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Bacterial preys and commensals condition the effects of bacteriovorus nematodes on *Zea mays* and *Arabidopsis thaliana*

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ABSTRACT

In this study, we analyzed the nematode-bacteria-plant interaction by using bacteriovorus nematodes isolated from maize rhizosphere and plant growth promoting rhizobacteria to evaluate their effect on plant growth and root development of maize and *Arabidopsis thaliana*. Two bacterial feeding nematodes, identified as *Oscheius tipulae* and *Mesorhabditis* sp., displayed distinct attraction patterns for seven rhizosphere bacteria with a strong bias towards *Rhizobium* strains. We tested the effect of these two nematodes on both plant growth and root morphology using native *Zea mays* and found that the co-inoculation of *Rhizobium phaseoli* Ch24-10, a plant growth promoter, and maize rhizosphere nematodes, increased dry weight of corn plants compared to plants treated only with bacteria or the nematode. Additionally, we found that the nematodes reduced the weight and length of the primary root and increased the number of emerging lateral roots. We further analyzed the effect of nematodes and bacteria using the model plant *A. thaliana* and observed similar effects on roots as those found in native corn. Nematodes reduced the primary root growth and increased the number of emerging lateral roots. The model nematode *Caenorhabditis elegans* N2 alone had no effect on *A. thaliana* plants, it became capable of affecting root morphology when fed with *Acinetobacter pittii,* which was originally isolated from the maize rhizosphere nematodes. Our results suggest a synergistic effect of nematodes and their associated bacteria both in plant development and root morphology, highlighting the importance of nematode-bacteria interactions in shaping plant growth and root morphology. A model of nematodes as Trojan horses carrying bacteria is proposed.

1. Introduction

Nematodes are one of the most abundant and ubiquitous multicellular organisms on Earth, inhabiting a broad range of habitats such as freshwater, marine and terrestrial environments among others (Ladygina et al., 2009). These worms are particularly abundant in soil, exhibiting densities ranging from 1 to 10 million ind. m^{-2}, thereby representing a significant fraction of the microbial biomass in this ecosystem (Blanc et al., 2006). Consistent with their ability to inhabit a broad range of environments, nematodes also exhibit different lifestyles, such as free-living or parasitic organisms of animals and plants, and types of feeding, since some feed on bacteria, fungi, algae, protozoa or even other nematodes (Kiontke and Fitch, 2013). Among these, bacterial-feeders are the main population of nematodes in the soil, representing 20–50% of total nematode classes isolated from mixed deciduous forest samples (Patra et al., 1987).

Nematodes can sense dozens of odors through several olfactory sensory neurons (Bargmann et al., 1993), showing attraction, aversion and/or memory for many of these odors (Colbert and Bargmann, 1995), which allows them to identify potential prey and avoid pathogens during bacterial grazing (Choi et al., 2015; Pradel et al., 2007). As an indirect result, bacterial grazing by nematodes modifies soil microbial communities, which in turn can also influence plant development as has been previously reported for tomato, peanut, and rice (Eller and Frenzel, 2001; Jiang et al., 2012). Additionally, in *Arabidopsis thaliana,* a model system used to study plant-microbe interactions because of its short life cycle (Jiang et al., 2012), bacterial feeding nematodes also modify root morphology in an auxin dependent way (Mao et al., 2007).

It has been proposed that the effect of nematodes on plant development may depend on nematode associated bacteria (Mao et al., 2006;

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Xu et al., 2015), and various species of rhizobia, such as *Rhizobium* sp., have been found to be part of the microbiota of these worms (Baquiran et al., 2013; Berg et al., 2016; Niu et al., 2016). *Rhizobium* spp. is a well-known plant growth-promoting bacterium that forms nitrogen-fixing nodules on legume roots. However, in addition to its symbiotic interaction with legumes, *Rhizobium* sp. has also been reported as a plant growth promoter in other non-nodulating crops, such as maize (Celador-Lera et al., 2017; Rosenblueth and Martínez-Romero, 2004).

Maize (*Zea mays*) is one of the most important cereal crops for human consumption and animal feed worldwide, both as silage and as crop residue (Godfray et al., 2010). In addition, maize is also processed into a variety of food and industrial products, including starch, sweeteners, oil, beverages, glue, industrial alcohol, and fuel ethanol (Ranum et al., 2014). Mexico is considered the site of origin of maize-based agriculture and the center of its genetic diversity (Perales and Golicher, 2014), making it an ideal place to study the effect of soil nematodes on native maize growth. Most of the crop lands in Mexico are managed by small-scale farmers and their yields depend heavily on soil fertility, and abiotic environmental conditions such as seasonal rains, temperature, soil pH, among others. Although various studies have addressed the diversity of bacterial communities in corn rhizosphere, and its role in promoting plant growth (Jurgens and Gude, 1994; Lin et al., 1983; Peiffer et al., 2013; Pereira et al., 2011; Piromyou et al., 2011; Rosenberg et al., 2009), the role of nematode-bacteria interactions in shaping the bacterial community in *Z. mays* rhizosphere, as well as their influence during maize development, have been scarcely studied.

