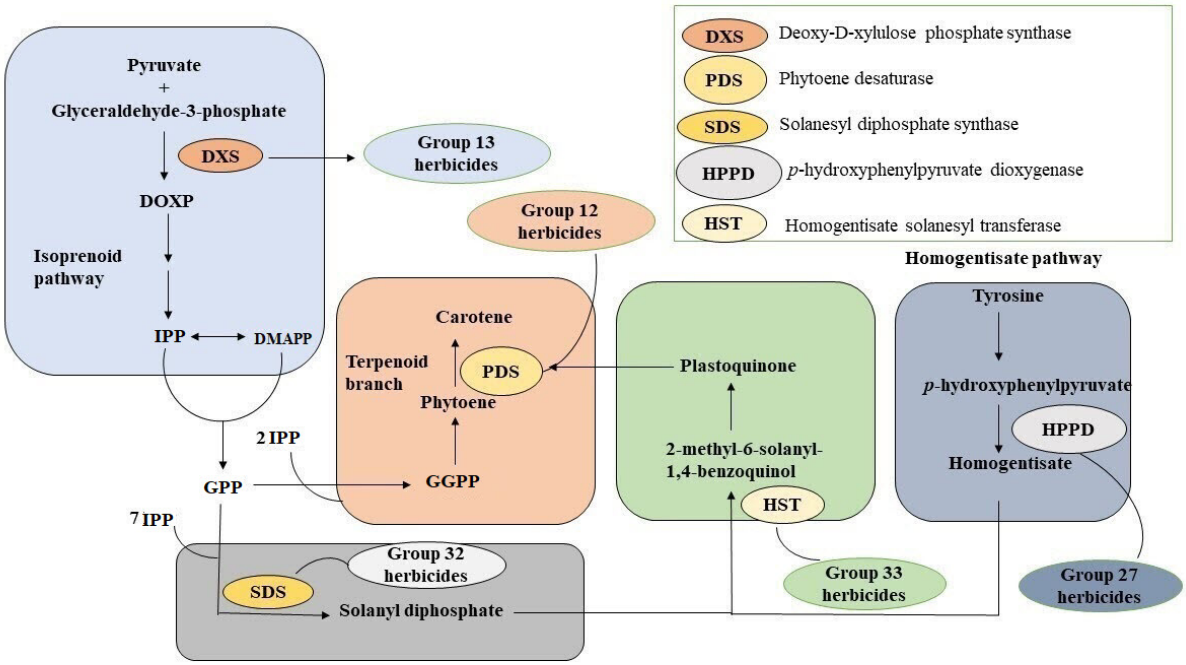

Fig. 1. Herbicide targets in isoprenoid, carotenoid, plastoquinone, and homogentisate pathways. Inhibition of key enzymes disrupts pigment biosynthesis, leading to chlorosis and growth suppression. DOXP, 1-deoxy-D-xylulose 5-phosphate; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; GPP, geranyl diphosphate; GGPP, geranylgeranyl diphosphate. All figures were prepared by us using Microsoft PowerPoint.

these herbicides. In 2007, the claims suggesting that ROS were directly involved in the mechanism of ALS inhibition were disproven [22]. The levels of antioxidant enzymes remained largely stable, and the lipid peroxidation observed after application of the ALS herbicide did not compromise membrane integrity. Herbicides targeting the isoprenoid, carotenoid, plastoquinone, and homogentisate biosynthetic pathways inhibit key enzymes involved in pigment biosynthesis, thereby disrupting the normal flow of intermediates such as DOXP, IDP, DMAPP, GPP, and GGPP, which ultimately leads to chlorosis and growth suppression in target plants (Fig. 1). Although the treated plants might not have mounted a response, the oxidative stress generated was insufficient to disrupt the antioxidant system. This study suggests that oxidative stress arises as a secondary effect of ALS inhibition.

4. Impact of Pre- and Post-Emergence Herbicides on Human Health

Glyphosate is well recognized for possessing antibacterial properties, primarily due to the observed inhibition of enolpyruvylshikimate-3-phosphate synthase [23]. Furthermore, various studies have shown that glyphosate tends to have a greater effect on strains considered "beneficial" compared to those deemed "pathogenic" [24]. The *Clostridium* species, known to cause disease, exhibited glyphosate resistance over 50 times higher than that of *Bifidobacteria*, which are typically regarded as beneficial. Glyphosate might also indirectly increase the levels of harmful microbes. For example, exposure to glyphosate levels that do not inhibit *Clostridium* growth has been associated with a reduction in the ability of *Enterococcus* to suppress Clostridial toxin production, including botulinum neurotoxin production [25]. Additionally, glyphosate has been shown to promote the development of antibiotic resistance in *Salmonella* species at concentrations exceeding regulatory limits but below application levels [26]. Since glyphosate suppression does not affect enolpyruvylshikimate-3-phosphate synthase in *Staphylococcus aureus*, this bacterium may grow more rapidly than other species that could be more beneficial. The complex characteristics of microbiome composition stability and varying sensitivity, along with the observation that most inhibitory levels seem to exceed regulatory thresholds, may point to a dysbiosis that increases the likelihood of inflammation and potential carcinogenic effects.

Notably, there is currently no strong evidence specifically linking glyphosate to intestinal cancers. Additionally, as previously mentioned, the suppression of short-chain fatty acids (SCFAs) produced by microbes represents another potential mechanism for this connection. DNA sequencing revealed no changes in the microbiota of young mice that were briefly exposed to a glyphosate-containing

diet for two weeks. However, a significant, dose-dependent reduction in fecal pH was observed, suggesting that the ability of beneficial bacteria to produce SCFAs was negatively affected [27]. In a subsequent study, the Nielsen research team found that a sufficient supply of aromatic amino acids in the diets of test subjects limited the impact of glyphosate on the composition of the gut bacterial community. Even in these circumstances, exposure to glyphosate was associated with reduced levels of detectable SCFAs, especially acetic acid. The synthesis of folate by bacteria also relies on the shikimate pathway, which glyphosate inhibits. Probiotic bacteria play a crucial role in folate production within the gut [28]. Folate deficiency has been directly linked to human genotoxicity, carcinogenicity, and events involving chromosomal breakage [29]. In human cells, these deficiencies increase the likelihood of ionizing radiation-induced DNA strand breaks [30]. Therefore, exposure to glyphosate might also affect cancer risk through this alternative pathway. In addition to glyphosate, the adjuvant surfactants and emulsifiers present in glyphosate-based herbicides (GBHs) also alter the microbiome. Glyphosate alone does not inhibit the growth of the lactic acid bacterium *Lactococcus cremoris*, even at concentrations of 200 ppm (parts per million) [31]. Furthermore, emulsifiers and surfactants have been shown to independently induce colitis in a mouse model, a condition associated with an increased risk of colon cancer [32,33]. Intriguingly, rather than directly harming beneficial bacteria, emulsifiers, such as Tween 80, seem to promote dysbiosis by creating conditions favorable to pathogenic bacteria that produce flagellin. In this context, exposure to food-grade emulsifiers has been shown to increase the population of bacteria associated with low-grade, chronic inflammation [34]. The findings from these surfactant studies suggest an interesting hypothesis: GBHs may exert a dual, synergistic effect on the gut microbiome through the combined actions of the associated components. Emulsifying agents might foster an environment that supports pathogen growth; meanwhile, glyphosate hinders the development and disease-fighting abilities of beneficial strains, thereby elevating the risk of inflammation and the associated consequences. Glyphosate inhibits beneficial bacteria only at concentrations well above those that the gut typically encounters. Nevertheless, this does not eliminate the possibility of differential inhibition effects within the gut, which is a much more intricate environment than standard growth media. Even if no specific factor can be pinpointed as the direct cause of a decline in a particular strain population, minor influences, such as dietary changes, can shift the balance of strain ratios and push the gut ecosystem closer to a dysbiotic state [35]. Glyphosate could be a major inhibitor of certain other strains.

4.1 Breast Cancer

Exposure to certain herbicides has been associated with multiple human health risks, making herbicide exposure a major concern in agriculture. Research indicates that some herbicides may increase the risk of breast cancer, possibly through mechanisms involving DNA damage, oxidative stress, or hormonal disruption [36]. Moreover, there were an estimated 2.3 million new breast cancer cases in 2020, which represented 11.7% of all new cancer diagnoses [37]. Breast cancer is the most commonly diagnosed cancer among women globally. Furthermore, breast cancer constitutes one in four cancer diagnoses and one in six cancer-related deaths among women worldwide, while this disease is projected to be responsible for 685,000 deaths. Since the majority of breast cancer cases arise from non-genetic factors, considerable interest exists in identifying potential environmental and lifestyle risk factors [38]. To our knowledge, the sole human study that employed biomonitoring to explore the possible connection between glyphosate or aminomethylphosphonic acid (AMPA) and breast cancer found that elevated urinary AMPA levels were associated with a heightened risk of developing breast cancer [39]. Conversely, glyphosate and AMPA have been related to various physiological and cellular processes, including oxidative stress, endocrine disruption, and epigenetic changes, which may contribute to breast carcinogenesis. This review will encompass these findings; for example, recent research has investigated the potential effects of glyphosate and AMPA exposure during pregnancy, a time of increased susceptibility to pesticide exposure [40]. Meanwhile, a relationship between maternal urine levels of glyphosate and AMPA and increased anogenital distance (AGD) in female neonates was observed, indicating potential endocrine disruption (Fig. 2) [41]. Additionally, urinary AMPA levels have been associated with markers of oxidative stress in urine [42]. Oxidative stress can contribute to DNA damage, which may facilitate carcinogenesis [43].

4.2 Thyroid Diseases

Herbicide exposure has been linked to thyroid disorders, including alterations in hormone production and receptor activity, reflecting the potential endocrine-disrupting effects associated with herbicides [44]. Thus, recognizing these diverse health impacts is essential for linking human health outcomes to broader discussions of soil microbial health and agricultural sustainability. Thyroid disease ranks as the second most common cause of endocrine disorders [45]. In areas with sufficient iodine levels, thyroid dysfunction primarily results from autoimmune thyroiditis [46]. The most prevalent types of this ailment include Graves' disease, known as autoimmune hyperthyroidism, and Hashimoto's disease, generally referred to as chronic autoimmune thyroiditis or autoimmune hypothyroidism [47,48]. Additional factors contributing to autoimmune thyroiditis, aside from excessive or insufficient io-

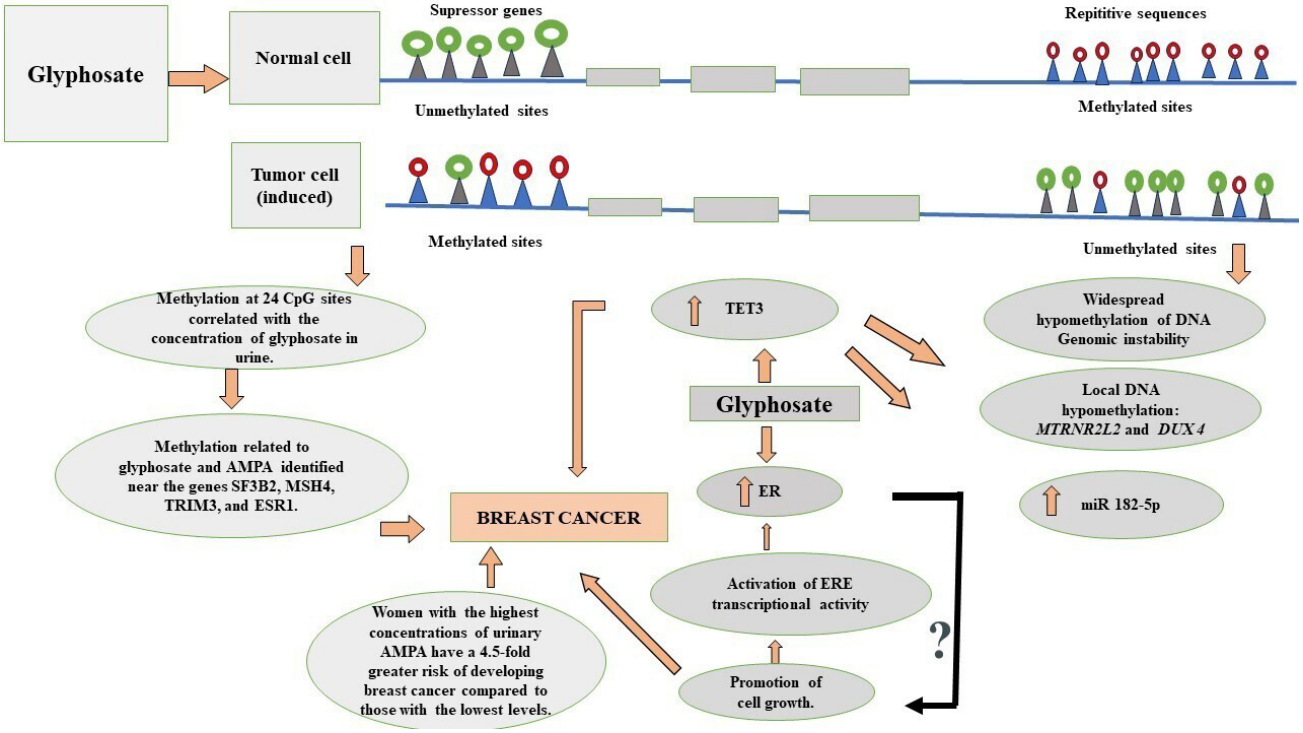

Fig. 2. Schematic representation of glyphosate pathway–promotes breast cancer through altered estrogen signalling, oxidative stress, DNA damage, epigenetic modifications, apoptosis dysregulation, and immune–microbiome interactions. TET3, tet methylcytosine dioxygenase 3; CpG, cytosine-phosphate-guanine; AMPA, aminomethylphoshonic acid; ER, estrogen receptor; ERE, estrogen response element.

