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Interaction between soil microbiome and nematode activity in soybean crop

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Thesis presented to obtain the degree of Doctor in Science. Area: Soil and Plant Nutrition

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SUMMARY

RESUMO

Interação entre o microbioma do solo e a atividade de nematoides na cultura da soja

A comunidade de nematoides é um importante componente da biodiversidade do solo, a qual possui uma variedade de funções ecológicas, que vão da ciclagem de nutrientes ao parasitismo das plantas. Embora muito esforço já tenha sido feito para descrever as comunidades de nematoides parasitos de plantas associadas à cultura da soja, pouco se sabe sobre as interações entre os nematoides e o microbioma do solo. Neste estudo, dois experimentos em condições controladas e análises de 178 amostras de solo coletadas nas principais áreas de produção de soja do Brasil, cobrindo quatro diferentes biomas (Amazônia, Mata Atlântica, Cerrado e Pampa), foram utilizadas para investigar as relações entre as comunidades de bactérias do solo e as comunidades de nematoides (parasitos de plantas e de vida livre) na cultura da soja. As comunidades de bactérias e nematoides totais dos solos foram analisadas pelo sequenciamento dos genes 16S rRNA e 18S rRNA, respectivamente; enquanto as comunidades de nematoides parasitos de plantas foram analisadas por microscopia óptica. Além disso, a influência das propriedades físicas e químicas dos solos sobre a comunidade de nematoides parasitos de plantas e sobre a atividade microbiana do solo foi investigada por meio da análise de 216 amostras de solo coletadas a partir de campos de soja em sistema de plantio direto. Os resultados experimentais demonstraram o efeito supressivo da diversidade de bactérias contra o parasitismo de *Meloidogyne javanica* em plantas de soja e apontaram grupos de bactérias potencialmente antagônicas a esse nematoide, tais como *Pseudomonas* spp. e *Microbacterium* spp. Os resultados amostrais mostraram correlações negativas significativas entre a densidade populacional de *Pratylenchus* sp. e a abundância relativa de vários gêneros de bactérias que ocorrem naturalmente nos solos cultivados com soja, como *Streptomyces* e *Paenibacillus*, os quais são conhecidos antagonistas de nematoides parasitos de plantas. Os resultados da pesquisa também demonstraram que a comunidade de nematoides nos solos cultivados com soja no Brasil é predominantemente composta pelas ordens Rhabditida, Tylenchida, Dorylaimida, Triplonchida e Enoplida; enquanto a comunidade de nematoides parasitos de plantas é predominantemente composta pelos gêneros *Meloidogyne*, *Pratylenchus*, *Heterodera*, *Rotylenchulus*, *Helicotylenchus*, *Scutellonema* e *Xiphinema*. Ademais, foi observada correlação positiva entre a riqueza de nematoides totais (predominantemente composta por nematoides de vida livre) e a produtividade da soja, o que mostra a importância positiva da comunidade de nematoides de vida livre na manutenção e elevação da produtividade de grãos. Além disso, observou-se que o teor de argila e o pH são as principais propriedades físico-químicas que modulam a comunidade de nematoides parasitos de plantas em solos cultivados com soja em sistema de plantio direto, enquanto o teor de matéria orgânica e o pH são as principais propriedades que modulam a atividade microbiana nesses solos. Este estudo é o primeiro a fornecer informações sobre as interações entre a diversidade bacteriana do solo e a comunidade de nematoides na cultura da soja no Brasil, as quais podem ser úteis para a idealização de novas estratégias de manejo desses organismos e para o desenvolvimento de novos produtos biológicos à base de bactérias potencialmente antagonistas aos nematoides parasitos de plantas.

Palavras-chave: 16S rRNA, 18S rRNA, Supressividade do solo, Sequenciamento de nova geração, Enzimas do solo

ABSTRACT

Interaction between soil microbiome and nematode activity in soybean crop

The nematode community is an important component of soil biodiversity, which has a wide range of ecological functions, from nutrient cycling to plant parasitism. Although much effort has been made to describe the plant-parasitic nematode communities associated to soybean crop, little is known about the interactions between nematodes and soil microbiome. Here, two experiments under controlled conditions and analyses of 178 soil samples collected from the main soybean producing areas in Brazil, in four different biomes (Amazon Rainforest, Atlantic Rainforest, Cerrado, and Pampa), were used to assess the relationships between soil bacterial communities and (plant-parasitic and free-living) nematode communities in soybean crop. Soil bacterial and total nematode communities were analysed by sequencing of the 16S rRNA and 18S rRNA genes, respectively, while plant-parasitic nematode communities were analysed by light microscopy. Furthermore, we investigated the influence of soil physicochemical properties on the plant-parasitic nematode community and microbial activity by analysing 216 soil samples collected from no-till soybean crops. Our experimental results demonstrated the suppressive effect of soil bacterial diversity against *Meloidogyne javanica* in soybean plants and highlighted some groups of bacteria potentially antagonistic to this plant-parasitic nematode, such as *Pseudomonas* spp. and *Microbacterium* spp. Our sampling results showed significant negative correlations between population density of *Pratylenchus* sp. and relative abundance of several bacterial genera that naturally occur in soybean cultivated soils, such as *Streptomyces* and *Paenibacillus*, which are known antagonists of plant-parasitic nematodes. Our results also showed that the total nematode community in soils cultivated with soybean in Brazil is predominantly composed of the orders Rhabditida, Tylenchida, Dorylaimida, Triplonchida and Enoplida, while the plant-parasitic nematode community is predominantly composed of the genera *Meloidogyne*, *Pratylenchus*, *Heterodera*, *Rotylenchulus*, *Helicotylenchus*, *Scutellonema*, and *Xiphinema*. We also observed a positive correlation between the total nematode richness (predominantly composed by free-living nematodes) and soybean yields, which highlights the positive importance of the free-living nematode community in maintaining and increasing grain productivity. In addition, our results demonstrated that the clay content and the soil pH are the main soil physicochemical properties modulating the plant-parasitic nematodes community in soybean cultivated soils under the no-till system and the soil organic matter content and the pH are the main properties modulating the microbial activity in those soils. Our study is the first to provide insights into the interactions between soil bacterial diversity and nematode communities in soybean crop in Brazil, which may be useful for the design of new strategies to manage these organisms and for the development of new biological products based on bacteria potentially antagonistic to plant-parasitic nematodes.

Keywords: 16S rRNA, 18S rRNA, Soil suppressiveness, Next-generation sequencing, Soil enzymes

1. NEMATODE COMMUNITIES: FROM AGRICULTURAL BENEFITS TO PLANT PATHOGENS AND THEIR COMPLEX RELATIONSHIP WITH SOIL MICROORGANISMS

1.1. Importance of the nematode community for soil functioning and quality

Nematodes are microscopic organisms belonging to the phylum Nematoda, which measure between 0.3 to 3.0 mm in length and are ubiquitous in different habitats (*i.e.,* soil, plants, animals, insects, water, etc.). Currently, there are more than 23,000 described nematode species, with one million species yet to be discovered, and approximately 35% of them inhabit the soil (MOURA; FRANZENER, 2017; SEESAO *et al.*, 2017). Among the soil nematodes, about 25% are herbivores (also called plant-parasitic nematodes) and can cause damage to plants (VAN DEN HOOGEN *et al.*, 2019, 2020). Although most research has been conducted on plant-parasitic nematodes, most nematode species are free-living that perform beneficial functions for soil processes. Nematodes actively participate in the decomposition of soil organic matter and in the regulation of biogeochemical cycles, regulating the food web by controlling soil microorganism populations (NEILSON *et al.*, 2020), and have a role in vegetation dynamics (GEBREMIKAEL *et al.*, 2016; SONG *et al.*, 2017). Given their contribution to essential processes for ecosystem functioning and because they are sensitive to environmental variations, nematodes are considered excellent indicators of soil quality and can be used to assess the impact of management practices in agriculture (GUTIÉRREZ *et al.*, 2016; MACHADO *et al.*, 2022).

Ito *et al.* (2015) evaluated the effects of tillage systems on soil nematode community in soybean crop and found higher abundance of all trophic groups of nematodes in the no-till system when compared to conventional tillage. Soil tillage leads to deterioration of the soil physical properties (*i.e.,* density, porosity, mechanical resistance, etc.), decreasing diversity and abundance of free-living nematodes and promoting the occurrence of plant-parasitic nematodes (CARDOSO *et al.*, 2016; SONG *et al.*, 2017). Leslie *et al.* (2017) studied the effect of cover crops on soil fauna in soybean crops and recorded higher diversity and complexity of free-living nematode community in soils under crop rotation when compared to fallow. Vegetation homogeneity in agriculture is the main adversity to soil nematode abundance and diversity (FRANCO-NAVARRO; GODINEZ-VIDAL, 2017).

The nematode community is also influenced by soil characteristics. Soil attributes (*i.e.* pH, clay content, organic matter, and nutrient availability) are considered the main variables determining the nematode community in the soil (CHOWDHURY; YAN; FRISKOP, 2020). Freitas *et al.* (2017) determined the influence of soil properties on nematode population dynamics in soybean crops. They found that soils with low fertility show higher occurrence of plant-parasitic nematodes (FREITAS *et al.*, 2017).

1.2. Plant-parasitic nematodes and their impacts on soybean crops

Soybean (*Glycine max* (L.) Merrill) is a plant of the Fabaceae family, subfamily Papilionoidae, which originated from East Asia and has been cultivated as food for thousands of years in China and other countries of the Asian continent, comprising an important and traditional diet in that region (BERK, 1992). Soybean is one of the main commodities in the world, used as a protein source and in oil and biofuel production (OECD-FAO, 2016). Brazil is the world's major soybean producer. In the 2021/2022 growing season, the crop area reached 41,452 thousand hectares, yielding 125.55 million tons of grains with an average yield of 3,029 kg ha^{-1} (CONAB, 2022).

More than 100 species of plant-parasitic nematodes are reported to be associated to soybean. In Brazil, the main nematodes causing damages to the soybean crop are root-knot nematodes (*Meloidogyne* spp.), root-lesion nematodes (*Pratylenchus* spp.), soybean cyst nematodes (*Heterodera glycines*), and reniform nematodes (*Rotylenchulus reniformis*). In addition, other species (*i.e., Aphelenchoides* sp., *Tubixaba tuxaua, Scutelonema brachyurus*, and *Helicotylenchus dihystera*) have emerged as threats to soybean crops (LIMA *et al.*, 2017; MACHADO, 2014).

Root-knot nematodes are the most economically important group of plant-parasitic nematodes worldwide. There are more than 100 species of root-knot nematodes described, which are capable of parasitizing more than 3,000 plant species. The main symptom caused by root-knot nematode infection is the gall formation on the roots, although it does not occur in all parasitised plant species. Galls are structures formed by cells that quickly grow and divide in the neighbouring giant cells, which are hypertrophied parenchymal cells that occur at the feeding site of the nematode. The root damage reduces absorption of water and nutrients, resulting in stunted plants with low productivity (BERNARD; EGNIN; BONSI, 2017; XIANG; LAWRENCE; DONALD, 2018).

Root-lesion nematodes are migratory endoparasites capable of causing losses estimated up to 30% in soybean production, mainly in sandy soils and in regions with irregular rainfall. Root-lesion nematodes have become important for soybean because of the no-till farming system and crop rotation, which benefit the multiplication of this nematode due to its high degree of polyphagy (DEBIASI *et al.*, 2016). Root-lesion nematodes move intercellularly through the root cortex, destroying cells and thus facilitating infection by bacteria and fungi. Infected plants show root necrosis and discoloration, rickets, chlorosis, and wilt, resulting in productivity loss (LIMA *et al.*, 2015).

Soybean cyst nematodes (SNC) are considered among the most economically destructive pest of soybean crops in Brazil. SNC can cause yield losses that can exceed 30%. Infected plants show chlorosis and dwarfism, which can be confused with nutritional deficiency symptoms, herbicide toxicity, and drought stress. *H. glycines* is a sedentary endoparasite of soybean, characterized by the formation of protective structures (cysts) of high viability in the soil (6-8 years). The cysts are formed by the retention of eggs inside the body of mature female after its death (LIMA *et al.*, 2017).

Reniform nematodes are sedentary semi-endoparasites commonly found in subtropical and tropical regions (LOPEZ-NICORA *et al.*, 2018). Reniform nematodes are characterised by the attachment of females with their egg mass to plant roots and absence of typical swellings observed for root-knot nematodes (SIKORA, 2021). *R. reniformis* causes significant economic losses in soybean production, especially when it occurs collectively with *H. glycines* and *M. incognita* (XIANG *et al.*, 2018).

1.3. Microbial community and suppressiveness of root diseases caused by plant-parasitic nematodes

Soil suppressiveness to plant diseases is the condition when establishment and/or persistence of the pathogen is inhibited by the presence of antagonistic microorganisms. In addition, soil suppressiveness can minimize the damage caused by the disease even when the pathogen is successfully established (WELLER *et al.*, 2002). This soil property is attributed to the collective activity of its microbial community and can be divided into two types: general suppressiveness, which is related to the general competition, parasitism, and antibiosis mechanisms between

the microorganisms and the pathogen; and specific suppressiveness, which is related the activity of specific groups of microorganisms that interfere at some stage of the soil pathogen's life cycle (EXPÓSITO *et al*., 2017).

Several studies have evaluated the effects of soil microbial community on the occurrence of soilborne diseases caused by fungi (MENDES, 2011; SIEGEL-HERTZ *et al*., 2018; XIONG *et al*., 2017) and bacteria (SHE *et al*., 2017; WEI *et al*., 2015a). On the other hand, due to the scarcity of nematode taxonomists and the lack of interaction between nematologists and microbiologists, studies evaluating the effect of microbial community on the suppressiveness of root diseases caused by plant-parasitic nematodes are still little reported in the literature. Wei *et al*. (2019) found that the addition of non-infested soil microbiome to the rhizosphere of tomato plants reduced *Meloidogyne* spp. abundance, while the addition of rhizosphere microbiome from infected plants increased the disease occurrence. Elhady *et al*. (2018a) found that inoculation of maize rhizosphere microbiome into soil cropped with tomato decreased parasitism of *Pratylenchus penetrans* and *Meloidogyne incognita*. This result was associated to the high functional diversity of maize rhizosphere microbiome as well as the presence of nematode antagonistic and tomato beneficial bacteria. These beneficial bacteria may be considered plant growth-promoting bacteria, which can act in soil suppressiveness through the availability of nutrients and plant hormones (ARAÚJO *et al*., 2020).

Regarding the use of isolated microorganisms in the biological control of plant-parasitic nematodes, bacteria such as *Pasteuria penetrans* (BHUIYAN *et al*., 2018), *Pseudomonas fluorescens* (KHAN *et a*l., 2016), and *Bacillus* spp. (CHINHEYA; YOBO; LAING, 2017), and fungi (DIAS-ARIEIRA *et al*., 2018; KATH *et al*., 2017) have been used effectively to control *Meloidogyne* spp. and *Pratylenchus* spp. However, the effects of soil microbial community on suppression of these pathogens still need elucidation.

1.4. Microbial community in soils affected by plant-parasitic nematodes

Studies assessing the microbial community of soils affected by plant-parasitic nematodes are incipient. Hussain *et a*l. (2018) recorded lower bacterial diversity in soil infested with *H. glycines* and cultivated with soybean compared to uninfested soil. In addition, the nematode-inoculated soil presented higher abundance of Proteobacteria than the healthy soil. The bacterial community in the soil rhizosphere of soybean changes in response to infection by *H. glycines*, due to the selection of specific groups of bacteria in the soil (MWAHEB *et al*., 2017).

Liu *et al*. (2018) found higher abundance and diversity of bacteria and fungi (including known plant-parasitic nematode antagonists, such as *Trichoderma* and *Pseudomonas*) in soil grown with soybean free of *H. glycines*, when compared to soils with medium and high population densities of the nematode. In contrast, according to Hamid *et al*. (2017), soybean monoculture for several successive years selects soil microorganisms capable of suppressing *H. glycines*, leading to the enrichment and dominance of microorganisms antagonistic to the nematode (*i.e., Pseudomonas* spp., *Pochonia*, *Fusarium*, *Burkholderia*, and *Streptomyces*). It is important to mention that successive soybean monoculture can result in negative effects on the soil microbial community, with substantial losses of functions essential for the functioning of the ecosystem (PAN *et al*., 2021).

Adam *et al*. (2014) found more diverse fungal and bacterial communities in suppressive soil when compared to communities associated to juveniles (J2) of *Meloidogyne hapla*, indicating the association of nematode-specific microorganisms, which may be responsible for pathogen suppressiveness. According to Elhady *et al*. (2017), nematodes are colonised by specific bacteria and fungi selected from the soil microbiome, which have their

abundance increased on the nematode surface and can perform particular ecological functions, such as induction of plant defence.

In conclusion, the current state of the art of interactions between the soil microbial community and nematode activity (occurrence and parasitism) is summarised in Figure 1.

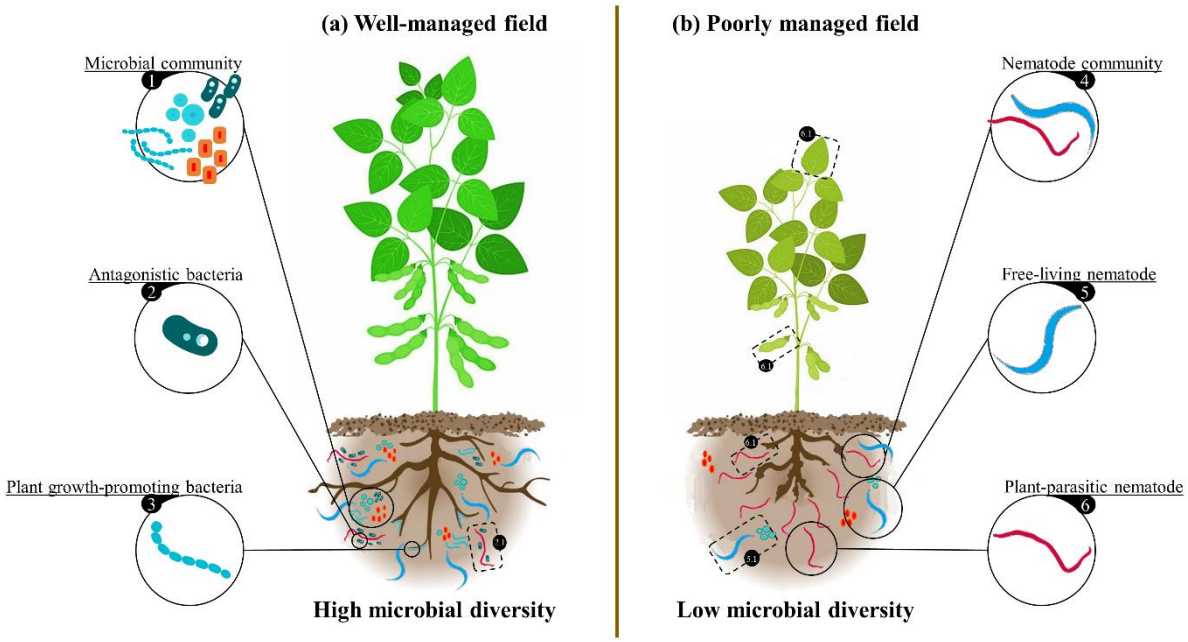

Figure 1. Graphical Abstract: Interaction between soil microbial community and nematode activity in soybean crop. (a) Well-managed field; (b) Poorly managed field. Details are listed from 1 to 6.

The soil microbial community is composed of diverse microorganisms that interact with nematodes and plants (Figure 1, detail 1). Antagonistic bacteria to plant-parasitic nematodes (Figure 1a, detail 2) and plant growth-promoting bacteria (Figure 1a, detail 3) occur naturally in soils with high diversity, suppressing plant-parasitic nematodes. Antagonist bacteria act directly on plant-parasitic nematodes by parasitizing juveniles and eggs, and through antibiosis (Figure 1a, detail 2.1). Plant growth-promoting bacteria provide nutrients and plant hormones that help mitigate the negative effects of infection by plant-parasitic nematodes (Figure 1a, detail 3). The soil nematode community is composed of free-living nematodes (Figure 1, detail 5) and plant-parasitic nematodes (Figure 1, detail 6). Free-living nematodes feed on bacteria (Figure 1b, detail 5.1), fungi, protozoa, and other nematodes, assisting in plant nutrition through nutrient mineralization. In addition, free-living nematodes compete for space with plant-parasitic nematodes. Plant-parasitic nematodes feed on plants, causing symptoms such as root deformation and necrosis, chlorosis, and reduced productivity (Figure 1b, detail 6.1). In well-managed fields (Figure 1a), with soils of high microbial diversity, the nematode community is predominantly composed of free-living nematodes. Whereas in poorly managed fields (Figure 1b), with soils with low microbial diversity, plant parasitic nematodes predominate in the nematode community. In this context, soil management practices adopted in well-managed fields, such as no-tillage farming, crop rotation, cover crops and organic matter addition, benefit the diversity of the soil microbial and faunal community (CHAVARRÍA et al., 2106; MOURA; FRANZENER, 2017; SROUR *et al.*, 2020), which can favour the suppression of plant-parasitic nematodes.

However, understanding the relationship between soil microbiome and the occurrence of plant-parasitic nematodes may be useful for the development of new strategies to control these pests, which may encourage the adoption of management practices that benefit the biological quality of the soil in soybean crops.

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2. INTERACTIONS BETWEEN SOIL BACTERIAL DIVERSITY AND PLANT-PARASITIC NEMATODES IN SOYBEAN PLANTS[1]

Abstract

Plant-parasitic nematodes are an important group of pests causing economic losses in agriculture worldwide. Among the plant-parasitic nematodes, the root-knot (*Meloidogyne* spp.) and root-lesion nematodes (*Pratylenchus* spp.) are considered the two most important ones affecting soybeans. In general, they damage soybean roots causing a reduction of about one third in productivity. The soil microbial community can exert a suppressive effect on the parasitism of plant-parasitic nematodes. Here, we investigated the effects of soil bacterial diversity on *Meloidogyne javanica* (*Meloidogyne*-assay) and *Pratylenchus brachyurus* (*Pratylenchus*-assay) suppression by manipulating microbial diversity using the dilution-to-extinction approach in two independent experiments in controlled conditions. Furthermore, we recorded the changes in the soil microbial community induced by the plant-parasitic nematodes infection. In *Meloidogyne*-assay, microbial diversity reduced the population density of *M. javanica* and improved plant performance. In *Pratylenchus*-assay, microbial diversity sustained the performance of soybean plants even at high levels of parasitism of *P. brachyurus*. Each nematode population affected the relative abundance of different bacterial genera and altered the core microbiome of key groups within the bacterial community. Our findings provide fundamental insights into the interactions between soil bacterial diversity and plant-parasitic nematodes in soybean plants.

Keywords: Dilution-to-extinction, 16S rRNA, Soil suppressiveness, *Meloidogyne javanica*, *Pratylenchus brachyurus*

2.1. Introduction

Nematodes are microscopic organisms belonging to the phylum Nematoda, which measure between 0.3 to 3.0 mm in length and are ubiquitous in different habitats (*i.e.,* soil, plants, animals, insects, water, etc.). Currently, there are more than 23,000 described nematode species, and approximately 35% of them inhabit the soil (MOURA; FRANZENER, 2017; SEESAO *et al.*, 2017). Among the soil nematodes, about 25% are herbivores (also called plant-parasitic nematodes) and can cause damage to plants (VAN DEN HOOGEN *et al.*, 2019, 2020). Although only a small portion of the soil-dwelling nematodes is plant-feeding, plant-parasitic nematodes are among the main pests causing economic losses in agriculture. According to Ha (2017), plant-parasitic nematodes cause about 10 to 14% of total losses in world agricultural production. In Brazil, losses reach approximately US$ 6.5 billion per year, and it is estimated that approximately US$ 3.0 billion per year are lost in the soybean crop (*Glycine max* (L.) Merrill) (MACHADO *et al.*, 2015).

More than one hundred species of plant-parasitic nematodes are reported to be associated with soybean. Among them, *Meloidogyne* spp. (root-knot nematodes) and *Pratylenchus* spp. (root-lesion nematodes) are considered as the two most important plant-parasitic nematodes affecting soybeans (JONES *et al.*, 2013). The root-knot nematodes are the most economically important group of plant-parasitic nematodes in the world. There are more than 100 species of root-knot nematodes described, which are capable of parasitizing more than 3,000 plant species. For the soybean crop, the *Meloidogyne javanica* is one of the most harmful species. The main symptom caused by root-knot nematode infection is the gall formation on the roots at the nematode's feeding site. The root damage causes a

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reduction in the absorption of water and nutrients, resulting in stunted plants with low productivity (BERNARD; EGNIN; BONSI, 2017; XIANG; LAWRENCE; DONALD, 2018). *Pratylenchus brachyurus* is a migratory endoparasite capable of causing losses estimated up to 30% in soybean production, mainly in sandy soils and in regions with irregular rainfall. Root-lesion nematodes move intercellularly through the root cortex, destroying cells and thus facilitating infection by bacteria and fungi. Infected plants show root necrosis and discoloration, rickets, chlorosis, and wilt, resulting in loss of productivity (LIMA *et al.*, 2015).

The use of chemical nematicides and crop rotation are the most adopted management practices in the control of plant-parasitic nematodes in soybean crops (LUAMBANO *et al.*, 2015). Due to their negative impact on the environment and human health, many traditional nematicides were banned from the market (DESAEGER; WRAM; ZASADA, 2021). This ban opened the door to the use of more environmentally-friendly control methods, such as integrated management practices (*i.e.*, use of cover crops, crop rotation, use of resistant cultivars) capable of promoting the suppressiveness of soilborne diseases (GINÉ *et al.*, 2016; SILVA; MEDEIROS; CAMPOS, 2018), and the use of microorganisms that act in the biological control of plant-parasitic nematodes (GAMALERO; GLICK, 2020).

Biological soil suppressiveness to plant diseases is defined as the condition in which the establishment and/or persistence of the pathogen is inhibited by the presence of disease antagonistic. Also, biological soil suppressiveness can minimize the damage caused by the disease even when the pathogen is successfully established (WELLER *et al.*, 2002). This soil property is attributed to the collective activity of its microbial community and can be divided into two types: general suppressiveness, which is related to the general competition, parasitism, and antibiosis mechanisms between the microorganisms and the pathogen; and specific suppressiveness, which is related the activity of specific groups of microorganisms that interfere at some stage of the soil pathogen's life cycle (EXPÓSITO *et al.*, 2017).

Studies indicated that the diversity of soil microbial communities can alter the ability of pathogens to colonize the soil (BENDER; WAGG; VAN DER HEIJDEN, 2016), playing a vital role in plant-parasitic nematode suppression (ELHADY *et al.*, 2018b). Although the soil microbial community can exert a suppressive effect on the incidence and parasitism of plant-parasitic nematodes, there is evidence that infection by plant-parasitic nematodes can also alter the composition of the soil microbial community (HUSSAIN *et al.*, 2018; NEILSON *et al.*, 2020; TOPALOVIĆ; VESTERGÅRD, 2021). However, the relationship between the soil microbiome (its composition, diversity, and function) and the parasitism of plant-parasitic nematodes is still poorly understood and needs to be better elucidated for the development of new environmental-friendly control strategies.

In this study, we hypothesized that soil bacterial diversity can act in the suppression of *M. javanica* and *P. brachyurus* in soybean plants. Also, we argue that the infestation by these nematodes can lead to changes in the soil microbial community structure and composition. Our objectives were (i) to evaluate the level of infestation by *M. javanica* and *P. brachyurus* in soybean plants grown in soil with distinct levels of bacterial diversity; (ii) to assess plant productivity on these soils; and (iii) to evaluate the effect of nematode inoculation on the diversity and composition of the bacterial community in a sterilized soil, which was naturally recolonized by microorganisms.

2.2. Materials and Methods

2.2.1. Soil description

Approximately one ton of soil was collected from the 0.00 to 0.20 m topsoil layer of an agricultural field located at the 'Luiz de Queiroz' College of Agriculture (ESALQ/USP), in the municipality of Piracicaba (22°43'S, 47°38'W, and 546 m a.s.l), in the state of Sao Paulo, Brazil. The soil in this area is classified as Ferralsol (Dystrophic Red Yellow) with a sandy loam texture (790 g kg^{-1} of sand, 35 g kg^{-1} of silt, and 175 g kg^{-1} of clay). Over the past years, this agricultural field has been cultivated with soybean (*Glycine max*). After sampling, the soil was separated into two parts: (i) for chemical characterization (Table S7) and (ii) for the mesocosm experiment.

2.2.2. Mesocosm preparation

Lime and fertilizers were applied to the soil in accordance with the soybean nutritional needs. A soil sample in its natural condition (not sterilized) was used to obtain the microbial inoculum. For this, about 500 g of soil the soil was 1:10 diluted in sterile water and passed through a 25-µm sieve to eliminate native nematodes. The remaining soil collected was subjected to three autoclaving for 60 minutes at 120 ºC, with the purpose of sterilization. After 15 days of drying under shade, approximately 1 kg of sterilized soil was placed in clean and disinfected pots (capacity of 1 dm^3). Then, the 1:10 sieved solution was serially diluted in sterile water up to the 10^{-6} factor, and the soil:water solutions obtained from the 10^{-1}, 10^{-3}, and 10^{-6} dilutions were used to inoculate the sterile soil (160 mL of solution per pot). This allowed us to create different levels of microbial diversity using the dilution-to-extinction approach (VAN ELSAS *et al.*, 2012). After inoculation of the diluted microbial communities, the mesocosms were incubated for 15 days to promote the establishment of the soil microbiome.

2.2.3. Experimental design and treatments

Two mesocosm experiments were carried out in a greenhouse in a completely randomized design. Each experiment consisted of five treatments, as follows: three dilutions of the microbial community (10^{-1}, 10^{-3}, and 10^{-6}), plus two control treatments - Infested Sterilized Soil (ISS) and Sterilized Soil (SS). ISS consisted of sterilized soil infested with nematodes. Ten biological replicates (10 different pots) were used per treatment, a total of 50 experimental units in each experiment. In each experiment, one species of plant-parasitic nematode (*Meloidogyne javanica* or *Pratylenchus brachyurus*) was introduced to artificially infest the soil. The nematode populations were acquired from the collections of the Nematology Laboratory of the ESALQ/USP.