In this study, we hypothesize that nematodes isolated from rhizospheric corn soils will show a strong feeding preference towards *Rhizobium* strains that may represent an abundant food source in the rhizosphere, which in turn might influence bacterial fitness, and thus, the plant growth promoting capabilities of these bacteria. On the other hand, considering that environmental nematodes carry a vast and diverse microbiota (adult worms contain ~10,000 bacterial cells, Berg et al., 2016), which in this case may also derive from maize rhizosphere, we hypothesize that bacteria carried by these nematodes may also play a role in plant development. Finally, considering the genetic homogeneity of *A. thaliana* compared to native corn, as well as its wide use as a model in root development analysis, we also used *A. thaliana* to further characterize the effects of nematodes and their associated bacteria in root morphology. Our findings reveal a novel synergistic effect in which co-inoculation of environmental nematodes and rhizobial strains increases plant growth in maize and modifies root morphology in both native corn and the plant model *A. thaliana*. Also, we showed that *Acinetobacter pittii,* a bacterium isolated from the nematodes, is capable of modifying root morphology, emphasizing the importance of nematode and their associated bacteria in plant development. A model of nematodes as Trojan horses carrying bacteria is proposed.

2. Materials and methods

2.1. Isolation and identification of nematodes

Nematodes used in this study were isolated from corn rhizosphere of young plants (vegetative stage V4) in a small agricultural field in Mexico City, and of senescent corn plants (reproductive stage R6) from an industrial cornfield in Guanajuato, Mexico. Nematodes were isolated following the protocol previously described by Barrière and Félix (2006). Briefly, a small portion (1 g) of soil was placed on the edge of nematode growth media (NGM) plates containing an *Escherichia coli* DH5α culture in the middle. The NGM medium is composed of 14 g Bacto-Agar (BD Difco™ New Jersey, USA), 2.9 g NaCl, 2.5 g Bacto-Peptone (BD Difco™ New Jersey, USA), 5 µg/ml cholesterol, 1 mM $MgSO_4$, 25 mM KH_2PO_4 (pH 6) and 1 mM $CaCl_2$ per liter of water. Plates were incubated at room temperature until the nematodes reached the bacteria in the middle of the plate. Individual nematodes were collected, categorized by morphology using a stereomicroscope (Olympus, Tokyo, Japan), and serially transferred to new NGM plates for culturing purposes. Nematodes were maintained at 20 °C on NGM fed with *E. coli* DH5α until use.

To identify the isolated nematodes, DNA was purified from worms using the DNaesy Blood and Tissue Kit (Qiagen Germatown, USA), and 18S rRNA genes were PCR amplified and sequenced using the primers described in Table 1, and Phusion High Fidelity DNA polymerase (ThermoFisher Scientific MA, USA). The following PCR conditions were used: 95 °C for 5 min, 30 cycles at 95 °C for 30 s, 60 °C for 1 min 30 s, 72 °C for 2 min, and one final cycle at 72 °C for 10 min. The resulting PCR products were purified, sequenced and compared against GenBank database using NCBI BLAST.

2.2. Bacteria

The bacterial strains used in this study were: *Bacillus subtilis* sp. CGE2031 (this study), *E. coli* DH5α (Taylor et al., 1993), *E. coli* OP50 (Brenner, 1974), *Metylobacterium extorquens* Am1 (Peel and Quayle, 1961), *Rhizobium etli* CFN42 (Quinto et al., 1985), *R. etli* CFN42 DsRed (Torres Tejerizo et al., 2015), *Rhizobium phaseoli* Ch24-10 (Rosenblueth and Martínez-Romero, 2004), *Rhizobium tropici* CIAT 899 (Martínez-Romero et al., 1991), *Streptomyces* sp. (this study), and *A. pittii* (this study). All bacterial strains were grown at 30 °C in peptone yeast medium (PY) with gentamicin (30 mg/ml) or spectinomycin (30 mg/ml) when required. The PY medium is composed of 5 g Bacto-Peptone (BD Difco™ New Jersey, USA), 3 g Bacto-Yeast extract (BD Difco™ New Jersey, USA), and 700 mM $CaCl_2$, per liter of water (Encarnación et al., 1995)

2.3. Bacterial attraction assay

In order to test the bacterial feeding preferences of the isolated nematodes to different bacterial strains, we carried out bacterial attraction assays in vitro. For this, bacteria were grown in 50 ml of liquid PY medium at 30 °C until cultures reached an optical density of 0.6 at 600 nm. 50 µl (µl) of each culture were separately placed on the edge of an NGM plate and incubated overnight at 25 °C. Then, 100 adult nematodes were placed in the middle of the plate, and after one hour, the number of worms on each bacterial lawn was determined (Supplementary 1). We used *E. coli* DH5α, the nematode food source

Table 1
Oligonucleotide sequences for primers used in this study.

