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Protorhabditis nematodes and pathogen-antagonistic bacteria interactively promote plant health

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Abstract

Background Fertilization practices control bacterial wilt-causing *Ralstonia solanacearum* by shaping the soil microbiome. This microbiome is the start of food webs, in which nematodes act as major microbiome predators. However, the multitrophic links between nematodes and the performance of *R. solanacearum* and plant health, and how these links are affected by fertilization practices, remain unknown.

Results Here, we performed a field experiment under no-, chemical-, and bio-organic-fertilization regimes to investigate the potential role of nematodes in suppressing tomato bacterial wilt. We found that bio-organic fertilizers changed nematode community composition and increased abundances of bacterivorous nematodes (e.g., *Protorhabditis* spp.). We also observed that pathogen-antagonistic bacteria, such as *Bacillus* spp., positively correlated with abundances of bacterivorous nematodes. In subsequent laboratory and greenhouse experiments, we demonstrated that bacterivorous nematodes preferentially preyed on non-pathogen-antagonistic bacteria over *Bacillus*. These changes increased the performance of pathogen-antagonistic bacteria that subsequently suppressed *R. solanacearum*.

Conclusions Overall, bacterivorous nematodes can reduce the abundance of plant pathogens, which might provide a novel protection strategy to promote plant health.

Keywords Bio-organic fertilization, Ralstonia solanacearum, Bacterivorous nematode selective predation, Pathogen-suppressing Bacillus spp, Nematode-bacteria trophic interaction

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Background

Every year, plant pathogens globally cause around 10–20% crop losses [1, 2]. One of the most well-known plant pathogens is *Ralstonia solanacearum*, which causes devastating bacterial wilt in a wide range of plants, including tomatoes, potatoes, bananas, and other crops [3]. Bacterial wilt is traditionally controlled by synthetic chemicals, such as bactericides [4]. While these approaches are usually effective in the short term, they are non-sustainable and may negatively impact the environment. Frequent and extensive use of synthetic chemicals may also lead to the development of resistance in plant pathogens [5]. Therefore, environmentally friendly approaches to control plant pathogens are urgently needed to sustain crop yields and to ensure future ecological integrity.

So far, many sustainable practices are in practice, such as optimized crop rotations, and minimized chemical inputs and performance of conservation tillage [6–9]. Among them, one of the most important ones to control plant pathogens is applying "green fertilizers" containing organic matter and plant probiotics [10, 11]. Organic matter derived from especially plant residues is essential to soil health, as it contributes to maintaining soil structure, water retention, nutrient cycling, and plant health, mainly through supporting the activity of beneficial soil microorganisms [12]. Furthermore, using plant probiotics to combat plant diseases has been proposed as a sustainable approach for pathogen control and for supporting plant performance [13, 14]. For instance, mature compost containing biocontrol agents (e.g., *Bacillus subtilis* and *Pseudomonas stutzeri*) might replace chemical fertilizers by providing equivalent levels of nutrients but at the same time improving soil health [15].

Green fertilizers also modify the soil microbiome in favor of indigenous soil microbes, such as *Bacillus* and *Pseudomonas* bacteria that enhance plant disease suppression [16, 17]. In addition, other trophic levels in the soil food web are stimulated by bio-organic fertilizer application [18]. Previous research showed that bio-organic fertilizer application increased abundances of protists, especially bacterivorous protists, which subsequently promoted plant growth and controlled *Fusarium* wilt by regulating the soil microbiome [19, 20]. Myxobacteria increased by bio-fertilizer management were also shown to control cucumber *Fusarium* wilt by stimulating soil antagonistic bacteria, such as *Microvirga* and *Cupriavidus* [21]. However, current soil suppression mechanisms induced by different fertilization practices concentrate on the microbiome, while links among bio-organic fertilizers, soil suppressiveness, and larger soil fauna remain unknown.

Among soil fauna, nematodes are by far most numerous and represent major players of ecosystem functions like nutrient cycling that positively affects plant performance [22]. The main functional groups of nematodes are bacterivores, fungivores, herbivores, omnivores, and predators [23]. Most focus has been on herbivores due to their direct negative role on plant health and as catalyzers of other plant pathogenic microorganisms such as bacteria, fungi, and viruses [24, 25]. However, more and more researchers have found potential benefits of free-living nematodes for plants. Among them, bacterivorous nematodes have been associated with fostering nutrient cycling [26–28]. Predation of bacterivores nematodes on bacteria has been shown to increase P cycling to result in enhanced plant growth [29]. Nematodes belonging to the genus *Protorhabditis* are among the most numerous bacterivores in soils, especially in moist and organic-rich soils [30]. *Protorhabditis* spp. can stimulate the overall abundance of bacteria, thereby accelerating organic matter mineralization leading to increased plant-available nutrients, such as nitrogen and phosphorus [31]. Furthermore, organic matter amendments can enhance bacterivores by improving microbial prey abundance, as well as the structure and nutrient status of soils [32]. A study analyzing the metabolic footprint of nematodes at a local scale also showed that carbon addition inhibited the abundance of herbivorous nematodes and stimulated the abundance of bacterivorous nematodes [33]. Bacterivores normally have special food preferences, with not all bacterial taxa being equally preyed upon. This selective feeding can alter bacterial community composition [34–36]. This nuanced interaction highlights the potential of nematodes as regulators of microbial diversity and structure within soil ecosystems. However, the role of nematodes in mediating soil microbiota to promote plant health remains unknown. Understanding how nematodes influence the intricate web of soil microbiota and its subsequent impact on plant health could unveil novel strategies for sustainable agriculture and ecosystem management.

Here, we aimed to identify nematode taxa and functional groups linked to disease suppression and plant health (Fig. 1). We used Illumina amplicon sequencing and qPCR to investigate the diversity and community composition of nematodes but also bacteria as major disease and biocontrol agents in tomato rhizospheres in fields treated with no-, chemical- and bio-organic fertilization regimes. We performed subsequent in vitro and greenhouse experiments to validate the interactive effects of bacterivorous nematodes with nine genera of bacteria on tomato health, including pathogenic *R. solanacearum* and pathogen-antagonistic *Bacillus*

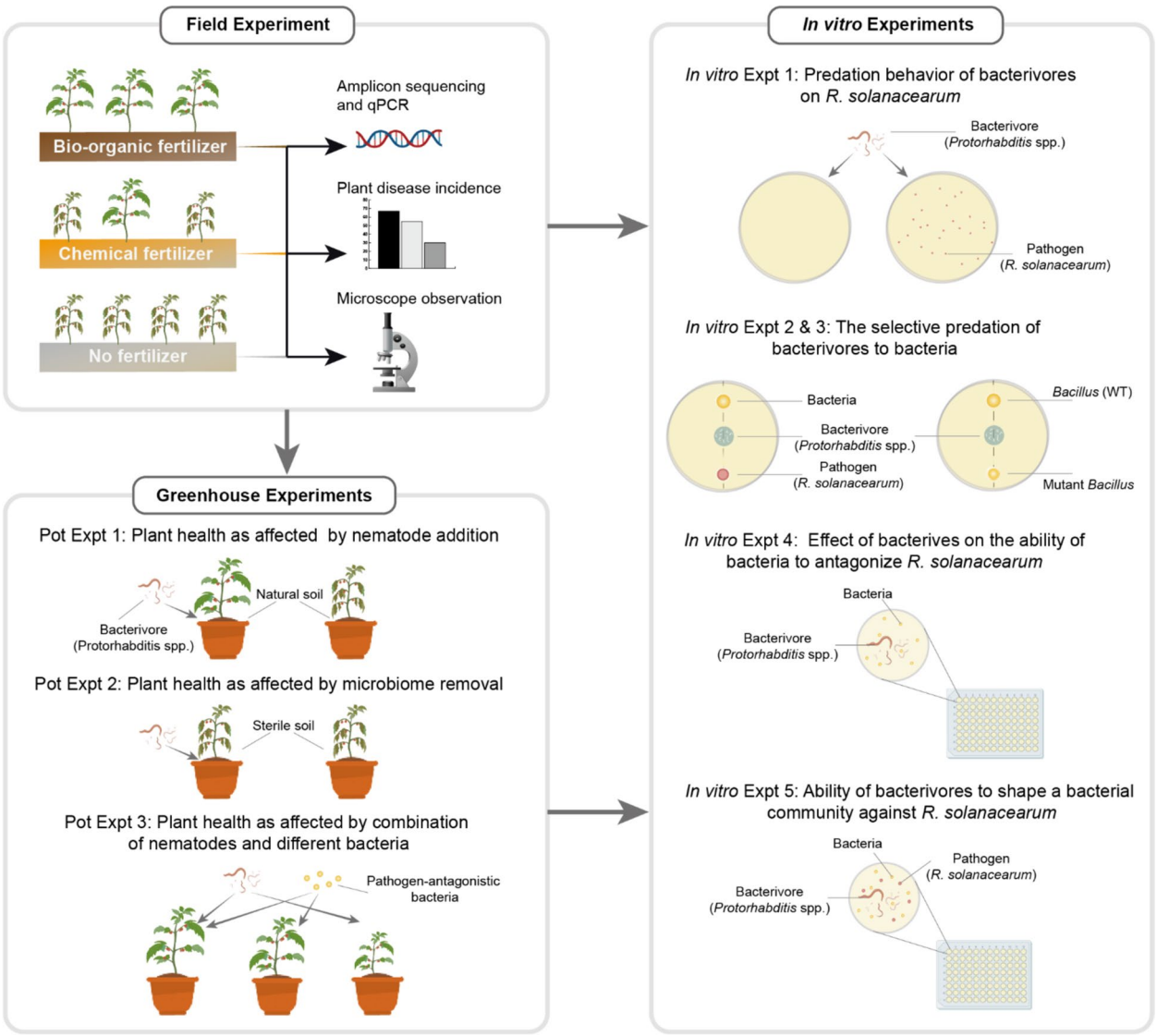

Fig. 1 Flow chart of the experiments. The study comprised several experiments: one field experiment, three greenhouse experiments, and five in vitro experiments. In the field experiment, we investigated whether bio-organic fertilizer application changes nematode communities and enhances their abundance. From there, we isolated different anti-pathogenic bacteria and bacterivorous nematodes. We then tested the importance of the bacterivores (greenhouse expt. 1) and the microbiome (greenhouse expt. 2) in pot experiments and assessed the importance of bacterivores nematodes in co-colonization with *Bacillus* for promoting plant health (greenhouse expt. 3). We also examined direct predation of bacterivorous nematodes on pathogenic *R. solanacearum (*in vitro expt. 1) and selective predation of nematodes on bacteria with different anti-pathogenic abilities (in vitro expts. 2–3). Finally, the co-cultural experiment validated the effects of bacterivores that promoted the expression in anti-pathogenic abilities of *Bacillus*, leading to a decrease of *R. solanacearum* (in vitro expts. 4–5)

spp. We hypothesized that as follows: (1) Bio-organic fertilizer application promotes nematode abundances, specifically bacterivorous nematodes, (2) bacterivorous nematodes change microbiome composition by favoring pathogen-antagonistic bacteria, and (3) bacterivorous nematode-induced microbiome changes will suppress *R. solanacearum* and tomato bacterial wilt disease.

