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Bioinoculant-induced plant resistance is modulated by interactions with resident soil microbes

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Abstract

Background Entomopathogenic fungi are increasingly used as bio-inoculants to enhance crop growth and resistance. When applied to rhizosphere soil, they interact with resident soil microbes, which can affect their ability to colonize and induce resistance in plants as well as modify the structure of the resident soil microbiome, either directly through interactions in the rhizosphere or indirectly, mediated by the plant. The extent to which such direct versus indirect interactions between bio-inoculants and soil microbes impact microbe-induced resistance in crops remains unclear. This study uses a split-root system to examine the effects of direct versus indirect (plant-mediated) interactions between an entomopathogenic fungus, *Metarhizium brunneum*, and resident soil microbes on induced resistance in tomato against two-spotted spider mites. Additionally, the study explores how these interactions influence the composition and diversity of soil fungal and bacterial communities.

Results Resident soil microbes reduced the efficacy of *M. brunneum* to induce resistance against spider mites. This reduction occurred not only when resident microbes directly interacted with the bio-inoculant but also when they were spatially separated within the root system, indicating plant-mediated effects. *M. brunneum* inoculation did not affect rhizosphere microbial diversity but led to changes in fungal and bacterial community composition, even when these communities were not in direct contact with the inoculant.

Conclusions This research highlights the impact of both direct and plant-mediated interactions between bio-inoculants and resident soil microbes on bio-inoculant-induced pest resistance in crop plants and underscores the importance of assessing potential adverse effects of fungal bio-inoculants on native soil communities.

Keywords Entomopathogenic fungi, Tomato, Arthropod pests, Soil microbial communities, Spider mites

Background

The use of entomopathogenic fungi (EPF) has become a sustainable strategy in pest control, reducing our heavy reliance on chemical pesticides. While these fungi are primarily known for their ability to cause disease in arthropod pests through mycosis [1, 2], they represent various lifestyles, including entomopathogens, saprotrophs and plant endophytes, that are not mutually exclusive even within genera and species. Entomopathogenic fungi with an endophytic lifestyle can provide a range of benefits to plants [3], including provisioning of nutrients [4], growth promotion [5], and mitigation of abiotic and biotic stress [6, 7]. Recently, there has been an increasing interest in using EPF living as plant endophytes for pest control and many species have been shown to enhance plant resistance to both below- and aboveground

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arthropod pests when inoculated in plants [8–10]. The effects of endophytic EPF on pest resistance are primarily mediated by activation of induced systemic resistance (ISR), associated with systemic alterations in defense gene expression and the downstream production of specific defense metabolites [11–13]. Despite the promising role of EPF as endophytes in pest control, two concerns have been raised regarding their use in sustainable agriculture and horticulture.

The first concern is that, in general, the effects of inoculations with beneficial microbes on pest resistance are highly variable and context-dependent [14], making them less reliable than pesticides. One of the important factors contributing to this context-dependency is the interaction between inoculated beneficial microbes and the native microbial community (NMC). Although EPF such as *Metarhizium sp.* have evolutionarily evolved from a lineage of plant symbionts and are assumed to have basic rhizo-competence [15], the competition for nutrients or root space, as well as antibiosis effects exerted by members of NMC, could reduce their colonization success and thereby their potential to induce resistance. In addition, induction of pest resistance by inoculated EPF could be hampered by indirect, plant-mediated interactions with NMC. For instance, triggering of ISR by beneficial microbes such as endophytic EPF is mediated by phytohormonal signaling through the jasmonic acid dependent pathway, whereas attack by biotrophic pathogens commonly activates phytohormonal signaling through the salicylic acid pathway. Due to commonly observed negative crosstalk between the jasmonic and salicylic acid pathways [16], plant responses to pathogenic members of the NMC could, therefore, interfere with the activation of ISR in response to inoculation with EPF [17]. However, potentially, interactions between EPF and NMC could also positively affect EPF-induced pest resistance due to facilitative interactions in the rhizosphere, for instance, through stimulation of root exudation by NMC, benefitting EPF, or through synergistic effects on plant defense signaling. To our knowledge, no studies have yet attempted to disentangle direct from plant-mediated effects of NMC on EPF-induced resistance. For the first time, in this study, we use a split root system to explicitly test for such direct versus indirect modulation of EPF-induced pest resistance by NMC.

A second concern related to the use of EPF as endophytes for sustainable pest control is the need for a high propagule density during soil inoculations to ensure its successful establishment in the plant. This has raised concerns about the potential negative effects of inoculations on native soil community composition, biodiversity and functioning [18–21]. Recent reviews and meta-analyses indicate that introduced microorganisms indeed commonly alter NMC composition, even to the extent that these communities do not return to their initial state [22, 23]. Thus far, studies that have assessed the effects of inoculations with EPF on NMC have shown inconsistent results, ranging from no to strong effects on NMC [24–26]. The mechanisms by which introduced microbes can alter NMC include both direct (competition, antibiosis, synergism, antagonism) and plant-mediated (e.g., altered root exudation) effects [23]. However, only a few studies have tried to disentangle the impact of such direct versus indirect effects of inoculants on NMC. In this study, we use a split root system to separate direct from plant-mediated effects of EPF on NMC composition and diversity.

Effects of microbial inoculants on NMC are often highly dependent on environmental factors [22]. Recently, it has been shown that the magnitude of the effect of introduced microbial strains on NMC diversity depends on the level of biotic stress experienced by host plants. In particular, Lee Díaz et al. [27] showed that bacterial and fungal inoculants affected the rhizobacterial diversity of herbivory-free plants but not of plants infested by a leaf-chewing herbivore, whereas the presence of the herbivore overall had a strong effect on NMC. Aboveground herbivory commonly alters the root exudation patterns, which in turn affects NMC composition. An increasing number of studies have shown that this can lead to the recruitment of beneficial microbes able to induce ISR against herbivores, interpreted as a "cry for help" by the plant [28–31]. In our study, we therefore also assess whether plant infestation with an aboveground pest results in changes in EPF colonization, changes in the NMC, and changes in the effect of EPF on the NMC.

In a previous study, we showed that the inoculation of tomato plants with the EPF, *Metarhizium brunneum* (Clavicipitaceae), induced resistance to an important agricultural pest (the two-spotted spider mite, *Tetranychus urticae*) [26]. *Metarhizium* is an extensively studied entomopathogenic genus regarding ecology, life history, and pathogenicity towards arthropod pests [32]. As endophytes, several *Metarhizium* species have shown efficacy in the promotion of plant growth and suppressing herbivores, including pestiferous spider mites [11, 33, 34]. In this paper, we aim to test whether the extent of induction of resistance by *M. brunneum* is affected by the type of interaction (no, direct, indirect) with NMC. In addition, we test how soil inoculation with *M. brunneum* affects the NMC, and whether this effect depends on the type of interaction (direct, indirect) and biotic stress of the host plant. Specifically, we address the following questions: (1) Does *M. brunneum* enhance growth and induce systemic resistance of tomato to spider mites and can we detect associated metabolic changes in

leaves? (2) Do resident soil microbes modulate *M. brunneum*-induced spider mite resistance and growth promotion? (3) Do these modulations only occur when resident microbes are in direct contact with *M. brunneum* in the rhizosphere, or can these effects also be mediated by the plant? (4) Does soil inoculation with *M. brunneum* affect the soil bacterial and fungal community? (5) Does spider mite infestation affect the soil microbial community, *M. brunneum* colonization, and the extent to which *M. brunneum* affects the soil microbial community?

Methods

A split-root experiment was conducted in a greenhouse at the Netherlands Institute of Ecology in spring 2023. Tomato plants were cultivated with their root system divided between two pots under five different inoculation treatments to investigate the effects of direct versus indirect (plant-mediated) interactions between resident soil microbes and *Metarhizium brunneum* bio-inoculum.

The five inoculation treatments (Fig. 1) were: (1) Control treatment (C) in which both pots contained sterilized soil, (2) Native microbial community treatment (NMC), in which one pot contained sterilized soil and the other pot non-sterilized soil, (3) *M. brunneum* treatment (Mb), in which both pots contained sterilized soil and *M. brunneum* was added to one of them, (4) *M. brunneum* and the native microbial community in separate pots treatment (NMC-Mb), in which one pot contained non-sterilized soil, and the other contained sterilized soil inoculated with *M. brunneum* (to test effects of potential indirect interactions between NMC and Mb via the plant) and (5) *M. brunneum* and the native microbial community in the same pot treatment (NMC + Mb), in which one pot contained sterilized soil, and the other non-sterilized soil inoculated with *M. brunneum* (to test effects of potential direct interactions between NMC and Mb in the soil).