Name	Sequence	Amplified region	Reference
F43	5′- ATTAAGCCATGCATGTC-3′	527 bp of 18S rRNA	Hadziavdic et al. (2014)
R570	5′-GGAGGGCAAGTCTGGTGCC-3′		
F566	5′-CAGCAGCCGCGGTAATTCC-3′	634 bp of 18S rRNA	
R1200	5′-CCCGTGTTGAGTCAAATTAAGC-3′		
NemF	5′-GGGGAAGTATGGTTGCAAA-3′	500 bp of 18S rRNA	Sapkota and Nicolaisen (2015)
18Sr2b	5′-TACAAAGGGCAGGGACGTAAT-3′		
fD1	5′-CCGAATTCGTCGACAACAGAGTTTGATCCTGGCTCAG-3′	1500 bp of 16S rRNA	Weisburg et al. (1991)
rD1	5′-CCCGGGATCCAAGCTTAAGGAGGTGATCCAGCC-3′		

after isolation, as a positive control in these assays.

For supernatant attraction assays, bacteria were grown as described above, cultures were centrifuged at 8000 rpm for 10 min, and the supernatants were collected and filtered using Millex® syringe filter units (pore size 0.22 µm). 50 µl of each supernatant were placed on NGM plates and preference assays were performed following the same procedure as for bacterial lawn attraction assays.

2.4. Confocal microscopy

To evaluate consumption of bacteria by nematodes, adult worms were placed on NGM plates and fed on the reporter strain *R. etli* CFN42 DsRed or *R. etli* CFN42 (as a control) during seven days at room temperature. After this period, the nematodes were recovered from the plates, fixed with ethanol 70% (Jones and Gwynn, 1991) and visualized in a Zeiss LSM 510 Meta Confocal Microscope. The images were captured using phase contrast at 20X magnification and analyzed using the software ImageJ (https://imagej.nih.gov/ij/).

2.5. Corn growth conditions

Native ("criollo de Hidalgo") maize seeds were sterilized with 70% ethanol and 20% bleach, as described in Rosenblueth and Martínez-Romero (2004). Sterilized seeds were then germinated in semi-solid Fahräeus agar (7 g Bacto-Agar (BD Difco™ New Jersey, USA), 0.1 g $CaCl_2$, 0.12 g $MgSO_4.7H_20$, 0.1 g KH_2PO_4, 0.15 g Na_2HPO_4, 0.005 g Fe-citrate, and traces of Mn, Cu, Zn, B and Mo per liter of water) (Fahraeus, 1957) during 48 h in a climate-controlled room (20 °C 16/8 h light/dark photoperiod). Seeds were then transplanted in flasks containing sterile vermiculite and Fahräeus solution (0.1 g $CaCl_2$, 0.12 g $MgSO_4.7H_20$, 0.1 g KH_2PO_4, 0.15 g Na_2HPO_4, 0.005 g Fe-citrate, and traces of Mn, Cu, Zn, B and Mo per liter of water) for corn development assays, or semi-solid Fahräeus agar for root morphology analyses. Plants were incubated in a climate-controlled room during three days before inoculation with nematodes and/or bacterial strains.

2.6. Z. mays development assays

To evaluate the influence of nematode-bacteria interactions in corn development, plants were inoculated with nematodes and/or bacteria as follows: bacterial cultures grown in PY medium (OD_{600} = 0.6) were centrifuged at 5000*g*/5 min, washed twice with sterile phosphate buffered saline (PBS) pH 7.2 and suspended in the same buffer ~10^6 colony-forming units (CFU) per gram of vermiculite were used for inoculation. For nematode inoculations, worms were cultured in NGM Petri dishes containing an *E. coli* OP50 lawn during two days at 25 °C, centrifuged at 1000*g*/1 min, washed six times with sterile water, and ~30 adult nematodes per gram of dry vermiculite were used for inoculation. The different treatments included: 1) control plants (without bacteria or nematodes); 2) plants inoculated with *R. phaseoli* Ch24-10; 3) plants inoculated with soil-isolated nematodes; and 4) plants co-inoculated with both bacteria and the corresponding nematode. After 28 days, plants were harvested and foliage was separated from the root. Height and dry weight of the foliage was measured as previously described (Piromyou et al., 2011).

2.7. Root morphology analysis in Z. mays and A. thaliana

To evaluate the effect of the bacterial-feeding nematodes on root development in corn plants, germinated sterile seeds were grown in flasks containing 200 ml of semi-solid Fahräeus agar in a climate-controlled room during one week. Then, bacterial suspensions (10^7 CFU) and/or nematodes (~3000 adult worms) were used for inoculation as described above. One week after, plants were harvested and root portions were collected to determine total root length and dry weight as previously described (Piromyou et al., 2011).

For root morphology analyses in the plant model *A. thaliana*, Col-0 ecotype seeds were dry sterilized during 2 h in contact with vapors of 50 ml of commercial hypochlorite and 1.5 ml of HCl, and then imbibed in sterile distilled water during two days at 4 °C. Then, plants were grown in Petri dishes (oriented vertically) with 0.2x Murashige and Skoog (MS) medium (Phyto Technology Laboratories, Lenexa), supplemented with 1% sucrose, vitamins (0.5 mg L^{-1} pyridoxine, 0.5 mg L^{-1} nicotinic acid) and 0.8% agar. Plants were maintained at 21 °C, 16/8 h light/dark photoperiod until germination (determined when the primary root was first visible under a stereo microscope). Four days after germination, plants were inoculated with nematodes (~1000 adult worms) and/or bacteria (10^6 CFU) at four centimeters from the root tip. Primary root length and emerged lateral roots for the same plant treatments as previously described were determined from scanned images using the ImageJ software (http://rsb.info.nih.gov/ij/).

