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Plant ectoparasitic nematodes prefer roots without their microbial enemies

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Abstract Root-feeding nematodes are major soil-borne pests in agriculture. In natural ecosystems, their abundance can be strongly controlled by natural enemies. In coastal foredune soil, the abundance of the ectoparasitic nematode *Tylenchorhynchus ventralis* is controlled by local interactions with soil micro-organisms. If not controlled, *T. ventralis* reduces growth and performance of the host plant *Ammophila arenaria*. In the present study, we examine if the nematodes may sense the presence of soil micro-organisms and, if so, they are able to actively avoid their enemies. First, using Petri dishes with agar medium we examined if *T. ventralis* can choose between *A. arenaria* seedlings inoculated with or without soil microorganisms. We observed that there was a trend (although non-significant) in nematode migration towards the non-inoculated plants. If the seedlings were not present, the nematodes did not make any choice and stayed in the centre of the Petri dish. Then, using Y-tubes filled with sterilized dune soil, we examined if *T. ventralis* could choose between *A. arenaria* roots with or without micro-organisms. We also included treatments of microbial suspensions without plants and a microbe-free filtrate. We observed that the nematodes preferred roots without microorganisms. Microorganisms alone or roots with microbial filtrate did not influence nematode choice significantly. We conclude that the nematode *T. ventralis* is able to choose roots without soil microorganisms when having roots with them as alternative. Such avoidance could explain why biological control of nematodes in field is not always effective, especially when microbial antagonists accumulate in specific parts of the rhizosphere.

Keywords Biological control · Coastal dunes · Root-feeding nematodes · Soil microorganisms · Tri-trophic interaction · Y-tubes

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Introduction

Root-feeding nematodes are the main belowground herbivores in (semi-)natural grassland ecosystems and take up as much as a quarter of the net primary production (Stanton 1988). Besides their influence

on plant productivity, root-feeding nematodes also influence plant community composition (de Deyn et al. 2004; de Deyn et al. 2007; Verschoor et al. 2002). In agricultural systems, root-feeding nematodes are major soil-borne pests and cause severe crop losses (Bird and Kaloshian 2003). Nematodes are difficult to be suppressed and the use of nematicides may lead to environmental contamination (Oka et al. 2000), which causes opposition against chemical nematode control (Doran et al. 1996). On the other hand, biological control is still unreliable and, therefore, not widely practiced (Alabouvette et al. 2006; Kerry 2000; Kerry and Hominick 2002). Studying how the abundance of root-feeding nematodes is controlled in natural ecosystems may help to improve the biological control of nematodes in agroecosystems (van der Putten et al. 2006).

In coastal foredunes, root-feeding nematodes are suppressed in a species-specific way. For example, temporal development of *Meloidogyne maritima* is delayed towards the end of the growth season by competition (Brinkman et al. 2005). Arbuscular mycorrhizal fungi can suppress the root lesion nematode *Pratylenchus* spp. when added two or three weeks in advance (de la Peña et al. 2006). The plant can control the cyst nematode *Heterodera arenaria* (van der Stoel et al. 2006) and microbial enemies are a major factor in suppressing the ectoparasitic nematode *Tylenchorhynchus ventralis* (Piśkiewicz et al. 2007). Thus far, in the studies mentioned above and other papers on root feeders, very little attention has been given to nematode behavior in relation to their natural enemies. Here, we examine the capacity of *T. ventralis* to avoid its natural enemies. Such avoidance could reduce the effectiveness of biological control.

Below ground, when searching for suitable feeding sites, root-feeding nematodes are attracted to plant roots by diverse chemical cues, for example CO_2, inorganic ions and salts (Abou-Setta and Duncan 1998; Le Saux and Queneherve 2002; Robinson 1995). Without the presence of attraction cues, parasitic nematodes move more randomly. Contact with the chemosensory stimulants or deterrents produced by host roots, determines if a nematode will commence feeding or whether it keeps searching for a better feeding site (Johnson and Gregory 2006). Nematodes sense environmental cues by a specialized nervous system that includes chemo, thermo-, and mechanosensory neurons (Bird and Bird 1991). There is some evidence that nematodes are able to change their olfactory preferences following exposure to harmful organisms. For example, *Caenorhabditis elegans* is able to avoid odors from the pathogenic bacteria, whereas it is attracted by odors from familiar, but non-pathogenic bacteria (Zhang et al. 2005). The question is whether root-feeding nematodes may also express such avoidance behavior.

In a previous study, we have shown that microorganisms from the rhizosphere of the coastal foredune grass *Ammophila arenaria* (marram grass) are able to control the ectoparasitic root-feeding nematode *T. ventralis* (Order *Tylenchida* Pearse 1942; Genus *Tylenchorhynchus* Cobb; 1913 former name *Telotylenchus ventralis* Loof 1963 (Fortuner and Luc 1987) (Piśkiewicz et al. 2007). These nematodes are key root herbivores in natural coastal foredunes. They are able to strongly decrease the biomass of *A. arenaria* (de Rooij van der Goes 1995a), if not controlled by their microbial enemies (Piśkiewicz et al. 2007). In the field *T. ventralis* usually does not reach population levels that can affect plant growth (van der Stoel et al. 2002), but numbers vary locally (de Rooij van der Goes et al. 1995b). In the present study, we examine if plant ectoparasitic nematodes *T. ventralis* are able to sense and avoid microbial communities that include their natural antagonists. Such avoidance would be of importance since the suppressive effects of microorganisms on *T. ventralis* are expressed locally (Piśkiewicz et al. 2008). At least some microorganisms can attack the nematodes directly (Piśkiewicz et al. 2007), although local induction of plant defenses cannot be excluded (Piśkiewicz et al. 2008).

