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RESEARCH

Bacterial Composition of *Meloidogyne exigua* Egg Masses from Symptomatic and Asymptomatic Coffee Plants

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Abstract

Root-knot nematodes (*Meloidogyne* spp.) threaten global agricultural production. Bacteria that inhabit the nematode egg mass have not been well explored. Using a metataxonomic approach based on sequencing the 16S rRNA gene of bacteria communities associated with *Meloidogyne exigua* egg masses, we found significant differences in bacterial composition and diversity in the egg masses of symptomatic coffee plants compared with asymptomatic ones for the first time in field conditions. The families *Pseudomonadaceae*, *Burkholderiaceae*, *Flavobacteriaceae*, *Rhizobiaceae*, *Micrococcaceae*, and *Bacteroidaceae* were more abundant in egg masses sampled from asymptomatic plants, and *Chitinophagaceae*, *Glycomycetaceae*, *Micropepsaceae*, *Beijerinckiaceae*, and *Enterococcaceae* were more abundant in samples from symptomatic plants. The genera *Pseudomonas*, *Sphingobacterium*, *Flavobacterium*, *Corynebacterium*, and *Virgibacillus* were found in greater abundance in egg masses from asymptomatic plants, and only *Tumebacillus* and *Bacillus* were significantly more abundant in samples from symptomatic plants. The reproduction and infectivity of *M. exigua* was tested in tomato plants. The reproduction index of *M. exigua* (nematodes eggs per gram of roots) was significantly lower when applying nematode inocula from asymptomatic coffee plants compared with inocula from symptomatic plants. The root weight of tomato plants infected with inocula from asymptomatic coffee plants was significantly higher than that of plants infected with inocula from symptomatic plants. However, there was no significant difference in the infectivity index (number of galls per root system) of tomato plants when inoculated with inocula from either source ($P \leq 0.05$). This study showed a differential bacterial community colonizing coffee plants with different levels of nematode infections, which opens the door for future nematode biological control.

Keywords: biological control, bionematicide, *Coffea arabica*, microbiome, soil microbial ecology

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Author contributions: D.d.B., V.P.C., and W.C.T. conceived the study. D.d.B., V.P.C., and W.C.T. developed the methodology. D.d.B., R.G.T., and D.d.S.M. conducted the formal analysis. D.d.B., L.L.d.P., and W.C.T. conducted the investigation. V.P.C. and W.C.T. provided the resources. V.S.P. curated the data. D.d.B. and W.C.T. wrote and prepared the original draft of the manuscript. D.d.B., W.C.T., D.d.S.M., V.N.K., S.M., V.S.P., and T.S.d.C. reviewed and edited the manuscript. W.C.T. and V.P.C. supervised the study. V.P.C. and W.C.T. performed the project administration. V.P.C. acquired the funding. All authors have read and agreed to the published version of the manuscript.

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The cultivation of coffee (*Coffea arabica*) in Brazil is important from economic and social perspectives, as the country is the largest producer and exporter of the grain in the world. In 2024, the estimated production reached 42.10 million 60-kg bags, with a cultivated area of 1.8 million hectares and gross revenue of $21.26 billion (CONAB 2024). Despite the historical relevance of coffee production for the country, the development of this crop has always been threatened by plant-parasitic nematodes, particularly by species belonging to the root-knot nematodes (*Meloidogyne* spp.) (Campos and Villain 2005).

Among the *Meloidogyne* spp., *M. exigua* is the most widely disseminated root-knot nematode in coffee production areas in Brazil (Castro et al. 2008; Salgado and Terra 2021). This soilborne pathogen generally causes small to large, rounded galls, mostly on new, whitish roots, which turn dark brown as the root becomes older (Villain et al. 2018). Coffee plants infected with *M. exigua*

show reduced absorption of water and nutrients (Pereira et al. 2021). Damage levels vary but can reach a 45% reduction in productivity, as observed by Barbosa et al. (2004) in a plantation in the state of Rio de Janeiro.

The *M. exigua* life cycle occurs in two distinct environments: in the soil, during the migratory juvenile stage, the second-stage juvenile (J2), and inside the host tissue, during the sedentary life stages. In these environments, the nematode interacts with several microorganisms; thus, several ecological relationships can occur, such as antibiosis, predation, and parasitism, which affect both the nematode and its host plant (Costa et al. 2015; Topalović et al. 2020). During the adult stage of *Meloidogyne* spp., nematode females lay eggs in a gelatinous matrix composed mainly of glycoprotein (Sharon and Spiegel 1993) and lectin (Spiegel and Cohn 1982), which can hold more than 500 eggs. The gelatinous matrix is produced in the female's rectal glands and released through the nematode's anal opening (Maggenti and Allen 1960). Orion et al. (2001) demonstrated the role of the gelatinous mass surrounding the *M. incognita* egg mass in resistance to egg-predatory microorganisms.

Over the past two decades, researchers have made important progress in understanding the microbial composition associated with *Meloidogyne* egg masses using culture-dependent methods (Papert et al. 2004). For example, Kok and Papert (2001) isolated and identified 70 bacteria species associated with the *M. fallax* egg mass. Similarly, Costa et al. (2015) and Estupiñan-López et al. (2018) isolated fungi and bacteria from egg masses of *M. exigua* and *M. paranaensis* from coffee plants. However, simply isolating microorganisms in culture media is not enough to unravel all the diversity that occurs in egg masses. In recent years, cultivation-independent methods based on profiling of marker genes or high-throughput metagenome sequencing have made it possible to understand a broader composition of microbial communities associated with different development stages of plant-parasitic nematodes (Topalović et al. 2022).