2.8. Estimating the effect of nematode-isolated bacteria in root size

To isolate nematode-associated bacteria, soil nematodes were grown in sterile NGM medium for three days (until they reached the adult phase) and then washed six times with sterile water. Then, ~50 adult worms were mechanically lysed by vortexing in 1 ml of sterile water with 0.5 g of zirconium beads, and serial dilutions from this suspension were plated in agar NGM to obtain isolated colonies. As control, colonies were also isolated from the model nematode *C. elegans* N2 that was maintained under laboratory conditions with routine bleach sterilization in monoxenic cultures with *E. coli* OP50. Then, we selected ten morphologically different colonies per nematode for identification by 16S rRNA sequence analysis using the universal primers fD1 and rD1 (Table 1) (Weisburg et al., 1991) and Phusion High Fidelity DNA polymerase (ThermoFisher Scientific MA, USA). The following PCR conditions were used: 95 °C for 5 min, 30 cycles at 95 °C for 30 s, 55 °C for 1 min 30 s, 72 °C for 2 min, and one cycle at 72 °C for 10 min.

To evaluate the effect of the isolated bacteria in root morphology, we inoculated *A. thaliana* plants with 10^6 CFU of *A. pittii*, a bacterial isolate that was commonly recovered from both nematodes, and/or ~1000 *C. elegans* worms (to avoid the effect of other commensal bacteria present in the environmental nematodes). After seven days, primary root length and emerged lateral roots were determined as described in 2.7.

2.9. Statistical analysis

Statistical analyses were performed using GraphPad Prism software version 5.0 (GraphPad Software Inc.). To test if the data show a Gaussian distribution we performed D'Agostiono-Person, Shaphiro-Wilk and/or Kolmogorov–Smirnov tests. Statistical analysis were then performed using one-way ANOVA (for parametric samples) or Kruscal-Wallis test (for non-parametric samples), followed by Tukey's HSD test (parametric) or Dunn's test (non-parametric) as post hoc tests. Dunnett's post hoc test was used for parametric samples if comparing a control vs various treatments.

3. Results

3.1. Attraction assays of isolated nematodes towards rhizospheric bacteria

We isolated two different nematodes from corn rhizosphere, which were identified as *O. tipulae* (from young plants, V4) and *Mesorhabditis* sp. (from senescent plants, R6). Phylogenetic analysis based on 18S rRNA sequences revealed that these nematodes are closely related to *C. elegans* and belong to the family *Rhabditidae* (Supplementary 2). We found that both nematodes are preferentially attracted *in vitro* to *Rhizobium* strains over other rhizospheric bacteria such as *M. extorquens*, *B. subtilis* and *Streptomyces* sp. (Fig. 1A–B). However, whereas *O. tipulae*

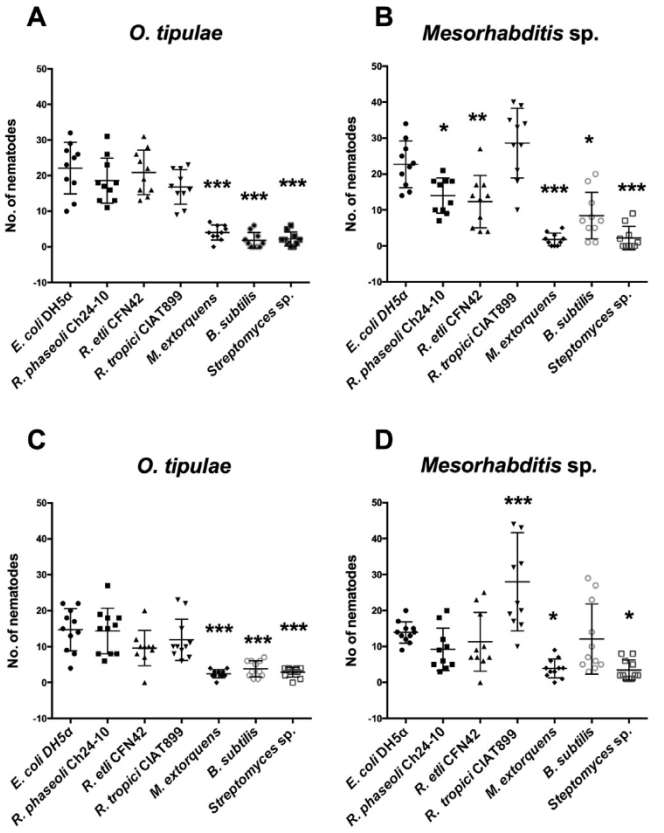

Fig. 1. Attraction of nematodes *O. tipulae* and *Mesorhabditis* sp. to different rhizobacteria (A and B, respectively) and their supernantants (C and D, respectively). Statistically significant differences with respect to *E. coli* DH5α are indicated by $^{**}(P < 0.01)$ or $^{***}(P < 0.001)$ (Dunnet's pos hoc test $P \leq 0.05$). Representative results of three independent experiments are shown.

