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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

Editor Karyn N. Johnson, University of Queensland

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The authors declare no conflict of interest.

Received 9 June 2022
Accepted 2 August 2022
Published 24 August 2022

main pests causing economic losses in agriculture. According to Ha (5), 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) (6).

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 the two most important plant-parasitic nematodes affecting soybeans (7). 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 reduction in the absorption of water and nutrients, resulting in stunted plants with low productivity (8, 9). *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 (10).

The use of chemical nematicides and crop rotation are the most adopted management practices in the control of plant-parasitic nematodes in soybean crops (11). Due to their negative impact on the environment and human health, many traditional nematicides have been banned from the market (12). 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, etc.) capable of promoting the suppressiveness of soilborne diseases (13, 14), and the use of microorganisms for biological control of plant-parasitic nematodes (15).

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. In addition, biological soil suppressiveness can minimize the damage caused by the disease even when the pathogen is successfully established (16). 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 to the activity of specific groups of microorganisms which interfere at some stage of the pathogen's life cycle (17).

Studies indicated that the diversity of soil microbial communities can alter the ability of pathogens to colonize soil (18), playing a vital role in plant-parasitic nematode suppression (19). 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 (20–22). 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 environmentally 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. We also 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.

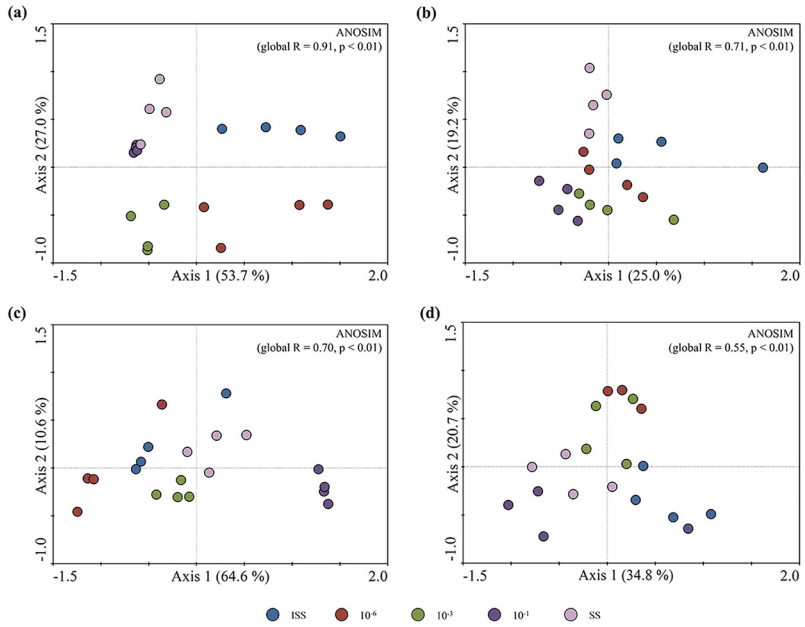

FIG 1 Principal-component analysis (PCA) analysis of the bacterial community in soil with different microbial diversity levels, inoculated with plant-parasitic nematodes. (a) Start and (b) end of *Meloidogyne*-assay; (c) start and (d) end of *Pratylenchus*-assay. ISS, Infested Sterilized Soil; SS, Sterilized 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.

RESULTS

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 $\cdot$ g^{-1} soil (Table S1 in the supplemental material). 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 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 ($P > 0.05$).

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 (Fig. 1a and b). On the other hand, in *Pratylenchus*-assay, we observed that the bacterial community was more strongly grouped according to the treatments at the start (Fig. 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 (Fig. S1 in the supplemental material).

The bacterial community diversity indices 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) index values compared to the 10^{-1} treatment ($P < 0.05$). We also 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).