Preparation of plants, fungi and spider mite cultures

Tomato (*Solanum lycopersicum* L. var. Moneymaker) seeds were obtained from Oranjeband Zaden, the Netherlands and surface sterilized by immersion in 70% ethanol for 1 min, followed by 1% NaClO for 10 min and rinsed five times with sterile double-distilled water. Sterilized seeds were air-dried for an hour in aseptic conditions and stored at 4 °C until needed. To confirm the effectiveness of seed sterilization, 100 µl of the final rinse water was plated on Sabouraud dextrose agar (SDA). The entomopathogenic fungus *M. brunneum* 1868, originally isolated from a diseased *Agriotes* sp. adult insect collected from an agriculturally utilized region in Slovenia [35], was sourced from the mycological collection of the Agricultural Institute of Slovenia. Fresh fungal cultures were propagated on SDAY/4 medium (16.25 g SDA, 2.5 g bacterial yeast extract, 11.25 g agar in 1 L of water) in the dark at 24 ± 1 °C for 2 weeks. Fungal suspensions were prepared by adding 10 mL of 0.01% Triton X-100 to the cultured SDAY/4 plates to scrape off fungal spores. The liquid was put in a 50 mL falcon tube, vortexed, and strained through multiple layers of sterile cheesecloth.

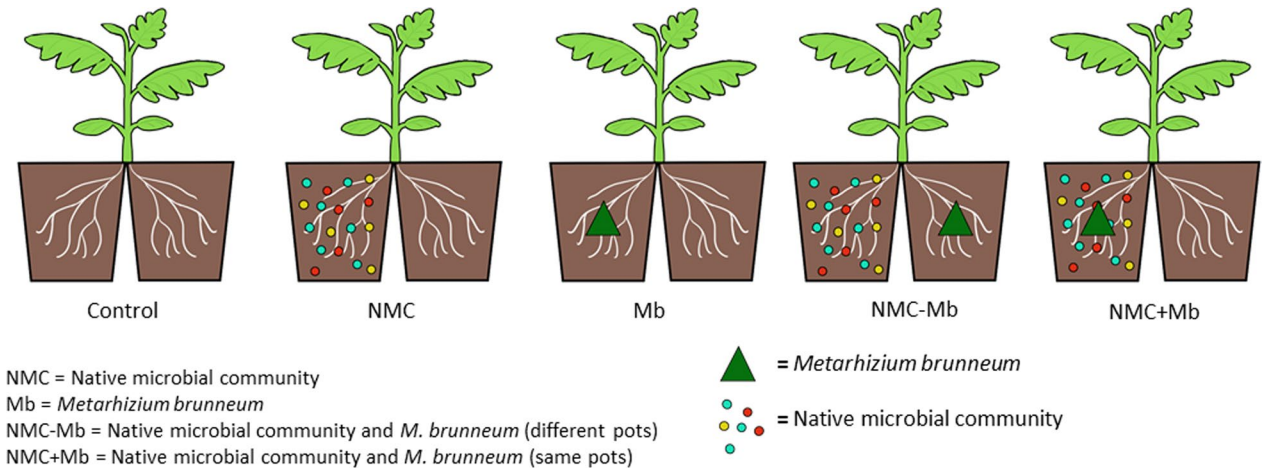

Fig. 1 Experimental setup with a split-root system to discriminate between effects of direct versus indirect interactions between a bio-inoculum (*Metarhizium brunneum*, Mb) and the native microbial community (NMC) on two-spotted spider mite (*Tetranychus urticae*) resistance in tomato. Three weeks old tomato seedlings were transferred to two adjacent pots with half of their roots in each of the pots. Direct interactions were enabled by putting the native microbial community and *M. brunneum* in the same pot (NMC + Mb), indirect (plant-mediated) interactions were enabled by putting the native microbial community and *M. brunneum* in different pots (NMC-Mb), no interactions were enabled with only *M. brunneum* (Mb) and only the native microbial community (NMC) treatments. A sterile soil treatment served as a control. Plants were either infested with spider mites or not

Spore concentrations in the filtered liquid were counted using a Fuchs-Rosenthal hemocytometer and suspensions were diluted to contain 1×10^8 spores ml^{-1} before inoculation. A colony of two-spotted spider mites (*Tetranychus urticae*) was acquired from the insect-rearing facility of the Department of Entomology at Wageningen University, the Netherlands and maintained on tomato plants for several generations.

Experimental setup

Seeds were germinated in trays containing sterilized coarse vermiculite for 21 days. The vermiculite was sterilized by autoclaving for 15 min at 121 °C. After germination, seedlings received 1/2 strength Hoagland nutrient solution twice a week. Three weeks after sowing, 180 similar-sized tomato seedlings (36 for each of the five treatments) were transplanted into the split-root setup (Fig. 1), consisting of two adjacent pots (9×9×10 cm Desch Plantpak), containing 800 g of soil: sand mix (sterilized or non-sterilized). The soil with the native microbial community was low-nutrient sandy soil collected from an arable field near Wageningen, the Netherlands. Soil was sieved through a 5 mm mesh and mixed with 0.71–1.25 mm coarse sand (Wildkamp, Lutten, the Netherlands) in a 3:1 ratio (w/w). Half of the soil: sand mix was sterilized by γ ray sterilization with a dosage of > 25 kGray (Isotron, Ede, The Netherlands). During transplantation, seedlings were carefully uprooted from the seedling trays, and vermiculite was removed by shaking. Holes were made in the soils on the sides of both pots and seedlings were transferred with half of their root system in one pot and the other half in another pot. As the short primary root cannot be split, half the plants within each treatment combination had their primary root placed in the inoculated pot and the other half in the non-inoculated pot. The roots were covered with soil, and vermiculite was sprinkled over the soil to reduce water evaporation. Transplanted seedlings were held up with two wooden sticks and a metal wire was loosely placed around the stem between the cotyledons and the oldest leaves for stability (Fig. S1). Plants were arranged in eighteen blocks each containing one replicate of each treatment combination.

Each pot was placed on a saucer (114×114×58 mm) to avoid cross-contamination and allow plant watering through capillary water uptake from the saucer. The plants were watered with 40 mL of 1/2 strength Hoagland nutrient solution once a week and with tap water twice a week. The greenhouse conditions were maintained at 25 °C during the 16 h day and 18 °C during the 8 h night, with 50–60% RH. One day after transplantation, pots were either inoculated with 2 mL of a 1×10^8 spores ml^{-1} fungal suspension (for pots assigned to inoculation with *M. brunneum*) or with 2 mL of 0.01% Triton X-100 by pipetting the liquid on the soil near the base of the stem.

Spider mite bioassay

Two weeks after inoculations, each treatment was divided into two groups of 18 plants, each group equally distributed with respect to the location of the primary root. One group per treatment was infested with spider mites and the other group was kept non-infested. To obtain the same-age spider mites for the *in-planta* bioassay, 60 adult female mites were carefully transferred from the general rearing to a 6-week-old tomato plant using a small paintbrush. After 24 h, adult mites were removed and the infested plant with freshly oviposited eggs was kept in a growth chamber for 14 days to allow the mites to develop into uniformly aged adults. For infestation, adult females were selected from the synchronized colony. Five mites were placed on the youngest fully developed leaf of each plant designated for mite treatment using a brush. Foam bands were secured around the stems of the infested leaves to prevent the mites from escaping (Fig. S1). The same foam bands were also placed on the leaves of plants without mites. The day following infestation, the number of mites was counted to assess the number of successfully established mites after release. The total number of (juvenile plus adult) spider mite offspring was counted on each infested plant at 4, 6, 10 and 15 days after infestation. Note that since *M. brunneum* infects roots and stems but not leaves of tomato, and spider mites were confined to leaf feeding, the effects of fungal inoculation on spider mites are expected to be mediated by systemically induced changes in leaf quality. After the final spider mite count the 7 weeks old plants were harvested to measure the parameters mentioned below.

