LUANA NASCIMENTO DA SILVA

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LUANA NASCIMENTO DA SILVA

HERBICIDAS AFETAM O MICROBIOMA DO SOLO QUE CONTROLA O NEMATOIDE FORMADOR DE GALHAS *Meloidogyne paranaensis* NO CAFÉ?

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APROVADO em 24 de janeiro de 2025

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LAVRAS -MG
2025

Dedico:

A minha Mãe Maria e meu Pai Adailton por todo amor e dedicação

Minha irmã Bianca pelo amor e amizade

DEDICATION

First, I'd like to thank God that has been putting all the dreams of mine inside of me and making them possible. When I am down, I lay my hands upon the ground and for the thousandth time I am calling him cause his earth is mine.

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RESUMO

O Brasil é o maior produtor de café do mundo tendo produzido 2.993.780 t em 1.836.741 ha, no ano de 2021. A produção vem oscilando nos últimos anos, em função de diversos problemas fitossanitários como nematoide de galhas *Meloidogyne paranaensis*. O microbioma de um solo supressivo atua diretamente contra infecções de patógenos radiculares. Os diversos herbicidas que são utilizados no cultivo do cafeeiro podem impactar a microbiota nativa do solo. Deste modo, o objetivo da dissertação foi avaliar o potencial efeito de cinco herbicidas na funcionalidade do microbiana de um solo proveniente de uma área com café em relação ao nematoide formador de galhas *Meloidogyne paranaensis.* O solo foi coletado de uma Agrofloresta de café sem histórico de aplicação de herbicidas. Os herbicidas foram 5: Saflufenacil 100g/ha; Pyroxasulfone 400ml/ha; Glifosato 3kg/ha; Flumioxazin 180ml/ha e Cletodim 0,9l/ha. As mudas foram inoculadas com 4000 ovos de *Meloidogyne paranaensis* e após 35 dias as plantas de tomate foram coletadas e contados o número de galhas, g-1, e ovos, g-1. Outro experimento para avaliar a quimiotaxia de *Meloidogyne paranaensis* em resposta a exposição do microbioma do solo aos herbicidas foi realizado. Além disso, realizou-se o isolamento e quantificação de bacterias totais, endosporogenicas e fungos totais provenientes do solo em diferentes tempos de exposição aos herbicidas (0 dias, 1 dia, 5 dias, 15 dias e 35 dias). O DNA total do solo foi extraído ao fim do experimento in-vivo a comunidade bacteriana foi avaliada. No experimento in-vivo verificamos que Saflufenacil e Pyroxasulfone aumentaram significativamente o número de ovos e galhas ($P < 0.05$) em comparação ao controle. Já o Glifosato, Cletodim e Flumioxazin não reduziram a supressividade do solo, não houve diferença estatística entre estes tratamentos e o controle inoculado. No experimento de quimiotaxia o microbioma exposto ao Glifosato foi repelente ao nematoide assim como o tratamento controle. Já o microbioma exposto ao Saflufenacil e Pyroxasulfone foi altamente atrativo ao J2 e diferenciaram do controle. Apenas Flumioxazin afetou a comunidade de fungos 15 dias após aplicação. O Saflufenacil reduziu a comunidade de bactérias totais 5 e 15 dias após aplicação, porém em 35 dias observou-se um aumento significativo em comparação ao controle. Clethodim, Piroxasulfona, Saflufenacil e Glifosato começaram a inibir as bactérias endosporogênicas 1 dia após a exposição. Já a análise de microbioma revelou a modulação do microbioma quando exposto aos diferentes herbicidas. Pela alfa diversidade, foi possível observar que a diversidade bacteriana foi menor no tratamento controle e maior nos solos expostos aos herbicidas. Proteobacteria, Bacteriodetes, Actinobacteria, Acidobacteria e Gemmatimonadetes foram os filos mais predominantes no solo. Ressalta-se a importância de entender o impacto dos herbicidas no microbioma supressivo a doenças radiculares.

Palavras-chave: Herbicidas; Controle biológico; Supressividade; Nematoides.

ABSTRACT

Brazil is the largest coffee producer in the world, having produced 2,993,780 tons on 1,836,741 ha in 2021. Production has been fluctuating in recent years, due to various phytosanitary problems such as the root-knot nematode *Meloidogyne paranaensis*. The microbiome of a suppressive soil can act against root pathogen infections. The various herbicides that are used in coffee cultivation can impact the soil's native microbiota. Therefore, the objective of the dissertation was to evaluate the potential effect of five herbicides on the microbial functionality of a soil from an area with coffee in relation to the root-knot nematode *Meloidogyne paranaensis*. The soil was collected from a coffee agroforest with no history of herbicide application. The herbicides were Saflufenacil 100g/ha; Pyroxasulfone 400ml/ha; Glyphosate 3kg/ha; Flumioxazin 180ml/ha and Cletodim 0.9l/ha. The seedlings were inoculated with 4000 *Meloidogyne paranaensis* eggs and after 35 days the tomato plants were collected and the number of galls, g-1, and eggs, g-1, were counted. Another experiment to evaluate the chemotaxis of Meloidogyne paranaensis in response to exposure of the soil microbiome to herbicides was done. Furthermore, the isolation and quantification of total bacteria, endosporogenic bacteria and total fungi from the soil were carried out at different times of exposure to herbicides (0 days, 1 day, 5 days, 15 days and 35 days). Total soil DNA was extracted at the end of the in-vivo experiment and the bacterial community was evaluated. In the in-vivo experiment we found that Saflufenacil and Pyroxasulfone significantly increased the number of eggs and galls ($P < 0.05$) compared to the control. Glyphosate, Cletodim and Flumioxazin did not reduce soil suppressiveness, there was no statistical difference between these treatments and the inoculated control. In the chemotaxis experiment, the microbiome exposed to Glyphosate was repellent to the nematode, as was the control treatment. The microbiome exposed to Saflufenacil and Pyroxasulfone was highly attractive to J2 and differentiated from the control. Only Flumioxazin affected the fungal community 15 days after application. Saflufenacil reduced the total bacterial community 5 and 15 days after application, but at 35 days a significant increase was observed compared to the control. Clethodim, Pyroxasulfone, Saflufenacil, and Glyphosate began to inhibit endosporogenic bacteria 1 day after exposure. Microbiome analysis revealed the modulation of the microbiome when exposed to different herbicides. Using alpha diversity, it was possible to observe that bacterial diversity was lower in the control treatment and higher in soils exposed to herbicides. Proteobacteria, Bacteroidetes, Actinobacteria, Acidobacteria and Gemmatimonadetes were the most predominant phyla in the soil. The importance of understanding the impact of herbicides on the microbiome that suppresses root diseases is highlighted.

Keywords: Herbicides; Biological control; Suppressiveness; Nematodes.

INDICADORES DE IMPACTO

O café é uma cultura de alto valor e tem sido cultivado globalmente. O café é uma fonte de grãos torrados que são usados para produzir bebidas populares consumidas em todo o mundo e sua produção aumenta a cada ano. O presente trabalho foi desenvolvido devido ao uso extensivo de herbicidas para controlar ervas daninhas que estão presentes em campos com plantações de café. Existe o risco de herbicidas para humanos devido à exposição cumulativa, além disso, evidências científicas mostram que esses produtos podem causar danos muitas vezes irreversíveis à saúde do solo, alterando uma comunidade microbiana benéfica do solo. Uma microbiota benéfica é aquela capaz de controlar o nematoide parasita de plantas usando vários modos de ação. Os resultados que obtivemos podem ajudar os produtores de café a escolher os herbicidas químicos que não impactam o microbioma natural do solo e melhorar o uso dos biológicos.

IMPACT INDICATORS

Coffee is a high-value crop and it has been cultivated globally. Coffee is a source of roasted beans that are used to produce popular beverages consumed worldwide and its production increases every single year. The present work was developed because of the extensive use of herbicides to control weeds that are present in fields with coffee crops. There is the risk of herbicides to humans due the cumulative exposure, additionally scientific evidence shows that these products can cause often-irreversible damage to soil health by changing a beneficial soil microbial community. A beneficial microbiota is that one capable to control the plant-parasitic nematode by using several modes of action. The results we obtained may help producers of coffee to choose the chemical herbicides that do not impact the natural soil microbiome and improve the use of biological ones.

SUMARY

INTRODUCTION

The Rubiaceae family brings together 128 genera and approximately 1417 species, being the genus Coffea sp. with more than 120 species, the most important is *Coffea arabica*,the most cultivated species in the world (Bento *et al*, 2021; Almeida & Antar, 2023; Nad & Maslin,2020). Brazil is the world's largest producer and consumer of coffee, having produced 2,993,780 t in 1,836,741 ha, in 2021 with an average yield of 1,630 t ha-1 (IBGE, 2021). The Southeast region is the main producer, and the states of Minas Gerais and Espírito Santo stand out as the main national producers (IBGE, 2021). Despite the consolidated importance of national coffee growing as a source of income, the production has been fluctuating in recent years, due to various phytosanitary problems (Muniz *et al*, 2019; IBGE, 2021). Among these, root-knot nematodes *Meloidogyne paranaensis* deserves to be highlighted, as they cause great damage to the crop (Gamboa-Becerra *et al*, 2021).

Coffee is a perennial crop that remains in the field for many years and allows nematode parasitism and considerable population increase during its cycles (Holderbaum *et al*, 2021). Thus, because it is a soil-borne pathogen, the root-knot nematode *Meloidogyne paranaensis* is one of the most important species that affects coffee production in Brazil (Campos & Vilain, 2005). The infection occurs in the root system and the pathogen induces formation of giant cells and eggs that cause a huge effect in the absorption of water and nutrients (Collange *et al*. 2011). Plants infected by the nematode show symptoms such as root deterioration, severe cracking, and cracking in the cortical tissue of the root (Arita *et al*, 2020). In addition to reducing productivity, damage to the root system also inhibits the defense mechanisms of the hosts, making the plants susceptible to attack by other phytopathogens (M. Mitreva-dautova, 2006). Some microorganisms that are present in the soil can cause plant disease, while others can be beneficial microbes as plant growth promoters and plant protection (Mendes *et al*., 2013).

In soils that suppress diseases, plant protection is mainly mediated by the microbiome, which acts directly gainst root pathogen infections (Chapelle *et al*., 2016), which is its first line of defense against root pathogens (Mazzola *et al*., 2007; Kyselková *et al*. 2009; Mendes *et al*. 2011).

Disease-suppressing soils are defined as "soils where the pathogen does not establish itself or persists, establishes itself but causes little or no harm, or establishes and causes disease for a time, but thereafter the disease is less important, although the pathogen may persist in the soil" despite ideal conditions for disease occurrence (Baker; Cook, 1974). In contrast, agricultural soils in which pathogens and parasites infect plants and cause disease are called non-suppressive or propitious soils (Weller *et al*., 2002; Garbeva *et al*, 2004). Various pesticides are needed during coffee cultivation, for this reason agricultural soils often contain pesticides in different concentrations. Pesticides are divided into groups according to the primary target and include herbicides, insecticides and fungicides (Gevao *et al*., 2000).

Most herbicides that are sprayed for a long time on weeds and shoots of crops, leachate directly into the soil and can affect soil microbial communities and consequently natural supressivity (Li *et al*., 2015; Georgieva *et al*., 2018; Gopalakrishnan *et al*., 2017) the herbicides can change the soil microbe's community composition (Canuto *et al*., 2024). Therefore, the present study is based on the hypothesis that natural suppressiveness of the soil is affected by herbicides. In this scenario, the general objective of the dissertation is to evaluate the potential effect of five herbicides on the microbial functionality of a soil in relation to the root-knot nematode *Meloidogyne paranaensis*.

1. LITERATURE REVIEW

1.1 Coffee sp (Coffea spp.)

Coffee (*Coffea* spp.) is one of the most important tropical crops that represents a significant source of finance for producers in many countries in Latin America, being responsible for generating employment and securing labor in Brazil (Andrade *et al*., 2009; Gosalvitr *et al*., 2023; Zaidan *et al*.,2022). *Coffea arabica* is the main species cultivated in the world and all Arabica cultivars have their natural origin from the Southwest (SW) Ethiopia (Senbeta & Denich, 2006; Beenhouwer *et al*., 2015). The wild populations of coffee are in the southwestern Afromontane rainforests, where coffee is a wild understory shrub that farmers have been harvesting for centuries (Schmitt *et al*., 2009). Brazil is the main exporter accounting for 44.6% of the world's coffee due to its edaphoclimatic conditions (Ico., 2023; Angeloni *et al*., 2019). Furthermore, Brazil is the world's largest producer and consumer of coffee, having produced 2,993,780 t in 1,836,741ha, in 2021 with an average yield of 1630 t ha-1 (IBGE, 2022).

