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ORIGINAL PAPER

Organic practices intensify the microbiome assembly and suppress root-knot nematodes

Julio C. P. Silva[1] · Thaisa C. S. Nunes[2] · Rafaela A. Guimarães[2] · Victor S. Pylro[3] · Lilian S. A. S. Costa[4] · Rafael Zaia[2] · Vicente P. Campos[2] · Flavio H. V. Medeiros[2]

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Abstract

Roots can recruit beneficial microorganisms to suppress plant pathogens. However, conventional and organic practices differently shape the soil microbiome and consequently the root protection. Here, we investigated the suppressive activity of soil microbiome against the root-knot nematode (RKN) *Meloidogyne incognita* in horticultural areas under organic or conventional practices and the microbiome profiles in non-inoculated (RKN-absent) and inoculated (RKN-present) rhizospheres. Soils were collected from neighboring areas under long-term conventional or organic practices, but physicochemically similar. After a set of bio-tests in autoclaved and non-autoclaved soils, we concluded that the soil suppressiveness was of biological origin. However, plant growth, RKN suppression and defense induction were higher in organic soils. Also, RKN was highly suppressed when the organic soil microbiome was transferred to infested substrates. We used Illumina MiSeq platform to determine bacterial and fungal profiles in organic and conventional tomato rhizospheres, inoculated or not with *M. incognita*. Our data suggest that despite the higher bacterial abundance in the conventional RKN-absent rhizosphere, the organic RKN-present rhizosphere recruited more efficiently antagonistic bacteria and fungi. Microbiome α-diversity increased in the organic RKN-present rhizosphere. Antagonistic bacterial genera *Pseudomonas*, *Serratia*, *Bradyrhizobium*, *Burkholderia* and *Azospirillum* and fungal genera *Beauveria*, *Clonostachys*, *Metarhizium*, *Purpureocillium* and *Arthrobotrys* were highly abundant only in the organic RKN-present rhizosphere. This work suggests the organic and conventional practices interfere in the rhizosphere microbiome composition and consequently in the suppression of RKN, where organic practices intensified the assembly of bacterial and fungal antagonists in the presence of *M. incognita*.

Keywords Plant-parasitic nematodes · Biocontrol · Bacterial community · Fungal community

Key messages

- Horticultural practices influenced the RKN density and rhizosphere microbiome composition.
- The highest plant growth promotion and *M. incognita* suppression were found in organic soils.
- Microbiome transference to a sterilized substrate suppressed *M. incognita*.
- Microbiome diversity increased in the organic rhizosphere inoculated with *M. incognita*.
- A higher antagonistic assembly was found in the organic inoculated rhizosphere.

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✉ Julio C. P. Silva
julio.c.silva@ufsm.br

[1] Department of Phytosanitary Defense, CCR, Universidade Federal de Santa Maria (UFSM), Santa Maria, Brazil

[2] Department of Plant Pathology, Universidade Federal de Lavras (UFLA), Lavras, Brazil

[3] Microbial Ecology and Bioinformatics Laboratory, Department of Biology, Universidade Federal de Lavras (UFLA), Lavras, Brazil

[4] Laboratory of Environmental Microbiology, Embrapa, Jaguariúna, Brazil

Introduction

The most widespread plant–parasitic nematode species in agricultural soils is the sedentary endoparasite root-knot nematode (RKN) *Meloidogyne incognita* (Jones et al. 2013). RKNs infect thousands of crops and cause annual losses of billions of dollars around the globe (Abad et al. 2003). Practices to reduce RKN populations are usually based on sustainable soil management combined or not with the conventional use of chemicals (Forghani and Hajihassani 2020). Consequently, differential practices affect the soil microbiome, leading to fluctuations in plant-pathogen populations, which are classified as levels of soil suppressiveness (Mendes et al. 2011; Topalović et al. 2020b; Weller et al. 2002).

Soil suppressiveness against RKN is measured by a simple comparison between different densities of their populations in different areas. In this case, soils with similar physicochemical conditions, but a wide fluctuation in RKN density, suggest a relevant involvement of the soil microbiome in RKN establishment (Elhady et al. 2018; Harkes et al. 2020; Mendes et al. 2013; Silva et al. 2018). For instance, some species of fungi, bacteria and free-living nematodes have been found in suppressive soils antagonizing RKNs, either directly by metabolites and parasitism, or indirectly by inducing plant defense and plant growth (Bakker et al. 2012; De Medeiros et al. 2017; Terra et al. 2018; Topalović et al. 2020a). Furthermore, plants have developed a strategy that involves the selective stimulation and enrichment of the antagonistic microorganisms in the rhizosphere (Hannula et al. 2019; Hussain et al. 2018). Knowledge concerning the plant's ability to recruit antagonists under different soil management practices will improve the basis for novel profitable and sustainable microbiome-based strategies for crop production (Liu et al. 2020).

Horticultural farming usually favors RKN establishment by using susceptible plants and providing convenient soil properties for nematode movement and infection (Tariq-Khan et al. 2017). At the same time, these conditions are propitious to an antagonistic microbiome development (Giné et al. 2013; Verdejo-Lucas et al. 2013). Most horticultural farmers apply toxic chemicals to reduce soilborne pathogens, whereas organic farmers overcome this situation by conservative practices that keep the soil biodiversity and stimulate the RKN antagonistic microbiome (Babin et al. 2021; Giné et al. 2013; Giné et al. 2016). Recently, researchers have demonstrated variations in the rhizosphere microbiome under different crop practices, where impressive low levels of plant pathogens were registered under long-term organic farming (Crowder et al. 2010; Giné et al. 2016; Harkes et al. 2020; Lupatini et al. 2017). Additionally, the microbial shifts in rhizospheres after RKN inoculation indicate that soil can be managed to recruit beneficial microorganisms (Topalović et al. 2020a; Zhou et al. 2019). The combined efforts of multiple mechanisms by the recruited microbiome should reduce RKN performance on plant roots. Consequently, the dynamics of the plant–microbiome relationship should be verified in the presence of *M. incognita* to investigate how RKN presence influences microbial recruitment in the rhizosphere (Elhady et al. 2021; Harkes et al. 2020; Yergaliyev et al. 2020).

Microbial communities in soil play an essential role in RKN suppression. However, the current understanding of how the microbiome is shaped in the soil to suppress RKN is limited for horticultural areas. Therefore, this study hypothesized that the microbiota structure and diversity are determined by the adoption of conventional or organic systems, which directly influence the suppression of RKN. In this work, we aimed to investigate plant-mediated effects of the microbiome from the RKN non-infested, organic or conventional horticultural soils against *M. incognita*. We also tested whether the soil suppressiveness could be transferred to the conducive (nematode-infested) soil. Finally, we determined the nematode communities in organic and conventional soils by their trophic levels and unraveled which bacterial and fungal taxa associated with the tomato roots grown in different soil systems with and without *M. incognita* by combining 16S rRNA and ITS amplicon sequencing.