dine, may encompass genetic predispositions, low selenium levels, infectious agents (such as Yersinia enterocolitica), and syndromic conditions, including Turner syndrome and Down syndrome. Globally, the occurrence of thyroid cancer is increasing, with the age-standardized incidence rate increasing by 20% between 1990 and 2013. Glyphosate is a non-selective post-emergent herbicide that effectively targets harmful weeds across a wide range of crops [49]. Studies have detected glyphosate in urine samples from individuals in both urban and rural environments who have been exposed through either occupations or recreational activities [50]. This finding raises concerns regarding the potential toxicity of the herbicide for children, pregnant women, and individuals of all ages. Glyphosate has also been associated with health issues, including thyroid disorders. Additionally, a positive correlation has been observed between the increasing occurrence of various intestinal disorders, such as celiac disease, and the rising use of glyphosate over time [51]. Celiac disease and thyroid issues often occur together, suggesting a potential link between glyphosate exposure and the development of both conditions [52]. Additionally, farmers who apply glyphosate may be at increased risk of thyroid disorders [53,54]. Nevertheless, regulatory agencies have not found any evidence to support claims that glyphosate acts as a carcinogen or an endocrine disruptor [51]. The zwitterionic form of glyphosate can bind anions in the acidic environment of the stomach, suggesting that iodide (I-) could interact with the herbicide. Indeed, the sodium–iodide symporter (NIS) in the gastrointestinal tract facilitates the conversion of dietary iodine and iodate into iodide, which is subsequently absorbed in the small intestine [55]. However, the NIS may not transport iodide-bound glyphosate, leading to increased fecal excretion and reduced serum levels. Additionally, the molecule is highly capable of forming complexes with cations at neutral and alkaline pH. Consequently, glyphosate has the potential to chelate vital minerals such as iodine, calcium, magnesium, selenium, iron, and zinc at the pH levels in the stomach, intestines, and overall internal environment, similar to the effects observed in plants [56]. These findings indicate that glyphosate can effectively form complexes with cationic macro and micronutrients in solution, which may influence nutrient uptake and metabolism in plants [57]. Furthermore, glyphosate disrupts the coupling between oxidation and phosphorylation in the metabolic cycle by increasing mitochondrial membrane permeability to protons and calcium ions, thereby hindering adenosine triphosphate (ATP) generation. For example, glyphosate reduces antioxidant defenses and induces oxidative stress in Sertoli cells, leading to necrotic cell death via a Ca^{2+}-dependent mechanism that activates endoplasmic reticulum receptors, such as inositol 1,4,5-trisphosphate (IP_3) receptors and ryanodine receptors, as well as L-type voltage-dependent calcium channels (LVDCC). From an exper-

imental standpoint, the numerous potential formulations of glyphosate-containing pesticides (commonly referred to as GBHs) introduce significant complexity in toxicological research. However, this is not the case from a regulatory perspective. Since cumulative-effect tests for the commercial formulations recommended by manufacturers are not performed, approval of commercial products relies solely on the active ingredient, which, in this instance, is glyphosate [51]. Consequently, potentially harmful, additive, or synergistic effects are overlooked. As noted, commercial glyphosate formulations are more toxic than glyphosate alone [56]. This suggests that the inert ingredients may be more toxic than the active ingredient, resulting in a more detrimental impact. Thus, research focused on actual pesticide formulations is crucial for tracking disease occurrences among populations exposed to these substances and for alerting regulatory bodies to the need to monitor and inspect subsequent use. This is particularly important, as both humans and the environment are exposed to GBH alongside glyphosate. The International Agency for Research on Cancer (IARC/WHO) has expressed concerns regarding the detection of glyphosate residues in soil, water, and urine samples from individuals living near or even far from agricultural areas. Based on available scientific data, the IARC has categorized glyphosate as "probably carcinogenic to humans" (group 2A) [58]. Prior research has indicated a connection between non-Hodgkin lymphoma rates and exposure to glyphosate [59,60,61]. Various substances that alter genes related to the regulation of the hypothalamic–pituitary–thyroid (HPT) axis have been associated with an increased risk of thyroid dysfunctions such as cancer, hyperthyroidism, and hypothyroidism [62,63,64,65]. Specifically, the hypothalamus and pituitary gland are affected by halogenated phenols, tributyltin, polychlorinated biphenyls, and surfactants. In contrast, the thyroid gland is influenced by perchlorate, nitrates, thiocyanates, parabens, and various pesticides [66,67]. Research has shown that the HPT axis is influenced by various chemicals, such as phthalates, bisphenol A (BPA), polycyclic aromatic hydrocarbons (PAHs), brominated and perfluorinated flame retardants (PBDEs), perfluorooctane sulfonate (PFOS), and perfluorooctanoate (PFOA) [67,68]. Additionally, thyroid autoimmunity may be associated with exposure to organochlorine insecticides, polychlorinated biphenyls, and heavy metals, including aluminum, mercury, and vanadium. Furthermore, both BPA and di-(2-ethylhexyl) phthalate (DEHP) have been associated with thyroid cancer and Hashimoto's thyroiditis. Meanwhile, exposure to flame retardants such as BDE-209 and tris(2-chloroethyl) phosphate (TCEP), along with cadmium and polybrominated diphenyl ethers, has been associated with a heightened risk of developing papillary thyroid cancer in humans [69,70]. Nonetheless, information on the toxicity of glyphosate remains limited, even though other herbicides have been shown to adversely affect the thyroid gland. Signs of thyroid poisoning were first observed in amphibians [63]. Hence, given that metamorphosis depends on thyroid hormones, amphibians are considered important indicators of environmental health for humans [71]. Tadpoles were subjected to various GBH formulations, including those with or without the polyoxyethyleneamine (POEA) surfactant, glyphosate, or solely POEA surfactant. The group treated with glyphosate and GBH without POEA did not show any changes in metamorphosis compared with the group exposed to GBH containing POEA surfactant, which did exhibit changes, or the group exposed solely to POEA, which also did [72]. In summary, POEA is detrimental to the HPT axis, whereas glyphosate is not. As mentioned earlier, regulatory agencies evaluated glyphosate rather than the associated formulation. Consequently, this study offered robust scientific evidence supporting the safety of glyphosate toward the endocrine system [72].

5. Impact of Pre- and Post-Emergence Herbicides on Soil Microbial Dynamics

Pre- and post-emergence herbicides affect soil microbial communities differently. Pre-emergence herbicides, such as cinmethylin, reduced microbial biomass carbon (MBC) by about 35% at 30 days and 50% at 60 days compared to untreated soil. Most microbes recovered to normal levels within 14 days after application, and soil pH decreased slightly by 0.3–0.5 units [73]. Post-emergence herbicides, such as imazethapyr and flumioxazin, had longer-lasting effects, reducing MBC by 25% at 15 days, while maintaining the level at about 20% lower than the control at 60 days [74]. These herbicides can alter microbial community composition and affect soil functions such as nutrient cycling, whereas pre-emergence herbicides mainly cause temporary changes. Hence, choosing the right herbicide and application timing is important for protecting beneficial soil microbes while managing weeds effectively. Agriculture faces numerous challenges, including soil contamination from chemicals, pests, and diseases that adversely impact plant health and productivity, as well as the loss of biodiversity and soil degradation [75]. In the twenty-first century, key factors influencing agricultural productivity include levels of microbial life in soil (Fig. 3). Soil microorganisms are vital for various interactions among soil, plants, herbicides, animals, and humans, as these microorganisms are essential for breaking down herbicides. The application of herbicides initially reduces the soil microbial community, but during the later stages of crop growth, this community begins to recover [1]. Despite the initial negative impact of herbicides on microorganisms, the process of co-metabolism, in which oxidizable carbon remains in the soil, leads to a significant increase in the microbial population [76]. Conversely, the fungal population tends to increase during herbicide breakdown, then decline. Consequently, the overall microbial community is initially affected by herbicide toxicity. As the breakdown occurs, the

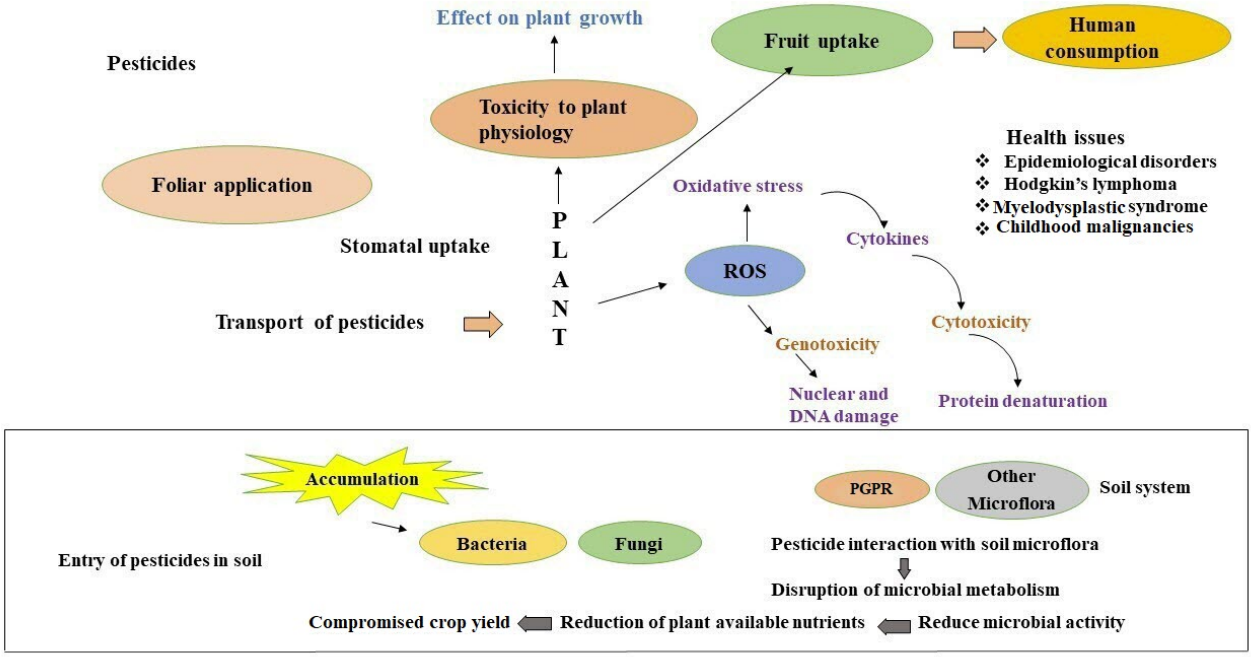

Fig. 3. Effect of pre- and post- emergence herbicides on microbial communities through biosynthetic regulations. ROS, reactive oxygen species; PGPR, plant growth promoting rhizobacteria.

available carbon promotes a modest increase in the microbial population once the toxicity subsides. Spraying herbicides after plant emergence significantly reduces bacterial and fungal populations, with little to no effect on actinomycetes. This phenomenon may be attributed to the resilience of actinomycetes, which are adept at degrading pesticides more effectively than either bacteria or fungi. At 60 days after sowing (DAS), a gradual increase in microbial population was observed across all herbicide-treated plots, bringing the populations closer to those in the untreated plots. However, the impact on all microbial communities was not deemed statistically significant. This phenomenon may stem from the capacity of the microbes to decompose and utilize pesticides as an energy source for subsequent physiological processes. Repeated herbicide application can induce microbial resistance, such as enrichment of specific degradation genes, thereby affecting long-term soil functions (Fig. 3). Metagenomic and enzyme activity studies complement CFU (colony-forming unit) data, providing stronger evidence of microbial functional recovery. Additionally, the combination of fenoxaprop-P-ethyl and ethoxysulfuron yielded the highest soil microbial counts at 86.07×10^6 CFU/g [77]. To minimize secondary damage, direct herbicidal applications aimed at controlling invasive species could be more effective if adjacent native or desirable plants are made less vulnerable to herbicide spread or are enhanced in their resistance to commonly used herbicides [78].