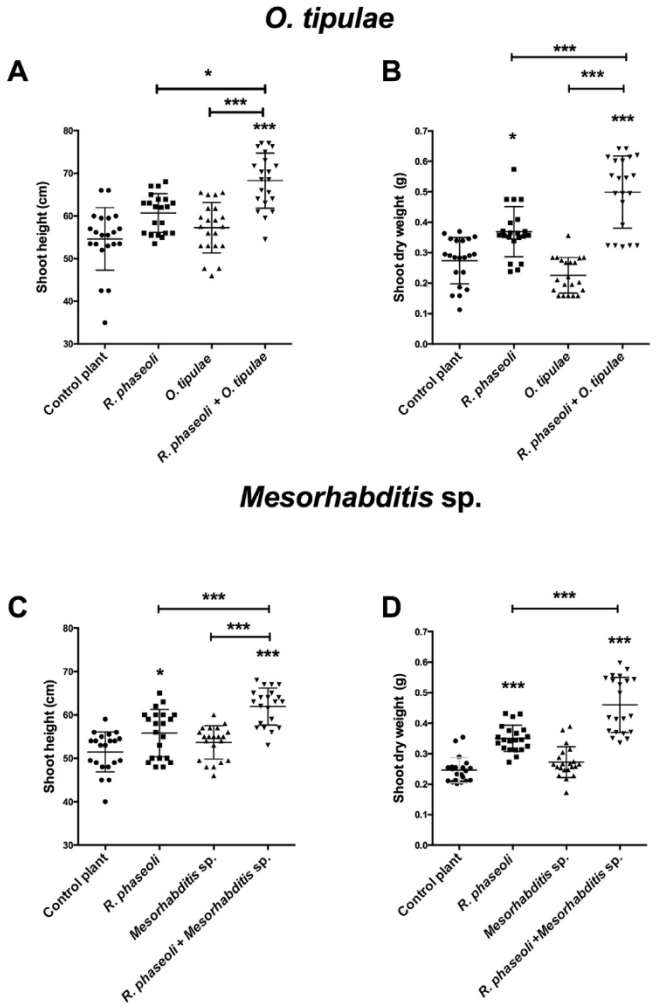

Fig. 2. Effect of nematodes and *R. phaseoli* Ch24-10 on corn shoot height (A and C) and shoot dry weight (D and B). Results of three independent experiments with seven replicate plants are shown. Statistically significant differences with respect to control plants are indicated by $^{*}(P < 0.05)$, $^{**}(P < 0.01)$ or $^{***}(P < 0.001)$ (Dunns' or Tukey HSD post hoc test $P \leq 0.05$) single asterisks represent comparisons against control plants, while comparisons among other groups are indicated with lines.

displayed similar attraction patterns for the three rhizobia strains used (Fig. 1A), *Mesorhabditis* sp. had a strong preference for *R. tropici* CIAT 899 (Fig. 1B). As expected, both nematodes showed a high preference for *E. coli* DH5α (their food source after isolation). We also evaluated the attraction of both nematodes towards bacterial supernatants, and even though nematode migration towards supernatants was generally lower compared to whole bacterial cultures, a similar pattern of preference was observed for both nematodes (Fig. 1C and D). Interestingly, the response of *Mesorhabditis* sp. towards *R. tropici* CIAT 899 supernatant was as strong as that previously observed for the bacterial lawn (Fig. 1D).

Fluorescence microscopy experiments confirmed that *Rhizobium* is a food source for *O. tipulae* and *Mesorhabditis* sp. since the reporter strain *R. etli* CFN42 DsRed was detected in the pharynx and along the intestine of both nematodes previously fed with the fluorescent bacterium, while worms fed with *R. etli* CFN42 wild type (negative control) did not show any signal (Supplementary 3).

3.2. Shoot biomass of corn plants inoculated with nematodes and Rhizobium

Based on the nematode preference towards *Rhizobium* strains, we decided to test the effect of nematode-*Rhizobium* interaction on maize development. We found that, although maize plants inoculated with *R. phaseoli* Ch24-10 showed higher shoot height and dry weight compared to control (untreated) plants, a significant further increase in these parameters was obtained with co-inoculations of *O. tipulae* or *Mesorhabditis* sp. plus *R. phaseoli* Ch24-10 compared to control plants (Fig. 2A–D). In contrast, we did not observe significant changes in growth of corn inoculated only with nematodes compared to control (untreated) plants.

3.3. Length and dry weight of Z. mays and A. thaliana roots inoculated with nematodes and Rhizobium

We also evaluated the effect of nematode-*Rhizobium* interactions on corn root morphology and found that *O. tipulae* treatment led to shorter primary roots compared to untreated plants, independently of the presence of *R. phaseoli* (Fig. 3A). However, in the case of *Mesorhabditis* sp., which also reduced primary roots, addition of *R. phaseoli* restored the root length to the levels observed in *R. phaseoli* Ch24-10 and control treatments (Fig. 3A).