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 plant roots. The ISS and 10^{-6} treatments showed a higher population density of *M. javanica* (12.4 ± 2.4 and 9.5 ± 2.6 thousand individuals $\cdot$ g^{-1} root, respectively) compared to 10^{-1} (6.7 ± 1.7 thousand individuals $\cdot$ g^{-1} root) (Fig. 2a). On the other hand, in *Pratylenchus*-assay, we observed a lower population

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[a]

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

[a]$R > 0.75$, well-separated groups; $R > 0.5$, groups with overlap but clearly differentiated; $R < 0.25$, not well-separated groups.
[b]ISS, Infested Sterilized Soil.
[c]SS, Sterilized Soil.
[d]Significant at the 0.05 probability level.
[e]Non-significant.

density of *P. brachyurus* in the 10^{-6} and 10^{-3} treatments (527 $\pm$ 285 and 605 $\pm$ 563 individuals $\cdot$ g^{-1} root, respectively) compared to ISS (1,388 $\pm$ 449 individuals $\cdot$ g^{-1} root). Furthermore, the 10^{-1} treatment (1,355 $\pm$ 857 individuals $\cdot$ g^{-1} root) presented a population density similar to the ISS treatment (Fig. 2b).

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

Soil bacterial community composition. T-RFLP (Terminal Restriction Fragment Length Polymorphism) was initially used as screening technique. For this, 10 biological replicates of each treatment were analyzed in each assay (see Fig. S2). Afterwards, we selected four biological replicates of each treatment (dilutions 10^{-1}, 10^{-3}, and 10^{-6}; and control treatments ISS and SS) in each assay for 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

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[a]

	Start			End		
Assay	Richness	Diversity (H′)	Evenness (J)	Richness	Diversity (H′)	Evenness (J)
Meloidogyne						
ISS[b]	1,441.75 ± 77.23 bc	5.30 ± 0.35 b	0.73 ± 0.04 b	1,667.00 ± 205.97 b	5.76 ± 0.35 c	0.78 ± 0.04 c
10^{-6}	1,288.50 ± 184.35 c	4.80 ± 0.12 c	0.67 ± 0.01 c	1,861.25 ± 102.16 b	6.07 ± 0.14 bc	0.81 ± 0.01bc
10^{-3}	1,603.50 ± 133.70 b	5.45 ± 0.28 b	0.74 ± 0.03 b	1,874.25 ± 143.50 b	6.16 ± 0.14 b	0.82 ± 0.02 b
10^{-1}	2,044.75 ± 206.23 a	6.25 ± 0.16 a	0.82 ± 0.01 a	2,186.50 ± 124.36 a	6.57 ± 0.06 a	0.85 ± 0.01 a
SS[c]	1,551.50 ± 273.42 bc	5.62 ± 0.28 b	0.77 ± 0.02 b	1,705.25 ± 227.82 b	6.01 ± 0.22 bc	0.81 ± 0.02 bc
Pratylenchus						
ISS[b]	1,282.25 ± 74.06 a	5.39 ± 0.15 bc	0.75 ± 0.02 b	1,511.75 ± 174.08 b	5.66 ± 0.10 b	0.77 ± 0.01 b
10^{-6}	1,277.50 ± 80.22 a	5.11 ± 0.04 c	0.71 ± 0.00 c	1,353.75 ± 102.49 b	5.58 ± 0.12 b	0.77 ± 0.01 b
10^{-3}	1,407.50 ± 109.14 a	5.57 ± 0.07 b	0.77 ± 0.01 b	1,933.50 ± 307.83 a	6.20 ± 0.31 a	0.82 ± 0.02 a
10^{-1}	1,414.75 ± 44.55 a	6.02 ± 0.05 a	0.83 ± 0.00 a	2,016.00 ± 189.81 a	6.27 ± 0.42 a	0.82 ± 0.05 a
SS[c]	1,274.00 ± 362.50 a	5.40 ± 0.48 bc	0.76 ± 0.04 b	1,491.00 ± 70.12 b	5.64 ± 0.10 b	0.77 ± 0.01 b