Using pyrosequencing, Cao et al. (2015) assessed the microbial composition of *M. incognita* at different life stages, including egg masses, and showed that microbial composition was very similar regardless of stage (female, J2, and egg masses). The bacterial communities of females and eggs of *M. paranaensis*, as well as coffee roots infested by the nematode, were found to be an important component of the disease caused by the nematode and may have favored its progress (Lamelas et al. 2020). Therefore, it is crucial to assess the microorganisms associated with *M. exigua* egg masses to enhance our understanding of the biology and ecology of this pathogen, thereby enabling a deeper insight into this pathosystem. Thus, we hypothesized that bacterial communities inhabiting *M. exigua* egg masses vary in structure and diversity and that this variation is host-dependent.

The objective of this study was to identify, through 16S rRNA amplicon sequencing, the bacterial community associated with the *M. exigua* egg masses collected from the roots of symptomatic and asymptomatic *Coffee arabica* plants.

Materials and Methods

Study area

Sampling of coffee roots and rhizosphere soil was done at the experimental area of the Federal University of Lavras (−21.23112, −44.99482), Lavras, Minas Gerais State, in the southeast region of Brazil (Fig. 1). This study area was chosen due to its nematological interest, as the coffee plants were infested with *M. exigua* at varying levels of severity. The purity of the root-knot nematode population was confirmed by the isoenzyme electrophoresis method (Carneiro and Almeida 2001). The area has been used for growing coffee (Topazio variety) for 12 years. The field undergoes conventional management, receiving regular applications of fertilizers, insecticides, and fungicides. The crops did not exhibit symptoms of fungal, bacterial, or viral diseases or attacks by insect pests. However, some plants showed different levels of *M. exigua* parasitism and different symptoms. To characterize these levels, the Boldini scale for coffee leaf coverage was used (Boldini 2001). This is a specific rating scale for coffee plants, ranging from 1 to 5, with a rating of 1 being given to well-developed plants with no symptoms, such as chlorosis or defoliation. A rating of 5 represents plants with severe defoliation and reduced size.

Soil and root sampling

Roots were collected in February 2020, during the rainy season. Six root samples from asymptomatic and six root samples from symptomatic coffee were collected from the borderline of the trees at a depth of 0 to 20 cm ($n = 12$). Each sample contained the roots

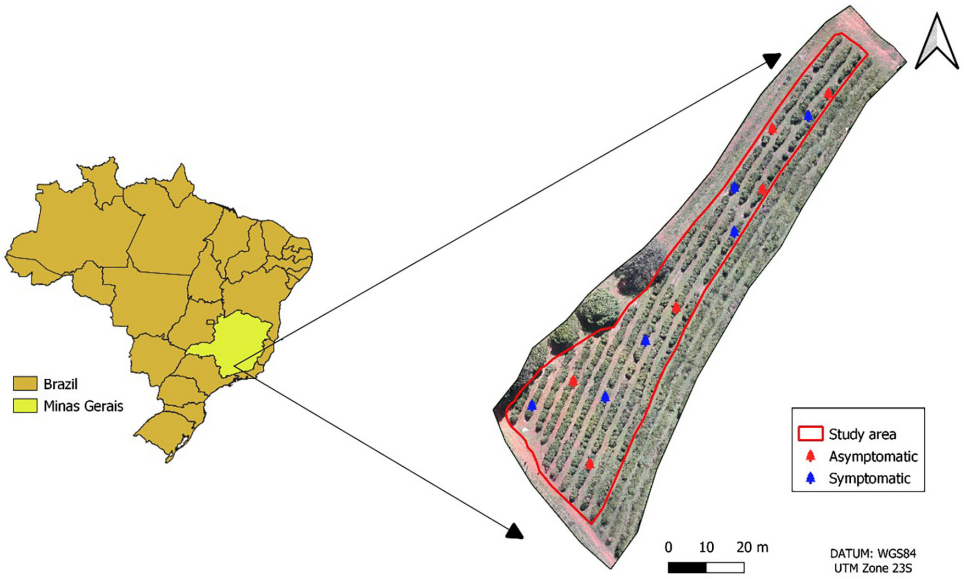

Fig. 1. Map of the study area in Minas Gerais State of Brazil on the left and, on the right, a representation of coffee root sites enclosed by a red line with red and blue dots pointing out symptomatic and asymptomatic sampling plants. WGS84 (World Geodetic System) is a global reference system, and UTM zone (Universal Transverse Mercator) is a coordinate system.

of two selected plants, with approximately 300 g of roots from each one. Additionally, rhizosphere soil was sampled from each plant to compare the bacterial composition in the two environments. For this, the same procedure described for roots was carried out for the rhizosphere collections, with approximately 100 g of soil being sampled in the root zone of each plant. After collection, the samples were stored in a refrigerator (4ºC) until subsequent analysis. To determine the physical and chemical properties of the soil, additional collections were carried out at each sampling point, at a depth of 0 to 20cm. The collected samples were sent to the Soil Analysis Laboratory in the Soil Department of the Federal University of Lavras, where they were processed following the Fertility Laboratory Quality Analysis Program coordinated by the Brazilian Agricultural Research Company (EMBRAPA).

Samples were evaluated for pH (potentiometric method: soil in water), cation exchange capacity (obtained by the sum of exchangeable bases + Al and H), organic matter (titration with potassium dichromate), base saturation (V [%], obtained by the sum of exchangeable bases × 100 divided per cation exchange capacity), aluminum saturation (m [%], obtained by the sum of exchangeable bases × 100 divided per Al), B (extraction with warm water), macronutrient composition (P, K, Mg, S, Ca), and micronutrients (Mn, Fe, Cu, Zn), extracted via the Mehlich method. The percentage of silt, clay, and sand was evaluated using the granulometric method.

Extraction of *M. exigua* egg masses from *C. arabica* roots

To remove egg masses from inside the roots, each root sample was carefully washed with tap water. Next, root fragments were immersed in 70% alcohol for 30 s, followed by immersion in 1% sodium hypochlorite for 1 min, then washed four times in sterilized water. Under a stereoscopic microscope, egg masses were collected from infected root tissues using a sterilized needle. Forty egg masses per sample were obtained from the roots and transferred to microtubes containing sterilized milli-Q water. The egg masses were subsequently used for DNA extraction right away. In addition, 15 egg masses were extracted for the infectivity experiment, conducted in a greenhouse (topic 2.5).