5.1 Impact on Soil Bacteria

A study examined the impact of the microbiological activity of pre-emergence herbicides pendimethalin and alachlor, as well as post-emergence herbicides imazethapyr and fenoxaprop-P-ethyl [77]. The analysis revealed that bacterial population densities in the root zone soil across different treatments did not differ significantly, corroborating earlier research indicating that these herbicides do not significantly affect bacterial populations in the soil [77]. Furthermore, the populations of nitrogen-fixing and phosphorus-solubilizing bacteria in the root zone soil showed no statistically significant differences among treatments, supporting the conclusion that herbicides do not adversely affect soil bacteria [79]. Repeated exposure to herbicides during weed management can select for herbicide-resistant bacterial strains. It is widely recognized that tolerance to herbicides can vary both between and within bacterial species. Additionally, herbicide tolerance may be developed through genetic changes in non-target genes associated with a broader capacity to endure stress. The mechanism behind this association may involve herbicide-induced changes in gene expression, potentially activating antibiotic resistance genes (ARGs) in bacteria. Previous research has shown that increased activity of efflux pumps can enhance the resistance of *Escherichia coli* to glyphosate and alter the antibiotic sensitivity of *Salmonella* and *E. coli* strains, such as typhimurium. If these same modifications

decrease bacterial susceptibility to both antibiotics and herbicides, then prolonged herbicide exposure could inadvertently promote the development of cross-resistance, thereby increasing antibiotic resistance [80]. Thus, recognizing the significance of selecting herbicides that target resistant microorganisms within complex soil microbial ecosystems is essential. Furthermore, additional ecologically relevant studies are necessary, as much of the existing evidence is based on simple laboratory tests utilizing single bacterial strains. In trials involving chili crops, the application of pendimethalin, oxyfluorfen, and propaquizafop at recommended doses diminished soil microbial populations by up to 15 days after application (DAA). Subsequently, treated plots exhibited a significant increase in microbial populations compared to the control. Oxyfluorfen demonstrated the highest level of inhibition, followed by pendimethalin and propaquizafop. Several other bacteria, such as *Thiobacillus*, *Bdellovibrio*, *Myxococcus*, *Polyangium*, *Azotomonas*, *Beijerinckia*, *Derxia*, *Rhizobium*, *Azospirillum*, *Pseudomonas*, *Arthrobacter*, *Clostridium*, *Bacillus*, *Achromobacter*, *Micrococcus*, *Agrobacterium*, *Nitrosomonas*, and *Nitrobacter*, have been reduced following the application of various pre- and post-emergence herbicides [81].

5.2 Impact on Soil Fungi

Compared with herbicide-treated plots, the soil fungus population was higher in the weedy check and hand-weeded plots. Meanwhile, plots treated with the fewest fungi were observed when fenoxaprop-P-ethyl was used alone or in combination with pendimethalin or alachlor. When combined with pendimethalin or alachlor, the herbicide fenoxaprop-P-ethyl greatly reduced the number of fungal species in the root zone soil, including the quantity of mycorrhizal spores and the capacity of these species to colonize black gram roots. Fenoxaprop-P-ethyl treatment inhibited soil fungi. Seven phyla were identified at the phylum level [79]. All five studied herbicides had significant and comparable impacts on the fungal community in the 0–20 cm rhizosphere soil, which was in contrast to the observed effects on the bacterial community. Within the stratum of 0–20 cm of the CK (control) soil, Ascomycota accounted for 45.0% of the overall fungal community, and was, thus, the most dominant fungal phylum [82]. Herbicides reduced the relative abundance of the phylum to 5.2%–7.9%. Additionally, there was a significant increase in the proportion of the fungal community “others” (not yet classified) from 9.6% in untreated soil to 44.0%–67.9% in treated soils following herbicide use [83]. Several other fungi, such as *Pythium*, *Alternaria*, *Fusarium*, *Sclerotium, Sclerotinia*, *Boletus*, *Endogone*, *Amanita*, *Rhizopus*, *Mucor*, *Cunninghamella*, *Chaetomium*, *Aspergillus*, *Penicillium*, *Verticillium*, and *Glomus*, have been reduced following the application of various pre- and post-emergence herbicides.

5.3 Impact on Mycorrhiza

The mutualistic, symbiotic relationship between fungi and the roots of higher plants is known as mycorrhiza, which is found in roots and organs that resemble roots [84]. Moreover, mycorrhiza serves as a conduit for energy transfer between the soil and plants [84]. Arbuscular and ectomycorrhizae are the most ubiquitous among the seven identified and named mycorrhizal species: ectomycorrhizae, ectendomycorrhizae, arbutoid, monotropoid, ericoid, orchidaceous, and arbuscular mycorrhizae. Over 80% of vascular plant species worldwide have mutualistic associations with arbuscular mycorrhizal fungi (AMF), which develop a mutualistic connection in which plants provide sugar to obligatory biotrophic fungi, and the fungi nourish plants with organic minerals, especially immobile nutrients such as zinc and phosphorus [2]. Plants that were not treated with herbicides exhibited a considerably higher colonization of black gram mycorrhizal roots. Meanwhile, applying pendimethalin and alachlor separately and in combination with imazethapyr did not considerably reduce the colonization of black gram mycorrhizal roots. However, using alachlor or pendimethalin in combination with fenoxaprop-P-ethyl greatly decreased the percentage of mycorrhizal colonization. In the rootzone soil, mycorrhizal spore numbers showed a pattern somewhat similar to that observed in the soil. Fenoxaprop-P-ethyl treatment dramatically decreased the number of spores and mycorrhizal root colonization in the wheat root zone soil. Compared with the recommended dose, nicosulfuron at 0, 10, 20, 100, and 1000 times the recommended dose significantly decreased both AMF and mycorrhizal colonization richness in the pot culture experiment. Thus, restricting AMF growth was postulated to result from two possible causes: the direct toxicity effects of the herbicide on AMF development and herbicide detoxification through colonized maize plants or indirectly through maize plants [85].

5.4 Impact on Actinomycetes

The morphology of the soil microbial community following two herbicide mixes, bispyribac sodium + metamifop and penoxsulam + cyhalofop butyl, showed that neither of the herbicide mixtures nor the tested doses had any inhibitory effect on the growth of actinomycetes at any point during the observation period. Given that neither of the studied herbicide mixtures had any negative impact on soil microflora, penoxsulam + cyhalofop butyl and bispyribac sodium + metamifop represent excellent herbicides that degrade rapidly into environmentally benign, harmless substances [86]. Several actinomycetes, such as *Streptomyces*, *Micromonospora*, *Nocardia*, and Thermoactinomyces, have been involved in this herbicide–soil microbial interaction. The most widely used herbicides in paddy were pyrazosulfuron ethyl, butachlor, pretilachlor, and 2,4-D. The results showed that butachlor had a substantially lower number of actinomycetes than pyrazosulfuron,

pretilachlor, 2,4-D etc. Actually, the brief 30-day inhibition was the maximum population observed, whereas 7 days was the minimum. The actinomycete population decreased following glyphosate spraying throughout the year. However, a subsequent laboratory investigation of the effects of applying glyphosate, metribuzin, and nicosulfuron at four rates (1 (recommended), 5, 10, and 50) showed that herbicide application temporarily inhibited actinomycete activity in soil. Furthermore, the 10 and 50 herbicide rates greatly reduced the amount of actinomycetes in the soil and the isolatory ability to develop *in vitro*. The most significant inhibition was observed with glyphosate, and effects became more pronounced at higher concentrations [87]. A similar brief suppression of the actinomycete population was observed with metribuzin and pendimethalin. The actinomycete population peaked on day 28 of treatment; glyphosate application also reduced the actinomycete population on day 7. The application of atrazine, atrazine + metolachlor, paraquat, and glyphosate resulted in a similar decline in soil actinomycete populations. Notably, herbicides inhibited actinomycetes more strongly under direct *in vitro* exposure than when actinomycetes were grown in soil.

6. Impact of Pre- and Post-Emergence Herbicides on Soil Physical Health and Soil Management

Herbicide applications affect soil microorganisms and the target organisms. Pesticides are important in modern agriculture because of the associated impacts on agricultural yield and profit ratios. Nevertheless, soil microbial populations are degraded by careless chemical applications. The two main biotic variables that affect weeds and pests reduce agricultural yield, productivity, and resource efficiency [88]. Herbicides are a class of pesticides that reduce or eliminate insect populations by specifically destroying plants and, as a result, killing targeted insects. Among the ten countries that utilize herbicides and insecticides worldwide, China is the most frequent user. Labor costs, the option to use pesticides, and the assurance of quick insect control have led to pesticide use being either permitted or widespread worldwide [89]. These products are applied directly or sprayed onto the soil surface and into the soil strata, where agricultural runoff into rivers and other waterways is caused by drifting sprays and excessive inputs that leach. The implementation of essential soil activities is frequently adversely affected by these unintended impacts on soil organisms. Likely adverse effects of using herbicides include disturbances to soil biochemical processes. In fact, these herbicides often slow the rate of biochemical reactions, interfering with microbial growth and soil enzymatic activity. Moreover, cycling of phosphorus, sulphur, and nitrogen, as well as the decomposition of biological materials, is significantly influenced by the soil microbial community. Meanwhile, the natural balance of the ground is disrupted by any variations in the quantity or function of soil microbes, which may have detrimental effects on the soil. Since these microbial communities support healthy plant growth, maintain soil health, and are essential to the proper operation of significant biogeochemical cycles, which, in turn, sustain the appropriate recycling of both inorganic and organic matter in the terrestrial environment, soil microbes are critical to the long-term survival of life on Earth [90]. Healthy plant microorganisms can substantially enhance plant well-being and fitness, thereby increasing yield and production. Mutually beneficial and parasitic microbes colonize both aboveground and underground plants. These living things can be divided into groups based on the associated colonized regions. For example, microorganisms that colonize the exterior of a plant are called epiphytes, whereas those that colonize the interior of the plant are called endophytes [91]. Therefore, changes in soil microbial communities caused by pre- and post-emergence herbicides not only affect ecosystem functions but also influence agricultural sustainability and food security. Shifts in microbial populations can alter nutrient cycling, soil fertility, and plant–microbe interactions, impacting crop growth and yield [92]. Thus, maintaining healthy, diverse soil microbiomes supports ecosystem resilience and plant health, underscoring the need for integrated management strategies that optimize herbicide use while preserving soil health and ensuring sustainable food production.