[a]Values are given as means ± standard deviation. Means with different letters within the same column differ significantly according to Duncan's multiple-range test ($P < 0.05$).
[b]ISS, Infested Sterilized Soil.
[c]SS, Sterilized Soil.

sample (operational taxonomic unit [OTU] table in the supplemental material). Overall, the soil bacterial community was comprised of 35 phyla and over 1,300 genera based on the SILVA database (Fig. S3 and S4). At the start of *Meloidogyne*-assay, we observed higher abundances 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 we analyzed the ISS and SS treatments, we observed that nematode inoculation resulted in consistent increases in the relative abundances of some bacterial genera (Fig. 4). The relative abundances of the genera *Massilia* and *Tuberibacillus* were increased at the start of Meloidogyne-assay and at the end of Pratylenchus-assay. Some increases in the relative abundances of bacterial genera were observed in ISS but did not occur in SS: *Laceyella*, *Pseudoflavitalea*, *Sinomicrobium*, and *Terrimonas*, associated with *M. javanica*; and Candidatus *Amoebophilus*, *Gemmata*, *Leptolyngbya*, *Niabella*, and *Roseiarcus*, associated with *P. brachyurus*.

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 (Fig. 5, Fig. S5) (Table S2). Furthermore, we identified the most relevant bacterial groups based on the values of betweenness centrality, defined as the number of times a node acts as a bridge along the shortest path between two other nodes (23), at the start and end of both experiments. At the start of *Meloidogyne*-assay, the lowest taxonomic levels 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).

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

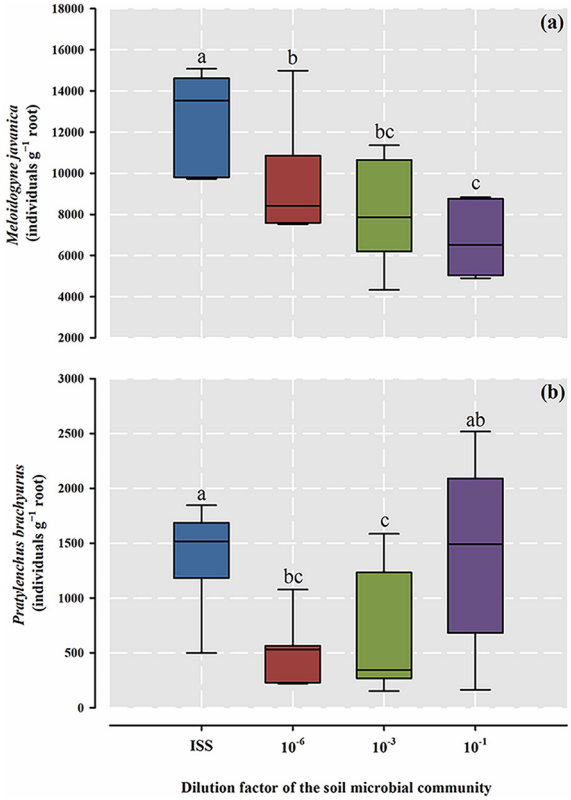

FIG 2 Nematode population density in roots of soybean plants grown in soil with different microbial diversity levels. (a) *Meloidogyne*-assay. (b) *Pratylenchus*-assay. Boxes with different letters on the same graph differ significantly according to Duncan's multiple-range test ($P < 0.05$). Data represent the mean of 10 biological replicate samples for each treatment in each assay.

bacterial genera across all treatments, in each assay, with nematode population density and plant growth measures. In *Meloidogyne*-assay, we observed strong negative correlations (Spearman < -0.6; $P < 0.05$) between bacterial genera and the population density of nematodes in soybean roots. Here, we highlighted the genera *Noviherbaspirillum*, *Devosia*, *Filimonas*, *Pseudomonas*, and *Jatrophihabitans*, which also presented strong positive correlations with grain biomass (Spearman > 0.6; $P < 0.05$) (Fig. 6). In *Pratylenchus*-assay, we did not observe negative correlations between bacterial genera and nematode population density.