Leaf chemistry

For chemical analysis, leaflets from the local (infested) leaf and from a systemic (non-infested) leaf (one younger than the local leaf) were individually sampled from 12 randomly selected replicate plants per treatment combination. Leaflets from plants within the same treatment combination were pairwise pooled, resulting in 6 biological replicates per treatment combination. The pooled leaves were immediately placed in 15 ml Falcon tubes, flash-frozen and stored at − 80 °C until further processing.

To assess changes in leaf quality in response to soil fungal inoculation we not only focus on secondary metabolites that can induce resistance to spider mites but also on primary metabolites, as reduced concentrations of soluble sugars or higher C/N ratios can also reduce the performance of leaf-feeding insect pests such as spider mites. To measure leaf concentrations of polyphenolics

(chlorogenic acid and rutin) and sugars (glucose), leaf extracts were made from all leaf samples using an extraction procedure modified from Pineda et al. [36]. Briefly, 800 µl of 70% methanol (MeOH, graded for high-performance liquid chromatography—HPLC) was added to 25 mg of freeze-dried finely ground leaf material. The mixture was vortexed for 30 s, sonicated at 20 °C for 30 min and centrifuged (Sartorius, Gottingen, Germany) at 10,000 rpm for 10 min. The supernatant was collected into a new centrifuge tube and extraction was repeated. Supernatants from the two extractions were pooled and vacuum-dried using Rota-vapor. The remaining pellet was re-dissolved in 1 ml of 70% MeOH, vortexed for 30 s, sonicated for 10 min and filtered through a 0.2 mm polytetrafluoroethylene syringe filter (Henske Sass Wolf GmbH, Tuttlingen, Germany) into glass vials for subsequent analysis. Analysis of phenolic compounds was performed by HPLC (ThermoFisher Scientific, Waltham, MA, USA) equipped with UV diode array detection following the method by Olszewska [37]. Analysis of glucose was performed by HPLC equipped with electrochemical detection (LC Bioinert 1260 Infinity, Decade elite ECD Antec.) according to van Dam and and Oomen [38]. Quantification was done using standard curves and concentrations were expressed as µg/g leaf dry weight. For analysis of C and N content, 2 mg of dried leaf subsample was weighted into tin foil cups and analyzed using a FLASH 2000 organic elemental analyzer (Brechbuhler Incorporated, Interscience B.V., Breda, The Netherlands).

Plant biomass

For biomass measurement, six infested and six non-infested plants per treatment were randomly selected. The aboveground plant parts were cut from the base of the stem and roots were thoroughly cleaned under running tap water. Both shoot and root sections were placed in paper bags, dried in the oven at 70 °C for 3 days and weighed to determine their dry weights.

Sequencing and bioinformatic analyses of rhizosphere soil microbial communities

For rhizosphere soil samples, the rhizosphere soils from the two adjacent pots per plant were collected separately. The soil adhering to the roots was gently collected in a 2 ml Eppendorf tube using a soft brush and spoon and stored at − 80 °C until further processing. In total, 87 samples were prepared for sequencing. For pots inoculated with *M. brunneum* (i.e., one of the pots in the Mb, NMC + Mb and NMC-Mb treatments) 24 out of the 36 replicates were randomly selected and pooled two by two per treatment combination, resulting in 36 samples. Similarly, for pots not inoculated with *M. brunneum* in the NMC, Mb, NMC + Mb, and NMC-Mb treatments, 16 out of the 36 replicates were randomly selected and pairwise pooled, resulting in an additional 40 samples. Finally, 4 pooled samples from the control treatment were included, as well as 4 non-sterile samples and 1 sterile sample from the soil that was used for inoculation with NMC, and one positive (mock) and negative (blank) control. No sequences were detected in the negative control while the positive control accurately detected the expected species (ZymoBIOMICS Microbial Community DNA Standards, Zymo Research, Irvine, CA, USA). Non-inoculated pots with originally sterile soil were naturally colonized during the experiment (see results). For sequencing of fungal and bacterial microbial communities in rhizosphere soil, DNA was extracted from ~ 0.25 g of moist rhizosphere soil using the Power Soil Pro DNA isolation kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. For fungal communities, the intergenic transcribed spacer (ITS2) region was targeted using the ITS4/ITS7 primer combination (5-TCCTCC GCTTATTGATATGC-3/5-GTGARTCATCGARTCTTT G-3) from Ihrmark et al. [39] with 300 bp coverage. For bacterial communities, the V4 region was amplified using 515F/806R primers (5-GTGYCAGCMGCCGCGGTAA-3/5-GGACTACNVGGGTWTCTAAT-3) from Caporaso et al. [40] with 250 bp coverage. Barcoded library preparations and Illumina MiSeq PE sequencing were performed at Genome Quebec Centre, Montreal, Canada.

The fungal (ITS) and bacterial (16S) amplicon sequencing data were processed using the DADA2 pipeline in R [41]. Amplicon sequence variants (ASVs) were generated by denoising and merging high-quality reads. Taxonomic assignments for the ITS and 16S sequences were determined through comparison with the UNITE (release 29.11.2022; [42] and SILVA (v. 132) databases, respectively. Only ASVs assigned as fungi were included in the ITS dataset and only ASVs assigned as bacteria were included in the 16S dataset. To ensure data quality, fungal data were filtered to remove samples with less than 500 sequences, resulting in the removal of 13 samples and samples with less than 10,000 sequences were filtered out from bacterial data, resulting in the removal of 5 samples. Finally, the downstream analysis was performed with 4,713 taxa of fungi and 21,885 taxa of bacteria.

Quantification of soil fungal biomass

Ergosterol quantification was performed to assess the fungal biomass in rhizosphere soil samples. Alkaline extractions of ergosterol were conducted following the protocol by de Ridder-Duine et al. [43]. Ergosterol concentrations were measured using LC-MSMS (UHPLC 1290 Infinity II and 6460 Triple Quad LC–MS, Agilent Technologies, CA, US).

Assessment of endophytic colonization

To assess the endophytic colonization of plants by *M. brunneum* in the Mb, NMC-Mb, and NMC + Mb treatments, samples were collected from 12 plants per treatment from both the spider mite-infested and non-infested groups. Six plants were sampled from the C and NMC treatments as a control. No colonization of Mb was found in the plant tissues from the C and NMC treatments. The colonization was examined in leaf, stem, and root samples. Roots from each of the two pots per plant were tested separately to evaluate the potential movement of colonized fungi from the roots in the inoculated pot to roots in the non-inoculated pot. From each of the selected plants, six leaf pieces (two 1.5–2 cm pieces of three leaves), six stem pieces (two 1.5–2 cm pieces from the base, middle and top of the stem, respectively), and six root pieces (three 1.5–2 cm pieces from the top and lateral roots, respectively) were sampled. All the samples were treated individually, washed under tap water and sterilized by immersing in 70% ethanol and 2% NaClO for 2 min, respectively, followed by three rinses with sterilized double distilled water and the edges were removed. This resulted in a total of 72 pieces per tissue per treatment combination. Samples from the C and NMC treatments underwent the same processing, resulting in 36 pieces per tissue per treatment. All the tissue pieces were placed in petri dishes containing selective media (as described by [13]) and incubated for 21 days at 25 °C in darkness. Stem pieces were carefully pressed into media to ensure the contact. Plant segments were inspected every 3 days until 21 days after incubation to check for endophytic outgrowth from internal tissue at the edges.

Statistical analysis

Statistical analyses for eco-physiological and microbiome data were conducted using R (R Core Team, 2023), and graphs were generated using the "ggplot2" package [44]. A Poisson generalized linear mixed model (*log link* function) was used to analyze the spider mite count data incorporating soil treatments with five categorical variables as a fixed effect and block as a random factor. A linear mixed model was fitted to plant biomass and chemical data with fungal treatment, presence/absence of spider mites, and their interaction as fixed effects and block as a random factor. A binomial generalized linear mixed-effect model (*logit link* function) was fitted to endophytic colonization data (presence/absence per plant piece and per plant tissue) using fungal treatment and plant tissue as fixed effects and block and plant id as random effects. The estimates for the confidence intervals were derived from the same models and percentage colonization was calculated by the proportion of plant pieces with observed fungal growth relative to the total number of plated plant pieces, expressed as a percentage [45]. The "lme4" package [46] was utilized for fitting mixed models, and *P*-values were computed using the "lmerTest" package based on the Satterthwaite's approximation [47]. Model fit was visually assessed through residual and quantile–quantile plots. Parameters exhibiting significant effects underwent pairwise comparisons using the Tukey post-hoc test using the "multcomp" package.