In the first experiment, the soil in each pot was infested with 3,000 nematodes (juveniles and eggs) of the species *M. javanica* (*Meloidogyne*-assay), while in the second experiment was infested with 1,200 nematodes (juveniles and adults) of the species *P. brachyurus* per pot (*Pratylenchus*-assay). In both experiments, the soil was infested by nematodes after the establishment of the microbiome, and soybean was sown immediately after soil infestation. For this, five soybean seeds of the cultivar M6410 IPRO were sown in each pot. The seeds were previously inoculated with *Bradyrhizobium japonicum* (strain 5079) using a density of 5 x 10^9 CFU per gram of peat, and

0.1g of peat per kilogram of seed was applied. Furthermore, an autoclaved sugar solution (10% w:y) was used to increase the adherence of the turf to the seeds. All treatments were inoculated, thus the possible effects of *B. japonicum* action on nematode suppression and on the microbial community were standardized. Soybean seedlings were thinned 12 days after sowing, keeping only two plants per pot. Soil moisture was regularly adjusted with sterilized distilled water to maintain moisture at 80% of the maximum water-holding capacity in the soil. Soil sampling was performed using a probe 24 hours after sowing (start) and in the end (end) of both experiments, when the plants were in the beginning of maturity stage (R7). Approximately (i) 2.0 g of soil were collected per pot and frozen (-80 ^{o}C) for molecular analysis, and (ii) 100 g were kept refrigerated at 4 ^{o}C and used for nematode extraction.

2.2.4. DNA extraction procedures

Samples collected 24 hours after sowing (start) and at the end of both experiments (end) were used. Total DNA was extracted from 0.4 g of soil using the DNeasy PowerSoil Kit (QIAGEN Laboratories, Carlsbad, CA, USA), following the manufacturer's instructions. The integrity of soil DNA was verified by 1.2 % agarose gel electrophoresis at 80 V for 40 min, in 1.0x TAE buffer (Tris, Acetate, EDTA) stained with GelRed™ (Biotium, CA, USA). Also, we used NanoDrop® 1000 spectrophotometry (Thermo Scientific, Waltham, MA, EUA) to check the DNA quality and quantity. DNA samples that showed well-defined bands and a 260/230 nm ratio close to 1.8 were considered suitable.

2.2.5. Bacterial community abundance

The bacterial 16S rRNA gene was quantified at the start and end of both experiments. The number of gene copies was quantified using a StepOnePlusTM Real-Time PCR System with 48-well plates (Applied Biosystems, Foster City, CA, USA). The 16S rRNA gene was amplified in reactions of 20 µL final volume with 10 µl absolute qPCR SYBR *Green/ROX qPCR Master Mix* (*2*×) (Abgene, Epsom, UK), 0.5 µl of each primer (10 µM), 0.3 µL of bovine serum albumin (BSA, 10 mg mL^{-1}), and 1 µL of DNA (approx. 10 ng). The primers and reaction conditions are presented in Table S8.

The reactions were performed in duplicate. Two negative controls were added in all quantifications for contamination monitoring. Standard curves were obtained using serial dilutions (10^{-1} to 10^{-8}) of template DNA amplicon with known copy numbers. All amplification reactions showed efficiency values between 92% and 110%, and the R^2 values of the standard curves were always higher than 0.99. The results were analyzed using the StepOnePlusTM Real-Time software version 2.2.2 (Applied Biosystems, Foster City, CA, USA).

2.2.6. High-throughput sequencing analysis

We used the T-RFLP (Terminal restriction fragment length polymorphism) approach (Supplementary Material) to test if the treatments showed significant differences in the microbial community structure. Afterward, we

select four biological replicates from each treatment for taxonomic profiling of the bacterial community. For this, we sequenced the V3-V4 region of the bacterial 16S rRNA gene (341F: CCTAYGGGRBGCASCAG, and 806R: GGACTACNNGGGTATCTAAT). In total, 80 DNA libraries were prepared using the Miseq Reagent Kit v3 (Illumina, San Diego, CA, USA), following the manufacturer's instructions for the Illumina MiSeq platform (2 × 250 bp paired-end).

The 16S rRNA gene paired-end reads were firstly merged using PEAR (ZHANG *et al.*, 2014). Then, the merged sequences were analyzed using QIIME 2 version 2021.4 (BOLYEN *et al.*, 2019). Firstly, the sequences were demultiplexed and quality control was carried out using DADA2 (CALLAHAN *et al.*, 2016), using the consensus method to remove any remaining chimeric and low-quality sequences. The samples were then rarefied to 95,500 sequences, according to the sample with the lowest number of sequences to eliminate the effect of sampling effort, and singletons and doubletons were removed. The taxonomic affiliation was performed at 97% similarity using the Silva database version 132 (QUAST *et al.*, 2013), and the generated matrix was further used for statistical analyses.

2.2.7. Nematode extraction and quantification

The nematodes were extracted from soil samples as described by Jenkins (1964), while the nematode extraction from roots followed Coolen & D'Herde (1972) method. Briefly, 50 g of soil and 2 L of water were mixed inside a Becker. Then, the soil:water solution was sieved through 20 and 400 mesh. The retained material on the 400-mesh sieve was poured into 50 mL tubes and centrifuged at 1800 rpm for 5 minutes. After centrifugation, the supernatant was discarded, and sucrose-water solution (400 g L^{-1}) was added into the tubes. Then, the tubes were centrifuged at 1800 rpm for 1 min. The supernatant was sieved through a 500-mesh sieve, from which the retained material was washed out and stored in glass jars. The final volume of the water-nematode suspension was 10 mL. To extract nematodes from roots, 10 g of roots were washed, dried with paper towel, cut into 1 cm pieces, and then crushed in a blender for 60 seconds. The root:water solution was sieved through 60 and 500-mesh. The retained material on the 500-mesh sieve was poured into 50 mL tubes, where kaolin was added. The tubes were centrifuged at 1800 rpm for 5 minutes. The following steps were the same for Jenkins (1964) method, with the addition of sucrose-water solution (400 g L^{-1}), centrifugation, at 1800 rpm for 1 min, and sieving through a 500-mesh sieve. The population density of *M. javanica* and *P. brachyurus* was estimated by counting using Peters' slides under an optical microscope.

2.2.8. Plant measurements

The soybean shoots and grains were collected at the end of both experiments. Later, they were dried in an oven at 65 ºC for 72 hours to determine the soybean shoot and grain dry biomass. Furthermore, we determined the roots biomass by weighing it prior to the extraction of nematodes.

2.2.9. Statistical analysis

The data were checked for the presence of outliers. The normality and homogeneity of the data were assessed using the Shapiro-Wilk and Levene tests, respectively. When necessary, data were transformed into logarithm or square root. Afterward, data were subjected to analysis of variance, and treatment means were compared by Duncan's multiple range test at 5 % probability, using the SAS program (SAS Institute, Cary, NC).

The bacterial community structure at the start and end of both experiments was subjected to Principal Component Analysis (PCA) using Canoco 4.5. The differences between treatments were evaluated by similarity analysis (ANOSIM) using the Paleontological Statistics freeware package (PASTv.3 - Hammer *et al.* (2001)). The ANOSIM-R statistic was used to indicate how different the groups are ($R > 0.75$: well-separated groups; $R > 0.5$: groups with overlap but clearly differentiated; $R < 0.25$: not well separated groups) (CLARKE, 1993). PASTv.3 was also used to calculate the richness, diversity (Shannon – H'), and evenness (Pielou - J') indexes.

To compare the differential abundance of bacterial groups between treatments, the OTU table was used as input in the software STAMP (PARKS; BEIKO, 2010). P-values were calculated based on two-sided Welch's t-test and correction using Benjamini-Hochberg FDR. The bacterial genera that showed the greatest difference between the SS and ISS treatments, with a confidence level of 95%, were presented in an overlapping bubble chart. Spearman correlation analysis was carried out to investigate the relationship between the relative abundance of the 150 most abundant bacterial genera (70% of the total sequences) and nematode population density and plant growth measurements, across all treatments, in each assay, using the CORR Procedure of SAS. The 20 most abundant genera that showed at least a significant ≥ 0.35 or ≤ 0.35 correlation ($p < 0.05$) were represented in a Heatmap. In addition, network analyses were performed to assess the complexity of the interactions among microbial taxa in each treatment (Supplementary Material).

2.3. Results

2.3.1. Soil bacterial community abundance

The abundance of bacteria ranged from 1.9×10^{10} to 4.6×10^{10} copies of the 16S rRNA gene g^{-1} soil (Table S1). At the start of both experiments, the abundance of bacteria was significantly higher in the Infested Sterilized Soil (ISS), 10^{-6}, 10^{-3}, and 10^{-1} treatments when compared to the Sterilized Soil (SS) ($p < 0.01$). However, no significant differences were observed among ISS, 10^{-6}, 10^{-3}, and 10^{-1}. At the End of both experiments, we did not observe a significant effect of the treatments on the abundance of bacteria ($p > 0.05$).

2.3.2. Soil bacterial community structure and diversity

Principal component analysis (PCA) revealed that the bacterial community was clustered according to the dilution treatments, at the start and end of *Meloidogyne*-assay (Figure 1a and b). On the other hand, in *Pratylenchus*-assay, we observed that the bacterial community was better grouped according to the treatments at the start (Figure 1c and d). Significant differences in the bacterial community structure among treatments and between times (start

and end) were confirmed by similarity analysis (ANOSIM). Also, we observed that these differences were more evident at the start of both experiments (Table 1). We also observed that the bacterial community differed significantly between the beginning and ending of each assay (Figure S1).

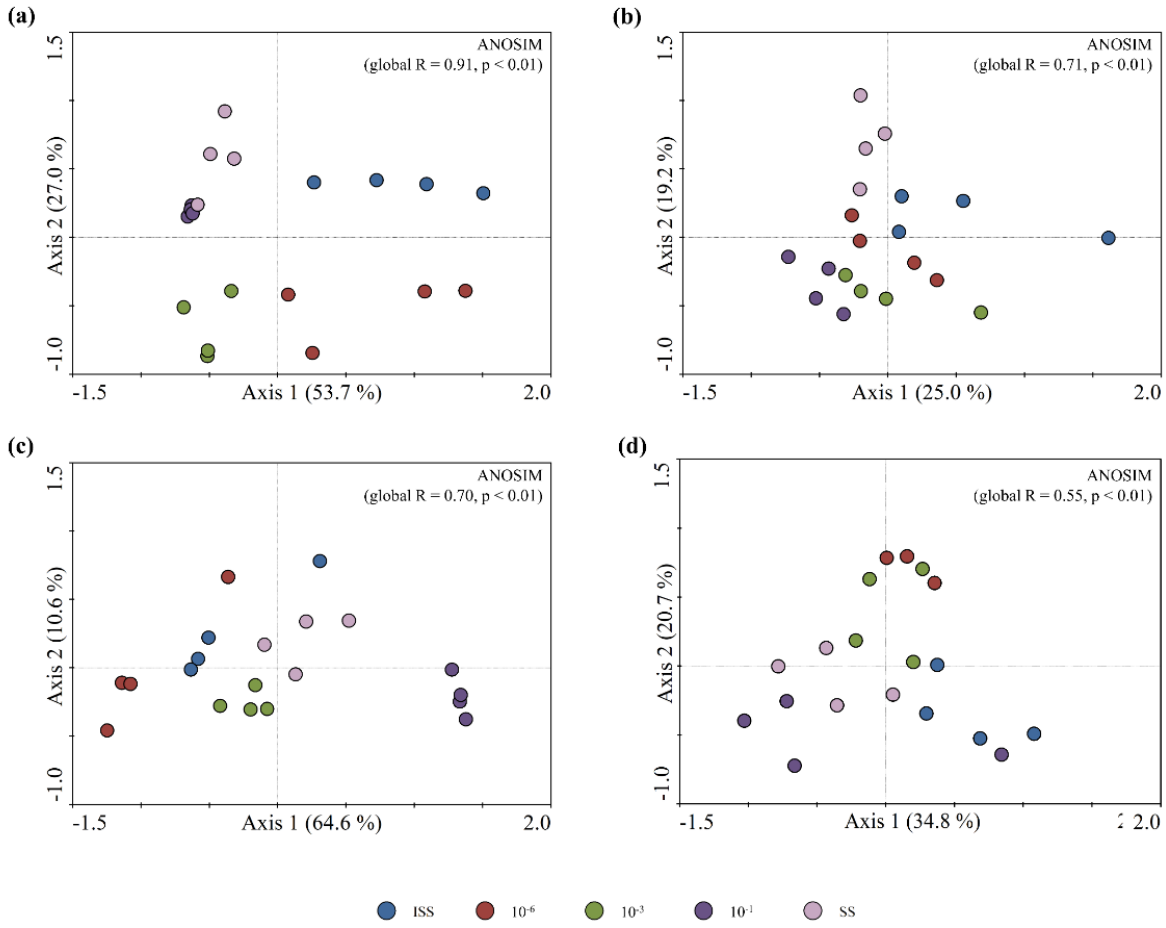

Figure 1. Principal component analysis (PCA) of the bacterial community in soil with different microbial diversity levels, inoculated with plant-parasitic nematodes. (a) start of Meloidogyne-assay, and (b) end of Meloidogyne-assay; (c) start of Pratylenchus-assay, and (d) end of Pratylenchus-assay. ISS: Infested Sterilized Soil. SS: Sterilized Soil. Global R > 0.75: well-separated groups; global R > 0.5: groups with overlap but clearly differentiated; global R < 0.25: not well-separated groups.

Table 1. R statistic values of the similarity analysis-pairing test of bacterial community structure in soil with different microbial diversity levels, at the start and end of Meloidogyne-assay and Pratylenchus-assay.

	Treatment (R value)			
	ISS	10^{-6}	10^{-3}	10^{-1}
Meloidogyne-assay				
Start				
10^{-6}	0.708*			
10^{-3}	1.000*	0.927*		
10^{-1}	1.000*	1.000*	0.875*	
SS	0.885*	0.947*	1.000*	0.958*
End				
ISS				
10^{-6}	0.270*			
10^{-3}	0.489*	0.625*		
10^{-1}	0.885*	0.937*	0.687*	
SS	0.666*	1.000*	0.979*	0.989*
***Pratylenchus*-assay**				
Start				
ISS				
10^{-6}	0.177ns			
10^{-3}	0.635*	0.760*		
10^{-1}	1.000*	1.000*	1.000*	
SS	0.250ns	0.541*	0.468*	1.000*
End				
ISS				
10^{-6}	0.364ns			
10^{-3}	0.781*	0.583*		
10^{-1}	0.614*	0.625*	0.385ns	
SS	0.645*	0.333*	0.760*	0,625*

R > 0.75: well-separated groups; R > 0.5: groups with overlap but clearly differentiated; R < 0.25: not well-separated groups. *Significant at the 0.05 probability level. nsNon-significant. ISS: Infested Sterilized Soil. SS: Sterilized Soil.

The bacterial community diversity indexes were significantly affected by the treatments. As expected, the treatments with a greater dilution of the microbial community (10^{-6}) had lower diversity (H') and evenness (J) indexes values when compared to the 10^{-1} ($p < 0.05$). Also, we observed that the 10^{-1} treatment presented higher diversity and evenness values in comparison to SS and ISS, at the start and end of both experiments (Table 2).

Table 2. Bacteria richness, diversity (H'), and evenness (J) in soil with different microbial diversity levels, at the start and end of the Meloidogyne-assay and Pratylenchus-assay.

	Start			End		
	Richness	Diversity (H')	Evenness (J)	Richness	Diversity (H')	Evenness (J)
***Meloidogyne*-assay**						
ISS	1441.75 ± 77.23 bc	5.30 ± 0.35 b	0.73 ± 0.04 b	1667.00 ± 205.97 b	5.76 ± 0.35 c	0.78 ± 0.04 c
10^{-6}	1288.50 ± 184.35 c	4.80 ± 0.12 c	0.67 ± 0.01 c	1861.25 ± 102.16 b	6.07 ± 0.14 bc	0.81 ± 0.01bc
10^{-3}	1603.50 ± 133.70 b	5.45 ± 0.28 b	0.74 ± 0.03 b	1874.25 ± 143.50 b	6.16 ± 0.14 b	0.82 ± 0.02 b
10^{-1}	2044.75 ± 206.23 a	6.25 ± 0.16 a	0.82 ± 0.01 a	2186.50 ± 124.36 a	6.57 ± 0.06 a	0.85 ± 0.01 a
SS	1551.50 ± 273.42 bc	5.62 ± 0.28 b	0.77 ± 0.02 b	1705.25 ± 227.82 b	6.01 ± 0.22 bc	0.81 ± 0.02 bc
***Pratylenchus*-assay**						
ISS	1282.25 ± 74.06 a	5.39 ± 0.15 bc	0.75 ± 0.02 b	1511.75 ± 174.08 b	5.66 ± 0.10 b	0.77 ± 0.01 b
10^{-6}	1277.50 ± 80.22 a	5.11 ± 0.04 c	0.71 ± 0.00 c	1353.75 ± 102.49 b	5.58 ± 0.12 b	0.77 ± 0.01 b
10^{-3}	1407.50 ± 109.14 a	5.57 ± 0.07 b	0.77 ± 0.01 b	1933.50 ± 307.83 a	6.20 ± 0.31 a	0.82 ± 0.02 a
10^{-1}	1414.75 ± 44.55 a	6.02 ± 0.05 a	0.83 ± 0.00 a	2016.00 ± 189.81 a	6.27 ± 0.42 a	0.82 ± 0.05 a
SS	1274.00 ± 362.50 a	5.40 ± 0.48 bc	0.76 ± 0.04 b	1491.00 ± 70.12 b	5.64 ± 0.10 b	0.77 ± 0.01 b

Means followed by the standard deviation. Means with different letters on the same column differ significantly according to Duncan's multiple range test ($p < 0.05$). ISS: Infested Sterilized Soil. SS: Sterilized Soil

2.3.1. Nematode population density

The population density of *M. javanica* and *P. brachyurus* were assessed at the end of *Meloidogyne*-assay and *Pratylenchus*-assay, respectively. In *Meloidogyne*-assay, the increase in soil microbial community dilution resulted in a significant increase in the number of nematodes in the plant roots. The treatments ISS and 10^{-6} showed a higher population density of *M. javanica* (12.4 ± 2.4 and 9.5 ± 2.6 thousand individuals g^{-1} root, respectively) when compared to 10^{-1} (6.7 ± 1.7 thousand individuals g^{-1} root) (Figure 2a). On the other hand, in *Pratylenchus*-assay, we observed a lower population density of *P. brachyurus* in the treatments 10^{-6} and 10^{-3} (527 ± 285 and 605 ± 563 individuals g^{-1} root, respectively) when compared to ISS (1,388 ± 449 individuals g^{-1} root). Furthermore, the treatment 10^{-1} (1,355 ± 857 individuals g^{-1} root) presented a population density similar to ISS (Figure 2b).

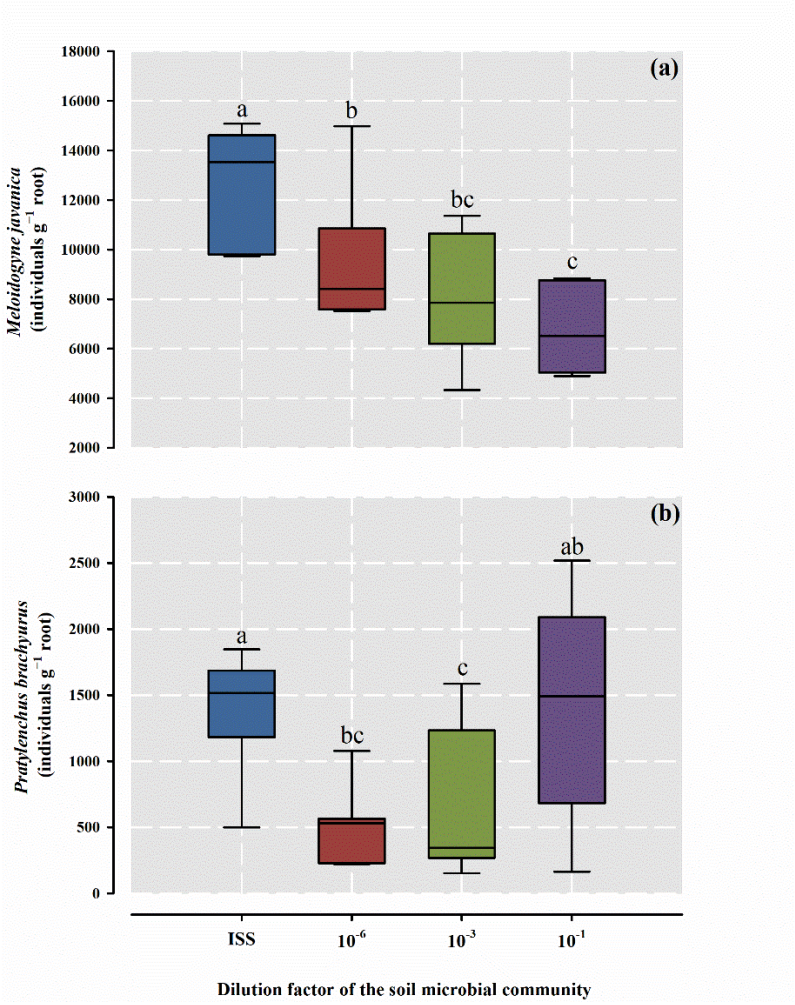

Figure 2. Nematode population density in roots of soybean plants grown in soil with different microbial diversity levels. (a) Meloidogyne-assay; (b) Pratylenchus-assay. Boxes with different letters on the same graph differ significantly according to Duncan's multiple range test ($p < 0.05$). ISS: Infested Sterilized Soil. Data represent the mean of ten biological replicate samples for each treatment in each assay.

2.3.2. Plant measurements

In *Meloidogyne*-assay, 10^{-1} and SS showed higher grain biomass (2.00 ± 0.19 g and 2.04 ± 0.12 g, respectively) when compared to 10^{-6} (1.78 ± 0.15 g) and ISS (1.68 ± 0.08 g) treatments. Also, we observed that the fresh mass of roots was significantly higher in the treatments that were inoculated with the soil microbial community, at all levels of diversity (mean of treatments: 12.82 ± 1.83 g) when compared to SS (9.39 ± 1.15 g) (Figure 3a). In *Pratylenchus*-assay, 10^{-1} showed the highest grain biomass (2.06 ± 0.19 g) when compared to the other treatments (mean of the other treatments: 1.61 ± 0.27 g). The highest fresh mass of roots in *Pratylenchus*-assay was observed in SS (8.99 ± 2.32 g) compared to all other treatments (mean of the other treatments: 5.90 ± 1.23 g) (Figure 3b). Lastly, there was no significant effect of treatments on shoot dry mass in both experiments.

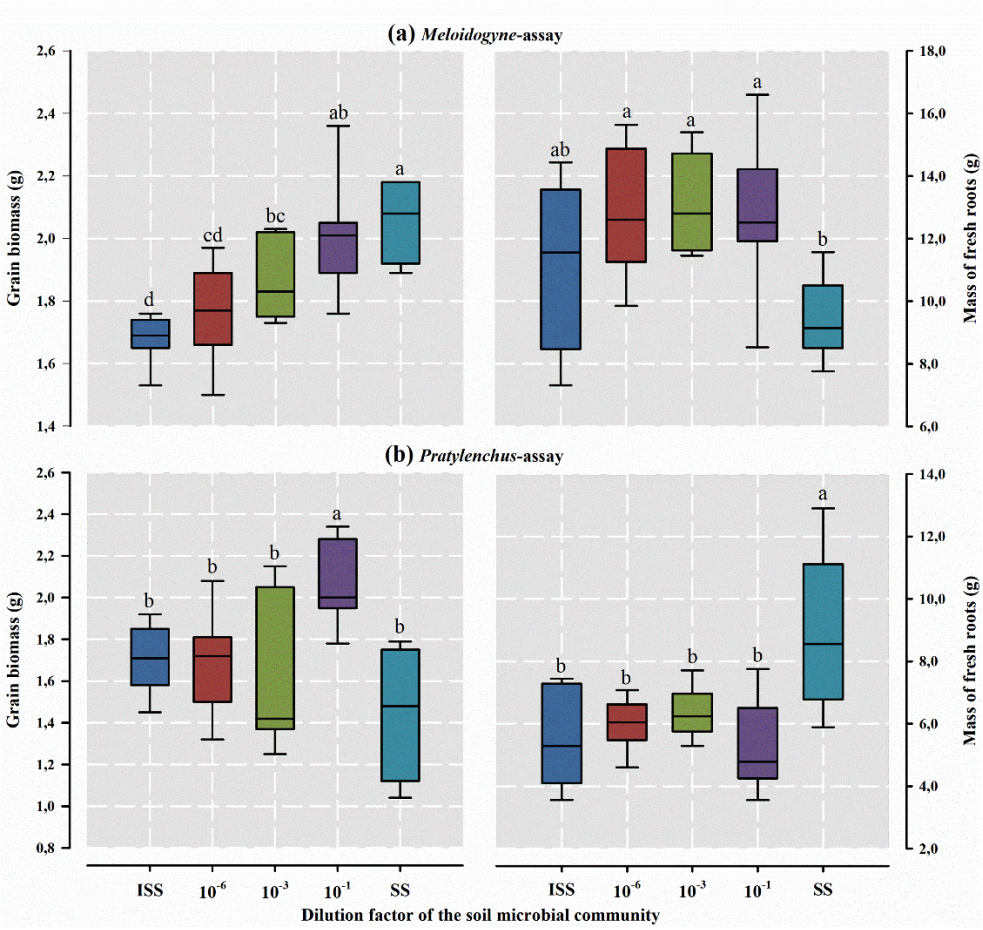

Figure 3. Grain biomass and mass of fresh roots of soybean plants grown in soil with different microbial diversity levels, inoculated with plant-parasitic nematodes. (a) Meloidogyne-assay; (b) Pratylenchus-assay. Boxes with different letters on the same graph differ significantly according to Duncan's multiple range test ($p < 0.05$). ISS: Infested Sterilized Soil. SS: Sterilized Soil. Data represent the mean of ten biological replicate samples for each treatment in each assay.

2.3.3. Soil bacterial community composition

The T-RFLP (Terminal Restriction Fragment Length Polymorphism) was initially used as screening technique. For this, ten biological replicates of each treatment were analyzed in each assay (Figure S2). Afterwards,

we selected four biological replicates of each treatment (dilutions 10^{-1}, 10^{-3}, 10^{-6}; and control treatments ISS and SS) in each assay for the taxonomic profiling of the bacterial community. Approximately 7,600,000 sequences were generated from 80 samples using 16S rRNA gene amplicon sequencing, with an average of 114,460 sequences per sample (OTU table in the supplemental material). Overall, the soil bacterial community was comprised of 35 phyla and more than 1,300 genera based on the SILVA database (Figure S3 and S4). At the start of *Meloidogyne*-assay, we observed a higher abundance of the genera *Lysobacter*, *Ralstonia*, *Chitinophaga*, *Devosia*, *Sinomonas*, *Sphingomonas*, and *Bradyrhizobium* in the treatments with greater microbial diversity ($p < 0.05$). At the end of *Meloidogyne*-assay, we observed a higher abundance of *Rasltonia* and *Rhodanobacter* in the treatments with greater microbial diversity ($p < 0.05$). At the start of *Pratylenchus*-assay, the genera *Caulobacter*, *Chitinophaga*, *Devosia*, *Lysobacter*, and *Variovorax* were enriched in the treatments with greater microbial diversity ($p < 0.05$). At the end of *Pratylenchus*-assay, the genera *Burkholderia* and *Paraburkholderia* presented a greater relative abundance in the treatments with less diversity ($p < 0.05$).

It is worth mentioning that in both experiments, when the ISS and SS treatments were analysed, we observed that the nematode inoculation resulted in consistent increases in the relative abundance of some bacterial genera (Figure 4). The relative abundance of the genera *Massilia* and *Tuberibacillus* were increased at the start of *Meloidogyne*-assay and at the end of *Pratylenchus*-assay. Some of the increases in the relative abundance of bacterial genera were observed in ISS but did not occur in SS, which were: *Laceyella*, *Pseudoflavitalea*, *Sinomicrobium*, and *Terrimonas*, associated with *M. javanica*; and Candidatus *Amoebophilus*, *Gemmata*, *Leptolyngbya*, *Niabella*, and *Roseiarcus*, associated with *P. brachyurus*.

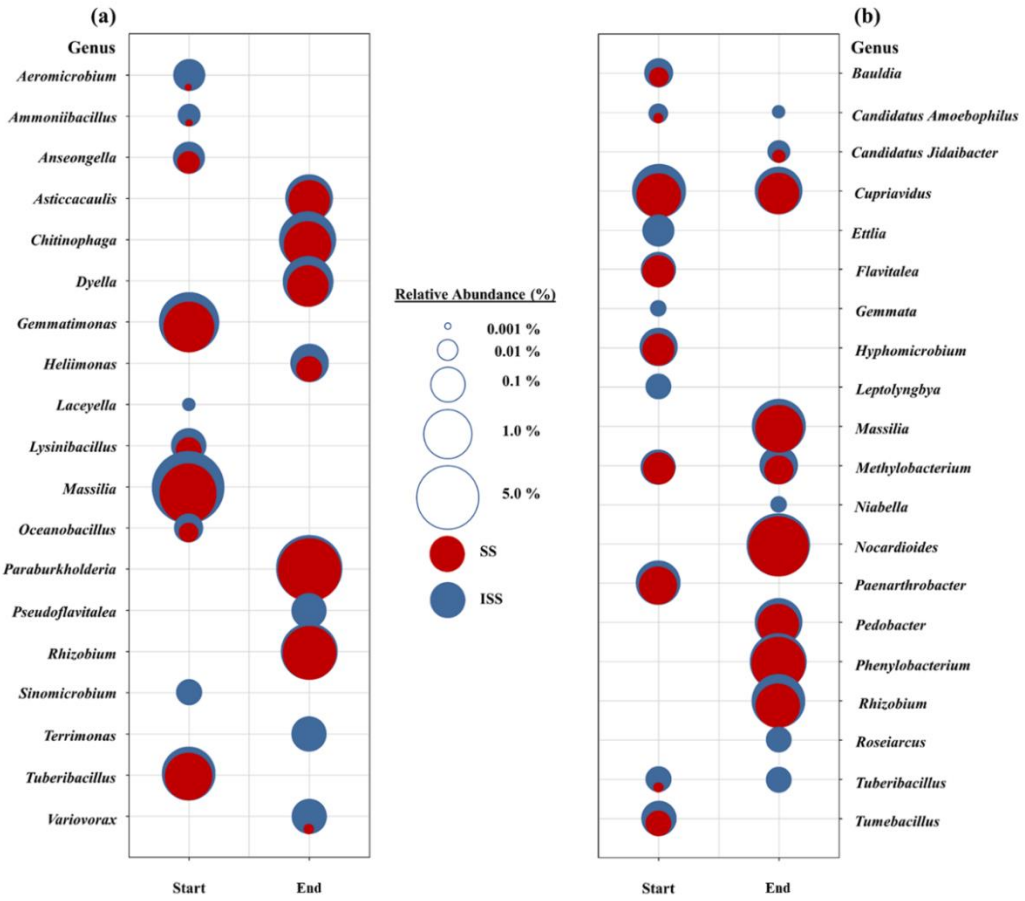

Figure 4. Increased bacterial taxa relative abundances with nematode inoculation between SS and ISS treatments, indicated by overlaid bubble plots that represent the relative percent abundance of a taxa at the start and end of Meloidogyne-assay (a) and Pratylenchus-assay (b).

