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Review

Neonicotinoid Effects on Soil Microorganisms: Responses and Mitigation Strategies

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Abstract: Pesticides play a critical role in pest management and agricultural productivity; however, their misuse or overuse can lead to adverse effects on human health and the environment, including impacts on ecosystems and contamination. Currently, neonicotinoids (NNIs) are the most widely used systemic insecticides and are questioned worldwide for their possible impacts on pollinators. After NNI application, a substantial portion is not absorbed by the plant and may accumulate in the soil, affecting the soil microbial community. In this review, we explore the main studies carried out either in the laboratory or in the field about this matter. The studies report that the application of NNIs affects soil microbial activity and can act on microbial communities differently due to their unique chemical properties, degradation in soil, soil type, effects on soil properties, and methods of application. NNIs alter the diversity, structure, and abundance of soil microbes, in some cases increasing or decreasing their representativeness in soil. Bacterial phyla like Pseudomonadota, Bacillota, Actinomycetota, and Nitrospirota increase after NNI exposure, just like the families Nitrosomonadaceae, Nitrososphaeraceae, Nitrospiraceae, Sphingomonadaceae, Streptomycetaceae, and Catenulisporaceae. At the bacterial genus level, *Nitrospira* was associated with a decrease in nitrification processes in soil. The bacterial genera *Sphingomonas*, *Streptomyces*, *Catenulispora*, *Brevundimonas*, *Pedobacter*, and *Hydrogenophaga are* related to NNI degradation after application. Microorganisms could minimize the impacts of NNIs in agricultural soil. Therefore, the use of bioinoculation as a bioremediation tool is explored as an alternative to contribute to agricultural sustainability.

Keywords: bioremediation; pesticides; microbial activity; bacterial community; microbes; nitrogen functional genes

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1. Introduction

The Green Revolution of the 1960s boosted plant productivity and involved the creation of public investment, hybrid crop development, and the application of synthetic fertilizers and pesticides in agriculture [1]. Now, it is widely accepted that chemical synthetic products such as pesticides play an important role in reducing pests, sustaining productivity, and maintaining agricultural production [2]. However, it is well documented that their widespread and improper management causes negative impacts on the environment (i.e., water resources and soil microbiomes) global ecosystem, and human health [3]. For these reasons, the current agronomic technologies include the concepts of integrated

pest management, agricultural sustainability, and regenerative agriculture, touted as solutions to improve soil quality and biodiversity while maintaining soil productivity and profitability [4–6].

Neonicotinoids (NNIs) are a group of new-generation systemic insecticides widely used in the past three decades. The preference for these compounds is attributed to their efficacy for insect control, easy application, and lower toxicity to mammals compared to other pesticides such as organophosphates, carbamates, and pyrethroids [7–9]. In agriculture, NNIs may be applied as foliar spray, soil drenches, or granules to a wide variety of crops. However, they are preferably used as a seed coating because the high persistence of these compounds offers long-term crop seed, seedling protection, and less risk of off-target exposure to non-target organisms [10]. Regardless of the use of NNIs, it is estimated that between 2 and 20% of the applied doses of NNIs are taken up by the roots, translocated to all parts of the plants, and can be present in pollen and nectar, making them toxic to bees [11]. Several studies have demonstrated that exposure of bees, as well as other pollinators, to sub-lethal doses of NNIs can have detrimental effects on their populations. For instance, it can affect the energy metabolism of bumblebees [12], pose mortality risks to foraging bees [13], and have significant negative impacts on bee health and colonies. NNIs have also been suggested as a possible factor in colony collapse disorder among bees [14]. Therefore, NNIs have become one of the most controversial pesticides due to their non-target effects on pollinators and natural enemies of pests [14].

NNI seed dressing can affect the activity of various soil organisms and reduce the decomposition of plant material [15]. On the other hand, a substantial portion of NNIs is not absorbed by the plant and may accumulate in the soil being subject to various distribution processes and effects. Therefore, the occurrence and fate of NNIs in the environment have become an important global issue [16] (Figure 1).

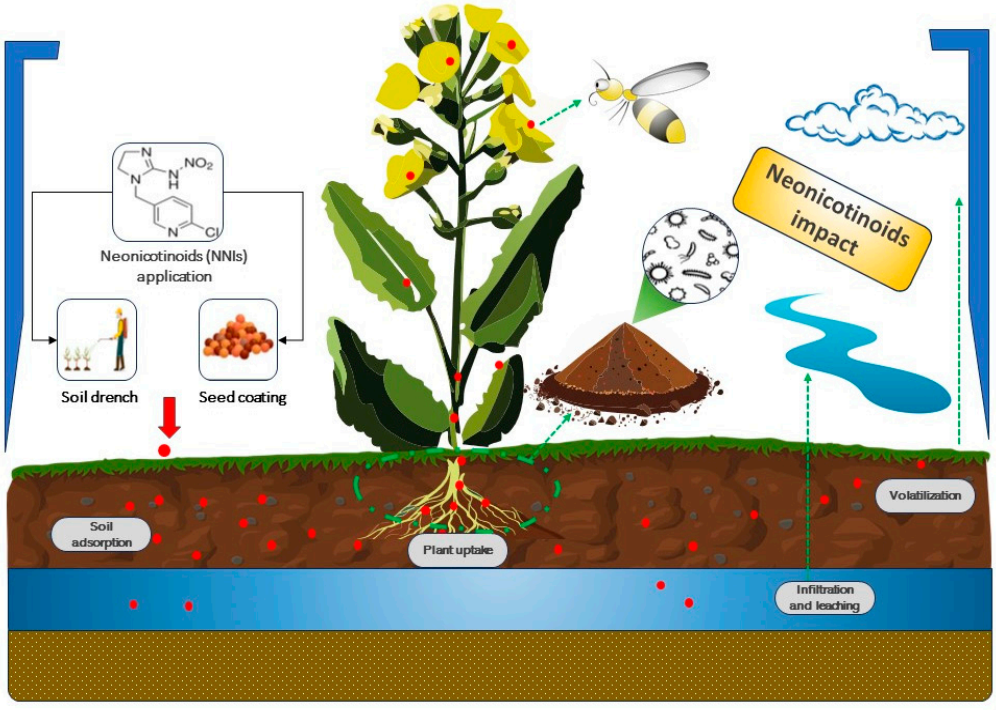


Figure 1. Effects of neonicotinoids (NNIs) on the environment and ecological processes. Red dot denoted NNI pesticides.

