

Interaction between Herbicides and the Nitrogen Cycle

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The interaction of herbicides in the nitrogen cycle and their consequences on soil health and agricultural production are essential topics in agronomic research.

soil health

sustainable agricultural practices

microbial respiration

1. Introduction

The interactions between herbicides and the nitrogen cycle involve complex processes of chemical, biological, and physical nature that can negatively affect the availability and cycling of this essential element in the soil ^[1]. From a molecular standpoint, herbicides can interact with nitrogen compounds, leading to their degradation or volatilization, thereby reducing the accessibility of these nutrients for plants ^[2]. Biologically, herbicides can interfere with the activity of nitrogen-fixing bacteria, diminishing their ability to convert atmospheric nitrogen into usable forms for plants ^[3]. This inhibition of biological nitrogen fixation directly affects the viability of this nutrient in the agricultural ecosystem ^[4]. Additionally, from a physical perspective, excessive herbicide application can cause modifications in soil structure, compaction and reduced porosity, leading to detrimental impacts on soil health ^[5] and the activity of nitrogen-fixing bacteria ^[6].

In order to provide a basis for decision-making in agricultural management, it is of utmost importance to understand the context of the interaction between the nitrogen cycle and herbicides.

2. Mechanism of Interaction

Interaction mechanisms play a pivotal role in crop development, exerting a critical influence on the intricate processes that orchestrate the nitrogen cycle. This intricate web of interactions encompasses Biological Nitrogen Fixation (BNF), carried out by both symbiotic bacteria ^[7] and those operating in a non-symbiotic manner ^[8], catalyzing the incorporation of nitrogen into the soil. In parallel, nitrification plays a crucial role in transforming organic nitrogen compounds into readily accessible inorganic forms for plants, while denitrification emerges as a key process, releasing atmospheric nitrogen from nitrogenous compounds ^[9]. Subsequent nitrogen assimilation by plants emerges as an essential process, playing a central role in the robust growth and development of these plant organisms ^[10].

All these mechanisms are essential for the proper and balanced functioning of the nitrogen cycle [10]. However, excessive and improper use of herbicides can potentially damage the efficiency of nitrogen cycling [1]. In a study conducted by Du et al. [11], it was observed that the presence of mesosulfuron-methyl had an effect on soil biodegradability, negatively influencing the capacity for decomposition and degradation of organic compounds in the soil. This interference is directly related to nitrogen transformation in the soil, encompassing crucial mechanisms such as nitrification, denitrification, and biological nitrogen fixation.

On the other hand, in a study by Hungria et al. [12], it was found that glyphosate had adverse effects on soybean plant nodulation. Nodulation is a crucial symbiotic mechanism for legumes, where an association occurs between plants and nitrogen-fixing bacteria, which are important for nitrogen fixation in the soil. Although the herbicide interfered with nodulation, the authors did not observe any effects of glyphosate on grain production.

These studies reveal the intrinsic complexity of these mechanisms for accurately assessing the effects of herbicides on nitrogen cycling dynamics. Such effects can have negative impacts on crop development, resulting in reduced nitrogen availability in the soil.

2.1. Nitrogen Fixation Inhibition

Nitrogen fixation in the soil is a biological process in which diazotrophic bacteria associated with leguminous plants, such as *Rhizobium* spp., have the ability to reduce atmospheric nitrogen (N_2) into ammonia (NH_3) through the enzyme nitrogenase [13]. Ammonia can then be converted into ammonium ions (NH_4^+) available for plants. For instance, the symbiosis between beans and the bacterium *Rhizobium leguminosarum* results in the formation of nodules on the roots, where nitrogen fixation occurs [14]. This process is crucial for providing nitrogen to plants, enhancing soil fertility, and contributing to agricultural productivity. Additionally, nitrogen fixation reduces the dependence on synthetic nitrogen fertilizers, which has direct implications for the environmental sustainability of agriculture.