7. Herbicidal Role in the Alteration of Composition and Diversity of Soil Microflora That Impact Soil Health and Ecosystem Function

Pre- and post-emergence herbicide applications can significantly modify soil microbial composition and diversity, disrupting nutrient cycling, soil fertility, and ecosystem functions [93]. These outcomes are consistent with contemporary soil health assessment approaches that evaluate microbial diversity, enzymatic activity, and soil functional indicators [94]. Based on how the herbicide functions, the effects on host- and soil-associated microorganisms may occur either directly—by influencing the survival and functionality of microbes—or indirectly, through their host or surroundings (Fig. 4). Consequently, glyphosate affects the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in the shikimate pathway, which provides essential amino acids found in most microorganisms and vegetation, and can, therefore, directly affect microbial survival. Additional herbicides that directly affect microbial metabolism include those that prevent the production of branched-chain amino acids by inhibiting ALS, those that inhibit the enzyme acetyl-CoA carboxylase (ACC), which prevents fatty acid formation, and those that inhibit the chemical glutamate, which interferes with nitrogen metabolism [95]. Another class of herbicides has a method of action that does not specifically target bacteria but instead affects plant hormone production, biosynthesis, and

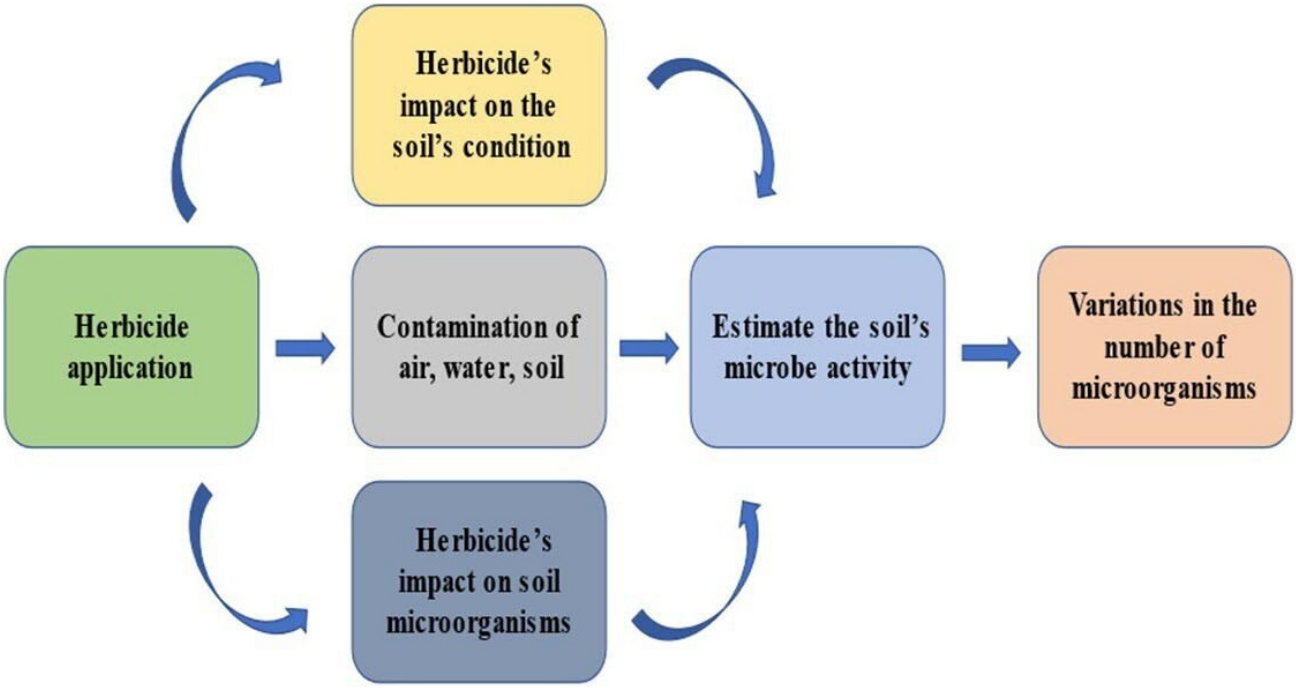

Fig. 4. Herbicides disrupt soil microbial communities, which increases oxidative stress and DNA damage, and reduces soil health and ecosystem functions.

photosynthesis. However, changes in plant features can alter how the plant and bacteria communicate. Ultimately, herbicide exposure will alter microbial populations in several ways [96]. Different bacteria are naturally more or less susceptible to herbicides. For example, specific amino acids affect glyphosate binding to the target enzyme and, subsequently, the microbiological sensitivity. Therefore, establishing the duration of a microbiological reaction to variations might also yield insights into the types of environmental variations to which the herbicide is suited [97].

Changes in the microbial response to herbicides can alter the population size of microorganisms. Many types of bacteria can digest herbicides, which can then serve as food sources. Some instances are as follows: Atrazine and glyphosate are metabolized by *Pseudomonas* and *Arthrobacter*, *Sphingomonas* breaks down dicamba and acetochlor, as well as paraquat, which is also metabolized by yeast *Lipomyces* [98]. Therefore, because of herbicide residues, the number of organisms in the population that process herbicides may increase. Chemicals may alter bacterial function, with repercussions for the community. Any of the aforementioned modifications may result in additional microbe-to-microbe communication. Long-term self-regulatory capacity can be sustained by healthy microbial communities [99]. Thus, changes induced by pesticide exposure could reduce communal efficiency. The system of rhizosphere and soil-associated microorganisms is essential to the ability of the soil to maintain important environments; the consequences of herbicide use for soil processes depend on herbicide chemical composition, mode of action, microbial susceptibility, edaphic variables, and climate. Thus, since these consequences depend on several, sometimes opposing processes, the effects of herbicides on soils remain uncertain. Nonetheless, soil parameters, agricultural management practices, soil chemical and physical properties, and weather patterns can jointly determine the impact of herbicides on microbial communities and the extent to which bacteria break down herbicides. Thus, various studies have examined how herbicides affect soil microbiomes (Table 1; Refs. [100,101]). For example, most bacterial shikimate pathways are negatively affected by the active ingredient glyphosate, while bacterial genetic resistance to glyphosate varies. Consequently, a portion of the resistant microorganisms that break down and utilize glyphosate as a nutrient source could become widespread. Similarly, atrazine might not affect the entire microbial community (active component); however, atrazine may decline in certain settings, *e.g.*, increased atrazine-degrading bacteria in the soil or increased soil microbe biomass, as a result of strong selection in their favor, which promotes atrazine decomposition. Within 7 to 30 days, herbicides can reduce the total microbial population, depending on the herbicide type [98]. Additionally, herbicides have an indirect, detrimental effect on microbial biodiversity by altering the associated physiology or metabolic processes. Consequently, herbicides influence enzymatic activity, protein production, the composition of cellular membranes, and the levels of growth regulators in plants (gibberellin production, transport of indoleacetic acid (IAA), ethylene concentration, etc.). It has also been documented that the use of

Table 1. The effects of commonly applied herbicides on soil microorganisms.

Herbicide	Doses	Impact on microorganisms in the soil	References
Nicosulfuron	0.3, 1.5, 3.0, and 15.0 mg/kg	The highest herbicide dosages (10× and 50×) reduced actinomycete numbers in both soil and *in vitro*.	[100]
Acetochlor	1.25, 1.50, 2.50, 3.125, and 5.0 L/ha	The fungus and actinomycetes were adversely affected by the population of bacteria. By harvest, the microbial population was similar across treatments, and, in some cases, significantly higher than the starting levels.	[100]
Propaquizafop	750 mL/ha		
Pendimethalin	3300 mL/ha	The microbial population was temporarily reduced by soil application.	[101]
Oxyfluorfen	850 mL/ha		

herbicides at excessive or higher doses can be fatal to several delicate microorganisms [99]. Agrochemical properties, such as soil texture, plant species, cropping system, and biological matter, as well as soil properties such as bioavailability, biological activity, determination, quantity, and toxicity, determine the extent to which chemical pesticides are harmful to soil microorganism diversity. Meanwhile, conventional tillage results in a greater reduction in soil microbial activity than no-till. After 12 days of applying mixtures of fluazifop-butyl + fomesafen and the herbicide fomesafen, mycorrhizal fungi colonization and soil microbial biomass carbon decreased under the traditional no-till method [102]. Indeed, several microbial communities are particularly susceptible to the contact effects of herbicides when combined with other compounds, compared with when a single herbicide is applied, as is the case with butachlor combined with cadmium. Following application, herbicides undergo physical and metabolic alterations, promoting a variety of additional molecules that are more lethal or durable to microbial populations that do not constitute the original targets. An example of this is the effect of 2,4-D and the associated byproducts on the Gram-negative bacterium *Burkholderia cepacia*. Apart from the active components (solvent and surfactant), the formulation also affects the herbicidal effect. Herbicides are more hazardous to bacteria when containing the surfactant polyoxyethylene amine than when only containing glyphosate acid. The detrimental effects of herbicides on soil biota may be mitigated by adding biochar to the soil [103]. The herbicidal effect can also be significantly influenced by soil type; the effect may be more severe in coarse-textured soils. For instance, in clay–sandy loam soils, herbicides were found to have a significant detrimental influence on chickpea microbial interactions [104]. Meanwhile, triazine herbicides pose a greater risk when applied over an extended period owing to the associated persistence in soil and residual activity. Repeated applications of atrazine can significantly reduce the frequency of soil bacteria. Likewise, the biodiversity of many actinomycetes and bacterial species in soil can be changed by atrazine and metolachlor.

8. Herbicidal Leading Role in the Reduction of Beneficial Soil Microbes Directly Affecting Nutrient Cycling and Soil Fertility

The current agricultural methods of most developing nations are unstable, and consequently, considerable amounts of hazardous effluents are discharged into water, land, and the atmosphere. The complexity of agricultural resources and the subsequent decomposition have increased with the development of nanoparticles and nanotechnology [105]. Nonetheless, considering how soil properties vary with geographic and climatic factors is essential [106]. The unwise utilization of several agricultural chemicals, including pesticides, fungicides, herbicides, nematicides, molluscicides, and rodenticides, has negatively impacted the current helpful soil microorganisms (Fig. 5). To achieve higher efficiency by eliminating or minimizing the insect population, herbicides (pesticides that specifically target herbs) and insecticides (pesticides that specifically target insects) are sprayed at random. Labor costs and the option of using pesticides, with the assurance of quick insect control, led to widespread global pesticide use [107]. According to American microbiologist Elaine Ingham, who founded Soil Foodweb, “the soil degrades as neither bacterium nor fungus disappears”. Microorganisms in the soil are significant only for their effects on soil fertility, structure, and activities [108]. Although the primary job of microorganisms is the breakdown of organic waste, these organisms also perform a wide range of additional tasks, including providing nutrients, which include nitrogen, phosphorus, and potassium through mineralization and fixation, thus promoting plant growth, eliminating toxic substances from the environment, suppressing organisms that cause disease, and producing compounds that might encourage plant growth. To increase crop output, soil bacteria also mineralize essential plant nutrients and produce plant hormones that enhance the system, promote development, and trigger stress responses [109]. Rhizobium, for example, changes atmospheric elemental N into biological matter. The quality of the soil is affected by factors ranging from the atmosphere to the surface, as well as changes in population dynamics, particularly in soils low in nitrogen. Legumes symbiosis with N_2-fixing rhizobia to obtain biologically

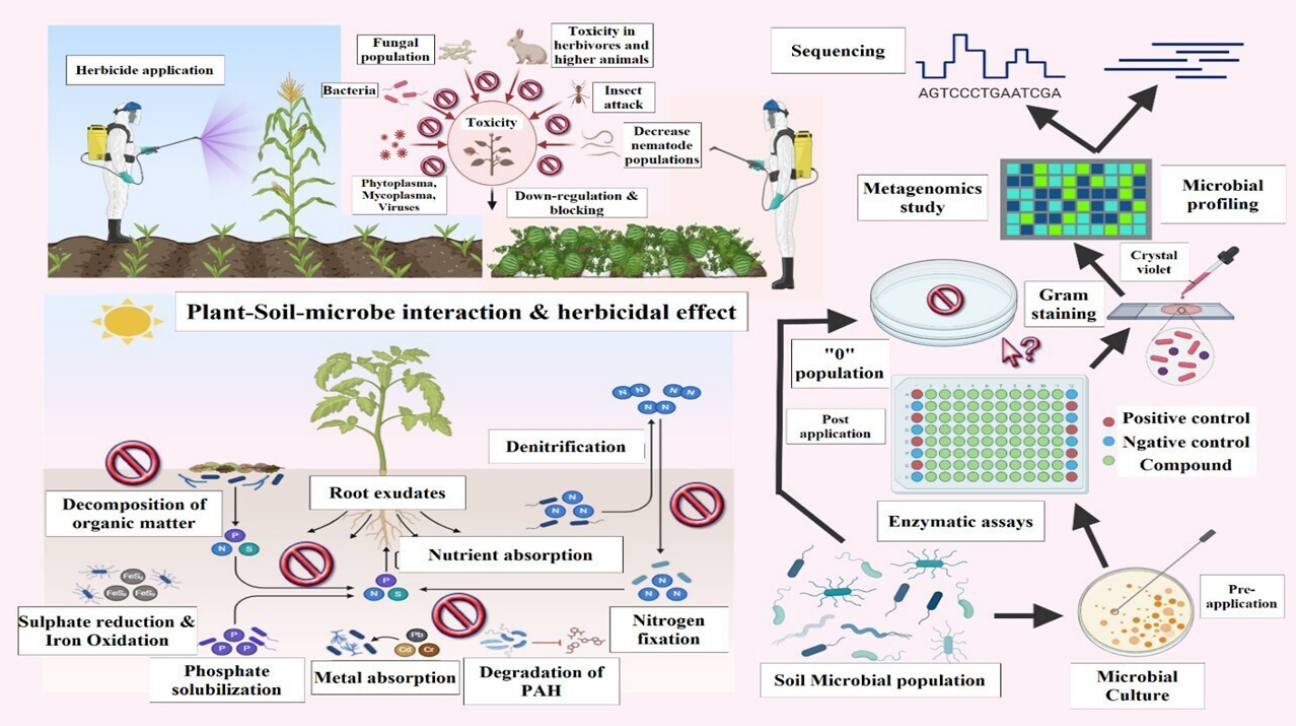

Fig. 5. Herbicide application and abnormal doses alter microbial diversity and activity, impair nutrient cycling and plant growth, and, ultimately, affect ecosystem health. PAH, polycyclic aromatic hydrocarbon.

available nitrogen, with higher nitrogen fertilizer deposition in agriculture, which "inflates" the concentration of nitrogen in the soil [110]. Recognizing the consequences of agrochemicals on soil microorganisms will require enhanced global efforts and a focus on sustainable soil management, health and nutrition, and food production and security. To maintain soil fertility and reduce soil biodiversity loss, an agriculturally productive, more environmentally friendly system is becoming increasingly necessary [111]. The main goals of most assessments are to provide a broad overview of how agrochemicals affect the health and sustainability of soil and to suggest management strategies that land managers, scientists, and policymakers may find helpful for combining organic and sustainable farming methods.