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 needs 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 (Tables 1 and 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 (24), which explains the presence of DNA in the SS treatment in both experiments. In addition, it is possible that relic DNA remained after autoclaving (25). The dilution-to-extinction approach favored the more abundant soil bacteria over the rarer bacterial taxa (data not shown) which are usually less abundant and may have important roles in the suppression of soil diseases (26). The biological origin of soil suppressiveness to plant-parasitic nematodes has been previously studied (13, 19, 27, 28).

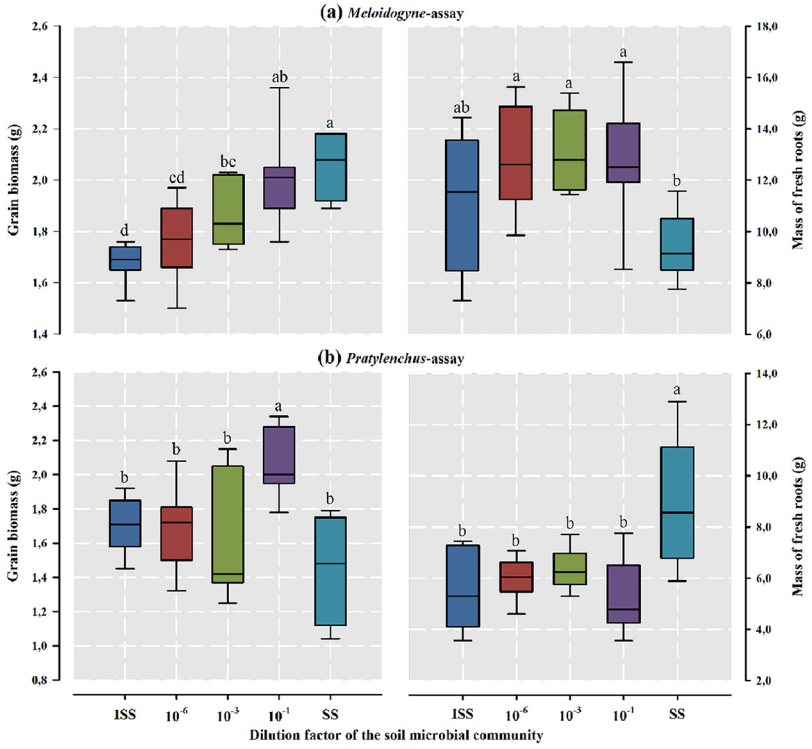

FIG 3 Grain biomass and mass of fresh roots of soybean plants grown in soil with different microbial diversity levels, inoculated with plant-parasitic nematodes. (a) *Meloidogyne*-assay. (b) *Pratylenchus*-assay. Boxes with different letters on the same graph differ significantly according to Duncan's multiple-range test ($P < 0.05$). Data represent the mean of 10 biological replicate samples for each treatment in each assay.

However, our study presents the suppressive effect of soil bacterial diversity on plant-parasitic nematodes 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 presence of potential antagonistic bacteria to nematodes in the 10^{-1} and 10^{-3} treatments resulted in less infestation by *M. javanica* compared to the 10^{6} and ISS treatments (Fig. 3a). Furthermore, the lower nematode infestation in the treatments with greater microbial diversity resulted in higher grain biomass (Fig. 4a). In general, high microbial diversity promotes functional redundancy and ecosystem services that can improve soil resilience (29). Also, higher microbial diversity enhances resource competition, which has been proposed as a key factor in the success or failure of pathogen invasion (30, 31).

On the other hand, in *Pratylenchus*-assay, although the highest diversity level was observed in the 10^{-1} treatment, the lowest *P. brachyurus* infestation levels were observed in the 10^{-3} and 10^{-6} treatments. Both treatments also presented a lower population density of the nematode compared to ISS (Fig. 3b). Even though the 10^{-1} treatment presented the highest population density of *P. brachyurus*, it presented the highest grain biomass among all treatments. This shows that soil suppressiveness to the nematode can minimize damage caused by the disease, even with the pathogen already established (16).

