

Plant domestication influences the distribution and host-location behavior of entomopathogenic nematodes

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ARTICLE INFO

Keywords:

Anomala orientalis
Biological control
Tri-trophic interactions
Root herbivory
Steinernema

ABSTRACT

Plant domestication is a powerful driver of ecological shifts in soil communities, yet its effects on tri-trophic interactions facilitating biological control involving entomopathogenic nematodes (EPNs) remain poorly understood. We investigated how the domestication of *Vaccinium corymbosum* (highbush blueberry) influences the distribution, composition, and host-location behavior of endemic EPN populations in cultivated blueberry fields and adjacent wild blueberry patches. We used quantitative PCR to characterize the EPN community and associated soil organisms across paired field sites. We also conducted olfactometer assays to test EPN behavioral responses to wild and domesticated plants with and without *Anomala orientalis* larvae. Our findings revealed a clear habitat partitioning, with *Steinernema glaseri* dominating cultivated fields and *Steinernema* spp. N7 and N10 being more prevalent in wild patches. Using the olfactometer assay, we observed distinct habitat-specific patterns: EPNs isolated from cultivated soils were more strongly attracted to domesticated blueberry roots, whereas those from wild soils responded preferentially to wild plants. Although neither group showed significant attraction to *A. orientalis* larvae alone, EPNs isolated from cultivated fields exhibited increased attraction to domesticated plants when insect larvae were present—consistent with a context-dependent ‘cry-for-help’ response. These findings suggest that domestication is likely to influence the structure and behavior of rhizosphere EPN communities, potentially by reinforcing long-term associations among plant genotypes, herbivores, and their natural enemies. Advancing our understanding of habitat-specific interactions will be essential to enhancing the specificity and efficacy of EPN-based biological control in agricultural systems.

1. Introduction

Soil ecosystems are among the most diverse on Earth, with a single gram of soil containing, for instance, up to 50,000 bacterial species (Ramirez et al., 2015; Roesch et al., 2007). Plants play a pivotal role in shaping soil communities, affecting their structure and function (Kulmatiski et al., 2008; Viketoft et al., 2005; Yeates et al., 1999). Because plants lack true immune systems and cannot physically escape attacks from belowground herbivores, they have evolved intricate interactions with surrounding soil communities to enhance their defenses against herbivores and soil-borne pathogens (e.g., Molina-Montenegro

et al., 2016; Rasmann and Turlings, 2016; Van Der Heijden et al., 2008).

In agricultural habitats, domesticated plants are typically bred to exhibit traits favored by humans, such as yield, taste, and other attributes (Purugganan and Fuller, 2009). Unfortunately, selection preferences can cause unfavorable outcomes for plants once they are established in cultivated soils (Fernandez et al., 2021). Among the intensified pressures in cultivated soils under monoculture agricultural production are root-feeding herbivores. They remain unseen until damage becomes visually apparent aboveground, which makes management decisions challenging and provides a logical rationale for applying prophylactic insecticide treatments (Johnson and Rasmann,

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2015). Furthermore, infestations can spread rapidly in modern monoculture farming systems, aggravating the problem (Gurr et al., 2016). Given these difficulties, there has been growing interest in understanding and manipulating rhizosphere interactions as a sustainable strategy to mitigate the impact of root-feeding herbivores (Stelinski et al., 2019).

A promising strategy for managing insect root herbivores is the use of entomopathogenic nematodes (EPNs), soil-dwelling organisms with proven efficacy in pest control across a wide range of insects, crops, and geographic regions (Blanco-Pérez et al., 2024). In their infective juvenile (IJ) stage, EPNs actively seek suitable insect hosts, penetrate their bodies, and release symbiotic bacteria into the hemocoel, killing the host within 24 to 72 h (Dillman et al., 2012). Thereafter, the EPNs develop inside the cadaver until they consume all nutrients, and new IJs emerge to seek new hosts (Tarasco et al., 2023). In some agricultural contexts, EPNs have already been implemented or developed as commercially viable tools for targeted control of specific root herbivores (Hiltpold, 2015; Lacey and Georgis, 2012; Ramakuwela et al., 2025). Regardless of insect presence, the IJs are known to locate insect-damaged roots across multiple systems, indicating that EPNs use changes in the volatile profile emitted by roots as cues to encounter potential hosts (Ali et al., 2011; Rasmann et al., 2005; Tarasco et al., 2023; van Tol et al., 2001). However, the species-specificity of these signals and their variability across target insects and plant systems remain poorly understood. Additionally, although plant species identity is assumed to shape nematode communities, the impact of genotypic variation in plants and EPN populations on such interactions is still uncertain (Brinkman et al., 2008; De Deyn et al., 2004; Sohlenius et al., 2011; Viketoft et al., 2005; Wardle et al., 2003).

Plant domestication presents a remarkable opportunity to investigate the effects of human selection on soil systems. While domesticated plants are tightly linked to managed environments, including cultivated soils, wild plants have evolved to thrive in their natural habitats (Pérez-Jaramillo et al., 2016). For example, comparative studies have shown that domestication can lead to consistent shifts in the composition and structure of soil microbial communities and plant-soil feedbacks, with consequences for belowground interactions and ecosystem functioning (Cordovez et al., 2019; Pérez-Jaramillo et al., 2018). This distinction highlights the challenge of studying plant-soil interactions in laboratory settings, where environmental differences are controlled but may fail to capture the complexity of natural systems (Chen et al., 2023). Nonetheless, laboratory studies remain valuable for understanding processes that warrant further exploration in more variable, naturalized contexts (Philippot et al., 2013). Previous research has shown that intense domestication selection can alter a plant's ability to respond to belowground herbivory, as observed in maize (Degenhardt et al., 2009; Rasmann et al., 2005). However, it remains unclear how these responses differ between wild and domesticated plants and whether the observed differences arise from variations in the plants, the insects, or their natural enemies.

We selected *Vaccinium corymbosum* L. (Ericales: Ericaceae) (northern highbush blueberry) as our study system due to its recent domestication and unique ecological context. Native to the understory of wooded forests in eastern North America, *V. corymbosum* ranges from Ontario and Nova Scotia (Canada) to Florida and eastern Texas (USA). The domestication of this rhizomatous shrub species began in the early twentieth century in the Pine Barrens region of New Jersey, USA (Eck, 1965; Rivera et al., 2015). Unlike many domesticated crops, which have undergone significant genetic modification and even speciation, domesticated *V. corymbosum* remains the same species as its wild relative, maintaining the capability, for instance, to cross-pollinate and produce viable offspring with its wild ancestor (Eck, 1965; Bian et al., 2014). In studies of the effects of domestication, this is particularly advantageous, as commercial cultivated blueberry fields within the Pinelands National Reserve are typically bordered by unmanaged, woody areas containing wild *V. corymbosum*, providing access to both

wild and cultivated plants for comparative studies. To investigate trophic interactions, we used the oriental beetle, *Anomala orientalis* (Waterhouse, 1875) (Coleoptera: Scarabaeidae), as the target insect species. *Anomala orientalis* is a generalist herbivore with root-feeding larvae that develop belowground and is a notable threat to commercial blueberry production, causing damage that ranges from yield loss to plant death (Polavarapu et al., 2007).