The high accumulation and persistence of some NNIs in soils (>100 days, DT_{50}) could induce changes in the structure, diversity, and functionality of the soil and rhizosphere microbiome [16–18], including important microbial communities involved in plant nutrition, such as the case of microorganisms involved in nitrogen (N) cycling [19,20]. It has

been reported that NNI application affects the nitrification and ammonification processes, influencing soil fertility, with a concomitant negative effect on plant productivity [16,20]. To date, and despite N being an essential nutrient and one of the most limited in agriculture [21], few studies have investigated the impacts of pesticides on soil microbial activity, soil community structure, and the abundance of key functional marker genes involved in the N cycle. The use of pesticides, especially when overused or repeatedly applied, can alter the composition and activity of soil microbial communities. However, when pesticides are used at recommended doses, the effects on soil microorganisms are generally transitive and do not significantly disturb microbial communities or activities [22]. The consequences of pesticide use can be far-reaching, influencing soil fertility, ecosystem health, and agricultural sustainability. Hence, it is essential to consider these potential impacts when managing pesticide applications to minimize adverse effects on soil microbial communities and functions. The study of NNI pesticides is crucial due to their widespread use and potential impacts on ecosystems. Therefore, the objective of this study is to offer a comprehensive and up-to-date review of the key findings related to the impact of this class of pesticides on soil microbial interactions and functions. The analysis encompasses results obtained under both laboratory and field conditions, shedding light on the specific microbiological groups implicated in responding to NNIs. Furthermore, this work aims to elucidate their role in mitigating the potential risks posed by this contentious category of pesticides.

2. Neonicotinoids and Their Use

The NNIs are a group of new-generation systemic insecticides widely used in recent decades in more than 120 countries [7], with annual sales that exceed $3.5 billion, and it is estimated to be >4.5 USD billion in 2028 [23]. They are synthetic compounds similar in structure to nicotine with a nitro-substituent group that confers insecticidal properties affecting the central nervous systems of insects. The preference for these compounds is attributed to their efficacy, easy application, and lower toxicity to mammals compared to other pesticides such as organophosphates, carbamates, and pyrethroids [7,8]. Currently, there are seven major NNIs distributed and used in agricultural activity, including acetamiprid (ACE), clothianidin (CLO), dinotefuran (DNF), imidacloprid (IMI), nitenpyram (NIT), thiacloprid (THA), and thiamethoxam (THM) [9] (Table 1).

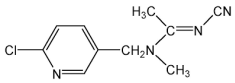
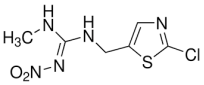
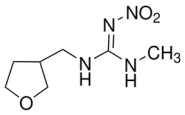
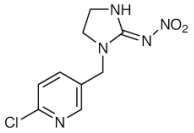
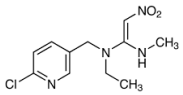
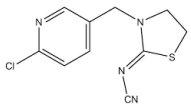
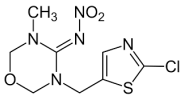
The NNIs compounds may be applied as a foliar spray, soil drenches, and granules in a variety of crops, including maize, soybeans, oilseed rape, sunflowers, cereals, beets, and potatoes, among many others. However, they are preferably used as a seed coating to reduce contact with non-target insects, to reduce the exposure of the insecticides to humans, and to prevent losses to the environment while still providing plant protection against insects [7,8]. Currently, nearly 100% of the maize planted in the United States and canola planted in Canada have a seed coating treatment that includes NNIs [7].

Regardless of the use of NNIs, between 2 and 20% of the applied doses are taken up by the roots and translocated to all parts of the plant [11], ensuring protection during all the growth stages of plant development against insect pests [24,25]. On the other hand, a substantial portion of NNIs is not absorbed by the plant and may accumulate in the soil where it is subjected to various dissipation processes, including adsorption–desorption and (bio)-degradation, the other two basic processes for determining the behavior of chemicals and fate [26].

NNIs are compounds highly soluble in water (184 to 570,000 mg L^{-1}) [27] with low adsorption onto soil particles but with a strong influence of the soil organic carbon (OC) amount [8,16]. According to the review by Pietrzak et al. [8], the adsorption capacities of NNIs onto soil particles are low, with reported values of K_d between 0.62 and 1.94 L/kg, 0.08 and 15.1 L/kg, 0.88 and 1.8 L/kg, and 0.17 and 35.9 L/kg for CLO, IMI, THM, and THD, respectively. Recently, Li et al. [28] studied the adsorption of seven NNIs in different agricultural soils characterized by a pH range of 5.6 to 7.2 and an OC content (%) range of 0.28 to 0.73. The results of this study showed that the K_d (≈2.0 L/kg) values for all NNIs were

closely related to the OC in the soils, and the calculated ΔG for all NNIs ranged from −14.6 to −19.5 kJ/mol, indicating that the adsorption occurs primarily through Van der Waals force, resulting in a weak and reversible adsorption process. Biodegradation may be affected by pesticide adsorption on soil, causing unavailability for microbial degradation [29,30]. The persistence of NNIs varies by compound and environmental conditions [25]. According to the Pesticide Properties Database (PPDB) [27], the typical half-lives (DT_{50}) vary from 0.88 d for THA to 545 d for CLO, although higher or lower values have also been reported under field conditions (Table 2). Recently, Li et al. [28] reported DT_{50} ranging from 33 to 305 d, in the next increasing order: CLO > THM > IMI > ACE > DNF > THA > NIT.

Table 1. Chemical structure of neonicotinoid insecticides currently used in agriculture [1].

NNIs	Chemical Structure	Target Pests	Application Mode	Formulation and Application Details
Acetamiprid (ACE)		Aphids; Thrips; Mirids; Spider mites	Foliar spray or used as a soil drench	Formulated as soluble granules for spray application
Clothianidin (CLO)		Corn rootworm, Southern corn billbug, Chinch flea beetle, corn leaf aphid	Seed treatment use on corn and canola	Flowable concentrate prepared for use as a seed treatment
Dinotefuran (DNF)		Whiteflies, Mealybugs, Thrips, Aphids	Soil incorporation, foliar application	Soluble concentrates, granules, soluble granules
Imidacloprid (IMI)		Sucking and soil insects, Plant hoppers, Aphids, Termites	Seed treatment	Granules that are mixed with water and applied as a spray
Nitenpyram (NIT)		Aphids; Thrips; Whitefly; Fleas; Ticks	Rice; Greenhouse crops; veterinary situations	Dusting powder, granules, drops
Thiacloprid (THA)		Aphids; Pollen beetles; Blossom midge	Seed treatments	Oil dispersions, soluble concentrates, and granules
Thiamethoxam (THM)		Aphids; Whiteflies; Thrips; Lacewings; Leafhoppers; Mealybugs; Wireworms	Vegetables, including brassicas, cucurbits, fruiting vegetables	Flowable concentrates for seed treatments, water dispersible granules, and suspension concentrates

[1] Information from the Pesticide Properties Database (PPDB) [27]. Available at: https://sitem.herts.ac.uk/aeru/ppdb/en/index.htm, accessed on 3 April 2024.