2.3.4. The network structure of the microbial community

We observed that the diversity gradient (dilution) affected the network complexity at the start and end of both experiments (Figure 5, Figure S5) (Table S2). Furthermore, we identified the most relevant bacterial groups based on the values of betweenness centrality, which is defined as the number of times a node acts as a bridge along the shortest path between two other nodes (POUDEL *et al.*, 2016), at the start and end of both experiments. At the start of *Meloidogyne*-assay, the lowest taxonomic level of the top three nodes with the highest betweenness centrality regardless of treatment were: *Chitinophaga*, *Massilia*, and Burkholderiaceae (Table S3). At the end of *Meloidogyne*-assay, the top three were: *Panacagrimonas*, Rhodanobacteraceae, and *Burkholderia* (Table S4). At the start of *Pratylenchus*-assay, the top three taxa with the highest betweenness centrality regardless of treatment were: *Chitinophaga*, *Dyella*, and *Pedobacter* (Table S5). At the end of *Pratylenchus*-assay, the top three were: Saccharimonadales, Acetobacteraceae, and Pedosphaeraceae (Table S6).

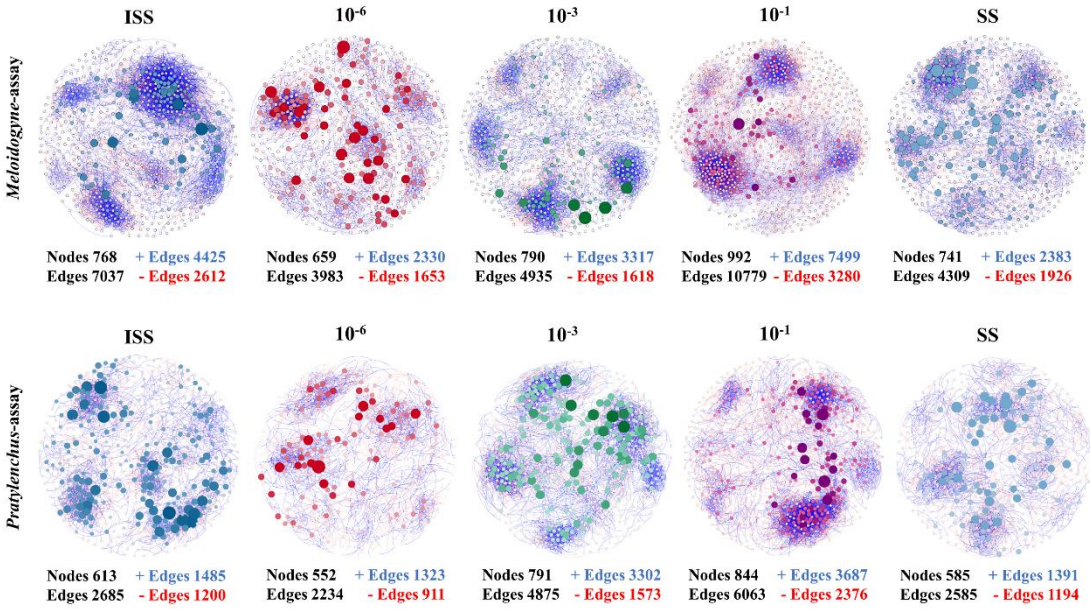

Figure 5. Network co-occurrence analysis of microbial communities of soil with different microbial diversity levels, inoculated with plant-parasitic nematodes at the end of the experiments. A connection stands for SparCC correlation with magnitude > 0.8 (positive correlation–blue edges) or < −0.8 (negative correlation–red edges) and statistically significant ($P \leq 0.01$). Each node represents taxa affiliated at OTU level and the size of node is proportional to the betweeness centrality value.

2.3.5. Correlation between nematode population density, plant growth parameters, and the main soil bacteria genera

Spearman's correlation analysis was performed to investigate the relationship between the relative abundance of the 150 most abundant bacterial genera across all treatments, in each assay, and nematode population density, and plant growth measures. In *Meloidogyne*-assay, we observed strong and negative correlations (Spearman < -0.6; $p < 0.05$) among bacterial genera and the population density of nematodes in soybean roots. Here, we highlighted *Noviherbaspirillum*, *Devosia*, *Filimonas*, *Pseudomonas*, and *Jatrophihabitans* genera, which also presented strong and positive correlations with the grain biomass (Spearman > 0.6; $p < 0.05$) (Figure 6). In *Pratylenchus*-assay, we did not observe negative correlations between bacterial genera and nematode population density.

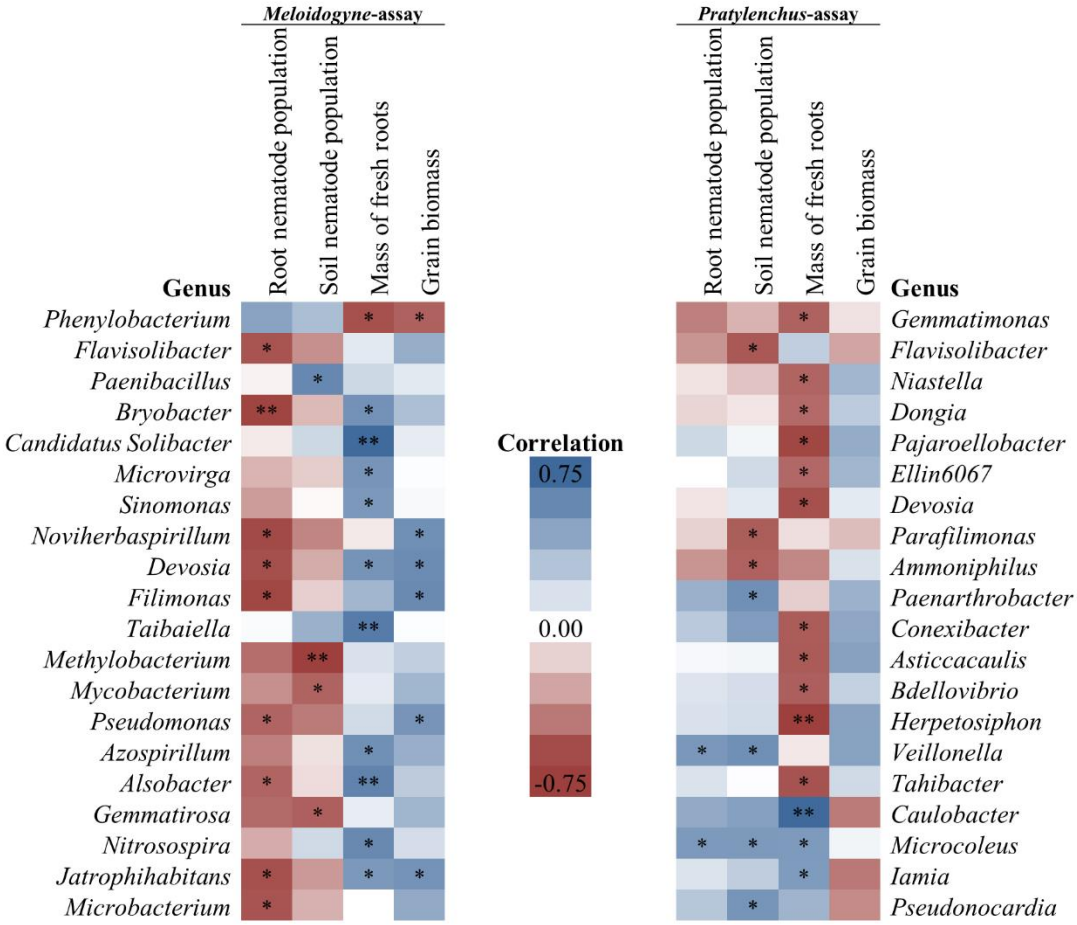

Figure 6. Figure 6. Heatmaps of Spearman's rank correlation coefficients of relative abundance of bacterial genus with nematode population density and plant measurements. *Significant at 0.05; **Significant at 0.01.

2.4. Discussion

Although the role of plant-parasitic nematodes is well known concerning losses in agriculture, the interplay between the occurrence of these organisms in soils and the resident microbiome remains to be better explored. Here we used two controlled experiments to assess correlations between the bacterial community diversity and composition, nematodes infestation, and effects on plants.

The dilution-to-extinction approach resulted in significant differences in the structure and diversity of the bacterial community across treatments (Table 1 and Table 2). Although not totally effective, heat sterilization significantly reduced the bacterial community, resulting in many empty niches. These niches became available for recolonization by other soil microorganisms (LI *et al.*, 2019), which explains the presence of DNA in the SS treatment in both experiments. In addition, it is possible that there was relic DNA after autoclaving (LENNON *et al.*, 2018). The dilution-to-extinction approach favoured the more abundant soil bacteria over the rarer bacterial taxa (data not shown) that are usually less abundant and may have important roles in the suppression of soil diseases (HOL *et al.*, 2015). The biological origin of the soil suppressiveness to plant-parasitic nematodes has been previously studied (ELHADY *et al.*, 2018b; GINÉ *et al.*, 2016; SONG *et al.*, 2016; WEI *et al.*, 2019). However, our study

presents the effect of soil bacterial diversity on plant-parasitic nematode suppression by manipulating microbial diversity in controlled conditions, avoiding the effects of environmental factors (*i.e.*, plant species, soil type and use, management, etc.), which can lead to different conclusions regarding diversity.

In *Meloidogyne*-assay, we argue that the greater soil microbial diversity and the presence of potential antagonistic bacteria to nematodes in the treatments 10^{-1} and 10^{-3} resulted in a lower infestation by *M. javanica* when compared to 10^{-6} and ISS (Figure 2a). Furthermore, the lower nematode infestation in the treatments with greater microbial diversity resulted in higher grain biomass (Figure 3a). In general, high microbial diversity promotes functional redundancy and ecosystem services that can improve soil resilience (WATSON; STRAUSS; DESAEGER, 2020). Also, a higher microbial diversity enhances the resource competition, which has been proposed as a key factor for the success or failure of pathogen invasion (MENDES *et al.*, 2018; WEI *et al.*, 2015b).

On the other hand, in *Pratylenchus*-assay, although the highest diversity level was observed in the 10^{-1} treatment, the lowest infestation levels by *P. brachyurus* were observed in 10^{-3} and 10^{-6} treatments. Both treatments also presented a lower population density of the nematode when compared to ISS (Figure 2b). Even though treatment 10^{-1} presented the highest population density of *P. brachyurus*, this treatment presented the highest grain biomass among all treatments. This shows that soil suppressiveness to the nematode can minimize the damage caused by the disease, even with the pathogen already established in this treatment (WELLER *et al.*, 2002).

Although soil microbial diversity is essential for the proper functioning of ecosystem processes, biodiversity by itself may not be enough to reduce the ability of pathogens to establish in the soil (BENDER; WAGG; VAN DER HEIJDEN, 2016). In our experiments, in addition to modulating soil microbial diversity, the use of the dilution-to-extinction approach altered the taxonomic composition of the bacterial community. At the start and end of both experiments, when analysed at the phylum and class level, the soil microbial community consisted of a common microbial core among most Brazilian soils (Figure S3 and S4) (ANDREOTE *et al.*, 2017; DE SOUZA; PROCÓPIO, 2021; MENDES *et al.*, 2014). However, when analyses were performed at the genus level, we observed significant differences among the treatments.

At the start of *Meloidogyne*-assay, the treatments with greater bacterial diversity showed a greater relative abundance of some bacterial genera capable of acting in the suppression of *Meloidogyne* spp. One of them is the genus *Lysobacter*, which presented a higher relative abundance in 10^{-1} treatment when compared to all others ($p < 0.001$) (Figure S6a). According to Chen *et al.* (CHEN *et al.*, 2006) and Lee *et al.* (LEE *et al.*, 2013), this bacterial genus can produce a variety of enzymes and/or toxins with known nematicide effects against *M. javanic*a and other nematodes. Other bacterial genera such as *Bradyrhizobium*, *Devosia*, and *Sphingomonas* presented greater abundance in 10^{-1} and 10^{-3} treatments. According to Topalović *et al.* (2020), these bacterial genera inhabit the soil, plant roots, and nematodes (*i.e.,* gut and/or body surface) in disease suppressive soils.

At the start of *Pratylenchus*-assay, *Lysobacter* and *Devosia* genera also presented greater relative abundance in the treatment with higher microbial diversity (10^{-1}) when compared to other treatments ($p < 0.01$) (Figure S6b). Although *Lysobacter* can produce compounds capable of acting against a variety of nematodes, including those of the genus *Pratylenchus* (Chen *et al.*, 2006), in our study its great relative abundance in the 10^{-1} treatment did not result in a reduction of *P. brachyurus* population (Figure 2b). Additionally, some studies indicate that the *Devosia* genus can produce auxins and siderophores, which promote plant growth (CHHETRI *et al.*, 2021). This may explain the high grain biomass observed in the 10^{-1} when compared to the other treatments (Figure 3b). The genus *Caulobacter* and *Variovorax*, which are also known as plant growth-promoting rhizobacteria (PGPR) (BERRIOS, 2021; HAN *et al.*, 2013), presented a higher relative abundance in 10^{-1} and 10^{-3} treatments. Different studies have reported

the presence of *Variovorax* in soils suppressive to plant-parasitic nematodes (ABALLAY *et al.*, 2012; CASTILLO; VIVANCO; MANTER, 2017; HAMID *et al.*, 2017; TOPALOVIĆ; HUSSAIN; HEUER, 2020).

The differences observed in the bacterial community structure between ISS and SS can be explained by the association between bacteria and nematodes. Foreign bacteria may have been introduced into the ISS soil together with the nematodes, and it may have positively influenced the survival and parasitism of the nematodes (TOPALOVIĆ; VESTERGÅRD, 2021). These differences persisted until the end of both experiments ($R > 0.6$, $p < 0.05$), indicating that the parasitism of nematodes may have affected the recruitment of microorganisms by the rhizosphere of soybean plants (HUSSAIN *et al.*, 2018). This suggestion is supported by the increase in the relative abundance of some bacterial genera in ISS compared to SS, and the occurrence of some bacterial genera only in ISS treatment (Figure 4). However, further studies using axenic nematodes are necessary to determine whether the difference in microbial community recruitment between nematode-free and infested soils is a plant effect mediated by nematode parasitism, or whether it is an effect of the microbial community conveyed by the inoculum.

Spearman's correlation analysis between the most abundant bacterial genera and the nematode population density and plant growth parameters showed that some bacterial genera have a high potential for the suppression of *M. javanica* in *Meloidogyne*-assay (Figure 8). Among them, the genera, *Devosia*, *Pseudomonas*, *Bryobacter*, *Noviherbaspirillum*, *Filimonas*, *Alsobacter*, and *Jatrophihabitans* presented strong negative correlations with the population density of *M. javanica* and strong positive correlations with plant growth parameters. It is worth mentioning that so far there are no studies about the potential biocontrol of these microorganisms. On the other hand, there are several studies in the literature about the biocontrol activity of *Pseudomonas* and *Microbacterium*. Bacteria belonging to the *Pseudomonas* genus, especially *Pseudomonas fluorescens*, have shown efficacy in *Meloidogyne* spp. control (ABD EL-AAL *et al.*, 2021; KHAN *et al.*, 2016; SIDDIQUI; HAAS; HEEB, 2005). Recent studies have also demonstrated the potential biocontrol of *Meloidogyne* spp. by bacteria belonging to the genus *Microbacterium* (TOPALOVIĆ *et al.*, 2019; ZHAO *et al.*, 2019).

In *Pratylenchus*-assay, we did not observe potentially suppressive bacteria to *P. brachyurus*. Conversely, we observed that *Veillonella* and *Microcoleus* were positively correlated with *P. brachyurus* population density. These bacteria may be related to parasitism by the nematode through protection against antagonistic microorganisms or by suppression of the plant's immune response (TOPALOVIĆ; VESTERGÅRD, 2021). Interestingly, *Devosia* is a PGPR characterized by its bioremediation activity and ability to fix nitrogen (TALWAR *et al.*, 2020). This genus showed the potential to suppress *M. javanica* but showed a negative correlation with the soybean mass of fresh roots in *Pratylenchus*-assay, which may indicate that *Devosia* acts on the specific suppressiveness of *M. javanica.*

Finally, the results of the co-occurrence network analysis revealed a higher complexity of connections (*i.e.*, number of nodes, edges, and communities) within the bacterial community of treatments with higher microbial diversity (10^{-1}) when compared to the other treatments, in both the experiments. According to Mendes *et al.* (MENDES *et al.*, 2018), a highly diverse microbial community exhibit a great number of interactions, with high competition for niche space, which results in great resistance to invasion by pathogens. This may explain the lower population density of *M. javanica* in the roots of plants in the 10^{-1} treatment, in *Meloidogyne*-assay.

The high complexity of the network observed in the ISS when compared to SS, at the end of both experiments, supports our hypothesis that parasitism by nematodes affects the recruitment of microorganisms by plants (WILSCHUT; GEISEN, 2021). We argue that the nematode invasion may increase the amount of ecological niches capable of being filled in the soil microbiome since nematodes can be parasitized by antagonistic bacteria, or even colonized by protective bacteria (ADAM *et al.*, 2014; ELHADY *et al.*, 2017; TOPALOVIĆ; VESTERGÅRD, 2021). Analyzing the rank of bacterial taxa with higher betweenness centrality in ISS and SS, at the start and end of

both experiments, we observed that the invasion by nematodes resulted in the alteration of the core microbiome of key groups within the community (BORGATTI, 2005). We argue that the invasion of the soil microbiome by a pathogen may replace key taxa and collapse the structure of the network (ALBRECHT *et al.*, 2014).

2.5. Conclusions

We demonstrate the suppressive effect of soil bacterial diversity against plant-parasitic nematodes on soybean plants. The microbial diversity, together with the presence of antagonistic bacteria to nematodes, are soil microbial factors capable of reducing the occurrence of *M. javanica* and sustaining the performance of soybean plants when parasitized by *P. brachyurus*. Furthermore, our results indicate that bacteria belonging to *Bryobacter*, *Noviherbaspirillum*, *Filimonas*, *Alsobacter*, and *Jatrophihabitans* genera are potential targets in studies of prospecting bacteria to act in the biological control of *M. javanica*.

Our results also support the hypothesis that plant-parasitic nematode infection leads to alterations in the soil microbial community. The genera *Laceyella*, *Pseudoflavitalea*, *Sinomicrobium*, and *Terrimonas* were induced in *Meloidogyne*-assay; and the genera Candidatus *Amoebophilus*, *Gemmata*, *Leptolyngbya*, *Niabella*, and *Roseiarcus* were induced in *Pratylenchus*-assay. Nonetheless, further studies are needed to understand whether the soil microbial community is altered by the presence of nematodes and their associated microorganisms, or whether this microbial community alteration is mediated by the plant's response to the infection.

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Supplementary Material

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MICROBIAL ECOLOGY

Interactions between Soil Bacterial Diversity and Plant-Parasitic Nematodes in Soybean Plants

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ABSTRACT Plant-parasitic nematodes are an important group of pests causing economic losses in agriculture worldwide. Among the plant-parasitic nematodes, the root-knot (*Meloidogyne* spp.) and root-lesion nematodes (*Pratylenchus* spp.) are considered the two most important ones affecting soybeans. In general, they damage soybean roots, causing a reduction of about one-third in productivity. The soil microbial community can exert a suppressive effect on the parasitism of plant-parasitic nematodes. Here, we investigated the effects of soil bacterial diversity on *Meloidogyne javanica* (*Meloidogyne*-assay) and *Pratylenchus brachyurus* (*Pratylenchus*-assay) suppression by manipulating microbial diversity using the dilution-to-extinction approach in two independent experiments under controlled conditions. Furthermore, we recorded the changes in the soil microbial community induced by plant-parasitic nematode infection. In *Meloidogyne*-assay, microbial diversity reduced the population density of *M. javanica* and improved plant performance. In *Pratylenchus*-assay, microbial diversity sustained the performance of soybean plants even at high levels of *P. brachyurus* parasitism. Each nematode population affected the relative abundance of different bacterial genera and altered the core microbiome of key groups within the bacterial community. Our findings provide fundamental insights into the interactions between soil bacterial diversity and plant-parasitic nematodes in soybean plants.

IMPORTANCE Root-knot and root-lesion nematodes cause losses of billions of dollars every year to agriculture worldwide. Traditionally, they are controlled by using chemical nematicides, which in general have a negative impact on the environment and human health. Fortunately, the soil microbial community may suppress these pests, acting as an environmentally friendly alternative to control nematodes. However, the effects of soil microbial diversity on the parasitism of plant-parasitic nematodes still poorly understood. In this study, we provide fundamental insight into the interactions between soil bacterial diversity and plant-parasitic nematodes in soybean plants, which may be useful for the development of new strategies to control these phytopathogens.

KEYWORDS dilution-to-extinction, 16S rRNA gene, soil suppressiveness, *Meloidogyne javanica*, *Pratylenchus brachyurus*

Nematodes are microscopic organisms belonging to the phylum Nematoda, which measure between 0.3 to 3.0 mm in length and are ubiquitous in different habitats (i.e., soil, plants, animals, insects, water, etc.). Currently, there are more than 23,000 described nematode species, and approximately 35% of them inhabit the soil (1, 2). Among the soil nematodes, about 25% are herbivores (also called plant-parasitic nematodes) and can cause damage to plants (3, 4). Although only a small portion of the soil-dwelling nematodes is plant-feeding, plant-parasitic nematodes are among the

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Supplementary Methods

Terminal restriction fragment-length polymorphism (T-RFLP) Analysis

T-RFLP fingerprinting was used to determine variations in bacterial community structure in all samples from both experiments (200 samples). The 16S rRNA gene was amplified with primers 8-FM (5`- AGA CTT TGA TCM TGG CTC AG - 3`) labeled with 6-carboxyfluorescein (6-FAM) and 926r (5`- CCG TCA ATT CCT TTR AGT TT – 3`) (SCHÜTTE *et al.*, 2009). The thermal cycling conditions were: 95°C for 4 min (initial denaturation), followed by 30 cycles of 95 °C for 30 s, 57 °C for 30 s, and 72 °C for 45 s, with a final extension step at 72 °C for 10 min. The final volume of the reactions was 50 µL, containing 6 µL of $MgCl_2$ (final $MgCl_2$ concentration of 3 mM), 0.2 µL (1 U) of Taq polymerase, 5 µL of Mg-free buffer (Sinapse Inc, São Paulo, Brazil), 4 µL of deoxyribonucleotide triphosphate mixture (dNTP) (0.2 mM of each nitrogenous base) (Invitrogen Corporation, Carlsbad, CA, USA), 0.1 µL of each primer (0.01 mM) and 1 µL of DNA template, and 33.6 µL of sterilized ultrapure water. The amplification products were digested with the endonuclease HhaI (5 U) (Invitrogen Corporation, Carlsbad, CA, EUA) and precipitated with 125 mM EDTA and 3 M sodium acetate. Then, the amplification products were resuspended using Hi-DiTM formamide (Applied Biosystems, Life Technologies) with GeneScanTM 1200 LIZ® marker (Applied Biosystems, Life Technologies) and analyzed on an ABI Prism 3500 automatic sequencer (Applied Biosystems, Life Technologies). T-RFLP profiles were analyzed using GeneMapper® 4.1 (Applied Biosystems, Life Technologies), then subjected to Two-dimensional non-metric multidimensional scaling (NMDS) using Primer 5. The differences between treatments were evaluated by similarity analysis (ANOSIM) using the Paleontological Statistics freeware package (PASTv.3 - Hammer *et al.* (HAMMER; HARPER; RAYAN, 2001)).

Co-occurrence network analysis

Network analyses were performed to assess the complexity of the interactions among microbial taxa in each treatment of both experiments. Non-random co-occurrence analyses were carried out using the Python module 'SparCC' (FRIEDMAN; ALM, 2012). For this, a table of frequency of hits affiliated at genus level was used for analysis. For each network, the SparCC correlations between microbial taxa were calculated and only strong (SparCC > 0.8 or < -0.8) and highly significant ($P < 0.01$) were selected. The nodes in the reconstructed network represent taxa at OTU level, whereas the edges represent significantly positive or negative correlations between nodes. The network graphs were based on a set of measures, such as number of nodes, number of edges, modularity, number of communities, average node connectivity, average path length, diameter and cumulative degree distribution. Networks visualization and properties measurements were calculated with the interactive platform Gephi (BASTIAN; HEYMANN; JACOMY, 2009).

Supplementary Tables

Table S1. Bacterial abundance in soil with different microbial diversity levels, at the start and end of the *Meloidogyne*-assay and *Pratylenchus*-assay.

	***Meloidogyne*-assay**		***Pratylenchus*-assay**	
	Start	End	Start	End
	---------- Number of 16S rRNA gene copies g^{-1} soil ----------			
ISS	3.5×10^{10} a	3.0×10^{10}	3.2×10^{10} a	2.3×10^{10}
10^{-6}	4.0×10^{10} a	3.0×10^{10}	3.7×10^{10} a	2.5×10^{10}
10^{-3}	3.6×10^{10} a	2.5×10^{10}	3.6×10^{10} a	2.5×10^{10}
10^{-1}	4.6×10^{10} a	2.8×10^{10}	3.7×10^{10} a	2.9×10^{10}
SS	1.9×10^{10} b	2.4×10^{10}	2.6×10^{10} b	2.5×10^{10}

Means with different letters on the same column differ significantly according to Duncan's multiple range test ($p < 0.05$). ISS: Infested Sterilized Soil. SS: Sterilized Soil.

Table S2. Topological characteristics of the networks among the treatments at the start and end of the Meloidogyne-assay and Pratylenchus-assay.

	Start					End				
	10^{-1}	10^{-3}	10^{-6}	ISS	SS	10^{-1}	10^{-3}	10^{-6}	ISS	SS
Meloidogyne-assay										
Network properties										
Number of nodes[a]	668	637	551	649	750	992	790	659	768	741
Number of edges[b]	3420	4153	2688	3677	4447	10779	4935	3983	7037	4309
Positive edges[c]	2299	2907	1603	2262	2626	7499	3317	2330	4425	2383
Negative edges[d]	1121	1246	1085	1415	1821	3280	1618	1653	2612	1926
Modularity[e]	1.659	1.502	2.768	2.521	2.939	1.431	1.638	2.700	2.126	4.767
Number of communities[f]	115	75	65	71	57	116	84	57	69	56
Network diameter[g]	12	9	8	10	11	15	16	9	16	12
Average path length[h]	2.686	2.643	2.547	2.408	2.786	3.558	3.337	2.599	3.617	3.179
Average degree[i]	5.12	6.52	4.878	5.512	5.929	10.866	6.247	6.044	9.163	5.815
Average clustering coefficient[j]	0.377	0.163	0.176	0.181	0.169	0.180	0.176	0.181	0.182	0.172
Pratylenchus-assay										
Network properties										
Number of nodes[a]	599	525	541	485	560	844	761	552	613	585
Number of edges[b]	3360	2236	3070	2653	4173	6063	4875	2234	2685	2585
Positive edges[c]	2202	1352	2119	1847	2704	3687	3302	1323	1485	1391
Negative edges[d]	1158	884	951	806	1469	2376	1573	911	1200	1194
Modularity[e]	1.738	2.302	1.563	1.392	1.839	2.316	1.608	2.709	4.711	5.644
Number of communities[f]	85	100	92	79	51	77	78	66	75	78
Network diameter[g]	11	8	9	10	11	13	13	9	10	12
Average path length[h]	2.786	2.275	2.459	2.497	3.2	3.006	3.100	2.709	2.563	2.794
Average degree[i]	5.609	4.259	5.675	5.47	7.452	7.184	6.406	4.047	4.38	4.419
Average clustering coefficient[j]	0.155	0.16	0.171	0.195	0.171	0.163	0.175	0.16	0.159	0.162

[a]Microbial taxon (at genus level) with at least one significant ($P < 0.01$) and strong (SparCC > 0.8 or < - 0.8) correlation;

[b]Number of connections/correlations obtained by SparCC analysis;

[c]SparCC positive correlation (> 0.8 with $P < 0.01$);

[d]SparCC negative correlation (< -0.8 with $P < 0.01$);

[e]The capability of the nodes to form highly connected communities, that is, a structure with high density of between nodes connections (inferred by Gephi);

[f]A community is defined as a group of nodes densely connected internally (Gephi);

[g]The longest distance between nodes in the network, measured in number of edges (Gephi);

[h]Average network distance between all pair of nodes or the average length off all edges in the network (Gephi);

[i]The average number of connections per node in the network, that is, the node connectivity (Gephi);

[j]How nodes are embedded in their neighborhood and the degree to which they tend to cluster together (Gephi).

Table S3. Top ten lowest taxonomic level with high betweeness centrality and number of correlations (that is, degree) for each treatment at the start of *Meloidogyne*-assay.