Sequencing data were normalized by total sum scaling (TSS) to mitigate potential biases and variations in sequencing depth [48]. Treatment differences (fungal treatments and presence/absence of spider mites) were visually represented using Non-metric Multidimensional Scaling (NMDS) for fungi and bacteria. The analysis employed a Bray–Curtis dissimilarity matrix and was further examined for statistical differences through permutational multivariate analysis of variance (perMANOVA) using the Adonis 2 function of the vegan package [49]. The analysis focused on the treatments in which native microbial community were present (analysing the compartments containing background microbial communities). Linear models were applied to test effects of inoculation treatments, spider mite presence and their interactions for top genera of fungi and bacteria and *p* values were corrected with the "False Discovery Rate" (FDR). In addition to analyses of treatment differences in the relative abundance of fungi and bacteria at fixed taxonomic levels, we performed heat tree analyses to visualize and test differences in relative abundance between treatments across the taxonomic hierarchy using the Metacoder package [50]. The comparison of differential abundances was based on log2 median proportions and the significance of differences between treatments was calculated by Wilcoxon rank-sum test and *p* values corrected with FDR.

Results

Spider mite population growth

After 15 days of infestation, the number of spider mites per plant was significantly affected by inoculation treatment (Fig. 2, $F_{4,68} = 3.14$, $P < 0.0001$). Inoculation with *M. brunneum* in sterile background soil reduced the number of spider mites per plant by 28%, indicating that *M. brunneum* induced resistance against spider mites. However, the magnitude of this *M. brunneum*-induced resistance was significantly diminished in the presence of the native microbial community. When *M. brunneum* was inoculated in the same root compartment as the native microbial community, the reduction in the number of spider mites per plant was only 17%. While this reduction was still significant, it indicates that *M. brunneum*-induced resistance was only half as effective in the presence of the native microbial community. Interestingly, the same

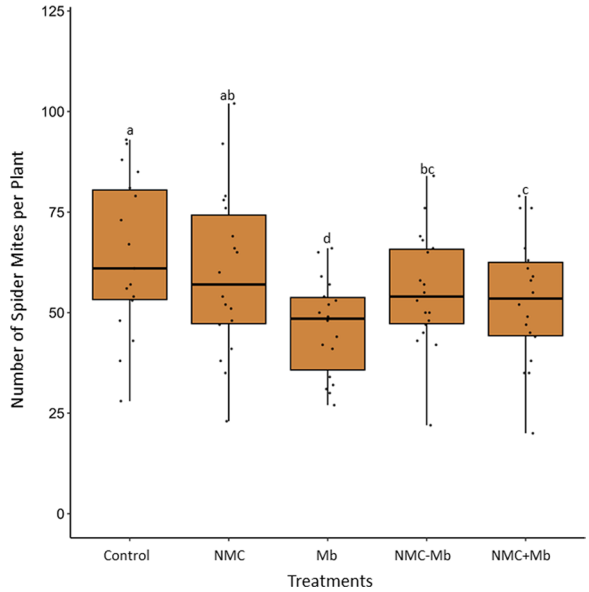

Fig. 2 Impact of direct versus indirect interactions between *Metarhizium brunneum* and native microbial community on population growth of two-spotted spider mites (*Tetranychus urticae*) on tomato plants. Each box in the plot corresponds to a specific treatment in the split-root setup, i.e., control with 0.01% Triton X-100 (C), only-native microbial community (NMC), only-*M. brunneum* bio-inoculum (Mb), native microbial community and *M. brunneum* in different pots (NMC-Mb) and native microbial community and *M. brunneum* in the same pot (NMC + Mb). Boxes not sharing a common letter are significantly different from each other (Tukey post-hoc test, $\alpha = 0.05$ following generalized mixed model). The median value for each treatment is represented by a thick horizontal line within its respective box

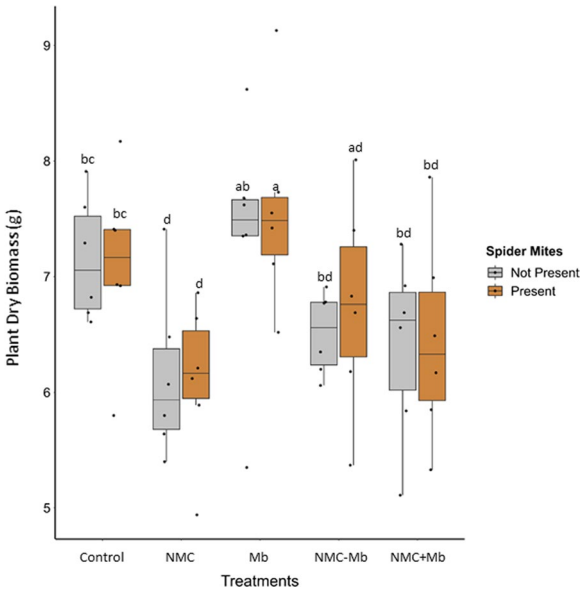

Fig. 3 Impact of direct versus indirect interactions between *Metarhizium brunneum* and native microbial community on total dry biomass of tomato plants in the absence (grey boxes) and presence (brown boxes) of two-spotted spider mites**.** Each box in the plot corresponds to a specific treatment in the split-root setup, i.e., control with 0.01% Triton X-100 (C), only-native microbial community (NMC), only-*M. brunneum* bio-inoculum (Mb), native microbial community and *M. brunneum* in different pots (NMC-Mb) and native microbial community and *M. brunneum* in the same pot (NMC + Mb). Boxes not sharing a common letter are significantly different from each other (Tukey post-hoc test, $\alpha = 0.05$ following linear mixed model). The median value for each treatment is represented by a thick horizontal line within its respective box

effect was seen when *M. brunneum* and the native microbial community resided in different root compartments, indicating that the mitigating effects of the native microbial community on *M. brunneum*-induced resistance were at least partly mediated by the plant.

The number of spider mites produced per successfully established female was also significantly affected by inoculation treatment ($F_{4,68} = 3.16$, $P < 0.001$) and was reduced by *M. brunneum* in the absence of the native microbial community but not in their presence (Fig. S2). This indicates that effects of *M. brunneum* on spider mites were at least partly mediated by differences in oviposition and hatching rates and not only by differences in the propensity of female spider mites to settle on plants with and without *M. brunneum*.

Plant biomass

Total biomass of tomato plants was significantly affected by inoculation treatment (Fig. 3, Table S1). Presence of the native microbial community reduced total biomass by 14%, whereas inoculation with *M. brunneum* did not significantly affect total biomass. However, the presence of *M. brunneum* partly mitigated the reduction in biomass by the native microbial community. Spider mite infestation did not affect total biomass. Treatment effects on shoot biomass were similar to those on total biomass, whereas no effects were observed on root biomass and root to shoot weight ratio (Fig. S3, Table S1).

Leaf chemistry

Local leaf concentrations of the two phenolic compounds, chlorogenic acid (CGA) and rutin were strongly affected by both soil inoculation treatment, spider mite infestation and their interaction (Fig. 4, Table S2, all $P < 0.01$). Whereas uninoculated control plants did not enhance local CGA and rutin concentrations in response to spider mite infestation, plants grown with *M. brunneum* or native microbes in their root environment enhanced local CGA concentrations by 120% and 155%, and rutin concentrations by 52% and 30%, respectively. This indicates that both *M. brunneum* and native microbes primed plants for herbivore-induced production of these phenolic compounds in the leaves. Interestingly, when plant