However, nitrogen fixation in non-leguminous crops differs from that in leguminous crops, as non-leguminous plants lack the intrinsic ability to form root nodules housing nitrogen-fixing bacteria, as seen in legumes. Instead, nitrogen fixation in non-leguminous crops involves symbiotic interactions with diazotrophic bacteria colonizing the rhizosphere (the soil region surrounding roots) to provide nitrogen to the host plants [15][16].

These bacteria facilitate nitrogen fixation through the enzyme nitrogenase, which catalyzes the conversion of N_2 into usable nitrogenous compounds [16]. In many cases, optimizing nitrogen fixation in non-leguminous crops can be achieved through sustainable agricultural practices, such as crop rotation and cover cropping. Introducing specific cover crops that host diazotrophic bacteria can enhance nitrogen availability in the soil for primary crops [15]. Moreover, due to the absence of symbiotic association in non-leguminous crops, the regular application of nitrogen fertilizers is commonly employed as a conventional approach to meet nutritional demands.

Some herbicides can inhibit the activity of nitrogen-fixing bacteria present in plant roots, resulting in reduced nitrogen availability in the soil [1]. A study conducted by Chen et al. [17] investigated the effects of butachlor at doses

of 0.15 and 1.5 kg ha⁻¹ on the diversity of diazotrophic bacteria in the soil. The results revealed changes in the diversity of these bacteria in response to exposure to butachlor. Initially, the authors observed a suppression in nitrogen fixation activity, as evidenced by a reduction in acetylene production. However, in a subsequent stage, an increase in the suppression of this activity was observed. These findings suggest that butachlor interferes with the soil diazotrophic bacterial community, negatively affecting its nitrogen fixation capacity. Similar results were found in the study by Angelini et al. [3], where S-metolachlor, diclosulam, glyphosate, imazethapyr, and imazapic were applied to peanuts, resulting in a reduction in the diversity and abundance of nitrogen-fixing bacteria in the soil. Additionally, a pronounced decrease in nitrogen fixation activity by these bacteria was observed. These results indicated that exposure to the mentioned herbicides has negative effects on the community of nitrogen-fixing bacteria, reducing nitrogen availability for peanut plants.

Despite the importance of the nitrogen fixation process in providing plants with a usable form of nitrogen, it is also crucial to recognize the significance of denitrification in maintaining the balance of this essential element in the soil–plant–environment relationship.

2.2. Inhibition of Denitrifying Bacteria

Denitrification is an essential step in the nitrogen cycle, where the bacteria transform nitrate (NO₃⁻) into gaseous nitrogen (N₂), releasing it into the atmosphere [18][19][20][21]. Some bacteria, such as *Pseudomonas denitrificans* [22][23] and *Paracoccus denitrificans* [24][25], are mainly responsible for this process. These bacteria are able to utilize nitrate as an alternative energy source under anaerobic conditions [10][18][19].

The inhibition of denitrifying bacteria can occur due to various factors, such as the presence of toxic chemicals in the soil, including herbicides [21]. These substances can interfere with the metabolism of denitrifying bacteria, compromising their ability to efficiently carry out denitrification [25].

The inhibition of these bacteria can have significant implications on the nitrogen cycle and nutrient balance in the soil. This can lead to the accumulation of nitrate in the soil and a reduction in nitrogen availability for plants. The excessive accumulation of nitrate in the soil can disrupt the absorption of other nutrients by plants, destabilizing the nutritional equilibrium and consequently diminishing the effective availability of essential nitrogen for healthy growth. This phenomenon arises from ion antagonism, which affects nutrient transport processes in plant roots. As observed by Yu et al. [26], the application of acetochlor at a concentration of 10 mg kg⁻¹ resulted in the inhibition of soil denitrification potentials. This inhibition could be attributed to a decrease in both the abundance and activity of denitrifying bacteria after the application of acetochlor. Similar results were found by Crouze et al. [27], where mesotrione increased the ammonium content in the soil and caused a decrease in nitrate content observed in treated soils. These functional impacts were mainly correlated with changes in the abundance of oxidizing bacteria or denitrifying bacteria.

Since herbicides have the potential to inhibit vital processes, one of the main impacts is the reduction of nutrient absorption by plants. This decrease in absorption can result in various issues, such as nutrient deficiency in the

plant and, consequently, a decrease in productivity.