Table 2. Chemical properties of neonicotinoid insecticides (molecular mass, water solubility, octanol-water partition coefficient, soil degradation (DT_{50}), and major soil metabolite) [1].

Pesticide	Molecular Mass (g mol^{-1})	Water Solubility (mg L^{-1})	Koc	Soil Degradation [2] (d)	Soil Metabolite
ACE	222.67	2950	200	3	6-chloronicotinic acid
CLO	249.7	327	123	121	N-methyl-N-nitroguanidine
DNF	202.21	39,830	26	75	None
IMI	255.66	610	ND	174	6-chloronicotinic acid
NIT	270.72	570,000	60	8	None
THA	252.72	184	ND	8	Thiacloprid-amide
THM	291.71	4100	56.2	39	Clothianidin

[1] Information from the Pesticide Properties Database (PPDB) [27]. Available at: https://sitem.herts.ac.uk/aeru/ppdb/en/index.htm, accessed on 18 January 2024, ND: There is no available data. [2] Aerobic soil degradation, reported as the half-life (DT_{50}), under field conditions.

Hydrolysis can play a crucial role in breaking down NNIs in soil, with a shorter half-life observed as the soil moisture content increases [8].

NNI biodegradation processes by *Bacillus*, *Pseudomonas*, *Burkholderia*, *Rhodococcus*, *Streptomyces*, etc. have been reported, favoring the transformation of the parent compound to 6-chloronicotinic acid, nitrosoguanidine, desnitro, and urea [31]. The interaction of pesticides with soil microorganisms is of fundamental importance to obtain a compressive understanding of their environmental fate and ecosystem functioning. In the next topic, a revision and analysis of the most recent studies conducted in the last year for NNIs is reported.

3. Effect of Neonicotinoid Pesticides on Soil Microorganisms

Soil microbial communities play a critical role in soil fertility and crop growth by regulating several important processes, such as organic matter decomposition, organic pollutant degradation, and nutrient transformation [19,32]. Despite some types of pesticides that have a "fertilizer effect" on the soil by increasing nutrient availability [33], many others could induce significant changes in the composition and metabolic activity of microbial communities, thus affecting the soil quality and functioning [29,34].

The persistence of NNIs in soil poses a threat to the soil microbiome and affects their functionality. NNIs change the microbial community composition at the phylum and genus level in soil [19,34,35]. Both the type of NNIs and the level of exposure, as well as the soil composition, influence these changes. Changes in soil microbial communities after exposure to NNIs were reviewed by Akter et al. [36] using Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA). The author reported 29 articles; 66% of these reports were for IMI, and about 45% of total studies reported that NNIs had impacts on soil microbial community structure, composition, diversity, functioning, enzymatic activity, and N transformation. In this review, we analyze and discuss the specific responses and microorganisms involved, their magnitude, and what would be influencing them.

3.1. Neonicotinoids' Impact on Soil Microbial Activity

The effect of pesticides on soil microorganisms is a topic of concern due to their potential impact on soil fertility and ecosystem health. Microorganisms exposed to pesticides have developed a wide range of aerobic and anaerobic catabolic strategies, enabling their adaptation to remove these organic compounds present in soils, using them as nutrient sources [37]. The responses of soil microorganisms to pesticides have been corroborated by several studies focused on evaluating and understanding the activity and trend of soil enzymes and microorganisms [38]. Soil enzyme activities are often used as the soil quality index because they are sensitive to environmental stress and changes [34].

The insecticide IMI was the first NNI introduced to the market by Bayer CropScience in 1991. The effects of IMI on the soil environment are a worldwide concern. In this context, most of the studies carried out for NNIs consider this contaminant. Cycón et al. [18] evaluated the effect of IMI on the metabolic activity of microbial communities in a loamy soil (pH 6.6) without previous pesticide application. The method used for microbial activity was average well-color development, which reflects the soil microbial oxidative capacity in EcoPlates^{TM}. The results showed that IMI applied to soil at 1 mg kg^{-1} and 10 mg kg^{-1} caused a significant decrease in the parameters iver time at the two IMI concentrations applied. This response could be indicative of a decline in the use of carbon sources by microorganisms as substrates to produce energy, affecting an important ecological cycle and the maintenance of soil quality. Wang et al. [39] evaluated in an orchard soil (pH: 5.83; OM: 52.28 g kg^{-1}) exposed to IMI and ACE at doses of 10 to 80 µg g^{-1} the metabolic activities and enzyme activities like dehydrogenase, alkaline phosphomonoesterase, arginine deaminase, and urease. The author reported that thermodynamic parameters such as Q_T, k, and $J_{Q/S}$ decreased with the decrease in biomass while increasing the concentration of IMI and ACE, meaning that these insecticides inhibited both proliferation and metabolism. Dehydrogenase, phosphomonoesterase, and urease activities decreased with the increase

in IMI and ACE, while arginine deaminase (ammonification) increased. Therefore, the results indicate that the microbial biochemical reaction can be inhibited but also stimulated by NNI application [39].

As observed before, insecticides such as NNIs have a potential risk to the soil's biochemical characteristics by being able to alter soil ecosystems. New NNIs have been developed, such as paichongding (IPP), an insecticide developed in China characterized as having 40–50 times higher insecticidal activity than IMI [34]. To evaluate the effect of IPP on enzyme activities in two soils (pH: 8.25, OM%: 2.48; pH: 6.70, OM%: 6.70), the author used soil enzymes such as protease, dehydrogenase, catalase, and urease. The results showed that IPP application led to an increase in protease activity between days 20 and 45 after application in both soils. Catalase activity increased by 133% and 154% in both soils compared to the control soil after 100 days. Dehydrogenase activity decreased significantly from days 10 to 30 and then increased above the activity of soils without IPP treatment. Urease activity either increased or decreased depending on the soil type.

To evaluate the effect of agricultural practices such as pesticides and soil amendment, Castillo et al. [40] studied the effect of vermicompost addition in a soil (OC%: 1.6) treated with 3 mg kg^{-1} IMI. The results showed that the addition of vermicompost increased the dehydrogenase activity, but when the soil was treated with IMI, a decline in soil activity appeared after 30 days. While IMI incorporation into the pre-amended soils showed no changes in urease activity after 30 days, maintaining this condition over time.

The study of two NNI_S showed a change in carbon source utilization in the exposed soils. As compared to the control soil, low exposure to THM stimulated the utilization of all six categories of carbon sources, while low exposure to DNF stimulated the utilization of three categories of carbon sources, i.e., amines, carbohydrates, and phenolic compounds. On the other hand, exposure to middle (0.2 mg kg^{-1}) and high (2.0 mg kg^{-1}) levels of both NNIs decreased the utilization of itaconic acid and glucose-1-phosphate; exposure to high levels of both NNIs decreased the utilization of 4-hydroxy benzoic acid and L-threonine [17]. Table 3 shows the trend of the main microbiological responses in soils exposed to NNI pesticides conducted on a laboratory scale.