Although soil microbial diversity is essential for the proper functioning of ecosystem processes, biodiversity alone may not be enough to reduce the ability of pathogens to establish in the soil (18). 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 analyzed at the phylum and class level, the soil microbial community consisted of a common microbial

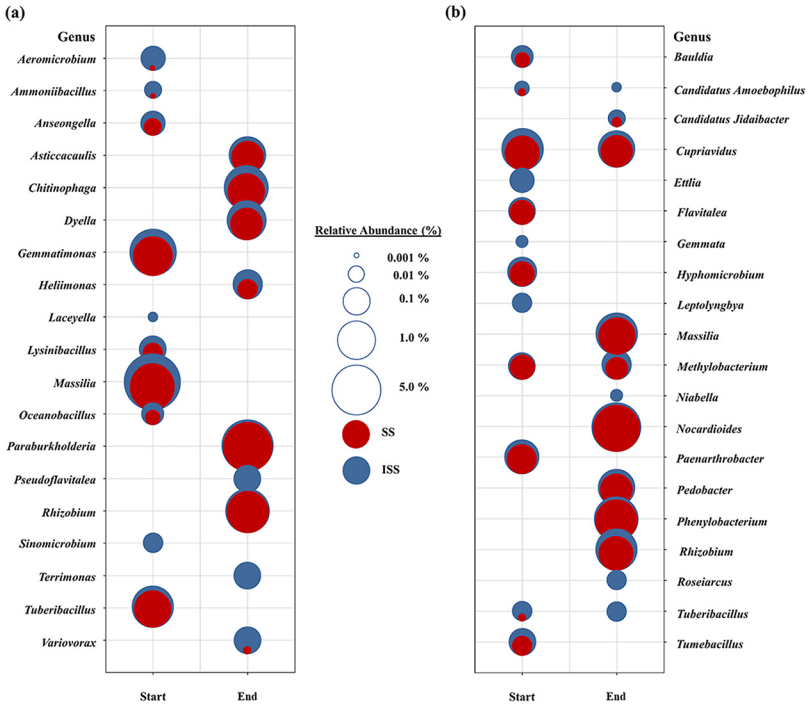

FIG 4 Increased bacterial taxa relative abundances with nematode inoculation between SS and ISS treatments, indicated by overlaid bubble plots that represent the relative percent abundance of a taxa at the start and end of (a) *Meloidogyne*-assay and (b) *Pratylenchus*-assay.

core among most Brazilian soils (Fig. S3 and S4) (32–34). 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 greater relative abundances of some bacterial genera capable of suppressing *Meloidogyne* spp. One of these is the genus *Lysobacter*, which presented a higher relative abundance in the 10^{-1} treatment than in all others ($P < 0.001$) (Fig. S6a). According to Chen et al. (35) and Lee et al. (36), this genus can produce a variety of enzymes and/or toxins with known nematicidal effects against *M. javanica* and other nematodes. Other bacterial genera, such as *Bradyrhizobium*, *Devosia*, and *Sphingomonas*, presented greater abundance in the 10^{-1} and 10^{-3} treatments. According to Topalović et al. (37), 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, the *Lysobacter* and *Devosia* genera also presented greater relative abundances in the treatment with higher microbial diversity (10^{-1}) compared to other treatments ($P < 0.01$) (Fig. S6b). Although *Lysobacter* can produce compounds which are active against a variety of nematodes, including those of the genus *Pratylenchus* (Chen et al. [35]), in our study, its high relative abundance in the 10^{-1} treatment did not result in a reduction in the *P. brachyurus* population (Fig. 3b). Additionally, some studies have indicated that the *Devosia* genus can produce auxins and siderophores, which promote plant growth (38). This may explain the high grain biomass observed in the 10^{-1} treatment compared to that in the other treatments (Fig. 4b). The genera *Caulobacter* and *Variovorax*, also known as plant growth-promoting rhizobacteria (PGPR) (39, 40), presented higher relative abundances in the 10^{-1} and 10^{-3} treatments. Different studies have reported the presence of *Variovorax* in soils to be suppressive to plant-parasitic nematodes (37, 41–43).