	Betweeness Centrality			Degree		
	Phylum	Lowest taxonomic level	Value	Phylum	Lowest taxonomic level	Value
10^{-1}	Bacteroidetes	*Chitinophaga*	858.21	Proteobacteria	*Sphingomonas*	45
	Proteobacteria	*Massilia*	707.71	Proteobacteria	Chromobacteriaceae	44
	Bacteroidetes	Chitinophagaceae	691.16	Proteobacteria	*Paracoccus*	43
	Proteobacteria	Burkholderiaceae	689.74	Proteobacteria	Burkholderiaceae	41
	Verrucomicrobia	Pedosphaeraceae	601.79	Proteobacteria	Myxococcales	41
	Firmicutes	*Cohnella*	494.47	Gemmatimonadetes	*Gemmatirosa*	40
	Proteobacteria	Chromobacteriaceae	463.27	Proteobacteria	*Sphingomonas*	40
	Proteobacteria	*Massilia*	438.65	Bacteroidetes	*Flavisolibacter*	40
	Firmicutes	*Domibacillus*	390.25	Proteobacteria	Xanthobacteraceae	39
	Verrucomicrobia	Pedosphaeraceae	384.28	Proteobacteria	Burkholderiaceae	39
10^{-3}	Chloroflexi	Thermomicrobiales	1009.47	Bacteroidetes	*Chitinophaga*	83
	Bacteroidetes	*Chitinophaga*	926.2	Proteobacteria	*Cupriavidus*	76
	Proteobacteria	Sandaracinaceae	826.16	Proteobacteria	Micropepsaceae	57
	Bacteroidetes	*Ignavibacteria*	735.54	Actinobacteria	Microtrichales	55
	Proteobacteria	*Massilia*	722.58	Bacteroidetes	Flavisolibacter	54
	Firmicutes	*Cohnella*	659.88	Proteobacteria	Micropepsaceae	51
	Proteobacteria	Micropepsaceae	615.25	Firmicutes	Sporolactobacillaceae	50
	Verrucomicrobia	*Chthoniobacter*	613.27	Proteobacteria	Sandaracinaceae	48
	Bacteroidetes	*Chitinophaga*	595.22	Gemmatimonadetes	Gemmatimonadetes	48
	Proteobacteria	Hyphomicrobiaceae	566.08	Verrucomicrobia	*Chthoniobacter*	46
10^{-6}	Firmicutes	*Tumebacillus*	1149.99	Actinobacteria	*Sinomonas*	37
	Firmicutes	*Paenibacillus*	548.01	Proteobacteria	Burkholderiaceae	34
	Proteobacteria	*Burkholderia*	514.5	Proteobacteria	Beijerinckiaceae	34
	Proteobacteria	Burkholderiaceae	454.61	Proteobacteria	Enterobacteriaceae	33
	Chloroflexi	*Anaerolineae*	379.61	Firmicutes	*Paenibacillus*	32
	Proteobacteria	Burkholderiaceae	319.84	Firmicutes	*Cohnella*	31
	Bacteroidetes	*Chitinophaga*	274.18	Proteobacteria	*Paucimonas*	30
	Actinobacteria	*Nocardioides*	266.64	Proteobacteria	*Massilia*	30
	Proteobacteria	Sphingomonadaceae	269.87	Bacteroidetes	*Chitinophaga*	29
	Verrucomicrobia	*Chthoniobacter*	257.75	Actinobacteria	*Nocardioides*	29
ISS	Proteobacteria	*Massilia*	500.02	Actinobacteria	Microbacteriaceae	48
	Bacteroidetes	Chitinophagaceae	321.69	Firmicutes	*Clostridiales*	46
	Bacteroidetes	*Niastella*	260.04	Firmicutes	*Paenibacillus*	44
	Proteobacteria	*Phenylobacterium*	252.8	Proteobacteria	*Massilia*	43
	Firmicutes	*Paenibacillus*	248.21	Proteobacteria	*Burkholderia*	42
	Firmicutes	Sporolactobacillaceae	247.23	Bacteroidetes	*Flaviaesturariibacter*	42
	Proteobacteria	Rhodospirillaceae	245.44	Bacteroidetes	Chitinophagaceae	41
	Proteobacteria	*Pantoea*	240.66	Proteobacteria	Burkholderiaceae	40
	Gemmatimonadetes	*Gemmatimonas*	232.64	Firmicutes	*Bacillus*	38
	Proteobacteria	*Phenylobacterium*	187.11	Proteobacteria	*Pusillimonas*	38
SS	Bacteroidetes	*Taibaiella*	1454.74	Bacteroidetes	Chitinophagaceae	52
	Bacteria	Hyphomicrobiaceae	1328.59	Firmicutes	*Paenibacillus*	51
	Proteobacteria	*Dongia*	824.88	Bacteroidetes	*Chitinophaga*	50
	Bacteroidetes	Chitinophagaceae	701.82	Firmicutes	*Bacillus*	47
	Bacteroidetes	Rhodothermaceae	588.98	Proteobacteria	*Pseudolabrys*	46
	Proteobacteria	Rhizobiaceae	562.72	Firmicutes	*Bacillus*	46
	Bacteroidetes	*Flavitalea*	543.9	Proteobacteria	Enterobacteriaceae	44
	Proteobacteria	*Burkholderia*	540.95	Proteobacteria	*Noviherbaspirillum*	44
	Bacteroidetes	*Mucilaginibacter*	531.1	Proteobacteria	*Burkholderia*	43
	Bacteroidetes	*Chitinophaga*	443.35	Proteobacteria	*Phenylobacterium*	43

Table S4. Top ten lowest taxonomic level with high betweeness centrality and number of correlations (that is, degree) for each treatment at the end of *Meloidogyne*-assay.

	Betweeness Centrality			Degree		
	Phylum	Lowest taxonomic level	Value	Phylum	Lowest taxonomic level	Value
10^{-1}	Proteobacteria	*Panacagrimonas*	7925.26	Proteobacteria	Micropepsaceae	118
	Proteobacteria	*Burkholderia*	4186.8	Proteobacteria	*Massilia*	105
	Cyanobacteria	*Sericytochromatia*	3712.64	Proteobacteria	Burkholderiaceae	105
	Acidobacteria	*Holophagae*	3270.59	Bacteroidetes	*Pedobacter*	90
	Proteobacteria	*Hyphomicrobium*	3254.1	Firmicutes	*Cohnella*	89
	Bacteroidetes	*Cytophaga*	3158.36	Bacteroidetes	*Mucilaginibacter*	86
	Proteobacteria	*Burkholderia*	2952.54	Cyanobacteria	*Oxyphotobacteria*	86
	Proteobacteria	*Massilia*	2884.05	Proteobacteria	*Dongia*	80
	Proteobacteria	*Nevskia*	2804.01	Proteobacteria	*Sphingomonas*	78
	Chloroflexi	*Ktedonobacteria*	2801.81	Acidobacteria	*Occallatibacter*	78
10^{-3}	Actinobacteria	Gaiellales	2888.12	Verrucomicrobia	Pedosphaeraceae	56
	Bacteroidetes	Chitinophagaceae	2854.08	Gemmatimonadetes	*Gemmatimonas*	54
	Proteobacteria	Micropepsaceae	2631.47	Actinobacteria	Acidimicrobiia	53
	Proteobacteria	*Noviherbaspirillum*	2542.09	Actinobacteria	*Intrasporangiaceae*	51
	Chloroflexi	*Ktedonobacteria*	1775.7	Bacteroidetes	*Filimonas*	51
	Proteobacteria	*Burkholderia*	1659.02	Actinobacteria	*Sporichthya*	49
	Proteobacteria	Fodinicurvataceae	1578.19	Actinobacteria	Frankiales	49
	Gemmatimonadetes	*Gemmatimonas*	1357.49	Bacteroidetes	*Mucilaginibacter*	48
	Actinobacteria	Gaiellales	1166.17	Proteobacteria	*Sphingomonas*	48
	Bacteroidetes	Sphingobacteriales	1094.57	Actinobacteria	*Sinomonas*	47
10^{-6}	Bacteroidetes	*Flavisolibacter*	475.583	Proteobacteria	*Noviherbaspirillum*	62
	Proteobacteria	Xanthobacteraceae	423.597	Actinobacteria	*Jatrophihabitans*	62
	Gemmatimonadetes	*Gemmatimonas*	412.239	Proteobacteria	*Burkholderia*	60
	Proteobacteria	Beijerinckiaceae	396.224	Firmicutes	*Planococcus*	60
	Proteobacteria	Sandaracinaceae	396.011	Proteobacteria	*Mesorhizobium*	58
	Patescibacteria	Saccharimonadales	393.51	Proteobacteria	Rhizobiales	56
	Proteobacteria	Alphaproteobacteria	385.583	Firmicutes	Sporolactobacillaceae	56
	Actinobacteria	*Mycobacterium*	369.542	Proteobacteria	Beijerinckiaceae	56
	Cyanobacteria	*Sericytochromatia*	350.002	Proteobacteria	*Burkholderia*	56
	Bacteroidetes	Saprospiraceae	347.693	Bacteroidetes	*Chitinophaga*	55
ISS	Proteobacteria	Rhodanobacteraceae	4551.26	Bacteroidetes	*Chitinophaga*	86
	Bacteroidetes	*Niabella*	4023.25	Proteobacteria	Burkholderiaceae	84
	Proteobacteria	Caulobacteraceae	3679.05	Proteobacteria	*Chelativorans*	83
	Proteobacteria	*Sphingomonas*	3470.7	Proteobacteria	Devosiaceae	82
	Firmicutes	*Paenibacillus*	3133.74	Bacteroidetes	*Terrimonas*	80
	Firmicutes	*Exiguobacterium*	2898.23	Bacteroidetes	Chitinophagaceae	80
	Proteobacteria	*Burkholderia*	2837.33	Proteobacteria	*Pseudomonas*	79
	Proteobacteria	Enterobacteriaceae	2398.8	Proteobacteria	Rickettsiales	74
	Proteobacteria	*Bdellovibrio*	2331.73	Firmicutes	*Exiguobacterium*	72
	Bacteroidetes	*Flavisolibacter*	2227.2	Bacteroidetes	*Pseudoflavitalea*	71
SS	Proteobacteria	*Novosphingobium*	1187.47	Gemmatimonadetes	Longimicrobiaceae	50
	Proteobacteria	*Burkholderia*	1170.61	Proteobacteria	*Burkholderia*	49
	Bacteroidetes	*Spirosoma*	901.222	Bacteroidetes	Chitinophagaceae	49
	Firmicutes	*Paenibacillus*	846.41	Firmicutes	*Cohnella*	46
	Proteobacteria	*Frateuria*	833.496	Proteobacteria	Burkholderiaceae	46
	Gemmatimonadetes	*Gemmatimonas*	799.492	Firmicutes	*Paenibacillus*	45
	Proteobacteria	Rhizobiaceae	770.279	Proteobacteria	Devosiaceae	43
	Verrucomicrobia	*Chthoniobacter*	745.856	Actinobacteria	Gaiellales	42
	Proteobacteria	*Novosphingobium*	1187.47	Proteobacteria	Caulobacteraceae	41
	Verrucomicrobia	Pedosphaeraceae	740.24	Actinobacteria	Solirubrobacteraceae	41

Table S5. Top ten lowest taxonomic level with high betweeness centrality and number of correlations (that is, degree) for each treatment at the start of *Pratylenchus*-assay.

	Betweeness Centrality			Degree		
	Phylum	Lowest taxonomic level	Value	Phylum	Lowest taxonomic level	Value
10^{-1}	Proteobacteria	Burkholderiaceae	920.56	Proteobacteria	*Massilia*	61
	Proteobacteria	*Dyella*	636.79	Proteobacteria	Burkholderiaceae	52
	Gemmatimonadetes	*Gemmatimonas*	598.35	Proteobacteria	*Novosphingobium*	52
	Proteobacteria	Rhizobiaceae	593.36	Firmicutes	*Bacillus*	51
	Proteobacteria	*Sphingomonas*	546.54	Proteobacteria	*Sphingomonas*	48
	Proteobacteria	*Paracaedibacter*	473.19	Proteobacteria	*Mesorhizobium*	48
	Firmicutes	*Paenibacillus*	434.32	Firmicutes	*Paenibacillus*	47
	Bacteroidetes	*Mucilaginibacter*	411.67	Bacteroidetes	Chitinophagaceae	46
	Proteobacteria	*Massilia*	385.15	Proteobacteria	*Rhizobium*	46
	Proteobacteria	*Burkholderia*	380.43	Proteobacteria	*Methylocella*	46
10^{-3}	Actinobacteria	*Streptomyces*	265.55	Firmicutes	*Paenibacillus*	40
	Firmicutes	*Cohnella*	245.83	Actinobacteria	*Streptomyces*	38
	Proteobacteria	*Burkholderia*	228.82	Bacteroidetes	*Chitinophaga*	38
	Verrucomicrobia	*Chthoniobacter*	205.93	Proteobacteria	*Sphingomonas*	38
	Firmicutes	*Paenibacillus*	158.12	Bacteroidetes	Chitinophagaceae	37
	Proteobacteria	Caulobacteraceae	154.55	Proteobacteria	Xanthobacteraceae	36
	Firmicutes	*Bacillus*	150.33	Actinobacteria	Gaiellales	36
	Bacteroidetes	*Mucilaginibacter*	142.39	Bacteroidetes	*Fluviicola*	35
	Bacteroidetes	*Chitinophaga*	127.64	Actinobacteria	*Nocardioides*	34
	Proteobacteria	Burkholderiaceae	115.57	Bacteria	*Burkholderia*	33
10^{-6}	Proteobacteria	*Asticcacaulis*	511.38	Proteobacteria	*Pseudomonas*	61
	Bacteroidetes	Chitinophagaceae	419.9	Proteobacteria	*Pantoea*	53
	Cyanobacteria	*Sericytochromatia*	390.75	Bacteroidetes	Chitinophagaceae	48
	Bacteroidetes	*Mucilaginibacter*	332.59	Cyanobacteria	*Sericytochromatia*	47
	Actinobacteria	*Paenarthrobacter*	315.67	Bacteroidetes	*Dyadobacter*	40
	Bacteroidetes	*Flavisolibacter*	309.46	Proteobacteria	*Asticcacaulis*	39
	Proteobacteria	Sphingomonadaceae	280.04	Proteobacteria	Micropepsaceae	35
	Proteobacteria	*Mesorhizobium*	268.42	Bacteroidetes	*Mucilaginibacter*	35
	Bacteroidetes	Sphingobacteriales	263.37	Proteobacteria	Rhodospirillaceae	33
	Proteobacteria	*Massilia*	246.43	Firmicutes	*Cohnella*	33
ISS	Proteobacteria	*Sphingomonas*	750.67	Proteobacteria	*Lysobacter*	43
	Firmicutes	*Paenibacillus*	421.69	Proteobacteria	Xanthobacteraceae	43
	Proteobacteria	*Massilia*	353.87	Firmicutes	*Paenibacillus*	42
	Proteobacteria	Burkholderiaceae	350.38	Firmicutes	*Bacillus*	41
	Firmicutes	*Bacillus*	287.3	Proteobacteria	*Massilia*	40
	Proteobacteria	*Pseudolabrys*	240.8	Bacteroidetes	*Pedobacter*	38
	Actinobacteria	Intrasporangiaceae	220.7	Proteobacteria	*Ramlibacter*	38
	Proteobacteria	*Haliangium*	218.3	Bacteroidetes	*Chitinophaga*	38
	Proteobacteria	*Pseudomonas*	217.63	Proteobacteria	*Sphingomonas*	38
	Proteobacteria	Xanthobacteraceae	194.36	Bacteroidetes	*Taibaiella*	37
SS	Bacteroidetes	*Chitinophaga*	1449.2	Bacteroidetes	*Chitinophaga*	74
	Proteobacteria	*Dyella*	1377.9	Proteobacteria	*Massilia*	73
	Bacteroidetes	*Pedobacter*	1302.1	Proteobacteria	Burkholderiaceae	62
	Firmicutes	*Ammoniphilus*	1283.7	Bacteroidetes	*Mucilaginibacter*	58
	Proteobacteria	Rhodanobacteraceae	1067.1	Proteobacteria	Beijerinckiaceae	58
	Firmicutes	Planococcaceae	1034.3	Bacteroidetes	*Arcticibacter*	57
	Firmicutes	*Paenibacillus*	998.08	Firmicutes	*Bacillus*	56
	Firmicutes	*Bacillus*	986.63	Proteobacteria	Rhodanobacteraceae	53
	Proteobacteria	*Massilia*	971.7	Proteobacteria	*Sphingomonas*	52
	Proteobacteria	*Sphingomonas*	957.94	Proteobacteria	Caulobacteraceae	46

Table S6. Top ten lowest taxonomic level with high betweeness centrality and number of correlations (that is, degree) for each treatment at the end of *Pratylenchus*-assay.

	Betweeness Centrality			Degree		
	Phylum	Lowest taxonomic level	Value	Phylum	Lowest taxonomic level	Value
10-1	Patescibacteria	Saccharimonadales	1874	Firmicutes	*Bacillus*	78
	Proteobacteria	Acetobacteraceae	1797.36	Proteobacteria	*Parasutterella*	77
	Verrucomicrobia	Pedosphaeraceae	1512.11	Bacteroidetes	*Parafilimonas*	76
	Proteobacteria	Burkholderiaceae	1450.38	Gemmatimonadetes	Gemmatimonadaceae	76
	Proteobacteria	Micropepsaceae	1357.86	Actinobacteria	Gaiellales	75
	Verrucomicrobia	*Chthoniobacter*	1235.04	Proteobacteria	Caulobacteraceae	74
	Gemmatimonadetes	*Gemmatimonas*	1157.4	Verrucomicrobia	Pedosphaeraceae	74
	Acidobacteria	*Granulicella*	1117.36	Firmicutes	*Lactobacillus*	74
	Proteobacteria	*Haliangium*	942.84	Bacteroidetes	Chitinophagaceae	72
	Bacteroidetes	*Parafilimonas*	886.56	Verrucomicrobia	*Opitutus*	70
10-3	Proteobacteria	*Noviherbaspirillum*	785.54	Actinobacteria	*Paenarthrobacter*	46
	Gemmatimonadetes	*Gemmatimonas*	764.4	Gemmatimonadetes	Longimicrobiaceae	46
	Bacteroidetes	*Niastella*	723.94	Cyanobacteria	*Sericytochromatia*	45
	Firmicutes	*Bacillus*	707.49	Actinobacteria	*Streptomyces*	43
	Cyanobacteria	*Sericytochromatia*	666.1	Actinobacteria	*Nocardioides*	43
	Proteobacteria	Burkholderiaceae	659.24	Chloroflexi	Kallotenuales	41
	Proteobacteria	Micropepsaceae	593.48	Gemmatimonadetes	Gemmatimonadaceae	40
	Gemmatimonadetes	Longimicrobiaceae	576.61	Proteobacteria	*Nitrosospira*	40
	Acidobacteria	Blastocatellaceae	569.28	Bacteroidetes	Chitinophagaceae	39
	Proteobacteria	*Rhodanobacter*	563.36	Actinobacteria	*Gaiellales*	39
10-6	Bacteroidetes	*Mucilaginibacter*	534.35	Firmicutes	*Paenibacillus*	31
	Proteobacteria	*Pseudomonas*	367.55	Gemmatimonadetes	*Gemmatimonas*	30
	Proteobacteria	*Sphingomonas*	361.38	Bacteroidetes	*Flavisolibacter*	29
	Proteobacteria	*Phenylobacterium*	359.89	Proteobacteria	Beijerinckiaceae	28
	Actinobacteria	*Nocardioides*	319	Proteobacteria	*Burkholderia*	28
	Proteobacteria	Beijerinckiaceae	274.57	Gemmatimonadetes	Longimicrobiaceae	28
	Bacteroidetes	Chitinophagaceae	267.15	Proteobacteria	Micropepsaceae	28
	Cyanobacteria	Obscuribacterales	260.77	Bacteroidetes	Chitinophagaceae	27
	Bacteroidetes	*Chitinophaga*	260.2	Actinobacteria	*Leifsonia*	27
	Proteobacteria	Micropepsaceae	209.22	Proteobacteria	*Noviherbaspirillum*	27
ISS	Actinobacteria	*Marmoricola*	313.77	Bacteroidetes	*Mucilaginibacter*	33
	Proteobacteria	*Pseudomonas*	306.66	Proteobacteria	*Massilia*	33
	Proteobacteria	Burkholderiaceae	287.53	Proteobacteria	*Inquilinus*	32
	Gemmatimonadetes	*Gemmatimonas*	286.3	Bacteroidetes	*Arcticibacter*	32
	Proteobacteria	*Haliangium*	276.05	Actinobacteria	*Marmoricola*	31
	Actinobacteria	*Paenarthrobacter*	268.99	Proteobacteria	*Pseudomonas*	31
	Firmicutes	*Bacillus*	265.7	Proteobacteria	*Dongia*	31
	Gemmatimonadetes	Longimicrobiaceae	248.83	Firmicutes	*Cohnella*	31
	Gemmatimonadetes	*Gemmatimonas*	243.75	Proteobacteria	*Rhizobium*	31
	Proteobacteria	Micropepsaceae	241.44	Cyanobacteria	Nostocales	31
SS	Firmicutes	*Cohnella*	495.14	Proteobacteria	*Burkholderia*	38
	Verrucomicrobia	Pedosphaeraceae	467	Firmicutes	*Paenibacillus*	36
	Verrucomicrobia	*Chthoniobacter*	398.9	Firmicutes	*Cohnella*	35
	Gemmatimonadetes	*Gemmatimonas*	325.05	Proteobacteria	*Sphingomonas*	35
	Proteobacteria	*Mesorhizobium*	323.11	Actinobacteria	*Nocardioides*	34
	Acidobacteria	Candidatus *Solibacter*	322.58	Actinobacteria	*Paenarthrobacter*	33
	Firmicutes	*Paenibacillus*	317.37	Proteobacteria	Xanthobacteraceae	33
	Bacteroidetes	*Flavisolibacter*	297	Gemmatimonadetes	Gemmatimonadaceae	31
	Bacteroidetes	Chitinophagaceae	284.09	Cyanobacteria	Nostocales	31
	Proteobacteria	Myxococcales	282.26	Actinobacteria	Frankiales	30

Table S7. Chemical properties of the soil used in *Meloidogyne*-assay and *Pratylenchus*-assay.

	pH	M.O.	P	K^+	Ca^{2+}	Mg^{2+}	H+Al	SB	T	V
	($CaCl_2$)	($g.dm^{-3}$)	($mg.dm^{-3}$)	---------- mmolc.dm^{-3} ----------						(%)
Meloidogyne-assay	5.1	17.5	25.0	1.0	15.7	4.7	14.2	21.5	35.7	60.1
Pratylenchus-assay	4.6	17.5	24.3	0.9	9.3	3.0	16.3	13.2	29.5	44.7

pH in $CaCl_2$ 0.01 mol L^{-1}; phosphorus (P) colorimetric method extracted with anion exchange resin; potassium (K^+) extraction with ion exchange resin and determination in an atomic emission spectrophotometer; calcium (Ca^{2+}) and magnesium (Mg^{2+}) extraction with ion exchange resin and determination in an atomic absorption spectrophotometer; potential acidity (H+Al) extracted with SMP buffer. M.O.: organic matter; SB: sum of bases; T: potential CEC; V: base saturation.

Table S8. Primers and cycling conditions used to amplify the target genes

	Primer sequence (5'-3')	Thermal cycling conditions
qPCR primers		
Total bacteria (16S rRNA)		
341f[1]	CCTACGGGAGGCAGCAG	95°C 10 min, 1 cycle; 94ºC 30 s, 55ºC 30 s, 72ºC 30 s, 35 cycles
518r[1]	ATTACCGCGGCTGCTGG	
T-RFLP primers		
8F-FAM	5'-AGAGTTTGATCCTGGCTCAG-3'	95°C 4 min, 1 cycle; 95ºC 30 s, 57ºC 30 s, 72ºC 40 s, 30 cycles
926r	5'-CCGTCAATTCCTTTRAGTTT-3'	

[1] Muyzer (1993); Schütte *et al.* (2009)

Supplementary Figures

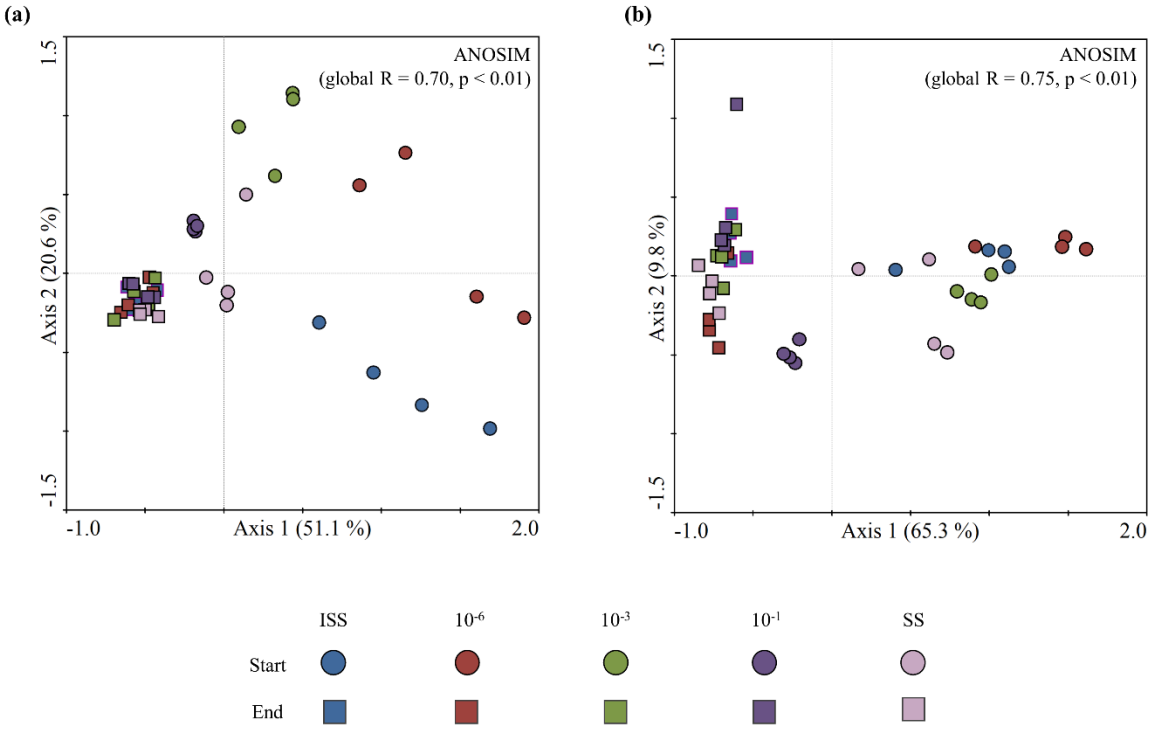

Figure S1. Principal component analysis (PCA) analysis of the bacterial community in soil with different microbial diversity levels, inoculated with plant-parasitic nematodes. (a) start and end of Meloidogyne-assay; (b) start and end of Pratylenchus-assay. ISS: Infested Sterilized Soil. SS: Sterilized Soil. Global R > 0.75: well-separated groups; global R > 0.5: groups with overlap but clearly differentiated; global R < 0.25: not well-separated groups.

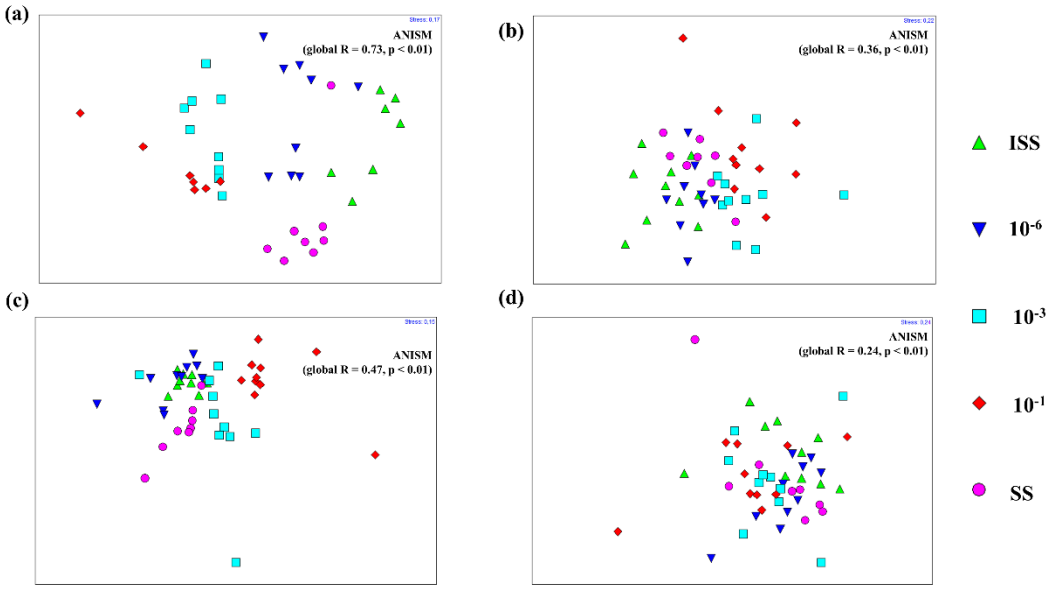

Figure S2. Two-dimensional non-metric multidimensional scaling (NMDS) plots of the bacterial community in soil with different microbial diversity levels, inoculated with plant-parasitic nematodes, based on Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis. (a) start of Meloidogyne-assay, and (b) end of Meloidogyne-assay; (c) start of Pratylenchus-assay, and (d) end of Pratylenchus-assay. ISS: Infested Sterilized Soil. SS: Sterilized Soil.