Methods

Horticultural sites and soil sampling

The soils were collected from horticultural areas cropped under plastic greenhouses at Universidade Federal de Lavras (UFLA)—Hortagro Property in Ingai, Minas Gerais—Brazil. At this property, greenhouses have been using conventional or organic practices side by side, cropping sweet pepper (*Capsicum annuum* cv. 'Magali') for 4 years (2–3 cycles per year). Although the cultivar of sweet pepper is susceptible to *M. incognita*, the areas are not affected by RKNs. Each greenhouse consisted of three rows of sweet pepper plants spaced 30 cm from each other. Soil physicochemical analyses were performed at the Laboratory of Soil Analysis of UFLA. The areas presented clay loam soils, where the pH were 6.9 and 7.2 for organic and conventional areas, respectively (Supplementary Table S1). The final composite samples collected from organic and conventional areas were separated and stored at cold chambers (6 °C) until further use in the experiments. Soil samples were collected after harvesting, before planting and new soil practices. The samples were collected twice at the end of two consecutive

sweet pepper cycles to repeat the experiments. More details of practices, soil sampling (number and collection of subsamples and other details) and soil properties can be found in the supplementary methods and results (Supplementary Information).

Root-knot nematode acquisition and nematode community evaluation

The second-stage juveniles (J_2) and eggs used in all experiments were obtained from pure populations of *M. incognita* race 3 multiplied in tomato plants maintained in a greenhouse at the Laboratory of Nematology—UFLA. The eggs were obtained according to the Hussey and Barker technique (Hussey and Barker 1973). For nematode community estimation, we used the centrifugation technique (Jenkins 1964) to extract the nematodes and the number of nematodes in each trophic level was estimated according to the ecological classification of Yeates (Yeates et al. 1993). Details are described in supplementary methods (Supplementary Information).

Beneficial effects of soil microbiome against *Meloidogyne incognita*

Growth promotion and *M. incognita* suppression

The sampled soil was placed in plastic cups (200 mL). Soil from each system (i.e., conventional and organic) was used to fill the cups in two different ways. Each soil was represented by cups with natural soil and control cups containing soil sterilized by autoclaving three times (200 °C for 25 min, three following days). For both organic and conventional samples, natural and sterilized soils were used. In each cup, a 15-day-old tomato seedling (*Solanum lycopersicum* cv. 'Regina') was transplanted. The tomatoes grew for 45 days in a greenhouse and received water according to technical recommendations (Terra et al. 2018). Then, 45-day-old tomato plants were removed from the cup, washed and dried at room temperature. Thereafter, the roots were separated from the shoots (whole above-ground part) placed in paper bags and transferred to an oven at 70 °C. The material was left in the oven until it reached a constant dry weight (about 3 days). Finally, the material was weighed to obtain the dry weight of the shoots and roots.

Similar to the growth promotion experiment, we used the natural soils and sterilized soils from conventional and organic greenhouses. The soils were transferred to 200-mL plastic cups, where 20-day-old seedlings (to provide young roots enough for J_2 infection) of tomato cv. 'Regina' were transplanted. Three days after transplanting, an aqueous suspension containing 200 J_2 of *M. incognita* was inoculated to the soil of each cup. The tomato plants received irrigation and fertilization for 45 days. Then, the root system was removed and weighed. The galls were counted, and the eggs were extracted (Hussey and Barker 1973) and also counted in an inverted microscope using Peter's chamber. The numbers of galls and eggs were estimated per gram of the root system.

Defense induction and enzymes activity estimation

The capability of the rhizosphere microbiome to induce systemic resistance was verified in a split-root assay (De Medeiros et al. 2017). Two neighboring cells of a Styrofoam tray (90 cm^3 each cell) were arranged as one inducer cell and one responder cell. A 20-day-old tomato seedling cv. 'Regina' was transplanted between cells and fixed by a sterilized toothpick as a seedling tutor. Half of the root system was placed inside the inducer cell and the other half inside the responder cell. Each inducer cell was filled with the natural soils from the organic or conventional greenhouses, and the responder cell was filled with a sterilized artificial substrate (60% pine bark, 15% vermiculite and 25% humus; Terra do Paraíso, Holambra, SP, Brazil). The control consisted of plants with sterilized soil in the inducer cell and sterilized artificial substrate in the responder cell. Despite being different than sampled soils, the substrate in the responder cells is a good alternative to bioassays with tomato, as it provides an optimal environment for J_2 movement and infection (De Medeiros et al. 2017; Gomes et al., 2020). After 3 days, the responder cell was inoculated with 200 J_2. The plants were watered daily according to recommendations (Terra et al. 2018). After 45 days, the roots were weighed and the number of galls and eggs was determined in the responder cell.

Five extra seedlings of each treatment and control were used to estimate the activity of defense-related enzymes at different periods. In this case, roots of the responder cell were collected: before transplanting the seedlings in the Styrofoam (day 0); just before the J_2 inoculation (day 3); 4 days after inoculation to provide enough time for J_2 infection (De Medeiros et al. 2017; Shukla et al. 2018) (day 7) and 10 days after inoculation (day 17). Roots from the responder cell were gently washed by distilled water to remove any substrate residue and promptly immersed in liquid nitrogen and kept frozen in a deep freezer (−80 °C) until the extraction of the enzymes phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) and guaiacol peroxidase (POX, EC 1.11.1.7). More details are described in supplementary methods (Supplementary information).

Microbiome transference to *M. incognita* infested substrate

To extract soil microbiomes, we applied the techniques of Zhou et al. (2019) and Elhady et al. (2018) with minor modifications. Fifteen grams of each natural soil sample

was blended three times with 150 mL sterile NaCl water solution (0.85%) at the highest speed of an electric blender for 60 s to break up soil aggregates and mix adequately the solution. In each extraction, soil particles were sedimented and the microbial suspensions of the supernatant were passed through a 200-mesh screen sieve (0.07 mm) to remove remaining particles, macro-organisms, and root debris. The extracted microbiome was pelleted for 10 min at 2000 rpm and resuspended in 45 mL sterile water providing the soil slurry. Then, a 100-mL plastic cup was filled with the sterilized artificial substrate and a 20-day-old tomato seedling cv. 'Regina' was transplanted to it. The day after transplanting, each cup received 0.0, 5.0, 10.0 or 15.0 mL of the soil slurries per cup (0, 5, 10 or 15%). The concentration of 0% consisted of 15 mL sterile water, representing the control. The transplanted microbiomes were established for three days. Then, a suspension with 200 J_2 of *M. incognita* was inoculated to the soil of each cup. The tomato plants received irrigation and fertilization according to technical recommendations for 45 days. The galls were counted, and the eggs were extracted and also counted. The number of galls and eggs was estimated per gram of the root system.