Table 3. Microbiological response based on the activity of soils exposed to NNIs.

Pesticide	Application Rate	Condition	Microbiological Response	Reference
IMI	1 and 10 mg kg^{-1}	Laboratory	The total biomass was reduced on days 1 and 14 with the low dose of IMI, and on days 1, 14, and 28 with the high dose. In addition, the higher dosage induced changes in the composition of microbial communities and their metabolic activity.	[18]
IMI-ACE	0 to 80 mg kg^{-1}	Laboratory	ACE showed higher toxicity than IMI with a dose–response relationship. Microbial activity was reduced over a short period of time. ACE and IMI reduced dehydrogenase activity by 40% and 30%, respectively. Urease activity declined by 21% and 30% with IMI and ACE-treated soil, respectively, after two days.	[39]
IPP	10 mg kg^{-1}	Laboratory	Protease activity increased two times after IPP application at 20, 30, and 45 days. Catalase activity increased 133–155% at day 100. Dehydrogenase activity was decreased, and urease was increased.	[34]
IMI	3 mg kg^{-1}	Laboratory	Vermicompost-amended soil increased dehydrogenase activity 2 and 4-fold after 30 days of pesticide application, while urease decreased. IMI induces changes in abundance, structure, and activity with a better tolerance in amended soil.	[40]

In general, the NNIs affect soil microbial activity. The dehydrogenase activity has been the most widely used among the studies conducted so far for this type of pesticide. Soil dehydrogenases are the major representatives of the oxidoreductase enzyme class; they occur intracellularly in all living microbial cells, and they serve as an indicator of the microbiological redox systems. Therefore, they are considered a good and adequate measure of microbial oxidative activity in soil [41]. These processes may include the decomposition of organic matter, the transformation of chemical compounds, and other biological activities involving electron transfer and substrate oxidation that could be affected by the presence of NNIs in soil.

3.2. Neonicotinoids' Impact on Soil Microbial Composition

Soil microbial composition refers to the types and abundance of microorganisms, such as bacteria, fungi, and archaea, present in the soil. Understanding the composition of soil microbial communities can provide insights into soil fertility, land quality, and ecosystem functioning. Pesticides can significantly impact the composition of soil microbial communities, causing shifts in their population and structure [42]. Such changes in soil microbial composition, following pesticide application, can generally affect carbon and nutrient cycling processes. A study conducted to evaluate the impact of twenty pesticides on soil carbon microbial functions and community composition found that insecticide application significantly increased the abundance of certain genes related to carbon degradation in acidic soil [43]. Pesticides have been shown to decrease microbial diversity in various soil types. A study on irrigated rice fields found that the use of pesticides resulted in a decrease in bacterial diversity and abundance [44]. The long-term application of pesticides can have persistent effects on soil microbial composition as can the repeated application of these compounds [45]; therefore, understanding the effects of pesticides on soil microbial composition is crucial for sustainable agriculture and soil management.

Molecular ecological tools can offer a comprehensive level of coverage and resolution when studying microbial community diversity and metabolic activities without culturing the microbes [46]. Studies conducted specifically on NNI pesticides have shown effects on bacterial communities, diversity, and community composition. Cai et al. [19] studied the effect of 10 mg kg^{-1} IPP on soil. The main results showed that IPP affected soil microbial species diversity, and bacteria were more diverse in the soil with higher OM. The study showed differences in community composition at both the phylum and genus levels after applying IPP to soils. Specifically, the phylum Pseudomonadota, Bacillota, and Chloroflexota were stimulated to increase with IPP application, while the phyla Bacteroidota, Actinomycetota, and Acidobacteriota were inhibited. These results were related to the incubation time and the complicated degradation of IPP.

Zhang et al. [16] studied the effects of 5 mg kg^{-1} of THA, IMI, and CLO in four agricultural soils (pH 3.8 to 7.2, OC% 0.11 to 3.08). The authors reported that compared to the control soil, the Chao, ACE, and Shannon indices significantly decreased at day 20 in the soil (pH 7.2, OC% 3.08) contaminated with IMI and in the soil (pH 4.9, OC% 1.26) contaminated with CLO and THA. Therefore, the three insecticides changed the soil microbial community structures at the phylum level among treatments, soils, and the incubation time. In this context, soil with a lower OC% content was more affected by the pesticides. According to the authors, the microbial community and abundance in soil exposed to NNIs are influenced by different degradation rates over time in the different soils.

Zhang et al. [47] assessed the impact of THM on agricultural soils (pH 4.37–8.29; OM 1.00–5.24%) sampled from five vegetable fields, exposing them to pesticide levels ranging from 1.5 mg kg^{-1} to 4.0 mg kg^{-1}. The authors reported that the effect of THM on bacterial diversity varied by soil type and pesticide concentration. Actinomycetota increased in the majority of THM-treated soil, while Nitrospirota diminished in the richest soil but increased in the most acidic soils. Soil bacterial co-occurrence network complexity was reduced with the increasing concentration and the soil physicochemical properties (pH,

OM, cation exchange capacity, and silt content). Similarly, in an interesting work, Wu et al. [48] indicated that THM altered the bacterial community composition in farmland soils with varying pH and organic matter content (pH 8.06, OM 14.70 g kg^{-1}; pH 6.59, OM 38.40 g kg^{-1}). The diversity of bacteria on days 7, 28, and 56 decreased at a pesticide rate of 1.8 and 18 mg kg^{-1}, but on day 56, diversity increased with the higher pesticide rate. The main phyla at all sampling points in the two soils treated with THM were Pseudomonadota, Patescibacteria, Acidobacteriota, Actinomycetota, Chloroflexota, and Bacteroidota. In both soils, the abundance was slightly different, being influenced by THM application and soil pH.

Garg et al. [46] assessed the impact of IMI on the bacterial community diversity in mango orchard soil (pH 7.6; organic carbon 0.43%). Soil samples, both treated and untreated with IMI, were collected and underwent metagenomic analysis. The results showed that at the phylum level, Pseudomonadota, Planctomycetota, Chloroflexota, and Verrucomicrobiota decreased in soil treated with IMI, while Actinomycetota increased. Also, the results indicate that in control soil, total microbial population abundance was higher but diversity was lesser, while in IMI-treated soil, an inverse response was observed, probably due to the adaptation of potential IMI-degrading microorganisms. The effect on the soil microbial community after exposure to 0.02 mg kg^{-1}, 0.2 mg $kg^{-1,}$ and 2.0 mg kg^{-1} THM and DNF was evaluated by Yu et al. [17] in an urban soil (pH: 6.2, OC%: 1.1). The main results showed degradation of both contaminants, and after 112 days, the phyla Pseudomonadota and Acidobacteriota were dominant. The microbial community differed significantly between the control soil and soils exposed to the two NNIs, with both the type of NNI and the level of exposure influencing these changes. The relative abundances of the phyla Gemmatimonadota and *Candidatus* Paceibacterota decreased, while Chloroflexota and Nitrospirota increased under most exposed conditions.