Our previous studies in the Pinelands region have examined the distribution of plant-parasitic nematodes and EPNs in cultivated and wild environments (Rivera et al., 2016; Rivera et al., 2015). Soil baiting revealed putative novel EPN species that were exclusively associated with wild habitats, while *Steinernema glaseri* (Steiner, 1929) (Rhabditiida: Steinernematidae) was more prevalent in cultivated fields (Rivera et al., 2016). However, this traditional technique, which relies on the highly susceptible *Galleria mellonella* (Linnaeus, 1758) (Lepidoptera: Pyralidae) larvae as hosts, may have biased these findings by favoring EPN populations that are better suited to this artificial host, which is not naturally found in soils (Griffin, 2015). To enhance the resolution of our return sampling effort, we employed qPCR, a highly sensitive molecular tool (Blanco-Pérez et al., 2025c), to reassess the five previously sampled sites with the highest EPN abundance. Using species-specific primers-probe sets, we resampled EPN diversity in soil to better understand differences potentially missed by traditional baiting techniques (Campos-Herrera et al., 2016a; Campos-Herrera et al., 2011b). Additionally, qPCR enabled the simultaneous assessment of other soil community components, such as free-living nematodes (FLNs), nematophagous fungi (NF), and ectoparasitic bacteria like *Paenibacillus* (Bacillales: Paenibacillaceae), all of which may interact with or compete against EPNs in the soil ecosystem (Campos-Herrera et al., 2012; Campos-Herrera et al., 2011a; Pathak et al., 2012).

Our central aim was to comprehend the impact of the domestication of both plants and soil on soil communities and trophic interactions, particularly within the context of biological control. We hypothesized that IJs would show a behavioral preference for plant types characteristic of their habitat of origin—wild or cultivated—reflecting potential local adaptation to distinct rhizosphere environments driven by plant genotype and the possibility for differences in plant response to root feeding. As such, we also hypothesized EPN populations would exhibit stronger attraction to plants hosting root-feeding *A. orientalis* larvae, consistent with previous findings on plant-mediated recruitment of belowground natural enemies (Ali et al., 2011; Rasmann et al., 2005; van Tol et al., 2001). Our main objectives were (i) to assess how domestication alters the composition and diversity of the EPN community and associated soil organisms and (ii) to test the effectiveness of endemic EPN populations in enhancing the biological control of *A. orientalis* under natural and cultivated conditions through the detection of root-feeding. This study provides valuable insights into the ecological consequences of plant domestication and its impact on soil communities, with implications for sustainable pest management in agricultural systems.

2. Material and methods

2.1. Field collection

To build on earlier research by Rivera et al. (2015, 2016), we selected five previously sampled sites (Supplementary Table S1, Supplementary Fig. S1) within the Pineland National Reserve of New Jersey (USA) that hosted the most persistent EPN communities. Following the methodology described by Rivera et al. (2015), we collected soil samples in May 2015, pairing those from wild areas with wild blueberries with adjacent commercial cultivated blueberry fields growing domesticated varieties. Briefly, at each location, we randomly selected six plants per habitat type (wild vs. domesticated). We extracted eight vertical soil cores (30.5 cm L × 2.1 cm D) per plant within the predefined 15.24 m × 15.24 m sampling blocks used in prior studies. This design yielded 60

soil samples, 12 per site: six from each of the wild patches and cultivated fields. Samples were transported in an enclosed cooler to New Brunswick (NJ, USA) to be stored at 4 °C for two to four days before processing. In the laboratory, soil cores from each plant were homogenized, and 200 g subsamples from each composite sample were preserved for further analysis.

2.2. Isolation of nematodes and associated organisms

Following Campos-Herrera et al. (2019), nematodes and other soil organisms were co-extracted from 200 g (Wiesel et al., 2015) of each of the 60 soil samples through sucrose centrifugation (Jenkins, 1964). This method allows the extraction of EPNs along with key associated organisms, specifically, FLNs that might compete with EPNs for cadaver resources (Campos-Herrera et al., 2015a; Duncan et al., 2003), NF that prey on nematodes (López-Llorca et al., 2007), and ectoparasitic *Paenibacillus* bacteria, whose spore attachment to the cuticle can limit nematode mobility (El-Borai et al., 2005; Enright and Griffin, 2005). The recovered samples were collected in 50 mL Falcon tubes and left overnight at 4 °C to allow sedimentation. The concentrated material at the bottom of each tube, containing organisms and residual soil, was then transferred to 1.5 mL Eppendorf tubes through a series of successive centrifugation and evacuation steps. The final pellet was lyophilized and stored at 4 °C until DNA extraction.

2.3. Quantification of soil communities

Lyophilized nematodes retrieved from soil samples were resuspended in 50 mL of distilled water before DNA extraction. To ensure proper disaggregation, lyophilized samples containing isolated soil organisms were mechanically disrupted for 15 s using sterile blue pestles attached to a Kontes™ Pellet Pestle™ motor (DWK Life Sciences GmbH, Mainz, Germany). We followed the DNA extraction protocol outlined by Campos-Herrera et al. (2016b), using the UltraClean™ Soil DNA Extraction Kit (MOBIO Laboratories, Inc., Carlsbad, CA, USA) with the maximum yield protocol and a final elution volume of 60 µL. DNA quality and concentration were assessed in duplicate using a NanoDrop 2000C spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Dilutions were prepared to final concentrations of 0.2 ng/µL for EPN and FLN quantifications and 10 ng/µL for NF and bacterial quantifications. DNA aliquots were stored at –80 °C for further analysis.

We screened for 14 EPN species employing molecular approaches (Table 1), including two putative new species from the *S. glaseri* sub-group endemic to the region (Rivera et al., 2016), as well as *S. feltiae* (Filipjev, 1934), *S. carpocapsae* (Weiser, 1955), and *Heterorhabditis bacteriophora* (Poinar, 1976) (Rhabditida: Heterorhabditidae), which are widely distributed cosmopolitan species (Bhat et al., 2020; Hominick, 2002). Additionally, we included species *S. kraussei* (Steiner, 1923), *S. intermedium* (Poinar, 1985), *S. intermedium-group*, *S. affine* (Bovien, 1937), *S. arenarium* (Artyukhovsky, 1967), and *H. megidis* (Poinar, Jackson & Klein, 1987), which are commonly reported in temperate regions (Hominick, 2002; Jaffuel et al., 2018), while *H. zealandica* (Poinar, 1990) is often associated with warm, freshwater environments (Hominick, 2002; Nguyen and Hunt, 2007). The two included the putative new *steinernematid* species isolated from the study sites, which are closely related to *S. phyllophagae* (Nguyen & Smart, 1994), and were designated *Steinernema* sp. N7 and *Steinernema* sp. N10 (Table 1) after being identified with de novo designed species-specific primers-probe sets (Table 2). To confirm their identification and determine suitable regions for molecular tool development, we performed molecular characterization of the ITS and D2D3 rDNA regions and integrated these findings into previously established qPCR approaches (see details in Supplementary Data 2). The FLNs screened in this study—*Acroboloides* spp. (Rhabditida: Cephalobidae), *Oscheius onirici* (Torrini et al., 2016) (Rhabditida: Rhabditidae), and *O. tipulae* (Lam & Webster, 1971) (Table 1)—were selected based on their documented co-emergence with

Table 1

Summary of soil organisms analyzed, including entomopathogenic nematodes (EPNs), free-living nematodes (FLNs), nematophagous fungi (NF), and ectoparasitic bacteria, along with their respective populations, GenBank accession numbers (ACNO) for the ITS region, and references for primer and probe sequences.