DNA extraction and polymerase chain reaction (PCR)

Total DNA from egg masses and soil samples (0.25 g per sample) was obtained using the DNeasy PowerSoil Pro Kit (Qiagen) according to the manufacturer's recommended protocol. DNA quality was verified by spectrophotometry in NanoDrop (Thermo Fisher Scientific). After the extraction step, the DNA samples were stored at −80°C until further processing. The DNA was amplified in a 25-µl reaction using the Invitrogen Platinum Hot Start PCR Master Mix (Thermo Scientific) according to the manufacturer's protocol. The V4 region of the 16S rRNA gene was amplified with the barcoded primers 515F (CCTACGGGAGGCAGCAG) and 806R (CCTACGGGAGGCAGCAG) (Caporaso et al. 2012). The PCR products were evaluated using electrophoresis on a 1.2% (wt/vol) agarose gel and stained with red gel dye in 1× TAE buffer. The PCR products were purified to remove primers and short fragments using magnetic beads (MagSi-DNA NGSPREP Plus, Magtivio). The multiple PCR products were quantified using a Qubit 2.0 fluorometer (Invitrogen) and dsDNA BR Assay Kit (Invitrogen), then combined in equimolar ratios. This composite sample was concentrated in 25 ng and used for library preparation with the Ion OneTouch 2 System fitted with the Ion PGM Hi-Q View OT2 400 Kit using the Ion 318 Chip v2 (Thermo Fisher Scientific), following the manufacturer's recommendations.

Bioinformatics analysis

The bioinformatics analysis of the sequences was performed following the guidelines of the Brazilian Microbiome Project (Pylro et al. 2014), using the BMP Operating System (BMPOS) (Pylro et al. 2016). Preprocessing of 16S rRNA gene data and diversity estimation were performed with VSEARCH ver. 2.3.4 (Rognes et al. 2016) and QIIME ver. 1.9.1 (Caporaso et al. 2012), respectively. Sequence clustering followed the UPARSE method and was classified into operational taxonomic units (OTUs) at an identity threshold of 97% similarity (Edgar 2010). Representative sequences of OTU groups were used to assign the taxonomic category using the SILVA database (Quast et al. 2013). The 16S rDNA datasets were rarefied to the same number of sequences per database (Lemos et al. 2011) and used to construct dissimilarity matrices generated by Binary and Bray-Curtis distances using the 'phyloseq' package in R. The statistical significance among treatments was calculated using permutational multivariate analysis of variance with 10,000 permutations using the 'Adonis' function. The dataset was summarized at the family level, and changes in microbial diversity were measured using the alpha diversity metric, Chao1, Simpson, Shannon, and Observed indexes (Magurran 2004); beta diversity (principal coordinate analysis) of the bacteria in egg masses and the rhizosphere was compared using a nonparametric test of analysis of similarity (Anderson 2001). Community composition was analyzed using the 'phyloseq' (McMurdie and Holmes 2013) and 'metacoder' (Foster et al. 2017) R packages. For the metacoder R package, the OTUs of the bacterial community between symptomatic and asymptomatic samples were compared. All data were analyzed using R software (R Core Team 2020) and the Microbiome Analyst platform (https://www.microbiomeanalyst.ca) (Botina et al. 2023; Chong et al. 2020).

Greenhouse experiments for infectivity and reproduction of *M. exigua* in tomato plants

For this experiment, tomato plants were inoculated with egg masses from symptomatic and asymptomatic coffee plants. Tomatoes were chosen because of their quick development and wide use in experiments with nematodes. For this, a tomato seedling of the Santa Clara variety, approximately 15 days old, with three pairs of true leaves, was transplanted into a 300-ml plastic cup filled with Multiplant substrate. Five days after transplanting, the 15 egg masses were inoculated into the substrate containing the tomato plants. Six replications were used for symptomatic plants and six replications for asymptomatic plants. To ensure that each tomato plant received the general penetration rate of *M. exigua*, which is 3,000 eggs, first, 5 egg masses were used to quantify the average number of eggs in each egg mass. It was found that each egg mass had approximately 200 eggs. Therefore, it was necessary to collect 15 egg masses to have a total of 3,000 *M. exigua* eggs per plant for the experiment. The egg masses were stored in sterilized test tubes containing sterilized water at room temperature.

The experiment was repeated in July 2020 (called experiment 2), using inocula from the same area and collected from the same plants, as previously described. The inoculum for experiment 1 was collected in February during the summer season. Greenhouse conditions were approximately 25ºC, and plants were fertilized as needed.

For the inoculum control, which represented the quality and viability of the nematode inoculum, just eggs were used, as recommended in the methodological protocol. Coffee roots containing galls were collected from symptomatic and asymptomatic plants. Then, using the Hussey and Barker (1973) method, eggs were released from the gelatinous matrix (egg masses), and 3,000 eggs were inoculated in tomato plants, with six replications. After

45 days of inoculation, the number of nematode eggs per gram of root and number of galls in tomato plants were evaluated.

Statistical analysis

In the greenhouse assay, the experiments were conducted in a completely randomized design, and the results of the repetitions of each experiment were subjected to combined analysis. The data were previously subjected to a normality test (Shapiro-Wilk) and homogeneity test (Bartlett). Next, the F test was applied, using analysis of variance. When the F test was significant ($P < 0.05$), the means for the different treatments were compared using the Tukey test ($P < 0.05$). The R software (R Core Team 2020) was used to run the statistical tests.

Results

Physical and chemical soil properties

There was low variability among soil properties collected in symptomatic and asymptomatic coffee samples (Table 1). Slightly lower pH was observed in soil with asymptomatic plants compared with soil with symptomatic plants. A similar trend was observed for macronutrients, micronutrients, and textural variables.