9. Interruption of the Mutually Beneficial Relationship Between Soil Fungi and Plant Roots, Impeding Plant Development and Resilience

Mycorrhizas are mutually beneficial relationships between root structures and fungi that promote soil aggregate stability and the uptake of essential nutrients, particularly ammonium (NH_4^+) and nitrate (NO_3^-). Some herbicides can actually support beneficial interactions between plants and microbes. For example, bispyribac sodium has been shown to enhance arbuscular mycorrhizal (AM) associations in the soil, helping plants absorb nutrients more efficiently [112]. Similarly, low concentrations of glyphosate can increase AM fungal colonization, promoting plant growth [113]. Even azoxystrobin, a widely used fungicide, may improve plant growth by stimulating AM fungi activity [114]. Spore germination and mycorrhizal propagation are negatively affected by these herbicides, including trifluralin, oxadiazon, and oryzalin. Alternatively, oxadiazon and oxyfluorfen considerably broaden the range of microorganisms and may increase P mobility in rice. Under greenhouse conditions, glyphosate reduces the cell, seed, and spore densities of soil AMF by 40% and root mycorrhization [115]. Glyphosate can immediately alter the environment and the production of active metabolites in plants, potentially negatively affecting AMF root colonization. Glyphosate can also indirectly affect the growth of intraradical mycelium and the formation of arbuscules, thereby controlling the quantity of AMF. Thus, the impact of glyphosate on AMF, and, as a result, the associated application, has been shown to greatly boost phosphorus efficiency via the use of microbial spores [113]. Enhanced alachlor rates of application dramatically decreased the spore count and severity of persistent infection in the vesicular–arbuscular interior spores of mycorrhizae. The harmful effects of the chemicals acetochlor and prometryn are widely established [98]. At interaction and for AMF rates between 0.1 and 10 mg/L, prometryn is more toxic than acetochlor. Fatty acids, 16:1ω5 neutral membrane fatty acid and 16:1ω5 protein fatty acid, which represent hyphal density and spore density in soil, can be used as measures of AM survival and growth, and, accordingly, reflect

the detrimental effects of herbicides on fungal live growth. The administration of a pyrethrum, terpenes, and spinosad combination did not significantly alter the AM structure or root colonization capacity of the fungal population during the evaluation of several biological pesticides in conjunction with chemical fungicides [114]. Nevertheless, the application of carbendazim to pots entirely inhibited mycorrhizal colonization. Meanwhile, applying herbicides at higher rates should be performed only after careful assessment. The effects of the herbicide metribuzin were assessed on the three AMF species in barley and maize, compared with non-inoculated treatments. AMF inoculation significantly enhanced chlorophyll development and amount in maize and barley at reduced herbicide doses (175 g a.i. ha^{-1}) [116]. Therefore, higher metribuzin doses are not recommended, and it is proposed that mycorrhizal fungi can promote plant growth to alleviate agricultural stress at lower metribuzin doses. However, when applied to maize pot cultures, the nematicide carbofuran 3G (Furadan) showed a favorable effect; thus, Furadan use was proposed for cleaner pot culture management. As a result, herbicides on the AMF differ based on the specific situation, and the overall cause and effect may be influenced by several variables, including the host, the composition, plant fitness, and the type of connections that occur between vegetation and mushrooms that result in reciprocal rewards [117]. Additionally, it has been noted that in both regulated and agricultural environments, phenotypic variability in arbuscular mycorrhizal symbiosis (AMS) is regulated by plant–fungal phospholipid metabolism, simultaneously, external factors, and nutrient cycling, which may result in observed differences in traits among different combinations of plants and arbuscular mycorrhizal fungi (AMF) [118]. A plant must carefully navigate a variety of immune, health-related, and symbiotic cues to maximize its own expansion, welfare, and growth and development, which occur through the roots, since roots serve as the main source of water and nutrients for plants and are a key point of exposure to microorganisms [119].

10. Development of Herbicide-Resistant Microbial Strains for the Repeated Use of Herbicides, Leading to Complications of Soil Management Practices

Weed species may become resistant to herbicides if the species are used repeatedly and consistently, especially if the herbicides share a similar mode of action. However, there is always a chance that weed varieties that become resistant to a certain herbicide will also develop resistance to other, similarly acting herbicides. The primary rationale is to possess a comparable binding location. Herbicide resistance in weeds may continue to evolve, increasing yield loss. Herbicides are currently a crucial component of weed control. In industrialized nations, there have been more documented incidences of herbicide resistance. The accidental or intentional application of herbicides at sub-lethal levels alters the susceptibility of biotypes to development, metabolism, and survival, particularly in self-pollinating vegetation such as wild oats [120]. The frequency with which weeds exhibit herbicide resistance, the difficulty of managing them, and the associated costs are steadily rising worldwide. Herbicide-resistant weeds are not a recent problem, as resistance was developed by biotypes as early as the 1950s [121]. However, in the 1980s, traits were adopted to address weed resistance linked to pesticide resistance in crops [122]. Subsequently, 523 distinct reports of weed resistance worldwide have presently been documented across 269 weed species (115 monocots and 154 dicots). Herbicide-resistant weeds have been reported in 99 crops across 72 countries, involving resistance to 167 herbicides representing 21 of the 31 known modes of action. Furthermore, compared with 195 species in 2010, 321 distinct species are now included in the classification of herbicide-resistant weeds in just 11 years [123]. Moreover, the situation has worsened over the past three decades; there has been no breakthrough in chemistry, nor has a new site of action for herbicides been identified. This could be because a significant (USD 250 million+) investment was made in developing novel herbicide chemicals, redirecting more money toward fungicides and insecticides. Additionally, the rapid development of herbicide resistance represents a key driver of this change. Therefore, to prevent yield declines and maintain sustainable crop production, there needs to be a concerted effort to implement timely, effective weed control techniques amid the growing prevalence of herbicide resistance. Controlling herbicide resistance cannot be achieved using a single method. However, implementing management strategies is challenging. Consequently, a variety of weed control tools is required to address the issues, concerns, and difficulties associated with various production methods [124]. Regions, cropping systems, and management techniques, as well as access to economic solutions, are essential to implementing management strategies. Moreover, ongoing work is required to establish a community-wide, multidisciplinary approach to understanding how difficult controlling weeds is across the entire agricultural enterprise and to convey the necessity of tackling herbicide resistance.

11. Long-Term Viable Effects on Microbial Communities, Potentially Leading to Decreased Soil Biodiversity

Almost all complex agrochemical compounds ultimately accumulate in soil zones that are toxic to beneficial soil bacteria, even though these bacteria are not the intended targets. Since herbicides and insecticides are sprayed evenly across the entire field, regardless of damaged regions, 95% of sprayed herbicides and 98% of sprayed insecticides enter non-target soil microbes rather than the intended targets [116]. Therefore, only about 0.1% of all in-

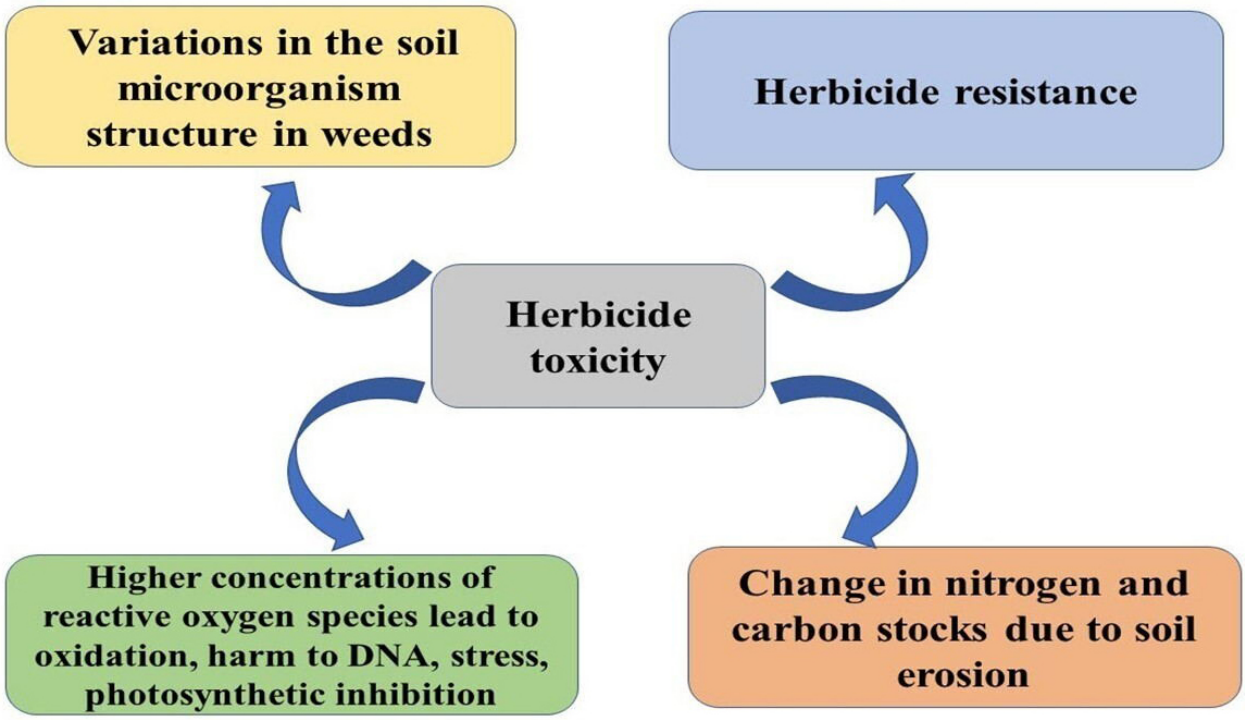

Fig. 6. Herbicidal co-suppression and co-dominance affect multiple physiological, biochemical, and molecular pathways, which influence both plant and soil system functions.

secticides target the intended species, with the remainder polluting the environment and the soil. In addition to disrupting soil biodiversity, this careless application of pesticides has adverse effects on soil microcosms, which comprise soil microfauna in field communities and soil ecosystems. Excessive levels of herbicides that leach underground directly affect the soil microorganisms which influence how plants develop and grow and are a natural measure of the fertility of the soil (Fig. 6). Similarly, a lot of research has documented the outcomes of several pesticides for suppressing the activity of soil enzymes, such as hydrolyzes the enzymes urease, nitrate reductase, oxidoreductases, nitrogenases as well as dehydrogenases, which influence the nutrient status of soil. Moreover, pesticide applications affect biological nitrogen fixation and related biotransformations, such as S-oxidation, phosphorus solubilization, nitrification, denitrification, and ammonification. Furthermore, heavy pesticide use in modern agriculture reduces the functional diversity of many soil microbial communities that lack targets, as well as decreasing microbial biomass carbon (MBC). Biochar-based fertilizers are not only a source of essential plant nutrients but can also help improve and modify specific chemical and physical soil properties [125].