Species	Population	GenBank ACNO	Primers-probe sets' reference
EPNs			
<i>Heterorhabditis bacteriophora</i>	(commercial)	KJ938576	Campos-Herrera et al. (2011a)
<i>H. indica</i>	Btw	KJ938571	Campos-Herrera et al. (2011b)
<i>Steinernema affine</i>	CH	KJ938567	Torr et al. (2007)
<i>S. carpocapsae</i>	DOK-83	KJ818295	Campos-Herrera et al. (2011a)
<i>S. feltiae</i>	RS-5	KJ938569	Campos-Herrera et al. (2011a)
<i>S. glaseri</i>	CH	GU173998	Campos-Herrera et al. (2011b)
<i>S. intermedium</i>	82	AF171290	Campos-Herrera et al. (2015a)
<i>S. intermedium-group</i>	VAD-1067	KJ696684	Campos-Herrera et al. (2015a)
<i>S. kraussei</i>	OS	KJ696686	Campos-Herrera et al. (2015a)
<i>Steinernema</i> sp. N7	N7	MG711658	Current study
<i>Steinernema</i> sp. N10	N10	MG711660	Current study
FLNs			
<i>Acroboloides-group</i>	RT1-R15C	JQ237849	Campos-Herrera et al. (2012)
<i>Oscheius onirici</i>	MG67 P20	KJ938578	Campos-Herrera et al. (2015b)
<i>O. tipulae</i>	MG68 P29	KJ938579	Campos-Herrera et al. (2015b)
NF			
<i>Arthrobotrys dactyloides</i>	H55	KJ938574	Pathak et al. (2012)
<i>A. musiformis</i>	11	KJ938572	Pathak et al. (2012)
<i>A. oligospora</i>	8	KJ938573	Pathak et al. (2012)
<i>Catenaria</i> sp.	1D	JN585805	Pathak et al. (2012)
<i>H. rhossiliensis</i>	2931	–	Zhang et al. (2006)
<i>Purpureocillium lilacinum</i>	9357	KJ938575	Atkins et al. (2005)
Ectoparasitic bacteria			
<i>Paenibacillus nematophilus</i>	NEM2	AF480936	Campos-Herrera et al. (2011a)

IJs from EPN-infected host cadavers in insect-baiting assays conducted in previous surveys (Blanco-Pérez et al., 2022; Campos-Herrera et al., 2019; Campos-Herrera et al., 2012; Jaffuel et al., 2018). Additionally, ten EPN and one FLN species were included to validate the qPCR assays, ensuring specificity by preventing cross-amplification and adapting previously published protocols to our experimental conditions (Supplementary Table S2). Most nematode species were maintained in pure cultures, with EPNs reared in *G. mellonella* larvae (Woodring and Kaya, 1988) and FLNs cultured on 1% nutrient agar (Difco, MD, USA), feeding on associated bacteria. For *S. intermedium* and *S. phyllophagae*, for which live material was unavailable, plasmids containing their complete ITS region sequences (AF171290 and FJ410327, respectively) were used as templates (Supplementary Table S2). EPN and FLN populations were stored in tap water and Buffer M9, respectively, at 12 °C in dark conditions.

We screened fungal species representing the three NF main groups (Nordbring-Hertz et al., 2006): the nematode-trapping fungi *Arthrobotrys dactyloides* (Drechsler, 1937) (Orbiliomycetes: Orbiliaceae), *A. musiformis* (Drechsler, 1937), and *A. oligospora* (Fresenius, 1852), the endoparasites *Hirsutella rhossiliensis* (Minter & Brady, 1990) (Hypocreales: Ophiocordycipitaceae) and *Catenaria* sp. (Rhizophydiales: Catenariaceae), and the egg-parasitic fungi *Purpureocillium lilacinum* (Thom, 1910) (Hypocreales: Ophiocordycipitaceae) (Table 1). Due to their widespread occurrence (Gray, 1987), these NF are also known to associate

Table 2

Species-specific primers—forward F and reverse (R) and TaqMan® probes (P) designed for the detection of two putative new steinernematid species (populations N7 and N10), including primer and probe sequences, amplicon length, primer/probe concentrations (M), and annealing temperature (Ta).

Population	Primers and probe (5'-3')	Length	Primers/probe M	Ta
N7	F TACAATGGCCACCACCATCG R GCCGTAAAGCCCAACACTG P FAM-TGGCGCGTTTCTTGCCAGCT-MGB	164 bp	250/100 mM	66 °C
N10	F TGCATGTTGCAGCATTGTAG R ATGTCCTTCACACAGCCAC P FAM-GCGACCGTACCAACAACGGCT-MGB	113 bp	250/100 mM	66 °C

with EPNs in uncultivated and agricultural soils (Pathak et al., 2017; Pathak et al., 2012). Most of the NF species were maintained in pure cultures on Corn Meal Agar (Fluka analytical, Sigma-Aldrich, CO, USA) (Pathak et al., 2012), except for *Catenaria* sp., for which a plasmid was used to insert the entire ITS region (Table 1). Finally, we screened the ectoparasitic bacterial species *Paenibacillus nematophilus* (Enright and Griffin, 2005) using a plasmid carrying a fragment from the 16S rDNA region (Table 1).

Species-specific primers and TaqMan® probes (5' fluorogenic reporter dye FAM; 3' end quencher BQH-1) were synthesized by Eurofins Scientific SE (Luxembourg). Following the MIQE procedure (Bustin et al., 2009), Real-time qPCR reactions were performed with a Corbett Research RG-6000 PCR Thermocycler Qaigen Roto-Gene Q (Mortlake, Australia). We used optical 100-well gene discs (Biolabo, Scientific Instruments, Switzerland) and KAPA TaqMan Fast qPCR Kit Universal Master Mix (Labgene, Switzerland). The thermal cycling consisted of 2 min at 60 °C and 5 min at 95 °C, followed by corresponding cycles of 95 °C for 5 s and the adjusted temperature for 50 s per organism. The number of cycles was 38 for EPNs and FLNs and 50 for the NF and the bacteria. All samples were run in duplicate (unknowns, positive/standard curves, and negative controls) using a 10-fold dilution of the DNA. In contrast, undiluted total DNA was used to identify and quantify NF and ectoparasitic bacteria (Campos-Herrera et al., 2015b). A correction factor derived from the dilution series enabled us to convert qPCR data into specific organism counts (units per organism type described in Supplementary Data 2). Additionally, we conducted a preliminary experiment to evaluate the suitability and accuracy of the methods for identifying and quantifying the two putative new EPN species (Supplementary Table S3; Supplementary Fig. S1).