The differences observed in the bacterial community structure between the ISS and SS treatments 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 may have positively influenced their survival and parasitism (21). These

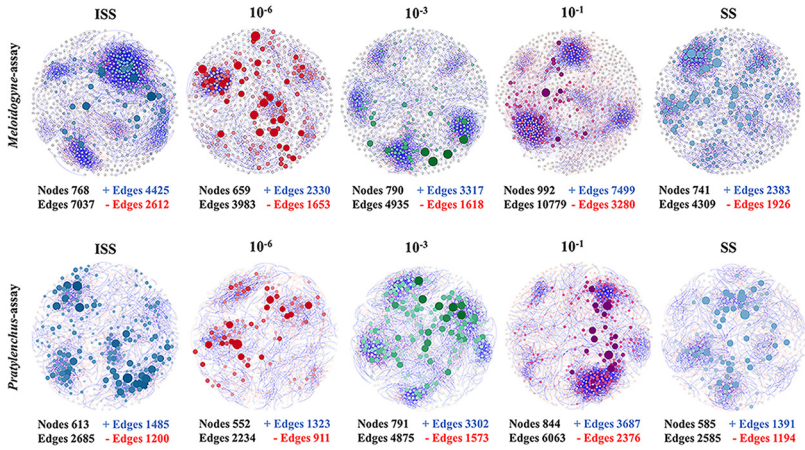

FIG 5 Network co-occurrence analysis of microbial communities of soil with different microbial diversity levels, inoculated with plant-parasitic nematodes, at the end of the experiments. A connection indicates SparCC correlation with magnitude > 0.8 (positive correlation: blue edges) or < -0.8 (negative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

differences persisted until the end of both experiments ($R > 0.6$, $P < 0.05$), indicating that the nematode parasitism may have affected the recruitment of microorganisms by the rhizospheres of soybean plants (20). This suggestion is supported by the increases in the relative abundances of some bacterial genera in the ISS treatment compared to those in the SS treatment, and the occurrence of some bacterial genera only in the ISS treatment (Fig. 2). 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 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 high potential to suppress *M. javanica* in *Meloidogyne*-assay (Fig. 6). Among these, the genera *Devosia*, *Pseudomonas*, *Bryobacter*, *Noviherbaspirillum*, *Filimonas*, *Alsobacter*, and *Jatrophihabitans* presented strong negative correlations with the *M. javanica* population density and strong positive correlations with plant growth parameters. It is worth mentioning that so far, there have been no studies regarding potential biocontrol by 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 controlling *Meloidogyne* spp. (44–46). Recent studies have also demonstrated the potential biocontrol of *Meloidogyne* spp. by bacteria belonging to the genus *Microbacterium* (47, 48).

In *Pratylenchus*-assay, we did not observe potentially suppressive bacteria against *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 suppression of the plant's immune response (21). Interestingly, *Devosia* is a PGPR characterized by its bioremediation activity and nitrogen-fixing ability (49). 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}) compared to the