Similarly, whereas *O. tipulae* did not change root dry weight significantly compared to control plants, and co-inoculations of *O. tipulae* and *R. phaseoli* were similar to single *R. phaseoli* treatment (Fig. 3B), plants treated with *Mesorhabditis* sp. showed a higher root dry weight, which was further increased with the addition of *R. phaseoli* compared to untreated plants or *R. phaseoli* single treatments. These results correlated with an increased outgrowth and elongation of lateral roots and total area observed in plants treated with the nematode-*Rhizobium* mixtures compared to single treatments (Supplementary 4A). We did not observe significant differences in shoot biomass and plant height

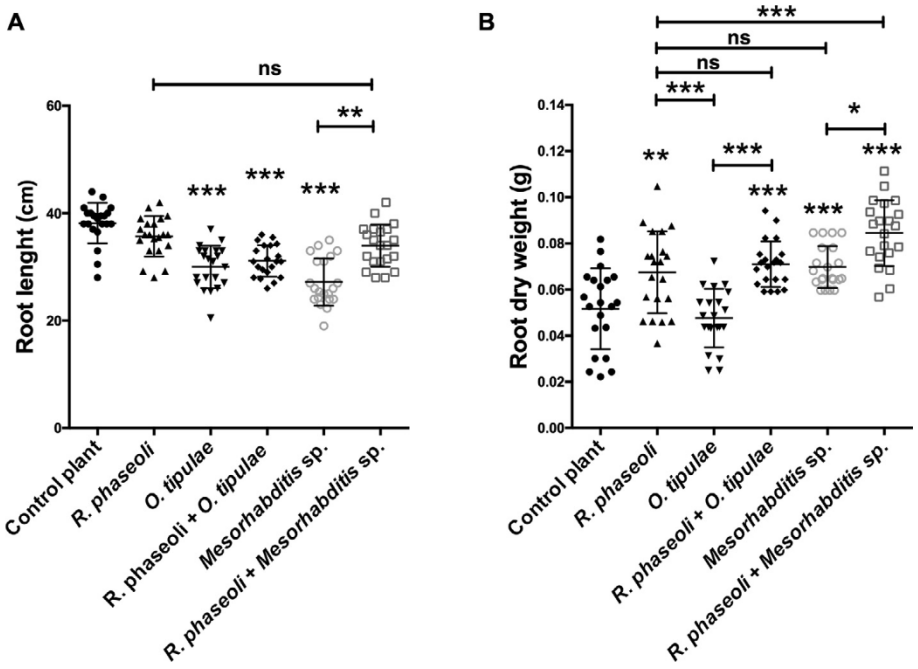

Fig. 3. Effect of *Mesorhabditis* sp., *O. tipulae* and *R. phaseoli* Ch24-10 on corn root length (A) and root dry weight (B). Results of three independent experiments with seven replicate plants are shown. Statistically significant differences are indicated by $^{*}(P < 0.05)$, $^{**}(P < 0.01)$ or $^{***}(P < 0.001)$ (Dunn's or Tukey HSD post hoc test $P \leq 0.05$), single asterisks represent comparisons against control plants, while comparisons among other groups are indicated with lines.

among the different treatments at this early time point sampled (seven days) (Supplementary 4B and C).

We performed similar root morphology analysis in the plant model *A. thaliana,* and we also observed shorter primary roots in plants to which nematodes were added. Plants treated with *O. tipulae* or *Mesorhabditis* sp. were ~50% and ~37% shorter compared to control plants, respectively (Supplementary 5A). Similarly, a slight increase in the number of emerged lateral roots was also observed with co-inoculations of nematode-*Rhizobium* compared to single *Rhizobium* treatments, however, such differences did not reach significance (Supplementary 5B–C).

3.4. Influence of nematode microbiota on A. thaliana root morphology

In addition to the role of nematode-*Rhizobium* interactions described above, we also decided to evaluate the effect of nematode-associated (commensal) bacteria on root morphology. We found two bacteria that were commonly isolated from both *O. tipulae* and *Mesorhabditis* sp., which were closely related to *Stenotrophomonas maltophilia* KUGK130 and *A. pittii* (Table 2), whereas as expected, *E. coli* was the only bacterium isolated from *C. elegans* that was regularly fed with this bacterium. Then, we tested the role of one of these bacterial isolates, *A. pittii*, in *Arabidopsis* root morphology. We found that, whereas single inoculations of the bacterium or the nematode *C. elegans* N2 had no affect *Arabidopsis* root morphology (Fig. 4A and B), roots inoculated with *C. elegans* N2 previously fed with *A. pittii* showed a reduction of the primary root length and an increase in emerged lateral roots (Fig. 4B). This phenotype was similar to that previously observed for roots of plants treated with the environmental nematodes (Supplementary 5).