2.4. Two-choice olfactometer assays

To investigate the association between EPNs isolated from wild patches vs cultivated fields, wild and domesticated *V. corymbosum*, and a potential root-feeding herbivore, *A. orientalis*, we conducted a series of two-choice olfactometer assays under laboratory conditions. We produced IJs from field-isolated stock batches collected from domesticated (*Steinernema glaseri*; Hammon, NJ, USA; 39.59254, -74.77338) and wild (Tabernacle, NJ, USA; 39.825374, -74.634985) sites, harvested from reinfected *G. mellonella* larvae, and maintained at room temperature (21–24 °C) for up to two weeks before use. Wild and domesticated *V. corymbosum* (var. Bluecrop) plants were propagated from softwood cuttings (Eck, 1965) in the spring of 2011 and 2012. Wild plants were sourced from three isolated populations within the Pinelands National Reserve, and 10 plants were selected from each site for propagation. All plants were maintained in a breeding greenhouse at the Philip E. Marucci Center for Blueberry and Cranberry Research (NJ, USA), where they received standard horticultural treatments, including fertilization and winter chilling. Plants were grown 30–36 months before use in experiments to ensure the required root development. Plants were repotted into silica sand, with the pH adjusted to 5–6, and allowed to rest for 72 h after repotting before use in the experiments. Third-instar *A. orientalis* were collected from turfgrass areas at the Rutgers Horticultural Farm No. 2 (40°47' N, 74°42' W; North Brunswick, NJ, USA) in fall 2014 and spring 2015. Larvae were maintained at 10 °C for up to 10 weeks prior to

experiments and acclimated at room temperature for 12 h before use. In assays with both the plant and insect, larvae were allowed to feed on plant roots for 24 h before the trials were conducted.

We assessed EPN behavioral preferences using a glass two-choice olfactometer (Fig. 1; Supplementary Fig. S3). The setup consisted of a central horizontal chamber (25 mm diameter, 152 mm length) with a central opening for nematode introduction, which was connected to two removable 2.5 cm pieces attached to the center. These pieces connected to two treatment chambers, where insect larvae and/or plants were placed in sand. A borosilicate glass frit (medium porosity, 6.35 mm thick) was placed on each side of the chamber, preventing nematode movement into the side chamber with the treatment while allowing volatile cues to diffuse. Plants were potted in glass treatment chambers with sand from Whitesbog, NJ, USA (sand/silt/clay 93/7/0, OM 5.1%, pH 3.9; Koppenhöfer and Fuzy, 2007) and allowed to acclimate for at least 48 h before use in experiments. In choice tests, *A. orientalis* larvae infesting plants were introduced 24 h before the start of the experiment. A total of 1000 ± 100 IJs of *S. glaseri* (from cultivated fields) or *Steinernema* sp. N7 (from wild patches) were applied to the central chamber and allowed to move freely at room temperature (21–24 °C) for 40 h. Fresh IJ batches harvested no more than two weeks before were used for each experiment. Each experimental run included six two-choice olfactometers arranged on a laboratory bench. When testing domesticated and wild plants, three olfactometers were assigned to each plant type per run, with randomized placement along the bench to minimize positional bias. Each treatment combination in Figs. 4, 5, and 6 was replicated 12 times.

To determine IJ attraction, the two connector pieces were carefully removed from the central and treatment chambers, and the substrate was emptied into separate beakers for each connector. IJs were then extracted using the sieve-and-decant method and counted under a dissecting microscope. To do this, the sand removed from the choice chambers was submerged in 1000 mL of water and agitated for one minute. Then, the solution was decanted over a (no. 400) mesh sieve, and then the contents were washed into a 100 mL beaker. This process

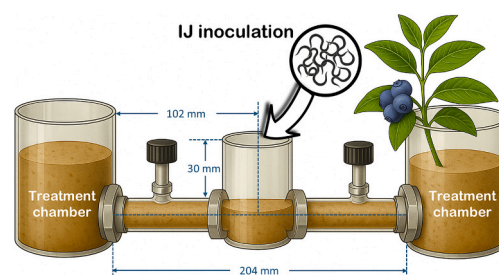


Fig. 1. Schematic representation of the glass two-choice olfactometer used in behavioral assays. The device consists of a central chamber where infective juveniles (IJs) of entomopathogenic nematodes are inoculated, symmetrically connected to two peripheral chambers containing the experimental treatments. Chambers are connected by glass tubes fitted with medium-porosity glass frits, allowing diffusion of chemical cues while preventing nematode movement between compartments. Main dimensions of the olfactometer components are indicated.

was repeated twice for each sample to ensure all nematodes were removed.

2.5. Statistical analyses

We assessed differences in the abundance of EPNs and associated soil organisms between uncultivated wild blueberry patches and commercial blueberry fields, as well as nematode directional movement in two-choice olfactometer assays. All quantifications of soil organisms from DNA samples were standardized per 100 g dry soil. EPN and bacterial abundances were expressed as IJ and plasmid copy numbers, respectively. FLN and NF abundances were normalized to a 0–1 scale by dividing each recorded value (n_i) by the highest observed value for that species (n_{max}), using the equation $100 \times n_i/n_{max}$ (Jongman et al., 1995). This normalization minimized variation caused by intrinsic differences, such as the substantial copy-number disparities between FLN and NF species. Given that many NF species can persist in the soil as saprophytes (Nordbring-Hertz et al., 2006), we assumed most NF species isolated via sucrose centrifugation were in the infectious phase. Thus, NF abundance was expressed as an infection rate (IR), calculated as the ratio of the DNA quantity of each NF species to the total DNA extracted (Campos-Herrera et al., 2012). In two-choice olfactometer assays, nematode preference was quantified as the percentage of IJs that moved toward each choice from the center release point into the 2.5 cm piece attached to the center, which connected to the treatment chamber, calculated relative to the total number of IJs introduced.

We used parametric and nonparametric statistical tests, depending on the data distribution, in SAS 9.3 (Proc Univariate). Firstly, data normality was assessed using the Shapiro-Wilk test. Differences in soil organism abundances between wild and cultivated blueberries were assessed using parametric *t*-tests, excluding target organisms present in fewer than 10% of the sites (Lepš and Hadincová, 1992). Resulting counts of IJs from the two-choice olfactometer tests were recorded along with the total number of IJs extracted from the central chamber where they were applied. The central chamber data and the two-choice chamber data were used to correct for extraction efficiency by converting the choice counts to percentages of the total number of IJs extracted. The percentages in either choice section were then analyzed. First, data were analyzed for normality (SAS 9.3, Proc Univariate). Because not all datasets were normally distributed, both parametric and nonparametric methods were necessary. We used one-tailed paired *T*-Tests and Wilcoxon matched-pairs signed-rank tests where appropriate (GraphPad Prism 6). To compare the amount of total movement toward the treatments, unpaired nonparametric Mann-Whitney tests were used. All statistical analyses were performed at a significance level of $p < 0.05$.