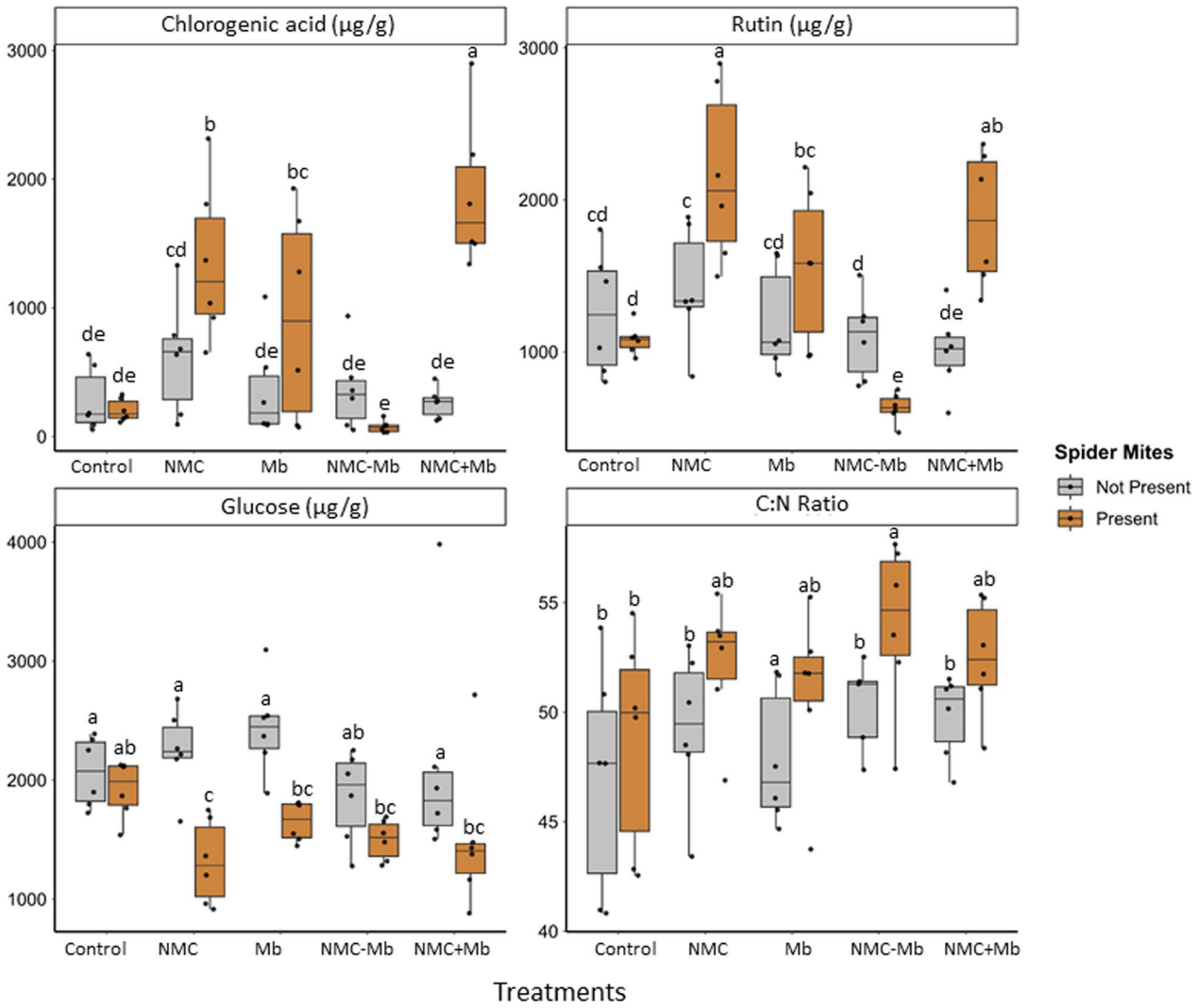

Fig. 4 Impact of direct versus indirect interactions between *Metarhizium brunneum* and the native microbial community on the concentration of chlorogenic acid, rutin, glucose and C:N ratio of local leaves in the absence (grey boxes) and presence (brown boxes) of two-spotted spider mites. Each box in the plot corresponds to a specific treatment in the split-root setup, i.e., control with 0.01% Triton X-100 (C), only-native microbial community (NMC), only-*M. brunneum* bio-inoculum (Mb), native microbial community and *M. brunneum* in different pots (NMC-Mb) and native microbial community and *M. brunneum* in the same pot (NMC + Mb). Boxes not sharing a common letter are significantly different from each other (Tukey post-hoc test, α = 0.05 following linear mixed model). The median value for each treatment is represented by a thick horizontal line within its respective box

roots were exposed to both *M. brunneum* and native microbes, their joint effects on leaf phenolics depended on whether they occurred in the same or in different root compartments. When they co-occurred, their impact on leaf phenolics was more or less additive, whereas their effects were strongly antagonistic, completely abolishing any priming effect, when they resided in separate compartments. Changes in rutin concentrations of systemic leaves in response to inoculation treatments and spider mite infestation were similar to those of local leaves, whereas changes in CGA concentrations were less strong and more variable than in local leaves (Fig. S4, Table S2). Despite the clear microbiome-dependent changes in the concentrations of these phenolic compounds in response to spider mite infestation, neither the leaf concentration of CGA, nor those of rutin were significantly correlated with spider mite numbers ($r = -0.23$ and $r = -0.15$, respectively, $n = 29$, $P > 0.2$), hence there is no support for their role in the observed microbially induced defense.

Glucose concentrations in local leaves were overall reduced by spider mite infestation (Table S2, $P < 0.001$) but the extent of the reduction varied with inoculation

treatment (Table S2, soil treatments × spider mites, $P = 0.05$). Specifically, spider mites did not affect leaf glucose concentrations in uninoculated control plants but reduced them by 42% and 32% in plants that harboured native microbes and *M. brunneum* in their roots, respectively (Fig. 4). However, the microbiome-induced repression of glucose concentrations in local leaves was diminished when *M. brunneum* and native microbes were jointly present, either in the same or in different compartments (Fig. 4).

In systemic leaves, changes in glucose concentrations in response to microbial inoculations and spider mite infestations were generally in the same direction as for local leaves, but no significant interaction between effects of inoculation treatments and spider mite infestation was observed (Fig. S4, Table S2). C:N ratios of local leaves were overall high due to the relatively low levels of leaf nitrogen (0.86% dry weight). Both spider mite infestation ($P < 0.001$) and microbial inoculations ($P < 0.01$) independently increased the C:N ratio of local leaves (Fig. 4, Table S2). Effects of treatment on C:N ratio of systemic leaves were qualitatively similar but less strong than those observed in local leaves (Fig. S4, Table S2).

M. brunneum colonization

M. brunneum readily colonized the whole root system of plants, as evidenced by the absence of significant differences between the percentages of root colonization in the root compartments that were and were not inoculated with the fungus ($P = 0.12$). The percentage of root or stem colonization was also not affected by the sampling location (top, mid, bottom) within these tissues ($P = 0.36$ and $P = 0.72$, respectively, Table S3). However, the extent of overall root colonization was significantly affected by the presence and location of native microbes (Mb, NMC-Mb, NMC + Mb; $P < 0.001$) and spider mite infestation ($P < 0.001$, Table S3). Similarly, stem colonization by *M. brunneum* was significantly affected by fungal treatments ($P = 0.01$) and spider mites ($P < 0.001$, Table S3). Notably, spider mite infestation overall enhanced the percent colonization that was detectable in roots and stems (Table S4). By contrast, the presence of native microbes tended to reduce root and stem colonization, especially in the roots of non-infested plants and in the stems of infested plants when they occurred in the opposite compartment (Table S4).

Soil fungal biomass

The ergosterol concentrations of rhizosphere soils did not significantly differ between treatments ($F_{2,\ 23} = 1.44$; $P = 0.25$), indicating that total fungal biomass was similar across treatments and that differences in the relative abundance of fungal groups among treatments roughly reflect differences in absolute abundance.

Microbial community composition

Fungal communities—Multivariate analysis showed that fungal communities of root compartments with sterilized soil were colonized by fungi during the runtime of the experiment but that these communities clustered separately from the soils with native microbes and those inoculated with *M. brunneum* (Fig. S5). In total, 312 fungal genera were detected in the rhizosphere soil collected from compartments with a native microbial community, viz. NMC, NMC-Mb, and NMC + Mb. Three genera, *Emericellopsis, Chrysosporium* and *Penicillium,* comprised more than half of their total abundance (Fig. 6b). *Metarhizium* ASVs were virtually absent from native microbial samples (relative abundance (RA) = 0.02%, Fig. 6b). When *M. brunneum* was inoculated into native fungal communities, it became a dominant part of the fungal community, reaching an average RA of 47% (range 12–86%), and thereby significantly affecting overall fungal community composition (Fig. 5a, $P = 0.001$). Colonization of *M. brunneum* in the inoculated root compartment generally resulted in good colonization of roots growing in the corresponding non-inoculated root compartments as well (see colonization data), but did not result in substantial colonization of the soil of non-inoculated compartments (RA = 0.2%), indicating that it did not significantly proliferate from the roots into the rhizosphere of the non-inoculated soil compartments. Despite the dominant presence of *M. brunneum* in inoculated soil compartments, after excluding *M. brunneum* ASVs from the fungal communities, only a marginally significant effect of *M. brunneum* inoculation on fungal community composition was observed (Fig. 5b, $P = 0.06$). Similarly, despite the significant effect of spider mite infestation on *M. brunneum* root colonization rates (see above), spider mites did not significantly affect soil fungal community composition (Fig. 5b, $P = 0.09$).