Methods

Field experiment design

To investigate the effects of different fertilization on soil nematode and microbial communities, we performed a long-term field experiment. The study site was (starting

in March 2013) located at the Nanjing Institute of Vegetable Science in a subtropical monsoon climate region in China (31°43′ N, 118°46′ E). The annual average temperature is 16.7 °C, and precipitation is 959 mm. Soil is silty clay. Three treatments of the field experiment were selected for this study: (1) Control with no fertilizer (Ctrl); (2) chemical fertilizer treatment (CF), where a total of 120 kg ha^{-1} nitrogen (N), 180 kg ha^{-1} phosphorus (P), and 120 kg ha^{-1} potassium (K) were added in the sample collection season; and (3) bio-organic fertilizer treatment (BF), where 7500 kg ha^{-1} of bio-organic fertilizer was applied, corresponding to a total nutrient input of 138.75 kg ha^{-1} N, 60 kg ha^{-1} P, and 109.5 kg ha^{-1} K. The bio-organic fertilizer was produced by inoculating 5% (v/dw) *Bacillus amyloliquefaciens* SQR9 [37], which was isolated in our laboratory, into chicken manure compost, and then fermented for 7 days. In addition, the nutrient differences (nitrogen, phosphorus, and potassium) among fertilizers applied to each treatment were tested and compensated using mineral fertilizers (urea, superphosphate, potassium sulfate). Each treatment had 3 independent replicate plots, and each replicate contained 40 tomato plants (*Solanum lycopersicum*). The tomato variety used in this experiment was a commercial cultivar, "Shi Ji Hong Guan," one of the early ripening tomato cultivars with round, large, and red fruits. A bioassay for disease incidence was performed at the end of the experiment during the harvest season in October 2019. The assessment was based on observations of typical wilt symptoms, including necrosis and leaf drooping [37]. The disease incidence was calculated by counting the number of tomato plants with bacterial wilt among the total number of tomato plants in each plot. One value was obtained from each plot; thus, each treatment had three disease incidence replicates.

Soil sampling

Soil (bulk and rhizosphere) samples from all treatments were collected during the harvest season in October 2019. In brief, 6–8 healthy tomato plants were randomly extracted in each treatment plot. Loosely adhering soil was gently shaken off before rhizosphere soil was collected, according to Deng et al. [13]. Soils were passed through 2-mm meshes to remove roots, stones, and larger soil animals and then were stored at − 80 °C and 4 °C prior to DNA extraction and physicochemical analyses. Soil nematodes were extracted from 100-g field-moist soil by a modified Baermann method, and total nematode abundance was counted under a microscope (Nikon ECLIPSE Ts2) at 40 × magnification [38]. Soil pH, moisture, total carbon (TC), total nitrogen (TN), total phosphorus (TP), total potassium (TK), nitrate (NO_3^--N), ammonium (NH_4^+-N), available phosphorus (AP), and available potassium (AK) were determined according to the methods described in Dong et al. [39].

DNA extraction, Illumina MiSeq sequencing, and real-time qPCR analysis

Soil DNA was extracted from 10-g soil using the DNeasy® PowerMax® Soil Kit (Qiagen, Germany), according to the manufacturer's instructions. For MiSeq sequencing, the DNA extracted served as a template for the amplification of the V4 hypervariable regions of the bacteria 16S rRNA genes targeting primer pairs were 515F (5′-GTG CCAGCMGCCGCGGTAA-3′)/806R (5′-GGACTA CHVGGGTWTCTAAT-3′), which produces an amplicon of ~ 250 base pairs (bp) [40] and nematode 18S rRNA genes using the paired primers NF1-F (5′-GGT GGTGCATGGCCGTTCTTAGTT-3′)/18Sr2b (5′-TAC AAAGGGCAGGGACGTAAT-3′) [41] and the eukaryote-wide V4_1f (5′-CCAGCASCYGCGGTAATWC C-3′)/TAReukREV3 (5′-ACTTTCGTTCTTGATYRA -3′) [42], which both produce an amplicon of ~ 350 base pairs (bp). Detailed information regarding the PCR program and barcode strategy for each sample is provided in the supplementary material. The amplification product was sequenced on an Illumina MiSeq platform at Personal Biotechnology Co., Ltd. (Shanghai, China). The fliC gene was used to quantify the copy number of *R. solanacearum* with specific primers (forward, 5′-GAACGC CAACGGTGCGAACT-3′; reverse, 5′-GGCGGCCTT CAGGGAGGTC-3′) [43]. The abundance of *Bacillus* was quantified by qPCR with specific primer (forward, 5′-ATGTTAGCGGCGGACGGGTGAG-3′; reverse, 5′-AAGTTCCCCAGTTTCCAATGACC-3′) [44]. qPCR analyses were performed with a qTOWER 2.2 system of Analytik Jena (Jena, Germany) using SYBR green I fluorescent dye detection in 20-μL volumes, which contained 2 μL of template, 10 μL of SYBR Premix Ex Taq (TaKaRa Bio Inc., Japan), and 0.4 μL of both forward and reverse primers (10 μM each) in 96-well plates. All qPCRs were performed using the standard temperature profile [45]. In brief, the qPCR conditions were as follows: 30 s at 95 °C, 40 cycles consisting of 5 s at 95 °C, 34 s at 60 °C, and 60 s at 72 °C, 1 s at 95 °C. Each sample was analyzed in three replicates, and the results were expressed as $\log_{10}$ values (target copy number per gram soil in each treatment).

Bioinformatic analysis

Bacterial and nematode raw sequences were split according to their unique barcodes. Furthermore, adaptors and primer sequences were trimmed using cutadapt (https://github.com/marcelm/cutadapt). Subsequently, the trimmed bacterial and nematode sequences were processed with the UPARSE pipeline according to previously established protocols [46]. Bacterial sequences

with expected errors > 1.0 or a length shorter than 200 bp and nematode sequences with expected errors > 1.0 or a length shorter than 350 bp were removed. After discarding singletons, the remaining bacterial and nematode sequences were respectively categorized into zOTUs and zOTUs at 100% similarity [47, 48]. The RDP database (http://rdp.cme.msu.edu/) was used to assign the taxonomic identity of each phylotype of bacteria [49], and the PR2 database was used to assign the taxonomic identity of phylotypes for nematode [50]. We further assigned taxonomic nematode zOTUs into different trophic groups (bacterivores, fungivores, plant parasites, and omnivores/predators) according to their feeding mode [41].

Culturable nematodes, bacterial strains, and their growth conditions

Bacterivorous nematodes, *Protorhabditis*, were isolated from filed soil in December 2019 by the Ecological Laboratory of Nanjing Agricultural University and cultivated in nematode growth medium (NGM) agar plates [51] at 20 °C for 1 week by feeding on *E. coli* OP50. Prior to utilization, nematodes were washed with disinfectant (containing 0.1% streptomycin sulfate and 0.002% cycloheximide) and sterilized water to minimize the effects of *Escherichia coli* on the nematode characteristic, including predation and movement [52].

Through high-throughput isolation and identification, bacterial isolates from tomato rhizosphere soil were performed according to previously described protocols [13, 53]. In brief, tomato roots were washed in PBS on a shaking platform for 30 min at 170 rpm. For limiting dilution, homogenized rhizosphere soils were sedimented for 15 min, and the supernatant was empirically diluted, distributed, and cultivated in 96-well microliter plates with 200-mL tryptic soy broth (TSB) medium in each well. Then, a two-step barcoded PCR protocol in combination with Illumina HiSeq was adopted to define the sequences of bacterial 16S rRNA genes of rhizosphere bacteria. Afterward, sequences were clustered into zOTUs with greater than 99% 16S rRNA gene similarity and submitted to the RDP database for taxonomic identification. Finally, 42 isolate genera were obtained in this step, and according to high-throughput sequencing analysis results, the activated *R. solanacearum* and a variety of taxonomic bacteria strains (*Arthrobacter*, *Bacillus*, *Fictibacillus*, *Paenibacillus*, *Pedobacter*, *Phycicoccus*, *Lysinibacillus*, *Mesorhizobium*, and *Microvirga*) were selected for subsequent experiments. The ability of all bacterial isolates to inhibit the growth of *R. solanacearum* was tested according to previous research [54].

In vitro experiment systems and setup

In vitro experiment 1

Predation behavior of bacterivorous nematodes on *R. solanacearum* was observed. A total of 5 μL of *R. solanacearum* solution (concentration: 10^6 cfu mL^{-1}), and about 100 *Protorhabditis* individuals, were placed on two sides of nematode growth medium. Within 24 h, nematodes' moving and feeding behavior towards *R. solanacearum* was observed and recorded under a 40× inverted microscope (Nikon ECLIPSE Ts2).

Growth of bacterivorous nematode abundance through preying on *R. solanacearum* was also evaluated. Furthermore, 10 μL of *R. solanacearum* (concentration: 10^6 cfu mL^{-1}) solution was spread evenly on nematode growth medium, and the nematode growth medium without *R. solanacearum* coating was set as control. All mediums were placed in a 30 °C incubator for 5 h to ensure the *R. solanacearum* growth, and then 100 *Protorhabditis* individuals were inoculated at the center of the medium at 20 °C. After 6-, 12-, 18-, and 24-h cultures, the nematode abundances were counted, respectively. All treatments were performed with six biological replicates.

In vitro experiment 2

To investigate the selective predation of bacterivorous nematodes to bacteria, we performed an in vitro predation test experiments. A total of 5 μL of each targeted strain (concentration: 10^6 cfu mL^{-1}) and 5 μL of *R. solanacearum* with the same concentration were placed on opposite sides of the nematode growth medium. One-hundred individual nematodes were placed on the center of nematode growth medium, and then nematode growth medium was placed in a 20 °C incubator for 6 h to count the nematode abundances consuming bacteria on both sides of the plate. The nematode predation preference index was calculated as $(A-B)/(A+B)\times 100\%$, where A and B denote nematode abundances on the test solution side and *R. solanacearum* solution side, respectively. All treatments were performed with six biological replicates.

In vitroexperiment 3

Based on the result of the second in vitro experiment, the mutant *Bacillus* strain (*Mut_B*, disrupted in the bacillomycin D pathway) and wild-type *Bacillus* strain (*WT_B*), which were stored in Jiangsu Provincial Key Lab of solid Organic Waste Utilization of Nanjing Agricultural University, were used to confirm the effect of the anti-pathogen ability of bacteria on bacterivorous nematode predation. A total of 5 μL of mutant *Bacillus* strain and wild-type *Bacillus* strain (concentration: 10^6 cfu mL^{-1}) were placed on opposite sides of nematode growth medium. One-hundred individual nematodes

were inoculated at the center of the medium at 20 °C. After 6-h culture, the nematode abundance that preys on bacteria was counted on both sides of nematode growth medium. All treatments were performed with six biological replicates.