2.3. Reduced Nitrogen Uptake by Plants

The reduction in nitrogen uptake by plants can be attributed to multiple factors, including decreased soil microorganism activity, direct interference of herbicides with plant membrane transporters, and modulation of enzymes involved in nitrogen metabolism [28]. Nitrogen uptake by plants is a complex phase and vital for their growth and development. It involves the uptake of nitrate (NO_3^-) and ammonium (NH_4^+) from the soil by the roots, followed by transport to other parts of the plant [29]. This process primarily takes place in the roots, where membrane transporters play a pivotal role by facilitating the selective transport of nitrogen ions [30]. The reliance on these transporters is pivotal for the effectiveness of nitrogen uptake, exerting a substantial influence on nutritional equilibrium and plant productivity [30].

Nitrogen assumes a vital role for plants, fulfilling a dual function in synthesizing pivotal compounds, including amino acids, proteins, nucleotides, and other nitrogenous substances within plant cells [30]. These essential compounds significantly contribute to plant structure and functionality, encompassing critical processes such as growth, development, reproduction, and the plant's adaptive response to environmental stresses. Furthermore, the potential compromise in plant growth due to the reduction of assimilable nitrogen forms may consequently impact the entirety of these fundamental functions. [10][30].

Furthermore, herbicides can interfere with membrane transporters, which are responsible for the active or passive transport of nitrogen ions within plant cells. This interference can compromise the efficiency of nitrogen uptake, affecting the availability of this essential nutrient for plants [28].

Another noteworthy aspect is the potential influence of herbicides on nitrogen metabolism enzymes in plants. Inhibition of these enzymes could impact the assimilation of nitrate and ammonium, impairing the conversion of these compounds into usable forms for amino acid and protein synthesis [31]. This would result in an imbalance in nitrogen metabolism, directly impacting plant growth and development [31].

Therefore, the reduction in nitrogen uptake may be attributed to a combination of factors, including decreased microbial activity in the soil, direct interference with plant membrane transporters, and disruptions in enzymes involved in nitrogen metabolism.

Due to the impact of herbicides on processes essential for nitrogen availability [1], significant interference with plant development occurs. This interference includes the reduction of the bacterial population responsible for converting the element into a form assimilable by plants and the formation of root nodules [32]. According to Zablotowicz and Reddy [32], glyphosate interfered with the symbiosis between soybean and nitrogen-fixing bacteria, compromising the uptake of this nutrient by the crop. In addition, Singh and Wright [33], when evaluating terbutylazine, simazine, prometryn, and bentazone in pea crops, observed a decrease in plant growth due to the impairment of root nodule formation, which made it impossible to absorb nitrogen from the soil.

2.4. Changing the Rate of Organic Matter Decomposition

Soil organic matter (SOM) is made up of a variety of organic compounds, both polymers and monomers, which exhibit different sizes and levels of decomposition. These compounds interact with their surrounding environment, and it is these physicochemical interactions that play a key role in microbial dynamics in the rhizosphere [34]. These interactions are responsible, for example, for the decomposition of organic molecules and, consequently, for their persistence in the soil [35][36]. Interactions between herbicides and soil organic compounds can occur through several physical forces, such as hydrogen bonds, van der Waals forces, electrostatic forces, covalent bonds and hydrophobic interactions. These interactions act simultaneously on the sorption of herbicide molecules in soil colloids [37][38].

The use of organic compounds in the soil presents different responses in the retention of herbicides. For example, Mendes et al. [39] evaluated the addition of cow bone char on sorption–desorption and mobility of hexazinone, metribuzin and quinclorac applied to sandy loam soil under laboratory conditions. The results obtained proved that bone char was an excellent sorbent for reducing the mobility of the three herbicides due to the high sorption in the soil. On the other hand, Prata et al. [40] evaluated the effect of stillage addition on the behavior of diuron and ametryn in sandy and clayey soils. The authors reported that the addition of stillage did not affect the sorbed amount of these molecules and did not result in changes in the organic carbon content of the soil after four days of application.