A closer examination of the top 20 fungal genera, representing 85% of the total RA (Fig. 6a, b), revealed that the addition of *M. brunneum* in the same root compartment as the native microbes (NMC + Mb vs. NMC) strongly enhanced the RA of *M. brunneum,* reducing the RA of most other fungal genera, whereas addition of *M. brunneum* to the other root compartment (NMC-Mb vs. NMC) had only minor effects on the native fungal community. Across all treatments, only three of the twenty top genera, *Pseudeurotium*, *Talaromyces* and *Clonostachys* were significantly affected by inoculation treatment in addition to the inoculated *Metarhizium* (Table S5). Furthermore, although the overall effect of spider mite infestation on overall fungal RA was only

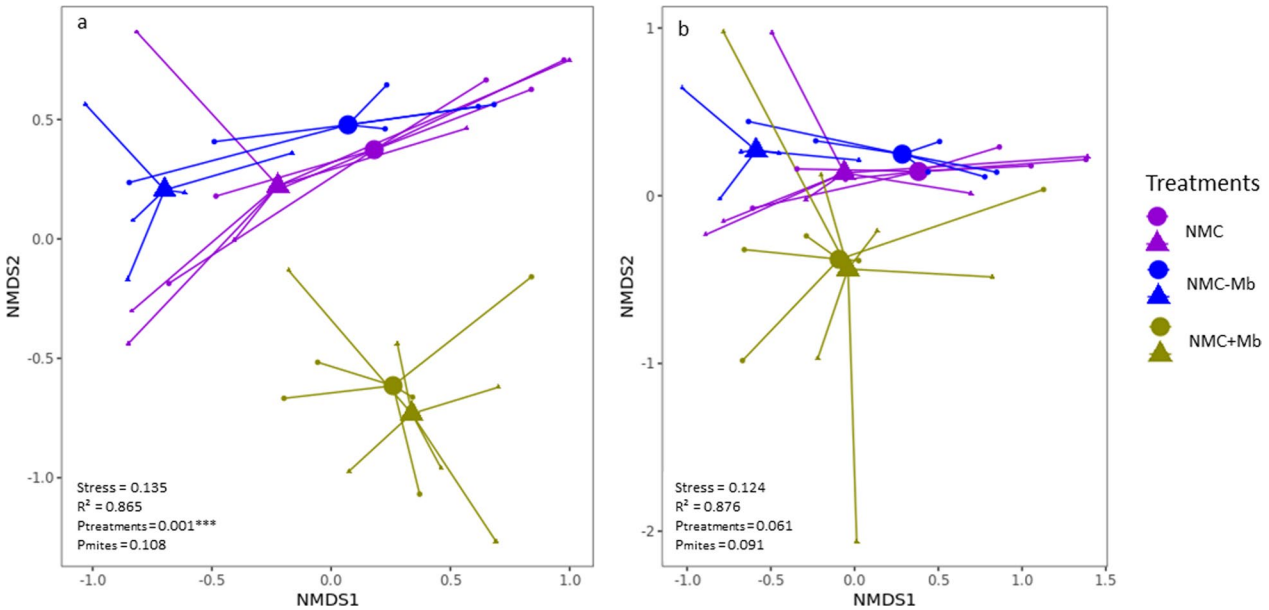

Fig. 5 Impact of direct versus indirect interactions between *Metarhizium brunneum* (Mb) and native microbial community (NMC) on resident fungal communities. Non-metric multidimensional scaling (NMDS) plots (using Bray–Curtis dissimilarity) showing the impact of three inoculation treatments on fungal communities: (i) only NMC when not inoculated with Mb (purple symbols), (ii) when NMC inoculated with Mb in a separate root compartment (blue symbols), or (iii) when NMC inoculated with Mb in the same root compartment (green symbols) in the absence (circles) and presence (triangles) of two-spotted spider mites. Plot **a** shows the fungal communities including *Metarhizium* ASVs and plot **b** shows the fungal communities excluding *Metarhizium* ASVs

marginally significant, its effect on the top 20 genera tended to be strongest in plants that harboured *M. brunneum* and native microbes in different root compartments (NMC-Mb), where the tendency of spider mites to reduce the RA of the most dominant genus *Emericellopsis* was most strongly observed, enhancing the RA of most other genera (Fig. 6a).

Heat trees, used to visualize pairwise differences in fungal abundances between inoculation treatments across taxonomic levels (Fig. S6), corroborated the significant impact of inoculation with *M. brunneum* on the structure of the native fungal community, both when it was inoculated in the same compartment (Fig. S6a) and when it was inoculated in the opposite compartment (Fig. S6b). In agreement with the results discussed above, the heat trees show that inoculation with *M. brunneum* led to asymmetric changes in the fungal community, where the relative abundance of taxa in only a few branches were enhanced by inoculation, whereas taxa in many branches showed decreased relative abundances, both when *M. brunneum* was inoculated in the same and when it was inoculated in a different compartment as the native microbes.

Bacterial communities—Bacterial communities of root compartments with sterile soil were clustered separately from the soils with native microbes (Fig. S7). In total, 43 bacterial phyla and 725 bacterial genera were detected in the rhizobacterial communities of root compartments with native microbes (NMC, NMC-Mb, NMC + Mb). Six dominant phyla, Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Planctomycetes and Firmicutes, accounted for almost 90% of the total abundance. Inoculation with *M. brunneum* significantly impacted the overall rhizobacterial community composition (Fig. 7, $P < 0.01$). The treatment involving direct interactions with *M. brunneum* (NMC + Mb) showed a more distinct separation from the treatment with only native microbes than the treatment in which native microbes could only indirectly interact with *M. brunneum* (NMC-Mb, Fig. 7). Similar to the results described above for the impact of spider mite infestation on fungal communities, infestation of host plants by spider mites did not exert a significant overall effect on rhizobacterial composition, but its effects tended to be stronger in treatments where the native bacterial community could only indirectly interact with *M. brunneum* (NMC-Mb) than in the other treatments (Figs. 6c, 7).

In contrast to what was observed for fungal communities, a closer examination of the top 20 bacterial genera, representing 42% of the total RA (Fig. 6c, d), revealed that the majority of overall dominant bacterial genera had a higher RA in the treatment with direct interactions with *M. brunneum* (NMC + Mb) than in the treatment with only native microbes (NMC) regardless of mite presence.