Although the economic importance with a comprehensive global market, this crop still faces various diseases that affect its productivity. *M. paranaensis* is a plant parasitic nematode that stands out as an important species of nematode to coffee, because it limits the implementation of coffee crops in infested areas and the productivity of those already planted (Gonçalves & Silvarolla 2007). The plants infected by *M. paranaensis* do not form characteristic galls, it causes root cracking, necrosis, leaf fall and finally the plant death (Goulart *et al*., 2019; Oliveira & Rosa 2018). In addition, this species is widely spread in *Coffea arabica* and considering Minas Gerais State as the main producer in Brazil, it is necessary to invest in the management to ensure the production in the state (Castro *et al*., 2008; Salgado *et al*., 2015). The need to manage the weed community in coffee fields is considerable due the susceptibility of the crop to compete for basic environmental resources necessary for its growth, development and production such as water, nutrients, light and CO2 (Melloni *et al*., 2018; Zaidan *et al*., 2023; Fialho *et al*., 2011).

The weed competition is higher during the period of crop and fruiting formation (Fialho *et al*., 2010). During these stages, the cultivation rows are supposed to be without weeds (Netto Acácio *et al*., 2021) and the main weed control strategy is chemical by applying herbicides. However, increased use of herbicides can disturb the soil environment, leading to disturbances in

the beneficial microbial community and microfauna that are responsible for soil suppressiveness against plant-parasitic nematodes (Guimarães *et al*., 2017, Benachour & Seralini, 2009; Gandhi *et al*., 2021). The microorganisms living in the soil such as bacteria, fungi, protozoa and microfauna, are the living part of soil organic matter, can act as natural enemies of plant-parasitic nematodes (Tótola & Chaer, 2002). The chemical herbicides can be considered a stress factor in the soil by exerting an influence on physical, chemical, and biological properties (Babin *et al*., 2019)

1.2 Root-knot nematode *Meloidogyne paranaensis*

Nematodes are members of a diverse group of animals that are able to inhabit all ecosystems, many have developed parasitic lifestyles (Mitreva *et al*., 2006; Vlaar *et al*., 2021). There are approximately 4100 nematode species that parasitize plants, between them Root-knot nematodes (RKNs) are considered one of the most important agricultural pathogens (Decraemer *et al*., 2006; Jones *et al*., 2013) and they are all the nematodes of the genus *Meloidogyne*. Root-knot (RKN) are responsible for damaging most of the cultivated crops, with annual crop losses estimated in $US 157 billion (Coyne *et al*., 2018).

This obligate plant pathogen nematode is a biotrophic endoparasite that establishes complex relationships with its host plants (Kumar & Miyara, 2022; Keçici *et al*., 2022) and requires living hosts to complete its life cycle (Hussain *et al*., 2024). However, RKN can cause yield losses, mainly when there is a high population of inoculum in the area that disorder the root development and plant production (Hussain *et al*., 2024).

When it comes to Coffee plants, several species of *Meloidogyne* are capable to infect them, but *Meloidogyne paranaensis* stands out hindering plant growth and causing losses of approximately 35% in yield (Carneiro & Cofcewicz., 2008; Lopez-Lima *et al*., 2015).

The sexual dimorphisms in root-knot nematodes happens because the females are pear-shaped that stays inside of the roots and the males are free living (Khan *et al*., 2023). The life cycle takes 25-28 days to be completed and has different stages (eggs, juveniles and adults) (Collett *et al*., 2024). It begins with the eggs where the embryonic development forms the J1, that remains inside of the eggs. After the first molt, J1 turns into J2, which is the infective stage of RKN's life cycle. After the third nematode stage (J3), only males are motile and leave the roots (Antil *et al*., 2023; Mandal *et al*., 2021; Pulavarty *et al*., 2021). Following the hatching of eggs, the J2 comes out to the soil to infect the host by penetrating the root cells walls by using its

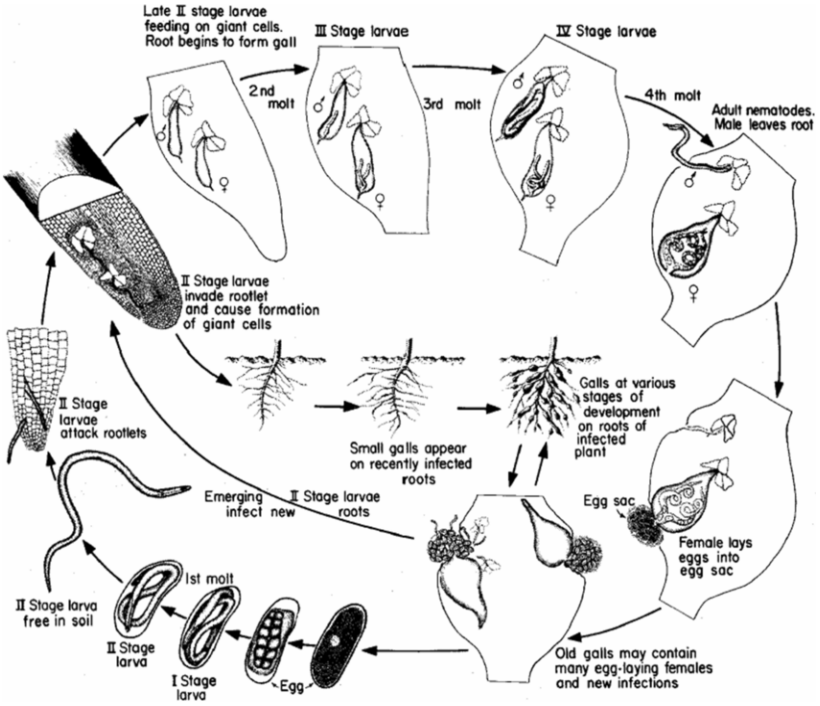
stylet (Li *et al*., 2015; Cavalcanti *et al*., 2024). After infection the J2 migrate in the vascular cylinder of the root cells and a site of feeding is established by inducing the cells that are around to become multinucleated giant cells (Tian *et al*., 2015). The nematode injects secretions into the giant cells to feed itself by extracting nutrients (Vanholme *et al*., 2004). The sedentary females produce eggs, which are mainly deposited outside the root surface in a gelatinous matrix (Papadopoulou & Triantaphyllou 1982; Jones *et al*., 2013).

The main symptom of root-knot disease are the galls. These galls are a result of the root cells hyperplasia that leads to an irregular root growth, primarily they are white and become brown after root maturation (Salgado *et al*., 2015; Campos & Villain, 2005). In addition, the nematode infection and the root galling causes yellowing of leaves, fragile roots, nutrient deficiency that reduces plant growth and resistance against biotic and abiotic stresses (Ralmi *et al*., 2016). *Meloidogyne* species don't have any structure of resistance and survive in an infested area because of the wide host range (Moens *et al*., 2009).

There are more than 100 species of root-knot nematodes (*Meloidogyne* spp.) that are capable to parasite more 3000 host plants (Khan *et al*., 2022) Chemical nematicides are the most widely used method for managing *Meloidogyne* spp. However, many of them have been restricted because of the impacts on the environment and human health (Kim *et al*.,2018; Schouteden *et al*., 2015).

The use of biological control organisms is an eco-friendly alternative to reduce root-knot nematodes. Several rizospheric microbes including Fungi and Bacteria of the genus *Arthrobotrys*, *Pochonia*, *Catenaria*, *Purpureocillium*, *Trichoderma*, *Pasteuria*, *Bacillus*, *Pseudomonas*, *Arthrobacter* and *Streptomyces* have been reported to control root-knot nematodes (Jamal *et al*., 2017; Martínez-Medina *et al*., 2017; Topalovic *et al*., 2020). These microbial antagonists of plant-parasitic nematodes possess mechanisms of their antagonism, such as competition for food space and colonization (Hunziker *et al*., 2015) antibiosis (Gómez Expósito *et al*., 2017), hyperparasitism (Mcneely *et al*., 2017), induction of systemic resistance, predation, volatile compounds and via releasing molecules that change behavior of nematodes (Liu *et al*., 2022; Antil *et al*., 2023)

Figure 1: Life cycle of Meloidogyne spp.



Source: (Stirling & Reay 2002)

1.3 Soil and Plant Microbiome

The soil is a complex community of organisms, that are determined by chemical and physical characteristics of the soil. The diversity and functionality of microbial communities in the soil determine the soil health that will provide a sustainable agriculture (Haney *et al*., 2015; Berg *et al.,* 2016). The microbiome is composed of several different types of organisms, including bacteria, fungi, protozoa, archaea, and viruses (Mueller & Sachs, 2015).

In agriculture the soil microbiome does ecosystem and physiological processes as the promotion of plant growth and development, litter decomposition, nutrient cycling and soil fertility (Bounaffaa *et al*., 2018; Tamayo-Velez & Osorio, 2018; Dubey *et al*., 2019). Most microbes of the soil microbiome are clustered around the tissue of plants root in a zone called rhizosphere (Afridi *et al*., 2022). The rhizosphere is 1-3 mm of soil around the roots that is a site for a complex interaction between the plant and the micro and macro biodiversity of the soil (bacteria, fungi, oomycetes, viruses, protists, archaea and nematodes (Egamberdieva *et al*., 2008).

The plants exudates (organic compounds) attract some microbes and improve the biodiversity on it (Fahad *et al*., 2022). This number of microorganisms that reside in the rhizosphere, colonize the different plant parts, and assist the host plant under adverse conditions. There are beneficial microbes in the rhizosphere that are able to help the plants to absorb nutrients, grow and stimulate the plant resistance (Liu *et al*.,2021). On the other hand, there are plant parasitic fungi, bacteria and nematodes that inflict disease in the crops (Adesina *et al*., 2009; Botelho *et al*., 2019; Buddrus-Schiemann *et al*., 2011). It means that not all the microbiome is involved in the functions of controlling pathogens, this positive functional role of the microbiome happens because of the cooperative effects of one or more strains (Rojas-Solís *et al*., 2018).

The beneficial and parasitic microbes are struggling for space and food in the rhizosphere, as a result the plants may get healthy and protected or diseased (Geller & Levy., 2023). As an example, the plant parasitic nematodes are able to influence the plant health and the soil microbial community in two ways and at the same time in the soil (Hussain *et al*., 2024).

The main mechanisms of action of the pathogen are affected by the production of microorganisms' metabolites (antibiotics, cell wall-degrading enzymes). In addition, microorganisms in rhizosphere can induce systemic resistance against pathogens and promote the resilience of the host against environmental stressors (Mhlongo *et al*., 2018; Cordovez *et al*., 2019). Competition, hyperparasitism, antibiosis, production of extracellular enzymes, and induction of resistance are established mechanisms (Raymaekers *et al*. 2020).

All these beneficial microbes associated with the roots are referred to as plant growth-promoting biocontrol agents. Various studies have proven that plants secrete small molecules (photosynthetic carbon) that recruit the beneficial microflora (Busby *et al*., 2014). Additionally, the diversity and population ratio of plant pathogens and beneficial microorganisms are linked to the amount and quality of plant root exudates and microbial interactions in the rhizosphere (Somers *et al*., 2004).

1.4 Soil suppressiveness

In a disease suppressive soil, a soil-borne pathogen doesn't establish, establish but not cause disease a lot or no disease at all, or establish and cause disease and after a certain time the disease severity reduces (Baker & Cook, 1974; Weller *et al*., 2002). On the other hand, a soil in which pathogens are capable to infect and cause disease in plants are considered conducive soils

(Garbeva *et al*., 2004).

There are two types of soil suppressiveness, the general and specific soil suppressiveness. A general soil suppressiveness is supposed to control a wide range of soil-born pathogens as nematodes, fungi and bacteria (Nishioka *et al*., 2022) and the presence of organic matter and root exudates can initiate the suppression of soil-borne pathogens by releasing nutrients on the soil that feed the beneficial microbiota (Cretoiu *et al*., 2013).

However, the general soil suppressiveness cannot be transferred to a conducive soil (Cook & Baker, 1983; Weller *et al*., 2002). On the other hand, specific suppression is a result of arranged activities of specific groups of soil microbes, it is transferrable (Siegel-Hertz *et al*., 2018; Borneman & Becker, 2007) and can be induced in field soils after the disease outbreak (Chen., 2007). To transfer the microbes that implies in suppressiveness, it is necessary that these beneficial microbes multiply in the conducive soil **(**Topalovic *et al*., 2020).