In vitro experiment 4

To investigate the effect of bacterivorous nematodes on the ability of bacteria to antagonize *R. solanacearum*, we performed an *in vitro* co-culture experiment. A total of nine targeted strains (*Arthrobacter*, *Bacillus*, *Fictibacillus*, *Paenibacillus*, *Pedobacter*, *Phycicoccus*, *Lysinibacillus*, *Mesorhizobium*, and *Microvirga*) was selected to test. Among these strains, *Bacillus*, *Fictibacillus*, and *Lysinibacillus* were stimulated by bacterivorous nematodes in fields, which also has been reported as biocontrol bacteria in agricultural production [54–56]. Other six bacterial strains were selected randomly in soils to test as non-antagonistic bacteria. These bacterial solution with the final concentration of 10^6 cfu mL^{-1} and *Protorhabditis* with a concentration of 1500 ind. mL^{-1} were supplied in 10% nutrient agar (NA) liquid medium in a 96-well plate system (final volume of 200 µL per well), which were cultured for 24 h with six replicates per treatment at 20 °C. There were 19 treatments, including the above 9 isolation bacterial strains and their pairs with *Protorhabditis* (Table S1). After 72-h culture, the liquid medium was centrifuged at 4000 rpm for 10 min to get the supernatant of each treatment. In order to obtain the sterilized supernatant, the supernatant of each treatment was filtered by a 0.22-µm sterile filtration membrane. Then, 2 µL of *R. solanacearum* with a concentration of 10^6 cfu mL^{-1} and 20-µL sterilized supernatant were added to each well, and 10% NA medium was supplemented to the whole volume of 200 µL. After 24 h, the suspension turbidity (OD_{600}) was measured as the pathogen-antagonistic ability of each treatment by using SpectraMax M5 (Sunnyvale, CA, USA) at room temperature. All treatments were performed with six biological replicates.

In vitro experiment 5

To investigate the alteration of bacterivorous nematodes on the assembly of a *R. solanacearum*-suppressing bacterial community against *R. solanacearum*, we performed an in vitro co-culture experiment. Twenty microliters of nutrient agar (NA) medium with *R. solanacearum* (concentration: 10^6 cfu mL^{-1}), each targeted strain (concentration: 10^6 cfu mL^{-1}), and nematode *Protorhabditis* (1500 ind. ml^{-1}), was added to 140-µL fresh nutrient broth medium in 96-well plates. There were 20 treatments, including the above 9 isolation bacterial strains in the fourth in vitro experiment and their pairs with *Protorhabditis* and *R. solanacearum* (Table S2). The suspensions were sprayed on the *R. solanacearum* growth selective medium, and the amount of *R. solanacearum* was quantified after 24-h culture. All treatments were performed with six biological replicates.

Greenhouse experiment systems and setup

Soils collected from the experiment field in chemical treatment were passed through 2-mm meshes to remove the impurities (e.g., roots and stones) and then divided into two parts. One part was sterilized by 75-kGy gamma rays at Nanjing Xiyue Technology Co., Ltd., Nanjing, China. The other part was used to prepare soil suspensions at a volume ratio of 1:10 between soil and sterilized water and passed through a 20 -µm mesh sieve to remove nematodes [57]. The absence of nematodes and eggs in the suspensions was ensured by microscopic observation. The nematode-free suspensions were amended into sterile soil at a volume ratio of 1:2 to produce nematode-free soils. These soils were stored in sealed plastic culture bottles at 25 °C for more than 1 month [58], and the soil moisture was adjusted to 60% of its maximum holding capacity by adding distilled water. In this study, nematode-free soils were obtained as background soils for all greenhouse experiments.

Greenhouse experiment 1

The effect of bacterivorous nematodes *Protorhabditis* on tomato bacterial wilt was investigated. Nematodes with different abundance levels (0, 200, 400, and 800 *Protorhabditis* individuals) were inoculated into 200 g of nematode-free soil per pot resulting in the following four treatments: (1) Ctrl, soil without nematodes; (2) Pro1, soil inoculated with 200 *Protorhabditis* individuals; (3) Pro2, soil inoculated with 400 *Protorhabditis* individuals; and (4) Pro3, soil inoculated with 800 *Protorhabditis* individuals. After 20 days of tomato plants transplanting and growth, *Protorhabditis* nematodes were inoculated, followed by *R. solanacearum* (10^5 cfu/g soil) 5 days later. Each treatment contained three components (six plants in each component). The disease incidence in this greenhouse experiment was calculated by counting the number of tomato plants appearing with bacterial wilt among the total number of tomato plants in each component. One value was obtained from each component; thus, each treatment had three incidence replicates.

Greenhouse experiment 2

The effect of bacterivorous nematodes on tomato disease incidence by directly consuming *R. solanacearum* was investigated. Bacterivorous nematodes (0, 800 *Protorhabditis* individuals) were inoculated into 200-g sterilized soil resulting in the following two treatments: (1) S_Ctrl, sterile soil without nematodes, and (2) S_P, sterile

soil inoculated with 800 *Protorhabditis* individuals. After 20 days of tomato plants transplanting and growth, *Protorhabditis* nematodes were inoculated, followed by *R. solanacearum* (10^5 cfu/g soil) added to all treatments 5 days later. The disease incidence was calculated as in the 1st greenhouse experiment.

Greenhouse experiment 3

The combinative effect of bacterivorous nematodes and pathogen-antagonistic bacteria on tomato bacterial wilt was investigated. According to our high-throughput sequencing analysis and in vitro experiments results, three bacterial isolates (*Bacillus*, *Lysinibacillus*, *Fictibacillus*) and bacterivores nematode (*Protorhabditis*) were selected to build combinations in soils. Eight-hundred *Protorhabditis* individuals and each bacteria solution (10^7 cfu/g soil) were introduced into 200 g of soil per pot following eight treatments: (1) Ctrl, soil without any nematode and bacteria inoculation; (2) Pro, soil inoculated with 800 *Protorhabditis* individuals; (3) Bac, soil inoculated with 5-mL *Bacillus* solution; (4) Pro + Bac treatment, soil inoculated with 800 *Protorhabditis* individuals and 5-mL *Bacillus* solution; (5) Lys, soil inoculated with 5-mL *Lysinibacillus* solution; (6) Pro + Lys, soil inoculated with 800 *Protorhabditis* individuals and 5-mL *Lysinibacillus* solution; (7) Fic, soil inoculated with 5-mL *Fictibacillus* solution; and (8) Pro + Fic, soil inoculated with 800 *Protorhabditis* individuals and 5-mL *Fictibacillus* solution. After 20 days of tomato plant transplanting and growth, *Protorhabditis* nematodes were inoculated, followed by bacterial isolate solution (10^7 cfu/g soil) 5 days later. Another 5 days later, *R. solanacearum* (10^5 cfu/g soil) was inoculated to all treatment soil. The disease incidence was calculated as in the 1st greenhouse experiment.

Statistical analysis

The α-diversity of bacterial and nematode communities in the field experiment was estimated using the non-parametric Shannon index. Bray–Curtis distance and Euclidean distance were calculated by "vegdist" function of vegan package on R 4.1.1 [59]. Differences in community structure between fertilization treatments were tested using permutational multivariate analysis of variance (PERMANOVA), which was performed using the vegan package (function: adonis) with 9999 permutations [59]. Unweighted UniFrac distance metrics were calculated based on phylogenetic trees for community comparisons by the UniFrac function in the GuniFrac package in R 4.1.1 and then visualized using principal coordinate analysis (PcoA) plots (PCoA function in ape) [60]. Comparisons among multiple treatments were performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple range test, while differences between two groups were analyzed using the *t*-test. All statistical analyses were conducted using IBM SPSS 23.0 software (SPSS Inc., USA). To investigate the correlation between soil physical–chemical properties and soil nematode composition with disease incidence, Mantel and partial Mantel test was performed with "mantel" and "mantel. Partial" function of vegan package on R 4.1.1 based on Euclidean distance of soil physical–chemical properties and Bray–Curtis distance of nematode composition. Hmisc package in R (version 4.1.1) was used to construct the correlation heatmap between bacterivorous nematodes zOTUs and bacterial zOTUs (average relative abundance > 0.01%) based on the Spearman correlation matrix [61]. As the bacterivorous nematode zOTUs were significantly enriched in low-disease treatment (BF) compared to other treatments (Ctrl and CF), we focused on nematode taxa zOTUs and bacterial taxa zOTUs and used Spearman's correlation coefficient to evaluate the correlation between significantly different bacterivorous nematodes zOTUs and bacterial zOTUs (average relative abundance > 0.01%). In addition, we calculated the relationship between these bacterial taxa zOTUs and disease incidence from the field experiment based on Spearman correlation. Structural equation model (SEM) analysis was conducted to test the direct and indirect effects of bacterivores, herbivores, bacterial community structure, and key bacterial zOTU 181 at multiple trophic levels on plant disease incidence. The bacterial community structure for the SEM analysis was represented by the first axis of principal coordinate analysis (PCoA) based on the Bray–Curtis distance. We used χ^2 test, *P*-value, degree of freedom(df), the root-mean-square error of approximation (RMSEA), and comparative fit index (CFI) to check the model fitness. The SEM analyses were conducted using Amos 16.0 software (Amos IBM, USA).

The variances of plant disease incidence and abundance of *R. solanacearum* among different treatments in greenhouse experiments were calculated using one-way analysis of variance (ANOVA) followed by Tukey's multiple range test. The relationship between disease incidence and abundance of *R. solanacearum* and *Protorhabditis* density in soil was calculated based on Spearman correlation.