Bacterial diversity and abundance in egg masses and rhizosphere soil from symptomatic and asymptomatic coffee roots infected with *M. exigua*

Sequencing of the 16S rRNA gene from egg masses yielded a total of 603,328 sequences after quality filtering. On average, each sample contained 50,277 sequences, representing 241 bacterial OTUs. A total of 97,718 sequences were obtained from the rhizosphere, representing 537 OTUs. The phyla Firmicutes and Actinobacteriota were among the most abundant in *M. exigua* egg mass samples; on the other hand, Proteobacteria, Actinobacteriota, Chloroflexi, and Acidobacteria were the most abundant in samples from rhizosphere (Fig. 2). A significant difference in the composition of the bacterial community was observed in *M. exigua* egg mass samples collected from symptomatic versus asymptomatic plants ($P < 0.003$; Fig. 3A). In contrast, no significant differences were detected in the samples from the rhizosphere ($P < 0.11$; Fig. 3B).

Several families were found to be more abundant in samples from asymptomatic plants (Fig. 4, branches and nodes colored in red), including *Pseudomonadaceae*, *Burkholderiaceae*, *Flavobacteriaceae*, *Rhizobiaceae*, *Micrococcaceae*, and *Bacteroidaceae*. In contrast, only a few families, including *Chitinophagaceae*, *Glycomycetaceae*, *Micropepsaceae*, *Beijerinckiaceae*, and *Enterococcaceae* were more abundant in symptomatic plants (Fig. 4, branches and nodes colored in blue). When comparing the taxonomic differences between bacterial communities in egg masses from symptomatic and asymptomatic plants, many taxa were found to be equally abundant (Fig. 5, branches and nodes colored in gray). However, there was a greater abundance of certain genera in asymptomatic samples, including *Pseudomonas*, *Sphingobacterium*, *Flavobacterium*, *Corynebacterium*, and *Virgibacillus* (Fig. 5, branches and nodes colored in red), compared with symptomatic plants, which were primarily represented by *Tumebacillus* and *Bacillus* (Fig. 5, branches and nodes colored in blue).

Regarding the alpha diversity of bacterial communities, samples from the egg masses of asymptomatic plants presented the highest diversity (Shannon; $P < 0.001$) (Fig. 6C) and richness (Observed and Chao1; $P < 0.001$) (Fig. 6A and B) indexes, followed by the Simpson ($P < 0.001$) (Fig. 6D) index. No significant differences were observed in the Observed ($P > 0.001$), Chao1 ($P > 0.001$), Shannon ($P > 0.001$), and Simpson ($P > 0.001$) indexes for rhizosphere samples.

Infectivity and reproduction of *M. exigua*

The infectivity and reproduction of *M. exigua* in tomato plants inoculated with egg masses from asymptomatic and symptomatic coffee plants was evaluated in a greenhouse experiment. In both assays, conducted at different times, the number of galls per gram of tomato root was similar in both treatments (symptomatic and asymptomatic plants) (Table 2). For the variables number of eggs per gram of root and root weight, a statistical difference was verified between treatments ($P < 0.05$) in the two assays. In the first one, after 45 days, there was a 61.3% reduction in the number of eggs and

TABLE 1
Soil properties from the experimental area of the Universidade Federal de Lavras, Minas Gerais, Brazil[z]

		$P_{Mehlich}$	S	K	Na	Ca	Mg	Al	OM	OC	B	Cu	Fe	Mn	Zn	V	m	Clay	Silt	Sand
Samples	pH H_2O	(mg/dm^3)				($cmol/dm^3$)			(g/dm^3)		(mg/dm^3)					(%)				
AS	4.9	5	45.6	0.12	0.01	1.1	0.5	0.2	3.7	2.1	0.26	3.3	34.9	4.5	2.4	23.93	10.36	75.3	5.6	19.1
AS	4.5	3.4	17.6	0.33	0.01	0.5	0.2	1	3.3	1.9	0.19	2.6	42.1	2.3	1.3	10.68	9.02	78.6	4.3	17.1
AS	4.9	4.9	30.4	0.2	0.01	1.9	0.5	0.2	3.6	2.1	0.17	2.8	30.1	8.1	3.7	31.41	7.12	73.6	6	20.4
AS	5.4	8.5	16.2	0.4	0.01	2.9	0.8	0.1	4.3	2.5	0.57	3.4	24.8	10.9	5	51.96	2.38	67	7.6	25.4
AS	4.9	5.1	38.9	0.16	0.01	1.8	0.5	0.1	3.6	2.1	0.35	3.2	31.1	8.1	3.6	30.99	3.89	73.5	6.1	20.4
AS	5	5.3	21.7	0.17	0.01	1.8	0.6	0.2	3.6	2.1	0.38	3	29.9	5.5	4.2	33.16	7.19	76.8	4.4	18.7
S	5.8	4.1	55.6	0.39	0.01	2.7	0.6	0	3.2	1.8	0.15	3	25.4	6.1	3.2	59.68	0	72.9	7.4	19.7
S	5.1	7.1	42.7	0.22	0.01	2	0.5	0.1	4.3	2.5	0.17	2.4	29.9	2.8	1.6	37.24	3.53	72.9	7.4	19.7
S	5.4	4.6	17.7	0.41	0.01	2.3	0.5	0	3.8	2.2	0.15	3.4	32.2	4.7	2.5	45.87	0	74.5	5.8	19.7
S	5.1	4.1	64.2	0.15	0.02	0.7	0.3	0.7	3.9	2.3	0.23	2.3	34	1.6	1.1	13.34	7.43	75.9	7.5	16.6
S	4.8	4.4	54	0.13	0.01	0.7	0.2	0.4	3.1	1.8	0.29	2.2	36.8	2.8	8	16.67	7.78	74.1	5	20.9
S	4.9	2.6	47.3	0.18	0	0.8	0.2	0.4	3.3	1.9	0.33	2.1	43.7	2.3	4.9	20.42	5.32	76.8	5.5	17.7