The addition of nitrogen sources can alter the decomposition rate of SOM. Nitrogen availability directly affects microbial activity in the soil, being a key factor in the decomposition of SOM [34]. Li et al. [41] reported that urea fertilization decreased the decomposition of SOM and maize straw, evidenced by the higher nitrogen and carbon content compared to soil without urea.

In addition, the application of mixtures with herbicides and nitrogen fertilizers can influence the development characteristics of plant species. Dupont et al. [42] evaluated the isolated and combined effect of glyphosate and nitrogen doses on *Tanacetum vulgare* plants. Based on the results obtained, the authors showed that the application of glyphosate nitrogen and their interactions affected the reproductive characteristics of the plants evaluated, mainly the density and flowering phenology. Flowering was severely delayed by glyphosate application (10.5 days delay per 100 g a.e. ha⁻¹ year⁻¹). Although nitrogen partially attenuated the reduction in floral abundance, the delay in flowering was amplified when nitrogen was added.

The use of nitrogen fertilizers can also interfere with the selectivity of herbicides. Langaro et al. [43] evaluated the selectivity of herbicides according to the time of nitrogen application in irrigated rice. The results obtained proved that the application of nitrogen, in general, resulted in an increase in the height and dry matter of rice plants, and the application of nitrogen before waterlogging and split applications (50% before and 50% after waterlogging) were favorable to the crop, consequently, the selectivity of the crop to herbicides. Bispyribac-sodium resulted in the greatest damage and reduction in rice plant height, followed by bentazone and carfentrazone-ethyl, while the lowest damage and highest grain yield were obtained with the quinclorac application.

Therefore, the addition of nitrogen sources to soils is still a point to be elucidated by the scientific literature in view of the variety of responses listed so far. The decomposition of SOM by providing an additional supply of nitrogen to soil microorganisms. This may result in a more rapid release of nutrients contained in the decomposing SOM, making them available to plants in a more readily usable form, thus influencing the behavior of herbicides in the soil.

2.5. Change in the Rate of Nitrogen Fixation by Symbiotic Bacteria

The use of symbiotic bacteria applied as inoculants can meet the nitrogen needs of the plant. Bacteria of the genus *Bradyrhizobium* are the most used in agriculture due to the high world production of soybeans, enabling the reduction in the use of industrialized nitrogen fertilizers [44].

However, the increased use of pesticides, especially herbicides, can interfere with the process of biological nitrogen fixation by plants, both with positive and negative effects. The positive effect is observed when herbicides stimulate the growth and development of the fixation nodules. On the other hand, the negative effect is manifested in the reduction of nitrogen fixation efficiency, resulting in lower plant growth and yield [45][46].

After application, herbicides may come into contact with rhizobia immediately or, in the case of herbicides with residual effect, throughout the development of the main crop. These herbicides have the ability to impact the rhizobia, the host plant and also the establishment and development of the symbiosis [47]. The use of herbicides with a residual effect on the soil is a technique that enhances weed management, as it allows control in the pre-emergence of weeds [48]. However, the problem involved in the use of these herbicides is the possibility of carryover, that is, the presence of bioavailable residues of the herbicide in the soil, which can influence the microbial activity of the soil [49].

The behavior of herbicides in the soil is complex since the reduction of the population of a certain species of microorganisms can occur in contrast to the development of another population, which is not so affected by the toxic effects of the product. The species or individuals that survive start to use nutrients, such as carbon, sulfur and nitrogen, as well as the chemical energy of the herbicide molecules, which are released during the degradation process. Barroso et al. [50] reported that *Bradyrhizobium* sp. strain BR 3901 was able to produce nitrogen to catalyze degradation reactions of diuron, sulfentrazone, oxyfluorfen, and 2,4-D.

In studies carried out by Vercellino and Gómez [51], the authors evaluated the growth parameters of 81 strains of different genera of rhizobia (*Rhizobium*, *Mesorhizobium*, *Ensifer* and *Bradyrhizobium*) when exposed to the application of glyphosate, 2,4-D and atrazine, in addition to the ability of these strains to degrade herbicides. The genera studied showed different responses to the herbicides evaluated, with the genus *Bradyrhizobium* showing a greater ability to transform herbicides into compounds of lower phytotoxicity. In addition, among the compounds evaluated, atrazine was the most used as an energy source for bacteria. The results obtained confirmed that *Bradyrhizobium* strains were able to both denitrify and use atrazine as an energy substrate.