The box plots illustrating the predation preference of *Protorhabditis* on bacteria and inhibition of bacteria to *R. solanacearum* in in vitro experiments were generated using the ggbeeswarm package [62]. The relationships between bacterivores abundance and disease incidence/*R. solanacearum* density in greenhouse experiments were calculated based on Spearman correlation. In the experiment assessing the increase in nematode *Protorhabditis* abundance due to predation on *R.*

Table 1 Statistical methods used and main outcomes of the field, greenhouse, and in vitro experiments

Experiment	Response variables	Statistical method	Test values	Degree of freedom	*p* value
Field exp	Disease incidence	ANOVA	F (49.208)	2	< 0.001
Field exp	Nematode abundance	ANOVA	F (13.171)	2	0.006
Field exp	*R. solanacearum* abundance	ANOVA	F (9.903)	2	0.013
Field exp	Nematode Shannon index	ANOVA	F (4.651)	2	0.06
Field exp	Nematode PCoA	PERMANOVA	F (3.136)	2	0.007
Greenhouse exp. 1	Disease incidence	ANOVA	F (68.017)	3	< 0.001
Greenhouse exp. 2	Disease incidence	t-test	t (1.255)	4	0.288
Greenhouse exp. 2	*R. solanacearum* density	t-test	t (2.592)	6.043	0.041
Greenhouse exp. 3	Disease incidence	ANOVA	F (7.433)	7	< 0.001
Greenhouse exp. 3	*R. solanacearum* density	ANOVA	F (28.016)	7	< 0.001
In vitro exp. 2	Predation preference	ANOVA	F (32.190)	8	< 0.001
In vitro exp. 2	Inhibition rate	ANOVA	F (76.997)	8	< 0.001
In vitro exp. 4	Inhibition rate of bacteria	ANOVA	F (9.785)	9	< 0.001
In vitro exp. 4	Inhibition rate of combination with bacteria and *Protorhabditis*	ANOVA	F (8.174)	9	< 0.001
In vitro exp. 5	*R. solanacearum* density	ANOVA	F (4.199)	8	0.005
In vitro exp. 5	*R. solanacearum* density in combination with bacteria and *Protorhabditis*	ANOVA	F (20.937)	8	< 0.001

solanacearum, both ANOVA and independent sample *t*-tests were used for statistical analysis. ANOVA was applied to examine the changes in *Protorhabditis* number on plates over different time points, in the presence or absence of *R. solanacearum*. Independent sample *t*-tests were used to compare nematode abundance between *R. solanacearum* — present and *R. solanacearum* — absent plates at the same time points. Similarly, for assessing the inhibitory effects of different bacterial strains on *R. solanacearum* with or without nematode presence, both ANOVA and independent sample *t*-tests were used. ANOVA was employed to compare the inhibitory effects of different bacterial strains on pathogens. Independent sample *t*-tests were used to compare the inhibitory effects of the same bacterial strain on pathogens with or without nematode presence. In all experiments, the relative change was calculated by subtracting the control group data from the treatment group data, dividing the result by the control group data, and multiplying by 100%. Statistical methods used and main outcomes of the field, greenhouse, and in vitro experiments were listed as Table 1.

Results

Field disease incidences and nematode abundance under different fertilization regimes (field experiment)

Disease incidence in bio-organic fertilizer treatment (BF) was 51.3% and 27.5% lower than those in non-fertilizer (Ctrl) and chemical fertilizer (CF) treatments, respectively (Fig. 2a and Table 1). Additionally, the nematode abundance in BF was 127.0% and 54.1% higher than in Ctrl and CF (Fig. 2b and Table 1). Furthermore, the abundances of *R. solanacearum* in Ctrl and CF were 32.2% and 23.1% higher than those in BF, respectively (Fig. 2c and Table 1). A strong negative correlation existed between nematode abundance and tomato disease incidence in fields based on the Spearman's rank correlation analysis ($R = -0.87$; $p = 0.0045$; Fig. 1d).

Bio-organic fertilizer promotes abundances of bacterivorous nematodes by increasing soil carbon matter (field experiment)

Bio-organic fertilizer in fields changed the amount of soil nutrients such as soil organic carbon, total nitrogen, available phosphorus, and available potassium (Table S3). Mantel test showed that soil organic carbon was the most important soil property influencing the nematode community structure, including bacterivorous nematodes and herbivore nematodes (Fig. S1a). In addition, soil nitrate nitrogen content affected the nematode community structure. Furthermore, Spearman correlation analysis showed a positive correlation between the relative abundance of bacterivorous nematodes and soil organic carbon ($R = 0.800$, $p < 0.001$; Fig. S1b), and there was a marginally significant negative relationship between soil organic carbon and herbivore nematodes ($R = -0.67$, $p = 0.059$, Fig. S1c).

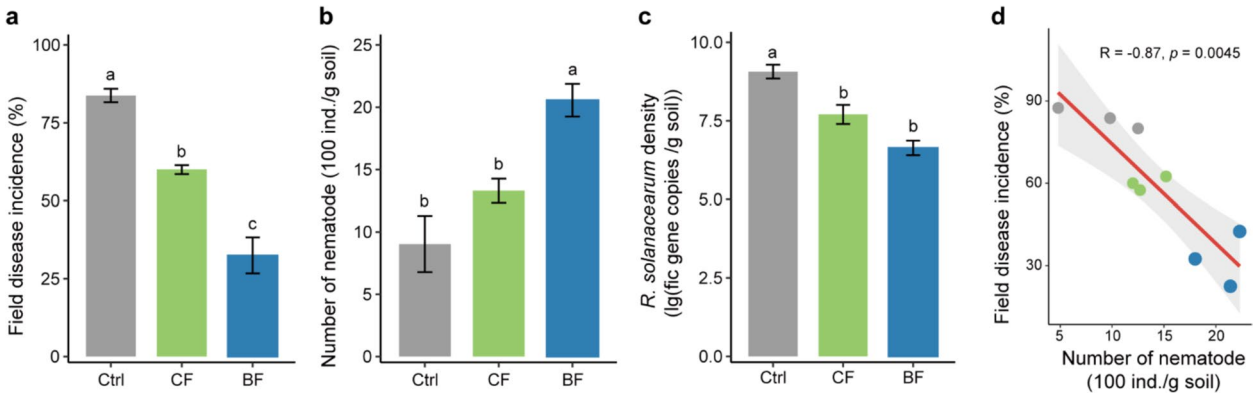

Fig. 2 Overall plant, disease, and biological patterns observed in the tomato fields under no fertilizer (Ctrl), applied with chemical (CF), or bio-organic fertilizer (BF). **a** Disease incidence analysis in experimental fields planted with tomato. **b** Nematode abundances in numbers $g.^{-1}$ under different fertilization regimes. **c** Abundance of *R. solanacearum* in rhizosphere soil. **d** Correlation between soil nematode abundance and tomato disease incidence, and gray shading denotes 95% confidence intervals. In **a**, **b**, and **c**, data are plotted as means ± SEs ($n = 3$), and bars with different letters indicate significant differences as defined by one-way ANOVA with Tukey's post hoc test ($p < 0.05$)

Underlying drivers of nematode and bacterial functional taxa linked to disease incidence (field experiment)

Alpha diversity of nematode communities was not affected by fertilization management (Fig. 3a, Table 1). However, fertilization management shaped the nematode community composition with three distinct clusters for non-fertilized, chemical, and bio-organic treatments, respectively (PERMANOVA test, $R^2 = 0.511$, $p < 0.001$; Fig. 3b and Fig. S2). Indicator species analysis revealed that five bacterivorous nematodes zOTUs were enriched in BF treatment, including zOTU 74 (*Cephalobus*), zOTU 112 (*Cephalobus*), zOTU 241 (*Protorhabditis*), zOTU 252 (*Protorhabditis*), and zOTU 731 (*Acrobeloides*). In contrast, three herbivore nematodes zOTUs (i.e., zOTU 18 (*Meloidogyne*), zOTU 529 (*Rotylenchulus*), zOTU 19 (*Meloidogyne*)) enriched in non-fertilized treatment (Fig. 3c). Among the five bacterivorous nematodes zOTUs, zOTU 112 (*Cephalobus*), zOTU 252 (*Protorhabditis*), and zOTU 731 (*Acrobeloides*) had negative relationships with disease incidence in the field experiment based on Spearman's correlation analysis ($R = -0.84$, $p = 0.005$; $R = -0.68$, $p = 0.046$; $R = -0.792$, $p = 0.011$; Fig. 3d).

Spearman's correlation analysis was used to identify further links between these bacterivorous nematode zOTUs and bacterial zOTUs. We found that these bacterivorous nematode zOTUs were positively correlated with bacterial zOTU 181 belonging to *Bacillus* spp. (zOTU74: $R = 0.81$, $p = 0.009$; zOTU731: $R = 0.74$, $p = 0.02$; zOTU241: $R = 0.80$, $p = 0.009$; zOTU252: $R = 0.96$, $p < 0.001$; zOTU112: $R = 0.75$, $p = 0.002$; Fig. 3d). Moreover, a strong negative correlation ($R = -0.70$, $p = 0.014$) was found between the relative abundance of zOTU 181 (*Bacillus*) and disease incidence based on the data in the field experiment (Fig. 3d). Relative abundance change of zOTU 181 (*Bacillus*) was the fifth most increased zOTU just after zOTU 65 (*Lysinibacillus*), zOTU 159 (*Nitrosospira*), zOTU 102 (*Methanomassiliicoccus*), and zOTU 67 (*Methylobacillus*). Structural equation model (SEM) indicated that bacterivorous nematodes could change bacterial community composition ($R = 0.588$, $p < 0.001$). Abundances of *Bacillus* zOTU (zOTU 181) were suggested to be increased by bacterivorous nematodes ($R = 0.354$, $p = 0.024$), linked to declines in tomato disease incidence (*Bacillus* OTU 181: $R = -0.765$, $p < 0.001$, bacterial community composition: $R = -0.662$, $p < 0.001$; Fig. 3e).

Pathogen suppression capability of bacterivorous nematodes (in vitro exp. 1 and greenhouse exp. 1 and 2)

The first in vitro experiment showed that *Protorhabditis* moved to *R. solanacearum* immediately after being introduced to nematode growth medium (Fig. S3 and Table S4). After 24 h, the abundance of nematodes in both treatments (Ctrl and + *R. solanacearum* treatment) was increased by 52.6% and 149.4% compared with the initial inoculation, respectively (Fig. S4). The abundance of nematodes on nematode growth medium cultured with *R. solanacearum* was 67.2% higher than in Ctrl after 24 h ($p < 0.001$, unpaired Student's *t*-test, Fig. S4).

The first greenhouse experiment estimated the effect of bacterivorous nematodes to decrease plant disease incidence in nematode-free soil. Results showed that although the *Protorhabditis* density initially inoculated was different, the *Protorhabditis* density in each treatment was not significantly different when the tomatoes in pots were harvested (Fig. S5a and Table S4). The *R. solanacearum* density in soils inoculated with 800