[z] Soil sampled in coffee plants infected with *Meloidogyne exigua*. OM, organic matter; OC, organic carbon; V, base saturation; m, aluminum saturation; macronutrient composition ($P_{Mehlich}$, K, Mg, S, Ca); micronutrients (Mn, Fe, Cu, B, Zn, Na); AS, asymptomatic sample; S, symptomatic sample. No significance ($P < 0.05$) was obtained by t test between means of asymptomatic and symptomatic treatments.

a 37% increase in the weight of tomato roots when tomato plants were inoculated with egg masses from asymptomatic coffee plants compared with plants that received egg masses from symptomatic plants in the first experiment. In the second assay (repetition), there was a 67.86% reduction in the number of eggs and a 24.79% increase in the weight of tomato roots inoculated with egg masses from asymptomatic coffee plant roots. For the inoculum control, high nematode infection and reproduction were verified, demonstrating the integrity of the inoculum; as no egg masses were used, this suggests the effect of egg masses in the development of nematodes (Table 2).

Discussion

The main objective of this work was to assess bacterial communities associated with *M. exigua* egg masses and compare the abundance and bacterial diversity from samples collected from coffee plants showing different health status. This is the first report to show the complexity of bacterial diversity in *M. exigua* egg masses using the next-generation sequencing approach.

In *Coffea arabica* plantations infested by *M. exigua*, it is common to find both affected and healthy plants within the same field plot (Costa et al. 2015). With this in mind, we selected an

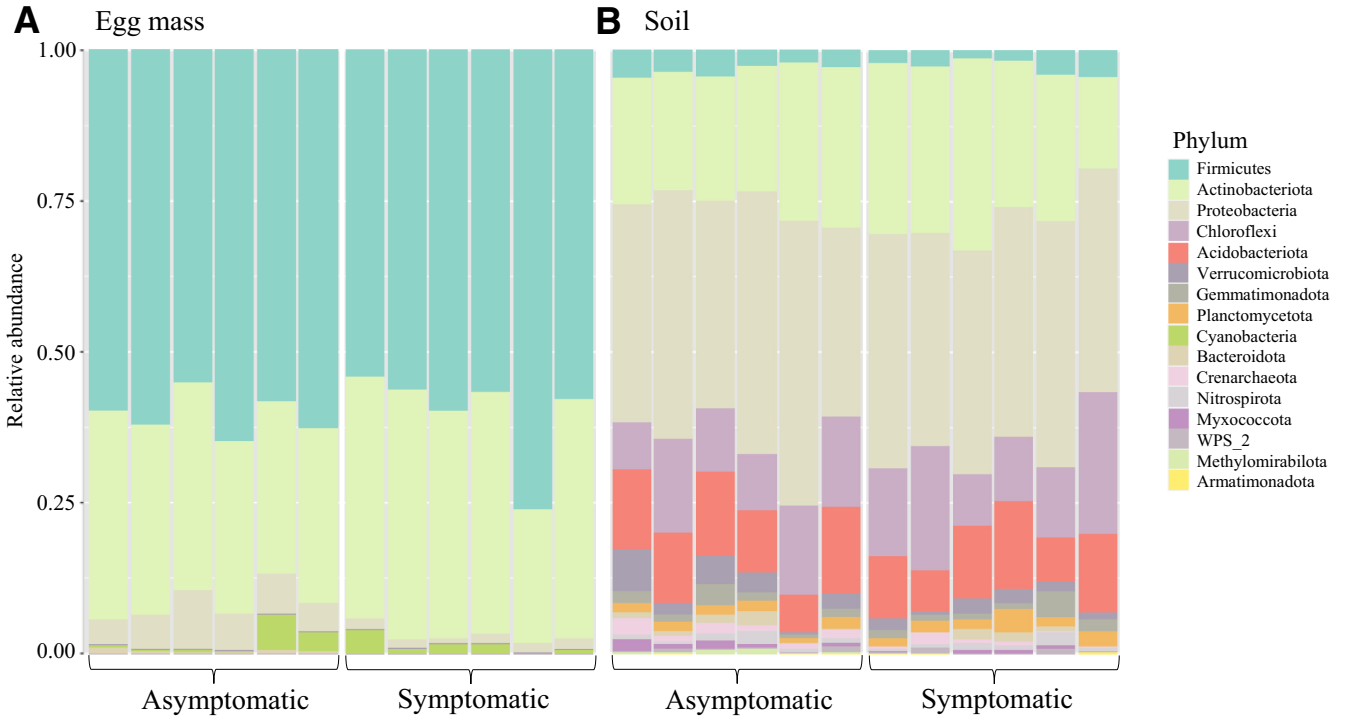

Fig. 2. Relative abundance of operational taxonomic units at the phylum level from **A,** egg masses and **B,** rhizosphere soil from symptomatic and asymptomatic *Coffea arabica* plants. WPS_2, *Candidatus* phylum Eremiobacterota.