This will totally depend on the interaction with the indigenous conducive soil biota, chemical and physical properties of the soil as pH, organic matter, clay content (Dias, 2002; Dias-Arieira *et al*., 2021). Soil suppressiveness have been identified against root-knot nematodes (Silva *et al*., 2022; Silva *et al*., 2018; Westphal & Xing., 2011).

The simple comparison between the densities of nematodes populations in the plants and soils from different areas is the method to evaluate the soil suppressiveness against *Meloidogyne* spp. (Elhady *et al*.,2018). If different soils with similar physicochemical conditions have a large fluctuation in density of RKN, it means that there is a significant collaboration of the soil microbiome in the RKs establishment (Mendes *et al*., 2013; Silva *et al*.,2018).

The microbes of a suppressive soil parasitize and kill different stages of the RKNs life-cycle by using their modes of action (Li *et al*., 2015). Fungi and bacteria have several strategies to prevent the nematode establishment, RKN nematode-trapping fungi have adhesive hyphal traps and rings constricting, endoparasite fungi uses adhesive conidia to parasite the J2, egg- and female-parasitizing fungi use hyphal tips, *Bacillus* and *Trichoderma* induce systemic resistance in plants hosts, and some fungi and bacteria produce toxins (Topalovic et al.,2020)

1.5 Herbicides' effects on the soil suppressiveness

Weeds are one of the major problems to the agriculture productivity. The chemical herbicides are the most common strategy for weed management, it happens because of its economic viability and easy application. However, these chemical herbicides have been

reported to impact the ecosystem functions and the soil environment by changing the microbial population of soils in quantitative and qualitative terms (Raj & Syriac 2017; Gandhi *et al*., 2021). The herbicide can stimulate or reduce the microbial growth and the enzymatic activities of them, but it'll totally depend on the active ingredient and its concentration in the herbicide, microorganism species and environmental conditions (temperature, humidity, type of soil) (Latha & Gopal 2010; Maheswari & Ramesh 2019).

A chemical is considered an ideal herbicide if it has the toxicity on the target weeds as well as detoxify into non-toxic compounds rapidly to avoid its toxicity toward any other organism. (Stanley *et al*., 2013). Considering the part of the chemical herbicides that affect non target macro and microorganisms, these herbicides will consequently reduce some soil functions that are perform by the living organisms such as the degradation of organic matter, nitrogen carbon, availability of carbon and nutrients, and therefore crop production (Sebiomo *et al*., 2011). The chemical herbicides are capable to influence the stability of soil borne pathogens and beneficial microbes in the soil, therefore the disease caused by the pathogen is the negative consequence (Rosenbaum *et al*., 2014)

Unfortunately, little is known about the impact of increased herbicide use on soil biota and the plant-parasitic nematodes that are supposed to be controlled by this microbiome. A comprehensive experiment testing different active ingredients is necessary to help farmers to identify where a change in herbicide management could protect the ecosystem services provided

3 Objectives

3.1General objectives

To evaluate if 5 herbicides that are commonly used to control weeds on coffee are able to affect the soil microbiome of a soil that is suppressive against *Meloidogyne parananensis*

3.2 Specific objectives

CHARPTER 1: "Do herbicides affect the functionality of soil microbiome that controls the root-knot nematode Meloidogyne paranaensis in Coffee?"

Evaluate the potential effect of five herbicides on the microbial functionality of a soil in relation to the root-knot nematode Meloidogyne paranaensis.

CHARPTER 2: "Do herbicides affect the composition of the soil microbiome from Coffee crops area?"

Evaluate the modulation of bacteria's taxa present in the soil microbiome caused by herbicides

and predict the contribution of these microbes on soil suppressiveness.

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Chapter 1-*Meloidogyne paranaensis* and chemical herbicides

"Do herbicides affect the soil microbiome that controls the root-knot nematode *Meloidogyne paranaensis* in Coffee?"

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Abstract

Chemical herbicides are proven to impact the indigenous soil microbiome however, little is known about the impact of these products in antagonistic microbiome that is responsible for soil suppressiveness against root-knot nematodes. Green house assay was conducted where the number of galls, eggs were evaluated. The application of Saflufenacil and Pyroxasulfone, showed the highest number of galls per gram of plants roots (scot-knot 5%) as compared to the control. As it was expected the Glyphosate was considered repellent to J2 of *Meloidogyne paranaensis*. On the other hand, Saflufenacil and Pyroxasulfone have turned the soil microbiome into very highly attractive CI ≥ 0.2. The result indicate that the chemical herbicide affected the soil microbiome, but it depends on the active ingredient.

1. Introduction

Coffee is a very important commodity to many countries around the world (Simões *et al*., 2020; Bohl *et al*., 2019). Coffee cultivation is fundamental to generate income and jobs (Muñoz-Rios *et al*., 2020). Brazil is the largest producer and exporter of coffee, and it has been producing around 30% of the world's coffee (Ico, 2022). However, with the expansion of the cultivation area, there is the need to invest in the management of the diseases and weeds that can reduce the productivity (Zaidan *et al*., 2023; Goulart *et al*. 2019). *Coffea arabica* is the main species produced in Brazil.

Meloidogyne paranaensis is a plant-parasitic nematode that causes injury to Coffee plant roots, in that way the plant is completely damaged and its production decreases a lot (Carneiro *et al*., 1996; Saucet *et al*., 2016)). Brazilian coffee producers don't have many alternatives to control *Meloidogyne paranaensis* because there aren't several resistant cultivars of *Coffea arabica* available on the market as well as chemical and biological products (Villain *et al*., 2018). *Meloidogyne paranaensis* has been spreading in the state of Minas Gerais that is the main producer of the crop in Brazil (Salgado *et al*., 2015; Terra *et al*., 2018).

This root-knot nematode induces the development of coffee corky-root disease and other symptoms are seen as the infection advances such as deep cracked cortical tissue and necrotic tips in older roots (López-lima *et a*l., 2020; Lamelas *et al*., 2020).

The beneficial soil microorganisms can be an alternative to control soil borne pathogens as *Meloidogyne paranaensis* if their mechanisms of action result in a natural and preexisting characteristic of the soil called suppressiveness (Schlatter *et al*., 2017). The microbiome can induce the plant defense responses, promote growth and reduce the number of galls and eggs by increasing plant resistance to nematode's infection (Gamalero and Glick, 2020). Nematodes are able to recognize stimuli through sensory organs, such as amphids and phasmids (Curtis, 2008;

Perry & Curtis, 2013; Rasmann *et al*., 2012). They can be attracted by sensory compounds from plants, bacteria, fungi and other nematodes (Stirling, 2014; Wu & Duncan *et al*., 2020).

In Brazil, several herbicides have been applied to control weeds in Agricultural systems with coffee plantations. Chemical control with herbicides is commonly used to manage weeds (Van Bruggen *et al*.,2018). In *Coffea arabica* plantations the management of weeds is obligatory, because the roots growing in the surface of soil (Zaidan *et al*.,2023). The weed control prevents the deficiencies of essential nutrients and water stress in coffee crops (Abdelaal *et al*., 2022)

Nowadays, there are 26 active ingredients registered to coffee crops in Brazil (Agrofit, 2024). Among these different herbicides, glyphosate-based ones are the most used in the country (Bytof *et al*.,2024). Structurally glyphosate is a phosphonomethyl derivative of glycine (N-(phosphonomethyl) glycine) that is a non-selective, systemic and post- emergence herbicide (Peixoto *et al*.,2024). Glyphosate is quickly translocated and the plants are not able to detoxify this herbicide, this is the explanation to its effectiveness and preference of many agricultural producers (Shaner, 2006). This herbicide has a large spectrum of action, that is capable to control mono and dicotyledonous weeds with annual or perennial cycles (Maria *et al*.,2018).

Glyphosate inhibits the activity of 5-enolpyruvylshikimate-3 phosphate synthase (EPSPS), which is a catalyst for one of the synthesis reactions of aromatic amino acids such as tyrosine, tryptophan and phenylalanine (Yamada & Abdalla, 2007). Clethodin is another post-emergence and systemic herbicide that has registration to be used in crop plantations. On the other hand, it belongs to the cyclohexanedione chemical group that with fatty acid biosynthesis through inhibition of the enzyme acetyl coenzyme-A carboxylase (ACCase) (Radwan, 2012)

Saflufenacil is another herbicide that has been recently registered in Brazil as an alternative to control glyphosate-resistant weeds (Gonçalves *et al*., 2016; B). It is used at post-emergence for controlling eudicotyledonous weeds and belongs to the pyrimidinedione chemical class, inhibiting the enzyme protoporphyrinogen oxidase (PROTOX) (Martins *et al*., 2012). Its absorption is made by both roots and leaves and the symptoms of toxicity appears in few hours as circular chlorotic injuries (Rodrigues *et al*., 2020). However, when Saflufenacil is applied directly on the soil its herbicidal activity is limited due the quickly degradation mediated by the microbiome (Patel *et al*., 2023) and the weak adsorption of it on soil's particles surface (Oliveira *et al*.,2024; Camargo *et al*., 2013).

Flumioxazin has the same mode of action of saflufenacil by inhibiting the enzyme

protoporphyrinogen oxidase (protox). This pre-emergence herbicide is absorbed by germinating seedlings and stops the first stages of development (Labonne & Capou, 1998). Weed species then start to bleach and rapidly die off.

Pyroxasulfone is relatively a new preemergence herbicide that it was first registered in 2012 (Yu *et al*., 2012). Pyroxasulfone inhibits 3-ketoacyl-CoA synthases and subsequently disturbs very-long-chain fatty acid synthesis, ultimately killing target weeds (Tanetani *et al*., 2011). It has good herbicidal activity against grass and broadleaf weed species (Nakatani *et al*., 2016).

The irrational use of these herbicides has been raising concerns about environmental contamination. The residual persistence of herbicides is able to affect the soil ecology, including the functional microorganisms that plays an important part in controlling plant- parasitic nematodes (Silva Canuto *et al*., 2024; Wang & Cernava, 2020). Therefore, the present study is based on the hypothesis that the natural suppressiveness of the soil is affected by herbicides. In this scenario, the objective of the dissertation is to evaluate the potential effect of five herbicides on the microbial functionality of a soil in relation to the root-knot nematode *Meloidogyne paranaensis*.

2. Material and methods

2.1 Soil collection site and soil sampling

The soil was collected from coffee agroforestry system in a property of Santana da Vargem, Mina Gerais, Brazil. At this property, any chemical herbicide has never been applied. The final composite samples collected from the area were sieved and then stored at cold chamber until the use in the experiments. The process of sieving was done to exclude plant debris and stones.

1.1. Inoculum preparation

The tomato plant (*Solanum lycopersicum* L. 'SantaClara'), was grown for the inoculum preparation and multiplication, containing a purely *Meloidogyne parananensis* population in the greenhouse of the Department of Plant Pathology Federal University of Lavras (UFLA) MG, Brazil. After 60 days, the infected root samples of tomato plants were collected, rinsed under running water, cut into 1-2 cm pieces, then blended for 60 seconds with a 0.5% sodium hypochlorite (NaOCl) solution. The material was loaded into a 200-mesh screen, thoroughly cleaned, then collected from a 500-mesh screen, washed properly, and collected the retained

eggs in water collected from a 500-mesh sieve (Boneti & Ferraz, 1981).

1.2. Evaluation of the sensitivity of the soil microbiome to herbicides

The soil was divided in two parts sterilized and non-sterilized. To prepare soil slurries containing the soil 100 g of soil sample (non-sterilized) was extracted in a soil dispenser with 1000 ml of sterile 0.85% NaCl at high speed for 20 minutes. After that, the soil slurry was passed through a 500-mesh sieve to remove the nematodes (eggs and J2), root debris and soil particles. The slurry was centrifugated for 10 minutes at 4000 rpm, supernatant was discarded and the pellet resuspended in 45 ml of sterile distilled water (SDW). The process of centrifugation was done again and the supernatant was discarded one more time. The pellet was diluted at the concentration of 10^{-1} by using SDW. All the methodology mentioned above was repeated until the necessary volume of slurry was acquired. Each 500ml plastic cup was filled with autoclaved soil and received a 10-days-old tomato seedling. Then each one of these cups received the soil slurry (75ml of slurry per 500 ml of autoclaved soil). The microbiome established in the soil and rhizosphere during 7 days until the application of herbicides. The treatments consisted of 5 herbicides and 7 replicates (7 plastic cups).