In consideration of the fact that pesticide seed treatments are common in agriculture and are considered an efficient and environmentally friendly method compared to traditional spraying, studies have been conducted to evaluate the effects of NNIs on the microbial community of the rhizosphere because it is one of the primary factors that determine plant health. Li et al. [49] studied the effects of IMI and CLO in a trial experiment conducted in sandy loam soil. The main observation according to Beta diversity indices was that the species richness of the fungal and bacterial community was suppressed by both insecticides in the wheat seedling stage when a pesticide concentration of 240 g a.i./100 kg seeds was added, whereas by the reviving period, stimulation of the soil microorganisms was observed. The main abundances of the bacterial group were Pseudomonadota, Acidobacteriota, Bacteroidota, Actinomycetota, and Gemmatimonadota, while the abundance of the fungal group was represented by the phyla Ascomycota, Basidiomycota, and Mortirellomycota. Similarly, Parizadeh et al. [35] studied the effect of 0.25 mg/seed THM in a 3-year rotation of soybean and corn in a clay loam soil type in Canada. According to the authors, THM showed complex effects on the composition of bacterial communities in the phyllosphere and soil. Specifically for soil, 294 bacteria, according to the identification of amplicon sequence variants (ASVs), were differentially abundant between the control and THM-treated samples. When the soil was treated with the pesticides, Actinomycetota and Chloroflexota were more abundant, while Pseudomonadota were less abundant. According to the same study, more than 60 genera were significantly impacted, some of them represented by *Ammoniphilus*, *Bacillus*, and *Rhizobacter*, among others. On the other hand, the genera favored with the THM addition were dominated by *Mycobacterium* and *Streptomyces*. In this report, the authors concluded globally that NNIs seed treatment has non-target effects on soil bacterial community structure and diversity over the growing season of soybean and corn ecosystems. In a recent field study by Parizadeh et al. [50], the impact of 0.25 mg/seed THM on soil microbial gene expression was assessed using metatranscriptomics. Results revealed changes in the expression of a limited number of microbial genes throughout the growing season and between years, including those related to heat shock proteins, regulatory functions, metabolic processes, and DNA repair. The in-

creasing application of metatranscriptomics could enhance our understanding of microbial community function, gene expression, regulation, and pathways in pesticide-affected soils, contributing to environmental sustainability [51]. Table 4 shows a summary of the main observations reported in different studies conducted with the objective of evaluating the effects of NNIs on the composition and structure of soil microbial communities.

Table 4. Reported observations for some NNIs applied at different rates and their influence on soil microbial composition.

Pesticide	Application Rate	Condition	Microbiological Response	Reference
IPP	10 mg kg^{-1}	Laboratory	The phyla Pseudomonadota, Bacillota, Planctomycetota, Chloroflexota, Armatimonadota, and Chlorobiota were stimulated. Phyla Bacteroidota, Actinomycetota, and Acidobacteriota were inhibited.	[19]
IPP	10 mg kg^{-1}	Laboratory	The genera *Pseudomonas* and *Pseudorhodoferax* increased from 0.3% to 21.4% and 0.1% to 14.3%, respectively, while *Thermomonas* decreased from 2.7% to 0.6%, after 60 days. In other soils, *Pseudomonas*, *Mycrovirga*, and *Brevundimonas* were stimulated to increase.	[34]
IMI-THA-CLO	5 mg kg^{-1}	Laboratory	Representative families of the phylum Pseudomonadota and Bacteroidota increased by at least 50% at days 20 and 60 after NNI application.	[16]
IMI	0.005%	Field	Phylum Pseudomonadota, Planctomycetota, Chloroflexota, and Verrucomicrobiota decreased, while Actinomycetota increased. The genus *Gemmata* totally disappeared in IMI treated soil, and microorganisms belonging to the genus *Prevotella* were present.	[46]
IMI-CLO	240 a.i. g/100 kg^{-1} seed	Field	The species richness of the bacterial and fungal communities was suppressed in the wheat seedling stage, but during the reviving period, stimulation of soil microorganisms was observed.	[49]
THM	1.5 to 4 mg kg^{-1}	Laboratory	The richness of the soil bacterial community in treated soils was reduced by about 20%. The plyla Pseudomonadota and Verrucomicrobiota increased, while Phyla Chloroflexota, Acidobacteriota, and Nitrospirota decreased after 60 days of THM application.	[47]
THM	0.25 mg $seed^{-1}$	Field	Pesticides affected the bacterial community structure (2.6%) and over time (2.4%). The phyla Actinomycetota and Chloroflexota were more abundant while Pseudomonadota were less abundant in THM-treated soil. More than 60 genera of soil bacteria were impacted, i.e., *Ammoniphilus*, *Bacillus*, and *Rhizobacter*.	[35]
THM	1.8 to 180 mg kg^{-1}	Laboratory	THM increased the bacterial abundance by 0.09 to 0.72 fold in one soil, but in another it was reduced. THM reduced the abundance of Actinomycetota and Chloroflexota. Bacteroidota and Bacillota increased in the basic soil, and Patescibacteria and Acidobacteriota increased in the acidic soil.	[48]
THM-DNF	0.2 to 2 mg kg^{-1}	Laboratory	The phyla Bacteroidota, Gemmatimonadota, and *Candidatus* Paceibacterota decreased at a rate > 10%. Chloroflexota and Nitrospirota increased at a rate > 10%. Pseudomonadota and Acidobacteriota also change (increased or decreased) at a rate < 10%.	[17]

According to the previous results, different NNIs can act on microbial communities differently due to their unique chemical properties, degradation in soil, effects on soil properties and microbial interactions, and the soil conditions in which they are applied. Each NNI has a unique chemical structure, which can influence its ability to interact with specific microorganisms in the soil. Species sensitive to NNIs could be replaced by more tolerant species, or some of them could evolve within the indigenous microflora to degrade compounds such as IMI, using them as an additional source of carbon and energy for development [18].