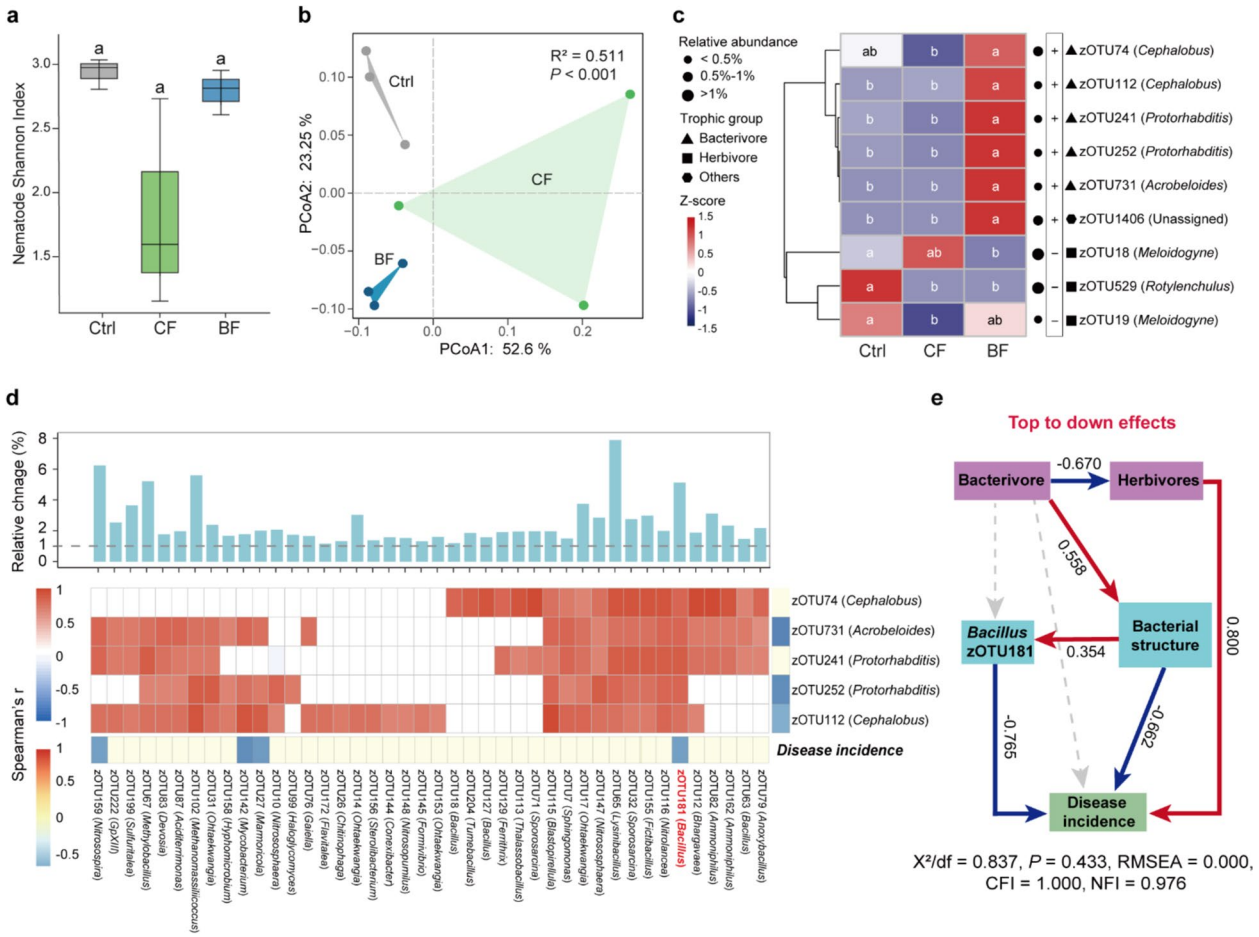

Fig. 3 Comparison of nematode community composition and correlations with bacteria and disease incidence under different fertilization treatments. **a**, **b** Nematode diversity and community structure in the field experiment shown using principal component analysis (PCA) based on Bray–Curtis distance. Different colors represent different fertilization treatments. **c** Heatmap showing the relative abundance of nematode zOTUs across various fertilization treatments. The color key indicates z-scores; plus and minus signs indicate positive and negative correlations with disease incidence. Significant differences are marked with different letters (ANOVA with Tukey's test, $p < 0.05$). **d** Heatmap depicting the relationship between the relative abundance of bacterivorous nematode zOTUs, bacterial zOTUs, and disease incidence. Only significant Spearman correlations ($p < 0.05$) are shown. **e** Structural equation model (SEM) illustrating how nematode and bacterial communities impact tomato disease incidence. Arrow width reflects the strength of the relationships: red arrows indicate positive correlations, blue arrows negative, and gray dashed lines indicate nonsignificant correlations. The numbers on the arrows are standardized path coefficients, and the model includes the proportion of explained variance. Model fit indices are also provided (χ^2, degrees of freedom (df), probability level (*P*), and root-mean-square error of approximation (RMSEA))

Protorhabditis individuals was lowest and was 21.80% lower than Ctrl treatment (Fig. 4a and Table 1). All treatments inoculated with *Protorhabditis* nematode (*Pro*1, *Pro*2, and *Pro*3) reduced 72.2%, 94.4%, and 77.8% tomato disease incidence compared to Ctrl treatment, respectively, and no significant difference among treatments inoculated with nematodes (*Pro2* and *Pro3*) was observed (Fig. S5b). A strong negative relationship was found between *Protorhabditis* nematode density in soils and tomato disease incidence (Fig. 4b). Spearman analysis also showed a negative correlation between *Protorhabditis* nematode density and *R. solanacearum* density (Fig. 4c). When sterilized soils were used in the second greenhouse experiment, no significant differences in tomato disease incidence and in the abundance of *R. solanacearum* were observed between the S_Ctrl treatment and S_P treatment ($p > 0.05$, unpaired Student's *t*-test; Fig. S6 and Table 1).

Bacterivorous nematodes together with *Bacillus* reduce Ralstonia solanacearum (in vitro exp. 2–5 and greenhouse exp. 3)

The results in the second in vitro experiment revealed that *Protorhabditis* could feed on the outer wall of

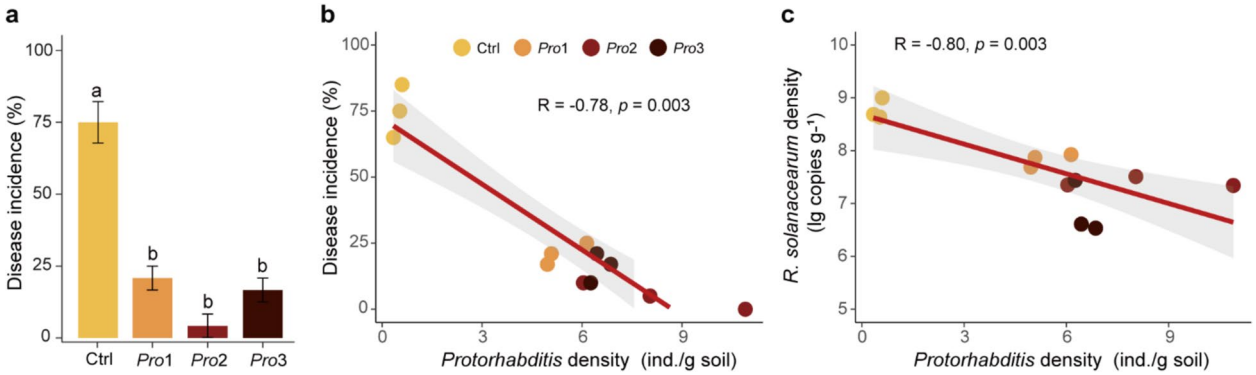

Fig. 4 Pathogen suppression capability of *Protorhabditis* nematodes in soil. **a** Effects of different inoculated *Protorhabditis* nematode abundance on tomato disease incidence. **b** Correlation between *R. solanacearum* density *Protorhabditis* abundances in pots. **c** Correlation between tomato disease incidence and *Protorhabditis* abundances in pots. In **a** and **b**, *p*- and R-values were calculated by Spearman's correlation test, and gray shading denotes 95% confidence intervals. In the Ctrl, no *Protorhabditis* nematode was inoculated. *Pro*1, soil inoculated with 200 individuals *Protorhabditis* nematodes. *Pro*2, soil inoculated with 400 individuals *Protorhabditis* nematodes. *Pro*3, soil inoculated with 800 individuals *Protorhabditis* nematodes

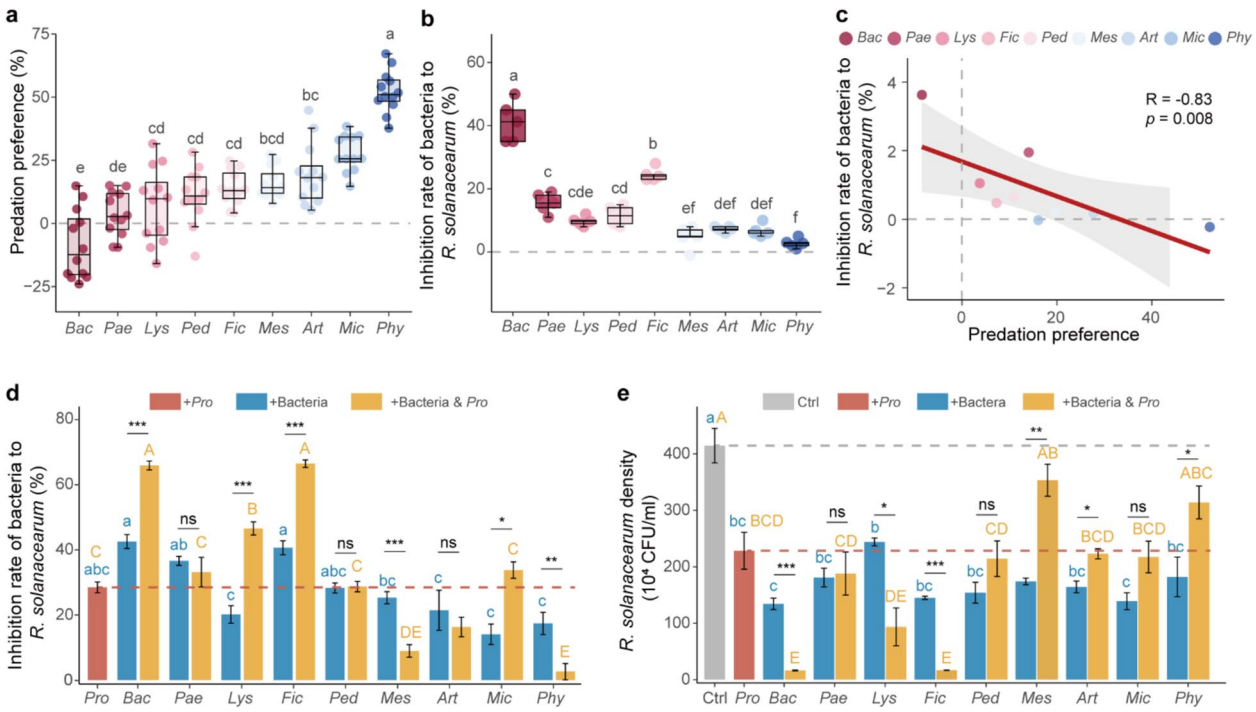

Fig. 5 Predation preference of *Protorhabditis* nematodes on different bacterial strains and effects of *Protorhabditis* nematodes and different bacterial strains antagonizing *R. solanacearum*. **a** Predation of nematodes on different bacterial strains; the 0 scale represents the comparison with pathogens as the standard; the lower the value, the less preyed by nematodes or the more preyed by nematodes ($n = 12$). **b** Inhibition effects of bacterial strains on *R. solanacearum* ($n = 6$). **c** Correlation between inhibition effects of bacterial strains and predation preferences of *Protorhabditis*; gray shading denotes 95% confidence intervals. **d** Inhibition rate to *R. solanacearum* of cultured supernatant of bacterivorous nematode *Protorhabditis*, bacterial strains alone, and the combination of strains and nematodes ($n = 6$). The red dotted line represents the average anti-*R. solanacearum* ability of nematode culture supernatant. **e***R. solanacearum* density in the absence (Ctrl) or presence of *Protorhabditis* nematodes, bacterial strains, and combinations of bacterial strains and *Protorhabditis* ($n = 6$). The gray and yellow dotted lines represent the average density of *R. solanacearum* in the Ctrl and + Pro treatments, respectively. Pro, *Protorhabditis* nematodes; Bac, *Bacillus*; Pae, *Paenibacillus*; Lys, *Lysinibacillus*; Fic, *Fictibacillus*; Ped, *Pedobacter*; Mes, *Mesorhizobium*; Art, *Arthrobacter*; Mic, *Microvirga*; and Phy, *Phycicoccus*. In all panels, boxes with different letters indicate significant differences as defined by one-way ANOVA with Tukey's post hoc test ($p < 0.05$). Significant differences in **d** and **e** by two-tailed unpaired *t*-test (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). In **d** and **e**, blue lowercase letters mean Tukey test results of the differences in (+ Bacteria) treatments, and yellow uppercase letters mean the Tukey test results of the differences in (+ Bacteria & Pro) treatments