12. Herbicidal Inhibition of Nitrogen-Fixing Bacteria Directly Causes Soil Nitrogen Imbalance

Different herbicides can disrupt the rhizobacteria–legume symbiotic relationship and impede essential N-fixation processes. Chemicals can harm the root hairs, where infections and stem development occur, disrupting the rhizobacterial infection process and impairing nodulation and, in turn, BNF in legumes. Furthermore, these chemicals might affect the phytochemical signaling of Rhizobium, which is essential to coordinating and controlling major BNF activities. Certain herbicides alter cell shape, leading to pleomorphic cells. Herbicides can also decrease bacteroids, dry plant matter, nitrogenase activity, and adenosine triphosphate (ATP) levels in Rhizobium, which, in turn, affects symbiotic N-fixation. *Bradyrhizobium* development and activity in soybeans can be inhibited by herbicide application. Herbicide use hinders *Bradyrhizobium japonicum* development in soybean cells *in vitro* and also affects nodulation in controlled greenhouse environments. However, chlorimuron ethyl does not affect *B. japonicum* growth, even at natural culture concentrations 150 times higher than suggested field rates. At concentrations above the recommended rate, triazines, such as terbutryn, simazine, prometryn, and bentazone, which are frequently employed, decrease bacterial activity. Conversely, suggested field rates of herbicides, including fluazifop-butyl, metolachlor, alachlor, and sethoxydim, had no adverse effect on soybean yields or BNF. Nonetheless, since glyphosate and paraquat include ethylamine, these chemicals are non-selective herbicides that could decrease the soybean N-fixation. The Rhizobium association method in agricultural plants can be slowed by applying the herbicide pendimethalin at 0.5–1.0 kg/ha. The widely used herbicide

2,4-D also often disrupts the metabolism of Rhizobium sp. in beans, thereby suppressing nitrification, BNF, and the growth and development of blue-green algae (BGA). Additionally, 2,4-D residues are detected in considerable quantities in the cytosol and cell wall of Rhizobium, indicating that 2,4-D affects the spread of rhizobacteria [103]. Azotobacter are free-living, aerobic soil microorganisms that fix nitrogen and are crucial to the N-cycle. However, Azotobacter are extremely vulnerable to herbicides, even after only 7–14 days of exposure. The type and dosage of herbicidal chemicals used determine the extent to which Azotobacter activity, population, growth, and development are inhibited [126]. During the 28-day herbicide treatment, the metabolic processes of Azotobacter and another bacterium were significantly decreased in prometryne-treated soybean and sunflower fields. *Rhizobium phaseoli* and *Azotobacter vinelandii* were suppressed by agroxone, atranex, and 2,4-D herbicides; the populations of these bacteria also declined as herbicide concentration increased. Similar results were observed in sugarbeet, where a 1.7 L/ha application of metolachlor and dimethenamid significantly reduced Azotobacter population levels. Additionally, the applied dimethenamid was reduced by 2% and 18% for metolachlor and dimethenamid, respectively, averaging 1.6 L/ha, compared with 1.4 L/ha. In comparison, the combination of trifluralin and flumetsulam was decreased by 2% at an average of 2 L/ha, instead of the recommended 1.7 L/ha [121]. The interpretation of comparative species decomposability may be affected by the selection of the breakdown index or the analytic method of gross breakdown data [127]. Nearly every living thing in the ecosystem is covered with a variety of microorganisms that together compose the microbial communities. Microbes provide hosts with vital functions, such as protection, nutrient provision, and food digestion, and impart a variety of behavioral features [128]. The diversity of the rhizosphere and root microbiota was affected by BX secretion, with the root fungus showing the strongest effects. The influence of the genetic background was not less significant than that of BX discharge, indicating that BX exudation is a crucial characteristic through which maize shapes its associated microbial community [129].

13. The Impact of Herbicides on Soil Microflora May Vary Based on the Types of Herbicidal Treatment, Soil Properties, and Environmental Conditions

According to American microbiologist Elaine Ingham, who founded Soil Foodweb, “the soil degrades if both bacteria and fungi disappear.” The only possible argument for the significance of soil microbes is that these microbes affect soil fertility, composition, and properties. Herbicides are incorporated into the soil both directly and indirectly during plant treatment through toxins or water from plants and animals. Following herbicide application, herbicides can evaporate (volatilize), be removed by surface runoff, be taken up by plants, be absorbed into the soil, or be exposed to chemical or microbiological degradation, or be leached into groundwater and the deep soil layer. A comprehensive overview of the chemical properties, mode of action, associated toxicological effects, and environmental impact of the herbicides discussed in this review is provided under Table 2 (Refs. [130,131,132,133,134,135,136,137,138,139,140,141,142,143,144,145,146,147,148,149,150,151,152,153,154,155]) and **Supplementary Table 1**. Herbicides can be toxic, but mixing several substances can create dangerous effects that harm both humans and the ecosystem. Rhizobium, for instance, transforms atmospheric elemental N into biological matter. Degradation of herbicides in soil can occur by photochemical, chemical, or microbiological processes. Soil microbiological diversification and the mutually beneficial interactions between soil organisms, which support nutrient absorption and other biological functions, directly impact plant biomass output [156]. Only a minor portion of pesticides break down photochemically in soil, but the process is predominant in air and water. Herbicides break down chemically in soil through oxidation, hydrolysis, and non-hydrolytic transformations. Microorganisms break down aliphatic and hydroxyl molecules effectively, whereas aromatic compounds are decomposed more slowly. The chemicals with the slowest decomposition rates are those with rings containing oxygen, sulphur, or nitrogen. Atrazine mineralization is slow (<2%) at moderate temperatures and with lower moisture content, and decreases further at deeper soil levels. This occurs when temperature and moisture levels increase over a 2- to 6-month period. Based on soil profile depth, 10–80% of 2,4-D was mineralized. The two main goals of ecological restoration with synthetic microbiological communities are the breakdown of contaminants and the restoration of biodiversity [157]. Although the primary job of these communities is the breakdown of organic waste, these organisms also perform a wide range of additional tasks, including providing nutrients, such as potassium, phosphorus, and nitrogen through mineralization and fixation, thus promoting plant growth, eliminating toxic substances from the environment, suppressing organisms that cause disease, and producing compounds that might encourage plant growth. To increase crop output, soil bacteria also mineralize nutrients plants require from the environment and release hormones that strengthen protective systems, promote growth, and trigger stress responses.

14. Promotion of Alternate Weed Management Practices With Reduced Herbicide Limits Can Enrich Soil Microflora and Maintain Soil Health

Adopting sustainable weed control strategies that reduce herbicide use can help restore microbial balance, improve soil health, and support ecosystem resilience [158].

Table 2. Effects of diverse herbicides: chemical properties, associative mode of action, toxicological effects, and their environmental impact.

Herbicide name	IUPAC name	Herbicide type	Mode of action	Formula (molecular weight)	Soil toxicity/other toxicity	Agriculture or another sector implementation	Effectivity (environmental/health hazard)	References
Fenoxaprop-P-ethyl	ethyl (*2R*)-2-[4-[(6-chloro-1,3-benzoxazol-2-yl)oxy]phenoxy]propanoate	Aryloxyphen-oxypropionates	Fatty acid synth-esis (ACCase)	$C_{18}H_{16}ClNO_5$ (361.8 g/mol)	Ability to influence soil microorganisms, altering microbial activity and overall soil health.	Used to control both annual and perennial grasses in crops such as soybeans, wheat, and barley.	It can persist in soil and contaminate water, potentially harming non-target plants and aquatic life.	[130]
Alachlor	2-chloro-*N*-(2,6-diethylphenyl)-N-(methoxymethyl)acetamide	Chloroacetamide	Very-long-chain fatty acid synthases	$C_{14}H_{20}ClNO_2$ (269.77 g/mol)	Alachlor may have toxic effects on soil microbiota, potentially altering nutrient cycling and overall soil health.	Used to control broadleaf weeds and annual grasses in peanuts, soybeans, and maize.	Alachlor can seep into water, potentially contaminating groundwater and harming aquatic life as well as non-target vegetation.	[131]
Propanil	*N*-(3,4-dichlorophenyl) propanamide	Amides	Photosynthesis photosystem II D1 protein	$C_9H_9C_{l2}NO$ (218.08 g/mol)	Impact on soil microorganisms, which could alter the microbial ecosystems of rice paddies.	Propanil is used to control grassy weeds in rice fields.	Propanil may persist in aquatic systems, posing risks to both non-target plants and wildlife.	[132]
Atrazine	1-chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine	Triazines	Photosynthesis photosystem II D1 protein	$C_8H_{14}ClN_5$ (215.68 g/mol)	Effects on soil microorganisms, altering their roles and diversity.	Broadleaf weeds in maize, sugarcane, and sorghum are controlled using atrazine.	Atrazine may persist in soil, contaminating groundwater and harming aquatic ecosystems and non-target plants.	[133]
Topramezone	4-[3-(4,5-dihydro-1,2-oxazol-3-yl)-2-methyl-4-methylsulfonylbenzoyl]-2-methyl-1H-pyrazol-3-one	Phenyl pyrazolyl ketone	HPPD	$C_{16}H_{17}N_3O_5S$ (363.4 g/mol)	Impact on soil microorganisms, which could interfere with microbial activity and overall soil health.	Controls grasses and broadleaf weeds in crops such as soybeans and maize.	Topramezone is a persistent contaminant that may pollute water sources and adversely affect aquatic species and non-target plants.	[134]
Paraquat	1-methyl-4-(1-methylpyridin-1-ium-4-yl)pyridin-1-ium	Bipyridyl	Photosystem I energy diversion	$C_{12}H_{14}N_2^{+2}$ (186.25 g/mol)	Effects on microbial diversity and soil health caused by disruptions to soil microbial populations.	Paraquat is a non-selective herbicide used to control weeds in a variety of crops and non-crop areas.	Paraquat can persist in soil and is extremely toxic to non-target plants and animals, raising serious long-term environmental concerns.	[135]

Table 2. Continued.

Herbicide name	IUPAC name	Herbicide type	Mode of action	Formula (molecular weight)	Soil toxicity/other toxicity	Agriculture or another sector implementation	Effectivity (environmental/health hazard)	References
Pendimethalin	3,4-dimethyl-2,6-dinitro-*N*-pentan-3-ylaniline	Anilines	Disruption of mitosis	$C_{13}H_{19}N_3O_4$ (281.31 g/mol)	Potential impacts on soil microorganisms, which could disrupt microbial activity and overall soil health.	Pendimethalin is used to control annual grasses and broadleaf weeds in crops including corn, soybeans, and various vegetables.	Pendimethalin can persist in the environment, contaminating water sources and harming aquatic life as well as non-target plants.	[136]
Glufosinate	(2RS)-2-amino-4-[hydroxy(methyl)phosphinoyl]butanoic acid or (RS)-2-amino-4-[hydroxy(methyl)phosphinoyl]butyric acid	Organophosphate	Glutamine synthase	$C_5H_{12}NO_4P$ (181.13 g/mol)	In soil, glufosinate, is rapidly broken down by microbes and is not highly active.	The broad-spectrum herbicide glufosinate is used to control weeds in a variety of crops, including cotton, soybeans, and corn.	It is quickly decomposed by microorganisms and is not highly active in soil; therefore, there is very little risk of water contamination.	[137]
Diuron	3-(3,4-dichlorophenyl)-1,1-dimethylurea	Methyl	Photosynthesis	$C_9H_{10}C_{l2}N_2O$ (233.09 g/mol)	Effects on soil microorganisms, which influence microbial populations and key soil processes.	Controls grasses and broadleaf weeds in cotton, sugarcane, and various other crops.	It may persist in water and soil, posing risks to aquatic life and non-target plants.	[138]
Bromoxynil	3,5-dibromo-4-hydroxybenzonitrile	Nitriles	Photosynthesis photosystem II D1 protein	$C_7H_3Br_2NO$ (276.91 g/mol)	By affecting the microbial balance and overall soil health, it can impact soil microorganisms.	Broadleaf weeds in maize, cereal crops, and non-crop areas are controlled using bromoxynil.	Bromoxynil can harm aquatic life and non-target plants due to its persistence in soil and water.	[139]
Acetochlor	2-chloro-*N*-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide	Chloroacetanilide	Geranylgeranyl pyrophosphate (GGPP)	$C_{14}H_{20}ClNO_2$ (269.77 g/mol)	Impact on soil microbes, which could alter microbial diversity and affect soil functioning.	In crops such as maize and soybeans, acetochlor controls broadleaf weeds and annual grasses.	It may contaminate water supplies and negatively impact aquatic life and non-target plants by leaching into groundwater.	[140]
Simazine	6-chloro-2-*N*,4-*N*-diethyl-1,3,5-triazine-2,4-diamine	Triazine	Photosystem II inhibitor	$C_7H_{12}ClN_5$ (201.66 g/mol)	Impact on soil microbes, which could alter microbial diversity and affect overall soil health and condition.	In both non-crop areas and crops such as maize, simazine controls grasses and broadleaf weeds.	It may persist in soil and water, polluting water sources and harming aquatic life as well as non-target plants.	[141]

Table 2. Continued.