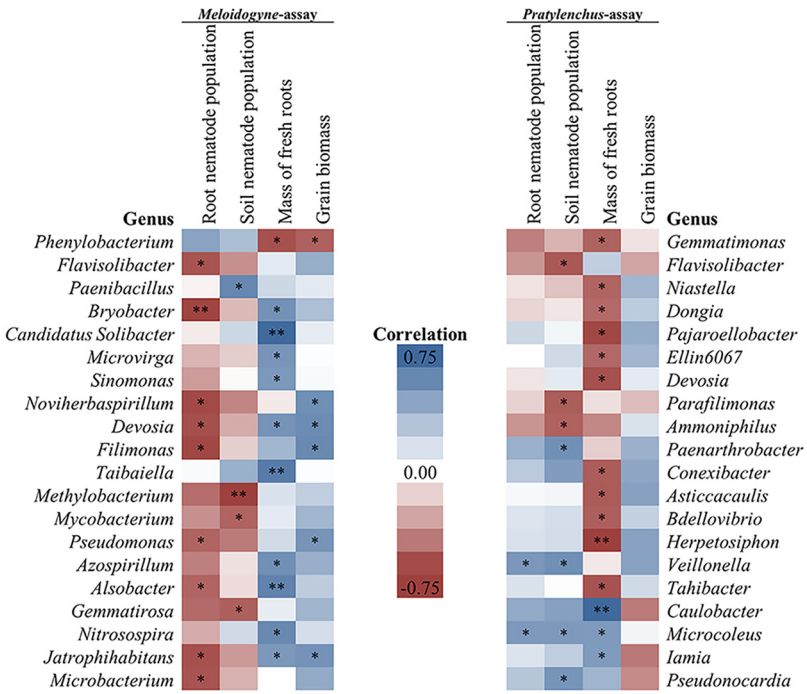

FIG 6 Heatmaps of Spearman's rank correlation coefficients of relative abundance of bacterial genus with nematode population density and plant measurements. *, significant at 0.05; **, significant at 0.01.

other treatments in both experiments. According to Mendes et al. (30), a highly diverse microbial community exhibits 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 *M. javanica* population density in the roots of plants in the 10^{-1} treatment in *Meloidogyne*-assay.

The high complexity of the network observed in the ISS compared to the SS treatment, at the end of both experiments, supports our hypothesis that nematode parasitism affects the recruitment of microorganisms by plants (50). We argue that 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 (21, 51, 52). In analyzing the ranks of bacterial taxa with higher betweenness centrality in the ISS and SS treatments, at the start and end of both experiments, we observed that nematode invasion altered the core microbiome of key groups within the community (53). We argue that invasion of the soil microbiome by a pathogen may replace key taxa and collapse the structure of the network (54).

Finally, 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 factors capable of reducing the occurrence of *M. javanica* and sustaining the performance of soybean plants parasitized by *P. brachyurus*. Furthermore, our results indicate that bacteria belonging to the genera *Bryobacter*, *Noviherbaspirillum*, *Filimonas*, *Alsobacter*, and *Jatrophihabitans* are potential targets for studies prospecting bacteria to use for 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.

MATERIALS AND METHODS

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 above sea level), 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} sand, 35 g · kg^{-1} silt, and 175 g · kg^{-1} clay). Over the past years, this agricultural field has been cultivated with soybean (*Glycine max*). After sampling, the soil was separated into two parts: one for chemical characterization (Table S7) and the other for the mesocosm experiment.

Mesocosm preparation. Lime and fertilizers were applied to the soil in accordance with 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 the soil was diluted 1:10 in sterile water and passed through a 25-μm sieve to eliminate native nematodes. The remaining soil collected was autoclaved three times for 60 min at 120°C, 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). Next, the 1:10 sieved solution was serially diluted in sterile water up to 10^{-6}, 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 (55). After inoculation of the diluted microbial communities, the mesocosms were incubated for 15 days to promote the establishment of the soil microbiome.

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

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); in the second experiment, each pot was infested with 1,200 nematodes (juveniles and adults) of the species *P. brachyurus* (*Pratylenchus*-assay). In both experiments, the soil was infested by nematodes after the establishment of the microbiome, and soybean was sown immediately after 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 5×10^9 CFU per g of peat, and 0.1 g of peat per kg 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* 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 of the soil. Soil sampling was performed using a probe 24 h after sowing (start) and at the end (end) of both experiments, when the plants were at the beginning of maturity stage (R7). Approximately 2.0 g of soil was collected per pot and frozen (–80°C) for molecular analysis, and 100 g was kept refrigerated at 4°C and used for nematode extraction.