There were two controls that consisted in the autoclaved soil microbiome non-exposed to any product, with and without inoculation of eggs. All the products were applied in the higher dose recommended. Glyphosate (Glifosato 72 WG Alamos) 3kg/ha; Clethodim (Select one pack) 0,9 l/ha; Flumioxazin (Pledge) 180 ml/ha; Saflufenacil (Heat) 100g/ha and Pyroxasulfone (Yamato) 400ml/ha. All the cups were placed on the workbench in a randomized complete design.

The inoculation of seedlings was performed 5 days after the herbicides application with 4000 eggs that were extracted from a pure inoculum of *Meloidogyne paranaensis.* The tomato plants received irrigation according to technical recommendations for 35 days. The galls were counted and the eggs were extracted and also counted. At the end the number of galls and eggs were estimated per gram of root system.

1.3. Chemotaxis of J2 towards the soil microbiome exposed to the herbicides

The chemotaxis was done by using the Bargmann *et al*. (1993) and Wang *et al*. (2019) with some changes. Petri dishes with 9 cm diameter containing 2% water-agar medium (WA) were divided into three areas: A) neutral area, located in the center of the plate; B) test area; C) control area. Soil with microbiome exposed to herbicide action for 5 days was diluted to a

concentration of 10^{-1} using sterile distilled water. A 20 µl aliquot of each dilution was placed in position b and another aliquot of 20 µl of soil without herbicide exposure was added in position c. The control treatment was the microbiome without exposure to the product in position B and sterile distilled water in position c. Then, the plates were incubated at 28 ◦C in the dark. After 24 h, the number of J2 in the test (B) and control (C) areas were quantified with the helping of an inverted optical microscope. The chemotaxis index (CI) was obtained by subtracting the number of J2 in the test area from the number of J2 in the control area and then dividing by the total number of J2 found outside the neutral area (Bargmann *et al*., 1993). If CI ≥0.2, soil was highly attractive and, if 0.1 ≤CI <0.2 was considered slightly attractive. If -0.1 ≤CI <0.1, as a random response. If -0.2 <IC < -0.1, soil was considered repellent and, a CI ≤ -0.2 as highly repellent (WANG *et al*., 2019). The experiment was performed with five replicates each.

1.4. Statistical analysis

All assays used a completely randomized design. All data were previously subjected to normality test (Shapiro-Wilk test) and then the F test was applied through analysis of variance (ANOVA). The means of the experiments were compared by Tukey test (P< 0.05). Sisvar software and Sigma plot Sigma Plot® version 11 were used for statistical analysis and artwork, respectively.

2. Results

2.1. Sensitivity of suppressive soil microbiome to herbicides

The manipulation of suppressive soil microbiome (soil slurry) with some herbicides has probably perturbed the microbiome functions where an increase of eggs and galls per gram of root was observed (P < 0.05) as compared to the control (Figure 1 A, B). The soil microbiome that was exposed to the chemical herbicides showed significant increase of the nematode's population. It means that the number of nematodes that penetrated the roots were significantly affected by the active ingredient applied in the microbiome. Saflufenacil and Pyroxasulfone were similar in increasing eggs and galls and showed a significant difference (P< 0.05) compared to the inoculated control (Figure 1A, B). Glyphosate, Clethodim and Flumioxazin didn't reduce the soil suppressiveness since there was no difference between these treatments and inoculated control (Figure 1A, B).

2.2. Chemotaxis of J2 towards the soil microbiome exposed to the herbicides

The microbiome exposed to Glyphosate was considered repellent to J2 of *Meloidogyne paranaensis* -0.2 <IC < -0.1. The control that consisted in the microbiome without herbicide was considered highly repellent CI ≤ 0.2. On the other hand, Saflufenacil and Pyroxasulfone have turned the soil microbiome into very highly attractive CI ≥ 0.2 (Table 1). There was no difference between the Control and Glyphosate (Figure 2). Saflufenacil and Pyroxasulfone had both the higher chemotaxis index and the control was different from these treatments (Figure 2).

3. Discussion

In this work we supplied a comprehensive understanding of the herbicides' effects on soil suppressiveness at both microbiological properties and nematode's population. The effect of herbicides on soil suppressiveness is influenced by a robust amount of biological and abiotic soil factors (Gan & Wickings, 2017; Matallo *et al*., 2014; Costa *et al*., 2023). In the present study, we investigated the effect of herbicides on microbial structure and function.

The Saflufenacil was the herbicide that most increased the number of eggs and galls per gram of soil, followed by Pyroxasulfone. Some hypotheses can be assumed for the cause of this behavior as the mode of action of these herbicides and their time of persistence on soil.

Saflufenacil is an inhibitor of the enzyme protoporphyrinogen oxidase (PROTOX) that is the last enzyme present in the route of chlorophyll's synthesis and this process of inhabitation totally depends on the light (Albrecht, 2023) the decrease in the photosynthesis rate is the physiological direct response. Considering that the exudation of photosynthates by the plants into the rhizosphere is responsible for shaping the plant-microbiome interaction and stability (Pang & Xu,2024), Saflufenacil has probably affected this establishment of the nematode's antagonistic microbes on the roots.

In addition, this product doesn't have a long time of persistence on the soil, because it is quickly absorbed by the plant and the symptoms of its toxicity is seen few hours after application **(**Johnson *et al*., 1978; Ashigh & Hall, 2010). This fact corroborates the non- direct effect of the Saflufenacil on soil microbiome in the presence of the host.

Saflufenacil and Flumioxazin didn't have similar results even with the same mode of action, since Flumioxazin did not increase the nematode's population as compared to the control. This result may be related to the high adsorption of Flumioxazin (cationic herbicide) to soil colloids and crop residues (Ferrell *et al*., 2005; Alister *et al*., 2008). On the other hand,

anionic herbicides such as Saflufenacil shows low adsorption to the soil component (Szmigielski *et al*. 2018) what would explain the results. The evaluability of the herbicide in the soil is direct related to the adsorption of it with soil particles surface (Khorram *et al*. 2017).

In chemotaxis experiment Pyroxasulfone and Saflufenacil (in the soil) were both attractive to the nematode as compared to the control ($P<0.05$) (Figure 2). These active ingredients have probably disturbed the soil microbiome and it turned into an attractive one to the nematodes. It is noticed that in the chemotaxis test, there is not the contribution of the plant absorption and the product would be available in the soil to affect the microbiome much more.

Many previous studies have confirmed the effects of pesticides on microbes' organisms (Qu *et al*.,2021; Ma *et al*.,2022; Yu *et al*., 2021) although the Pyroxasulfone effects on soil microbial community is not clearly understood. It has proven that Pyroxasulfone increases the soil organic matter concentration and the number of total bacteria in the soil (Wang *et al*.,2023). In this present work Pyroxasulfone was capable to drive the function of the soil microbiome (Figure 1) probably via selection of taxa that are not related to the control of nematodes.

When it comes to Glyphosate, it did not increase the number of eggs and galls (Figure 1 A, B), this result may be related to the adsorption of its metabolites in the soil. The molecular structure of Glyphosate is considered different as compared to other 96% herbicides, because Glyphosate has a linear carbon chain with a weaker bond without any aromatic ring in its structure (NCBI,2020) it affects the microbial activity, that increases due the utilization of Glyphosate as a source of Nitrogen and Carbon (nutrients).

On the other hand, the first and main precursor metabolite of Glyphosate is AMPA (aminomethylphosphonic acid) (Fenner *et al*.,2013). This metabolite has a higher toxicity than Glyphosate and longer half-life in the soil 23–958 days compared to glyphosate's (1–197 days), what is directly related to its adsorption in the soil particles (Ojelade *et al*.,2022).

The type of soil also determines the rate of Glyphosate's metabolization and in a soil with a large quantity of organic matter the degradation of the product is less intensive, it means that the AMPA, that is more toxic and persistent than Glyphosate is adsorbed on soil particles (Jiao *et al*., 2024). The soil used in the experiment came from an Agroforestry and this model of agriculture has been reported to increase the soil organic matter (Mondelaers *et al*., 2009; Gattinger *et al*., 2012; Tuomisto *et al*., 2012). This might suggest that the herbicide was inactivated by the soil adsorption what would explain its non-toxicity to the microbiome.

These results are corroborated by the chemotaxis responses where Glyphosate was repellent to the nematode (Figure 2) and it can be deduced that even after Glyphosate exposure the soil remains suppressive as well as the control. Glyphosate has been reported in the literature as a product that doesn't drive the structure of prokaryotic and fungal communities (Kepler *et al*., 2020; Lupwayi *et al*., 2020; Bottrill *et al*., 2020).

4. Conclusions

Our results indicate that the chemical herbicides have an impact in the soil microbiome that suppress the root-knot nematode *Meloidogyne paranaensis*, but it depends on which herbicide we are applying in the soil. The increase in the number of eggs and galls as an herbicide's consequence represents the changes in the suppressiveness. The effect in nematode's population, infection and chemotaxis were better performed in the soil with Saflufenacil and Pyroxasulfone than Glyphosate. The microbiomes' major influence was confirmed when their transference to a sterile substrate suppressed *M. parananensis* in tomato roots. However, the rhizosphere recruits antagonistic microbes more efficiently in absent of herbicides.

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Legends and figures

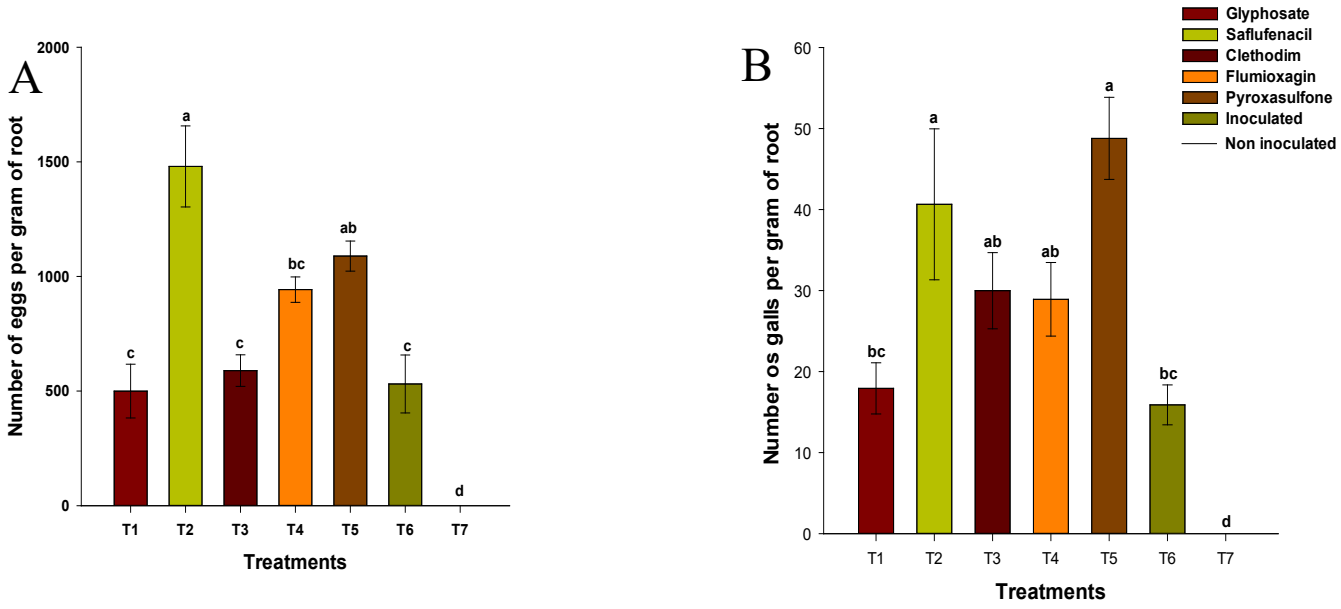

Figure 1. Influence of herbicides on soil microbiome solution in the number of eggs (A) and galls (B) of *Meloidogyne paranaensis*. The number of eggs and galls were quantified 35 days after the infestation of 4000 eggs on tomato root (*Solanum lycopersicum* 'Santa Clara'). Seven replicates were used in each treatment and bars with different letters indicate significant differences between different treatments by Tukey's test ($P < 0.05$)