Statistical analyses

All the previous experiments contained five replicates and were repeated twice. The experiment repetitions were submitted to analyses of variance (ANOVA; experiment 1 × experiment 2), and if there was no difference between them, a combined analysis was performed with 10 replicates. All data were previously submitted to normality (Shapiro–Wilk) and homogeneity (Bartlett) tests. Differences in sterilized and natural soil variables were tested by pairwise *t*-tests ($P < 0.05$) and between soil systems by a normal *t*-test ($P < 0.05$). Defense-related enzyme activity (PAL and POX) was distinguished through the days according to post hoc Tukey test ($P < 0.05$). The microbiome slurry applied in different concentrations was evaluated by nonlinear regression chosen by the model's best fit.

Microbiome assembly in conventional and organic rhizosphere inoculated or not with Meloidogyne incognita

To analyze the total DNA of the bacterial and fungal communities, we used part of the composite sample collected either from organic or conventional systems (sampling details in supplementary methods—Supplementary information). Only natural soils of the last sampling were used. A 20-day-old tomato seedling was transplanted into each cup (200 mL). After 5 days, a 100 µL suspension with 200 J_2 of *M. incognita* was inoculated in three of the cups through a pipette tip buried 5 cm in the soil, as near as possible of the roots (RKN-present rhizosphere). The suspension was applied very carefully inside the buried tip to avoid spreading the J_2 to other parts of the rhizosphere. Immediately after inoculation, we removed the tips to be sure that any suspension was retained inside the tips. In the other three cups, the tomato was transplanted to the soils without J_2 inoculation (RKN-absent rhizosphere). The seedlings were placed in a greenhouse and watered by spraying the soil surface with enough amounts of water (± 10 mL every 2 days) to keep the moisture at 60% field capacity without spreading J_2 in the inoculated cups. Fifteen days after inoculation, the roots were removed, and the attached rhizosphere soil was sampled. Inoculated samples were obtained from rhizosphere parts above and far enough from the inoculated spot to avoid any J_2 in the samples. All inoculated cups exhibited fresh galls, indicating J_2 infection.

Total soil DNA extraction

From the attached rhizosphere soil, the DNA was extracted from RKN-absent and RKN-present tomato rhizospheres. This region corresponds to the first line of defense against J_2 by the activity of the antagonistic microbiome (Silva et al. 2018; Topalović et al. 2020b). The DNA was extracted from 0.5 g of each sample using the PowerSoil DNA Isolation Kit (MO BIO Laboratories Inc.; Carlsbad, CA, USA) following the protocols suggested by Marty et al. (2012) and de Carvalho et al. (2016). Details are described in supplementary methods (Supplementary Information).

Fungal and bacterial DNA sequencing and metataxonomic analyses

There were three replicates of RKN-absent and three of RKN-present for both organic and conventional rhizospheres. The microbial community's structure in the soil samples was monitored using a metataxonomic approach. The 16S rRNA genes encoding bacteria and archaea were amplified via PCR using Bact_341F (5′ CCTACGGGNGGC WGCAG 3′) and Bact_806R (5′-GACTACHVGGGTATCTA ATCC-3′) recommended by the Brazilian Microbiome Project (Pylro et al., 2014), using the BMP Operating System (BMPOS) (Pylro et al., 2016), as this pair provides high coverage of the domain bacteria and good representation of the bacterial diversity down to genus. The fungal encoding intergenic region was amplified via PCR using the ITS1F (5′ CTTGGTCATTTAGAGGAAGTAA 3′) and ITS2 (5″ GCT GCGTTCTTCATCGATGC 3″) primers (Schoch et al. 2012) in an Illumina MiSeq sequencing platform (paired 2 × 250 pb) (Illumina; San Diego, CA, USA). Briefly, the OTU table was built using the UPARSE pipeline (Edgar 2013). The reads were truncated at 300 bp and quality filtered using a maximum expected error of 0.5 (meaning that on average

one nucleotide in every two sequences is incorrect). Filtered reads were dereplicated, and unique sequences (singletons) were removed. These sequences were clustered into OTUs with a 97% similarity cutoff. After clustering, the sequences were aligned and classified using the SILVA reference database (release SSU_Ref_132) using Qiime (Caporaso et al., 2010). Microbiome analyses were conducted by meta-analysis approaches using the MicrobiomeAnalyst platform based on QIIME and R replications, starting from marker gene abundance data (MDP) (Chong et al. 2020). The α-diversity, demonstrated for the adopted management system (conventional or organic) and nematode inoculation (RKN-absent or RKN-present), was calculated by Shannon index to represent community diversity and by the number of observed OTUs to represent community richness. The β-diversity was used to verify clusters and variances of communities in both organic and conventional rhizospheres by using principal coordinate analysis (PCoA), while the dissimilarity of the systems was measured by the Bray–Curtis method. The statistical significance of the samples was evaluated by PERMANOVA ($P < 0.05$). The differences in the abundance of genera were evaluated by performing a Gaussian fit test ($P < 0.05$, FDR < 0.05). All steps were applied to both 16S and ITS to verify bacterial and fungal abundance and diversity. Additional analyses are described in supplementary methods (Supplementary Information).

Results

Physicochemical and nematode profiles of conventional and organic soils

The physicochemical properties of the soils differed slightly, but not strongly in general (Supplementary Table S1). Briefly, the organic soils had more organic matter, while conventional had more available potassium, phosphorus and calcium amounts. Both systems had pH near to 7 and clay loam texture. The total number of nematodes (all trophic levels) was higher in organic soils than conventional ($t = 3.66$; $P = 0.02$), as well as the number of only bacterivorous ($t = 5.168$; $P < 0.001$) and only predators ($t = 4.71$; $P < 0.001$) (Supplementary Fig. S1).

Plant growth promotion and suppressiveness of soil microbiomes against Meloidogyne incognita

The growth promotion in tomatoes planted in organic or conventional soils without RKN was significantly higher in natural than sterilized soils (t values > 2.08, $P = 0.03$) (Fig. 1a). But the weights of shoots and roots from organic soils were higher than those from conventional soils ($t > 3.00$; $P = 0.01$). The natural organic soil provided a 20% increase in shoot weight and 30% in root weight compared to natural

