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Nematodes as suppressors and facilitators of plant performance

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Summary
Plant–nematode interactions are mainly considered from the negative aspect with a focus on plant-parasitic nematodes (PPNs), which is justified considering the agronomic losses caused by PPNs. Despite the fact that PPNs are outnumbered by nonparasitic free-living nematodes (FLNs), the functional importance of FLNs, especially with regard to plant performance, remains largely unknown. Here, we provide a comprehensive overview and most recent insights into soil nematodes by showing direct and indirect links of both PPNs and FLNs with plant performance. We especially emphasize the knowledge gaps and potential of FLNs as important indirect players in driving plant performance such as stimulating the resistance to pests via improving the disease suppressive activity of the rhizobiome. Together, we present a holistic view of soil nematodes as positive and negative contributors to plant performance, accentuating the positive but underexplored role of FLNs.

Introduction: importance of soil nematodes for plant performance
Plants are the basis of almost all life on Earth, especially that of animals including humans. We have modified most land on Earth for our need to grow human-essential plants in agriculture that led to fundamental increases in food production. However, constant food production is not guaranteed, partly because plant performance can be reduced by pests, here referring to all organisms, including pathogens that impair plant nutrition, growth, and defense (Kranz, 2005; Döring et al., 2011). These pests lead to c. 40% global crop yield losses annually (IPPC Secretariat, 2021). The pests are often controlled by pesticides, but at a cost: negative side-effects, such as losses of nontarget biodiversity and detrimental impacts on the environment might in the long-run outweigh the short-term benefits of plant stimulation (Oka, 2020). To promote a more sustainable plant protection, the European Commission aims to halve the current use of pesticides by 2030 (European Commission, 2022), putting yield at risk.

Among these pesticides are nematicides that act against plant-parasitic nematodes (PPNs). Plant-parasitic nematodes are notorious plant pests that cause annual yield losses of over 80 billion US$ worldwide (Abd-Elgawad & Askary, 2015). There are over 4100 currently described PPN species that parasitize virtually all plant species, including all crops (Decraemer & Hunt, 2006). Therefore, nematodes are mostly viewed as negative contributors to plant performance. However, nematodes can also positively contribute to plant performance. For example, non-parasitic free-living nematodes (FLNs) (here defined as nonparasitic soil nematodes excluding entomopathogenic nematodes as obligate insect parasites) are the most abundant soil animals that outnumber PPNs in terms of abundance and diversity (van den Hoogen et al., 2019). Free-living nematodes display different functions in the soil food web and can act, for example, as consumers of bacteria, fungi, protists, and other nematodes. Their rapid response to environmental disturbances makes them good bioindicators of soil health (Ferris et al., 2001). The abundance and trophic nature of FLNs are often used for measuring different nematode-based indicators that can reflect the ecosystem status of soil (Du Preez et al., 2022). Furthermore, FLNs can more or less selectively feed on members of the soil microbiome (Rønn et al., 2012; Liu et al., 2017), acquire microorganisms on their surface (Dirksen et al., 2016), transfer microbiomes to remote habitats (Finkel et al., 2017), and serve as a food to higher trophic groups (Stirling, 2014). These interactions may shape the rhizobiome, here defined as the biodiversity attached to and influenced by roots, and ensure nutrient transfer between microbiomes and plants to eventually shape plant performance (Trap et al., 2016). However, the functional links between FLNs and plant performance remain surprisingly little studied.
In this review, we collected reports and presented a multitude of pathways in which PPNs and FLNs inhibit and most importantly promote plant performance. More specifically, we show that direct links that are governed by nematode feeding on roots are prevalent between PPNs and plants and, thereby, negatively affect plant performance. On the contrary, indirect links, often being plant-beneficial, are more pronounced between FLNs and plants via interlinkages of FLNs with the rhizobiome through predation and dispersal. We especially highlight the knowledge gaps in understanding the role of FLNs to assist plant protection against pests and explore how their multitrophic position in the food web can contribute to this aspect.