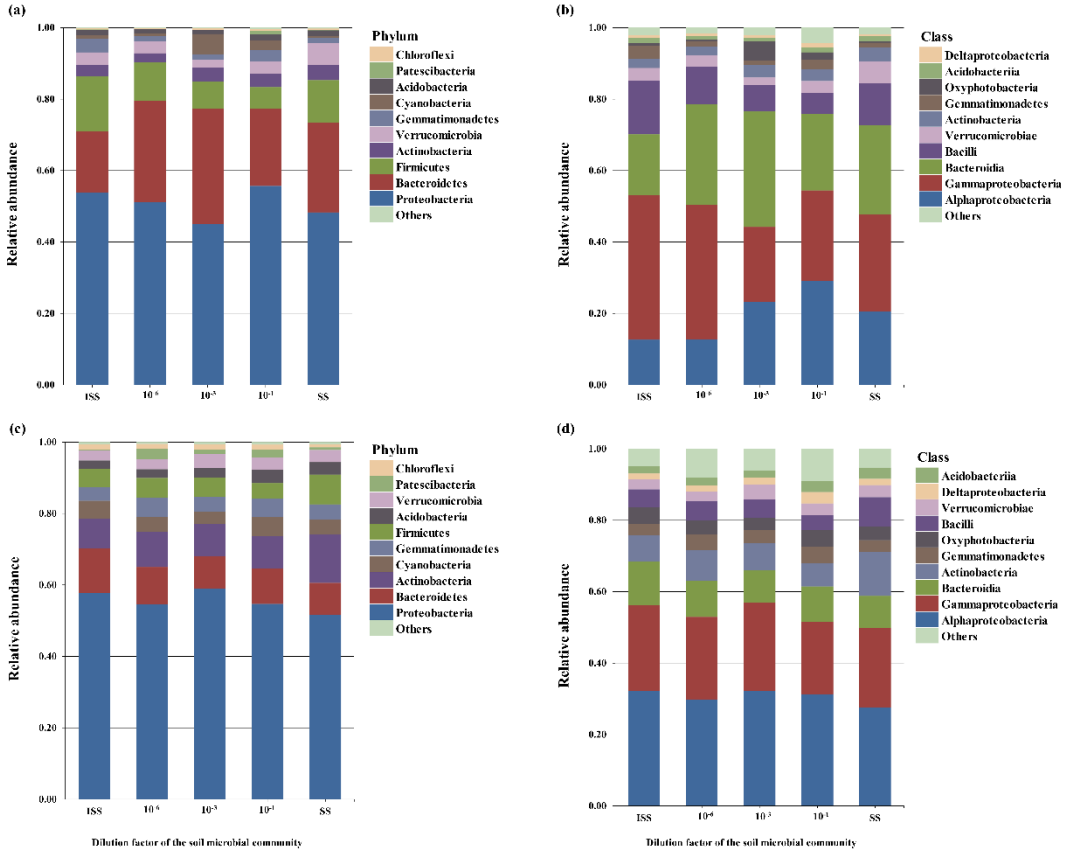

Figure S3. Relative abundance of bacterial taxa in soil with different microbial diversity levels, inoculated with Meloidogyne javanica, and cultivated with soybean plants. (a) Relative abundance of bacterial phyla at the start of the Meloidogyne-assay. (b) Relative abundance of bacterial classes at the start of the Meloidogyne-assay. (c) Relative abundance of bacterial phyla at the end of the Meloidogyne-assay. (d) Relative abundance of bacterial classes at the end of the Meloidogyne-assay. The same colours in each bar across different locations are indicate the same taxa. ISS: Infested Sterilized Soil. SS: Sterilized Soil.

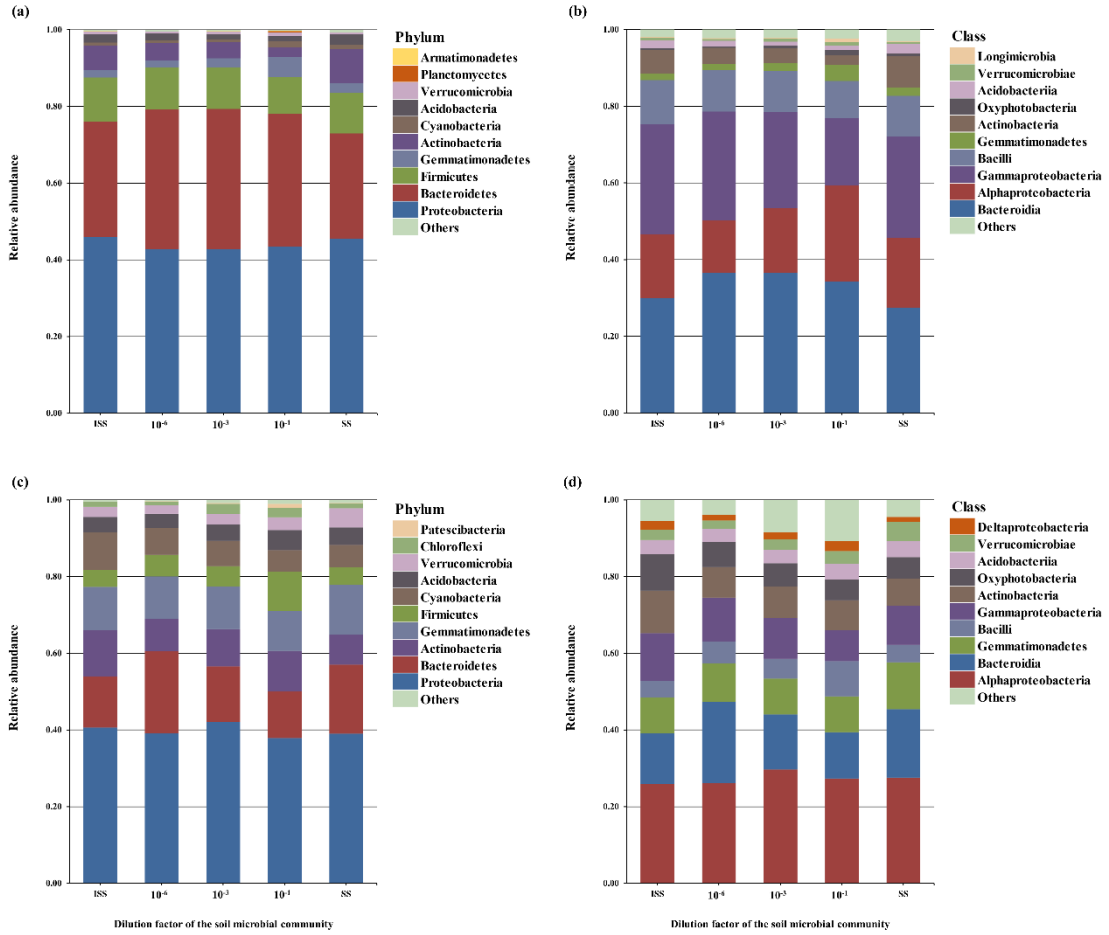

Figure S4. Relative abundance of bacterial taxa in the in soil with different microbial diversity levels, inoculated with Pratylenchus brachyurus, and cultivated with soybean plants. (a) Relative abundance of bacterial phyla at the start of the Pratylenchus-assay. (b) Relative abundance of bacterial classes at the start of the Pratylenchus-assay. (c) Relative abundance of bacterial phyla at the end of the Pratylenchus-assay. (d) Relative abundance of bacterial classes at the end of the Pratylenchus-assay. The same colours in each bar across different locations are indicate the same taxa. ISS: Infested Sterilized Soil. SS: Sterilized Soil.

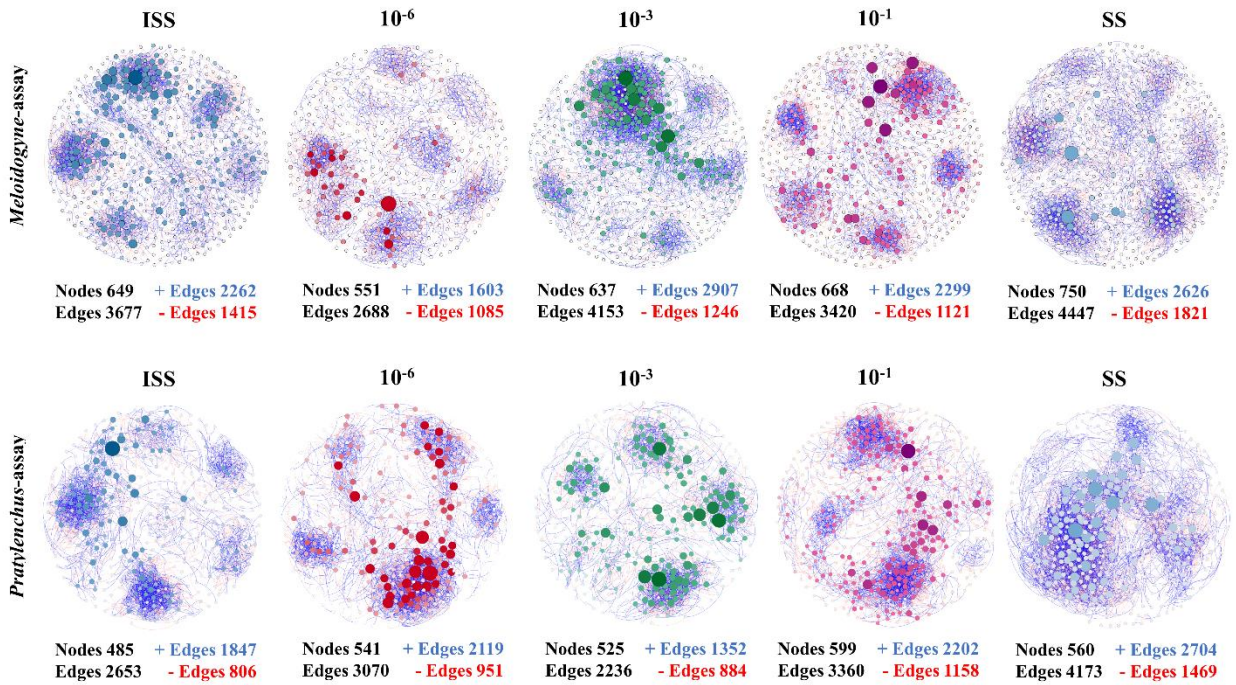

Figure S5. Network co-occurrence analysis of microbial communities of soil with different microbial diversity levels, inoculated with plant-parasitic nematodes at the start of *Meloidogyne*-assay and *Pratylenchus*-assay. A connection stands for SparCC correlation with magnitude > 0.8 (positive correlation–blue edges) or < −0.8 (negative correlation–red edges) and statistically significant ($P \leq 0.01$). Each node represents taxa affiliated at OTU level and the size of node is proportional to the betweeness centrality value.

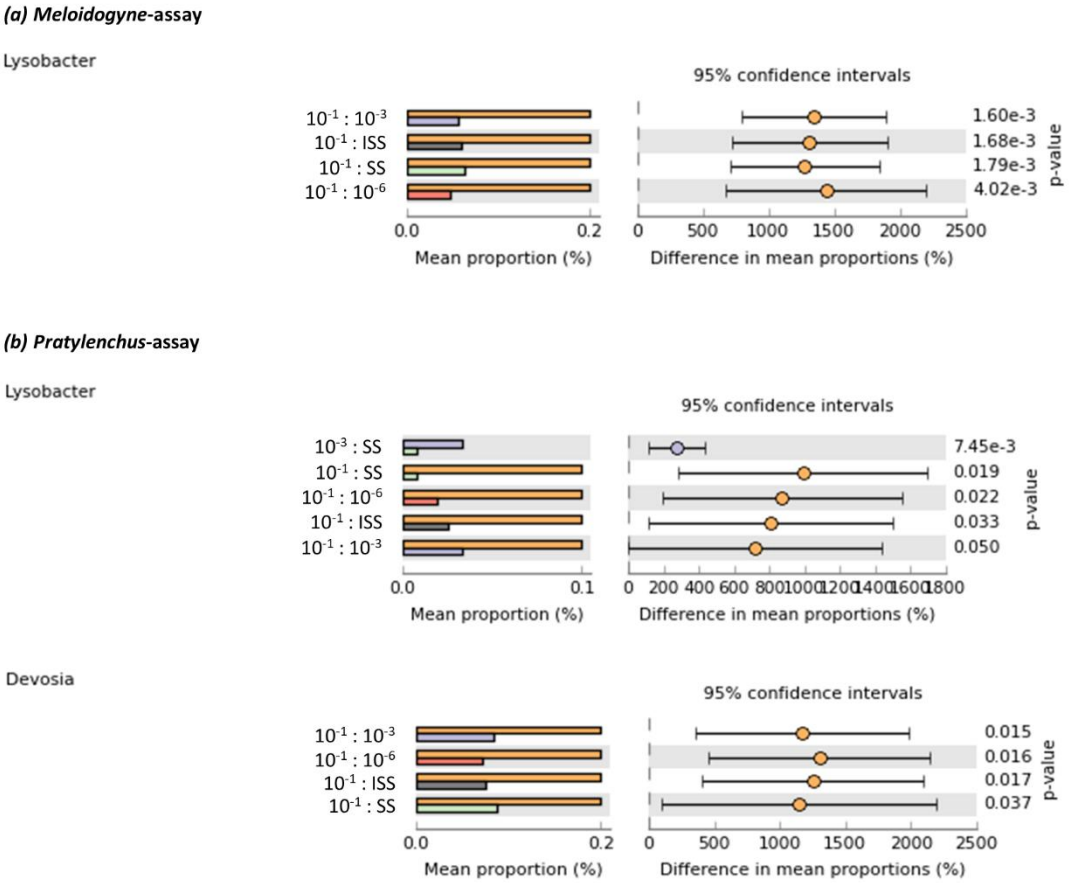

Figure S6. Welch's post-hoc test for relative abundance of Lysobacter at the start of the Meloidogyne-assay (a), and Lysobacter and Devosia at the start of the Pratylenchus-assay.

3. INTERACTIONS BETWEEN BACTERIAL AND NEMATODE COMMUNITY IN SOYBEAN CULTIVATED SOILS IN BRAZIL

Abstract

Brazil is the most megadiverse country in the world, where grain agriculture (especially soybean) has been widely practiced. Nevertheless, the biological diversity in soybean cultivated soils in Brazil is still poorly described. Some efforts have been made to characterise the bacterial communities, but little is known about nematode diversity and about the relationship between bacteria and nematodes in these soils. Here, we used 178 soil samples collected from the main soybean producing areas in four different biomes (Amazon Rainforest, Atlantic Rainforest, Cerrado, and Pampa) to characterise the bacterial and nematode (free-living and plant-parasitic) community in soybean cultivated soils in Brazil, and to investigate correlations between them. We observed that the bacterial classes Vicinamibacteria, Alphaproteobacteria, Bacilli, Actinobacteria, and Blastocatellia were predominant along the sampling sites. In the total nematode community, the orders Rhabditida, Tylenchida, Dorylaimida, Triplonchida, and Enoplida were predominant. The plant-parasitic nematode community was composed by the genera *Meloidogyne*, *Pratylenchus*, *Heterodera*, *Rotylenchulus*, *Helicotylenchus*, *Scutellonema*, and *Xiphinema*. We observed significant correlations between population density of *Pratylenchus* sp. and relative abundance of several bacterial genera in the Cerrado and Atlantic Rainforest. Conversely, bacterial diversity presented positive correlations with population density of *Pratylenchus* sp. Finally, we observed a positive correlation between total nematode richness and soybean yield in the Cerrado, highlighting the importance of free-living nematodes to maintain soil functions in agriculture.

Keywords: 16S rRNA, 18S rRNA, Next-generation sequencing, Plant-parasitic nematodes, Soil suppressiveness

3.1. Introduction

Brazil is a highly diverse country with an area of approximately 8.5 million km², encompassing six distinct biomes (Amazon Rainforest, Atlantic Rainforest, Cerrado, Caatinga, Pampa, and Pantanal) (MAIA *et al.*, 2020). Approximately 5% of this area (41,4520 km²) is cultivated with soybean, especially in the Cerrado and Atlantic Rainforest (CONAB, 2022; IBGE, 2022), where grain crops are increasingly replacing above-ground biodiversity. Nevertheless, belowground biodiversity in soybean fields in Brazil is still poorly described. Studies have investigated the bacterial community in soybean fields (CEZAR *et al.*, 2021; LEITE *et al.*, 2021; PROCÓPIO; BARRETO, 2021), but the nematode community has been overlooked and the relationship between bacteria and nematodes needs to be better understood.

Nematodes are the most abundant group of animals on earth, which occupy all trophic levels of the soil food web and occur in almost all environments, presenting great diversity in the soil (VAN DEN HOOGEN *et al.*, 2019). Nematodes can be classified according to their feeding habits, such as herbivores, fungivores, bacterivores, predators, and omnivores (YEATES *et al.*, 1993). Most nematode species are free-living, which perform beneficial functions for soil processes (MOURA & FRANZENER, 2017; VAN DEN HOOGEN *et al.*, 2019). Nematodes actively participate in the decomposition of soil organic matter and in the regulation of biogeochemical cycles, regulating the food web by controlling soil microorganism populations (NEILSON *et a*l., 2020), and have a role in vegetation dynamics (GEBREMIKAEL *et al.*, 2016; SONG *et al.*, 2017).

On the other hand, herbivorous (or plant-parasitic) nematodes are among the main crop pests, which cause significant economic losses in soybean production (MACHADO, 2014; MACHADO; AMARO; DA SILVA, 2019). In a previous study, we demonstrated the suppressive effect of soil bacterial diversity against plant-parasitic nematodes on soybean plants under controlled experimental conditions (BARROS *et al.*, 2022). However, we did not know the relationship between the bacterial community and nematode occurrence under field conditions.

In this study, we hypothesised that soil bacterial diversity is inversely related to the occurrence of plant-parasitic nematodes in soybean fields, while the occurrence of free-living nematodes is positively related. Our objectives were (i) to characterize the bacterial and nematode (free-living and plant-parasitic) community in soybean cultivated soils in Brazil; (ii) to investigate the correlation between the bacterial community (diversity, composition, and abundance) and the nematode community; and (iii) to study the correlation between bacterial diversity, total nematodes, and plant-parasitic nematodes and soybean yields.

3.2. Materials and Methods

3.2.1. Sampling locations and description of soil collections

This study used 176 soil samples that were collected from the main soybean producing areas in four different biomes in Brazil (Amazon Rainforest, Atlantic Rainforest, Cerrado, and Pampa) (Figure 1; Table S1) during the 13th Edition of the National Challenge of Maximum Soybean Productivity - 2020/21 growing season (CESB, 2020). The samples were collected between January and May 2021. Samplings were carried out using an auger, at a depth of 0 - 0.2 m, at the soybean line, in areas from 2.5 to 10 ha. In all sampled areas, each composite sample comprised five samples. The auger was sanitised between each sampling point.

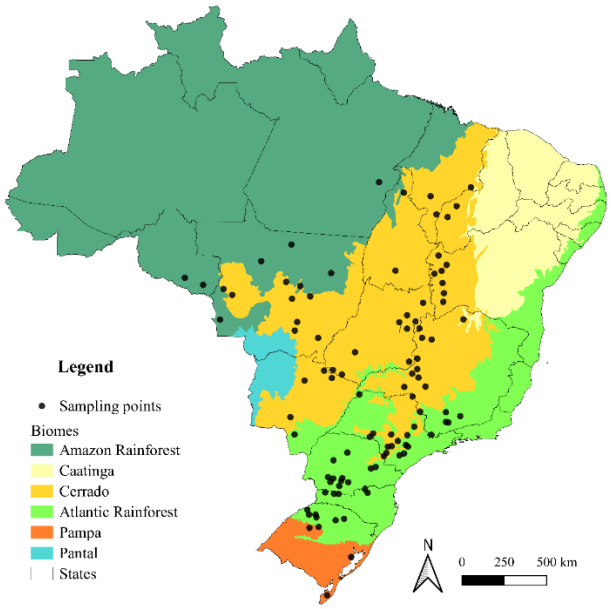

Figure 1. Map of sampling points along the Brazilian territory.

3.2.2. Environmental variables and soybean yield

Soil physicochemical properties: clay content, pH, soil organic matter (SOM) content, and available phosphorus (P) of the soil samples, in addition to soybean yields at each sampled site, were provided by the CESB research network. Briefly, the clay content was determined by the pipette method, the pH in $CaCl_2$ 0.01 mol L^{-1}, the SOM was determined by the colorimetric method, and the available P was extracted using ion-exchange resin and determined by the colorimetric method (EMBRAPA, 2009). Climatic data: average annual temperature (ºC) and total annual rainfall (mm) (30-year average observations) were taken from Climatempo database (CLIMATEMPO, 2022).

3.2.3. Bacterial community analysis

Total DNA was extracted from 0.25 g of soil using the DNeasy PowerSoil Kit (QIAGEN Laboratories, Carlsbad, CA, USA), following the manufacturer's instructions. The integrity of soil DNA was verified by 1.5 % agarose gel electrophoresis at 80 V for 40 min, in 1.0x TAE buffer (Tris, Acetate, EDTA) stained with SYBR® Safe ™ (Invitrogen, Carlsbad, CA). In addition, we used PicoGreen ® (Thermo Fisher Scientific) to check the DNA quantity.

We sequenced the V3-V4 region of the bacterial 16S rRNA gene. For that, 16S rRNA gene sequences were amplified from DNA samples using primers 515f (GTGYCAGCMGCCGCGGTAA) and 806r (GGACTACNVGGGTWTCTAAT) (APPRILL *et al.*, 2015; PARADA; NEEDHAM; FUHRMAN, 2016). The amplicon libraries were created following the standard Illumina metagenomic sequencing library preparation protocol. PCR conditions were 95°C for 3 min, followed by 25 cycles of 95°C for 20 sec, 55°C for 10 sec, and 72°C for 20 sec, with a final extension at 72°C for 5 min. All reactions (20 µl) were conducted with KAPA High-Fidelity DNA Polymerase with 5 µl of DNA as template. All samples were quantified using PicoGreen ®, pooled to equimolar amounts, and run on Illumina MiSeq platform with 300 base paired end readings.

Quantitative Insights Into Microbial Ecology (QIIME, version 2022.2) (BOLYEN *et al.*, 2019) was used to analyse the sequencing data. The q2 DADA2 (CALLAHAN *et al.*, 2016) plugin was used to trim sequences, denoise and create paired end readings. The resulting amplicon sequences variants (ASV) were assigned taxonomy using the q2 feature classifier (BOKULICH *et al.*, 2018) and Greengenes 13 reference database (MCDONALD *et al.*, 2012).

3.2.4. Nematode community extraction

The nematode communities were extracted from soil samples as described by the method presented by Jenkins (1964). Briefly, 50 g of soil and 2 L of water were mixed in a Becker. Then, the soil:water solution was sieved through 20 and 400 mesh. The retained material on the 400-mesh sieve was poured into 50 mL tubes and centrifuged at 1800 rpm for 5 min. After centrifugation, the supernatant was discarded, and sucrose-water solution (400 g L^{-1}) was added into the tubes. Then, the tubes were centrifuged at 1800 rpm for 1 min. The supernatant was sieved through a 500-mesh sieve from which the retained material was washed out and stored in glass jars. The final

volume of the water-nematode suspension was 10 mL. Nematode samples were separated into two parts: (i) for the analysis of total nematode community and (ii) for the analysis of plant-parasitic nematode community.

3.2.5. Total nematode community analysis

The nematode samples were lyophilized and then subjected to DNA extraction using the PureLink® Pro 96 well Genomic DNA Purification Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. 18S rRNA gene sequences were amplified from DNA samples using primers NF1 (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGTGGTGCATGGCCGTTCTTAGTT) and 18Sr2b (GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTACAAAGGGCAGGGACGTAAT) (MULLIN; HARRIS; POWERS, 2003; PORAZINSKA *et al.*, 2009). The amplicon libraries were created following the standard Illumina metagenomic sequencing library preparation protocol. PCR conditions were 95°C for 3 min, followed by 25 cycles of 95°C for 20 sec, 55°C for 10 sec, and 72°C for 20 sec, with a final extension at 72°C for 5 min. All reactions (20 µl) were carried out with KAPA High-Fidelity DNA Polymerase with 2 µl of DNA as template. All samples were quantified using PicoGreen ®, pooled to equimolar amounts, and run on Illumina MiSeq platform with 300 base paired end readings.

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3.2.6. Plant-parasitic nematode community analysis

The population each plant-parasitic nematode taxon was estimated by counting on Peters' slides using a light microscope. Temporary (formalin) and/or permanent (glycerin) slides were examined under a microscope with a clear camera for identification to species level. The identification was carried out based on morphological and morphometric characteristics. The free-living nematodes were counted in total.

3.2.7. Statistical analysis

The soil biological community (bacterial, total nematode, and plant-parasitic nematode community) structure in the samples were subjected to the Redundancy analysis (RDA) using the Canoco version 4.5 (LEPŠ; ŠMILAUER, 2003). The significance between environmental variables and biological community structure was verified using forward selection (FS) followed by the Monte Carlo permutation test. In addition, the Mantel tests were used to evaluate the linkages between the biological community structure and geographic distances, using geographical coordinates in the Paleontological Statistics freeware package (PASTv.4) (HAMMER; HARPER; RAYAN, 2001). The differences in the biological community structure between the biomes (Amazon Rainforest, Atlantic Rainforest, Cerrado, and Pampas) were evaluated by the PERMANOVA analysis using the PASTv.4, which was also used to calculate richness, biological indexes (richness, diversity (Shannon – H'), and evenness (Pielou – J).

To compare the relative bacterial abundances and total nematode groups between the biomes, the ASV tables were used as input in the software STAMP (PARKS; BEIKO, 2010). P values were calculated based on a two-sided Welch's *t* test and correction using the Benjamini-Hochberg false-discovery rate. To compare population density of plant-parasitic nematode taxa between the biomes, the data were subjected to the Kruskal-Wallis non-parametric analysis of variance, followed by the Dwass-Steel-Critchlow-Fligner test at 5% probability using the Jamovi 1.6 software.

The five most abundant classes of bacteria, the five most abundant order of total nematode, and the three most abundant taxa of plant-parasitic nematodes in the biomes were plotted on boxplots. The results at each sampling point and the averages by states in Brazil were presented in maps. The Spearman correlation analysis with the Bonferroni correction was carried out to investigate the relationship between the relative abundance of the 100 most abundant bacterial genera, bacterial ecological indexes, relative abundance of total nematode orders, plant-parasitic nematode population density, and soybean yield in the Cerrado and Atlantic Rainforest biomes, using PASTv.4. The bacterial genera that showed at least one correlation higher than 0.35 or lower than -0.35 were represented in heatmaps. We did not perform the correlation tests for the Amazon Rainforest and Pampa due to the small number of samples collected from these biomes.

3.3. Results

3.3.1. Environmental variables and soybean yield

The pH of the soil samples ranged from 4.4 to 6.5 with an average of 5.5. The clay content ranged from 63 to 697 g kg^{-1} with an average of 379.2 g kg^{-1}. The SOM content ranged from 9 to 106 g dm^3 with an average of 36.4 g dm^3. The available P content ranged from 5 to 203 mg dm^3 with an average of 62.0 mg dm^3. The average annual temperature ranged from 17.7 to 27.2 ºC with an average of 22.7 ºC. The total annual rainfall ranged from 778 mm to 2229 mm with an average of 1516.4 mm. Lastly, soybean yield ranged from 3,415 to 7,750 kg ha^{-1} with an average of 5,678 kg ha^{-1} among the sampling sites (Figure S1). The averages of environmental variables and soybean yield in each biome were presented in the supplementary material (Table S2).

3.3.2. Community structure of bacteria, total nematodes, and plant-parasitic nematodes and their relationships with environmental variables

The redundancy analysis (RDA) was used to determine the relationship between biological community (bacterial, total nematode, and plant-parasitic nematode community) structure and environmental variables. For the bacterial community, the Monte Carlo permutation test showed that the bacterial community structure was significantly related to total annual rainfall ($F = 3.26$; $p < 0.05$). The RDA plot revealed that the bacterial community was not well clustered according to the biomes (Figure 2a). On the other hand, the PERMANOVA analysis confirmed significant differences in the bacterial community structure between the Amazon Rainforest and the Atlantic Rainforest and the Cerrado (Table 1).

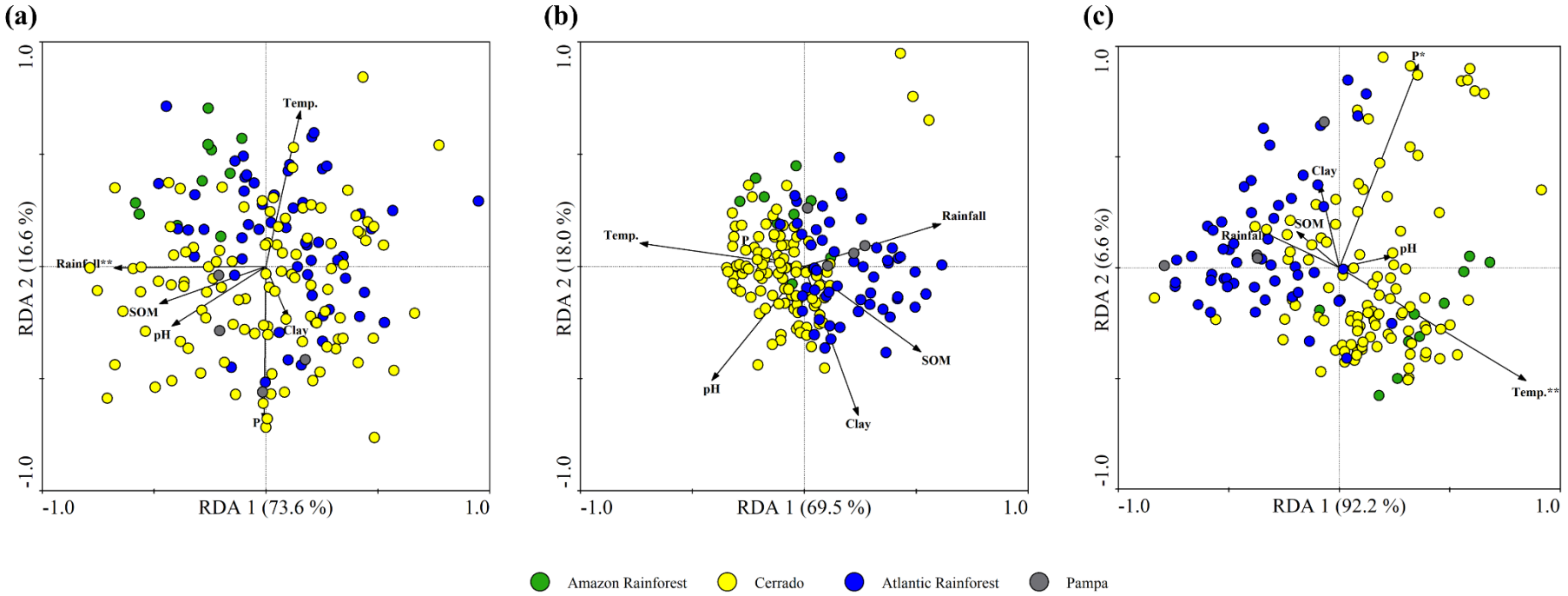

Figure 2. Redundancy analyses (RDA) of the bacterial community (a), total nematode community (b), and plant-parasitic nematode community (c) in soybean cultivated soils in four different biomes in Brazil. Arrows indicate correlation between environmental variables and biological structure of the communities. Clay: soil clay content; SOM: soil organic matter; P: available phosphorus; Temp.: average annual temperature; Rainfall: total annual rainfall. * Significant at 0.05 probability, and ** significant at 0.01 probability level, according to the Monte Carlo permutation test.