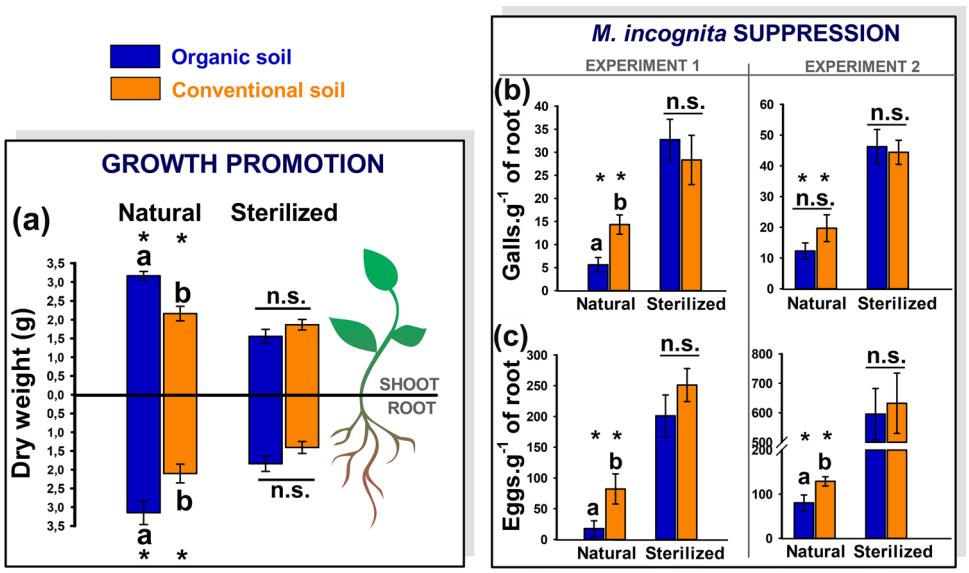

Fig. 1 Growth promotion and suppression of *Meloidogyne incognita* in tomatoes cultivated on sterilized or natural soils from organic and conventional horticultural areas. **a** Growth promotion of tomato shoots and roots by grams. **b** Infectivity of *M. incognita* is represented by the mean number of galls per gram of roots. **c** Reproduction of *M. incognita* is represented by the mean number of eggs per gram of roots. The evaluation of plant growth was made in *M. incognita*-free soils, while the suppression was evaluated in inoculated soils. Asterisks represent significant paired *t*-test values ($P < 0.05$) between sterilized and natural soils of each system. Different letters indicate significance ($P < 0.05$, n.s.: not significant) of *t*-test between means of organic and conventional systems. Bars show ± standard errors of the means. Results came from two combined experiments **(a)** or by each experiment repetition **b** and **c**

conventional soil. Similarly, the number of galls (Fig. 1b) and eggs (Fig. 1c) in tomato roots from soils inoculated with RKN was both reduced in natural soils compared to sterilized ones in the two repetitions (t values < -3.50; $P < 0.01$). The number of galls in natural organic soils was significantly lower than in natural conventional soils (t values < -3.08; $P < 0.01$) only in one experiment, with 67% fewer galls (Fig. 1b). However, egg formation was reduced by 80% in natural organic soils in both experiments (t values < -2.32; $P < 0.01$) (Fig. 1c).

Defense induction against Meloidogyne incognita and defense-related enzymes activity in tomato

To explore whether the rhizosphere microbiome was associated with defense-inducing mechanism, we used split-root bioassays (Fig. 2a). First, the defense was measured by estimating the galls and eggs formation and later by the activity of defense-related enzymes (PAL and POX). Despite that the number of galls and eggs of the conventional soils did not differ between natural and sterilized in the first experiment (t value $= 1.28$; $P = 0.08$) (Experiment 1), the galls and eggs in both experiments were lower in natural soils from the organic greenhouses (t values < 3.2; $P < 0.01$) (Fig. 2 b and c). The natural organic soils significantly induced defense (t values < -3.0; $P < 0.01$) by reducing the number of galls by 40% of (Fig. 2b) and eggs by 52% (Fig. 2c) compared to natural conventional soils**.** However, in the second experiment, the natural organic and conventional soils induced defense against *M. incognita* resulting in fewer galls and eggs compared to sterilized soils (t values < 2.0; $P < 0.01$) (Fig. 2b and c). In consequence, there was a significant increase in the activity of defense-related enzymes when the tomato seedlings were transplanted to both natural soils (Fig. 2d and e). The PAL activity increased significantly by transplanting the seedlings to natural organic ($P < 0.01$) and conventional ($P < 0.01$) soils, even before inoculating the J_2 (Fig. 2d). However, POX activity only increased before inoculation in natural organic soils ($P < 0.01$), while in natural conventional soils the POX activity increased only after nematode inoculation ($P = 0.02$) (Fig. 2e). Organic and conventional soils exhibited lower enzyme activity in sterilized soils than natural ones.

Microbiome transfer into the infested substrate

To guarantee an exclusive effect of the microbiome against RKN, we applied four different amounts of slurry (0–10%)

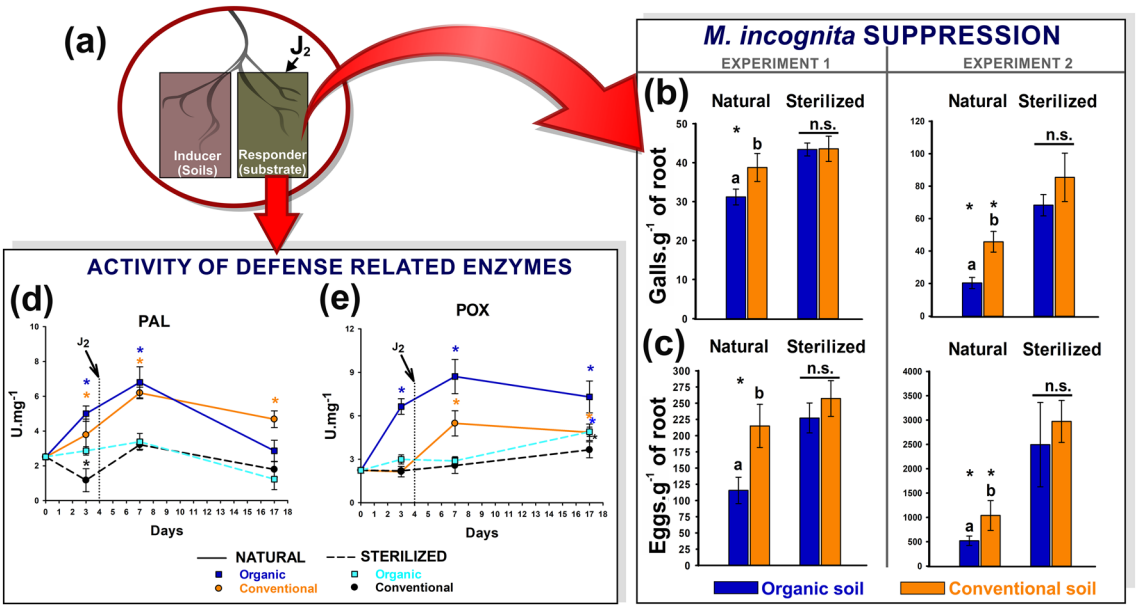

Fig. 2 Defense induction in tomato roots against *Meloidogyne incognita*. **a** Roots of tomatoes were transplanted in natural and sterile soil of organic and conventional greenhouses under the split-root technique. **b** Nematode infectivity by the mean number of gall per gram of roots. **c** Nematode reproduction by the mean number of eggs per gram of root. Activity of defense-related enzymes **d** phenylalanine ammonia-lyase (PAL) and **e** peroxidase guaiacol (POX). The activity was determined for each enzyme unit (U). Black arrows indicate second-stage juveniles (J_2) inoculation moment in the responder cell filled with substrate, while soil treatments were applied in the neighbor inducer cell. **b** and **c** Asterisks represent significant paired t-test values ($P < 0.05$) between means of sterilized and natural soils from each system. Different letters indicate the significance of t-test ($P < 0.05$, n.s.: not significant) between means of organic and conventional systems within the natural or sterilized condition. **d** and **e** Asterisk indicates means that are significantly different from day zero according to the Tukey test at 5% probability. Bars show ± standard errors of the means. The results came from two experiment repetitions, where enzyme activity results were joined