3.3. Effects of Neonicotinoids on the Metabolic Process of Soil Microorganisms

The influence of the NNIs on soil bacterial community composition has increased in the last few years because there is a great interest in knowing the positive or negative effect on beneficial soil microorganisms responsible for carrying out different metabolic processes. Some of the key metabolic processes of soil microorganisms include decomposition, mineralization, N fixation, nitrification, denitrification, the carbon cycle, respiration, and siderophore production. One of the prime microbe-mediated soil functions of agricultural concern is N cycling, a parameter widely used as an indicator of soil health due to its importance for plant productivity and health [43,52]. Soil N cycling includes microbial N fixation, which is the process of reducing atmospheric nitrogen (N_2) to biologically available ammonium (NH_4^+-N) by diazotrophic prokaryotes carrying nitrogenase activity and the nifH gene. Nitrification is an important process in the global N cycle, which converts NH_4^+-N via NO_2^--N to NO_3-N by bacteria carrying the amoA gene. Finally, denitrification is a microbial process that converts NO_3^--N to N gases. Bacteria carrying a variety of genes, including narG, napA, nirK, qnor, cnorB, nirS, and nosZ [32,52,53], are responsible for this last step. Communities involved in each step of N cycling are relevant because a slight disturbance might have a severe pivotal role in soil N cycling and regulating soil N available to plants [52]. These metabolic processes collectively contribute to the fertility, structure, and overall health of the soil. In this review, we focus on two processes related to the effect of NNIs on microorganisms, which are associated with the N cycle and biodegradation.

Pesticides in soil change the abundance of functional genes involved in N-fixation, nitrification, and denitrification [19,34,35]. Studies have shown that pesticides can have dose-independent effects on N microbial cycling and soil-specific effects [43]. N-fixing bacteria such as *Azospirillum*, the nitrifier *Nitrosomona*, and the denitrifier *Pseudomonas*, could be reduced temporarily in the presence of pesticides. Conversely, pesticides can increase the N-fixing bacterium *Rhizobium*, the nitrification bacterium *Nitrospira*, and the denitrification bacteria *Sphingobium* and *Streptomyces* [53]. Also, a negative correlation between the pesticide residues and the abundance of ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) genes involved in nitrification has been reported for pesticides applied at the recommended rate [54]. The effect of 20 commercial pesticides applied at their recommended dose and five times the recommended dose on N microbial cycling in three different agricultural soils was studied by Sim et al. [43]. They observed that the effects on N microbial cycling were related to a reduction of specific enzymatic activities and the potential for nitrification. Although the greatest pesticide effect was an increase in AOA coming from the family *Nitrososphaeraceae* compared to less dominant AOA and other nitrifiers such as *Nitrosospira* AOB lineages.

The evaluation of pesticide effects on non-target organisms in soils could be a useful tool to monitor soil health and quality, providing crucial information to be used for pesticide ecotoxicology and hazard assessment [47]. Unfortunately, there are not many studies specifically conducted to evaluate the effect of NNIs on microorganisms associated with the N cycle, despite N being an essential nutrient and one of the most limited in agriculture [21]. On the other hand, degradation genes related to NNI degradation have been poorly explored and are necessary to promote safety evaluation and degradation-related pathways [48].

One of the early works carried out in this line of research was conducted by Cycón et al. [20] that reported changes in the community structure of ammonia-oxidizing microorganisms after exposure to 1 and 10 mg kg^{-1} soil of IMI. The PCR-DGGE profile evidenced that the main factor involved was the pesticide concentration rather than the elapsed time. IMI increased the diversity and richness of the AOB community and decreased the AOA community. On the other hand, the pesticide affected the concentration of N-NO_3^- in the soil and increased the N-NH_4^+ concentration. These responses were attributed to two possible causes: archaea and/or bacteria involved in ammonia oxidizing were killed by the insecticide, N-NH_4^+ transformation to N-NO_3^- was paused, and the degradation process of IMI could have caused the production of N-NH_4^+ by killing sensitive microorganisms that are responsible for the mineralization process, consequently increasing ammonium concentration. Finally, the authors observed that sensitive species among ammonia-oxidizing microorganisms were replaced by those characterized by a higher tolerance to IMI and/or the ability to degrade the insecticide in soil.

Zhang et al. [16] evaluated the degradation of THA, IMI, and CLO and their effects on soil microorganisms. The authors reported that Pseudomonadota and Bacteroidota were the predominant microorganisms in soil exposed to THA, probably associated with the biodegradation of this contaminant. In the same study, NNI degradation via nitrate reduction and cyano- and amino- hydrolysis resulted in metabolites that influenced the nitrification process and the families associated with it. The author attributed these results to the metabolites of NNIs, which provide more substrates for microorganisms responsible for N-NH_4^+ to N-NO_3^- transformation.

Parizadeh et al. [35] suggest, according to their results obtained in a field experiment, that NNIs have negative effects on nitrification. Genera negatively affected by NNI application included some of the beneficial soil bacteria, such as plant growth-promoting rhizobacteria and those involved in the nitrogen cycle. On the other hand, genera potentially involved in NNI degradation increased. In this context, a decrease in Nitrospiraceae was also observed in a soil (pH 6.52; OM% 5.02) treated with 1.5 to 4.0 mg kg^{-1} THM but increased in another one (pH 4.37; OM% 4.84) [47]. In the same study, Actinomycetota and Chloroflexota were bacterial phyla that increased, while Pseudomonadota and Gemmatimonadota decreased, abundance of 30 predominant genera was observed and positively related to THM degradation. Cai et al. [19] reported that the genera *Brevundimonas*, *Pedobacter,* and *Hydrogenophaga* were the most abundant after IPP application and key for pesticide degradation.

Finaly, a study conducted using THM applied to two different soils at concentrations of 1.8, 18.0, and 180.0 mg kg^{-1} reported that gene copy numbers of the N cycle key enzymes, nitrite reductase, assimilated nitrate reductase, and ferritin nitrite reductase, were significantly reduced, as were N cycle-related enzymes such as nitrilase and formamidase. In addition, THM reduced the abundance of related carbon metabolism enzyme genes after a short period. On the other hand, a total of 18 biodegradation genes and 5 pesticide degradation genes were detected in soil samples. The most abundant subtypes of biodegradation genes were bphA1, benA, encoding dioxygenase, and the P450 gene, encoding cytochrome P450, which is an important enzyme in the metabolisms of NNIs [48].

Table 5 summarizes the main trends observed in soils exposed to NNIs, concerning the microbial responses related to the N cycle and biodegradation.

According to the review conducted on the most representative studies carried out during the last decade, NNI pesticides, like many other pesticides, affect the functionality, structure, and composition of soil microbiota. NNIs could decrease or increase the abundance of potentially beneficial soil bacteria belonging to several phyla, such as Pseudomonadota, Chlorofletota, Actinomycetota, Bacillota, and Bacteroidota, among others. Many of these phyla include representative families and genera of bacteria involved in both the pesticide degradation process [55] and various steps of the nitrogen cycle, such as nitrification [56] (Figure 2).

Table 5. Effects of NNI application on soil microbiological processes.