4. Discussion

Soil nematodes exhibit diverse dietary habits that mainly depend on food availability in their environment and preference for certain bacterial species over others (Bargmann et al., 1993; Choi et al., 2015; Yu et al., 2015). Here, we found that *O. tipulae* and *Mesorhabditis* sp., two bacterial-feeding nematodes isolated from the rhizosphere of corn, show attraction to *Rhizobium* strains over Gram-positive bacteria, thus suggesting that *Rhizobium* strains are a common food source for these nematodes in this ecosystem. However, the fact that *Mesorhabditis* sp. showed a higher preference for *R. tropici* over other *Rhizobial* strains also suggests that these nematodes may show subtle differences in feeding preferences in rhizospheric soils. Although nematode preference for Gram-negative bacteria has previously been reported for *Cephalobus brevicauda* and *C. elegans* (Salinas et al., 2007; Yu et al., 2015), this is the first study reporting attraction of soil nematodes to the plant growth promoter, *R. phaseoli* Ch24-10.

Various studies have demonstrated that bacterial grazing by nematodes modifies the endogenous microbial community and influences bacterial fitness and activity (Bonkowski et al., 2000; Irshad et al., 2011; Jousset et al., 2009). Accordingly, we found that maize growth promotion by *R. phaseoli* Ch24-10 was increased in the presence of soil-isolated nematodes, and that such phenotype was greater for *Mesorhabditis* sp. This result correlates with a previous study in which

Table 2
Assignment 16S rRNA sequences of the isolated bacteria from *O. tipulae* and *Mesorhabditis* sp.

Isolate	Origin	Closest NCBI match	Identity %	Number of isolates
Ost2	*O. tipulae*	*Pseudomonas alkylphenolia* strain KL28	99	1
Ost5	*O. tipulae*	*Ochrobactrum pituitosum* strain CCUG 50899)	99	1
Ost6	*O. tipulae*	*Stenotrophomonas maltophilia* strain KUGK130	99	1
Ost8	*O. tipulae*	*Acinetobacter pittii strain HUMV-6483*	99	2
Mes1	*Mesorhabditis* sp.	*Acinetobacter pittii* strain HUMV-6483	99	2
Mes2	*Mesorhabditis* sp.	*Stenotrophomonas maltophilia* R551	99	5
Mes10	*Mesorhabditis* sp.	*Achromobacter insolitus* strain DSM 23807	99	1

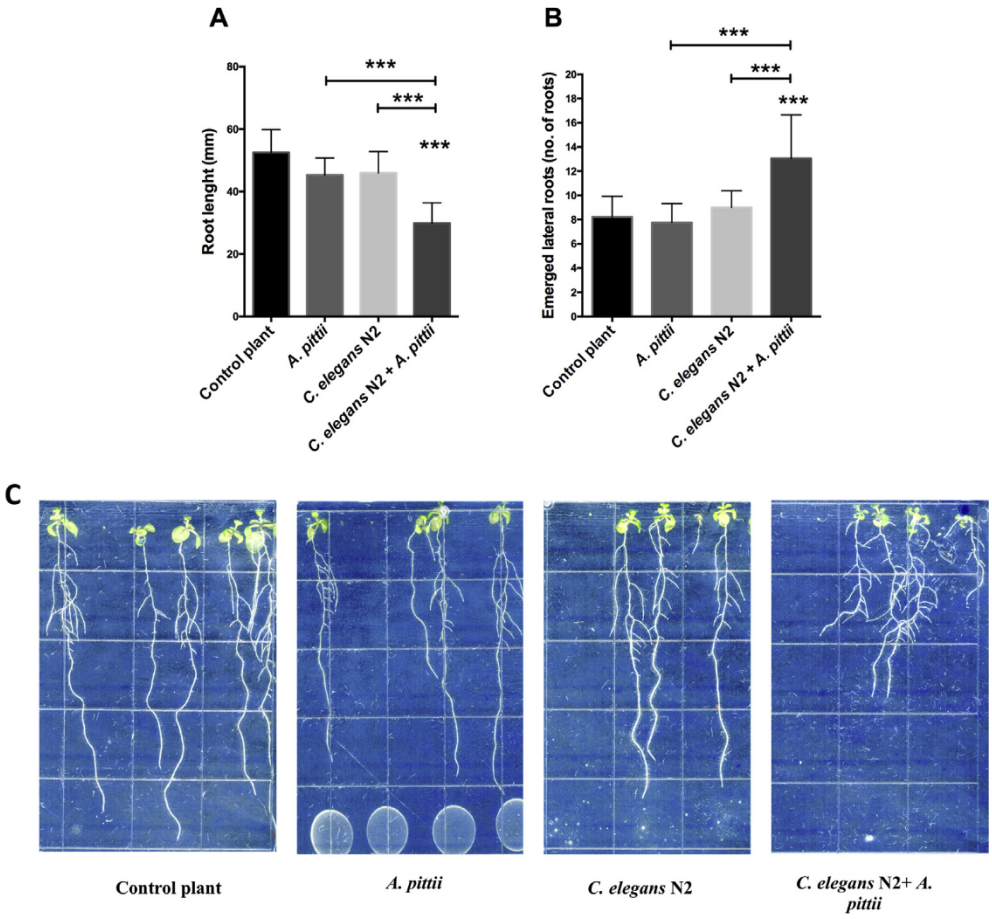

Fig. 4. Effect of *A. pitti* and *C. elegans* N2 on *A. thaliana* root length (A) and lateral emerged roots number (B). Representative images of the different treatments (C). Results of three independent experiments with 10 replicate plants are shown. Statistically significant differences with respect to the control plant are indicated by $^{*}(P < 0.05)$, $^{**}(P < 0.01)$ or $^{***}(P < 0.001)$ (Tukey HSD post hoc test $P \leq 0.05$).