3. Results

3.1. Detection and distribution of soil organisms

Four of the 14 EPN species screened were detected, with three—*S. glaseri*, *Steinernema* sp. N7, and *Steinernema* sp. N10—occurring in at least 5% of the samples. Contrary, *S. affine* was found only once in a single wild and cultivated paired site and was therefore excluded from further statistical analyses. Among the detected species, *S. glaseri* exhibited the highest abundance, predominantly in cultivated sites ($t_{58} = 33.02$, $p = 0.001$; Fig. 2A). In contrast, *Steinernema* sp. N7 was more prevalent in wild sites ($t_{58} = 29.00$, $p = 0.034$) but at lower densities (Fig. 2B). *Steinernema* sp. N10 was detected residually, with no significant differences in occurrence between wild and cultivated sites (Fig. 2C). Co-occurrence patterns revealed that *S. glaseri* and *Steinernema* sp. N7 were found together in three wild and two cultivated sites, with *S. glaseri* being the dominant species. Additionally, *Steinernema* sp. N7 and *Steinernema* sp. N10 co-occurred at a single wild site.

Regarding FLNs, two of four species were detected: *Acrobeloides*-group and *O. tipulae*. Only *Acrobeloides*-group showed a significantly higher abundance in cultivated sites ($t_{58} = 3.31$, $p = 0.002$; Fig. 3A). Similarly, among the six NF species screened, only *P. lilacinum* and *H. rhossiliensis* were detected (Fig. 3C, D). However, their occurrence did not significantly differ between habitat types, likely due to high data dispersion. Finally, the ectoparasitic bacterium *P. nematophilus* was found to co-occur exclusively with the EPN *S. glaseri* in one cultivated site.

3.2. Behavioral responses of entomopathogenic nematodes to plant type and host cues

When comparing IJ attraction to three *A. orientalis* larvae in the absence of plants, about 1.8× more *S. glaseri* (cultivated) IJs were attracted to the larvae than the sand alone, but the effect was not statistically significant ($W = 58$, $p = 0.074$; Fig. 4). Despite this, neither *S. glaseri* (isolated from cultivated sites) nor *Steinernema* sp. N7 (isolated from wild sites) exhibited a significant preference for third instar *A. orientalis* larvae over sand alone ($W = 54$, $p = 0.127$; Fig. 4).

Similarly, when given a choice between sand and either a domesticated ($W = 58$, $p = 0.081$) or wild plant ($W = 41$, $p = 0.455$), *S. glaseri* (cultivated) showed no significant preference for the plant (Fig. 5A, C). However, when comparing IJ movement between plant types, *S. glaseri* (cultivated) was significantly more attracted (about 2.2×) to domesticated than to wild plants ($U = 9.00$, $p = 0.040$; Fig. 5A, C). In contrast, when choosing between sand and a plant, *Steinernema* sp. N7 (wild) showed no attraction to domesticated plants ($W = 48$, $p = 0.248$; Fig. 5B) but significantly preferred wild plants ($t_{11} = 2.94$, $p = 0.009$; Fig. 5D). Unlike *S. glaseri* (cultivated), no significant differences were

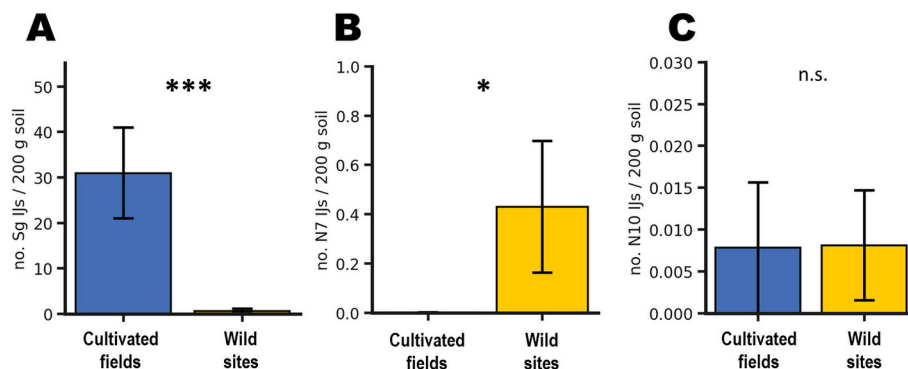


Fig. 2. Abundance ($\pm$ SEM) of infective juveniles (IJs) for the entomopathogenic nematode species (A) *Steinernema glaseri* (Sg), (B) *Steinernema* sp. N7, and (C) *Steinernema* sp. N10 in cultivated blueberry fields and adjacent wild blueberry patches. Statistical analysis was performed using a one-sample *t*-test (*** $p < 0.001$; * $p < 0.05$; n.s., not significant).