extracted from organic and conventional soils in sterilized substrates. By increasing the amount, the number of galls and eggs was significantly reduced by applying either organic ($P<0.01$) or conventional ($P<0.01$) slurries (Fig. 3). The numbers of galls and eggs were significantly more reduced by using organic slurry than conventional slurry. The number of galls was reduced by 85% when 10% of the organic slurry was applied, while 10% of the conventional slurry reduced the number of galls by 40% (Fig. 3b). The number of eggs was reduced by 88% when 10% of the organic soil slurry was applied, while 10% of conventional soil slurry reduced the number of eggs by 70% (Fig. 3b).

Bacterial and fungal diversity in organic and conventional rhizosphere inoculated or not with Meloidogyne incognita

For rhizosphere samples, 1.31 million reads were recovered after quality filtering for 16S and more than 1.64 million were recovered for ITS (Supplementary Table S2), representing 10 877 bacterial operational taxonomic units (OTUs) and 1594 fungal OTUs at 97% sequence similarity. For α-diversity, species-area curves were obtained for species richness (Supplementary Fig. S2). The bacterial diversity ($F=7.71$; $p<0.01$), represented by the Shannon index, was higher in the conventional rhizosphere than the organic rhizosphere. Bacterial richness ($t=6.41$; $P<0.01$), represented by the observed OTUs, was similar between the horticultural adopted systems in RKN-absent rhizospheres (Supplementary Fig S3a). However, in the organic RKN-present rhizosphere, there was an increment in both richness ($F=45.7$; $P<0.01$) and observed OTUs ($F=27.7$; $P<0.01$), while in the conventional rhizosphere they remained similar to RKN-absent samples (Fig. 4a). The richness of the fungal community was similar between organic and conventional rhizospheres ($t=0.22$, $p=0.14$) but with a different diversity, where the conventional rhizosphere presented a higher Shannon index ($P=0.003$; $t=4.0655$) in RKN-absent samples (Supplementary Fig. 3b). However, similar to bacteria, in the organic rhizosphere there was an increment in fungal diversity ($F=22.3$; $P<0.01$) and observed OTUs ($F=5.37$; $P=0.02$), in RKN-present samples (Fig. 4b). Regarding the β-diversity, PCoA revealed a significant effect of the adopted system for both bacterial and fungal communities, even in the RKN-absent rhizosphere (Supplementary Fig. S3). In general, only the kind of horticultural adopted system (organic or conventional) explained 89.6% and 74.8% of the total variability in the community composition of bacteria (PERMANOVA, $P<0.001$) (Fig. 4c) and fungi (PERMANOVA, $P<0.001$), respectively (Fig. 4d). Furthermore, organic and conventional samples were dissimilar from each other by Bray–Curtis analysis for both bacterial and fungal communities (Supplementary Fig. S4). However, the bacterial community (16S) in the organic RKN-present and RKN-absent rhizospheres was less similar (1.5 dissimilarity level) than in conventional soils (Supplementary Fig. S4a).

A total of 22 phyla of Bacteria were identified in all the samples, where Actinobacteria, Acidobacteria, Proteobacteria and Gemmatimonadetes accounted for more than 75% of the relative abundance (Supplementary Fig. S5a). Biocontrol genera of plant diseases as *Bacillus*, *Rhizobium*, *Acidovorax* and *Bradyrhizobium* were equally found in both conventional and organic rhizospheres, while *Burkholderia*, *Pseudomonas* and *Serratia* were only found in the organic rhizospheres (Supplementary Figs. S7 and S8). Excluding *Bacillus*, the abundance of all the biocontrol genera found

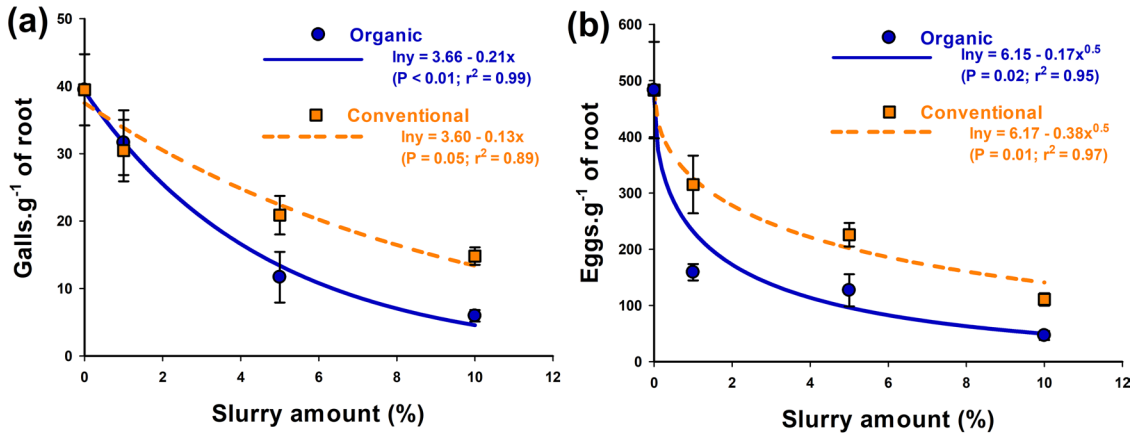

Fig. 3 Slurry containing the microbiome of organic and conventional soils in different concentrations applied against *Meloidogyne incognita*. **a** Infectivity of *M. incognita* is represented by the number of galls per gram of roots. **b** Reproduction of *M. incognita* is represented by eggs per gram of roots. The soil slurries contained soil microbiomes and were applied in sterilized substrates, where 20-day-old tomato seedlings were transplanted. Second-stage juveniles were inoculated in the tomato roots. Bars show ± standard errors of the means. The results came from two combined experiments