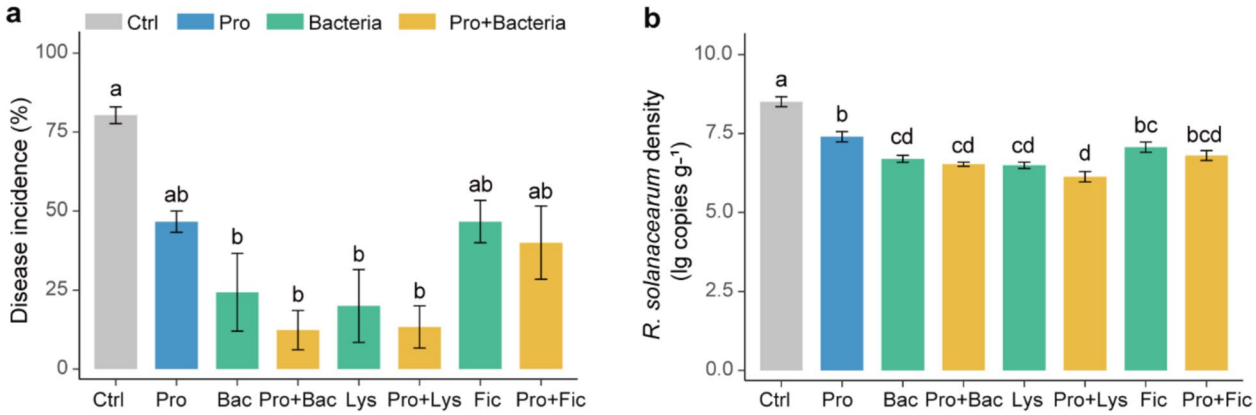

Fig. 6 Effect of combinations of *Protorhabditis* and bacteria on tomato health. **a** Plant disease incidence. **b** Soil *R. solanacearum* density. Data are plotted as means ± SEs ($n = 3$), and bars with different letters indicate significant differences as defined by one-way ANOVA with Tukey's post hoc test ($p < 0.05$)

bacterial colonies immediately after being placed on nematode growth medium cultured with each of the tested bacterial strains. *Protorhabditis* had the weakest predation preference for *Bacillus* (Fig. 5a and Table 1). The inhibition effect of *Bacillus* on *R. solanacearum* was 298.8% higher than the average value of other bacterial strains (Fig. 5b and Table 1). The Spearman correlation analysis showed the negative relationship between predation preference of *Protorhabditis* to bacteria and the inhibition rate of bacteria to *R. solanacearum* (Fig. 5c).

The result was similar in the third in vitro experiment, when *Bacillus* was disrupted in the bacillomycin D pathway, as the inhibition rate to *R. solanacearum* was 50.9% lower than in the *WT_Bac* treatment. On the contrary, the bacterivorous nematode abundance predation in *Mu_Bac* treatment was 276.80% higher than in the *WT_Bac* treatment (Fig. S7 and Table S5).

In the fourth in vitro experiment, the antagonistic ability of bacteria against *R. solanacearum* after co-cultivation of nematodes and bacteria was investigated. The results showed that the antagonistic ability of the liquid supernatant of *Protorhabditis* was 49.6%, 28.6%, and 42.7% lower compared to that of *Bacillus*, *Paenibacillus*, and *Fictibacillus* (Fig. 5d and Table 1). The antagonistic ability of liquid supernatants of *Bacillus*, *Fictibacillus*, and *Lysinibacillus* cultured together with *Protorhabditis* was 54.8%, 63.5%, and 131.2% higher than that of bacteria cultured alone (Fig. 5d and Table 1), and higher than other "bacteria-bacterivorous nematodes" combined treatments (Fig. 5d and Table 1).

In the fifth in vitro experiment, the co-culture experiments of bacteria strains, *R. solanacearum,* and *Protorhabditis* nematodes, were carried out to test the synergistic effect of bacterivorous nematodes and bacteria against the pathogen. Results showed that the abundance of *R. solanacearum* in Ctrl treatment was 44.9% higher than that in the *Protorhabditis* treatment and each bacterial strain treatment (Phy: 56.1%, Fic: 65.0%, Bac: 67.6%, Art: 60.4%, Mes: 58.0%, Ped: 62.9%, Pae: 56.4%, Mic: 66.5% and Lys: 41.2%; Fig. 5e and Table 1). The abundance of *R. solanacearum* in *Bacillus*, *Lysinibacillus*, and *Fictibacillus* cultured with *Protorhabditis* was 87.84%, 61.75%, and 88.51% lower than these in treatments just combined *R. solanacearum* and bacterial strains (Fig. 5e and Table 1). This finding was similar with results in the third in vitro experiment, indicating that nematodes cooperated with *Bacillus* produced stronger pathogen suppression ability than other treatments.

According to the fourth and fifth in vitro experiments, we further selected three bacterial strains (*Bacillus*, *Fictibacillus*, and *Lysinibacillus*) for the subsequent third greenhouse experiments. We combined *Protorhabditis* nematodes with pathogen-antagonistic bacteria: *Bacillus*, *Fictibacillus*, and *Lysinibacillus*, respectively. The results revealed that all bacteria-nematode combined treatments decreased tomato disease incidence (*P* + *Bac*: 84.7%; *P* + *Fic*: 20.2%; *P* + *Lys*: 83.4%, Fig. 6a) compared to the Ctrl treatment. Furthermore, the *R. solanacearum* density in the *P* + *Bac* treatment was 28.0% and 17.2% lower than those in Ctrl and *P* treatments (Fig. 6b). However, the *Protorhabditis* density in the *P* + *Bac* treatment was 49.4% lower than in the *P* treatment (Fig. S8 and Table S5). In addition, inoculation of *Protorhabditis* nematodes increased soil *Bacillus* densities. Specifically, the *Bacillus* density in the *P* treatment and the *P* + *Bac* treatment was 18.2% and 22.7% higher than that in the Ctrl treatment (Fig. 6d), which is similar to the first greenhouse experiment (Fig. S9 and Table S6).

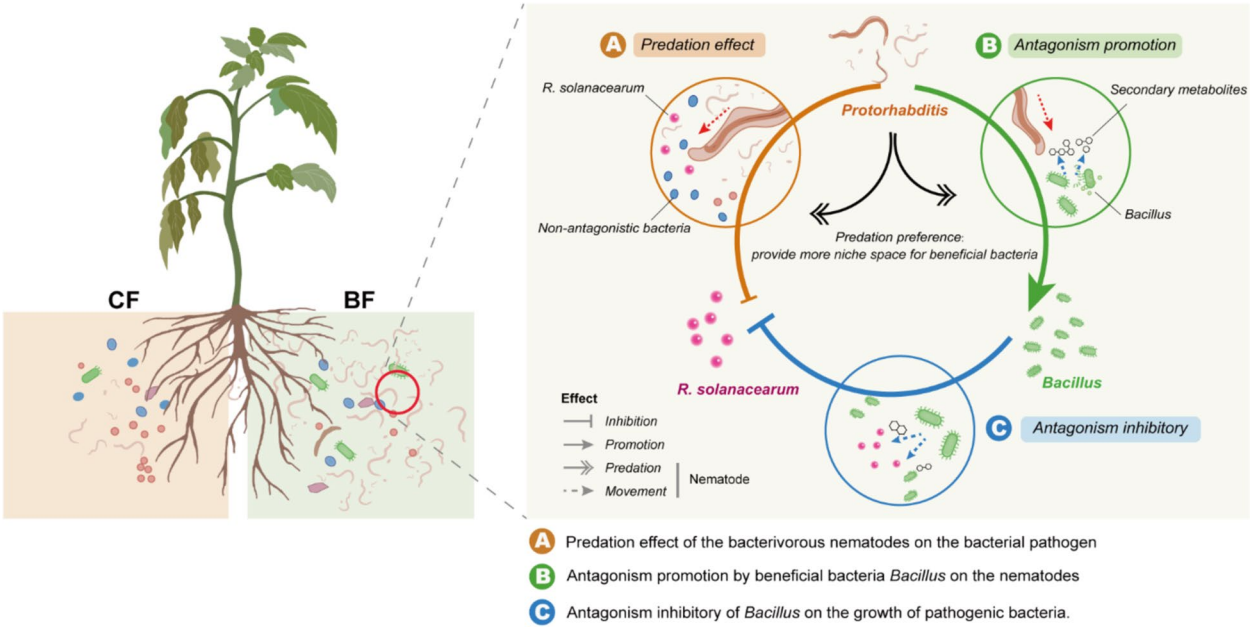

Fig. 7 Conceptual model. Conceptual model depicting the mechanisms illustrating how bacterivorous nematodes directly feed on pathogenic bacteria and promote by selective predation of a pathogen-antagonistic microbiome that benefits plant health

Discussion

Here, we demonstrated that the synergy of bacterivorous nematodes and pathogen-antagonistic bacteria stimulated by bio-organic fertilizer application changed microbiome composition and functioning that enhanced disease suppression (Fig. 7).

Application of organic fertilizers usually increases the soil organic matter content, which provides a rich source of nutrients for microbial growth [63]. In turn, microbial communities change, with abundances of certain bacteria, fungi, or other microorganisms increasing more than others, while also microbial interactions shift [64]. In our field experiment, we found that bio-organic fertilization increased absolute abundances of nematodes and relative abundances of bacterivorous nematodes, which is in agreement with hypothesis 1. Notably, the bio-organic fertilizer contained a rich organic component compared to the chemical fertilizer. This organic carbon likely is the basis for increased biodiversity gains, especially among nematodes as shown at the local and the global scale [23, 65, 66]. Previous research has demonstrated that the incorporation of carbon-rich materials, such as crop residues based on straw, enhances nematode abundances [67]. Indeed, our findings revealed that bio-organic fertilizers alter soil parameters, particularly within the realm of associated soil organic matter, which leads to changes in both nematode community structure and functional composition [68–70]. Prior studies illuminated that bio-organic fertilization establishes a favorable environment for bacteria and thereby their microbiome predatory protists [19]. Nematodes as main bacterial predators have also been shown to be increased with fertilizers [14, 39, 71], which we confirm here.