The relationship between herbicide application and symbiotic nitrogen fixation may have different effects depending on the crop variety, as well as the symbiotic strains used. Bossolani et al. [52] evaluated the effect of glyphosate doses on *Bradyrhizobium* strains (*Bradyrhizobium elkanii*—SEMIA 5019—and *Bradyrhizobium japonicum*—SEMIA 5079) and biological nitrogen fixation in soybean plants (BMX Potência RR). The authors reported that the inoculated RR soybean did not present alteration in the leaf chlorophyll index by the application of glyphosate, and regardless of the Inoculation, the soybean plants were able to recover from the application of glyphosate, not impairing their development.

The influence of herbicides on the nodulation of nitrogen-fixing bacteria is not a specific point of the soybean crop. Paniagua-López et al. [53] reported that the application of pendimethalin and clethodim reduced the ability of *Phaseolus vulgaris* and *Medicago sativa* to fix nitrogen by inhibiting root growth and modifying the composition of root exudates, as well as rhizospheric bacterial fitness. The authors cited 30% reductions in nodulation after clethodim application, while pendimethalin totally inhibited nodulation, causing reduced growth and motility of nitrogen-fixing nodules.

In another study, Khan et al. [54] evaluated bentazone, isoproturon, fluchloralin and 2,4-D, applied to soil, on growth characteristics, chlorophyll contents, nitrogen and protein contents, nodulation and seed yield in chickpea inoculated with *Mesorhizobium ciceri*. Fluchloralin and 2,4-D caused negative effects on chickpea seed yield.

In the study carried out by Santos et al. [55], the growth of *Rhizobium tropici* strains BR 322 and BR 520, used as inoculants in bean cultivation in Brazil, was analyzed in culture medium based on mannitol and yeast extract added with bentazone, metolachlor, imazamox, fluazifop-p-butyl, fomesafen, and paraquat. The authors found that paraquat was the herbicide with the highest growth inhibition of the strains evaluated, followed by the commercial mixture of fomesafen and fluazifop-p-butyl. As for the other herbicides, there was no reduction in growth. Overall, strain BR 520 showed greater tolerance to the herbicides tested, except for paraquat.

Thus, an efficient alternative for the remediation of contaminated soils may be the symbiosis between phytoremediating plants and microorganisms that possess enzymatic activity capable of metabolizing herbicides. Several groups of microorganisms degrade and utilize herbicide residues as a source of carbon and nitrogen. Bioremediation agents can absorb and degrade herbicides in secondary metabolic pathways or stimulate the soil microbiota to promote the transformation of these contaminants into compounds with lower or no toxicity [56][57][58].

Inoculation of symbiotic bacteria can contribute to the phytoremediation of herbicides in soil. Some *Bradyrhizobium* strains have the potential to degrade herbicides in soils, which is important for environmental decontamination purposes. Barroso et al. [59] evaluated the tolerance and in vitro growth of *Bradyrhizobium* sp BR 3901 in media without carbon and nitrogen sources, exposed to 2,4-D, oxyfluorfen, clomazone, glufosinate-ammonium, atrazine, ametryn, glyphosate, sulfentrazone, and diuron). The results obtained showed a reduction in nodulation.

In the study by Mielke et al. [59], the potential for positive interactions between symbiotic microorganisms (*Bradyrhizobium* sp. BR 2003—SEMIA 6156) and *Canavalia ensiformis* was evaluated, aiming at the

phytoremediation of the herbicide sulfentrazone in the soil. The authors highlighted that microbiological indicators showed satisfactory results, mainly for the dose of 400 g ha⁻¹. Thus, the symbiosis between herbicide-metabolizing microorganisms and phytoremediating plants can be an efficient alternative to remediate soils contaminated with herbicides.

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