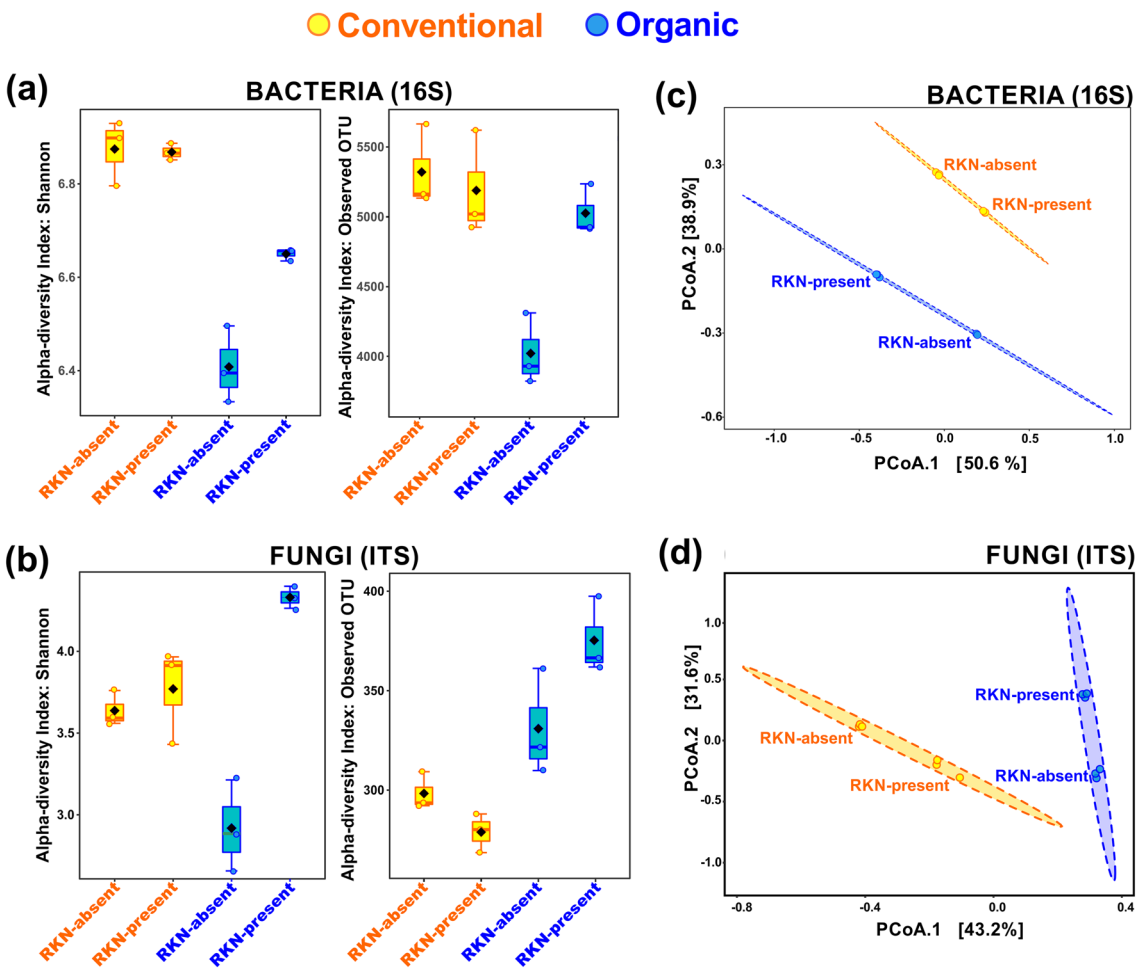

Fig. 4 Comparative analysis of the alpha (α) and beta (β) diversity of 16S and ITS rRNA sequences of the bacterial and fungal community from organic and conventional rhizosphere inoculated (RKN-present) or not (RKN-absent) with *Meloidogyne incognita*. α-Diversity metrics of **a** bacterial 16S rRNA and **b** fungal ITS gene fragments of tomato rhizosphere in four treatments shown by Shannon index and observed OTUs. Rarefaction curves were done in 5 steps. β-Diversity metrics of **c** bacterial 16S rRNA and **d** fungal ITS genes fragments from tomato rhizosphere in four treatments by using PCoA. Distances of PCoA were measured by Bray–Curtis method. Three samples of RKN-present or RKN-absent were used for each soil system

in the organic rhizosphere has increased in the RKN-present rhizosphere (Fig. 5a). For the conventional soil, there was a significant change in bacterial abundance in the RKN-present rhizosphere only for *Proteobacteria*, *Patescibacteria* and *Bacteroidetes* (Supplementary Table S3; Fig. 5b), where only the abundance of biocontrol agents *Acidovorax* and *Rhizobium* significantly increased (Fig. 5b).

There was a significant difference in 20 fungi orders between the organic and conventional samples in RKN-present rhizospheres, including orders with important biological control agents of RKN, such as Eurotiales, Hypocreales and Orbiliale, which were higher in organic rhizospheres ($P < 0.01$) (Supplementary Fig. S9b). Therefore, the 20 most abundant orders increased in organic RKN-present rhizospheres, while any increment happened at the order level in the conventional rhizosphere (Supplementary Table S4). The genera usually involved with biocontrol of RKN *Trichoderma*, *Clonostachys*, *Purpureocillium* and *Arthrobotrys* were found in both organic and conventional samples, but at higher abundance in organic rhizospheres (Supplementary Fig. S10). However, *Beauveria* and *Metarhizium* genera, which are also biocontrol agents, were only found in the organic rhizosphere (Supplementary Fig. S10). Yet, in the organic RKN-present rhizospheres, there was a significant increase in five RKN biological control genera of fungi (Fig. 6a), while for the conventional RKN-present rhizospheres, there was a significant increase in abundance only for *Trichoderma* (Fig. 6b).

Additional results are given in Supplementary Information.