DNA extraction procedures. Samples collected 24 h 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), 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 1.0× TAE buffer (Tris-acetate-EDTA) stained with GelRed (Biotium, CA). We also used NanoDrop 1000 spectrophotometry (Thermo Fisher Scientific, Waltham, MA) to check the DNA quality and quantity. DNA samples which showed well-defined bands and a 260/230-nm ratio close to 1.8 were considered suitable.

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 StepOnePlus real-time PCR system with 48-well plates (Applied Biosystems, Foster City, CA). 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, United Kingdom), 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 (approximately 10 ng). The primers and reaction conditions are presented in Table S8 in the supplemental material.

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 StepOnePlus Real-Time software version 2.2.2 (Applied Biosystems, Foster City, CA).

High-throughput sequencing analysis. We used T-RFLP (Supplemental Material) to determine whether the treatments showed significant differences in the microbial community structure (Fig. S1). Afterward, we selected 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, 806R: GGACTACNNGGGTATCTAAT). In total, 80 DNA libraries were prepared using the MiSeq Reagent Kit V3 (Illumina, San Diego, CA), following the manufacturer's instructions for the Illumina MiSeq platform (2 × 250-bp paired-end).

The 16S rRNA gene paired-end reads were first merged using PEAR (Paired-End reAd meRger) (56). Next, the merged sequences were analyzed using QIIME 2 version 2021.4 (57). First, the sequences were demultiplexed and quality control was carried out with DADA2 (58), using the consensus method to remove any remaining chimeric or 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 (59), and the generated matrix was further used for statistical analyses.

Nematode extraction and quantification. The nematodes were extracted from soil samples as described by Jenkins (60), while the nematode extraction from roots followed the methods of Coolen and D'Herde (61). Briefly, 50 g of soil and 2 L of water was mixed inside a Becker. Next, 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 1,800 rpm for 5 min. After centrifugation, the supernatant was discarded, and sucrose-water solution (400 g · L^{-1}) was added into the tubes. Next, the tubes were centrifuged at 1,800 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 root was washed, dried with paper towel, cut into 1-cm pieces, and crushed in a blender for 60 s. 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 1,800 rpm for 5 min. The following steps were the same as those of the Jenkins (60) method, with the addition of sucrose-water solution (400 g · L^{-1}), centrifugation at 1,800 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.

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

Statistical analysis. 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 using Canoco version 4.5. Differences between treatments were evaluated by similarity analysis (ANOSIM) using the Paleontological Statistics freeware package (PASTv.3, Hammer et al. [62]). The ANOSIM-R statistic was used to indicate the degree to which groups differed from each other ($R > 0.75$, well-separated groups; $R > 0.5$, groups with overlap but clearly differentiated, $R < 0.25$: not well-separated groups) (63). PASTv.3 was also used to calculate the richness, diversity (Shannon, H′), and evenness (Pielou, J) indices.

To compare the differential abundances of bacterial groups between treatments, the OTU table was used as input in the software STAMP (64). *P* values were calculated based on a two-sided Welch's *t* test and correction using Benjamini-Hochberg false-discovery rate. The bacterial genera which showed the greatest difference between the SS and ISS treatments, with a confidence level of 95%, are presented in an overlapping bubble chart. Spearman's 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 which showed at least a significant ≥0.35- or ≤0.35-correlation ($P < 0.05$) were represented in a heat map. In addition, network analyses were performed to assess the complexity of the interactions among microbial taxa in each treatment (see Supplemental Material).

Data availability. The amplicon data are available at the NCBI Sequence Read Archive under the ID number PRJNA832861.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 1.7 MB.

SUPPLEMENTAL FILE 2, XLSX file, 0.4 MB.

ACKNOWLEDGMENTS

This study was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) grant no. 2019/09460-0, and in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES; Finance Code 001) and a CNPq grant to F.D.A. (proc. 400761/2021-1).

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