**Negative impact of nematodes on plant performance**

**Direct negative impact**

Deeply studied, well-known and widely recognized and feared among farmers and gardeners are PPNs that directly damage plants via feeding mainly on roots. Among these PPNs, endoparasitic PPNs (especially migratory and sedentary groups that we feature below) cause the most damage as they are in the most intimate contact with the plant (Jones et al., 2013). The damage threshold for notorious root-knot nematodes was estimated to be only between 1 and 8 eggs per cm³ of root material (Greco & Di Vito, 2009). In addition to forming progenies that can amount to several hundreds of eggs per only one female, some PPNs have the highest spreading rate of all plant pests due to their wide host range of many thousands of plant species (Bebber et al., 2014). Endoparasitic PPNs use short stylets to penetrate the root and feed directly from the parenchymal cells in the vascular cylinder (Jones et al., 2013) (Fig. 1). Sedentary endoparasitic PPNs are represented by two subgroups: root-knot nematodes and cyst nematodes. Root-knot nematodes infect various plant species, including vegetables, grasses, shrubs, and trees. Some root-knot nematode species have a wider host range than others; for instance, *Meloidogyne incognita* is ubiquitous and infects >3000 plant species including major crops, while *Meloidogyne incognita* parasitizes mostly woody perennial plants. Cyst nematodes generally have a narrower host range than root-knot nematodes, but can be devastating to their host. *Heterodera schachtii* infections alone can cause 95% of the annual yield losses in sugar beet (Müller, 1999).

Sedentary endoparasitic PPNs have different life stages. Only the second-stage juveniles (J2s) infect plant roots. Once inside the root, the J2s take symplastic (cyst nematodes) or apoplastic (root-knot nematodes) routes to reach the permanent sites for feeding. For example, root-knot nematodes, being unable to break through the root endodermis, enter the root at the elongation zone of the root tips and use meristem tissue to pass to the vascular cylinder (Grundler et al., 1992), while cyst nematodes directly pass through the endodermis barrier (Holbein et al., 2016). At their destination, PPNs manipulate defense mechanisms in the host plant, including Fig. 1 Negative impact of nematodes on plant performance. Direct negative impact (parasitism): Nematode groups that feed on plants are sedentary endoparasitic nematodes, migratory endoparasitic nematodes, and ectoparasitic nematodes. Root-knot nematodes (RKN) form galls on the roots with hundreds of eggs in egg masses. Migratory endoparasitic nematodes reside outside and inside the roots, where they make necrotic lesions during moving and feeding. Ectoparasitic nematodes reside outside the roots and only insert the stylet within the root, while some of them are vectors of plant viruses. Indirect negative impact: All plant-parasitic nematodes (PPNs) create wounds for the entrance of secondary pathogens. PPNs and free-living nematodes (FLNs) can acquire plant-deleterious microorganisms endogenously or on their surface, transport them to the roots, and stimulate the formation of a pathobiome.
the repression of cell wall barrier defense and changes in diverse hormonal pathways (Holbein et al., 2016). Nematode feeding directly damages plant tissue, leading to necrosis and anatomical alterations of the roots, such as formation of root galls by root-knot nematodes. Deformations and impaired root growth can result in reduced water uptake, photosynthesis and plant growth.

Migratory endoparasitic nematodes, including root lesion nematodes (Pratylenchus spp.) and burrowing nematodes (Radopholus spp.), are also among devastating PPNs (Jones et al., 2013). All stages of migratory endoparasitic nematodes are mobile and vermiform. A yellow disease of black pepper caused by *Radopholus similis* in 1950s was responsible for the loss of 20 million black pepper trees in Indonesia (Hubert, 1957). The root lesion nematode *Pratylenchus thornei* was reported to cause between 25% and 28% of grain yield losses in wheat and barley when present at densities between 150 and 250 nematodes g^{-1} soil (Fanning et al., 2020).

In contrast to endoparasitic PPNs, ectoparasitic PPNs use their long stylets to feed on different root parts without invading the roots. Economically, they are less important than the endoparasitic nematodes, especially if they primarily feed on root hairs. However, they can also cause injuries to the outer layers of cortical tissue and progressive deterioration of the root system as in case of *Helicotylenchus* spp. (Riascos-Ortiz et al., 2020).

**Indirect negative impact**

Indirectly, PPNs can negatively affect plants via multifaceted interactions with microorganisms (Fig. 1). Plant-parasitic nematodes can form disease complexes with some plant-pathogenic members of the rhizobiome, including bacteria, fungi, and viruses (Back et al., 2002; Lamelas et al., 2020). Notable examples are diseases caused by synergistic interactions between *Meloidogyne* spp. and *Fusarium* wilt pathogens, or by *Pratylenchus* and *Globodera-Verticillium dahliae* complexes (Back et al., 2002). Besides using the wounds created by PPNs for the entrance into the roots, some secondary pathogens can also use PPNs for phoresy (being transported on the nematode body without harming the nematode). For instance, some ectoparasitic PPNs, such as *taxa in the families Trichodoridae* and *Longidoridae*, transmit plant-pathogenic viruses (nepoviruses and tobraviruses) via their stylet when feeding on roots (Decraemer & Robbins, 2007). Arguably, the most notorious pest on vineyards is the dagger nematode *Xiphiphena index* that transmits Grapevine fanleaf virus (Jones et al., 2013). In addition, some plant-pathogenic rhizobacteria seem to hitchhike on nematodes (Karimi et al., 2000; Adam et al., 2014). It was also shown that root-lesion nematodes can impair the symbiosis between the soybean roots and nitrogen fixing bacteria by impairing the formation of bacterial nodules (Elhady et al., 2020).