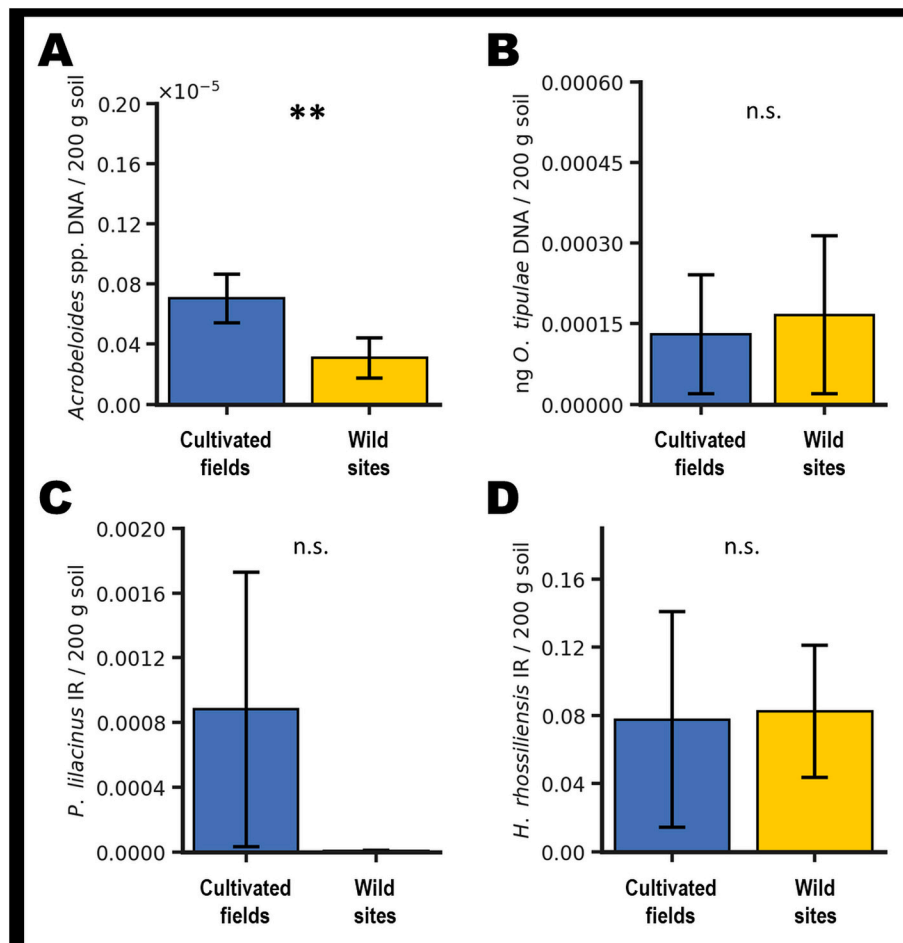


Fig. 3. Abundance ($\pm$ SEM) of the free-living nematodes—expressed as plasmid copy number (no. ng DNA)—(A) *Acroboloides* group and (B) *Oscheius tipulae*, and of the nematophagous fungi—represented as infection rate (NF IR)—(C) *Purpureocillium lilacinum*, and (D) *Hirsutella rhossiliensis* in cultivated blueberry fields and adjacent wild blueberry patches. Statistical analysis was performed using a one-sample t-test (** $p < 0.01$; n.s., not significant).

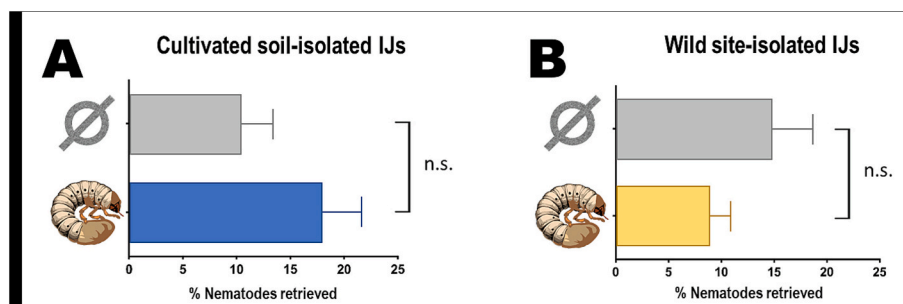


Fig. 4. Mean ($\pm$ SEM) percentage of entomopathogenic nematodes retrieved on the treatment side after 40 h in a two-choice assay comparing attraction to *Anomala orientalis* larvae in sand vs. sand alone. (A) *Steinernema glaseri* isolated from cultivated blueberry fields. (B) *Steinernema* sp. N7 isolated from adjacent wild blueberry patches. “n.s.” indicates not significant by one-sample t-test.

observed when comparing the number of *Steinernema* sp. N7 (wild) attracted to domesticated versus wild plants ($W = 52$, $p = 0.165$; Fig. 5B, D).

When including *A. orientalis* larvae into the system, *S. glaseri* (cultivated) showed a significantly higher attraction to domesticated plants with larvae than domesticated plants alone ($W = 72$, $p = 0.039$), a pattern not observed for wild plants (Fig. 6A, C). No significant preference for *Steinernema* sp. N7 (wild) was observed between plants alone and plants with larvae ($W = 52$, $p = 0.175$, regardless of plant type (Fig. 6B, D).

4. Discussion

This study examined the influence of plant domestication on soil communities and trophic interactions, with a particular focus on EPNs and their responses to *V. corymbosum* in both cultivated and wild environments. We hypothesized that EPN populations endemic to these habitats would exhibit differential attraction to plants, reflecting distinct adaptations to farming and wild soil conditions. Our findings confirmed that plant domestication influences EPN distribution and behavior, suggesting that agricultural practices shape EPN community

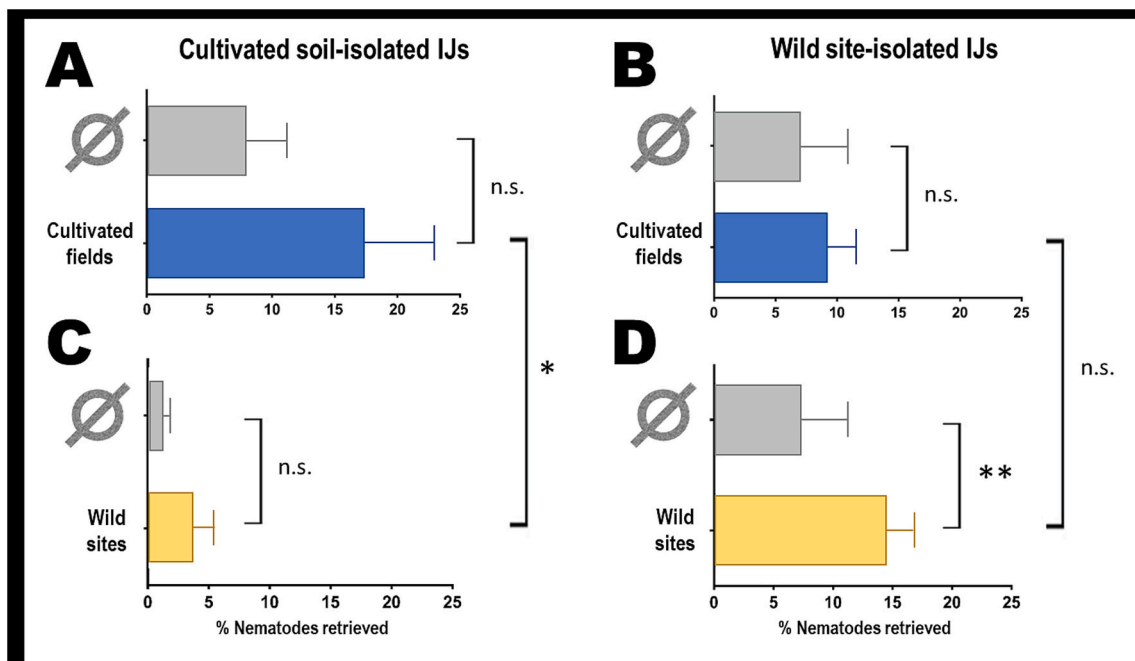


Fig. 5. Mean ($\pm$ SEM) percentage of entomopathogenic nematodes retrieved on the treatment side after 40 h in a two-choice olfactometer assay assessing attraction to domesticated (A, B) and wild (C, D) *Vaccinium corymbosum* plants vs. sand alone. (A, C) *Steinernema glaseri* isolated from cultivated blueberry fields. (B, D) *Steinernema* sp. N7 isolated from adjacent wild blueberry patches. Statistical analysis was performed using a one-sample *t*-test (** $p < 0.01$; * $p < 0.05$; n.s., not significant).