Pesticide	Application Rate	Condition	Microbiological Response	Reference
IMI	1 and 10 mg kg^{-1}	Laboratory	The nitrification rate was decreased by 25–65%, and the ammonification process was stimulated on days 14, 28, and 56. IMI applied at a dose of 10 mg kg^{-1} suppressed the AOA community members for 56 days. The diversity and richness of AOB decreased on days 1 and 14.	[20]
IMI-THA-CLO	5 mg kg^{-1}	Laboratory	Family Nitrosomonadaceae, Nitrososphaeraceae, and Nitrospiraceae increased after pesticide application.	[16]
THM	1.5 to 4 mg kg^{-1}	Laboratory	Bacterial genera *Sphingomonas*, *Streptomyces*, and *Catenulispora* were associated with biodegradation.	[47]
THM	0.25 mg $seed^{-1}$	Field	Genera such as *Ammoniphilus*, *Bacillus*, *Nitrospira*, *Nitrosospira, and Rhizobacter,* among others, were affected. The genera *Mycobacterium* and *Streptomyces* were dominant.	[35]
IPP	10 mg kg^{-1}	Laboratory	The genera *Bacillus*, *Pseudomonas*, *Azohydromonas*, and *Paenibacillus* increased with the pesticide. THe genera *Brevundimonas*, *Pedobacter,* and *Hydrogenophaga* were related to IPP degradation.	[19]

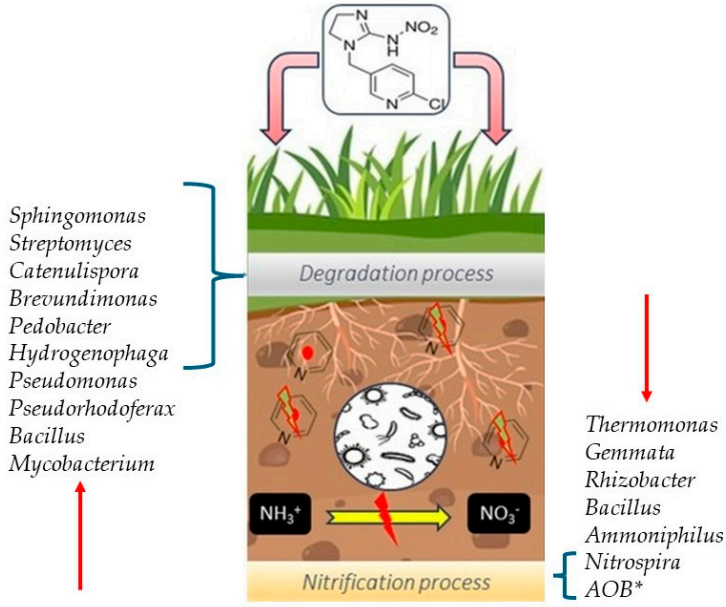


Figure 2. Genera of bacteria that have decreased (red down arrow) or increased (red arrow up) in response to exposure to NNIs in soil. The red arrow indicates the trend. * Ammonia-oxidizing bacteria.

4. Strategies for Mitigating Neonicotinoids Effects on Soil Health

While the issue of pesticides in the environment is a recurring theme in scientific research, it remains an unresolved challenge. Consequently, there is ongoing effort in the development of biotechnological tools to address this issue. In this context, bioremediation, known as a natural process that uses microorganisms to break down contaminants, has motivated various studies with the aim of finding new microorganisms with the ability to metabolize pesticides, including NNIs. A diverse group of microorganisms capable of degrading pesticides have been reported and isolated from different environments. According to Kumar et al. [55], it can be concluded that different phyla such as Actinomycetota, Bacteroidota, Basidiomycota, Chlorophyta, Cyanobacteria, Ascomycota, Bacillota, and Pseudomonadota are sources of microorganisms capable of degrading several pesticides. One of the earliest published articles focusing on the bioremediation of NNIs by Hussain et al. [31] reported that at that time, there were few publications on the biodegradation of NNIs by bacteria. In that review, the authors pointed out bacterial groups such as *Bacillus* sp.,

Burkholderia sp., *Mycobacterium* sp., *Pseudomonas* sp., *Rhodococcus* sp., and *Stenotrophomonas* sp., among others, that were able to degrade IMI, ACE, THA, and THM. In most cases, the microorganisms were isolated from agriculture soil and pesticide-contaminated soil, and the mode of action of NNIs was through catabolic and cometabolic processes. The authors also reported that metabolic pathways for microbial degradation of IMI and ACE can result in the formation of 6-chloronicotinic acid and continue until its mineralization. A review conducted by Ahmad et al. [57] expanded the list of microorganisms highlighted for their ability to degrade the seven most studied NNIs, with species of bacteria and fungi such as *Streptomyces* sp., *Fusarium* sp., *Phanerochaete* sp., *Rhizobium* sp., *Acinetobacter* sp., and *Sphingomonas* sp., among others. The biodegradation of THM by the white-rot fungus *Panerochaete chrysosporium* was studied by Chen et al. [58]. The main observations were a reduction in THM of 49% and 98% after 15 days and 25 days, respectively. The THM detoxification was performed through dichlorination, nitrate reduction, and C-N cleavage to convert the main product into lower biologically toxic metabolites. Moreover, the THM degradation was related to cytochrome 450.

An NNI that has received great attention in the last few years due to an increase in its use is ACE. In this context, studies on biodegradation have demonstrated the ability of several bacterial strains to degrade this insecticide. The actinobacteria strain *Streptomyces canus* CGMCC 13662 has been found to remove up to 70% of ACE from a solution containing 200 mg L^{-1} of the compound. The degradation of ACE occurs through the hydrolysis of the cyanoimine moiety, which is mediated by a novel nitrile hydratase [59], similar to the one reported by Sun et al. [60] for the plant growth-promoting rhizobacterium *Variovorax boronicumulans* CGMCC4969. In a similar study, Yang et al. [61] showed that *Pigmentiphaga* sp. strain D-2 uses an amidase enzyme to initiate the biodegradation of ACE. Lastly, Boufercha et al. [62] investigated the biodegradation of THM by *Labrys portucalensis* F11 isolated from contaminated sediment. The research found that the strain was able to remove between 41% and 100% of the insecticide when it was supplied as a different source of nutrients. The author proposed 12 degradation by-products, which were produced through nitro reduction, oxadiazine ring cleavage, and dichlorination processes. There are many more studies of this type, which have been summarized in different reviews [9,63–65]. In Table 6, a brief representation of some NNI-degrading bacteria is shown.

Table 6. Some representative genera of bacteria capable of degrading NNI.