Mesorhabditis sp. also caused a greater increase in plant growth of peanut in the presence of *Bacillus megaterium* compared to another soil-isolated nematode, *Cephalobus* sp. (Xu et al., 2015). *Cephalobus* sp. in turn, showed a higher effect in increasing soil basal respiration and indol 3-acetic acid (IAA) content in rice in the presence of *Burkholderia* sp. compared to the laboratory nematode, *C. elegans* (Cheng et al., 2011). These results highlight the differential effects in plant development among environmental nematodes.

It has been previously shown that bacterial feeding nematodes increase the production of bacterial IAA (Fu et al., 2005; Rosenberg et al., 2009), which in turn induces expression of diverse plant genes involved in cell elongation, leading to enhanced root growth (Guilfoyle, 2007). We also observed changes in root architecture, shorter primary roots in plants treated with nematodes and increased root dry weights in nematode-*R. phaseoli* treatments, both in native corn and *A. thaliana*. A similar phenotype was observed with another rhizobial strain, *Rhizobium sp*. IRGB74, which was able to colonize the roots of *A. thaliana*, thus inhibiting the growth of the main root and enhancing the formation of lateral roots in an auxin-dependent manner, however, the effect of *Rhizobium*-nematode co-inoculations was not evaluated in this work (Zhao et al., 2018). In addition, it has also been reported that high levels of auxins inhibit cell elongation in primary roots, and increase the number of lateral roots and root hairs in *A. thaliana* inoculated with *Bacillus amyloliquefaciens* UCMB5113 or *Rhizobium sp*. IRGB74 (Asari et al., 2017). Although we observed an enhanced auxin response in the root cap of *Arabidopsis* roots using a DR5:Gus reporter line, both in single and double treatments (Supplementary 6), such increased amount of auxins may explain the phenotype promoted by *R. phaseoli*, but not the differences observed in primary roots with nematode treatments, thus suggesting the presence of a multifactorial and more complex mechanism than previously described.

In this study, we also report that members of the nematode microbiota can also influence plant development when transferred to an innocuous nematode such as *C. elegans*, which indicates that nematode-associated bacteria condition the effects of nematodes, which may explain, at least in part, the diverse phenotypes observed in plants treated with different environmental isolates Similarly, it has also been reported that some nematodes have a mutualistic relationship with their bacterial preys. For example, the insect-pathogenic nematodes *Heterorhabditis* sp. and *Steinernema* sp. have independently evolved close relationships with bacteria of the genera *Photorhabdus* spp. and *Xenorhabdus* spp., respectively (Lacey and Georgis, 2012; Lacey et al., 2015; Torres-Barragan et al., 2011). These nematodes infect insects and release the symbiotic bacteria that multiply quickly and kill the host whereas are consumed again for the nematode as a food source.

Altogether, our results show a novel role of soil nematodes and their associated bacteria, including bacterial preys and commensals, in modulating root morphology and plant growth. We propose a model in which nematodes may act like Trojan horses, carrying rhizobial bacteria to plant roots, where bacteria are then able to colonize and multiply, indirectly increasing nematode food source and influencing plant fitness (Fig. 5). It is worth noting that such effects may depend on the type of nematode and its associated bacteria, as well as plant host, thus emphasizing the complex mechanism of plant growth-promotion by these organisms. This work and further studies may help to fully understand the environmental impact and ecological effects of bacteria and bacteria-feeding nematodes in soil, which in turn may lead to novel approaches to manage nematode populations, or their core microbiota, for agricultural purposes, for example, by developing novel nematode-based biofertilizers to increase the number of plant growth promoting bacteria in rhizosphere and/or improve root colonization by these bacteria.

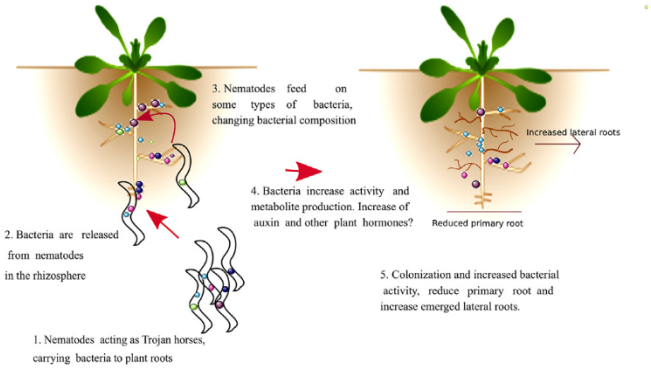

Fig. 5. Proposed model of nematodes as Trojan horses (see the text for further details).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.apsoil.2018.08.012.

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