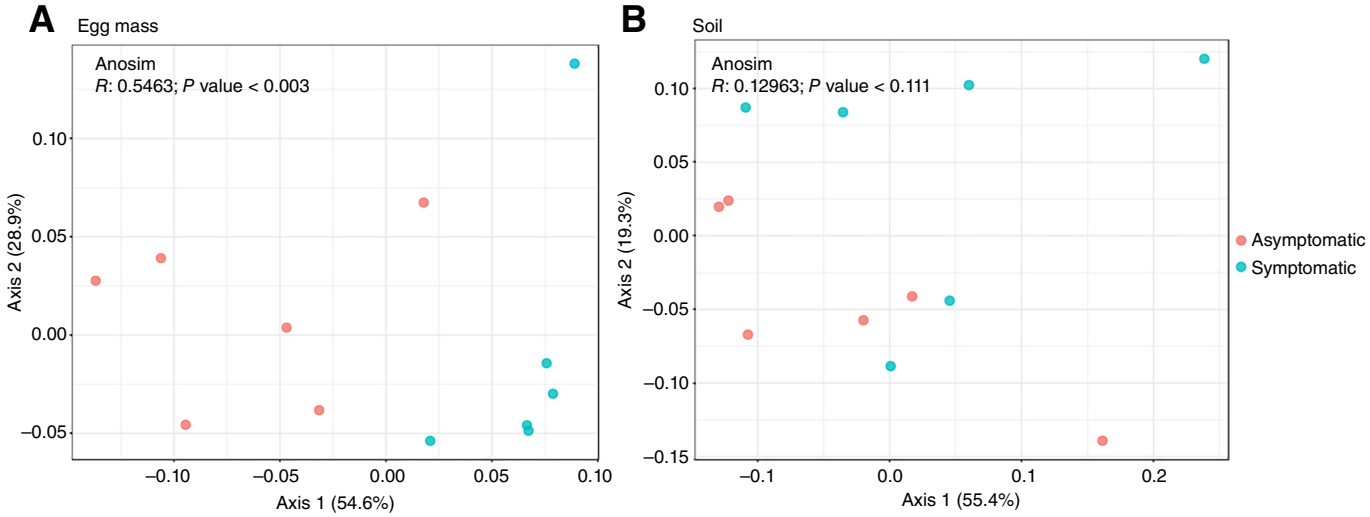

Fig. 3. Principal component analysis of the bacterial community in **A,** egg masses and **B,** rhizosphere soil from symptomatic (blue dots) and asymptomatic (orange dots) *Coffea arabica* plants. Global $R = 0.75$, well-separated.

M. exigua-infested coffee field for our study, with similar soil physicochemical conditions but varying levels of disease against the nematode. Previous research using culture-dependent methods has suggested that bacteria present in the *M. exigua* egg mass play a fundamental role in the disease's development in the field (Costa et al. 2015; Estupiñan-López et al. 2018). Recent technological advances have allowed for the study of egg mass microbiomes using culture-independent methods (Pent et al. 2018; Xia et al. 2019). Until now, only Lamelas et al. (2020) have investigated the bacterial microbiome associated with phytonematodes in *C. arabica* plants. Their study was the first to investigate bacterial communities at different stages of the *M. paranaensis* life cycle, including egg masses in healthy and infected coffee tissues. Unlike our study, the authors did not find significant differences in alpha diversity at any stage of the nematode's life cycle. Another study involving coffee infected with plant-parasitic nematodes and the microbiome was carried out by Hoang et al. (2020), but they explored the effect of endophytic bacteria and nematodes in *Coffea canephora* instead of *C. arabica*.

The dominant phyla found in the *M. exigua* egg masses were Firmicutes, Actinobacteria, and Proteobacteria. The Firmicutes phylum includes several genera that are important for the biological control of phytonematodes, such as *Bacillus* (Chinheya et al. 2017).

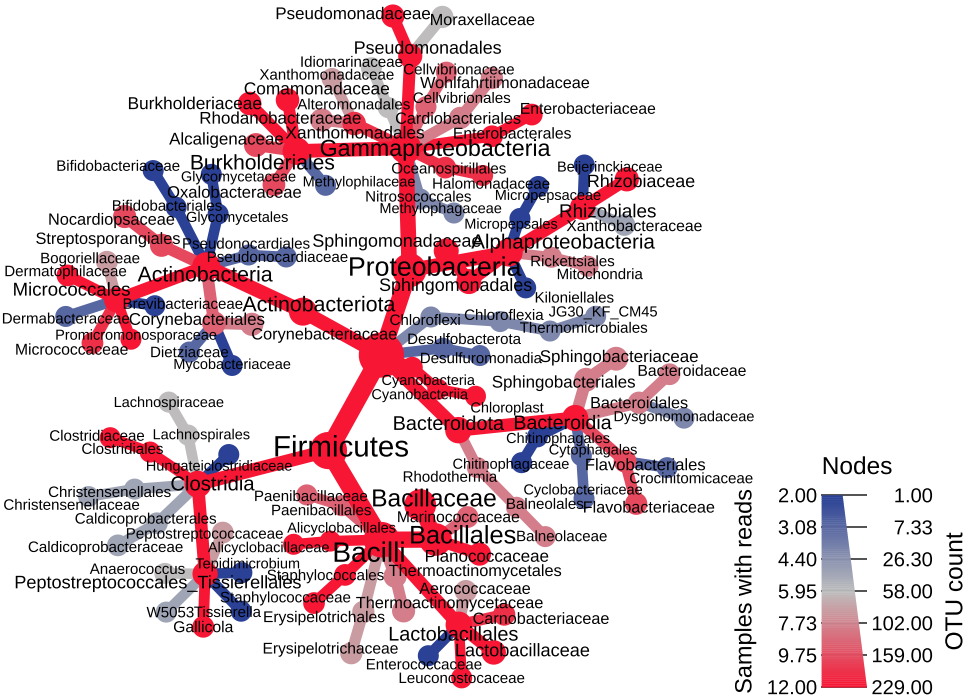

Fig. 4. Differential heat tree showing the relative abundance of bacteria among all the taxa, up to the family level, for egg masses between asymptomatic (colored in red) and symptomatic (colored in blue) coffee plants. For each taxon, a Wilcoxon rank sum test was used to assess differences between the median abundances of samples in each treatment (plant health status). The branches indicate the association between taxa, and the node sizes indicate the operational taxonomic unit (OTU) counts per taxon. Taxa colored in blue are more abundant in symptomatic plants, and those colored in red are more abundant in asymptomatic plants. Taxa colored in gray were equally detected in both symptomatic and asymptomatic plants.