Herbicide name	IUPAC name	Herbicide type	Mode of action	Formula (molecular weight)	Soil toxicity/other toxicity	Agriculture or another sector implementation	Effectivity (environmental/health hazard)	References
Metolachlor	2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(1-methoxypropan-2-yl)acetamide	Chloroacetamide	Very-long-chain fatty acid synthases	$C_{15}H_{22}ClNO_2$ (283.79 g/mol)	The impact of soil microbial activity has the potential to modify soil functions and microbial diversity.	Metolachlor is used in crops such as maize and soybeans to inhibit the growth of broadleaf weeds and annual grasses.	Metolachlor can persist in the environment, contaminating water and harming aquatic life as well as non-target plants.	[142]
Mesotrione	2-(4-methylsulfonyl-2-nitrobenzoyl)cyclohexane-1,3-dione	Triketones	HPPD	$C_{14}H_{13}NO_7S$ (339.32 g/mol)	In soil, mesotrione can reduce the number of bacteria, fungi, and actinomycetes. β-glucosidase and other soil enzyme activities may also be affected.	Manage broadleaf weeds in a range of crops, including popcorn, sweetcorn, and field corn.	Mesotrione exhibits longevity and long-term residues, soil health, meaning subsequent crops may be ecologically at risk. Mesotrione can also enter non-target plants through runoff, airborne dust, and spray drift.	[143]
Bentazone	2,2-dioxo-3-propan-2-yl-1*H*-2λ^6,1,3-benzothiadiazin-4-one	Benzothiadiazole	Photosystem II inhibitor	$C_{10}H_{12}N_2O_3S$ (240.28 g/mol)	Effect on soil microorganisms, which could change the health of the soil and microbial activity.	Reduces sedge and broadleaf weeds in crops such as soybeans, corn and rice.	It may persist in water systems and affect non-target plants and aquatic species.	[144]
Bipyrazone	1,3-dimethyl-4-(α,α,α-trifluoro-2-mesyl-p-toluoyl)-1H-pyrazol-5-yl 1,3-dimethyl-1H-pyrazole-4-carboxylate	Pyrazole	HPPD inhibitor	$C_{20}H_{19}F_3N_4O_5S$ (484.45 g/mol)	Effect on soil microbes, which could cause microbial populations and soil functions to become disrupted.	Controls grasses and broadleaf weeds in crops such as corn and soybeans.	It can persist in soil, where it may contaminate water and harm aquatic life and non-target plants.	[145]
Butachlor	*N*-(butoxymethyl)-2-chloro-*N*-(2,6-diethylphenyl)acetamide	Chloroacetamide	Long-chain fatty acid inhibitor	$C_{17}H_{26}ClNO_2$ (311.8 g/mol)	Impact on soil microbes, which could alter microbial diversity and soil health.	Butachlor is used to control broadleaf weeds and annual grasses in crops such as maize and rice.	Butachlor may contaminate water supplies and adversely affect aquatic life and non-target plants by seeping into groundwater.	[146]

Table 2. Continued.

Herbicide name	IUPAC name	Herbicide type	Mode of action	Formula (molecular weight)	Soil toxicity/other toxicity	Agriculture or another sector implementation	Effectivity (environmental/health hazard)	References
2,4-D	2-(2,4-dichlorophenoxy)acetic acid	Phenoxy carboxylates	F-box proteins	$C_8H_6Cl_2O_3$ (221.03 g/mol)	The microbial community in agricultural soil may undergo slight functional changes as a result of 2,4-D exposure. The community may also develop an intolerance to 2,4-D due to pollution.	Since the 1940s, herbicides have been used to control broadleaf weeds in turf, lawns, rights-of-way, aquatic sites, and forestry sites, and many agricultural, fruit, and vegetable crops. Herbicides can also be utilized to control citrus plant development.	Groundwater, surface water, drinking water, and agricultural catchments are all known to contain 2,4-D. Additionally, 2,4-D can be found in surfaces and indoor air.	[147]
Bensulfuron-methyl	methyl 2-[(4,6-dimethoxypyrimidin-2-yl)carbamoylsulfa-moylmethyl]benzoate	Sulfonylureas	ALS; branched-chain amino acids	$C_{16}H_{18}N_4O_7S$ (410.4 g/mol)	Reduces the number of beneficial microorganisms in the soil, which can inhibit the release of allelopathic chemicals and limit the activity of soil enzymes.	Effective control of a wide range of annual and perennial weeds in crops such as rice and wheat.	Bensulfuron-methyl is a herbicide that can poison fish, birds, beneficial insects, and non-target plants, as well as contaminate water, soil, and surrounding vegetation.	[148]
Pretilachlor	2-chloro-N-(2,6-diethylphenyl)-N-(2-propoxyethyl)acetamide	Chloroacetamide	Long-chain fatty acid inhibitor	$C_{17}H_{26}ClNO_2$ (311.8 g/mol)	Impact on microbial populations and processes in treated regions due to its mild toxicity to soil microorganisms.	Reduces certain broadleaf weeds, grasses, and sedges in rice paddies.	Has the ability to affect water quality and organisms through runoff and can be hazardous to aquatic life.	[149]
Dicamba	3,6-dichloro-2-methoxybenzoic acid	Phenoxy carboxylates	F-box proteins	$C_8H_6Cl_2O_3$ (221.03 g/mol)	Dicamba has a half-life of 1–4 weeks in soil, making it relatively persistent. Dicamba is highly mobile and soluble in water, and can contaminate groundwater.	Dicamba is a synthetic auxin herbicide that is used to manage broadleaf weeds.	Dicamba can damage non-target plants, birds, insects, fish, and pollinators, as well as contaminating neighboring streams and groundwater. *Salmonella enterica* and other human pathogens, such as *Escherichia coli*, may also develop antibiotic resistance as a result of its use.	[150]

Table 2. Continued.

Herbicide name	IUPAC name	Herbicide type	Mode of action	Formula (molecular weight)	Soil toxicity/other toxicity	Agriculture or another sector implementation	Effectivity (environmental/health hazard)	References
Bispyribac	2,6-bis[(4,6-dimethoxypyrimidin-2-yl)oxy]benzoic acid	Pyrimidinyl benzolates	ALS; branched-chain amino acids	$C_{19}H_{18}N_4O_8$ (430.4 g/mol)	Bispyribac can alter the diversity of functional microorganisms, enzyme activities, and the overall soil microbial community.	A broad spectrum, post-emergent herbicide for controlling grasses, sedges, and broadleaf weeds in rice crops.	Low to moderate harm to biological diversity and is unlikely to contaminate groundwater.	[151]
Imazethapyr	5-ethyl-2-[(RS)-4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl]nicotinic acid	Imidazolinones	ALS; branched-chain amino acids	$C_{15}H_{19}N_3O_3$ (289.335 g/mol)	Reduced soil microbial biomass and enzyme activity.	Effective grass and broadleaf weed control can protect a wide range of crops.	Imazethapyr can impact the quality of surface water, particularly in areas with poorly drained soils and shallow groundwater. Imazethapyr is toxic to the environment and poses a passive health hazard.	[152]
Tembotrione	2-[2-chloro-4-methylsulfonyl-3-(2,2,2-trifluoroet-hoxymethyl)benz-oyl]cyclohexane-1, 3-dione	Triketones	HPPD	$C_{17}H_{16}ClF_3O_6S$ (440.8 g/mol)	Tembotrione may be phytotoxic to crops grown in rotation. Additionally, its half-life can exceed ninety days in certain soils, posing a risk to subsequent crops. It degrades faster in alkaline environments, meaning decomposition is pH-dependent.	A selective, post-emergence herbicide designed to manage a wide range of grassy and broadleaf weeds in maize.	Fish, invertebrates, and algae are examples of non-target species that may be poisoned by tembotrione and the associated parent compound and brokendown derivatives.	[153]
Glyphosate	2-(phosphonometh-ylamino)acetic acid	Organophosphate	EPSPS; shikimate pathway function	$C_3H_8NO_5P$ (169.073 g/mol)	The balance of fungi and bacteria in the soil can be altered by glyphosate.	An extremely strong broad-spectrum herbicide that controls a range of weeds.	Glyphosate is considered a potential carcinogen and that may be hazardous to humans, affecting the liver, embryos, placenta, and plasma as well as epithelial cells.	[154]

Table 2. Continued.

Herbicide name	IUPAC name	Herbicide type	Mode of action	Formula (molecular weight)	Soil toxicity/other toxicity	Agriculture or another sector implementation	Effectivity (environmental/health hazard)	References
Penoxsulam	2-(2,2-difluoroethoxy)-*N*-(5,8-dimethoxy-[1,2,4] triazolo[1,5-c]pyrimidin-2-yl)-6-(trifluoromethyl) benzenesulfonamide	Triazolopyrimidine sulfonamide	ALS; branched-chain amino acids	$C_{16}H_{14}F_5N_5O_5S$ (483.4 g/mol)	Numerous soil-dwelling bacteria can degrade penoxsulam, and certain fungi, such as *Aspergillus flavus* and *Aspergillus niger*, can also contribute to this process.	Management of broadleaf weeds, sedges, and grasses in transplanted rice.	It is toxic to aquatic plants and can be hazardous to soil and the environment.	[155]

IUPAC, international union of pure and applied chemistry; HPPD, 4- hydroxyphenylpyruvate dioxygenase; ALS, acetolactate synthase; EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase.

However, regulatory frameworks could be enhanced by including microbial and ecological criteria to guide sustainable herbicide application. Research suggests that compared with monoculture systems, incorporating leguminous plants into a cropping system can improve total yields and income stability [159]. Nonetheless, differences exist between the various kinds of legumes. Short-season legumes, such as groundnuts, are easier to market and better suited to agricultural systems focused on producing goods for the market [160]. Conversely, long-season legumes, such as pigeon pea, improve maize yields by fixing a significantly higher amount of nitrogen, an essential component in systems based on maize, and increasing the availability of phosphorus [159]. Over time, three zero-till systems within a single system that included three residue-producing crops: mungbean, wheat, and rice, showed consistent superiority. This system includes zero-till summer mungbean with wheat debris, zero-till direct-sown rice with summer mungbean debris, and zero-till wheat with rice debris. Compared with conventional rice-wheat systems and ploughing, this system achieved about 33.5% higher total productivity (measured as wheat equivalent yield). Additionally, this system led to a 25% reduction in nitrogen use for wheat and rice cultivation [160]. The rice–wheat system, based on sustainable farming practices, is a crucial tactic for climate change adaptation and shows potential as a substitute for the widely used traditional tilled method. However, identifying a crop that can effectively contribute to economic viability and replace rice as an essential crop to expand the dominant Indo–Gangetic Plains pattern of rice and wheat cultivation has proven difficult. In terms of total system productivity, the permanent zero-till broad bed and flatbed approaches with debris regularly outperformed traditional tillage in all three systems. When combined with 100% nitrogen treatment, the broad bed and flat bed with debris from zero till permanent in the cotton–wheat system resulted in greater system productivity than the conservation tillage method by 15% and 13%, respectively. This wheat–cotton combination, based in California, has the potential to be a better option than the rice–wheat system, a crucial climate change adaptation method. Zero till permanent broad beds with debris and flat beds with and without debris surpassed the traditional system productivity of farming techniques, expressed in wheat equivalent yield (WEY), under a conservation tillage-based pigeon pea–wheat system. Comparing zero-till permanent broad beds with debris and flat beds with and without debris to conventional tillage, the results showed increases in system production of about 19%, 18%, and 11%, respectively. Similar outcomes were also noted in the system, including maize and wheat [161]. Over time, the populations of dominant weeds and weed seedbanks in the rice–wheat system decreased when legume combinations were included, as in wheat–rice–mungbean or rice–wheat–chickpea. Remarkably, compared to the rice–wheat system, *Phalaris minor* was identified as the most troublesome weed in the Indian Indo–Gangetic region, and its incidence was reduced by 42% when the rice–chickpea system was integrated into the rice–wheat system [162]. Furthermore, adding legumes (mungbean and chickpea) to the maize–wheat rotation introduced diversification and increased legume diversity, thereby reducing the dominance of above-ground and soil seedbank weeds and promoting ecological weed management [163]. Legumes can enhance yields in rice-based ecosystems, and the long-term implementation of zero-tillage residue management in the rainy seasons following puddled rice cultivation has been shown to reduce weed flora and seed density in upland areas [164]. The efficiency of solarization in eliminating weeds in crops such as maize has been reported to reach up to 98%. However, agricultural damage caused only by weeds has been shown in non-solarized control plots to reach 90% [165]. Globally, solarization has been effective in controlling annual weeds such as redroot pigweed, *Setaria spp*., *Portulaca spp*., *Digitaria spp*., *Barnyard grass*, *cogongrass*, *Ageratum spp*., and *Amaranthus spp*. Meanwhile, eliminating weeds also prevents harmful insects and bacteria from growing and spreading, which could otherwise complete their life cycles on uncontrolled vegetation. During summer, uncontrolled vegetation such as *Convolvulus arvensis* and *Cyperus spp*. has demonstrated greater resistance to soil solarization, whereas winter-season wild flora are generally more susceptible to eradication. The spatial and temporal variability in soil moisture conditions in irrigated environments offers opportunities to improve weed control efficiency. When the topsoil is dry, crops can have an early edge against weeds through large-seeded crop cultivation in wet conditions, such as deep soil. The location and density of weeds can also be affected by the selected watering technique. For example, planting wheat in dry conditions helps limit weed emergence and competitiveness since wheat germinates better in dry conditions than many weeds thrive. Surface irrigation, often using the flooding approach, is the primary irrigation technology worldwide. The proportion of weed species in rice crops has changed following the switch to direct sowing. Notably, certain species have successfully adapted to rice directly seeded situations, including *Fimbristylis miliacea*, *Cyperus difformis*, *Ischaemum rugosum*, and *Echinochloa* spp [166]. Different post-sowing water regimes cause these species to respond differently in terms of germination and establishment, providing important information for selecting weed constituents for weed communities. In contemporary agriculture, overuse of fertilizer and water can unintentionally promote weed growth. Modern production systems must account for the effect of irrigation on weed development, even though these systems are planned and operated with a specific crop in mind. Thus, within the context of integrated weed management, ensuring the appropriate water supply, drainage, and flow methods are used is crucial.