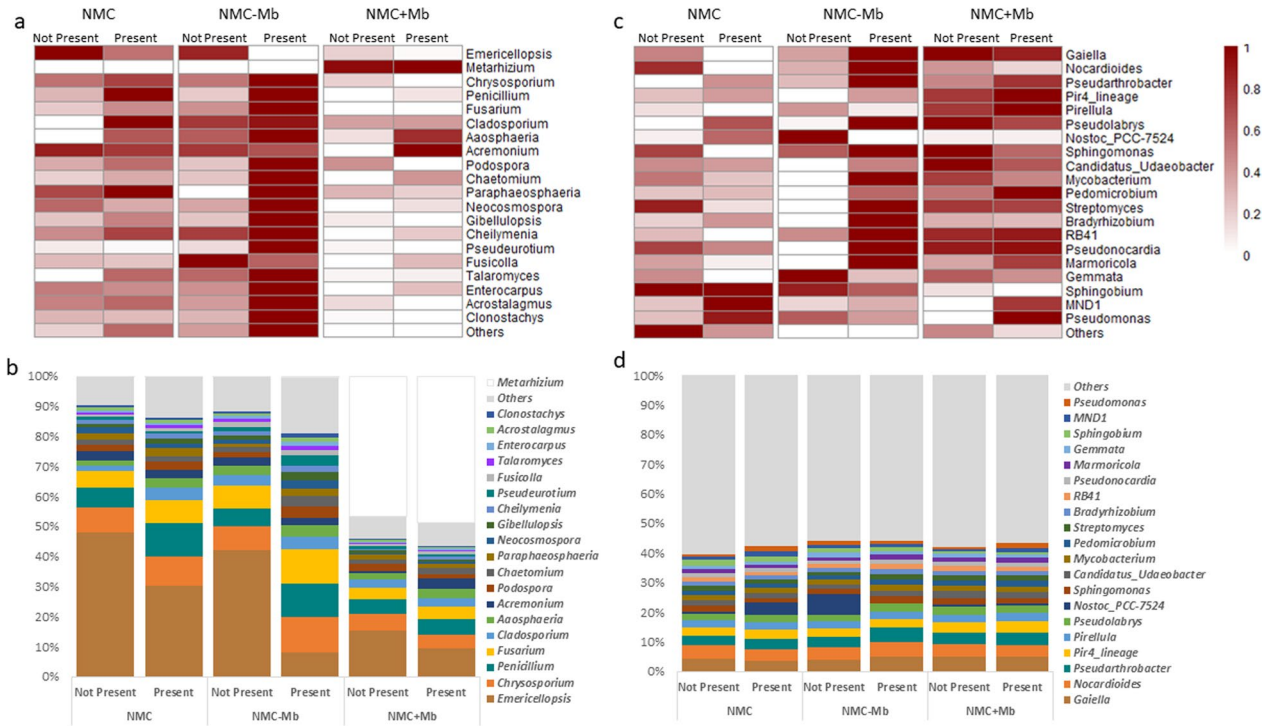

Fig. 6 Impact of direct versus indirect interactions between *Metarhizium brunneum* and the native microbial community on top 20 most abundant fungal and bacterial genera in the rhizosphere for the treatments with only-native microbial community (NMC), native microbial community and *M. brunneum* in different pots (NMC-Mb) and native microbial community and *M. brunneum* in the same pot (NMC + Mb) when the spider mites were present or not present on the plants. **a** Heat map comparing the relative abundance of the top 20 fungal genera among treatments, **b** relative abundance graph of the top 20 fungal genera, **c** heat map comparing the relative abundance of the top 20 bacterial genera among treatments, and **d** relative abundance graph of the top 20 bacterial genera

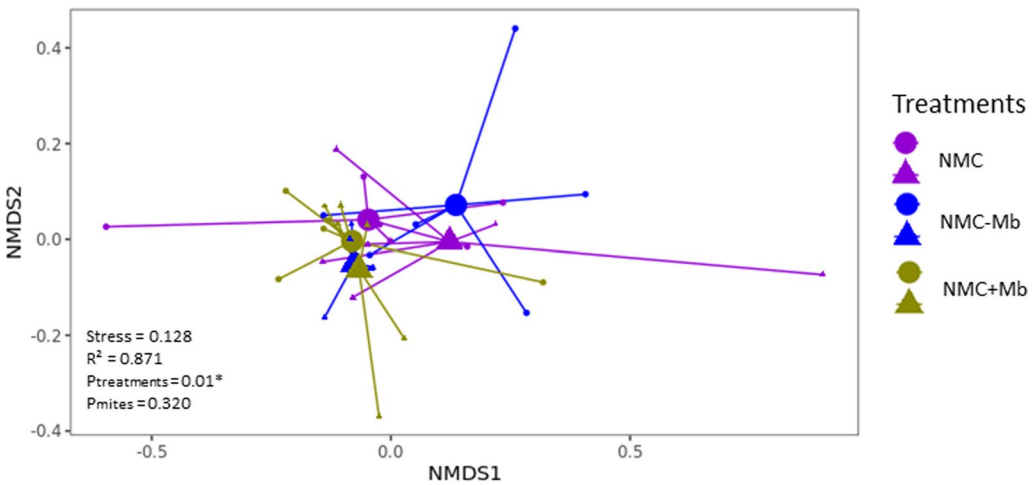

Fig. 7 Impact of direct versus indirect interactions between *Metarhizium brunneum* (Mb) and native microbial community (NMC) on resident bacterial communities. Non-metric multidimensional scaling (NMDS) plots (using Bray–Curtis dissimilarity) showing the impact of three inoculation treatments on bacterial communities: (i) only NMC when not inoculated with Mb (purple symbols), (ii) when NMC inoculated with Mb in a separate root compartment (blue symbols), or (iii) when NMC inoculated with Mb in the same root compartment (green symbols) in the absence (circles) and presence (triangles) of two-spotted spider mites

In the treatments with indirect interactions (NMC-Mb), most genera showed a higher RA only in the presence of spider mites (Fig. 6c). However, there was one genus, *Sphingobium,* that showed a significantly lower abundance in the presence of *M. brunneum*, especially when *M. brunneum* could directly interact with the native bacterial community (Table S6).

Heat trees (Fig. S8), showed that interactions with *M. brunneum* led to asymmetric shifts in taxonomic groups. In contrast to the results for the 20 most abundant genera, groups across the taxonomic classification that significantly differed between inoculated and non-inoculation treatments predominantly showed reduced relative abundance when in direct (Fig. S8a) or indirect (Fig. S8b) contact with *M. brunneum*.

Discussion

The impact of entomopathogenic fungal inoculants on plant growth and defense can be affected by the native microbial community, either through direct microbial interactions in the rhizosphere, or indirectly, through modulation of the plant's response to the inoculant by resident microbes. In this study, we show that *M. brunneum* enhances the resistance of tomato plants to spider mites but that the extent of this induced resistance is affected both by direct and plant-mediated interactions with the rhizosphere soil microbiome. In addition, we show that the entomopathogenic fungal inoculant affects the native microbial rhizosphere community not only through direct interactions with native microbes in the rhizosphere but also indirectly through modulation of the host plant.

Interactive effects of the inoculated entomopathogen and the native microbial community on plant resistance

Inoculating tomato plants with *M. brunneum* reduced the number of spider mites compared to uninoculated control plants both in the presence and absence of NMC, indicating that *M. brunneum* broadly induced resistance against spider mites. Inoculation of plants with *M. brunneum* has been shown to induce systemic resistance against a range of arthropod pests from different taxa and feeding guilds. For instance, soil inoculations with *M. brunneum* significantly reduced the reproduction of green peach aphids in sweet peppers [51], decreased spider mite numbers in common bean [52] and minimized leaf consumption in cauliflower by the larvae of a specialist lepidopteran pest, *Plutella xylostella* [53]. However, the effectiveness of these responses can be isolate-dependent, as certain isolates of *M. brunneum* have shown positive effects on herbivores like aphids and spider mites [54–56]. The isolate of *M. brunneum* used in the present study has previously shown the ability to negatively affect the population growth of spider mites in soil containing native microbes [26]. Yet, the present study suggests that these effects are more pronounced under sterile soil conditions.

In the present study, the magnitude of *M. brunneum*-induced resistance was significantly diminished in the presence of NMC compared to sterile conditions. This decline in effectiveness may have resulted from NMC hindering the establishment of the inoculated microbe in the rhizosphere, which is essential for achieving beneficial effects [57]. However, the observed reduction in resistance against spider mites in the presence of NMC occurred even in the absence of direct interactions between the inoculant and the NMC because they resided in different root compartments. In fact, *M. brunneum*-induced resistance was reduced from 28 to 17% when it was directly interacting with the NMC in the rhizosphere, but even reduced to 14% when *M. brunneum* and NMC resided in separate root compartments. This suggests that the attenuation of *M. brunneum*-induced resistance by the presence of NMC can be mainly attributed to plant-mediated interactions rather than due to a reduced rhizosphere competence of the inoculum through direct interactions with the native microbial community. Effects of entomopathogens on pest resistance are primarily mediated by induced systemic resistance responses [11, 12, 58]. This involves the activation of phytohormonal signaling pathways upon recognition of the beneficial fungus, followed by the downstream activation of defense responses, such as the production of defense metabolites. However, plants tailor their responses to environmental challenges by integrating and prioritizing multiple signals from their environment through positive or negative crosstalk between various triggered phytohormonal signaling pathways. Therefore, one of the possible explanations for the reduced extent of *M. brunneum*-induced spider mite resistance in plants that additionally interacted with the NMC could be that the phytohormonal signal transduction pathways triggered by pathogenic members of the NMC interfered with the activation of signal transduction pathways in response to *M. brunneum* [17], resulting in reduced activation of spider mite defense. Future molecular studies of defense activation of plants in response to *M. brunneum* inoculation in the presence and absence of NMC would be required to test such hypotheses.