Recent insights into plant–nematode interactions reveal that root infections by endoparasitic PPNs shift microbial functions such as enhancing degradation of root compounds that increase PPN performance and plant damage (Tian et al., 2015; Yergalıyev et al., 2020). Collectively, PPNs and microorganisms that support PPN development can be referred to as a pathobiome (Bass et al., 2019). The precise interactions within this potential pathobiome and the mechanisms of how nematodes may facilitate the spread of other pathogens remains unknown. It has been suggested that the specific rhizobiomes that are transmitted via the cuticle of endoparasitic PPNs into the roots can protect PPNs against the PPN-antagonistic root endophytes (Brown, 2018; Topalović & Vestergard, 2021). Better understanding the microbial role in the life cycle of PPNs on plants would be pivotal for disrupting the optimal host conditions for PPN development.

In contrast to PPNs, there is little information on how FLNs may negatively impact plant performance. Several studies suggested that FLNs can also serve as vectors of plant deleterious microorganisms. *Caenorhabditis elegans* and *Pristionchus* sp. were shown to transmit soft-rot bacteria endogenously and on their surface to the plant roots (Nykyri et al., 2013). Diplogaster sp. was reported as a vector of several plant pathogens including *Agrobacterium tumefaciens, Erwinia amylovora, Erwinia carotovora, Pseudomonas phaseolicola*, and a phage of *A. tumefaciens* (Chantano, 1969). It was shown that nematodes feed on these bacteria, which then survive the passage through the nematode digestive tract and get excreted outside. More recently, Kanfra et al. (2018) reported that an increased abundance of some FLNs, such as *Acrobacter* and *Acroboloides*, and their associated microbes in apple replanted disease field soils, was associated with more serious disease symptoms (Kanfra et al., 2018). However, the exact mechanisms behind this interaction remain to be determined.

**Positive impact of nematodes on plant performance**

**Direct positive impact**

Unlike arbuscular mycorrhizal fungi (Bennett & Groten, 2022) and some nitrogen (N) fixing bacteria (Schroeder et al., 2020) that directly enhance nutrient levels in plants and increase stress tolerance, such direct positive relationship between plants and nematodes likely does not exist.

**Indirect positive impact**

Indirectly, nematodes can stimulate plant performance by promoting the activity of plant growth-promoting rhizobacteria (PGPR) including biocontrol agents via root puncturing (PPNs), microbe-induced carbon sequestration (FLNs), and regulation of pests and pathogens (FLNs) (Fig. 2). In some cases, the roots punctured by sedentary endoparasitic nematodes are less attractive to other herbivores due to nematode-induced systemic defense responses in plants (Wondafrash et al., 2013; Arce et al., 2017). However, this happens only in primed plants or plants resistant to sedentary endoparasitic nematodes and cannot be regarded as a positive contribution of PPNs to plant defense. There is evidence that microorganisms that attach to the surface of endoparasitic PPNs can induce an array of early plant defense responses against the invading nematodes, including expressions of jasmonic acid/ethylene- and salicylic acid-signaling genes and an oxidative burst (Topalović et al., 2020). On the contrary, it was shown that when the population of a clover cyst nematode *Heterodera trifolii* below the damage threshold of the clover, it may stimulate the release of...
root exudates and promote microbial growth in the rhizosphere. This can increase the biomass of the host and neighboring plants (Denton et al., 1998; Bardgett et al., 1999; Yeates, 1999). We lack insights into whether this positive PPN-plant link in natural systems might be applicable in agricultural settings.