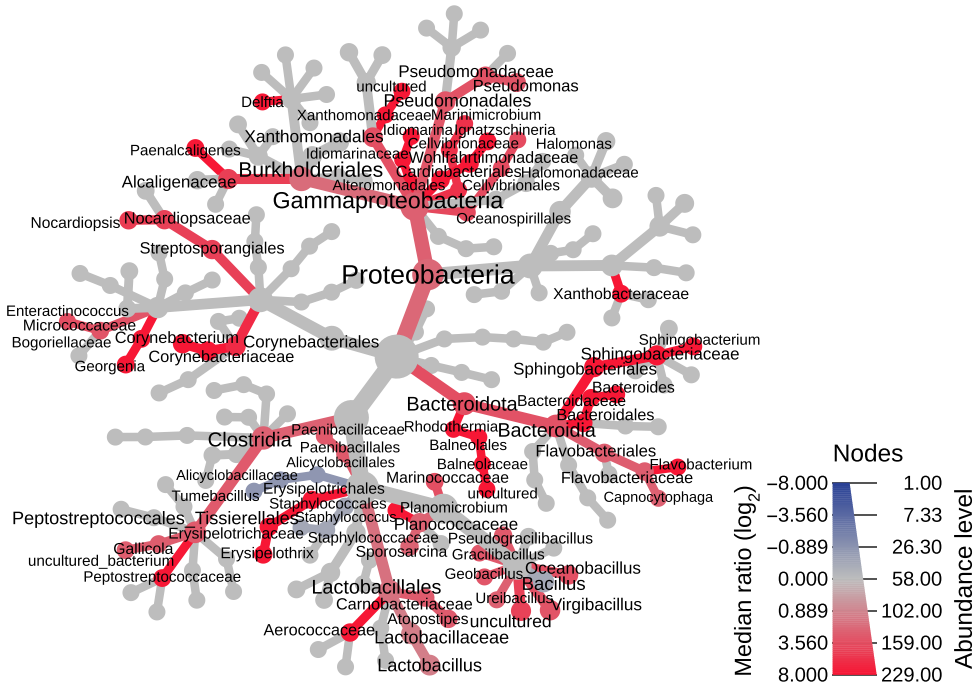

Fig. 5. Differential heat tree showing differences in bacterial composition to the genus level for egg masses. The comparisons were made among the health status of symptomatic and asymptomatic plants. For each taxon, a Wilcoxon rank sum test was used to test for differences between the median abundances of samples in each treatment. Taxa colored in red are statistically more abundant in the asymptomatic plants, and those colored blue are statistically more abundant in the symptomatic plants. The branches indicate the association between taxa, and the node sizes indicate the operational taxonomic unit counts per taxon. Taxa colored in in gray were equally detected in both symptomatic and asymptomatic plants.

Within the phylum Proteobacteria, classes such as Alphaproteobacteria and Gammaproteobacteria encompass bacterial genera known to be plant pathogens, nitrogen-fixing bacteria, and bacteria with potential for biological disease control. Particularly noteworthy within Proteobacteria is the bacterial family *Burkholderiaceae*, which comprises a diverse group of bacteria, including *Paraburkholderia* and *Pseudomonas*, with characteristics that promote plant growth and improve stress tolerance, such as the production of indole acetic acid, the solubilization of phosphates, the solubilization of potassium, and the production of 1-aminocyclopropane1-carboxylate deaminase (Rascovan et al. 2016). Nitrogen-fixing and cellulose-degrading bacteria are rhizobacteria or endophytic bacteria that have been previously associated with plant-parasitic nematodes (Li et al. 2023). In our study, this group of bacteria was found to be more abundant in egg mass samples from asymptomatic plants, including the cellulose-degrading bacterium *Sphingobacterium* within the *Sphingobacteriaceae* family. Also, *Corynebacterium*, a nematode-pathogenic bacterium within the phylum Actinobacteria, was found in greater abundance in egg mass samples from asymptomatic plants. Actinomycetes have been identified as associated with eggs and females of *Meloidogyne* spp. from various host plants using crop-dependent methods (Sun et al. 2006).

The egg mass and rhizosphere environments had different bacterial compositions, as expected. Rhizosphere samples had a greater predominance of the phylum Proteobacteria, a group commonly found in different types of soil. This was also found by Papert et al. (2004), who used the PCR-DGGE technique of the 16S rRNA gene to describe differences in the bacterial community between the egg mass of *M. fallax* and the rhizosphere soil of tomato and potato plants. According to the authors, this difference is likely due to differences in the nutritional composition of the nematode egg mass and root exudates found in the rhizosphere environment. Although