For the total nematode community, the RDA plot presented well defined groups, according to the biomes (Figure 2b), and significant differences between the Atlantic Rainforest and the Cerrado were confirmed by the PERMANOVA (Table 1). For the plant-parasitic nematode community, the Monte Carlo permutation test results showed that the community structure was significantly related to average annual temperature ($F = 11.12$; $p < 0.01$) and available P ($F = 4.74$; $p < 0.05$). The plant-parasitic nematode community was also clustered according to the biomes (Figure 2c) and significant differences between the Atlantic Rainforest and the Cerrado were confirmed by the PERMANOVA (Table 1).

The Mantel tests were used to evaluate the linkages between biological community (bacterial, total nematode, and plant-parasitic nematode community) structure and geographic distances between the sampling sites. No significant correlations were observed between the biological community structure and the geographic distances ($p > 0.05$).

Table 1. Differences in bacterial communities, total nematodes, and plant-parasitic nematodes in soybean cultivated soils between biomes in Brazil. The F statistic values represent the magnitude of change in the community structure.

	Bacteria		Total nematode		Plant-parasitic nematode	
	F	P value	F	P value	F	P value
Amazon Rainforest, Atlantic Rainforest	5.01	0.001	0.80	0.522	2.04	0.075
Amazon Rainforest, Cerrado	3.45	0.007	0.26	0.871	0.18	0.974
Amazon Rainforest, Pampa	0.93	0.430	0.50	0.741	2.12	0.062
Atlantic Rainforest, Cerrado	1.98	0.068	3.55	0.013	3.87	0.003
Atlantic Rainforest, Pampa	2.12	0.054	0.47	0.749	1.19	0.285
Cerrado, Pampa	1.86	0.082	0.28	0.864	2.09	0.073

3.3.3. Composition and diversity of bacterial, total nematode, and plant-parasitic nematode communities

The soil bacterial community was composed of 56 phyla, 146 classes, and 823 genera based on the Greengenes 13 reference database. The taxonomic composition analysis revealed that Vicinamibacteria (9%), Alphaproteobacteria (8%), Bacilli (7%), Actinobacteria (7%), and Blastocatellia (6%) were the predominant classes along the sampling sites (Figure 3a). We observed higher abundance of class Vicinamibacteria in the Atlantic Rainforest when compared to the Cerrado ($p < 0.05$) (Figure 3b). We also observed higher abundance of Alphaproteobacteria in the Atalntic Rainforest and the Cerrado when compared to the Amazon Rainforest ($p < 0.05$). The class Bacilli was more abundant in the Cerrado when compared to the Atlantic Rainforest ($p < 0.05$). The biomes did not affect richness, diversity, and equitability of bacteria ($p > 0.05$) (Figures S2a, S2b, and S2c).

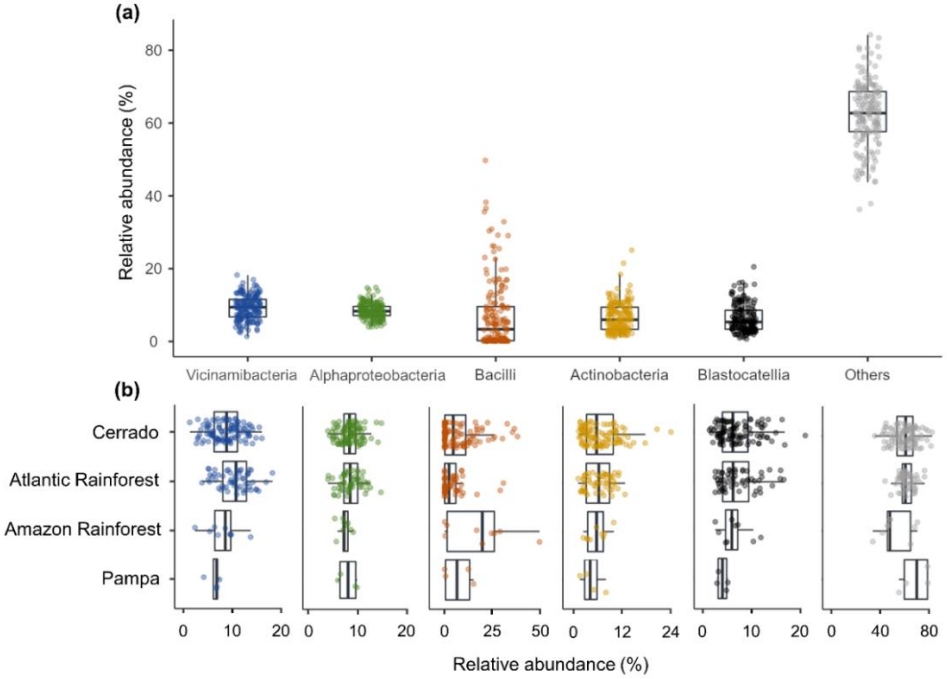

Figure 3. The median and interquartile range of bacterial relative abundance per class (a) and per biome (b).

The total nematode community comprised 11 orders based on SILVA database. The orders Rhabditida (45%), Tylenchida (29%), Dorylaimida (12%), Triplonchida (7%), and Enoplida (3%) were predominant along the sampling sites (Figure 4a). We observed higher abundance of the order Triplonchida in the Atlantic Rainforest when compared to the Amazon Rainforest and the Cerrado ($p < 0.05$) (Figure 4b). The biomes did not affect richness, diversity, and equitability of total nematode ($p > 0.05$) (Figures S2d, S2e, and S2f).

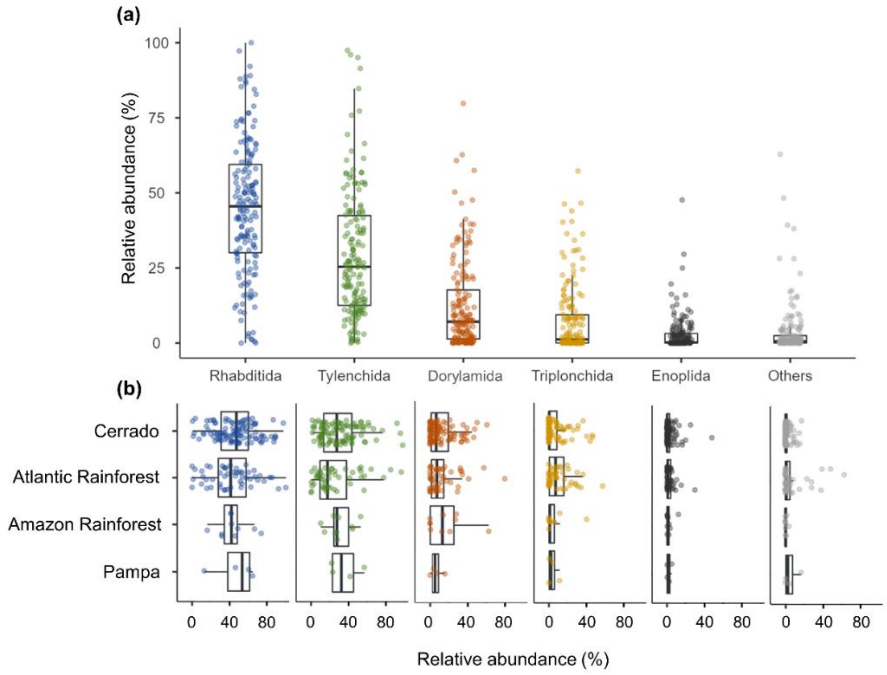

Figure 4. The median and interquartile range of relative abundance of total nematode per order (a) and per biome (b).

The plant-parasitic nematode community was composed of five families (Heteroderidae, Pratylenchidae, Hoplolaimidae, Longidoridae, and Criconematidae) and seven genera (*Meloidogyne*, *Pratylenchus*, *Heterodera*, *Rotylenchulus*, *Helicotylenchus*, *Scutellonema*, and *Xiphinema*). Among which, the species *Pratylenchus. brachyurus*, *P. penetrans*, *P. zeae*; *Heterodera glycines*, *Rotylenchulus reniformis*, *Helicotylenchus dihystera*, and *Scutelonema brachyurus* were identified. *H. dihystera* and *Pratylenchus* sp. were the nematodes with the highest abundance along the sampling sites (Figure 5c). The species *Helicotylenchus dihystera* was more abundant in the Atlantic Rainforest when compared to the Cerrado ($p < 0.05$) (Figure 5b). The biomes did not affect richness, diversity, and equitability of plant-parasitic nematode ($p > 0.05$) (Figures S2g, S2h, and S2i).

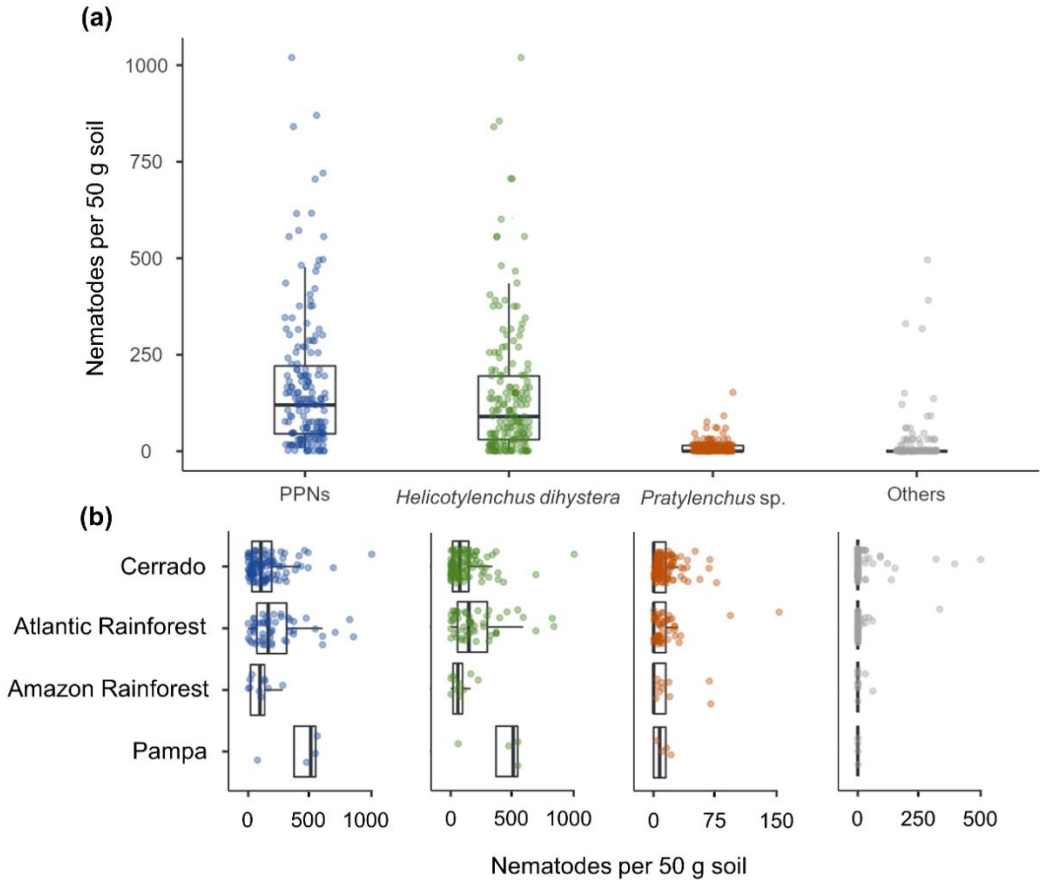

Figure 5. The median and interquartile range of plant-parasitic nematode abundances per taxon (a) and per biome (b).

The relative abundance of bacterial classes and total nematode orders, as well as population density of plant-parasitic nematodes, showed a wide variation even in sampling sites that were geographically near. In the bacterial community, the relative abundance of Bacilli varied up to 50%, Actinobacteria varied up to 21%, Blastocatellia varied up to 19%, Vicinamibacteria varied up to 13%, and Alphaproteobacteria varied up to 11% within the same state (Figure S3). In the total nematode community, the relative abundance of Tylenchida varied up to 97%, Rhabditida varied up to 96%, Dorylaimida varied up to 80%, Triplonchida varied up to 57%, and Enoplida varied up to 48% within the same state (Figure S4). In the plant-parasitic nematode community, *H. dihystera* varied up

to 1020 nematodes per 50 g of soil, while *Pratylenchus* sp. varied up to 150 nematodes per 50 g of soil within the same state (Figure S5).

3.3.4. Correlation between the main soil bacteria genera, relative abundance of total nematodes, and plant-parasitic nematode population density

The Spearman's correlation analysis was performed to investigate the relationship between the relative abundance of the 100 most abundant bacterial genera, ecological indexes, relative abundance of total nematode orders, and plant-parasitic nematode population density in each biome. We observed a large number of significant correlations ($p < 0.05$) between population density of *Pratylenchus* sp. and relative abundance of several bacterial genera in the Cerrado and Atlantic Rainforest (Figure 6). We highlighted *Paenibacillus*, *Streptomyces*, *Cohnella*, *Paenarthrobacter*, and *Aquisphaera*, which presented strong and negative correlations (Spearman's rho < -0.45; $p < 0.05$). In contrast to our hypothesis, diversity and evenness of bacteria presented positive correlations with population density of *Pratylenchus* sp. (rho = 0.33, $p < 0.05$; rho = 0.42, $p < 0.01$, respectively). Lastly, no significant correlations were observed between the bacterial ecological indexes and the relative abundance of total nematode orders ($p > 0.05$).

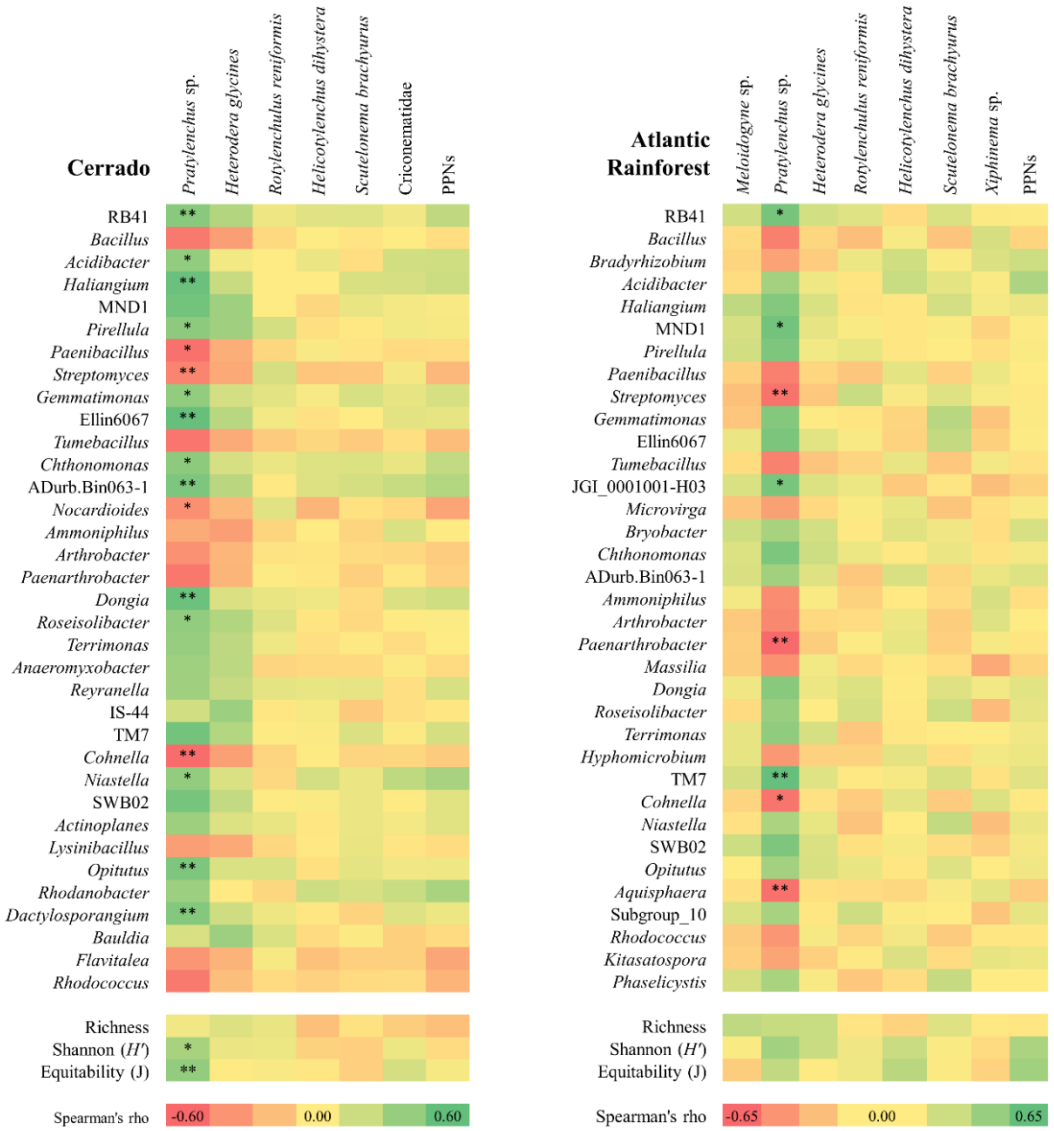

Figure 6. Heatmaps of the Spearman's rank correlation coefficients of relative abundance of bacterial genera and bacterial ecological indexes with nematode population density in soybean cultivated soils in the Brazilian Cerrado and the Atlantic Rainforest. * Significant at 0.05 probability and ** significant at 0.01 probability level.

3.3.5. Correlation between ecological indexes and soybean yield

The Spearman's correlation analysis was also performed to investigate the relationship between the ecological indexes and soybean yield. We observed a positive correlation between total nematode richness and soybean yield (rho = 0.31, $p < 0.05$) in the Cerrado (Figure S6). On the other hand, we observed a negative correlation between richness, diversity, and evenness of plant-parasitic nematodes and soybean yield (rho = -0.41, $p < 0.01$; rho = -0.40, $p < 0.01$; rho = -0.40, $p < 0.01$, respectively). Lastly, no significant correlations were observed between bacterial ecological indexes and soybean yield in any of the biomes evaluated ($p > 0.05$).

3.4. Discussion

We analysed the bacterial community, total nematode, and plant-parasitic nematode in 176 samples of soybean cultivated soils in four different biomes in Brazil (Amazon Rainforest, Atlantic Rainforest, Cerrado, and Pampa). To the best of our knowledge, our study is the first effort to characterize these biological communities in Brazilian soils on a national scale and to investigate the relationship between them.

The bacterial community structure, total nematodes, and plant parasitic nematodes did not show significant correlations with the geographical distances between the sampling points along the Brazilian territory. On the other hand, these communities were grouped according to the biomes, especially total nematode and plant-parasitic nematode communities. The Brazilian biomes have a wide variety of climates and soils with a wide diversity of different ecosystems (MAIA *et al.*, 2020). Thus, we argue that these biological communities may have been structured by deterministic, niche-based processes related to environmental factors (*e.g.*, rainfall, temperature, and soil properties) in each biome (LUAN *et al.*, 2020; RIGONATO *et al.*, 2018). This hypothesis may also be supported by the significant relationship between the bacterial community structure and total annual rainfall ($F = 3.26$; $p < 0.05$), between the plant-parasitic nematode community structure and average annual temperature ($F = 11.12$; $p < 0.01$), and available P ($F = 4.74$; $p < 0.05$).

The abundance of organisms, mainly the relative abundance of nematodes and population density of plant-parasitic nematodes, presented a wide variation in each biome studied, even in geographically near locations (Figure S3, S4, and S5). This reinforces the evidence that these communities are structured by deterministic processes. Although deterministic and stochastic processes act in a non-exclusive mutual manner, nematode communities are more structured by selection based on deterministic processes due to their lower dispersal rates compared to bacteria (LUAN *et al.*, 2020). Nematodes are capable of adapting to constant disturbances and environmental changes, many of which are caused by human activities in agricultural soils (LAZAROVA *et al.*, 2021).

The higher relative abundance of Vicinamibacteria observed in the Atlantic Rainforest when compared to the Cerrado may be related to the higher rainfall in the Atlantic Rainforest (Table S1). Vicinamibacteria (phylum Acidobacteria) is a class of drought-sensitive aerobic heterotrophic bacteria, which are found most abundantly in moist soils (HARTMANN *et al.*, 2017; HUBER *et al.*, 2022; RODRIGUEZ-RAMOS *et al.*, 2022). On the other hand, the lower rainfall and higher temperature in the Cerrado may have provided the highest relative abundance of Bacilli in this biome when compared to the Atlantic Rainforest. Species of Bacilli (phylum Firmicutes) are extremophiles in nature, which can survive extreme environmental conditions (*e.g.*, radiation, salinity, or even desiccation) (ROY *et al.*, 2022).

Regarding the total nematode community, we observed that the Silva 132 database was not able to affiliate the sequences to the family or genus level. A higher abundance of the order Triplonchida in the Atlantic Rainforest when compared to the Amazon Rainforest and Cerrado. The order Triplonchida includes the family Trichodoridae, which are polyphagous, ectoparasitic plant nematodes, with a worldwide distribution, and are important natural vectors of tobraviruses to plants (DECRAEMER; ROBBINS, 2007). Mattos *et al.* (2008) characterized the community of plant-parasitic nematodes in areas of native vegetation and agriculture in the Cerrado region of central Brazil. The authors observed the occurrence of Trichodoridae (*Paratrichodorus* and *Trichodorus*) only in agricultural areas, suggesting that these nematodes have been introduced to the Cerrado with agricultural activities (MATTOS *et al.*, 2008). We highlight that Triplonchida was not found in the microscopy analyses, which shows the importance of using molecular biology for the most complete description of the soil nematode community.

Regarding the plant-parasitic nematode community, we observed a higher population density of *H. dihystera* in the Atlantic Rainforest when compared to the Cerrado. *H. dihystera* is a migratory ectoparasite, or semi-endoparasite, often found in the soil, which can penetrate soybean roots and cause brown lesions. Epidemiology information of *H. dihystera* in tropical countries is still scarce (GARDIANO-LINK *et al.*, 2022). Our analyses have shown that this nematode can be found in different soil types (Chapter 4).

The Spearman's correlation analysis between the most abundant bacterial genera and the plant-parasitic nematode population density showed that some bacterial genera have a high potential for the suppression of *Pratylenchus* sp. (Figure 6). We observed significant negative correlations with *Streptomyces* and *Paenibacillus*, which act in the suppression of plant-parasitic nematodes (MARIN-BRUZOS *et al.*, 2021; SAMAC; KINKEL, 2001; TRIPATHI, 2018), as well as other genera of bacteria (*e.g.*, *Nocardioides*, *Cohnella*, *Paenarthrobacter*, and *Aquisphaera*) that could be potential targets in studies of bioprospecting bacteria for the control of *Pratylenchus* sp. We also observed positive correlations between some genera of bacteria and *Pratylenchus* sp. These positive correlations were expected to occur, since some bacteria can associate with nematodes and act to protect against antagonistic microorganisms or suppress the plant immune response (TOPALOVIĆ; VESTERGÅRD, 2021).

We observed positive correlations between bacteria diversity and population density of *Pratylenchus* sp. These results corroborate our previous study in which we observed a higher population density of *Pratylenchus brachyurus* in treatments with a higher bacterial diversity in a controlled experiment under greenhouse conditions (BARROS *et al.*, 2022). We argue that the role of the bacterial community against nematodes of the genus *Pratylenchus* occurs through specific suppressiveness, which is related to the activity of specific groups of bacteria that interfere at some stage of the nematode life cycle, or minimize the damage caused by the disease (EXPÓSITO *et al.*, 2017; WELLER *et al.*, 2002).

We also investigated the correlation between the ecological indexes and soybean yield. The positive correlation between the total nematode richness and soybean yield highlights the importance of a complex soil food web to maintain and boost plant productivity (FERRIS, 2010; LESLIE *et al.*, 2017). It is possible that in areas with higher total nematode richness there is more intense mineralization of organic matter, due to microbial turnover caused by the feeding of microbivores nematodes (Trap *et al.*, 2016). In this context, soil management practices, such as no-tillage farming, crop rotation, cover crops and organic matter addition, which benefit the soil faunal community (MOURA; FRANZENER, 2017), may increase the yields of soybean fields. On the other hand, the negative correlation between the plant-parasitic nematode diversity and soybean yield may be related to positive interactions between different species of plant-parasitic nematodes in sites with higher diversity, which can result in greater damage to plants (FONTANA *et al.*, 2018; TOMAZINI; GUERREIRO FILHO; MARCELO DE OLIVEIRA, 2021).

3.5. Conclusions

Our results show that bacterial communities, total nematodes, and plant-parasitic nematodes in Brazilian soybean crops are different according to biomes. These biological communities are possibly structured by deterministic processes related to environmental variables in each biome and to agricultural management practices in each crop. Vicinamibacteria, Alphaproteobacteria, Bacilli, Actinobacteria, and Blastocatellia were the most abundant classes of bacteria found in these crops, while Rhabditida, Tylenchida, Dorylaimida, Triplonchida, and Enoplida were

the most abundant orders of nematodes found. The most abundant plant-parasitic nematodes were *Helicotylenchus dihystera* and *Pratylenchus* sp.

Our hypotheses were not confirmed. Conversely, we observed positive correlations between bacterial diversity and population density of *Pratylenchus* sp. While the abundance of specific genera of bacteria (*e.g.*, *Streptomyces, Paenibacillus, Nocardioides, Cohnella, Paenarthrobacter, and Aquisphaera*) showed a negative correlation to population density of the plant-parasitic nematode. These results support the conclusion that the role of the bacterial community against nematodes of the genus *Pratylenchus* occurs through specific suppressiveness.

In addition, our results showed that total nematode richness correlated positively to soybean yield, highlighting the importance of free-living nematodes to maintain soil functions in agriculture.

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Supplementary Material

Table S1. Locality (Region, State, and Municipality) and number of samples (n)

Region	State	n	Municipality
North	Pará	1	Curionópolis
	Rondônia	1	Corumbiara
	Tocantins	2	Peixe, Wanderlândia
Northeast	Bahia	24	Formosa do Rio Preto, São Desidério, Correntina, Jaborandi, Luís Eduardo Magralhães, Riachão das Neves, Barreiras
	Maranhão	4	Nova Colinas, Tasso Fragoso, São João dos Patos
	Piauí	6	Baixa Grande do Ribeiro, Uruçuí
Central-West	Distrito Federal	3	Brasília
	Goiás	14	Formosa, Água Fria de Goiás, Cristalina, Iaciara, Cabeceiras, Padre Bernardo, Aporé, Catalão, Montividiu
	Mato Grosso do Sul	12	Chapadão do Sul, São Gabriel do Sul, Maracaju, Costa Rica, Laguna Carapã
	Mato Grosso	16	Querência, Vila Bela da Santíssima Trindade, Novo Horizonte do Norte, Nova Ubiratã, Nova Guarita, Jaciara, Guiratinga, Sorrriso, Campo Verde, Paranatinga, Comodoro, Campos de Júlio, Santa Rita do Trivelato
Southeast	Minas Gerais	24	Coromandel, Monte Carmelo, Unaí, Patrocínio, Itacarambi, Bonfinópolis de Minas, Guarda-Mor, Ibiá, Uberaba, Minte Santo de Minas, Bonfinópolis de Minas, Madre de Jesus de Minas, Conceição do Rio Verde, Combuquira, Nepomuceno
	São Paulo	26	Itapeva, Itaberá, Paranapanema, Piracicaba, Itararé, Campos Novos Paulista, Itaí, Pedregulho, Lenções Paulista, Santa Clara d'Oeste, Capão Bonito, Capela do Alto, Tatuí, São Miguel Arcanjo, Pardinho, Leme, Palmital
South	Paraná	19	Pinhão, Candoí, Castro, Mangueirinha, Mamborê, Quedas do Iguaçú, Rio Bonito do Iguaçú, São João, Foz do Iguaçú, Carambeí, Kaloré
	Rio Grande do Sul	14	Cruz Alta, Nova Ramada, Santo Augusto, Independência, Santa Cecília do Sul, Passo Fundo, Horizontina, Tapes, Jóia, Santa Vitória do Palmar
	Santa Catarina	10	Campo Erê, Abelardo Luz, Canoinhas, São Domingos, Major Vieira

Table S2. Environmental variables (mean followed by standard deviation) in soybean fields in each biome studied.