Arguably, the largest proportion of positive contributions of nematodes to the plant come from FLNs. These contributions are driven by plant inputs in soil (bottom-up regulation) and multitrophic functions of FLNs, especially as predators of bacteria and fungi (Thakur & Geisen, 2019). Some predatory FLNs directly feed on PPNs, but as predatory FLNs hardly grow in high numbers and feed on other FLNs that may be plant beneficial, they have never reached a wider application (Khan & Kim, 2007). Microbivorous FLNs and protists feed on the microbiome through selective and nonselective consumption (Liu et al., 2017). Similar to protists, FLNs have a C : N ratio similar to that of their microbial prey that will be lowered in the predator through respiration of carbon (Ferris et al., 1997). The extra N and N rich compounds such as NH}_4–N are released and available for plant uptake (Ingham et al., 1985). Zheng et al. (2022) also revealed that nematode predation induces competitive interactions and a higher diversity of alkaline phosphomonoesterase (ALP)-producing bacteria and increased ALP activity in the rhizosphere of rapeseed plants that enhanced P availability. Another study has shown that selective predation of fungivorous FLNs can increase the plant colonization by arbuscular mycorrhizal fungi leading to enhances in plant P and productivity (Jiang et al., 2020).

Besides predation, bacterial cells and fungal spores in soil can attach to the surface of FLNs and be transported from nutrient-poor niches (away from rhizosphere) to nutrient-rich (rhizosphere) niches through phoresy, where some microorganisms can be beneficial to plant performance (Horiuchi et al., 2005; García-Sánchez et al., 2021). However, it is difficult to discern the ingested and surface-attached FLN-associated microbiomes in their natural environment (Dirksen et al., 2016), and most studies on microbial attachment to the nematode surface are performed with PPNs that do not ingest microorganisms (Elhady et al., 2017; Topalović et al., 2019; Mohan et al., 2020). Phoresy itself may not involve high specificity as compared to the attachment of parasites (Elhady et al., 2017) or nematode-protective microbiomes (Topalović & Vestergård, 2021), especially if microorganisms quickly detach from the nematode. Nonetheless, phoresy could play important roles in transport and activation of plant growth- and plant defense-promoting microorganisms. There is evidence, which shows that the plant itself mediates the phoretic events between FLNs and plant-beneficial bacteria. Horiuchi et al. (2005) have shown that the legume Medicago truncatula releases specific volatiles, such as dimethyl sulfide, which attract bacterivorous Caenorhabditis elegans to roots. Thereby, C. elegans bring nitrogen-fixing Sinorhizobium meliloti endogenously and on their cuticle closer to the roots. Another study reported that root colonization by the plant-growth-promoting rhizobacterium Pseudomonas fluorescence increased in the presence of soil nematodes (Knox et al., 2004).

More targeted approaches such as labeling and microscopically...
tracing microbial hitchhikers both in the rhizosphere and in planta would provide a more precise mechanistic understanding on the role FLNs play in an active microbial transport and its role in plant stimulation.

Although in our review, we exclude entomopathogenic nematodes from the definition of FLNs, it is important to mention their indirect positive contribution to plant health. The entomopathogenic rhabditid nematodes, *Steinernema* spp. and *Heterorhabditis* spp. form symbiotic complexes with bacterial species within the genus *Xenorhabdus* and *Photorhabdus* that jointly kill the insect host after entering its gut (Brivio & Mastore, 2018). The entomopathogenic nematode-microbial symbiotic complexes are used commercially against various insect pests (Koppenhöfer et al., 2020).

**How can FLNs assist plant resistance to pests?**

Although FLNs are among the most abundant soil organisms spanning across all trophic levels, their potential to suppress plant pests is underexplored beyond the well-known commercial use of entomopathogenic nematodes. For improved plant performance, the FLNs in the rhizosphere should promote activities of plant-beneficial microorganisms and reduce activities of plant-deleterious microorganisms (‘disease-suppressive FLNs’). One way to establish ‘disease suppressive FLNs’ in soil is by employing agricultural practices that facilitate their establishment. There are several empirical examples that display the correlation between the FLN community response to certain sustainable agricultural practices and plant disease suppression. For instance, Masson et al. (2020) have reported that the presence of *Meloidogyne graminicola* and some other PPNs in rice roots decreased 7 yr after converting conventionally managed rice field to conservation agriculture consisting of no-tillage, application of mulch and inclusion of cover crops. The reduction of PPNs in rice roots under conservation agriculture was correlated with increased microbial richness and diversity and increased relative abundance of different groups of nematodes, especially omnivores. In addition, Schmidt et al. (2020) have shown that a reduced incidence of pea root rot disease under long-term organic minimum tillage with cover cropping and mulch application was positively correlated with different nematode-based indicators including increased nematode abundances, higher metabolic enrichment, and higher bacterivore carbon footprints. However, although there are positive links between different nematode-based indicators and FLN groups with the suppression of plant pests in previous studies, we need more studies that combine laboratory assays with field experiments to unravel whether this relationship is causative and how it can be utilized for improved plant performance (see the Section Outstanding questions and solutions).