15. Periodical Monitoring of Soil Microflora Responses to Herbicide Application is Essential for Sustainable Agriculture and Environmental Conservation

Temporal factors and agricultural practices strongly influence the magnitude of herbicide effects on soil microbial communities. Microbial populations fluctuate seasonally due to changes in soil moisture, temperature, and organic matter availability, making the timing of herbicide application critical for interpreting microbial responses [94]. Agricultural practices such as tillage and crop rotation also modulate these effects; conservation tillage and legume-based rotations often enhance microbial diversity and resilience, potentially mitigating herbicide-induced disturbances [89]. Considering these factors provides a better context for assessing herbicide impacts on soil microbial dynamics and ecosystems. Breakdown of pesticides is greatly influenced by soil microorganisms [167]. Pesticides can be metabolized by soil-dwelling bacteria through various enzymatic processes. For instance, the response that a parent molecule may become oxidized, decreased, and hydrolyzed as a consequence of microbial activity, introducing functional groups including hydroxyl, carboxyl, and amine. As a result, the primary chemical undergoes biological transformation, acquiring new physical and biological characteristics. Conjugation is another reaction that occurs—the connection between the microbe and the activated metabolite triggers the biotransformation. Pesticides may be separated into several categories. One general way is by the types of insects they manage, such as nematodes, weed killers, insecticides, fungicides, and rodenticides. Using pesticides has several benefits, such as increased food production and quality, and decreased insect-borne illness. However, the use of pesticides has raised questions about potential environmental harm, particularly to water supplies. The persistent and ubiquitous characteristics of pesticides, which affect biodiversity, are primarily to blame for the following environmental repercussions [168]. Finally, the metabolite is sequestered or excreted by the bacteria. Additionally, there are synthetic processes that result in the oligomerization of the parent chemical or the secondary conjugation of the parent compound by microbes. The reaction biotransforms the parent compound, producing a metabolite with a higher affinity for water. Bacteria are the primary degraders in soil, which is why prokaryotes (bacteria and actinomycetes) and eukaryotic fungi are involved in the transformation of pesticides. Most pesticide breakdown occurs in the solution phase; however, if adsorption occurs in the soil matrix, the rate of degradation over time is reduced [169]. Due to neighboring industrial discharges and agricultural fields, pesticides can be found in rivers and lakes. The fact that high levels of agrochemicals are compatible with soil allows the soil mass to act as a container for pesticides. Resources associated with water above ground, including the proximity and interaction between the ground and water sources, make lakes, streams, estuaries, and groundwater susceptible to pesticide pollution. Tiny amounts of pesticides can accumulate in water, and the food chain can amplify the pesticidal effects and transfer these chemicals to aquatic organisms that may pose a risk to global health [168]. Agribusiness uses most of the pesticides [170]. Herbicides are chemical weed killers primarily used to regulate plant development. Insects are controlled in residential gardens, food storage facilities, and farms by applying insecticides; meanwhile, fungicides are used to protect crops or seeds from fungal contamination. Additionally, pesticides can be divided into groups based on their mode of action, such as agents that kill, repel, or mitigate [171]. Pesticide pollution of soil impairs soil performance and associated chemical or biological composition, thereby affecting crop productivity. In addition, by destroying the natural materials in the soil, pesticides harm the biodiversity of microorganisms and enzymatic activity; enhanced irrigation and precipitation have also increased border activity and soil pesticide discharge.

16. Balancing Herbicide Use With Ecological Considerations Might Mitigate Adverse Effects on Soil Microflora and Promote a Healthy Soil Ecosystem

Soil microbial functions can be monitored using molecular marker genes: *amoA* (ammonia monooxygenase) assesses nitrification, *nirK* and *nirS* (nitrite reductases) indicate denitrification, and *nifH* (nitrogenase reductase) reflects nitrogen fixation. Carbon cycling is tracked using *mcrA* (methanogenesis) and *mxaF* (methanotrophy), while *phoD* and *phoC* (phosphatases) monitor phosphorus mineralization. Furthermore, 16S rRNA and ITS reveal the structure of bacterial/archaeal and fungal communities. These genes, combined with culture-based methods, provide sensitive detection of herbicide effects on soil microbial dynamics [172]. Herbicide application to the soil is toxic to microorganisms, and can reduce soil heterotrophic activity, the process of biomass respiration by microbes, and the function of bacteria that break down organic matter and cycle nutrients [173]. Alternatively, herbicide application has frequently been shown to result in microbial populations and enzyme activities returning after an initial period of temporary suppression; this may be because the microbes become accustomed to the herbicides or because the chemicals are decomposed. In addition, plant debris provides a greater nutrient supply, sustaining the growth and activity of microorganisms in areas where plants die after herbicide treatment [174]. While the functions of proteases depend on the location of microbes that break down protein molecules, the rise in soil dehydrogenase activity in herbicide-applied topsoil occurs from the seventh to the twenty-eighth day, which can be attributed to an increase in the microbial population that can use the chemicals as sources of carbon. The proteome is the set of proteins encoded by the genetic mate-

rial of an organism, which changes over time and is used to characterize the location, interactions, modifications, and functional changes [175]. Conversely, pretilachlor spray increases respiration activity and biomass; the respiration rate was not considerably changed by glyphosate spraying at the recommended rate. The enzyme activity of soil dehydrogenase was not affected by typical application rates of butachlor, metolachlor, and alachlor. In alluvial-rich soil, all butachlor treatments (5–100 mg/kg) markedly reduced methane production rates [149]. Moreover, respiration was not significantly affected by the use of the sulfonylurea herbicide within the specified concentrations. The rate of soil oxidation of methane and the amount of methane antioxidants were not significantly affected by the use of a nicosulfuron, atrazine, and dimethenamid herbicide combination. In field-grown soybeans, the use of imazethapyr (0.14 kg/ha) had no effect on soil microorganisms, soil DH (dehydrogenase), or hydrolase function. The treatment increased the activities of hydrolase, protease, and catalase by 0.05 kg/ha of imazethapyr, while decreasing DH activity. Herbicides not only directly affect soil microbes through inadvertent toxicity, but also indirectly alter microbial communities through interactions among microbes. For instance, suppression of dominant bacterial or fungal species may relieve competitive pressure, enabling opportunistic or resistant taxa to proliferate, thereby altering functional networks and nutrient cycling [176]. Such shifts can also affect plant–animal interactions, influencing broader ecosystem processes such as herbivory and pollination [177]. Considering these indirect pathways provides a more holistic mechanistic understanding of herbicide-induced changes in microbial populations and ecosystem function, enhancing predictions of long-term soil health and resilience under intensive herbicide regimes [178].

17. Future Scope

Future research should focus on employing standardized molecular techniques, such as high-throughput sequencing (HTS), metagenomics, and quantitative polymerase chain reaction (qPCR), to precisely evaluate how pre- and post-emergence herbicides affect soil microbial communities [179]. Combining these molecular approaches with precision agriculture (PA) tools such as site-specific herbicide application, soil monitoring sensors, and real-time microbial assessment can provide mechanistic insights into microbial population dynamics and ecosystem functions, ultimately supporting sustainable herbicide management strategies [180]. Large-scale production areas and intensive agricultural systems present a significant barrier to non-chemical weed management. To achieve sustainable weed management, an integrated plan that incorporates agronomic, cultural, physical, and mechanical approaches is needed [181]. Intensive agriculture driven by the Green Revolution promoted several secondary and tertiary issues, including soil and water contamination and herbicide resistance. Thus, investigations into sustainable farming now prioritize the security of food, health, and diverse diets over food security. While herbicide-based weed control may appear practical for monocropping, this approach fails to meet the expectations of consumers for healthy food. Agronomical tools and methods for non-chemical weed control may enhance sustainability and food safety. To reduce the restrictions and difficulties posed by weed competition in agriculture, solutions for agricultural sustainability based on cultivation systems, such as biodiversity agriculture, are crucial. As a “second genome” of all living things, including plants, the microbial environment has a mutually beneficial connection with health as a whole [182]. Sustainable agriculture can be ensured through novel ideas and cutting-edge techniques, including the use of competing varieties, crop cover, yield enhancement, and environmentally friendly intensification systems. Ecosystems of fresh water provide important benefits to economic well-being, known as ecosystem services, but are today challenged, in particular, by ecotoxicological stress from pollutants entering the environment [183]. In an environmentally benign manner, a significant reduction in pesticide use may improve the security of the food supply, agricultural production, and the living standards of growers and consumers. Notably, it is important to remember that most herbicidal impact studies used one of two methods: either the herbicides were sprayed onto the ground immediately, or their ultimate impact was determined. The assessment was conducted using a soil microcosm — a small-scale, self-contained experimental system that retains the natural soil microbial and faunal community from field-collected soil — to provide a more detailed understanding of the ecotoxicological impacts of substances in soil habitats. The main disadvantage of the soil microcosm in a diversity assessment study is that results are skewed toward dominant, rapidly expanding species. The physicochemical properties of the soil, which are crucial in the fixation of herbicide molecules with clay particles, temperature, and moisture, which aid in dissipation and changes in the microfloral population, are among the other parameters that act and change when chemicals behave, as well as how they affect the bacteria in the soil in the field. These parameters are typically overlooked during these experiments. Low levels of clay and organic matter reduce herbicide adsorption and increase herbicide bioavailability to microorganisms.

18. Conclusion

Herbicides are essential for weed management in modern agriculture. Herbicide use has dramatically increased in recent years. There are two approaches used in herbicide impact assessment studies: either the herbicide is sprayed directly under field conditions, or its effects are assessed in a soil microcosm — a small-scale, controlled system that retains the natural soil community. Herbicide use alters the soil microbial population both quantitatively and

qualitatively. Actinomycetes, bacteria, and mycorrhizae are the most extensively researched microorganisms impacted by the herbicide. The herbicide affected the biological processes in soil, including nutrient recycling, enzyme activity, and soil microbial respiration. However, it is crucial to remember that the effects of chemicals upon biological processes are typically more noticeable at greater application rates than at the suggested rates. More long-term research with a greater number of herbicides and variable environments is required to improve the accuracy and understanding of the system and generalize the effects. The growth of agriculture aims to sustain the growing human population and provide it with sufficient nutrition without endangering the ecological system of the biosphere or the surrounding environments. Herbicides are often seen as miracle solutions in developing nations. Given that many soil ecosystems retain chemicals and their contaminants for extended periods, herbicides pose serious dangers to the environment, soil, and human health. Most herbicides have a deleterious impact on the metabolic activities, structure, and range of microorganisms. Pesticides alter soil fertility, with immediate effects on agricultural productivity. The application of herbicides must be performed with judgment and discrimination, as the majority of adverse effects result from doses above those recommended. To lessen the detrimental impact on both humans and the natural world, producers and wholesalers, businesses, legislators, and other stakeholders must receive appropriate training on the responsible application of herbicides. The long-term impacts of herbicides exposure on microorganisms, as well as their ecotoxicological consequences in the soil environment, require well-designed trials.

Author Contributions

PP, SD, and JTC designed the research. SD and SM developed the overall concept. SD and JTC integrated and refined the key highlights. PP and SM conducted the literature search. All authors contributed to critical review and edited the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

The authors are immensely thankful to ICAR (Indian Council of Agricultural Research) for providing overall support and Vice Chancellor, CUTM for providing infrastructure facility and research environment on the aligned domain.

Funding

This research received no external funding.

Conflicts of Interest

The authors declare no conflicts of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/FBE43532.

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