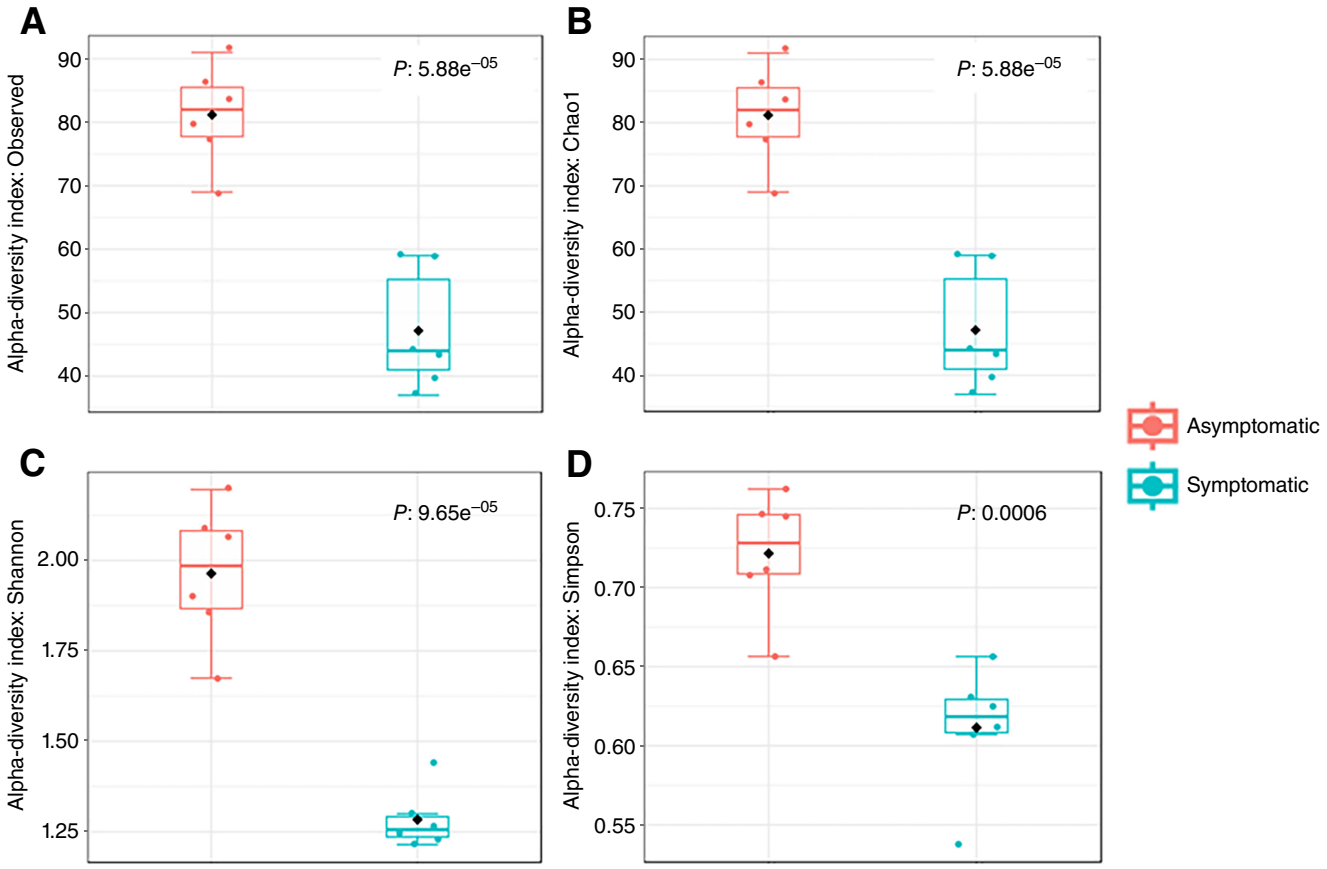

Fig. 6. Alpha diversity indices for the class of bacteria inhabiting the *Meloidogyne exigua* egg mass from symptomatic and asymptomatic *Coffea arabica* plants. **A,** Observed, $P = 5.88e^{-05}$; **B,** Chao1, $P = 5.88e^{-05}$; **C,** Shannon, $P = 9.65e^{-05}$; **D,** Simpson, $P = 0.0006$. Significant at the 0.001 probability level according to a *t* test.

TABLE 2
Infectivity and reproduction of *Meloidogyne exigua* in tomato plants, Santa Clara variety, inoculated with 3,000 *M. exigua* eggs from egg masses of symptomatic and asymptomatic *Coffea arabica* plant roots[z]

Treatments	Galls/g root		Eggs/g root		Root weight	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2
Inoculum control	20.66 a	39.69 a	1,149.33 a	1,015.33 a	12.31 ab	12.31 ab
Asymptomatic	7.66 b	17.33 a	411.14 b	207.31 c	14.59 a	13.79 a
Symptomatic	13.33 b	29.55 a	1,061.43 a	645.03 b	10.65 b	11.05 b

[z] Within columns, means followed by the same letter do not differ significantly from each other by the Tukey test ($P < 0.05$).

the data presented in this study demonstrated differences in the bacterial composition of the *M. exigua* egg masses from symptomatic and asymptomatic coffee plants, the same trend did not occur in rhizosphere soil samples collected from symptomatic and asymptomatic plants. This may be due to the rhizosphere being a more complex environment, with more factors involved, such as pH, exudates, temperature gradients, and different plants and microorganism species inhabiting this environment. These aspects are involved in the interaction with bacteria, resulting in a more buffered bacterial community (Bonito et al. 2019). In the egg mass environment, there are fewer factors involved in comparison with rhizosphere soil. An egg mass is a nutritional niche attracting specific bacteria that can be benefited by food source impacting or not the development of nematodes.

Interactions between nematodes and bacteria can be mutualistic, symbiotic, pathogenic, or parasitic (Proença et al. 2010). Studies on bacteria associated with plant-parasitic nematodes have mainly focused on antagonists due to their importance in regulating pathogen populations. From our greenhouse results, we can highlight that the findings are in line with the molecular analyses, which showed that several bacterial families with known biological control potential against *M. exigua* were more abundant in the egg mass collected from asymptomatic coffee roots (Fig. 4). Some isolates of these bacteria can act synergistically, enhancing the effect against nematodes through direct suppression, promoting plant growth, and facilitating colonization of the rhizosphere. In previous research, Costa et al. (2015) demonstrated that bacteria isolated from the egg mass of *M. exigua* produce volatile organic compounds toxic to its juveniles, causing greater J2 mortality compared with fungi evaluated in the same experiment. Although further studies are needed to understand the relationships between *M. exigua* and its associated bacteria, our results, particularly those regarding the infectivity and reproduction of tomato plants with *M. exigua* egg masses, provide new insights for nematode management, signaling the biotechnological potential of bacteria that inhabit the pathogen's egg mass as agents for biological disease control.

Based on recent studies with other plant-parasitic nematodes (Lartey et al. 2023; Topalović et al. 2022), we can suggest that bacteria associated with egg masses could protect the host by producing nematicide compounds or other mechanisms, such as volatile organic compounds (Wolfgang et al. 2019). Also, with our current results, the acquaintance of bacteria associated with *M. exigua* egg masses can be considered an important step in exploring microbial associations for potential use in nematode control in coffee plants.

In the present work, the key differences between bacterial communities in egg masses of symptomatic and asymptomatic coffee plants were shown. However, additional studies are required to explore the biological roles in nematode hosts and their potential as new targets for controlling plant-parasitic nematodes or enhancing the disease caused by them. Nevertheless, our data indicate that a complex network of bacteria-nematode-soil relationships defines the host health declining.

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