Another promising mechanism that can be exploited to make FLNs applicable is their potential to disperse immobile biocontrol agents (King & Bell, 2022). For that, FLNs could be added together with PGPR and thereby help their establishment and efficiency. Having this in mind, we propose how FLNs may assist direct and indirect PPN suppression via interacting with different microorganisms (Fig. 3). For microorganisms that require attachment to the PPN surface for PPN suppression, FLNs may transport these microorganisms closer to PPNs and increase the probability of microbial attachment to the PPN surface and PPN suppression (Fig. 3a,c). In addition, selective predation of FLNs on certain rhizobiome taxa can increase the abundance and activity of PPN antagonists by inhibiting their competitors (Fig. 3b,c). Finally, FLNs can transport some plant-beneficial rhizobiome taxa with plant-defense promoting functions closer to the roots and prevent root invasion by PPNs (Fig. 3d). Thus, through feeding and

**Fig. 3** Indirect contribution of free-living nematodes (FLNs) to plant defense against plant-parasitic nematodes (PPNs). FLNs transfer antagonists endogenically and via their cuticle close to PPNs (a). FLNs consume the competitors of PPN antagonists (b). FLNs increase the attachment of antagonists to the surface of PPNs (c). FLNs mediate the plant response to PPNs in the rhizosphere via modulating the rhizobiome (d).
Conclusions

Soil nematodes may positively and negatively affect plant performance. Plant-parasitic nematodes mostly negatively affect plant performance by direct feeding as endoparasites and ectoparasites and as facilitators of secondary pathogens in disease complexes and disease vectors. In turn, FLNs commonly indirectly stimulate plant performance via interactions with other soil biodiversity, especially bacteria and fungi. Via microbial feeding and phoresy, FLNs enhance microbial activity that might lead to increased carbon sequestration, nutrient cycling and plant growth. Furthermore, entomopathogenic FLNs are commercially available antagonists of insect pests.

However, the role of FLNs as plant beneficial organisms such as their role in suppressing pests remains poorly known despite evident links reviewed here, and therefore not exploited at all in applied products. This calls for an urgent need to expand ecological work on soil nematodes, particularly on their potential as plant promoting agents. A visionary way forward in using FLNs in enhancing plant performance in agricultural systems is to promote FLNs through adapted agricultural practices that are plant beneficial, either by catalyzing soil nutrient cycling or by manipulating the soil microbiome in favor of plant beneficial rather than pathogenic microorganisms. Another way can be in the application of FLNs as biological agents that shape the microbiome in a plant-enhancing manner. In any case, we are convinced that FLNs are key elements driving plant performance that should be more closely considered in both fundamental and applied research.

Outstanding questions and solutions

(1) How can we implement a more positive view on nematodes as plant promoting agents that extends the dominating negative view driven by PPNs?

Designing and conducting functional studies that focus on FLNs beyond simple compositional analyses.

(2) Which levels of PPNs can be beneficial for host and non-host plant species and under which conditions?

PPN-centered studies should involve diverse host and non-host plant species and varying levels of PPNs to test plant performance.

(3) How do we bridge scientific insights into the positive role of nematodes for plant performance to application?

Replicated field experiments to study nematodes in response to other management practices and identifying key nematode taxa linked to plant performance.

(4) What are the links between FLNs and plant diseases?

Screening the effects of FLN species with disease-causing microorganisms on different plant species in the greenhouse experiments.

(5) Which methods are best to examine the mechanisms underlying plant performance increases by FLNs?

Integrative methods including laboratory, greenhouse and field experiments followed by microscopy, transcriptomics, metagenomics and metabolomics to study the specificity and mechanisms of plant-FLN-microbe associations.

(6) How to find the most effective nematode-microbial species combinations that enhance plant performance?

Targeted cultivation and screening of the potential plant species-and genotype-specific FLN-microbial combinations that promote plant performance.
(7) How to bring FLNs as biological agents to the market? Standardization of protocols for a large-scale multiplication and application of FLN products in the field as exemplified by entomopathogenic nematodes.

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Author contributions
OT and SG contributed with the ideas, concepts, and writing. SG supervised and revised the manuscript.

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