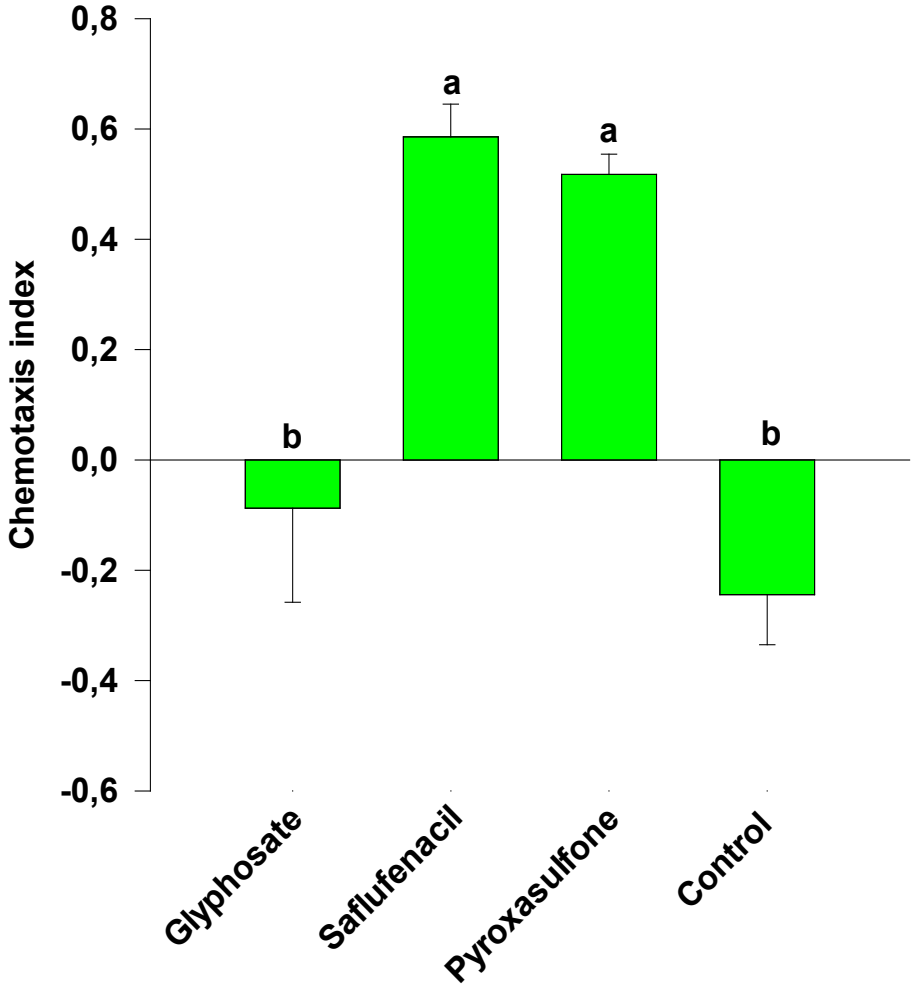

Figure 2. Chemotactic responses of *M.paranaensis* second-stage juveniles (J2) to soil microbiome exposed during 5 days to the herbicides Glyphosate, Saflufenacil and Pyroxasulfone. The bars represent the standard errors. Means followed by the same letters do not differ significantly from each other by the Tukey test ($P < 0.05$). There was difference between the treatments P = <0,001. Values are means of five replicates.

Table 1- Chemotactic responses of *M. paranaensis* second-stage juveniles (J2) soil microbiome exposed to herbicides.

Treatments	Chemotaxis index
I) Glyphosate	-0,0875
II) Saflufenacil	0,586
III) Pyroxasulfone	0,518
IV) Control	-0,244

I)Microbiome exposed to Glyphosate in position b and microbiome non exposed to any herbicide in position c; II) Microbiome exposed to Saflufenacil in position b and microbiome non exposed to any herbicide in position c; III) Microbiome exposed to Pyroxasulfone in position b and microbiome non exposed to any herbicide in position c; IV) Microbiome without herbicide position b and sterile distilled water

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CHAPTER 2-Chemical herbicides affecting the soil microbiome composition

"Do herbicides affect the composition of soil microbiome and concentration of culturable microbes from a suppressive soil?"

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Abstract

Chemical herbicides are proven to impact the indigenous soil microbiome. However, little is known about the impact of these products on antagonistic microbiome that is responsible for soil suppressiveness against root-knot nematodes. In vitro assay was conducted where effect of 5 herbicides in the community of culturable fungi and bacteria were evaluated. In addition, the 16S rRNA genes of the bacterial communities from soil from four different treatments, at the end of the in vivo assay, were sequenced. Only Flumioxazin affected the fungal community 15 days after application. Saflufenacil reduced the total bacterial community 5 and 15 days after application, but at 35 days a significant increase was observed compared to the control. Clethodim, Pyroxasulfone, Saflufenacil, and Glyphosate began to inhibit endosporogenic bacteria 1 day after exposure. Microbiome analysis revealed the modulation of the microbiome when exposed to different herbicides. Using alpha diversity, it was possible to observe that bacterial diversity was lower in the control treatment and higher in soils exposed to herbicides. Proteobacteria, Bacteroidetes, Actinobacteria, Acidobacteria and Gemmatimonadetes were the most predominant phyla in the soil.

1. Introduction

Coffee is a very important commodity to many countries around the world (Simões *et al*., 2020; Bohl *et al*., 2019). Brazil is the largest producer and exporter of coffee, and it has been producing around 2,99 million of tons per year (Ico, 2022; Fao, 2024). Minas Gerais is the main producer of coffee for its higher aptitude to this crop, with important producing regions for the agrobusiness to the state. However, there are so many abiotic and biotic factors that may affect the production of coffee quantitatively and qualitatively (Zaidan *et al*., 2022).

Weeds are among the biotic factors necessary to invest in the management, considering that they are strongly competitive for growth resources (water, light and nutrients) (Marcolini *et al*., 2009; Fialho *et al*., 2010). Plant-parasitic nematode is another biotic factor (pathogen) that causes injury to Coffee plant roots and it's responsible for compromising considerably the areas' production with crop infestation (Marques *et al*., 2024). Among these pathogens *Meloidogyne paranaensis* stands out as one of the most destructive and well distributed species of nematodes. *Meloidogyne paranaensis* has been spreading in the state of Minas Gerais that is the main producer of the crop in Brazil (Salgado *et a*l., 2015; Terra *et al*., 2018). *Meloidogyne paranaensis* is responsible for causing the typical symptoms of thickening with longitudinal cracks in the roots since the first biological cycle of the nematode (Guimarães *et al*., 2020), which commonly results in the death of the plant.

Soil suppressiveness is the natural ability of the soil to suppress pathogens and as a consequence increases plant productivity (Mendes *et al*.,2011). The suppressiveness is correlated

with the chemical, physical and biological properties of the soil (Trankner, 1992). A gram of soil is capable to contain tens of thousands different species of microorganisms (Rosselló-Mora & Amann, 2001). The soil microbial diversity is the main factor that confers plant protection by not allowing the pathogens to widespread and cause disease (Brussaard *et al*., 2007; Mendes *et al*., 2011). The microbiome can induce the plant defense responses, promote growth and reduce the number of galls and eggs by increasing plant resistance to nematode infection (Gamalero & Glick, 2020).

Herbicides are among the chemical often found in soils (Popp *et al*., 2013). Herbicides are intentionally applied to control weeds and guarantee the production and quality of coffee, by preventing deficiencies of essential nutrients and water stress (Zaindan *et al*.,2023). The herbicides in agriculture have been considered as a threat for soil microbiome and function (Eu, 2009).

When herbicides are applied to soil the abundance (the absolute abundance of total bacteria (16S rRNA) and fungi (nuclear Internal Transcribed Spacer (ITS) region) and diversity of different macro- and micro- organisms might be positively (stimulation) or negatively (inhibition) affected, and as a consequence the soil suppressiveness may also be altered (Chowdhury *et al*., 2008).

Microbes living in the rhizosphere include bacteria (Mendes *et al*., 2013), fungi (Nannipieri *et al*., 2007), archaea (Philippot *et al*., 2013), and viruses (Muscatt et al.,2021), together they comprise one of the most complex ecosystems (Zain *et al*., 2024). Throughout plant growth and development, the composition and proportion of rhizosphere microbial communities is keeping a dynamic stability, and the application of herbicides can disturb this stability, resulting in changes in rhizosphere microbial diversity and species (Dennis *et al*., 2018). Changes in the structure of microbial can affect plant health (Yu *et al*., 2024).

The quantification and isolation of microorganisms present in the soil is an assessment that helps to understand the impacts of herbicide application (Previati *et al*. 2012). Since biological indicators are more sensitive than chemical and physical indicators to detect in advance the changes that occur in the soil due to management practices (Stocker *et al*., 2017). The relative abundance or scarcity of some taxa can occur after infestation (Hussain *et al*.,2024) or after management measures such as the application of agrochemicals.

However, which taxa are crucial for the suppressiveness of Meloidogyne paranaensis

remains undefined. Advances in sequencing, bioinformatics, and functional genomics will help identify and quantify soil-associated microorganisms. Therefore, the present study is based on the hypothesis that the taxa not yet identified and that are part of the suppressive soil microbiome play an important role with specific mechanisms of action for control. In this scenario, the objective of this chapter is to evaluate the modulation of microbiome taxa caused by herbicide application, to identify and quantify soil-associated microorganisms and to discuss the role of taxa identified using previously characterized species as references.

2. Material and methods

2.1. Soil collection site and soil sampling

The soil was collected from coffee agroforestry system in a property of Santana da Vargem, Mina Gerais, Brazil. At this property, any chemical herbicide has never been applied. The final composite samples collected from the area were sieved and then stored at cold chamber until the use in the experiments. The process of sieving was done to exclude plant debris and stones.

2.2. Evaluation of the sensitivity of the soil microbiome to herbicides

The soil was divided in two parts sterilized and non-sterilized. To prepare soil slurries containing the soil 100 g of soil sample (non-sterilized) was extracted in a soil dispenser with 1000 ml of sterile 0.85% NaCl at high speed for 20 minutes. After that, the soil slurry was passed through a 500-mesh sieve to remove the nematodes (eggs and J2), root debris and soil particles. The slurry was centrifugated for 10 minutes at 4000 rpm, supernatant was discarded and the pellet resuspended in 45 ml of sterile distilled water (SDW). The process of centrifugation was done again and the supernatant was discarded one more time.

The pellet was diluted at the concentration of 10^{-1} by using SDW. All the methodology mentioned above was repeated until the necessary volume of slurry was acquired. Each 110 ml plastic sup was filled with autoclaved soil. Then each one of these cups received the soil slurry (16,5 ml de slurry per 110 ml of autoclaved soil). The microbiome established in the soil during 7 days until the application of herbicides. The treatments consisted of 5 herbicides and 5 replicates (5 plastic cups). There was one control that consisted in the autoclaved soil+ microbiome non-exposed to any product. All the products were applied in the higher dose recommended. Glyphosate (Glifosato 72 WG Alamos) 3kg/ha; Clethodim (Select one pack) 0,9 l/ha; Flumioxazin (Pledge) 180 ml/ha; Saflufenacil (Heat) 100g/ha and Pyroxasulfone

(Yamato) 400ml/ha. All the cups were placed on the workbench in a randomized complete design. The soil was watered with 15 ml of sterile distilled water (SDW) daily.

2.3. Enumeration of soil microbial population groups

Samples were obtained from each plastic cups with soil (5 treatments (herbicides) and the control X 6 replicates). At appropriated intervals (0 days, 1 day, 5 days, 15 days and 35 days after herbicides application), subsamples were taken to evaluate herbicidal effect. The exposure from 0 to 35 days was chosen in relation to the time necessary to fungi and bacteria growth, hours and days respectively. Numbers of culturable total bacteria, endosporogenic bacteria and fungi were determined by serial dilution and plating into selective media. The serial dilution was done until the concentration of 10^{-8} and all the dilution were plated to determine the colony-forming units (CFUs). Serial dilutions of soil samples (1 g fresh weight) were made with 9 ml of peptone water (total and endosporogenic bacteria) in a 15 ml tube (concentration of 10^{-1}). This first dilution was placed in an orbital shaker for 20 minutes at 230 rpm. The subsequent dilutions were made in 2 mL microcentrifuge tubes, by transferring 0,1 ml of the last dilution (10^{-1}) to 0,9 ml of peptone water, until the concentration of 10^{-8}.

Plating was performed based on the microdroplet technique (Romeiro,2001), and each Petri dish (filled with agar-nutrient) was divided into 8 parts and identified appropriately. All the 15 ml tubes were heated in a bain-marie at 80 ºC for 12 minutes, to inactivate the cells that are not considered endosporogenic ones and the dilution until 10^{-8} was performed again as well as plating. The total fungus community was determined following the same methodology mentioned above, but the culture medium was PDA (potato dextrose agar) with antibiotic and the soil dilution was performed by using a saline suspension 0.85%. Each dilution was plated with drops of 0,01 ml. The cells of fungus were incubated in a B.O.D at 25 °C, during 3 days at a photoperiod of 12 h. On the other hand, the bacteria cells were maintained in environmental temperature for 24 hours. After the incubation period, the colonies were counted to obtain the number of colony-forming units (CFUs).