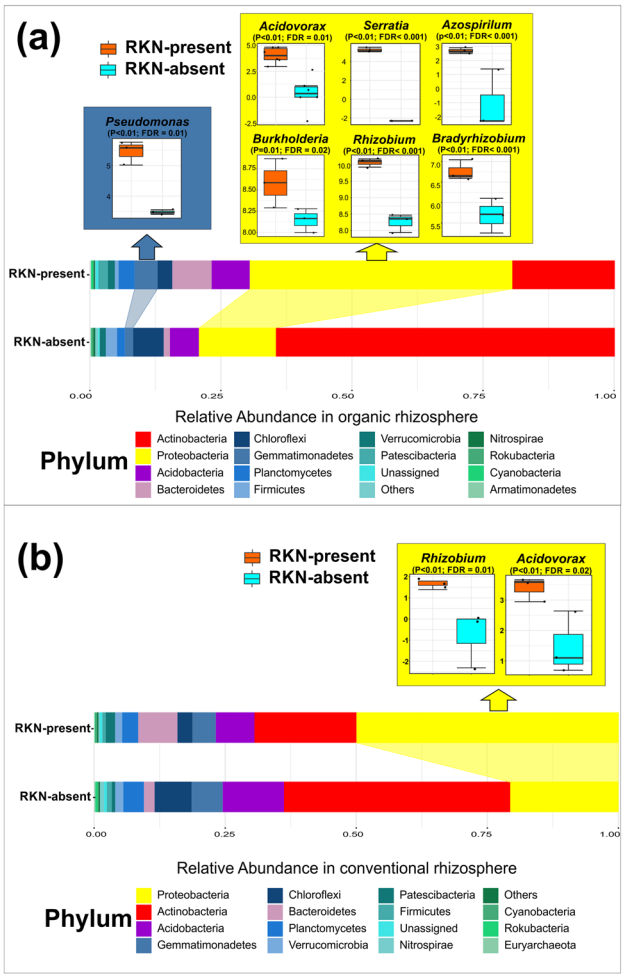

Fig. 5 Relative abundance at phylum level (%) and taxonomic distribution of bacterial OTUs, when assessed by 16S rRNA gene fragment sequencing from the rhizosphere of soils inoculated or not inoculated with *Meloidogyne incognita*. **a** Organic rhizosphere samples. **b** Conventional rhizosphere samples. The 15 most abundant phyla were shown for all genera of biocontrol agents with differentially abundant are shown. The differences in the abundance of genera were evaluated by metagenomeSeq performing Gaussian fit test ($P < 0.05$) after normalizing the data through CSS

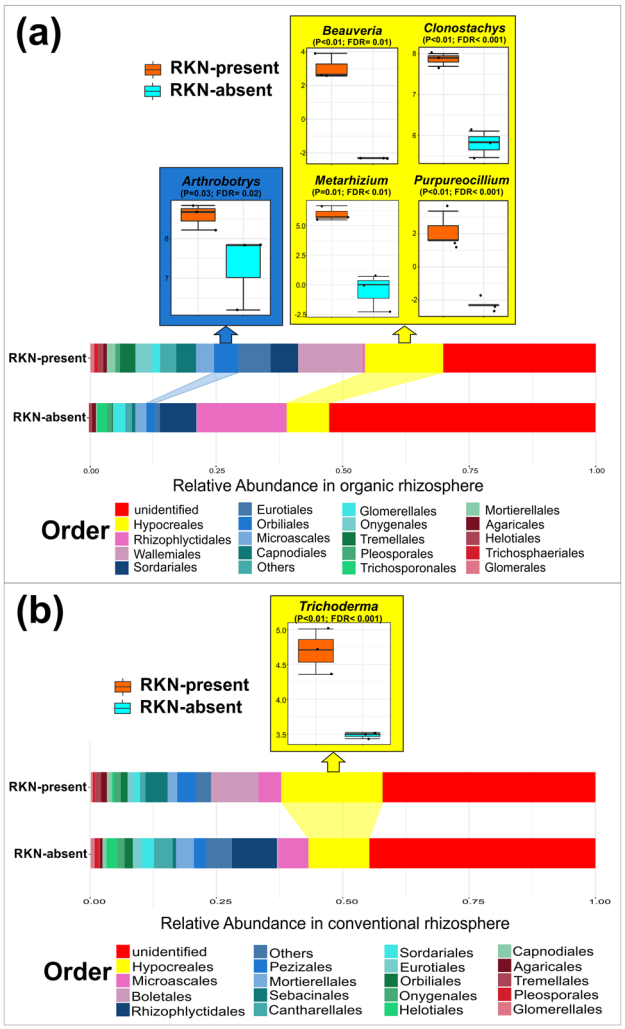

Fig. 6 Relative abundance at order level (%) and taxonomic distribution of fungal OTUs, when assessed by ITS gene fragment sequencing from the rhizosphere of soils inoculated or non-inoculated with *Meloidogyne incognita*. **a** Organic rhizosphere samples. **b** Conventional rhizosphere samples. Only the 20 most abundant orders were shown for each system. All genera of biocontrol agents with significantly different abundances are shown. The differences were evaluated by metagenomeSeq performing Gaussian fit test ($P < 0.05$) after normalizing the data through CSS

Discussion

In this work, we used RKN-free soils under long-term horticultural systems with crops susceptible to *M. incognita* and consolidated the hypothesis of the whole soil microbiome as the major driver in those soils' suppressiveness. We provided novel insights regarding the activity of the microbiome as a whole against the *M. incognita* from both organic and conventional horticultural soils. By evaluating mechanisms of growth promotion and defense induction in comparison with sterilized soils, we noticed the major relevancy of the microbiome in supporting the suppressiveness against *M. incognita*, as previously reported in other crop systems (Adam et al. 2014; Elhady et al. 2018; Tian et al. 2015; Topalović et al. 2020a; Weller et al. 2002). Usually, a wide range of organisms and biocontrol mechanisms are simultaneously antagonizing the RKN in the soil (Silva et al., 2018; Topalović and Heuer, 2019). However, the complexity of bacterial and fungal diversity in the rhizosphere of different horticultural systems was for the first time compared with RKN presence and absence.

Suppressiveness of the soil microbiome against Meloidogyne incognita

The long-term horticultural systems of this work were established in the same area, which provided similar soil physicochemical conditions, but a differential level of suppressiveness against the RKN. Physicochemical properties of the soils could be involved in the suppressiveness against RKN as we found loam clay soils and reasonable organic matter content in both soils. These physicochemical conditions are involved in plant-parasitic nematodes suppressiveness (Silva et al., 2018). However, both organic and conventional soil microbiomes were capable of promoting tomato growth, inducing systemic defense and directly affecting the *M. incognita* infection and multiplication by comparing to sterilized soils. Long-term conservative practices under similar conditions provide soil co-adaptation between the root and its associated microbiome, which may lead to more beneficial plant feedback (Babin et al. 2021; Goss-Souza et al. 2020). Under these circumstances, the establishment of antagonistic organisms is likely to be maximized because sufficient time has passed for adaptation among members of the microbiome (Qiu et al. 2014). Furthermore, when we transferred the soil microbiome in different concentrations to a sterile substrate, an increase of antagonistic effects was noticeable, turning a conducive soil into a suppressive one that was able to reduce the number of galls and eggs in tomato roots by about 90% (Fig. 3). Despite the suppressiveness of organic and conventional soils, the galls and eggs were reduced by more than 50% in the organic soil compared to the conventional soil (Fig. 1). The activity of defense-related enzymes increased in organic soils even before inoculation with *M. incognita* (Fig. 2). This evidence points to the most effective microbiome of organic soils in the specific suppressiveness against *M. incognita* for horticultural crops. Remarkably low levels of the RKN under prolonged organic practices have been described in the literature (Giné et al. 2016; Harkes et al. 2020). Additionally, the agrochemicals applied in the conventional system may interfere with the whole microbial community by inhibiting or eliminating certain groups of organisms and selecting members adapted to conventional practices (Lupatini et al. 2017; Stagnari et al. 2014). Antagonistic recruitment and establishment in the rhizosphere may explain the success of increasing suppressiveness by increasing microbial abundance (Qiu et al. 2014).