NNIs	Microorganisms	Response	Reference
ACE	*Sphingobium, Acinetobacter, Afipia, Stenotrophomonas, and Microbacterium*	Consortia was able to degrade completely 50 mg L^{-1} ACE in 144 h.	[66]
CLO	*Ochrobactrum anthropi, Acinetobacter johnsonii, Pseudomonas sp., and Stenotrophomonas maltophilia*	>79% of CLO (500 mg L^{-1}) was degraded by bacterial consortia.	[67]
DNF	*Pseudomonas monteilii FC02*	>92 DNF was removed after 14 days.	[68]
IMI	*Sphingomonas melonis*	Bioremediate the insecticide with an efficiency > 90%.	[69]
NIT	*Ochrobactrum* sp. strain DF-1	> 90.9% NIT (10 mg kg^{-1}) degradation was achieved, after two weeks.	[70]
THA	*Microvirga flocculans* CGMCC 1.16731	In soil, the bacterium transformed >92% of 80 µmol kg^{-1} soil THA in 9 d.	[71]
THM	*Bacillus aeromonas strain* IMBL 4.1 and *Pseudomonas putida* strain IMBL 5.2	>45 and 38% THM (50 µg mL^{-1}) was removed in 15 days.	[72]

Several reports have emphasized the ability and utility of bacteria and fungi as a potent and eco-friendly approach to getting rid of toxic NNIs [63–65]. Microorganisms are widely distributed in soil, present several morphological attributes, and their physiological versatility is often determined by the presence of a complex machinery of degradative genes/enzymes available to favor the metabolism of NNIs. Bioremediation of pesticide-contaminated soils is a long-standing, high priority goal in many countries and the subject

of numerous research studies. The inoculation of soil with microorganisms (bioaumentation) characterized by desired catalytic capabilities has been studied for some NNIs, resulting in promising results. Guo et al. [59] reported 90% of the ACE removal after 12 days in a soil contaminated with 5 mg kg^{-1} ACE and inoculated with *Streptomyces canus* CGMCC 13662, characterized by the presence of a nitrile hydratase that is overexpressed in the presence of ACE. Cai et al. [19] evaluated the inoculation of *Sphingobacterium* sp. P1–3 in a soil sprayed with 10 mg kg^{-1} IPP, observing a pesticide concentration that decreased rapidly under aerobic conditions. Yang et al. [61] evaluated the bioaugmentation of approximately 1.0×10^8 CFU of *Pigmentiphaga* sp. strain D-2 in a soil (pH 5.5) contaminated with 50 and 200 mg kg^{-1} ACE. The results showed a slow dissipation of ACE after 40 days without inoculation, with values of 30.2% and 24.3% for the lowest and highest concentrations applied, respectively, while the dissipation increased to 94.8 and 92.5%, respectively, with the inoculation of the strain. Other important results were that the bioaugmentation treatment improved the growth of bacteria associated with ACE biodegradation, reassembling the microbial community from the indigenous microbial consortia. The synergistic relationship between selected inoculants and indigenous microorganisms accelerated the mineralization of pesticides in soil, as the pesticide can provide a N source for microbial growth, as in the case of atrazine [33] and also the NNIs, which are composed molecularly of N and C mainly.

5. Conclusions and Future Prospects

After conducting this review, which complements existing studies, it has been observed that NNI pesticides, while replacing other toxic pesticides, have an impact on the community of microorganisms present in the soil. In general, the NNIs affect soil microbial activity. Moreover, these changes can lead to a decrease or increase in the population, diversity, and specific groups of microorganisms that play a crucial role in soil fertility through their unique roles. The literature suggests that NNIs have a negative effect on soil microorganisms due to the previously mentioned changes. However, different trends can be observed, such as an increase or decrease in the representative phyla of agricultural soil, such as Pseudomonadota, Chloroflexota, Bacteroidota, and Actinomycetota, among others. Various factors contribute to the effects of NNIs on soil microbial communities, including the type of NNI and the way degradation may occur. Microorganisms can evolve to become tolerant to NNIs and replace other sensitive microbial groups with complex chemical structures such as those of NNIs. Another important factor that influences the response of microbial communities to NNI exposure is soil type and its characteristics related to acidity, basicity, and organic matter content. In general, impacts or effects were minimized in nutrient-rich soils.

The degradation of neonicotinoids in soil can occur through different pathways, some of which could include degradation via nitrate reduction and cyano- and aminohydrolysis. During these transformations, it is observed that changes can occur in both communities and processes, but more research is required to explore the impact of NNI metabolites on soil. Despite families such as Nitrosomonadaceae, Nitrososphaeraceae, and Nitrospiraceae increasing after pesticide application, representative genera such as *Nitrospira*, associated with nitrification processes, may undergo a decrease with the application of NNIs, which could explain why the nitrification rate may decrease in some soils. According to these observations, much care is required when applying NNI and these compounds at the recommended doses to the soil to protect the soil's microbial composition, processes, and soil health. On the other hand, microorganisms from families such as Sphingomonadaceae, Streptomycetaceae, and Catenulisporaceae may be favored by the application of NNIs, leading to the development of degradative characteristics and genes. At the bacterial genus level, some stood out, such as *Sphingomonas*, *Streptomyces, Catenulispora*, *Brevundimonas*, *Pedobacter, and Hydrogenophaga*, among others. These microorganisms could play a relevant role in the degradation of these contaminants and prevent negative effects on the microbiome.

Various technologies can be used to treat NNIs, but they depend on the environment in which they are applied. The scientific community is continually searching for new microorganisms capable of degrading NNIs as a means of eliminating them from the environment. Different groups of microorganisms that can degrade NNIs have been described in various reviews, but there are not many studies showing their incorporation into the soil to minimize their impact. This is a vital aspect that needs to be explored in depth, along with other poorly understood factors like the relationship between NNIs, soil, and plants. Further research is necessary to understand the behavior of NNIs, as several factors determine their effects, despite their structural similarity. The use of pesticides, their responses, and associated problems are still unresolved issues that need to be explored further, especially with technological advances providing new insights into their biodegradative pathways, genomic, and molecular studies.

Current research on NNIs highlights their widespread use as insecticides and the potential risks they pose to the environment, human health, and wildlife. It is for this reason that, global research with NNI pesticides has progressed in several areas, including understanding their environmental risks, evaluating their cost-effectiveness, and developing methods to mitigate their impacts on nontarget organisms. The most common NNI pesticides used in agriculture include IMI, CLO, and THM, therefore, they have also been the most observed, studied, and regulated to prevent possible impacts on pollinators.

Studying the effect of NNI pesticides on soil is crucial for understanding their potential impacts on soil health, soil organisms, and the environment. This type of research can inform regulations, recommendations, and policies aimed at preventing negative effects, protecting soil health, promoting sustainable agriculture, and minimizing the ecological risks associated with NNI pesticides, which are products that will surely continue to be used for a long time.

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