2.4. Soil DNA extraction and quantification

At the end of the in vivo experiment, 35 days after inoculation, the soils corresponding to each treatment were collected. All samples were stored at -20°C until processed. Treatments with Saflufenacil, Pyroxasulfone, Glyphosate and Control (without exposure to herbicide) were selected. Seven replicates of each treatment were collected and homogenized. Subsequently, 3

replications of each treatment were used to DNA extraction and evaluate the microbiome. Total DNA extraction was conducted from the soil without any product (control) and the soil of plants treated with the chemical herbicides. The DNeasy Power Soil DNA Isola tion kit (MoBio, 12888) was employed for DNA extraction. For each treatment, 0.25 g of total soil was utilized for DNA extraction. The extracted DNA was quantified using a NanoDrop spectrophotometer and stored at − 80 ◦C until further processing.

2.5. Bacterial DNA sequencing and metataxonomic analyses

The microbial community structure in soil samples was monitored using a metataxonomic approach. The 16S rRNA genes encoding bacteria and archaea were amplified via PCR using Bact_341F (5′ CCTACGGGNGGCWGCAG3′) and Bact_806R (5′-GACTACHVGGGTATCTAATCC-3′) recommended by the Brazilian Microbiome Project (Pylro *et al*., 2014), using the System Operational BMP (BMPOS) (Pylro *et al*., 2016), as this pair provides high coverage of the bacteria domain and good representation of bacterial diversity up to the genus. Briefly, the OTU table was constructed using the UPARSE pipeline (Edgar, 2013). Reads were truncated at 300 bp and quality filtered using a maximum expected error of 0.5 (meaning that on average one nucleotide in every two sequences is incorrect). Filtered reads were dereplicated and unique sequences (singletons) were removed. These sequences were grouped into OTUs with a similarity cutoff of 99%. After clustering, sequences were aligned and classified using the SILVA reference database (version SSU_Ref_132) using Qiime (Caporaso *et al*., 2010). Microbiome analyzes were conducted by meta-analysis approaches using the Microbiome Analyst platform based on QIIME and R replications, from marker gene abundance (MDP) data (Chong et al. 2020). The α diversity, demonstrated for the treatments T1-Glyphosate, T2- Saflufenacil, T3- Pyroxasulfone and T4- Control, was calculated by the number of OTUs to represent the richness of the community.

The β-diversity was used to verify clusters and variances of communities in all the treatments by using principal coordinate analysis (PCoA), while the dissimilarity of the systems was measured by the Bray–Curtis method. The statistical significance of the samples was evaluated by PERMANOVA ($P<0.05$). Differences in the abundance of genera were evaluated by performing a Gaussian ft test ($P<0.05$, $FDR<0.05$). All steps were applied to 16S to verify bacterial abundance and diversity.

2.6. Data analysis

The in-vitro assays used a completely randomized design. All data were previously subjected to normality test (Shapiro-Wilk test) and then the F test was applied through analysis of variance (ANOVA) in the program Sigma Plot. The five times of soil samples were then distinguished through Tukey test ($P < 0.05$). The graphics were plotted using the software Past version 4.17

3. Results

3.1. Enumeration of soil microbial population groups

Effects of herbicides on culturable fungi community are shown in Figure 1. Differences among the fungal populations (Figure 1) in soil samples treated with all herbicides were not significant in the three first times of soil sampling (0 days, 1 day and 5 days), but Flumioxazin has increased considerably the number of fungal populations as compared to the control, in 15 days after the application. This effect of Flumioxazin didn't remain 35 days after application because there was no difference between the treatments (Figure 3).

The treatments didn't influence in community of total bacteria 0h after the herbicides application when compared with the control (Figure 3). One day after the herbicide's application only Pyroxasulfone and Clethodim were capable to reduce the total bacteria population (Figure 3) and had significant difference to the control. These results were amplified with the extend period of time of evaluation and some herbicides have remained reducing the total bacteria. Total bacteria numbers then decreased, with a significant reduction until the last time of soil collection. Clethodim have decreased the total bacteria only until 5 days as compared with the control. On the other hand, Flumioxazin, Saflufenacil and Glyphosate declined the total bacteria only 5 days after application (Figure 3) and the declining of Flumioxazin was the lower (Table 2). Saflufenacil was the only active ingredient that remained decreasing the total bacteria community even 15 days after its application. But intriguingly, Saflufenacil increased the total bacteria within 35 days of application, as compared to the control (Figure 3).

The effect of the five active ingredients on the endosporogenic bacteria is presented in Figure 3 and Table 3. There was no stimulation of herbicides on the endosporogenic bacteria 0 days after their application as compared to the control (Table 3). Clethodim, Pyroxasulfone, Saflufenacil and Glyphosate started to inhibit the endosporogenic bacteria in 1 day after application and these treatments remained the reduction within 5 days. The effect of

Flumioxazin only appeared 5 days after application and 15 days only this herbicide showed capacity of reduction as compared to the control (Figure 3). Subsequently, within 35 days the values of Glyphosate and Saflufenacil were the most diminished as compared to the control and the population tended to return to values very close to the time of 0 days.

3.2. Diversity and structure of the bacterial communities

Alpha diversity analysis revealed that microbiomes are variable between soils exposed to different herbicides. The result showed a gradient from the Glyphosate treatment (T1) to the other herbicide treatments Saflufenacil (T2), Pyroxasulfone (T3) and Control (T4). The median diversity was lower in the control treatment and higher in soils exposed to herbicides for the measurement of observed OTU diversity (Figure 4). The bacterial richness represented by the observed OTUs was higher in the soil exposed to Glyphosate (T1), while in the Saflufenacil (T2) and Pyroxasulfone (T3) treatments, the bacterial richness followed similar trend and had a higher diversity as compared to the control (Figure 4).

Regarding the β-diversity analyses, revealed a significant effect of the applied herbicide for bacterial communities' composition. In general, the active ingredients of the herbicides applied in the soil explained 59,9% of the total variability in the community composition of bacteria (PERMANOVA, $P<0.001$) (Figure 5). The differences in bacterial community structure were driven by the active ingredients of the herbicides (Figure 5). The bacterial community was clearly separated according to the four treatments, Glyphosate and Pyroxasulfone formed a distinct cluster from others (Figure 5).

A total of 30 phyla of Bacteria were identifed in all the samples, where Proteobacteria, Bacteriodetes, Actinobacteria, Acidobacteria and Gemmatimonadetes accounted for more than 75% of the relative abundance (Figure 7). The biocontrol phyla actinobacteria had a lower relative abundance in the treatment control (Figure 7).

Another biocontrol taxa, the genera *Bacillus*, was found in all the treatments but the abundance of it was higher in treatment with Saflufenacil (T2) Pyroxasulfone (T3) (Figure 6). Sedminibacterium, Pedobacter,Thermomonas, Chitinophaga were minority in all the the treatments with herbicides as compared with the control. On the other hand, some genera were favored by the application of herbicides, for instance Curpriavidus predominated in the treatment with Saflufenacil (Figure 6). The results were inconclusive to bacteria's genera because 49% of the them were not identified in the sequencing (Figure 7).

4. Discussion

There isn't a pattern of the herbicides' effect on microorganisms and how the chemical nature of the active ingredient influences its persistence in soil (Meena *et al*., 2020; Balasubramanian & Sankaran, 2001). In the present work, only Flumioxazin has increased the number of fungal populations at 15 days after application, indicating its stimulatory effect on fungal community (Figure 1). Probably, the increase in the fungal community abundance indicates its capacity of degrading and utilizing this herbicide as a source of carbon (C) and nitrogen (N) and that this herbicide does not have a toxic effect as it was reported by Pertile *et al*., 2020.

When it comes to Total bacteria, Clethodim and Pyroxasulfone were the active ingredients that primarily affected them negatively, only 1 day after exposure (Figure 3). Some active herbicide substances shortly after their application, are physically and biochemically modified into different secondary metabolites that can affect the soil microbiome negatively and may increase phytotoxic effects in plants (Meena *et al*., 2020).

The Pyroxasulfone had also a quickly effect on Endosporogenic Bacteria (EB) reduction (1 day after application) (Figure 5). However, the persistence of reduction has endured much more to the EB, until 5 days after application, this means that the EB is more sensitive to the active ingredient Pyroxasulfone than TB. Previous literature also indicated that Pyroxasulfone had a small effect on bacterial community and microbial functions (Wang *et al*.,2023).

Saflufenacil was the active ingredient with the highest time of persistence in the soil, considering that it reduced the total bacteria from 5 to 15 days after application (Figure 4). However, when the microbial population activities are recovered after a transient time of inhabitation; this probably happens due to the microbes' adaptation to these active ingredients or because of their degradation (Singh *et al*.,2020).This fact was observed in this work, considering that at a certain time of degradation (35 Days) Saflufenacil is the only product that the total bacteria community was able to mineralize, because we observed a significant increase on total bacterial community as compared to the control (Table 2).

On the other hand, the reduction on endosporogenic bacteria (Table 3) might has happened for the complicated metabolization of Saflufenacil by these microbes or its non-utilization as a source of nutrients (Castro Junior *et al*., 2006). All the herbicides have decreased the endosporogenic bacteria at 5 days after inoculation (Table 3).

In all the times of evaluation the endosporogenic bacteria community was decreased by the products, it may mean that the none of the herbicides were transformed and used as a source of nutrients by these bacteria (Table 3). In addition, the reduction in the abundance of microbes after herbicide application involve modifications in the enzymatic activity of microorganism, cellular membranes and composition of cell wall (Wolejko *et al*., 2022).

In this work, we applied herbicides in a soil from an Agroforest without a historical of any chemical pesticides and consolidated the hypothesis of the herbicides as a driver of the whole soil microbiome and suppressiveness against *Meloidogyne paranaensis*. By evaluating the microbiome, we noticed the relevancy of the chemical herbicides in the modulation of taxon, as previously reported in other systems (Gandhi *et al*.,2021; Torres *et al*.,2018; Caggìa *et al*., 2023; Ali *et al*.,2024).

Among the community of bacteria, the phyla Proteobacteria, Bacteriodetes, Actinobacteria, Acidobacteria and Gemmatimonadetes were the most abundant both treatments with herbicides and control (Figure 6) and are usually abundant in *Meloidogyne* spp. suppressive soils (Harkes *et al*. 2020; Zhou *et al.* 2019; Yergaliyev *et al*., 2020).

Numerous studies have showed no or transitory impacts of Glyphosate on microbial biomass, enzyme activity, respiration and biogeochemical process (Bünemann *et al*., 2006; Johnsen *et al*., 2001). However, as it was observed in this work (Figure 7 and 8) the changes in the microbial community composition and in the key functions mediated by these microbial populations are common impacts of Glyphosate (Imfeld & Vuilleumier, 2012; Mijangos *et al*.,2009; Widenfalk *et al*., 2008). The increase in the bacterial richness with the herbicides (Figure 7) might suggest an increase in root exudation as a plant response to the pesticide stressor, which in turn increases microbial biomass in the rhizosphere (Chen *et al*., 2017; Qian *et al*., 2011; Esperschütz *et al*., 2009).

Pyraxosulfone that has increased the bacterial diversity (Figure 7 and 8) is capable to impact the soil organic matter concentration (Gao *et al*., 2019; Wang *et al*., 2023) that is one of the most important physicochemical factors influencing the bacterial community (Bhattacharyya *et al*., 2022). Alterations to soil microbial community composition potentially have pronounced effects on soil quality as well as impact plant health (Bending *et al*., 2007; Lynch *et al*., 2004).

In this study Saflufenacil also increased, the microbial alpha diversity (Figure 4), which may be attributed to some soil microorganisms adapting to the pressure through Saflufenacil

residues. When xenobiotic compounds, are added into the system, various microbial compositions can suffer adaptive changes (Du *et al*., 2018). The bacteria community might use Saflufenacil as carbon and nitrogen sources, and even a competition for carbon sources may also be responsible for the community changed (Silva *et al*., 2023), which has been observed in other herbicide studies (Du *et al*., 2021; Ju *et al*., 2017; Pourbabaee *et al*., 2018).