Microbiome patterns in organic and conventional horticultural soils

In contrast to free-living nematodes (see supplementary discussion in Supplementary Information), the diversity of the bacterial community was higher in the RKN-absent conventional rhizosphere than in the organic rhizosphere, while fungal diversity diverged between the horticultural systems. However, the α-diversity of bacteria and fungi has increased by introducing the RKN in the organic rhizosphere, while in the conventional rhizosphere remained at the same level. Nevertheless, β-diversity distinguished both fungal and bacterial communities in different groups, which was highly explained by the kind of adopted system (more than 70% of the total variability). The modified rhizobiome after a pathogen attack protects the plant via multiple mechanisms in a 'crying for help' model (Rolfe et al. 2019). Therefore, the recruitment of microorganisms in infested soils selected antagonists of RKN more efficiently in organic horticultural soils, as already demonstrated in previous works (Adam et al. 2014; Elhady et al. 2017; Hussain et al. 2018; Topalović et al. 2020a). The impact of the practices in each horticultural system interferes with the bulk soil community and consequently in the capability of rhizosphere recruitment, where areas that adopt organic systems seem to respond better than conventional adopted areas (Bakker et al. 2018; Crowder et al. 2010).

Bacterial and fungal assembly in RKN-present or RKN-absent rhizospheres

The bacterial phyla Actinobacteria, Acidobacteria, Proteobacteria and Gemmatimonadetes were the most abundant in both organic and conventional soils and are usually abundant in RKN suppressive soils (Harkes et al. 2020; Zhou et al. 2019). Biocontrol and growth promoter bacteria, such as *Bacillus, Rhizobium, Acidovorax* and *Bradyrhizobium*, were found in both soil systems. These groups have been found in the resident community (bulk soil) of suppressive areas against RKN (Harkes et al. 2020). Despite that the conventional rhizosphere presented higher bacterial abundance, the number of exclusive bacterial genera was higher in the organic RKN-present rhizosphere (Supplementary Fig. S7, S8). Works suggest that bacterial shifts in inoculated soils may provide novel weapons for the attacked rhizosphere (Wolfgang et al. 2019). Additionally, chemical applications in conventional horticultural areas indirectly interfere with the shifts in the bacterial community, which is less affected in organic conservative systems (Lupatini et al. 2017). The increase of genera *Burkholderia*, *Pseudomonas* and *Serratia* was partly responsible for the enrichment of the bacterial abundance in the organic rhizosphere. These genera have been found in soils with significant suppressiveness to soilborne disease biocontrol including plant-parasitic nematodes (Bakker et al. 2018; Castillo et al. 2017; Elhady et al. 2017; Tao et al. 2020; Topalović et al. 2020b; Wolfgang et al. 2019; Zhou et al. 2019). Particularly, the *Pseudomonas* genus is often related to RNK antagonism by multiple mechanisms including plant defense induction (Nguyen

et al. 2010; Silva et al. 2018; Zhai et al. 2018). Furthermore, *Pseudomonas* and *Bradyrhizobium* can synergize and increase nutrient uptake, which promotes plant growth and defense against RKN (Khan et al. 2016). The recruitment of *Pseudomonas* and *Burkholderia* in the organic rhizosphere, in association with other bacteria found in both rhizosphere systems, may also improve the soil suppressiveness against RKN (Topalović et al. 2020a; Wolfgang et al. 2019). Despite the evaluation of resistance induction by enzyme activities in this work supporting such evidence, more mechanisms should be investigated to fully understand the involvement of the whole microbiome in RKN suppression.

Most of the fungi identified in this work were distributed in the phyla Ascomycota (Supplementary Fig S5b). Orders containing important biological control agents, such as Eurotiales, Hypocreales and Orbiliales, were found in both systems, but similar to the bacterial community, the organic soil presented an increasing number of fungal OTUs in the RKN-present rhizosphere. In contrast to bacteria, chemical applications in conventional systems directly interfere with the fungal community (Crowder et al. 2010; Lupatini et al. 2017). The fungicides azoxystrobin + difenoconazole and copper hydroxide, applied in the conventional greenhouses sampled in this work, disturb any of those Ascomycota. The genera *Trichoderma*, *Clonostachys*, *Purpureocillium* and *Arthrobotrys*, usually involved with biocontrol of RKN, were found in both systems but were usually more abundant in organic RKN-present rhizosphere (Supplementary Fig. S10 and Fig. S11). The *Trichoderma* had increased in abundance in both RKN-present rhizospheres and might strongly assist the plant against the infection of RKN (De Medeiros et al. 2017; Kiriga et al. 2018; Pocurull et al. 2020). *Arthrobotrys*, *Trichoderma* and *Purpureocillium* prey on juveniles and eggs by producing chitinase and usually increase in the presence of nematodes (Topalović and Heuer 2019). Organic soils usually preserve these nematophagous fungi (Harkes et al. 2020). Furthermore, the genera *Beauveria* and *Metarhizium*, only found in the organic rhizosphere, are also chitinase producers, growth promoters and plant defense inducers (Chairin and Petcharat 2017; Raad et al. 2019). Therefore, these genera are certainly involved in the advantages of organic soil suppressiveness over conventional soils in RKN-present rhizospheres.

Concluding remarks

The soil microbiome was the major responsible for the suppressiveness against *M. incognita* in the organic and conventional soils of the horticultural areas sampled for this work. Suppressiveness changed between the crop systems. Effects in growth promotion, galls and egg formation and defense induction were better performed in the organic than conventional soils. The microbiomes' major influence was confirmed when their transference to a sterile substrate suppressed *M. incognita* in tomato roots. Free-living nematodes were most abundant in organic soil, while a higher diversity in the bacterial community was found in the conventional rhizosphere. However, the organic rhizosphere recruits antagonistic bacteria and fungi more efficiently in the presence of *M. incognita*. Therefore, we provided evidence of the organic practices efficiently suppressing the *M. incognita* and intensifying the assemblage of the antagonistic microbiome in the presence of RKN in horticultural soils.

Data deposition

The sequenced amplicon libraries were deposited in the National Center for Biotechnology Information (NCBI) and are publicly accessible under the accession bioproject number PRJNA624777.

Author contributions

JCPS, FHVM and VPC conceived the study. JCPS, TCSN, RAG and RZ conducted the experiments. JCPS, VP and LSASA analyzed data. All authors read and approved the manuscript.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal participants This article does not contain any studies with human participants or animals (vertebrates) performed by any of the authors.

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