To obtain more insight into microbially induced changes in secondary metabolites that are putatively involved in plant defense responses to spider mites, we examined changes in the leaf concentrations of two phenolic compounds, chlorogenic acid (CGA) and rutin. We showed that both *M. brunneum* and native microbes primed plants for the herbivore-induced production of

these compounds. Both compounds have been shown to possess anti-herbivory activities against a broad range of pests, either by themselves or in combination with other compounds from the same or different biochemical classes [59–61]. However, our study did not provide any evidence to suggest that the enhanced production of these compounds contributed to the observed microbe-induced resistance, as in the current experiment, there was no significant correlation between spider mite abundance per plant and leaf levels of CGA or rutin. Nonetheless, it is interesting to note that *M. brunneum* and NMC independently and additively primed the production of these phenolics in spider mite-infested plants when these microbes resided in the same root compartment but that no priming was observed when they resided in different root compartments. This suggests a plant-mediated antagonism between the effects of these two groups of microbes, but only when their signals come in from distant parts of the root system. The mechanisms underlying such spatial effects are currently unknown. Both *M. brunneum* and NMC reduced the leaf glucose concentration and increased the leaf C/N ratio in the presence, but not in the absence of spider mites. Both changes indicate that these microbes reduced the quality of the leaf tissue for spider mites, which might have impacted their population growth.

Interactive effects of the inoculated entomopathogen and the native microbial community on plant growth

In contrast to the beneficial effects of *M. brunneum* on spider mite resistance, inoculation with this entomopathogenic fungus did not overall enhance plant growth. While several strains of entomopathogenic fungi have demonstrated growth-promoting effects in tomato plants [62–64], this particular strain of *M. brunneum* did not show such effects under our experimental conditions. However, *M. brunneum* did partially mitigate the negative impacts of the NMC on tomato growth. Plants growing in soil with a native NMC produced significantly less biomass than plants grown in sterile soil, indicating that the effects of pathogenic members of this community originating from a former agricultural soil outweighed the impact of beneficial members. Similar to what has been shown for *M. robertsii* [65], *M. brunneum* might have suppressed the pathogenic members of NMC. *M. brunneum* has been shown to produce antimicrobial volatile organic compounds, potentially protecting plants against rhizosphere pathogens and promoting healthy growth [66]. Interestingly, the partial mitigation of negative effects of NMC on growth was also observed when *M. brunneum* resided in a different root compartment, indicating that part of this mitigation might have been mediated by the plant.

Effects of the inoculated entomopathogen and spider mite infestation on the soil microbial community

Bio-inoculants can cause cascading effects on the functions of resident microbes, raising concerns about their applications in agroecosystems [20]. Amplicon sequencing analysis showed that the inoculation with *M. brunneum* through soil drenching did not reduce microbial diversity in the rhizosphere but led to significant changes in the overall composition of fungal and bacterial communities. The absence of effects of EPF inoculation on microbial diversity is in line with the previously observed pattern that fungal inoculations generally tend to have less strong effects on soil microbial diversity than bacterial inoculations [22]. In contrast to the negligible effects on microbial diversity, inoculation with *M. brunneum* significantly affected the community composition of both the fungal and bacterial rhizosphere communities. Differences in fungal community composition were mainly due to the increased abundance of the inoculant, since after removing *Metarhizium* ASVs from the analysis, the effects on the composition of the non-inoculated fungal community members were only marginally significant. Mayerhofer et al. [25] similarly reported that *M. brunneum* application caused only minor shifts in fungal communities under greenhouse conditions whereas it didn't impact indigenous prokaryotic and fungal communities under field conditions. In contrast, shifts in fungal and bacterial communities under greenhouse conditions were reported when comparing the effects of different inoculation methods with *M. brunneum* [26].

Interestingly, in our study, alterations in the fungal and bacterial community composition were observed even when the native soil community was not in direct contact with the inoculum, suggesting that these changes were mediated by plant responses to inoculation, for instance, through altered root exudation patterns or rhizodeposition in response to inoculation. Several studies have shown that plants alter their root exudation patterns in response to biotic interactions with the environment, resulting in the recruitment of beneficial microbes in the rhizosphere that can subsequently confer benefits in terms of growth promotion or systemic resistance [29–31, 67, 68] However, our results do not provide evidence for the recruitment of growth-promoting rhizosphere microbes, nor for the recruitment of systemic resistance inducing beneficial microbes in response to spider-mite infestation. Spider-mite effects on rhizosphere composition were overall nonsignificant, and the observed changes in major bacterial and fungal taxa in response to our inoculation and spider mite treatments did not encompass shifts in important taxa known to be involved in growth promotion or induced systemic resistance such as Pseudomonads, Bacilli, or AM fungi. Analyses of the

most dominant genera revealed that inoculation of *M. brunneum* in the same root compartment as the NMC tended to decrease the relative abundance of the majority of the top 20 most dominant resident fungal genera, while it tended to increase the relative abundance of the majority of the top 20 resident bacterial genera, both in the presence and absence of spider mites. This suggests that *M. brunneum* might outcompete the most abundant fungal genera to establish itself in the rhizosphere, while it favoured the most dominant bacterial genera. A notable exception was the relative abundance of the common bacterial genus *Sphingobium*, which was overall suppressed by *M. brunneum* inoculation. This genus harbors many species capable of degrading various aromatic and chloro-aromatic compounds, including many phenolic compounds [69], which may reduce both harmful toxic metabolites and beneficial signaling compounds in the rhizosphere. It would therefore be interesting to assess the consequences of the specific *M. brunneum*-mediated suppression of this group of bacteria in future studies.

Conclusions

We conclude that the efficacy of the bioinoculant *M. brunneum* in activating spider mite resistance in tomato host plants was significantly affected by the native rhizosphere community. In the specific arable soil that we used in this study, the presence of the native microbial community did not enhance, but actually decreased, the extent of *M. brunneum*-induced resistance. Interestingly, the decrease was not only observed when native microbes could directly interact with the inoculant, but also when they were spatially separated within the root system. This indicates that the mitigation of *M. brunneum*-induced resistance by the NMC was not simply due to lower rhizosphere competence of the inoculum as a result of competition with native microbes, but that it was at least partly mediated by the plant, i.e., that the native microbes interfered with the plant's activation of induced systemic resistance to spider mites in response to *M. brunneum*. Similarly, the priming of the production of leaf phenolic metabolites (CGA, rutin) in response to spider mite infestation by *M. brunneum* was affected by the presence of native microbes, although our results do not provide support that these compounds were involved in defense against the spider mites in the current experiment. Finally, *M. brunneum* inoculation in the rhizosphere did not reduce microbial diversity in the soil but led to small but significant changes in the composition of the bacterial community, even when that community was not in direct contact with the inoculum, indicating that these changes were mediated by plant responses to inoculation, for instance through altered root exudation patterns or rhizodeposition. Whether such changes subsequently feedback on plant resistance to aboveground organisms should be investigated in follow-up studies.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40793-025-00667-9.

Supplementary material 1

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Author contributions
All authors were involved in the conception of this research idea. MG and SR performed the experiment and took samples and processed the collected samples for chemical and molecular analysis. SR analyzed the data and EH provided support for the analysis of molecular data for rhizosphere microbial communities. SR wrote the original draft, and AB and EH contributed, reviewed, and edited/added substantially to the manuscript. All authors have reviewed and approved the submission of our manuscript.

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Availability of data and materials
The sequencing data presented in this study were deposited at the European Nucleotide Archive: https://www.ebi.ac.uk/ena: under the accession PRJEB79238 (ERP163425). All other datasets generated and analyzed in this study are available on DRYAD at https://doi.org/10.5061/dryad.9ghx3ffsd. The link for peer review purposes is available here: http://datadryad.org/stash/share/MuOxW4afysgI680F7Xmyv8O4TcXgfmj_0PVoa6sBvEE.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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