Saflufenacil is an inhibiting protoporphyrinogen oxidase herbicide, that catalyzes the formation of protoporphyrin IX, the last precursor of chlorophyll in its biosynthesis (Grossmann *et al*., 2019). Interestingly, some Phyllum of bacteria have isofunctional Protox enzymes such as Firmicutes, Proteobacteria and Cyanobacteria (Thiour-Mauprivez *et al*., 2019). Intriguingly, none of the taxa cited above have reduced with the application of Saflufenacil (Figure 9) what might suggest a natural tolerance to Saflufenacil.

The abundance of Proteobacteria increased considerably in the treatments with herbicides as compared to the control (Figure 9), in general Proteobacteria in soils has been related to an increase in microbial population in contaminated soils (Spain & Elshahed, 2009). In addition, proteobacteria is a signature of disturbance, and grow fast in perturbed environments (Singleton *et al*., 2006). The isolates of biological control agents attached to the surface coat of hatched J2 using them as carries to the plant roots (Topalović *et al*.,2023).

In the present work, taxa considered important to the soil suppressiveness of plant parasitic nematodes were more abundant in the treatments with herbicides, such as Bacillus (Figure 10) and Actinobacteria (Figure 9). These results might suggest that the constant selection of microbial taxa community happens in the rhizosphere and it's mediated by own pathogen presence in the soil (Siddiqui & Mahmood,1999; Topalović *et al*., 2019; Topalović *et al*., 2020). Considering that *Bacillus* is reported to stimulate the plant growth and induce resistance in the plants (Park *et al*., 2015; Raza *et al*., 2016; Tahir *et al*., 2017) the higher number of eggs and galls in the treatments with Saflufenacil and Pyroxasulfone may be related with the evaluability of roots to feed the pathogen since *Bacillus* was more abundant in these treatments (Figure 9).

Conclusions

This work allows us to conclude: Only Flumioxazin had an impact in the fungi community isolated by serial dilution. None of the active ingredients were capable to change neither bacterial nor fungal community 0 days after application. There was an increase in the

bacterial richness with the herbicides that might suggest an increase in root exudation as a plant response to the pesticide stressor. In the present work, taxa considered important to the soil suppressiveness of plant parasitic nematodes were more abundant in the treatments with herbicides, such as Bacillus and Actinobacteria.

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Legends and figures

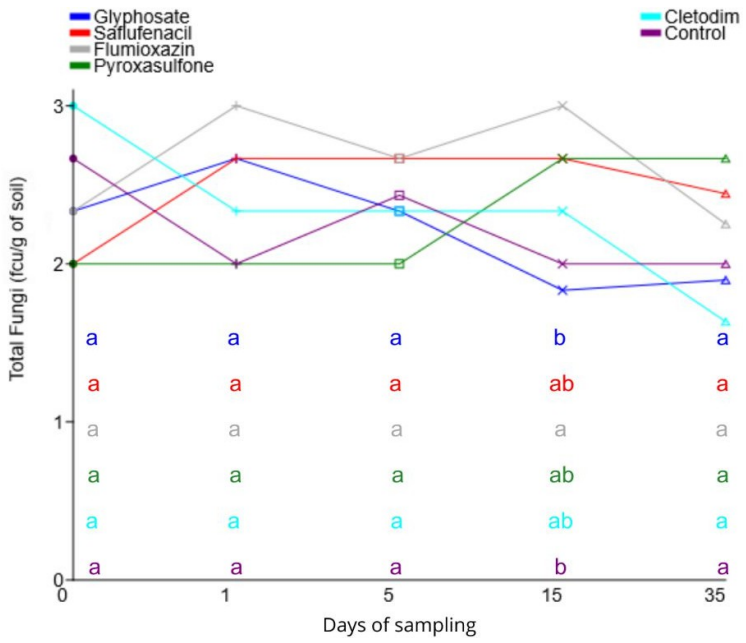

Figure 1. Effect of herbicides on total fungi from a suppressive soil microbiome exposed during 35 days to the Glyphosate, Saflufenacil, Flumioxazin, Pyroxasulfone, Clethodim and non-exposed (control). Means followed by the same letters do not differ significantly from each other by the Tukey test ($P < 0.05$). There was difference between the treatments $P = <0{,}001$. Values are means of five replicates.

Table 1. Total fungi

Treatments	0 h	24 h	5 dias	15 dias	35 dias
Glyphosate	2,33 [a]	2,67 [a]	2,33 [a]	1,83 [b]	1,90 [a]
Saflufenacil	2,00 [a]	2,67 [a]	2,67 [a]	2,67 [ab]	2,44 [a]
Flumioxazin	2,33 [a]	3,00 [a]	2,67 [a]	3,00 [a]	2,25 [a]
Pyroxasulfone	2,00 [a]	2,00 [a]	2,00 [a]	2,67 [ab]	2,67 [a]
Clethodim	3,00 [a]	2,33 [a]	2,33 [a]	2,33 [ab]	1,63 [a]
Control	2,67 [a]	2,00 [a]	2,43 [a]	2,00 [b]	2,00 [a]

Figure 2. Means followed by equal lowercase letters in the columns do not difer from each other by the Tukey test ($p \leq 0.05$)

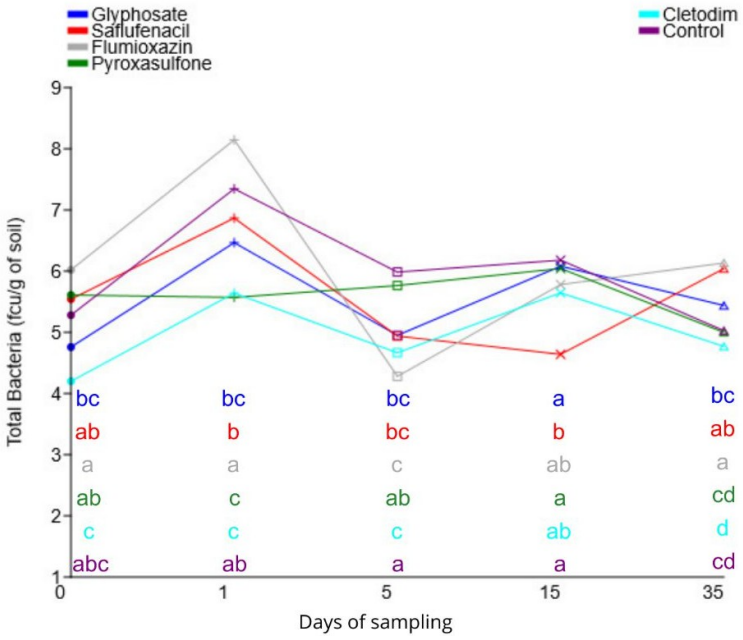

Figure 3. Effect of herbicides on total bacteria from a suppressive soil microbiome exposed during 35 days to the Glyphosate, Saflufenacil, Flumioxazin, Pyroxasulfone, Clethodim and non-exposed (control). Means followed by the same letters do not differ significantly from each other by the Tukey test ($P < 0.05$). There was difference between the treatments P = <0,001. Values are means of five replicates

Tabela 2. Total bacteria

Treatments	0 h	24 h	5 dias	15 dias	35 dias
Glyphosate	4,76 ^{bc}	6,47 ^{bc}	4,95 ^{bc}	6,08 ^{a}	5,44 ^{bc}
Saflufenacil	5,54 ^{ab}	6,87 ^{b}	4,94 ^{bc}	4,64 ^{b}	6,04 ^{ab}
Flumioxazin	6,02 a	8,15 ^{a}	4,28 ^{c}	5,78 ^{ab}	6,13 ^{a}
Pyroxasulfone	5,61 ^{ab}	5,57 ^{c}	5,77 ^{ab}	6,04 ^{a}	5,00 ^{cd}
Clethodim	4,20 c	5,64 ^{c}	4,67 ^{c}	5,65 ^{ab}	4,77 ^{d}
Control	5,28 ^{abc}	7,35 ^{ab}	5,98 ^{a}	6,18 ^{a}	5,03 ^{cd}

Figure 4. Means followed by equal lowercase letters in the columns do not difer from each other by the Tukey test (p≤0.05).

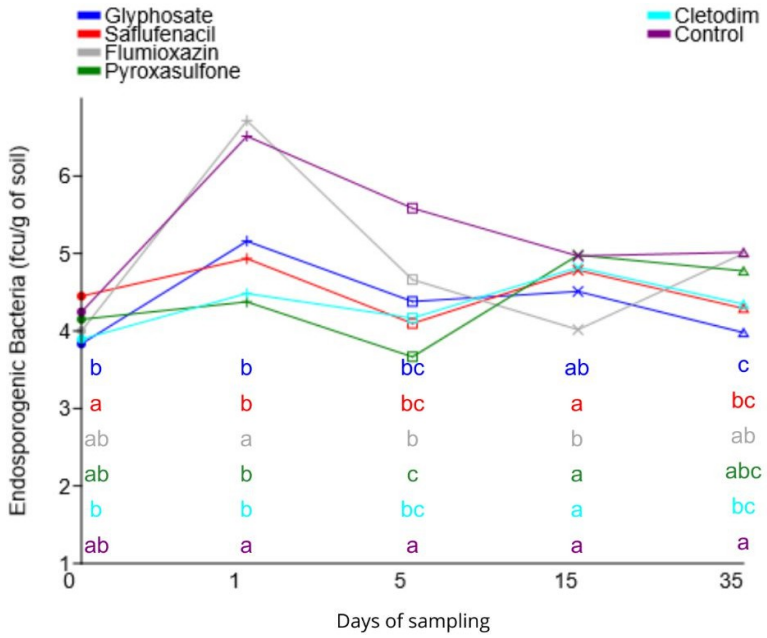

Figure 5. Effect of herbicides on endosporogenic bacteria from a suppressive soil microbiome exposed during 35 days to the Glyphosate, Saflufenacil, Flumioxazin, Pyroxasulfone, Clethodim and non-exposed (control). Means followed by the same letters do not differ significantly from each other by the Tukey test (P < 0.05). There was difference between the treatments P = <0,001. Values are means of five replicates.

Tabela 3. endosporogenic bacteria

Treatments	0 h	24 h	5 dias	15 dias	35 dias
Glyphosate	3,83 [b]	5,15 [b]	4,38 [bc]	4,51 [ab]	3,97 [c]
Saflufenacil	4,44 [a]	4,93 [b]	4,09 [bc]	4,78 [a]	4,29 [bc]
Flumioxazin	4,00 [ab]	6,71 [a]	4,66 [b]	4,01 [b]	4,99 [ab]
Pyroxasulfone	4,15 [ab]	4,37 [b]	3,66 [c]	4,97 [a]	4,77 [abc]
Clethodim	3,89 [b]	4,40 [b]	4,16 [bc]	4,82 [a]	4,34 [bc]
Control	4,25 [ab]	6,51 [a]	5,58 [a]	4,97 [a]	5,0 2[a]

Figure 6. Means followed by equal lowercase letters in the columns do not difer from each other by the Tukey test ($p \leq 0.05$)

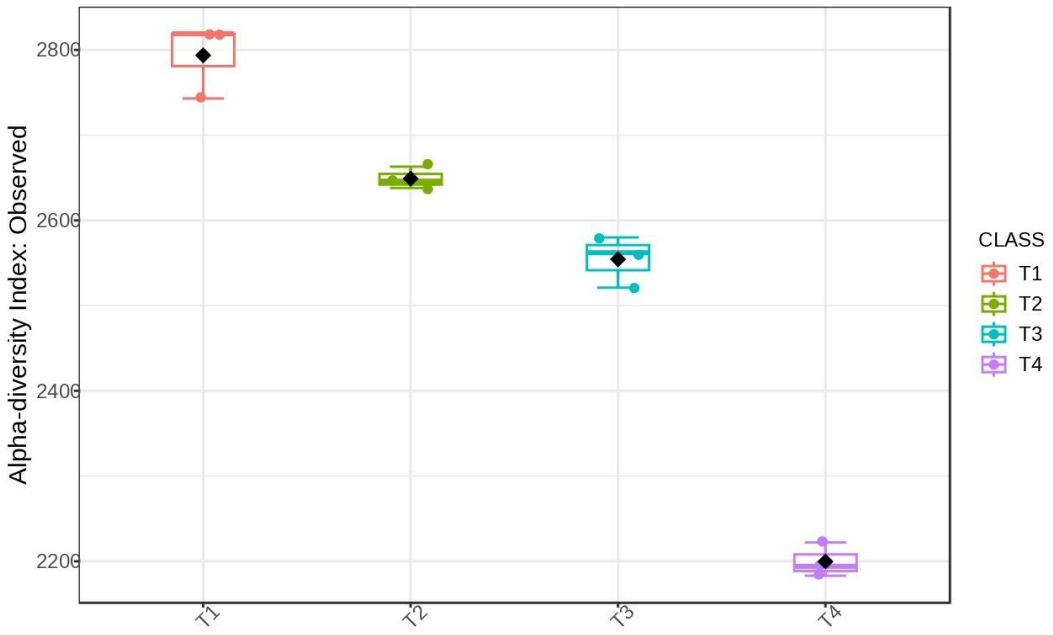

Figure 7. Alpha (α) diversity analysis of 16S sequences of the bacterial community from the soil treated with four herbicides active ingredients with tomato plants inoculated with *Meloidogyne paranaensis*. T1- Gliphosate; T2: Saflufenacil; T3: Pyroxasulfone and T4: Control. Alpha diversity measures observed OTUs, revealed significant differences in richness among four treatments. The Wilcoxon rank-sum test with Bonferroni correction was used. ****$p < 0.0001$