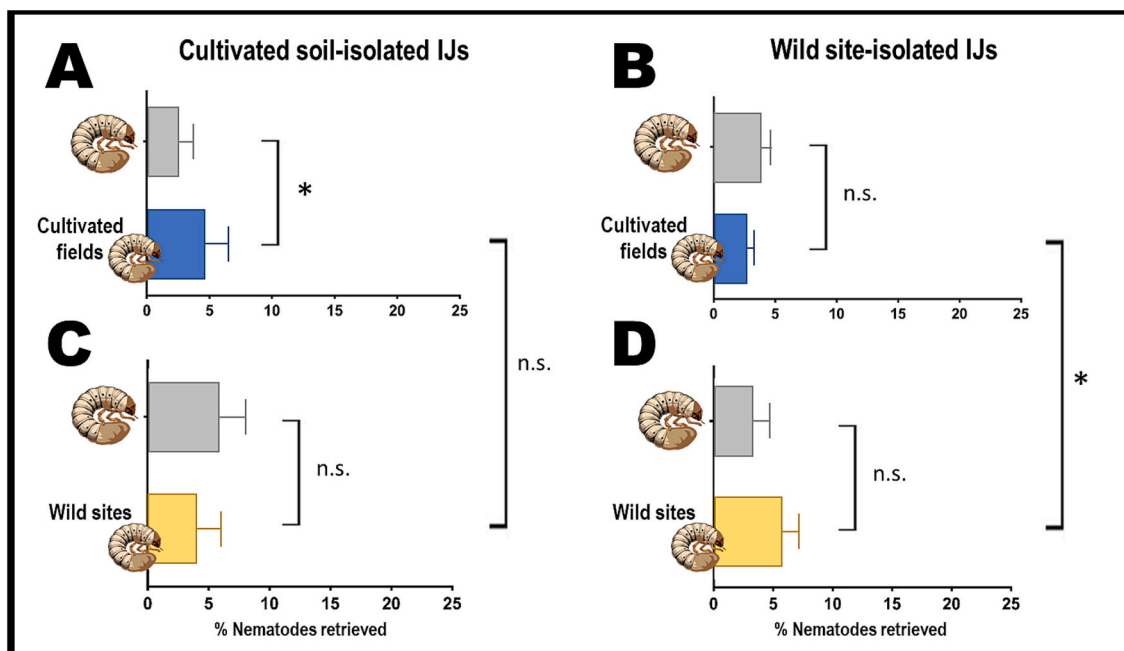


Fig. 6. Mean ($\pm$ SEM) percentage of entomopathogenic nematodes retrieved on the treatment side after 40 h in a two-choice olfactometer assay assessing attraction to domesticated (A, B) and wild (C, D) *Vaccinium corymbosum* plants with *Anomala orientalis* larvae vs. plants alone. (A, C) *Steinernema glaseri* isolated from cultivated blueberry fields. (B, D) *Steinernema* sp. N7 isolated from adjacent wild blueberry patches. Statistical analysis was performed using a one-sample *t*-test (* $p < 0.05$; n.s., not significant).

composition. Moreover, EPN populations from cultivated soils were more attracted to domesticated plants, mainly when *A. orientalis* larvae co-occurred, while those from wild areas preferentially responded to wild plants. These findings support the hypothesis that plant domestication influences plant-mediated interactions in the rhizosphere, potentially shaping and thereby enhancing the efficacy of biological control strategies in agricultural systems.

Our study also considered whether traditional insect baiting could underestimate EPN diversity, particularly in cultivated environments dominated by *S. glaseri*. Since its introduction by Bedding and Akhurst (1975), this widely used method has been essential for isolating and quantifying EPNs from soil, remaining the preferred approach for identifying new or unexpected species or populations (Koppenhöfer et al., 1998). Consistent with previous studies, our comparison of

traditional and molecular approaches confirmed that *G. mellonella* baiting is a reliable method for estimating EPN population abundance (Blanco-Pérez et al., 2022; Campos-Herrera et al., 2019; Jaffuel et al., 2018). However, molecular techniques such as qPCR and sequencing provide a valuable complement that may be overlooked due to host specificity or interspecific competition during baiting (Blanco-Pérez et al., 2025c). The detection of *Steinernema* sp. N10 in cultivated soils exemplified this limitation, as it had been missed by Rivera et al. (2016), likely due to its similarity to *S. glaseri* in infecting *G. mellonella*. Such underestimations can significantly affect diversity analyses, particularly metrics such as species richness, evenness, and relative abundance, which weight rare species differently (Hill, 1973).

The significantly higher abundance of EPNs in cultivated *V. corymbosum* fields compared to uncultivated soils aligns with Rivera et al. (2016), who attributed this pattern to increased host availability associated with greater *A. orientalis* pressure in cultivated habitats. Blueberry monocultures, propagated vegetatively and maintained with irrigation and fertilization, promote plant uniformity and vigor, likely supporting higher herbivore populations and, in turn, providing a more consistent resource base for EPNs. Because the historical prevalence of *A. orientalis* guided our site selection, its herbivory likely plays a key role in shaping EPN distribution. The near-exclusive prevalence of *S. glaseri* in cultivated fields, previously reported by Rivera et al. (2016) through baiting, further supports this interpretation, underscoring its competitive dominance, persistence, and high mobility relative to typically widespread steinernematid species (e.g., *S. feltiae*) or heterorhabditids (Grewal et al., 1994; Koppenhöfer et al., 1996; Lewis et al., 1995). Although *S. glaseri* has been relatively infrequent in an untargeted but extensive survey across New Jersey (Gaugler et al., 1992), our findings suggest that this EPN species thrives in cultivated environments, likely due to its adaptability and efficacy against *A. orientalis* (Rivera et al., 2016) and potentially other hosts, displacing other wild EPN populations.

Incorporating molecular tools in this study enabled a more comprehensive assessment of soil organisms within the EPN trophic network. Among the analyzed soil organisms, significant differences were observed only for *Acrobelloides* spp., bacterial-feeding FLNs from the family Cephalobidae. Classified as cp-2 on the colonizer-persister (cp) scale (Bongers and Bongers, 1998; Sieriebriennikov et al., 2014), *Acrobelloides* spp. are opportunistic nematodes capable of rapidly exploiting environments with high microbial activity, such as those in intensively managed agricultural soils (Pervez, 2011). Their increased abundance in cultivated soils may be linked to higher organic matter inputs and increased nitrogen availability, which favor low-cp bacterivore nematodes (Wei et al., 2021; Zhang et al., 2019). This effect is expected to be stronger in high-nutrient cycling systems, such as annual crops. For instance, Salas and Achinelly (2020) found that *Acrobelloides* species were absent from natural soils but highly abundant in tomato plantations. However, Blanco-Pérez et al. (2025a) identified Cephalobidae, particularly *Acrobelloides*, as the most abundant nematode taxa among 114 detected in vineyard soils through metabarcoding. An additional explanation is their potential ecological association with *S. glaseri*. Previous studies have shown that *Acrobelloides* species can exploit EPN-infected cadavers as a reproductive resource (Campos-Herrera et al., 2012; Duncan et al., 2003). Given that *S. glaseri* is the dominant EPN in these sites, a greater availability of infected hosts may have indirectly favored *Acrobelloides* populations.