Predators play a crucial role in shaping ecosystems by controlling prey populations, which in turn affects the entire food web [72, 73]. Predators can also stabilize entire food webs against disturbances [74]. It is remarkable that even microbial predators can shift the balance within soil micro-food webs, showcasing their importance in maintaining ecosystem functionality. Our findings highlighted this insight and confirmed hypothesis 2 that increased relative abundances of bacterivorous nematodes can change microbiome composition and function by favoring pathogen-antagonistic bacteria. Here, we identified the key bacterivorous nematode *Protorhabditis* that positively affected the relative abundance of pathogen-antagonistic bacteria in soil, such as *Bacillus*. Previous studies found that bacterivorous nematode communities dominated by *Protorhabditis* could enhance bacterial abundance and composition in the plant rhizosphere through moderately feeding on bacteria [31]. In our laboratory and greenhouse experiments, we could show that *Protorhabditis* preferentially preyed on those bacteria with limited pathogen-antagonistic ability, which enhanced the success of pathogen-antagonistic bacteria. It is likely that mechanisms underlying pathogen-suppression equally defend against predation, while other confounding physiological or chemical factors

(differences in morphology, biofilm production or differential secondary metabolite production) might play a role [75]. Indeed, certain secondary metabolites confer resistance against protist predation, including violacein, polyketide antibiotics, hydrogen cyanide, the exoprotease AprA, and cyclic lipopeptides [76], which likely also explains different feeding of nematodes on bacteria found here.

Predators tend to select prey that are less well-defended—those that are slower, less camouflaged, or lacking in other defense mechanisms. This selective feeding behavior, driven by the presence or absence of effective prey defenses, demonstrates how predation shapes the evolution of survival strategies in prey populations [77]. This idea is consistent with hypothesis 3, as nematodes reshaped bacterial community with increased pathogen-antagonistic functioning resulting in increased plant health. In our laboratory and greenhouse experiments, *Protorhabditis* directly preyed on *R. solanacearum*, thereby likely minimizing their abundance compared to other bacteria in the microbiome [78]. We actually found that pathogen-antagonistic bacteria, such as *Bacillus*, *Lysinibacillus*, and *Fictibacillus*, benefited from the presence of *Protorhabditis.* Moreover, we showed that the combination of *Protorhabditis* and *Bacillus* led to the lowest bacterial wilt disease incidence rather than the application of *Protorhabditis* or *Bacillus* alone, supporting former findings that interactions of different players (e.g. organisms, resources) might disproportionally increase soil function compared to single players [79]. Therefore, our findings support the assumption that predator-induced pathogen suppression leads to plant-beneficial side effects [20]. We further confirm that predation by *Protorhabditis* stimulates the expression of pathogen-antagonistic functions by promoting the production of *Bacillus*-derived secondary metabolites, resulting in prey shifting to pathogens or other bacteria leading to inhibited pathogen growth. Results from our third greenhouse experiment indicated that the addition of nematodes stimulated densities of indigenous *Bacillus* in the soil. However, when both nematodes and *Bacillus* were inoculated together, the density of *Bacillus* was lower compared to the treatment with only *Bacillus* inoculation. Despite this, the incidence and abundance of *R. solanacearum* were lowest in the combined inoculation treatment. These findings support the evolutionary theory that prey tends to enhance their ability to avoid predators under predation [80]. Therefore, we propose that microbial secondary metabolite biosynthesis stimulated by predator pressure induces soil pathogen suppression and enhances plant health.

Our findings can be translated into practical agricultural applications by promoting the use of *Protorhabditis* nematodes together with already often-applied *Bacillus* spp. These organisms together might stimulate the individual known function of *Bacillus* spp. to enhance soil health by fostering beneficial microbial communities and suppressing pathogens, thereby potentially reducing the need for chemical inputs. However, variability in soil types and environmental conditions across regions may affect the efficacy of these bio-organic fertilizers. Moreover, introducing *Protorhabditis* nematodes and *Bacillus* spp. into soils may alter overall soil biodiversity, with resulting consequences for other soil functions like nutrient cycling and organic matter decomposition. Further research should focus on these aspects to ensure that the introduction of this combination can actually become a widely used product to positively contribute to a more sustainable agriculture.

Conclusions

In summary, we uncovered the pivotal role of nematode predation on microbiome composition and functioning towards a pathogen-antagonistic microbiome and increased plant health. The finding of the regulatory effect of nematode predation on plant bacterial wilt provides an important advancement to our understanding of plant-pathogen interactions in soils. Our findings demonstrated that nematode microbiome predators should be added to the common microbial-centered studies on the mechanisms and sources of plant bacterial wilt and plant pathogens in general. This work might pave the way for the development of upgraded sustainable agricultural practices that harness beneficial nematodes to naturally control plant diseases, thereby reducing reliance on chemical treatments. Future research could explore the specific interactions between different nematode species and soil microbes to develop targeted biocontrol strategies.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40168-024-01947-1.

Supplementary material 1.

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Authors' contributions

X.X., R.L., C.T., W.X. and S.G. wrote the manuscript. X.X., R.L., S.G., S.L., M.D., X.D., C.T. and Q.S. developed the ideas and designed the experimental plans. X.X., R.J., X.W., X.L., H.M., Z.N. and N.L. performed the experiments. X.X., R.L., W.X. and S.G. analyzed the data. All authors reviewed the manuscript.

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Data availability
All raw sequence data (bacteria and nematode) have been made available in the NCBI Sequence Read Archive (SRA) database under the BioProject PRJNA1029785 and PRJNA1029901.

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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References

1. Dean R, Van Kan JAL, Pretorius ZA, Hammond-Kosack KE, Di Pietro A, Spanu PD, et al. The top 10 fungal pathogens in molecular plant pathology. Mol Plant Pathol. 2012;13:414–30.
2. Raaijmakers JM, Paulitz TC, Steinberg C, Alabouvette C, Moënne-Loccoz Y. The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant Soil. 2009;321:341–61.
3. Mansfield J, Genin S, Magori S, Citovsky V, Sriariyanum M, Ronald P, et al. Top 10 plant pathogenic bacteria in molecular plant pathology. Mol Plant Pathol. 2012;13:614–29.
4. Spencer DM. Fungicides in agriculture and horticulture. Nature. 1961;190:593–4.
5. Comont D, Lowe C, Hull R, Crook L, Hicks HL, Onkokesung N, et al. Evolution of generalist resistance to herbicide mixtures reveals a trade-off in resistance management. Nat Commun. 2020;11:3086.
6. Qiao C, Penton CR, Xiong W, Liu C, Wang R, Liu Z, et al. Reshaping the rhizosphere microbiome by bio-organic amendment to enhance crop yield in a maize-cabbage rotation system. Appl Soil Ecol. 2019;142:136–46.
7. Zhao J, Chen J, Beillouin D, Lambers H, Yang Y, Smith P, et al. Global systematic review with meta-analysis reveals yield advantage of legume-based rotations and its drivers. Nat Commun. 2022;13:13.
8. Govaerts B, Mezzalama M, Sayre KD, Crossa J, Nicol JM, Deckers J. Long-term consequences of tillage, residue management, and crop rotation on maize/wheat root rot and nematode populations in subtropical highlands. Appl Soil Ecol. 2006;32:305–15.
9. Pan F, Xu Y, McLaughlin NB, Liu W, Han X, Zhan L, et al. Influence of different fertilization regimes on plant-parasitic nematodes in the black soil region of Northeast China. Phytoparasitica. 2013;41:355–63.
10. MacLaren C, Mead A, van Balen D, Claessens L, Etana A, de Haan J, et al. Long-term evidence for ecological intensification as a pathway to sustainable agriculture. Nat Sustain. 2022;2022:1–10.
11. Palansooriya KN, Ok YS, Awad YM, Lee SS, Sung JK, Koutsospyros A, et al. Impacts of biochar application on upland agriculture: a review. J Environ Manage Academic Press. 2019;234:52–64.
12. Li HY, Wang H, Wang HT, Xin PY, Xu XH, Ma Y, et al. The chemodiversity of paddy soil dissolved organic matter correlates with microbial community at continental scales. Microbiome. 2018;6:6.
13. Deng X, Zhang N, Li Y, Zhu C, Qu B, Liu H, et al. Bio-organic soil amendment promotes the suppression of Ralstonia solanacearum by inducing changes in the functionality and composition of rhizosphere bacterial communities. New Phytol. 2022;235:1558–74.
14. Fu L, Penton CR, Ruan Y, Shen Z, Xue C, Li R, et al. Inducing the rhizosphere microbiome by biofertilizer application to suppress banana Fusarium wilt disease. Soil Biol Biochem. 2017;104:39–48.
15. Jin L, Jin N, Wang S, Li J, Meng X, Xie Y, et al. Changes in the microbial structure of the root soil and the yield of Chinese baby cabbage by chemical fertilizer reduction with bio-organic fertilizer application. Microbiol Spectr. 2022;10:e01215.
16. Xiong W, Guo S, Jousset A, Zhao Q, Wu H, Li R, et al. Bio-fertilizer application induces soil suppressiveness against Fusarium wilt disease by reshaping the soil microbiome. Soil Biol Biochem. 2017;114:238–47.
17. Tao C, Li R, Xiong W, Shen Z, Liu S, Wang B, et al. Bio-organic fertilizers stimulate indigenous soil Pseudomonas populations to enhance plant disease suppression. Microbiome. 2020;8:1–14.
18. Jing J, Cong WF, Bezemer TM. Legacies at work: plant–soil–microbiome interactions underpinning agricultural sustainability. Trends Plant Sci. 2022;27:781–92.
19. Guo S, Xiong W, Hang X, Gao Z, Jiao Z, Liu H, et al. Protists as main indicators and determinants of plant performance. Microbiome. 2021;9:1–11.
20. Guo S, Tao C, Jousset A, Xiong W, Wang Z, Shen Z, et al. Trophic interactions between predatory protists and pathogen-suppressive bacteria impact plant health. ISME J. 2022;16:1932–43.
21. Ye X, Li Z, Luo X, Wang W, Li Y, Li R, et al. A predatory myxobacterium controls cucumber Fusarium wilt by regulating the soil microbial community. Microbiome. 2020;8:8.
22. Wilschut RA, Geisen S. Nematodes as drivers of plant performance in natural systems. Trends Plant Sci. 2021;26:237–47.
23. van den Hoogen J, Geisen S, Routh D, Ferris H, Traunspurger W, Wardle DA, et al. Soil nematode abundance and functional group composition at a global scale. Nature Nature Publishing Group. 2019;572:194–8.
24. Rutter WB, Franco J, Gleason C. Rooting out the mechanisms of root-knot nematode-plant interactions. Annu Rev Phytopathol. 2022;60:43–76.
25. Björsell P, Edin E, Viketoft M. Interactions between some plant-parasitic nematodes and Rhizoctonia solani in potato fields. Appl Soil Ecol. 2017;113:151–4.
26. Brondani M, Plassard C, Ramstein E, Cousson A, Hedde M, Bernard L, et al. Morpho-anatomical traits explain the effects of bacterial-feeding nematodes on soil bacterial community composition and plant growth and nutrition. Geoderma. 2022;425:116068.
27. Bonkowski M, Villenave C, Griffiths B. Rhizosphere fauna: the functional and structural diversity of intimate interactions of soil fauna with plant roots. Plant Soil. 2009;321:213–33.
28. Berlinches de Gea A, Li G, Chen JO, Wu W, Kohra A, Aslan SK, et al. Increasing soil protist diversity alters tomato plant biomass in a stress-dependent manner. Soil Biol Biochem. 2023;186:109179.
29. Jiang Y, Wang Z, Liu Y, Han Y, Wang Y, Wang Q, et al. Nematodes and their bacterial prey improve phosphorus acquisition by wheat. New Phytol. 2023;237:974–86.
30. Jiang Y, Qian H, Wang X, Chen L, Liu M, Li H, et al. Nematodes and microbial community affect the sizes and turnover rates of organic carbon pools in soil aggregates. Soil Biol Biochem. 2018;119:22–31.
31. Jiang Y, Liu M, Zhang J, Chen Y, Chen X, Chen L, et al. Nematode grazing promotes bacterial community dynamics in soil at the aggregate level. ISME J. 2017;11:2705–17 Nature Publishing Group.
32. Briar SS, Fonte SJ, Park I, Six J, Scow K, Ferris H. The distribution of nematodes and soil microbial communities across soil aggregate fractions and farm management systems. Soil Biol Biochem. 2011;43:905–14.
33. Pan F, Han X, Li N, Yan J, Xu Y. Effect of organic amendment amount on soil nematode community structure and metabolic footprints in soybean phase of a soybean-maize rotation on Mollisols. Pedosphere. 2020;30:544–54.
34. Fierer N, Jackson RB. The diversity and biogeography of soil bacterial communities. Proc Natl Acad Sci U S A. 2006;103:626–31.
35. Rousk J, Bååth E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG, et al. Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME J. 2010;4:1340–51.