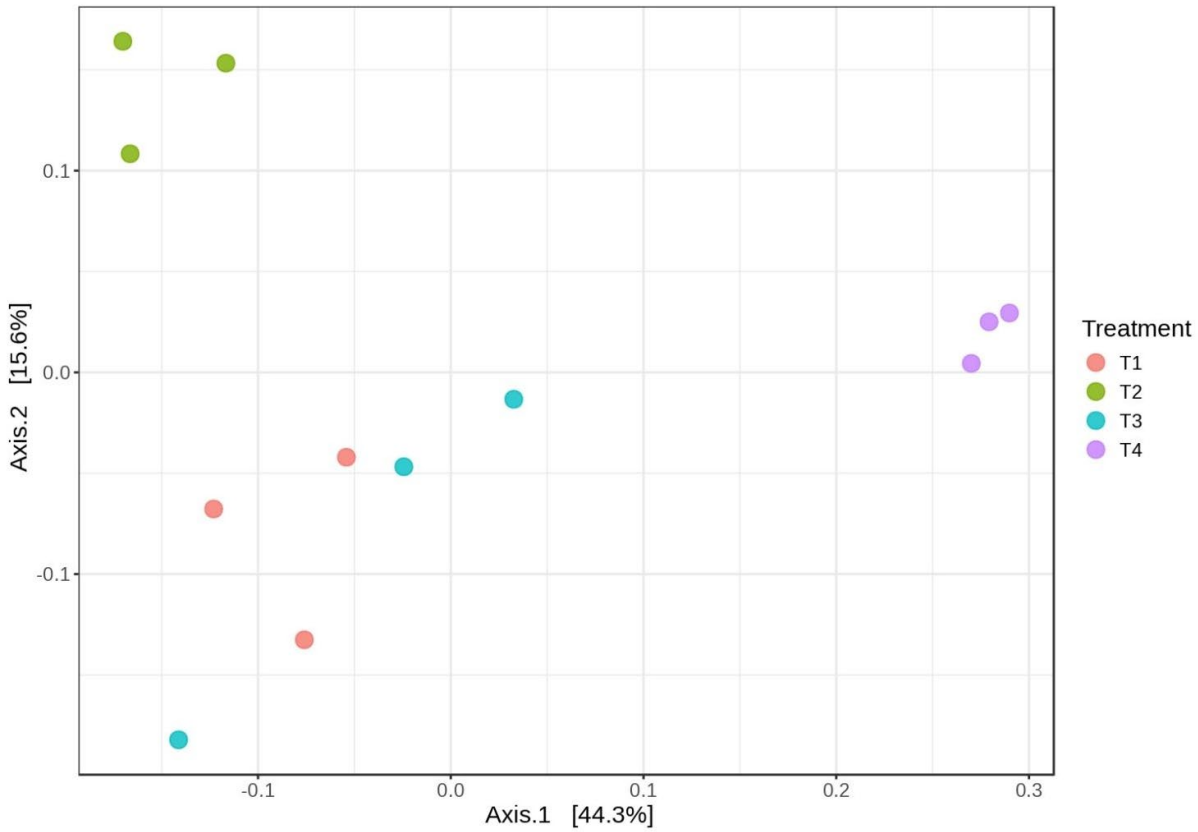

Figure 8- βeta (β) diversity analysis of 16S sequences of the bacterial community from the soil treated with four herbicides active ingredients with tomato plants inoculated with *Meloidogyne paranaensis*. T1- Gliphosate; T2: Saflufenacil; T3: Pyroxasulfone and T4: Control.Distances of PCoA were measured by Bray–Curtis method. Three samples each treatment was used.

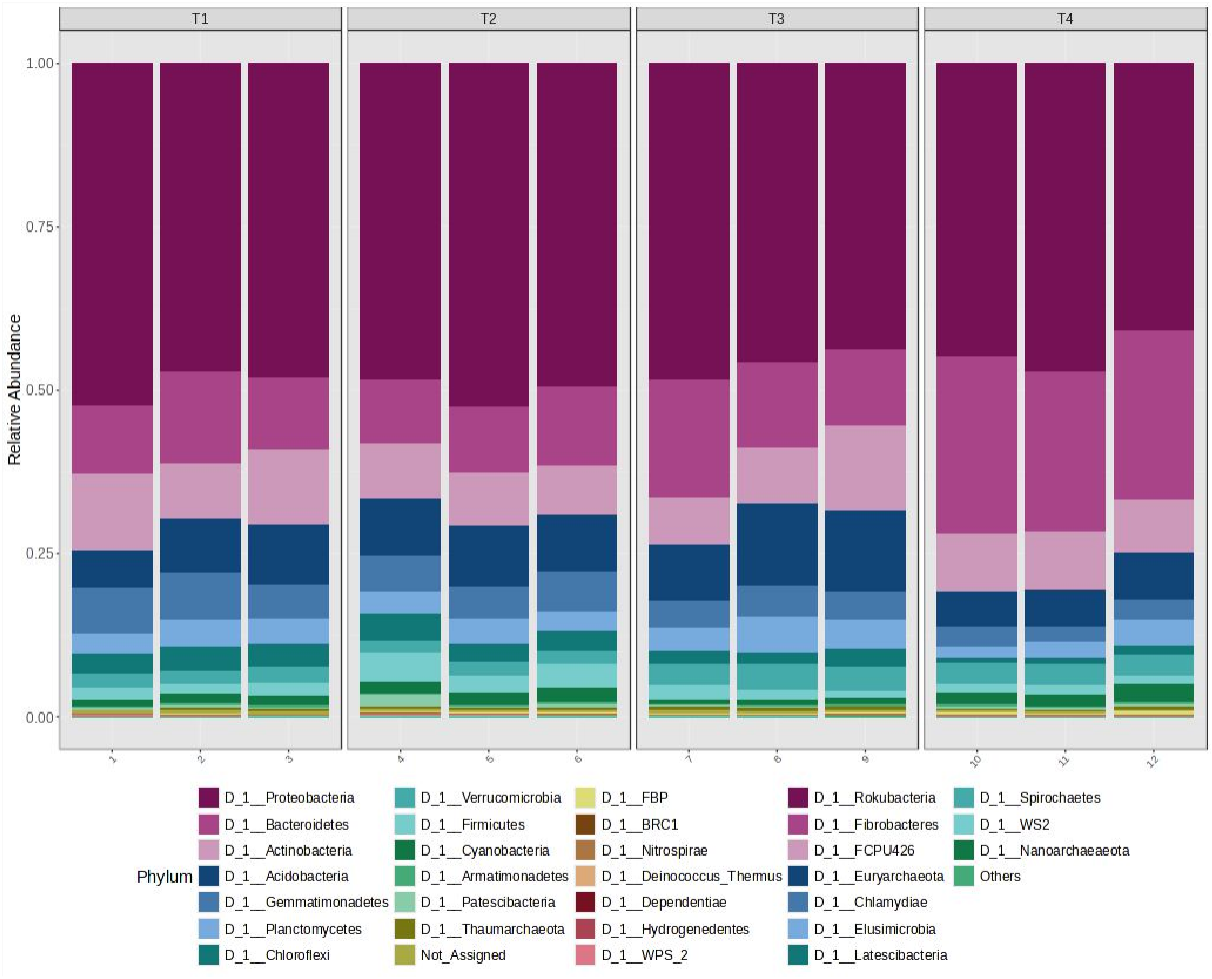

Figure 9. Distribution of bacterial communities in soil microbiome exposed to herbicides T1-Gliphosate; T2: Saflufenacil; T3: Pyroxasulfone and T4: Water Control. The figure depicts the relative abundance of bacterial communities at the Phylum level. Bacterial community composition is shown in bar charts (only significant taxa greater than 1% are shown).

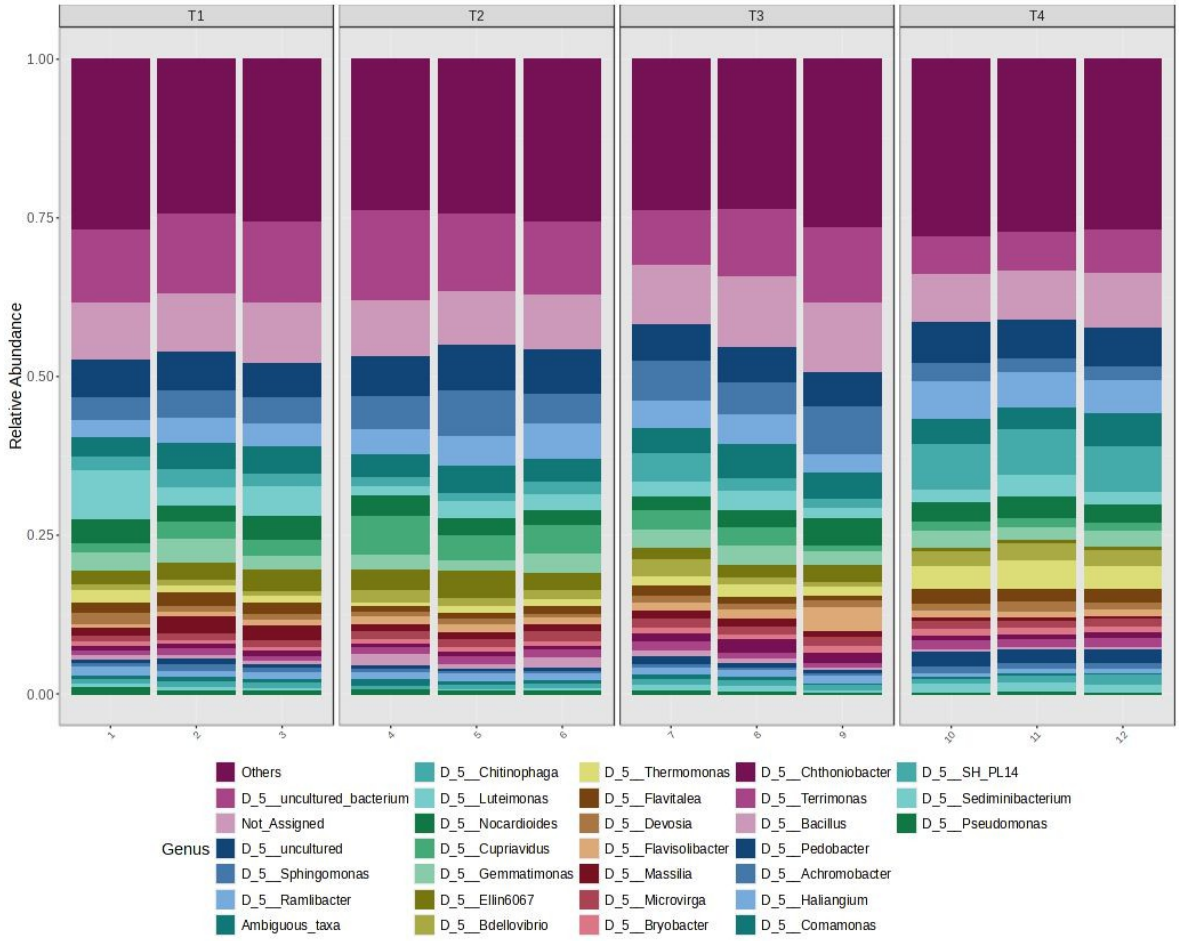

Figure 10. Distribution of bacterial communities in soil microbiome exposed to herbicides T1-Gliphosate; T2: Saflufenacil; T3: Pyroxasulfone and T4: Water Control. The figure depicts the relative abundance of bacterial communities at the Genus level. Bacterial community composition is shown in bar charts (only significant taxa greater than 1% are shown).

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