	Clay (g kg^{-1})	pH	SOM (g dm^3)	P (mg dm^3)	Temperature (^oC)	Rainfall (mm)	Yield (kg ha^{-1})
Amazon Rainforest	283.80 ± 91.20	5.41 ± 0.63	34.20 ± 12.24	49.60 ± 33.95	26 ± 1	1717 ± 76	5316 ± 396
Atlantic Rainforest	419.18 ± 142.29	5.49 ± 0.27	46.14 ± 16.50	58.95 ± 41.06	20 ± 2	1765 ± 307	6019 ± 840
Cerrado	370.72 ± 172.85	5.54 ± 0.44	31.43 ± 13.12	64.81 ± 52.84	24 ± 2	1364 ± 301	5508 ± 807
Pampa	275.25 ± 109.42	5.25 ± 0.31	33.75 ± 9.00	64.00 ± 52.54	19 ± 1	1567 ± 280	6346 ± 1708

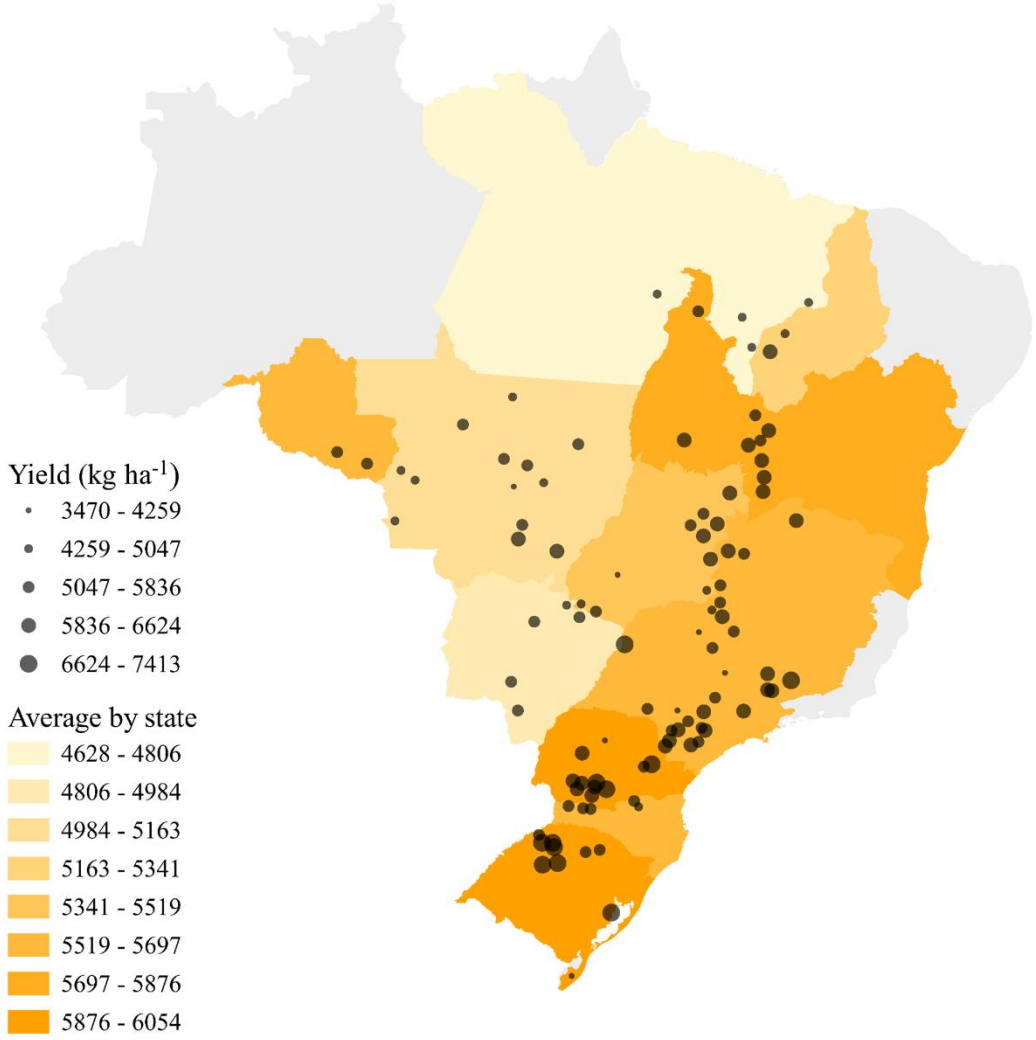

Figure S1. Grain yield map of soybean (kg ha^{-1}) in Brazil for the 2020/2021 growing season. Point sizes represent the yield at each municipality. Colour intensity represents the average yield in each state.

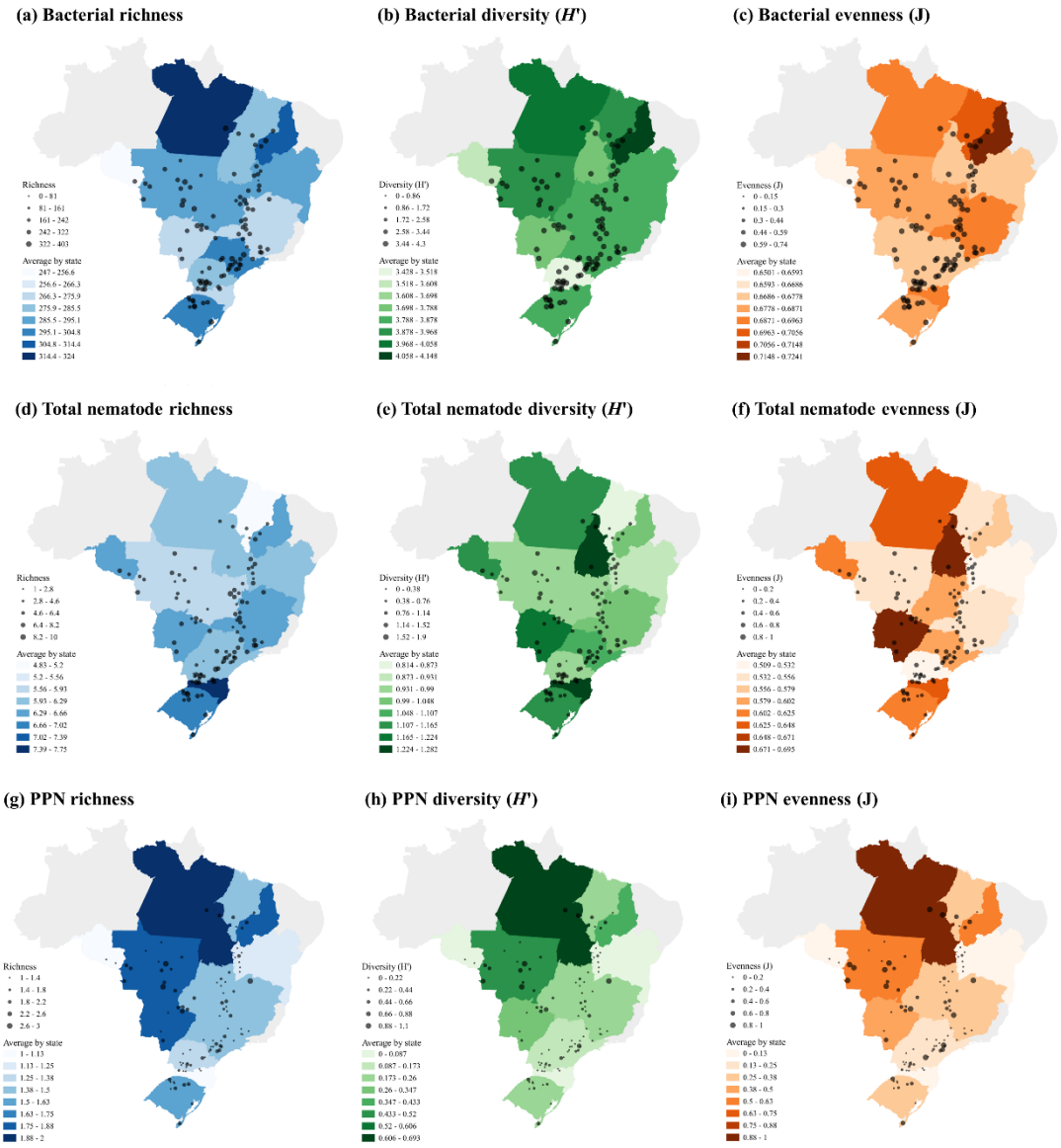

Figure S2. Maps of the ecological indexes (richness, diversity, and evenness) of bacteria, total nematode, and plant-parasitic nematode (PPN) in soybean cultivated soils in Brazil. Point sizes represent the value of the ecological index at each municipality. Colour intensity represents the value of the ecological index in each state.

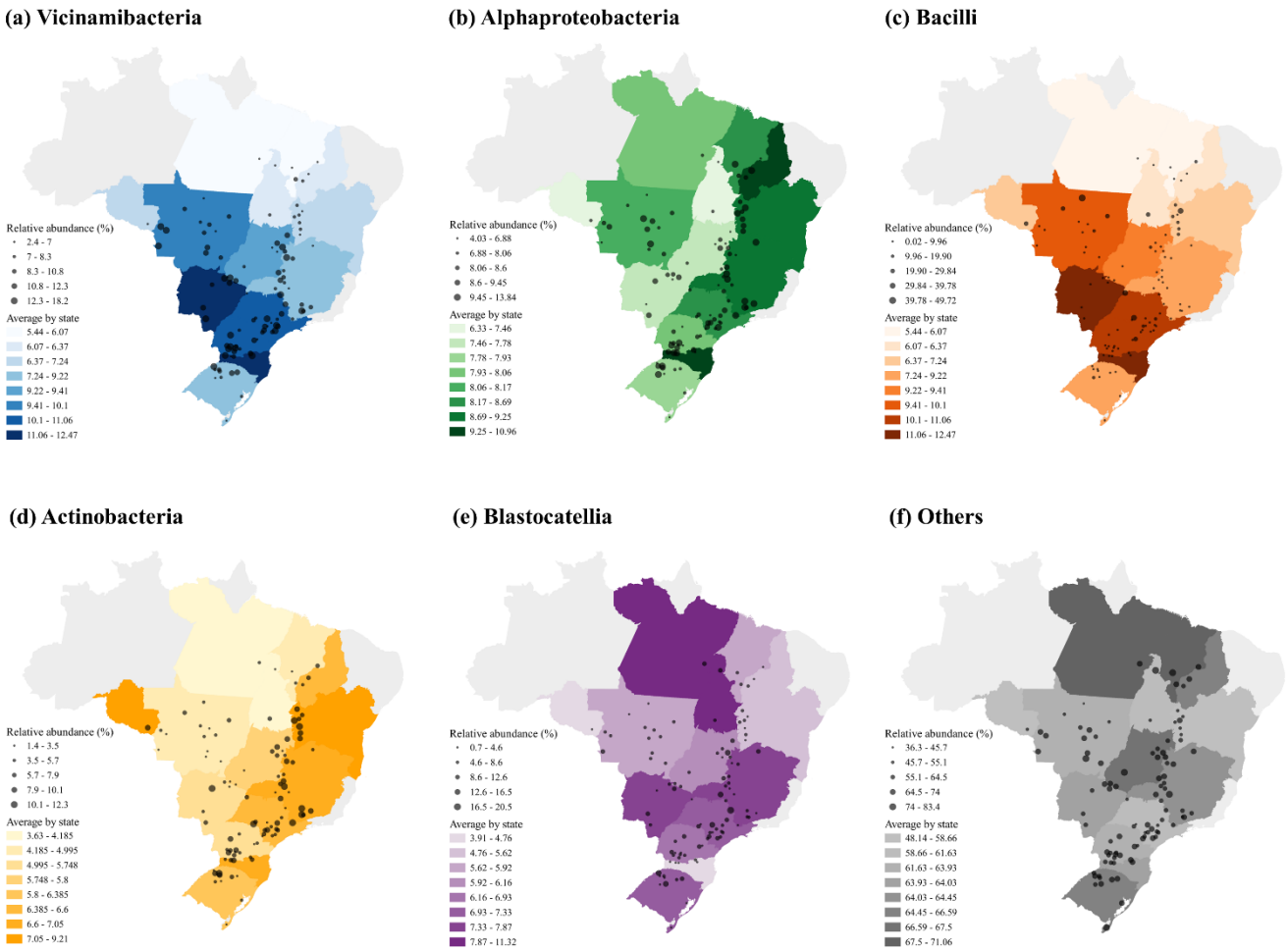

Figure S3. Maps of relative abundance of bacterial classes in soybean cultivated soils in Brazil. Point sizes represent the relative abundance at each municipality. Colour intensity represents the average relative abundance in each state.

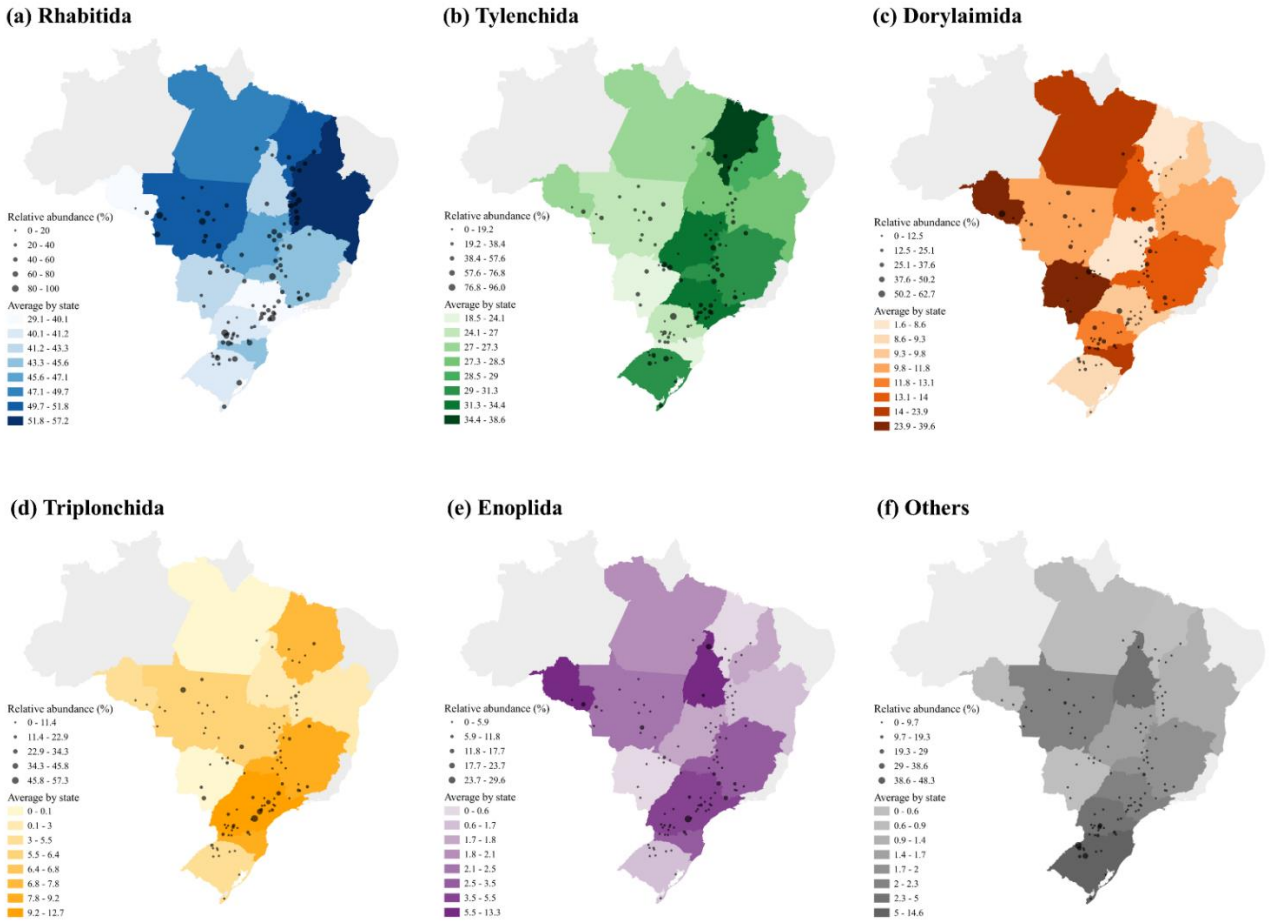

Figure S4. Maps of relative abundance of total nematode orders in soybean cultivated soils in Brazil. Point sizes represent relative abundance at each municipality. Colour intensity represents average relative abundance in each state.

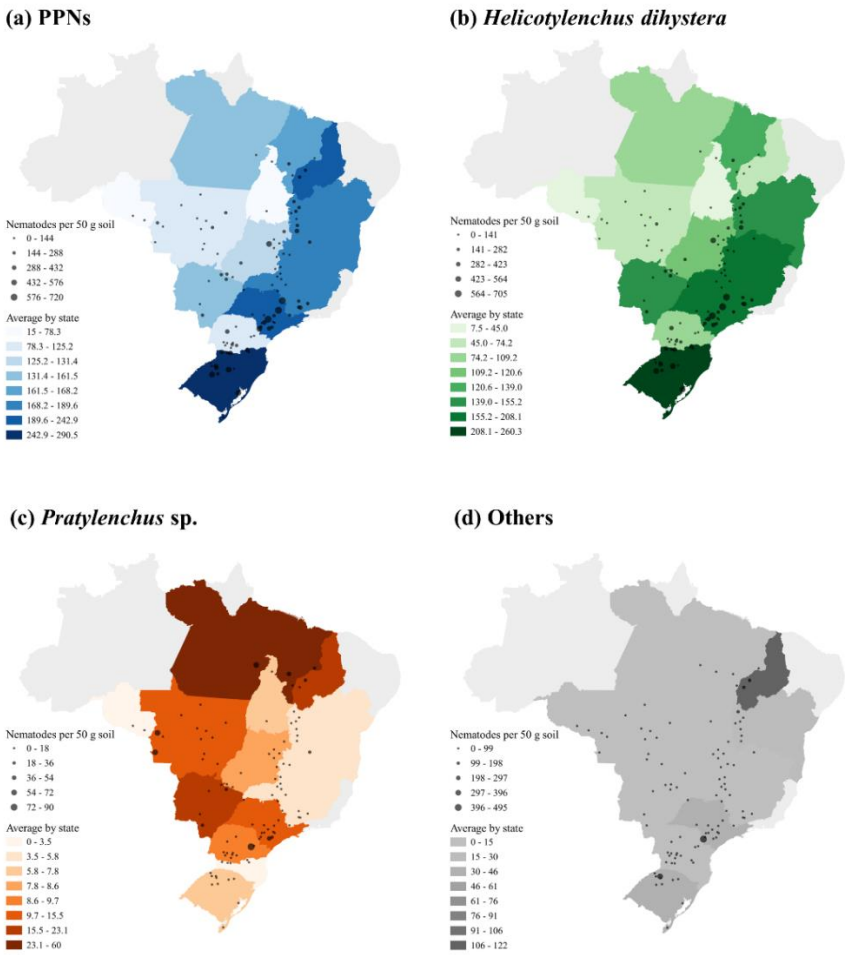

Figure S5. Maps of population density of plant-parasitic nematodes in soybean cultivated soils in Brazil. Point sizes represent population density at each municipality. Colour intensity represents average population density in each state.

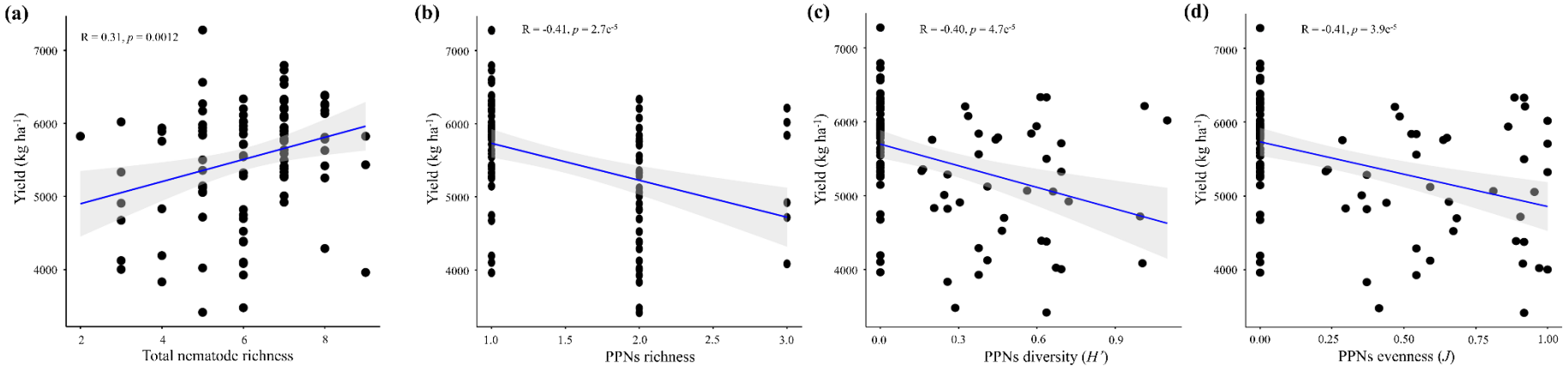

Figure S6. Scatter plot of the Spearman's rank correlation coefficient between total nematode richness and soybean yield (a), plant-parasitic nematode (PPNs) richness and soybean yield (b), PPNs diversity and soybean yield (c), and PPNs evenness and soybean yield (d).

4. PLANT-PARASITIC NEMATODE COMMUNITY AND MICROBIAL ACTIVITY IN SOILS UNDER NO-TILL SOYBEAN CROPS IN BRAZIL

Abstract

Soybean is the largest agricultural crop in Brazil, distributed along a variety of climates and soil conditions, and it is subjected to a large number of pests, among which, plant-parasitic nematodes are highly important. In general, the occurrence of plant-parasitic nematodes in soybean is influenced by soil physicochemical properties, such as the pH, texture, and nutrient content. These soil properties may determine the nematodes community composition and affect soil quality and functioning, which can be monitored by the analysis of microbial enzymes activity. In this sense, the analysis of β-glucosidase and acid phosphatase enzymes is recommended to assist decisions in the sustainable management of soybean cultivated soils in Brazil. In this study, we used 216 samples collected from no-till soils cultivated with soybean across Brazil. The samples were used to investigate the influence of soil physical and chemical properties on the plant-parasitic nematode community and enzymatic activity. The nematode genera *Meloidogyne*, *Pratylenchus*, *Heterodera*, *Rotylenchulus*, *Helicotylenchus*, *Scutellonema*, and *Xiphinema* were identified. The clay content and soil pH were the main properties modulating the plant-parasitic nematode community. We observed a higher abundance of plant-parasitic nematodes in highly acidic soils when compared to slightly acidic. The soil organic matter content and the pH were the main properties modulating microbial activity in the soil. No significant correlations were observed between enzymatic activity and nematode population density. This is the first study to provide insights into plant-parasitic nematode community composition and microbial activity in soybean cultivated soils on a national scale in Brazil.

Keywords: Soil properties, *Pratylenchus*; Soil enzymes; β-glucosidase; Acid phosphatase

4.1. Introduction

Soybean (*Glycine max* [L.] Merrill) is the largest and most important agricultural crop in Brazil, reaching approximately 125.5 million tons with more than 41,452 thousand ha planted in the 2021/22 growing season (CONAB, 2022). Soybean crops are grown in all five regions of the country with widely varying yields; however, with an average of only 3,029 kg ha^{-1}, due to a wide range of soil and climate conditions (CONAB, 2022; IBGE, 2022). Approximately 80% of the soybean area in Brazil is cultivated in no-till farming (FEBRAPDP, 2021), a system that reduces soil erosion (MERTEN *et al.*, 2015), reduces greenhouse gas emissions (SIQUEIRA-NETO *et al.*, 2021) and increases soil biodiversity (SROUR et al., 2020). Increase in soybean yield in Brazil has been stimulated by the National Challenge of Maximum Soybean Productivity, a competition that has been organized by the Brazilian Soybean Strategic Committee (CESB) since the 2008/09 growing season (CESB, 2020).

The need to deal with the wide range of pests that damage soybean crops are some of the challenges faced by farmers to keep production at current levels and increase crop yield (BUENO *et al.*, 2021). Among these pests are plant-parasitic nematodes, which cause losses estimated at US$ 3.0 billion per year (MACHADO *et al.*, 2015). The main nematodes that cause damage to soybean crops in Brazil are the soybean cyst nematode (SCN) (*Heterodera glycines*), root-knot nematodes (*Meloidogyne* spp.), root-lesion nematodes (*Pratylenchus brachyurus*), and the reniform nematode (*Rotylenchulus reniformis*) (MACHADO, 2014). In addition, other species (*e.g.*, *Scutellonema brachyurus* and *Helicotylenchus dihystera*) are emerging as threats to the crop (MACHADO; AMARO; DA SILVA, 2019).

The occurrence of plant-parasitic nematodes in soybean crops is influenced by crop management practices and soil physicochemical properties (FREITAS *et al.*, 2017; MACHADO *et al.*, 2022). Soil properties (*e.g.*, soil texture, pH, and organic matter) are considered the main variables that determine the nematode community in similar managed farming systems (CHOWDHURY; YAN; FRISKOP, 2020; SIMON *et al.*, 2018). The soil organic matter (SOM) has presented as an important suppressor of plant-parasitic nematodes (ABD-ELGAWAD, 2021; SILVA; MEDEIROS; CAMPOS, 2018). Decomposition of SOM is promoted by the release of enzymes, such as β-glucosidase and acid phosphatase, which are widely produced by members of the soil microbial community. It is known that quantification of these enzymes serves as an indicator of the microbial activity (LOPES *et al.*, 2018), which is capable of reducing the population of plant-parasitic nematodes (MARIN-BRUZOS; GRAYSTON, 2019). However, monitoring the activity of these enzymes on a national scale had not been carried out yet.

In this exploratory study, our objectives were (i) to characterize the plant-parasitic nematode community in soybean cultivated soils under no-till system across Brazil, (ii) to evaluate the activity of microbial enzymes in these soils, (iii) to investigate the influence of soil attributes on the plant-parasitic nematode community and enzymatic activity, and (iv) to investigate the relationship between the occurrence of plant-parasitic nematodes and soybean yield.

4.2. Materials and Methods

4.2.1. Sampling sites and description of soil collections

This study comprised 216 soil samples collected in soybean crops under no-till system. The samples were collected during the 13th Edition of the National Challenge of Maximum Soybean Productivity - 2020/21 growing season, promoted by CESB. Soils were sampled in the main soybean producing areas in the five regions of Brazil between January and May 2021 (Table 1). Briefly, soil samples were collected using an auger, at a depth of 0 - 0.2 m, at the soybean line, in areas from 2.5 to 10 ha. In all sampled areas, each composite sample comprised five samples. The auger was sanitized between each sampling point.

4.2.2. Soil physicochemical properties and soybean yield

Soil physicochemical properties: clay content, pH, soil organic matter (SOM) content, and available phosphorus (P) of the soil samples, in addition to soybean yield at each sampled site, were obtained from CESB research network. Briefly, the clay content was determined by the pipette method, the pH in $CaCl_2$ 0.01 mol L^{-1}, the SOM was determined by the colorimetric method, and the available P was extracted using ion-exchange resin and determined by the colorimetric method (EMBRAPA, 2009).

4.2.3. Nematode community analysis

The nematodes were extracted from soil samples as described by Jenkins (1964). Briefly, 50 g of soil and 2 L of water were mixed inside a Becker. Then, the soil:water solution was sieved through 20 and 400-mesh. The

retained material on the 400-mesh sieve was poured into 50 mL tubes and centrifuged at 1800 rpm for 5 min. After centrifugation, the supernatant was discarded, and sucrose-water solution (400 g L^{-1}) was added into the tubes. Then, the tubes were centrifuged at 1800 rpm for 1 min. The supernatant was sieved through a 500-mesh sieve, from which the retained material was washed out and stored in glass jars. The final volume of the water-nematode suspension was 10 mL. The population estimate of each plant-parasitic nematode taxon was obtained by counting on Peters' slides using a light microscope. Temporary (formalin) and/or permanent (glycerin) slides were examined under a microscope with a clear camera for identification to species level. The identification was carried out based on morphological and morphometric characteristics. The free-living nematodes were counted in total.

4.2.4. Soil microbial activity analysis

The soil microbial activity was assessed by the enzymes β-glucosidase and Acid phosphatase according to the method proposed by Tabatabai (1994). Briefly, 1.0 g of fresh soil was added to 10 mL test tubes, in which 2 mL of MUB buffer solution (pH 6 for β-glucosidase and pH 5 for Acid phosphatase, respectively) was added. The solutions containing soil were stirred and incubated (37 ^{o}C, 1 h) with 0.05 M p-nitrophenyl buffer solution (p-nitrophenyl-β-d-glucopyranoside for β-glucosidase and p-nitrophenyl phosphate for Acid phosphatase, respectively). The determination of the enzymatic activity was based on the colorimetric determination (400 nm) of p-nitrophenol released.

Table 1. Locality (Region, State, and Municipality) and number of samples (n)

Regions	States	n	Municipality
North	Pará	4	Curionópolis, Dom Eliseu, Paragominas, Redenção
	Rondônia	2	Alta Floresta, Corumbiara
	Tocantins	6	Alvorada, Araguaçu, Darcinópolis, Figueirópolis, Formoso do Araguaia, Wanderlândia
Northeast	Bahia	25	Barreiras, Correntina, Formosa do Rio Preto, Jaborandi, Luís Eduardo Magalhães, Riachão das Neves, São Desidério
	Maranhão	2	Nova Colinas, Tasso Fragoso
	Piauí	7	Baixa Grande do Ribeiro, Bom Jesus
Central-West	Distrito Federal	3	Brasília
	Goiás	15	Água Fria de Goiás, Aporé, Bela Vista de Goiás, Cabeceiras, Catalão, Chapadão do Céu, Cristalina, Iaciara, Montividiu, Padre Bernardo
	Mato Grosso do Sul	19	Caarapó, Chapadão do Sul, Costa Rica, Dourados, Laguna Carapã, Maracaju, Ponta Porã, São Gabriel do oeste
	Mato Grosso	21	Campos de Júlio, Comodoro, Diamantino, Guiratinga, Jaciara, Nobres, Nova Guarita, Nova Mutum, Nova Ubiratã, Novo Horizonte do Norte, Paranatinga, Querência, Sorriso, Vila Bela da Santíssima Trindade
Southeast	Minas Gerais	29	Arinos, Bonfinópolis de Minas, Buritis, Cambuquira, Campestre, Candeias, Conceição do Rio Verde, Coromandel, Delfinópolis, Guarda-Mor, Ibiá, Iguatama, Iraí de Minas, Itacarambi, Iturama, Luminárias, Machado, Madre de Deus de Minas, Monte Carmelo, Monte Santo de Minas, Nepomuceno, Passos, Patrocínio, Tupaciguara, Uberaba
	São Paulo	27	Altinópolis, Assis, Avaré, Bernardino de Campos, Buritama, Campos Novos Paulista, Capão Bonito, Capela do Alto, Itaberá, Itaí, Itapeva, Itararé, Leme, Palmital, Paranapanema, Pardinho, Piracicaba, São Miguel Arcanjo, Tarumã, Tatuí
South	Paraná	20	Candói, Carambeí, Castro, Foz do Jordão, Guarapuava, Kaloré, Mamborê, Mangueirinha, Marilândia do Sul, Pinhão, Quedas do Iguaçu, Rio Bonito do Iguaçu, São João
	Rio Grande do Sul	19	Coronel Bicaco, Cruz Alta, Gentil, Horizontina, Independência, Jóia, Mato Castelhano, Nova Ramada, Passo Fundo, Santa Cecília do Sul, Santo Antônio do Planalto, Santo Augusto, São Luiz Gonzaga, Tucunduva
	Santa Catarina	15	Abelardo Luz, Bela Vista do Toldo, Campo Erê, Campos Novos, Canoinhas, Mafra, Major Vieira, São Domingos

4.2.5. Statistical analysis

The redundancy analysis (RDA) was used to determine the relationship between plant-parasitic nematodes community structure and soil physicochemical properties, using Canoco 4.5. The significance between soil physicochemical properties and plant-parasitic nematodes community structure was assessed using the forward selection (FS) followed by the Monte Carlo permutation test. P values lower than 0.05 were considered statistically significant. In addition, we performed the permutational multivariate analysis of variance (PERMANOVA) (ANDERSON, 2001) to test whether soil physicochemical properties categories harboured significant differences in plant-parasitic nematodes community structure.