36. Ingham RE, Trofymow J, Ingham ER, Coleman DC. Interactions of bacteria, fungi, and their nematode grazers: effects on nutrient cycling and plant growth. Ecol Monogr. 1985;55:119–40.
37. Liu H, Xiong W, Zhang R, Hang X, Wang D, Li R, et al. Continuous application of different organic additives can suppress tomato disease by inducing the healthy rhizospheric microbiota through alterations to the bulk soil microflora. Plant Soil. 2018;423:229–40.
38. Liu M, Chen X, Qin J, Wang D, Griffiths B, Hu F. A sequential extraction procedure reveals that water management affects soil nematode communities in paddy fields. Appl Soil Ecol. 2008;40:250–9.
39. Dong M, Zhao M, Shen Z, Deng X, Ou Y, Tao C, et al. Biofertilizer application triggered microbial assembly in microaggregates associated with tomato bacterial wilt suppression. Biol Fertil Soils. 2020;56:551–63 Springer.
40. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 2012;6:1621–4.
41. Sapkota R, Nicolaisen M. High-throughput sequencing of nematode communities from total soil DNA extractions. BMC Ecol. 2015;15:3.
42. Bass D, Silberman JD, Brown MW, Pearce RA, Tice AK, Jousset A, et al. Coprophilic amoebae and flagellates, including Guttulinopsis, Rosculus and Helkesimastix, characterise a divergent and diverse rhizarian radiation and contribute to a large diversity of faecal-associated protists. Environ Microbiol. 2016;18:1604–19.
43. Schönfeld J, Heuer H, Van Elsas JD, Smalla K. Specific and sensitive detection of Ralstonia solanacearum in soil on the basis of PCR amplification of fliC fragments. Appl Environ Microbiol. 2003;69:7248–56.
44. Mori K, Iriye R, Hirata M, Takamizawa K. Quantification of Bacillus species in a wastewater treatment system by the molecular analyses. Biotechnol Bioprocess Eng. 2004;9:482–9.
45. Hu J, Wei Z, Friman VP, Gu SH, Wang XF, Eisenhauer N, et al. Probiotic diversity enhances rhizosphere microbiome function and plant disease suppression. MBio. 2016;7:10.
46. Edgar RC. UPARSE: Highly accurate OTU sequences from microbial amplicon reads. Nat Methods. 2013;10:996–8.
47. Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 2010;26:2460–1.
48. Sikder M. Genetic disruption of Arabidopsis secondary metabolite synthesis leads to microbiome-mediated modulation of nematode invasion. ISME J. 2022;1–12:2230.
49. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol. 2007;73:5261–7.
50. Guillou L, Bachar D, Audic S, Bass D, Berney C, Bittner L, et al. The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote small sub-unit rRNA sequences with curated taxonomy. Nucleic Acids Res. 2013;41:597–604.
51. Brenner S. The genetics of Caenorhabditis elegans. Genetics. 1974;77:71–94.
52. Ortiz A, Vega NM, Ratzke C, Gore J. Interspecies bacterial competition regulates community assembly in the C. elegans intestine. ISME J. 2021;15:2131–45.
53. Bai Y, Müller DB, Srinivas G, Garrido-Oter R, Potthoff E, Rott M, et al. Functional overlap of the Arabidopsis leaf and root microbiota. Nature. 2015;528:364–9.
54. Sun X, Xu Z, Xie J, Hesselberg-Thomsen V, Tan T, Zheng D, et al. Bacillus velezensis stimulates resident rhizosphere Pseudomonas stutzeri for plant health through metabolic interactions. ISME J. 2022;16:774–87.
55. Yuan J, Zhao J, Wen T, Zhao M, Li R, Goossens P, et al. Root exudates drive the soil-borne legacy of aboveground pathogen infection. Microbiome. 2018;6:6.
56. Naureen Z, Ur Rehman N, Hussain H, Hussain J, Gilani SA, Al Housni SK, et al. Exploring the potentials of Lysinibacillus sphaericus ZA9 for plant growth promotion and biocontrol activities against phytopathogenic fungi. Front Microbiol. 2017;8:1477.
57. Wagg C, Bender SF, Widmer F, Van Der Heijden MGA. Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proc Natl Acad Sci U S A. 2014;111:5266–70.
58. Calderón K, Spor A, Breuil MC, Bru D, Bizouard F, Violle C, et al. Effectiveness of ecological rescue for altered soil microbial communities and functions. ISME J. 2017;11:272–83.
59. Oksanen J, Simpson G, Blanchet F, Kindt R, Legendre P, Minchin P, et al. Package 'vegan' data - function reference - ggplot2. 2024.
60. Chen J, Bittinger K, Charlson ES, Hoffmann C, Lewis J, Wu GD, et al. Associating microbiome composition with environmental covariates using generalized UniFrac distances. Bioinformatics. 2012;28:2106–13.
61. Harrell FE, Dupont C. Hmisc: Harrell Miscellaneous. R package version 5.0-0. 2023.
62. Clarke E, Shvetsov A. ggbeeswarm: Categorical Scatter (Violin Point) Plots. R package version 0.7.2. 2023.
63. Bastida F, Eldridge DJ, García C, Kenny Png G, Bardgett RD, Delgado-Baquerizo M. Soil microbial diversity–biomass relationships are driven by soil carbon content across global biomes. ISME J. 2021;15:2081–91.
64. Harkes P, Suleiman AKA, van den Elsen SJJ, de Haan JJ, Holterman M, Kuramae EE, et al. Conventional and organic soil management as divergent drivers of resident and active fractions of major soil food web constituents. Sci Rep. 2019;9:1–15.
65. Frederiksen HB, Rønn R, Christensen S. Effect of elevated atmospheric CO2 and vegetation type on microbiota associated with decomposing straw. Glob Chang Biol. 2001;7:313–21.
66. Liu T, Chen X, Hu F, Ran W, Shen Q, Li H, et al. Carbon-rich organic fertilizers to increase soil biodiversity: evidence from a meta-analysis of nematode communities. Agric Ecosyst Environ. 2016;232:199–207.
67. Ferris H, Venette RC, Lau SS. Dynamics of nematode communities in tomatoes grown in conventional and organic farming systems, and their impact on soil fertility. Appl Soil Ecol. 1996;3:161–75.
68. Puissant J, Villenave C, Chauvin C, Plassard C, Blanchart E, Trap J. Quantification of the global impact of agricultural practices on soil nematodes: a meta-analysis. Soil Biol Biochem. 2021;161:108383.
69. Zhou J, Wu J, Huang J, Sheng X, Dou X, Lu M. A synthesis of soil nematode responses to global change factors. Soil Biol Biochem. 2022;165:108538.
70. Li Y, Cao Z, Hu C, Li J, Yang H. Response of nematodes to agricultural input levels in various reclaimed and unreclaimed habitats. Eur J Soil Biol. 2014;60:120–9.
71. Zhang F, Gao C, Wang J, Lu Y, Shen Z, Liu T, et al. Coupling sugarcane yield to soil nematodes: implications from different fertilization regimes and growth stages. Agric Ecosyst Environ. 2017;247:157–65.
72. Perkins DM, Hatton IA, Gauzens B, Barnes AD, Ott D, Rosenbaum B, et al. Consistent predator-prey biomass scaling in complex food webs. Nat Commun. 2022;13:4990.
73. O'Gorman EJ, Zhao L, Kordas RL, Dudgeon S, Woodward G. Warming indirectly simplifies food webs through effects on apex predators. Nat Ecol Evol. 2023;7:1983–92.
74. Brose U, Archambault P, Barnes AD, Bersier LF, Boy T, Canning-Clode J, et al. Predator traits determine food-web architecture across ecosystems. Nat Ecol Evol. 2019;3:919–27.
75. Wang X, Wei Z, Li M, Wang X, Shan A, Mei X, et al. Parasites and competitors suppress bacterial pathogen synergistically due to evolutionary trade-offs. Evolution (N Y). 2017;71:733–46.
76. Gao Z, Karlsson I, Geisen S, Kowalchuk G, Jousset A. Protists: puppet masters of the rhizosphere microbiome. Trends Plant Sci. 2019;24:165–76.
77. Nair RR, Vasse M, Wielgoss S, Sun L, Yu YTN, Velicer GJ. Bacterial predator-prey coevolution accelerates genome evolution and selects on virulence-associated prey defences. Nat Commun. 2019;10:1–10.
78. Zhang P, Zhang W, Hu S. Fungivorous nematode Aphelenchus avenae and collembola Hypogastrura perplexa alleviate damping-off disease caused by Pythium ultimum in tomato. Plant Soil. 2023;482:175–89.
79. Trap J, Bonkowski M, Plassard C, Villenave C, Blanchart E. Ecological importance of soil bacterivores for ecosystem functions. Plant Soil. 2016;398:1–24.
80. Wade MJ. The co-evolutionary genetics of ecological communities. Nat Rev. 2007;8:185–95.

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