The contrasting behavioral responses of EPNs from cultivated and wild habitats are consistent with habitat-specific foraging strategies shaped by plant-mediated cues. Rather than responding strongly to *A. orientalis* larvae alone, EPNs appeared to rely primarily on chemical signals emitted by plant roots (Rasmann et al., 2012), signals that may differ between domesticated and wild *V. corymbosum*. This supposition is in line with previous work showing that long-distance host location in soil often depends more on plant-derived volatiles than on generic host cues such as CO₂, particularly for cruiser EPN species like *S. glaseri*

(Ishibashi and Kondo, 1990; Lewis et al., 1993; Grewal et al., 1994). Root systems may also transmit substrate-borne vibrations produced during herbivory, further integrating mechanical and chemical channels of belowground signaling (Griffin, 2015; Lewis et al., 2024). These mechanisms help explain why plant context strengthened EPN responsiveness, while larvae did not stimulate strong movement. The disposition of EPNs to respond most strongly to the plant types characteristic of their native environments is consistent with selection favoring sensitivity to familiar volatile blends (Hiltpold et al., 2010; Willett et al., 2017) and with evidence that prior exposure can “train” nematodes to respond to specific chemical cues (Gaffke et al., 2023).

These patterns are consistent with a co-evolved framework in which plant genotypes emit cues that enhance the host-finding efficiency of their associated natural enemies. The increased sensitivity of cultivated-soil EPNs to domesticated plants under herbivory aligns well with the ‘cry-for-help’ model described in other plant-EPN systems (Ali et al., 2011; Rasmann et al., 2005). Differences between wild and cultivated habitats may help explain this specificity: in diverse natural systems, EPNs may benefit from responding to broad, non-specific root cues to maximize host encounter rates (van Tol et al., 2001), whereas agroecosystems dominated by a single plant genotype and a persistent herbivore can select for nematode lineages particularly attuned to a narrower range of volatiles (Turlings and Erb, 2018). The prominence of *S. glaseri* in cultivated soils is consistent with this idea, suggesting that locally adapted populations may exploit the chemical homogeneity of managed environments to maintain high population densities.

While our study offers new insights into the interactions between plant domestication, root herbivory, and EPN behavior, it also highlights some limitations that future research should address. For instance, although *A. orientalis*, like many root-feeding scarab larvae, is a relevant pest in cultivated blueberry systems, its advanced defenses (including sieve-like spiracles, antimicrobial peptides, and grooming behavior) can reduce susceptibility to EPN infection (Haji Allahverdi-pour and Karimi, 2021). In our assays, we used third instars to maximize the likelihood of eliciting a strong plant response. However, this choice may have reduced the detectability of behavioral responses in local EPN isolates. Furthermore, in naturally occurring populations and plant-insect interactions, there is likely variability between EPN species and scarab larvae, with second- and third-instar *A. orientalis* not differing in overall susceptibility to *S. glaseri* (Koppenhöfer and Fuzy, 2007; Koppenhöfer and Sousa, 2024). As demonstrated for *Diabrotica virgifera* (LeConte, 1868) (Coleoptera: Chrysomelidae) (Kurtz et al., 2009), earlier instars can be more vulnerable to infection, but this is unknown for our case study. Furthermore, it remains possible that *A. orientalis* secretes salivary compounds that suppress plant defense responses, as documented in other insect species (Hogenhout and Bos, 2011; Musser et al., 2002), thereby potentially disrupting the emission of volatiles involved in EPN recruitment.

Although plant domestication appears to be a major driver of the patterns observed in our study, differences in soil origin between cultivated and wild habitats likely modulate EPN performance by altering microbial communities, nutrient availability, and disturbance regimes (Philippot et al., 2013; Pérez-Jaramillo et al., 2018; Blanco-Pérez et al., 2024). Such edaphic factors may help explain the contrasting distribution and behavioral responses of EPNs and warrant explicit consideration under field conditions. A key priority for future work is to clarify how herbivore-induced root volatiles operate in natural settings and how soil community complexity affects their effectiveness in EPNs. Additional open questions include the extent to which temporal variation in root signals, driven by plant developmental stages or environmental heterogeneity (Agelopoulos et al., 1999), shapes belowground chemical signaling, as suggested by studies documenting ontogenetic-linked shifts in volatile emission and EPN activity (Erb and Lu, 2013; Furey and Tilman, 2021; Blanco-Pérez et al., 2025b). Unraveling how these biotic and abiotic factors jointly regulate plant-EPN interactions will be essential for translating ecological mechanisms into more

effective, context-adapted EPN-based biocontrol strategies.

5. Conclusions

This study showed that plant domestication can significantly affect the composition and functional behavior of EPN communities inhabiting crop soils. We found a clear habitat partitioning, with *S. glaseri* dominating cultivated blueberry fields, while wild habitats supported distinct endemic lineages (*Steinernema* sp. N7 and N10). These patterns indicate that domestication-driven changes in plant traits and soil conditions contribute to shaping EPN community structure. Our behavioral assays further illustrated that EPN populations exhibit habitat-specific host-location responses: isolates from cultivated soils were more strongly attracted to domesticated plants, particularly when *A. orientalis* damaged roots, whereas isolates from wild habitats preferred to wild blueberry plants. These findings suggest that the environmental history of EPN populations modulates their sensitivity to root-emitted cues and may influence their potential efficacy as biological control agents.

By integrating molecular diagnostics with behavioral experiments, this work provided a more complete understanding of how belowground trophic interactions differ between wild and domesticated systems. Overall, our results supported the view that domestication not only affects plant performance but also reshapes the evolutionary framework governing plant–EPN–herbivore interactions, reconfiguring multitrophic dynamics in the rhizosphere, and contributing to the development of more targeted, habitat-adapted EPN-based biocontrol strategies. Future research should aim to identify the chemical nature of the plant signals involved and to determine how soil biota and soil physico-chemical conditions jointly influence EPN recruitment under natural conditions. Addressing these complex, multitrophic interactions will improve our understanding of belowground signaling processes and help optimize the use of native EPN populations in sustainable agricultural management.

CRedit authorship contribution statement

Monique J. Rivera: Writing – review & editing, Writing – original draft, Visualization, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Albrecht M. Koppenhöfer:** Writing – review & editing, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Cesar Rodriguez-Saona:** Writing – review & editing, Resources, Project administration, Funding acquisition, Conceptualization. **Rubén Blanco-Pérez:** Writing – review & editing, Writing – original draft, Visualization, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, Microsoft Copilot, provided through the CSIC institutional platform, was used to assist with language refinement, structural organization, and clarity of expression. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Declaration of competing interest

The authors confirm that no conflicts of interest are associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome.

Acknowledgments

The authors thank the New Jersey blueberry growers for granting us

access to their farms. We also acknowledge the financial support received from the USDA Sustainable Agriculture Research and Extension (SARE) Program (Award No. GNE11-027 to MJR), and the MCIN/AEI/10.13039/501100011033 and the European Union NextGenerationEU/PRTR, which supported RBP *Juan de la Cierva* Postdoctoral Fellowship (JDC2022-048978-I). The Government of La Rioja (Spain) provided additional support for RBP through the CAR Postdoctoral Grant (Resolution 198/2025, call 2025–2028).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.apsoil.2026.106959>.

Data availability

Data will be made available on request.

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