Population density of each nematode taxon was subjected to the Shapiro-Wilk normality test. Since the data did not present a normal distribution, the results were subjected to the Kruskal-Wallis non-parametric analysis of variance, followed by the Dwass-Steel-Critchlow-Fligner test at 5% probability using Jamovi 1.6 software. The plant-parasitic nematode incidence (%) was calculated as follows: (soil samples in which the taxa was detected/total number of soil samples analysed) x 100.

The effect of soil physicochemical properties on the microbial activity was analysed by the multiple linear regression using Jamovi 1.6. For β-glycosidase the variables SOM, pH, and clay content were selected and used as predictors, while for Acid phosphatase, the predictor variables were SOM, pH, clay content, and available P content.

The Spearman correlation analysis was performed to investigate the relationship between nematode population density, soil physicochemical properties, microbial activity, and soybean yield using Jamovi 1.6. The Spearman's rank correlation coefficients were represented in heatmaps.

4.3. Results

4.3.1. Soil physicochemical properties and soybean yield

The pH of the soil samples ranged from 4.4 to 6.6 with an average of 5.5. The clay content ranged from 74 to 736 g kg^{-1} with an average of 381.6 g kg^{-1}. The SOM content ranged from 9 to 106 g dm^3 with an average of 37 g dm^3. The available P content ranged from 4 to 197 mg dm^3 with an average of 53.7 mg dm^3. Lastly, soybean yield ranged from 2,580 to 7,740 kg ha^{-1} with an average of 5,280 kg ha^{-1} among the sampling sites.

4.3.2. Soil nematode community structure and soil properties in soybean fields

According to the Monte Carlo permutation test, the structure of the plant-parasitic nematode community was significantly related to soil pH and clay content (Figure 1). The species–environment correlations of axes 1 and 2 were 0.20 and 0.24, respectively. The cumulative percentage variance of species-environment explained by the two first axis was 93.8%. Thus, it was possible to classify the samples according to the pH values into the following categories: highly acidic soils (pH ≤ 5.5 = 115 samples) and slightly acidic soils (pH ≥ 5.6 = 101 samples) (RAIJ *et al.*, 1996). Regarding the clay content, samples were classified into the following categories: sandy soils (< 30% clay = 67 samples), loamy soils (30 – 50% clay = 90 samples), and clayey soils (> 50% clay = 59 samples). The PERMANOVA

results confirmed the differences in the nematode community structure, according to soil acidity ($F = 4.34$; $p < 0.01$) and clay categories ($F = 3.11$; $p < 0.01$).

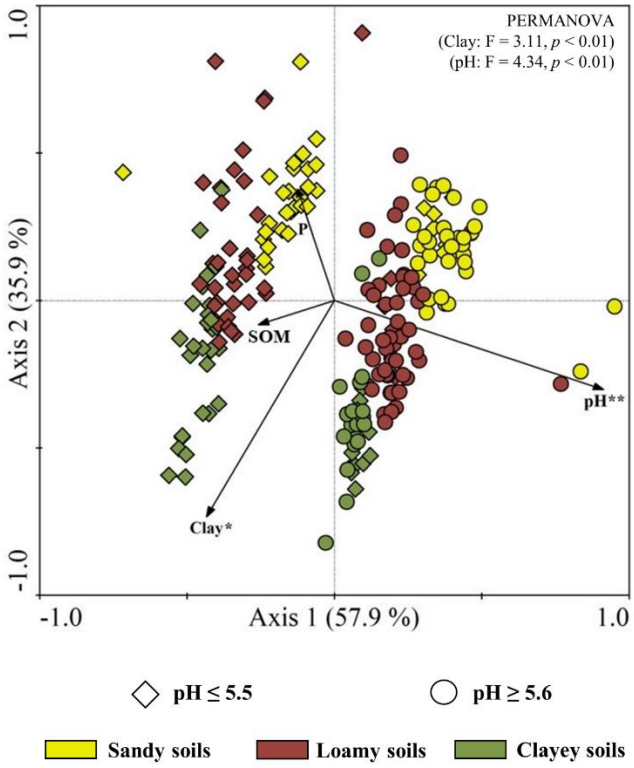

Figure 1. Redundancy analyses of the relationship between the nematode community structure and soil physicochemical properties. SOM: soil organic atter; P: available phosphorus. * Significant at 0.05 probability, and ** significant at 0.01 probability level according to the Monte Carlo permutation test.

4.3.3. Incidence and population density of plant-parasitic nematodes

The plant-parasitic nematode genera *Meloidogyne*, *Pratylenchus*, *Heterodera*, *Rotylenchulus*, *Helicotylenchus*, *Scutellonema*, and *Xiphinema* were identified in 216 soil samples from the different soybean crops. These genera belong to four families (Heteroderidae, Pratylenchidae, Hoplolaimidae, Longidoridae). In addition, we also identified nematodes of the family Criconematidae, which were not identified at the genus level.

Soil acidity significantly affected the occurrence of plant-parasitic nematodes (PPNs). In highly acidic soils, PPN abundance ranged from 15 to 720 PPNs per 50 g, while in slightly acidic soils abundance ranged from 15 to 345 PPNs per 50 g. Highly acidic soils presented a higher population density of *Helicotylenchus dihystera* when compared to slightly acidic soils ($p < 0.01$).

The genus *Meloidogyne* was found only in the highly acid soils of sandy and loamy texture, both of which showed an incidence of less than 3%. Three species belonging to the genus *Pratylenchus* were identified: *P. brachyurus*, *P. penetrans*, and *P. zeae*. In highly acidic soils, *Pratylenchus* sp. was identified in more than 40% of samples from sandy soils. Conversely, in loamy and clayey soils, *Pratylenchus* sp. was identified in less than 20% and 27% of samples, respectively (Table 2). In sandy soils, the family Criconematidae was identified in approximately 12% of samples. On the other hand, in loamy and clayey soils, the family Criconematidae was identified in less than 10% and 6% of

samples, respectively. In clayey soils, *Heterodera glycines* was present in approximately 8% of samples, while in sandy and loamy soils, *H. glycines* was present in less than 3.0% of samples. Sandy soils showed a higher population density of *Pratylenchus* sp. (14.11 ± 3.86 per 50 g) when compared to loamy soils (4.88 ± 1.84 per 50 g) ($p < 0.05$). On the other hand, population density of *H. dihystera* was higher in loamy soils (300.00 ± 52.00 per 50 g) when compared to sandy soils (149.11 ± 28.46 per 50 g) ($p < 0.05$).

In slightly acid soils, *Pratylenchus* sp. was identified in approximately 12% of samples from sandy soils. Conversely, *Pratylenchus* sp. was present in more than 26% of samples in loamy and clayey soils (Table 3). The family Criconematidae was observed in 12% of samples from sandy soils. On the other hand, in loamy soils, the family Criconematidae was present in less than 2.5% of samples, and members of this family were not found in clayey soils. In clayey soils, *H. glycines* was observed in 9.5% of samples, while in sandy and loamy soils, *H. glycines* was present in less than 3.0% of samples. Clayey soils showed a higher population density of *H. dihystera* (152.14 ± 24.01 per 50 g) when compared to loamy (97.02 ± 14.60 per 50 g) and sandy soils (103.18 ± 24.74 per 50 g).

4.3.4. Soil microbial activity

The β-glucosidase and acid phosphatase enzymes were used to assess the soil microbial activity. The β-glucosidase activity ranged from 4.4 to 203.8 mg p-nitrophenol kg^{-1} soil h^{-1}, with an average of 90.0 mg p-nitrophenol kg^{-1} soil h^{-1}. The acid phosphatase activity ranged from 122.7 to 2047.8 mg p-nitrophenol kg^{-1} soil h^{-1}, with an average of 628.5 mg p-nitrophenol kg^{-1} soil h^{-1}. The values of activities of β-glucosidase and acid phosphatase enzymes observed in each sample and their locations are shown in the supplemental material (Table S1). The multiple linear regression model for β-glucosidase activity indicated that SOM was the best predictor of the enzymatic activity (t-value = 6.251; $p < 0.001$) among the soil properties analysed (Table 4). For the acid phosphatase activity, the multiple linear regression model indicated that the pH (t-value = -3.549; $p < 0.001$) and SOM (t-value = 5.010; $p < 0.001$) were the best predictors of the enzymatic activity.

4.3.5. Correlation between soil properties, nematode population density, soil microbial activity, and soybean yield

In sandy soils, we observed a negative correlation between the pH and population density of *Pratylenchus* sp. (Spearman's rho = -0.30; $p < 0.05$) (Figure 2). Interestingly, we observed a positive correlation between population density of *H. dihystera* and soybean yield (Spearman's rho = 0.33; $p < 0.01$). On the other hand, population density of Criconematidae correlated negatively with soybean yield (Spearman's rho = 0.29; $p < 0.05$).

In loamy soils, we observed a negative correlation between the pH and total abundance of PPNs (Spearman's rho = -0.37; $p < 0.01$). In general, the pH correlated negatively with *H. dihystera* (Spearman's rho = -0.34, $p < 0.01$) and Criconematidae (Spearman's rho = -0.22, $p < 0.05$). Interestingly, the available P correlated positively with population density of *Scutellonema brachyurus* (Spearman's rho = 0.40, $p < 0.01$).

Table 2. Population density of plant-parasitic nematodes (individuals per 50 g of soil ± SE) in highly acidic soils (pH ≤ 5.5) under no-till soybean crops in Brazil.

	Mean ± SE			Range			Incidence (%)		
	Sandy	Loamy	Clayey	Sandy	Loamy	Clayey	Sandy	Loamy	Clayey
Meloidogyne sp.	0.44 ± 0.44	0.34 ± 0.34	0 ± 0	0-15	0-15	0-0	2.9	2.3	0.0
Pratylenchus sp.	14.11 ± 3.86 a	4.88 ± 1.84 b	7.50 ± 1.96 ab	0-150	0-60	0-75	41.2	18.6	26.3
Heterodera glycines	0.44 ± 3.86	0.34 ± 1.36	2.36 ± 1.53	0-15	0-15	0-60	2.9	2.3	7.9
Rotylenchulus reniformis	0 ± 0	0 ± 0	3.55 ± 3.29	0-0	0-0	0-135	0.0	0.0	2.6
Helicotylenchus dihystera	149.11 ± 28.46 b	300.00 ± 52.00 a	175.65 ± 36.69 ab	0-600	0-1740	15-870	85.3	97.7	100.0
Scutelonema brachyurus	1.32 ± 1.32	11.51 ± 11.51	0 ± 0	0-45	0-495	0-0	2.9	2.3	0.0
Criconematidae	26.02 ± 14.65	6.97 ± 4.12	4.34 ± 2.82	0-390	0-150	0-90	11.8	9.3	5.3
Xiphinema sp.	0.44 ± 0.44	1.39 ± 1.39	1.18 ± 1.64	0-15	0-60	0-30	2.9	2.3	5.3

Values with different letters in a row are significantly different according to Dwass-Steel-Critchlow-Fligner pairwise comparisons ($p < 0.05$). Incidence: (number of samples containing PPNs in the sample group/total number of samples in the group) x 100.

Table 3. Population density of plant-parasitic nematodes (individuals per 50 g of soil ± SE) in slightly acidic soils (pH ≥ 5.6) under no-till soybean crops in Brazil.

	Mean ± SE			Range			Incidence (%)		
	Sandy	Loamy	Clayey	Sandy	Loamy	Clayey	Sandy	Loamy	Clayey
Meloidogyne sp.	0 ± 0	0 ± 0	0 ± 0	0-0	0-0	0-0	0.0	0.0	0.0
Pratylenchus sp.	2.27 ± 0.83	6.70 ±2.13	5.71 ± 1.94	0-30	0-60	0-45	12.1	27.9	23.8
Heterodera glycines	0.27 ± 0.84	0.31 ± 1.58	2.14 ±1.56	0-9	0-15	0-30	3.0	2.3	9.5
Rotylenchulus reniformis	0 ± 0	0 ± 0	0 ± 0	0-0	0-0	0-0	0.0	0.0	0.0
Helicotylenchus dihystera	103.18 ± 24.74 b	97.02 ± 14.60 b	152.14 ± 24.01 a	0-660	0-450	0-465	84.8	97.8	95.2
Scutelonema brachyurus	0 ± 0	7.02 ± 7.02	0 ± 0	0-0	0-330	0-0	0.0	2.3	0.0
Criconematidae	15.90 ± 10.44	0.63 ± 0.63	0 ± 0	0-315	0-30	0-0	12.1	2.3	0.0
Xiphinema sp.	0 ± 0	0 ± 0	0 ± 0	0-0	0-0	0-0	0.0	0.0	0.0

Values with different letters in a row are significantly different according to Dwass-Steel-Critchlow-Fligner pairwise comparisons ($p < 0.05$). Incidence: (number of samples containing PPNs in the sample group/total number of samples in the group) x 100.

Table 4. Multiple linear regression models for the effects of soil chemical properties on β-glycosidases and the acid phosphatase activity in soils under no-till soybean crops in Brazil.

Variable	Estimate	SE	t-value	*p*-value
β-glycosidases model: R^2_{adj} = 0.166; F = 15.3; *p*-value < 0.001				
Intercept	5.6502	35.8506	0.158	0.875
SOM	1.1735	0.1877	6.251	< 0.001
pH	8.3370	6.2228	1.340	0.182
Clay content	-0.0133	0.0188	-0.705	0.481
Acid phosphatase model: R^2_{adj} = 0.206; F = 14.9; *p*-value < 0.001				
Intercept	1120.373	239.060	4.687	< 0.001
SOM	6.271	1.252	5.010	< 0.001
pH	-147.129	41.462	-3.549	< 0.001
Clay contet	0.126	0.128	0.985	0.326
Available P	0.698	0.410	1.703	0.090

SOM: soil organic matter

In clayey soils, we observed a negative correlation between the available P and total abundance of PPNs (Spearman's rho = -0.33; $p < 0.05$). In general, the available P correlated negatively with population density of *H. dihystera* (Spearman's rho = -0.30, $p < 0.05$).

As expected, we observed a positive correlation between SOM and the activity of β-glucosidase and acid phosphatase enzymes for most soil types (sandy soils: 0.41 and 0.56, respectively, $p < 0.01$; loamy soils: 0.33 and 0.35, respectively, $p < 0.01$; clayey soils: 0.26 and 0.10, $p < 0.01$ and $p > 0.05$, respectively). Here, we highlight that we did not observe a significant correlation between SOM and the activity of acid phosphatase only in clayey soils. Furthermore, we observed a negative correlation between the soil pH and the acid phosphatase activity in sandy soils (Spearman's rho = -0.28; $p < 0.05$) and loamy soils (Spearman's rho = -0.22; $p < 0.05$).

Soybean yield showed a positive correlation with the available P in sandy (Spearman's rho = 0.25; $p < 0.05$) and loamy soils (Spearman's rho = -0.33; $p < 0.01$). In addition, we observed a positive correlation between soybean yield and SOM (Spearman's rho = 0.21; $p < 0.05$), and between soybean yield and acid phosphatase (Spearman's rho = 0.23; $p < 0.05$) in loamy soils. Lastly, no significant correlations were observed between enzymatic activity and nematode population density in any soil category ($p > 0.05$). Nematode *Pratylenchus* sp. showed the highest correlations with the enzymatic activity, which presented a negative correlation with β-glycosidase (Spearman's rho = -0.22; $p = 0.08$) and a positive correlation with acid phosphatase (Spearman's rho = 0.21; $p = 0.09$) in sandy soils. All other nematodes showed irrelevant correlations (-0.2 < Spearman's rho < 0.2) in all soil categories studied.

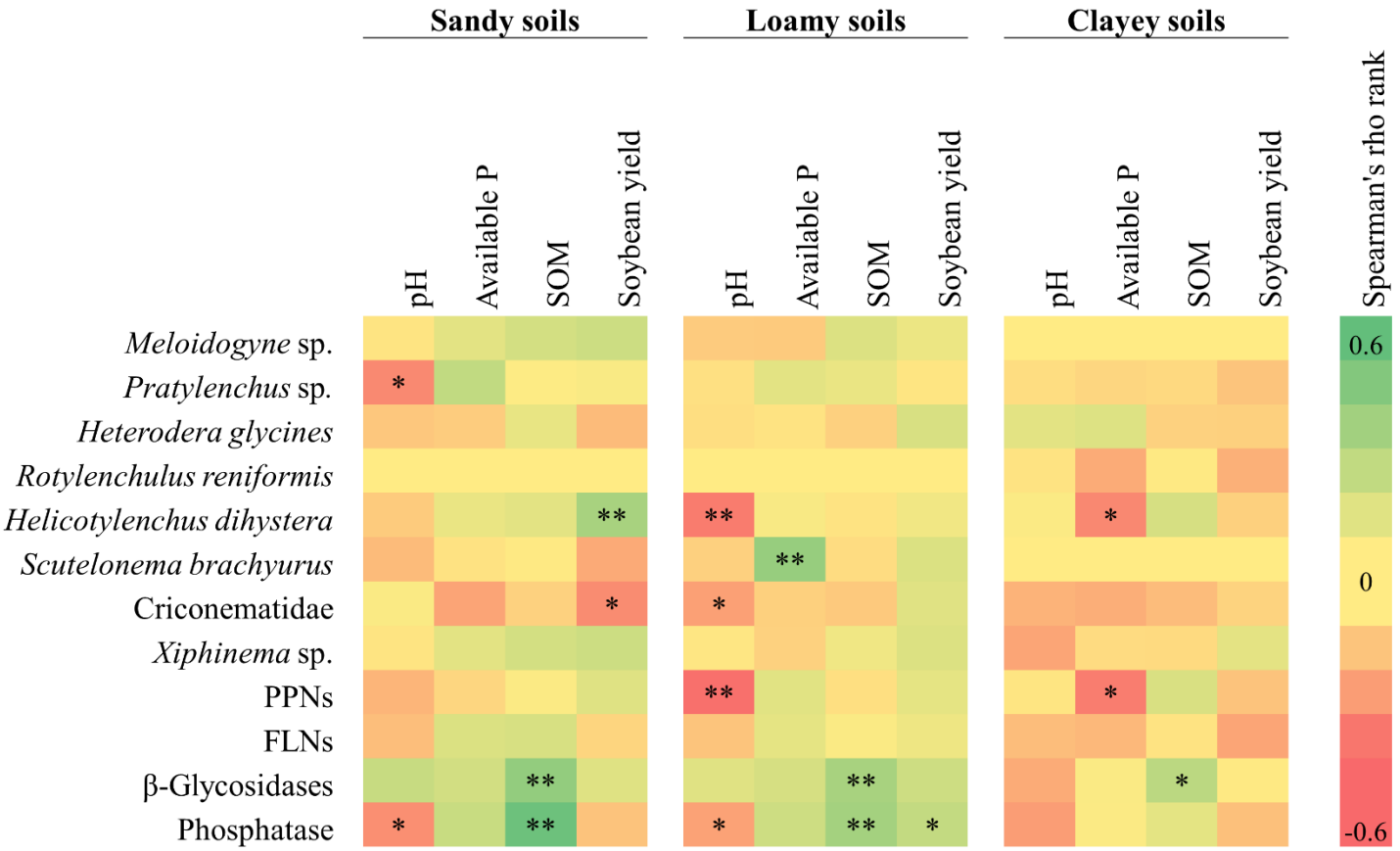

Figure 2. Heatmaps of the Spearman's correlation coefficients of nematode population density, soil physicochemical properties, microbial activity, and soybean yield in no-till soybean crops in Brazil. *Significant at 0.05; **Significant at 0.01. PPNs: total plant-parasitic nematode density; FLN: free-living nematodes density; SOM: Soil organic matter.

4.4. Discussion

We analysed the community of plant-parasitic nematodes in 216 samples of soybean cultivated soils in different regions of Brazil. Among the nematodes identified, we detected the main nematodes that cause damage to soybean (*e.g.*, *Heterodera glycines*, *Meloidogyne* sp., *Pratylenchus* sp., and *Rotylenchulus reniformis*), as well as other nematodes considered as emerging threats (*e.g.*, *Scutellonema brachyurus* and *Helicotylenchus dihystera*) (LIMA *et al.*, 2017; MACHADO, 2014). Regardless of the soil pH, nematodes *H. dihystera* and *Pratylenchus* sp. presented the highest incidence. *H. dihystera* is a migratory ectoparasite, or semi-endoparasite, often found in the soil, which can penetrate soybean roots and cause brown lesions (GARDIANO-LINK *et al.*, 2022). Dissemination and population density of *H. dihystera* have increased in recent years, making this nematode a potential pathogen for soybean in Brazil (MACHADO; AMARO; DA SILVA, 2019). *Pratylenchus* (root-lesion nematodes) is one of the most widespread plant-parasitic nematodes in soybean crops in Brazil (CASTANHEIRA *et al.*, 2020). This nematode is a migratory endoparasite that may be favoured by the no-till farming system, due to the continuous presence of roots that increases food availability for the nematode (CRUZ; ASMUS; GARCIA, 2020).

The plant-parasitic nematode community was significantly influenced by the soil pH and the clay content (Figure 1). Chowdhury *et al.* (2020) indicated that soil physicochemical properties (including soil texture) are important variables modulating the nematode community. According to the authors, soil physicochemical properties in general have a greater influence on the nematode community when compared to agricultural practices. Corroborating with our results, Simon *et al.* (2018) observed that the soil pH can affect nematodes directly and/or indirectly by i) changing the nutrient status and root structure of host plants, ii) altering soil microbial activity, iii) negatively affecting the ability of nematodes to regulate their osmotic pressure, and/or iv) simply directly killing them. Lastly, soil texture can affect prevalence, population density, reproduction, migration, penetration ability, and pathogenicity of plant-parasitic nematodes (UPADHAYA *et al.*, 2019).

Regardless of the clay content, we observed a higher abundance of PPNs in highly acidic compared to slightly acidic soils. This inverse relationship between soil acidity and PPNs abundance was also observed by the correlation analysis in loamy soils in which *H. dihystera* and *Criconematidae* showed negative correlations with the pH (Figure 2). According to Norton and Hoffmann (1974), the pH can be a useful variable to predict occurrence of PPNs. However, the pH effect may vary according to the nematode species. Our results indicate that raising the soil pH, which can be achieved by liming, can be useful to reduce the occurrence of PPNs in soybean crops in Brazil. Moreover, keeping the soil acidity close to neutral may favour the occurrence of microorganisms that suppress these pathogens (SILVA; MEDEIROS; CAMPOS, 2018).

In highly acidic soils, population density of *Pratylenchus* sp. was higher in sandy soils when compared to loamy soils (Table 2). This result was supported by the negative correlation between the pH and *Pratylenchus* observed in sandy soils (Figure 2). Our results corroborate previous studies that showed that this nematode may occur more easily in acidic and well-aerated soil conditions (CADET; BERRY; SPAULL, 2004; KAWANOBE *et al.*, 2020). According to Leiva *et al.* (2020), high porosity and high acidity (associated to low fertility) promote optimal conditions for *Pratylenchus* movement and reproduction in soybean crops.

Most PPNs thrive in sandy soils due to the higher proportion of macropores, which facilitates their movement towards host plants (MARANHÃO *et al.*, 2018). Curiously, our results indicated that in highly acidic soils *H. dihystera* presented a higher population density in loamy soils when compared to sandy soils (Figure 2). Furthermore, in slightly acidic soils, *H. dihystera* was more abundant in clayey soils when compared to sandy and

loamy soils. Epidemiology information on *H. dihystera* in tropical countries is still scarce (GARDIANO-LINK *et al.*, 2022). However, some studies performed in temperate countries (*e.g.,* the United States of America) have shown that this nematode can be found in soils with different texture classes (CHOWDHURY; YAN; FRISKOP, 2020; SIMON *et al.*, 2018).

Root-knot nematodes (*Meloidogyne* sp.) had a low incidence and population density in sandy and loamy soils with high acidity. This result suggests that, in well managed fields, the root-knot nematodes are not a problem for soybean, which may be attributed to the effective application of cultural, chemical, and biological methods for the management and control of these nematodes in the soybean crops evaluated (CHINHEYA; YOBO; LAING, 2017; MAZZETTI *et al.*, 2019; ZIRAKPARVAR, 2022). For SCN, the highest incidence of *H. glycines* occurred in clayey soils, regardless of the pH. Furthermore, we observed that *H. glycines* presented a negative correlation with the available P. Our results are in agreement with Bao *et al.* (2013), which indicate a high occurrence of *H. glycines* in clayey soils of low fertility (BAO *et al.*, 2013).

We observed a significant correlation between *H. dihystera* and Criconematidae occurrence and soybean yield in sandy soils. More productive plants usually have more roots, which can increase food availability for *H. dihystera* that can multiply at high rates near the roots without causing major problems to plant growth due its ectoparasitic habit (GARDIANO-LINK *et al.*, 2022). Nematodes of the Criconematidae family (*e.g.*, *Mesocriconema*, and *Discocriconemella*) are ectoparasites of little importance to soybean, but they are widely distributed in Brazilian soils (MACHADO *et al.*, 2022; MÁRQUEZ *et al.*, 2021).

The activities of β-glucosidase and the acid phosphatase enzymes were determined in 216 samples of soybean cultivated soils in the five regions of Brazil. The multiple regression analysis indicates that the activity of the β-glucosidase enzyme was determined by SOM, while the activity of the acid phosphatase enzyme was determined by SOM and the soil pH. These results were expected, since the potential of hydrolysis of these enzymes is directly linked to substrate availability (labile organic compounds in SOM) and the soil pH (SINSABAUGH *et al.*, 2008). Lopes *et al.* (2013) developed interpretative classes for soil microbial indicators (including β-glucosidase and acid phosphatase) as a function of the soil organic carbon (SOC) content. According to the authors, the positive correlation between SOC and enzyme activity can be attributed to the function of SOC as a source of energy and nutrients for microbial communities. Furthermore, SOC has a role in the physical protection of enzymes within soil aggregates (LOPES *et al.*, 2013).

The effects of SOM and the pH on the activity of β-glucosidase and acid phosphatase was also observed in the correlation analyses. The positive correlation between enzymes (mainly β-glucosidase) and SOM may indicate the change in the SOC content before it can be detected by other routine techniques (ADETUNJI *et al.*, 2017). Since SOM is considered the best indicator of soil quality, enzymes β-glucosidase and the acid phosphatase have been recommended to assist in decisions for environmental and economic sustainability regarding soil management in soybean cultivated areas in Brazil (MENDES *et al.*, 2021).

The absence of significant correlations between population density of plant-parasitic nematodes and the activity of microbial enzymes indicates that the occurrence of these pathogens is related to factors even more complex than the soil microbial activity alone. However, it is known that each microbial enzyme catalyses a particular reaction (ADETUNJI *et al.*, 2017). Thus, the occurrence of PPNs may be related to other enzymes, such as proteases and chitinases, which can degrade the cuticle of juveniles or parasitise on nematode eggs (GENG *et al.*, 2016; GORTARI; HOURS, 2008). In a previous study, under controlled conditions, we observed negative correlations

between population density of *Meloidogyne javanica* and relative abundance of bacteria with potential to produce these enzymes (BARROS *et al*., 2022).

4.5. Conclusions

Our results demonstrated that *Helicotylenchus dihystera* and *Pratylenchus* sp. were the most abundant PPNs in soybean crops. In general, the clay content and the soil pH were the main soil physicochemical properties modulating the PPNs community in soybean cultivated soils under no-till systems in Brazil. Furthermore, our results indicated that acid soils were prone to the occurrence of PPNs that cause economic damage to soybean crops. In addition, we observed that the SOC content and the pH are the main soil properties modulating the microbial activity in soybean cultivated soils under no-till systems. The SOM may improve the activity of β-glucosidase and acid phosphatase enzymes. Lastly, no significant correlations were observed between the enzymatic activity of β-glucosidase and acid phosphatase and the nematode population density.

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Supplementary Material

Table S1. https://www.dropbox.com/s/gjdqd4csyxhvwef/Table%20S1%20-%20CESB%20I%20-%20Barros%20et%20al.%20Apsoil.xlsx?dl=0

5. FINAL REMARKS

Research on the interaction between soil microbiome and nematode community activity is incipient. In this thesis, we have taken the first steps towards elucidating interactions between soil bacterial communities and nematode activity in soybean crops. We demonstrated the suppressive effect of soil bacterial diversity against plant-parasitic nematodes on soybean plants under controlled experimental conditions and pointed some groups of bacteria that are potentially antagonistic to plant-parasitic nematodes. Future research may focus on isolating and multiplying these bacteria so that they can be tested against plant-parasitic nematodes. In addition, our results corroborated the knowledge that plant-parasitic nematode infection leads to changes in the soil microbial community.

In our field research, we observed that bacterial communities, total nematodes, and plant-parasitic nematodes in Brazilian soybean crops are different according to biomes. Our experimental and sampling results together showed that the role of the bacterial community against plant-parasitic nematodes depends on the nematode taxa and may happen through general suppressiveness, specific suppressiveness, or even acting to benefit the nematodes. The results of our field research also showed that the total nematode community (predominantly composed of free-living nematodes) may increase soybean crop yield.

Furthermore, we investigated the influence of soil physical and chemical properties on the plant-parasitic nematode community and the microbial activity. Our results demonstrated that the clay content and the soil pH are the main soil properties modulating the plant-parasitic nematodes community in soybean cultivated soils under the no-till system and that the soil organic matter content and the pH are the main soil properties modulating the microbial activity in those soils.

Future approaches should be used to increase our knowledge of the interactions between the nematode community and the entire soil microbial community, not restricted to bacteria. For that purpose, further studies should evaluate the metagenome of soils affected by plant-parasitic nematodes and inhabited by free-living nematodes in different crops of agricultural interest. A deeper knowledge of the relationships between the nematode community and the soil microbiome allows the development of new strategies to manage the microbial community to control plant-parasitic nematodes and stimulate free-living nematodes to boost yield of agricultural crops.