To examine if the ectoparasitic nematodes avoid their microbial enemies, we first studied the choice of *T. ventralis* on agar in Petri dishes with or without *A. arenaria* seedlings inoculated with or without soil microorganisms. Our null hypothesis stated that there was no effect of microorganisms on attraction of *T. ventralis* to plant roots. Alternatively, we expected that *T. ventralis* would be attracted more to roots without than to roots with soil microorganisms. We also studied whether the soil microorganisms alone (without seedlings) or a microbial filtrate without soil microorganisms would give the same result as a combination of plant roots and microorganisms. We present and discuss new evidence that in dune soil the

ectoparasitic nematode *T. ventralis* is able to avoid plant roots with microorganisms when non-inoculated roots are the alternative.

Materials and methods

We designed two experiments to study the choice behavior of the nematode *T. ventralis*.

Experiment 1 was performed in Petri dishes on agar medium, allowing us to observe the nematode behavior in a non-destructive way. In this experiment, we examined the choice of *T. ventralis* between *A. arenaria* seedlings inoculated with microorganisms or microorganisms-free seedlings. We also checked nematodes attraction to microbial inoculum alone (without plants in Petri dishes).

Experiment 2 was performed in Y-tubes filled with sterilized dune soil, which simulated semi-natural conditions more effectively than the agar Petri dishes (Boff et al. 2001). In this experiment we examined the choice behavior of *T. ventralis* between *A. arenaria* without or inoculated with microorganisms originating from the natural foredune soil. We also used a microbial filtrate which did not contain any microorganisms, but only the compounds that could be extracted from the soil by water, including those produced by soil organisms.

Soil

In summer 2005, soil samples were collected from the natural coastal foredune at Voorne. The soil was collected from ten transects parallel to the beach and 50 m apart. At each sampling point, 10 kg of soil was collected from the youngest root zone of *A. arenaria* (van der Putten et al. 1988). The soil was sieved (0.5 cm mesh size) to remove plant parts and debris, and stored in plastic bags at 4°C until used (van der Stoel et al. 2002).

Plants

In summer 2005, seeds of *A. arenaria* were collected from a natural coastal foredune at Voorne, The Netherlands (latitude 51°55′ N–longitude 04°05′ E) and stored dry until used.

For experiment 1, the seeds were separated from their seed coat and subsequently sterilized by soaking in 95% ethanol and 8% bleach (85 ml bleach water, 92 ml H_2O, 150 µl tween-20) for 2 and 15 min, respectively. Sterilized seeds were washed five times for at least 2 min with sterilized tap water and then dried. In order to obtain *A. arenaria* seedlings, the seeds were placed on 30 Petri dishes of 8.5 cm diameter, each containing 10 ml of 0.5% microbial agar (Merck kGaA, De.). The seeds were placed on both sides of the dish, 2.5 cm away from the center. Seedlings on the agar matrix were grown for 8 days to create a diffusion gradient of root exudates. The experiment 1 was performed in a climate chamber with a 16/8 h light/dark regime at a temperature of 25/15°C, respectively.

In order to obtain seedlings for the experiment 2, the seeds were germinated for 2 weeks on moist glass beads in a climate chamber at a 16/8 h light/dark regime and 25/15°C to provide day/night conditions. When the first leaf was 2–3 cm long, the seedlings were transplanted to the arms of vertically positioned Y-tubes filled with sterilized dune soil. The soil moisture was adjusted to 10% $w{\cdot}w^{-1}$ and maintained at this level until the nematodes and microorganisms were added. After addition of soil organisms, the tubes were moistened with 2 ml of demineralized water once a day. This amount of water prevented that the migrating nematodes were flushed back to the bottom of the Y-tube. The experiment was carried out in a greenhouse at a day temperature of 21±2°C (day length 16 h) with additional light (to maintain a minimum of 225 µmol m^{-2} s^{-1} PAR with SON-T Agro lamps) and a night temperature of 16°C. These temperatures are comparable to summer conditions in the field and they are optimal for both plant and nematode development (S.R. Troelstra and R. Wagenaar, unpublished results).

Nematodes and soil microorganisms

Specimen of *T. ventralis* were collected from the same coastal foredune area as plants and soil and cultured on the roots of *A. arenaria* plants in a greenhouse (Piśkiewicz et al. 2007). One day before usage, the nematodes were extracted from the cultures by Cobb's decantation method (Oostenbrink 1960).

Microorganisms were extracted from the collected dune soil by shaking 100 g soil samples with demineralized tap water (1:1 $w{\cdot}w^{-1}$) for 10 min and filtering the supernatant through a 20 µm mesh

(Bezemer et al. 2005; Klironomos 2002). Prepared microbial solutions were checked by microscope to establish that they did not contain nematodes or spores of arbuscular mycorrhizal fungi.

The microbial filtrate was prepared by filtering a microbial solution by 0.2 μm sieve. This filtrate did not contain any nematodes and microorganisms, but only the compounds that could be extracted from the soil by water, including those produced by soil organisms.

Experimental setup

Experiment 1—choice of T. ventralis in Petri dishes

In case of the Petri dishes with and without seedlings, the treatments consisted of the following combinations: Control (no organisms added)↔Control; Control↔Microorganisms; Microorganisms↔Microorganisms. On each half of the Petri dish, depending on the inoculation treatment, 80 μl of microbial suspension or sterile tap water was applied using a pipette to the roots of 8 days-old seedlings. Each treatment combination was carried out in five replicates. Three days after microorganisms were inoculated, 40 *T. ventralis* were added in a 100 μl water suspension at the center of the Petri dishes. Migration of the nematodes from the centre of the agar plate towards the edge was recorded 3 and 16 h, 1 day, 2, 4 and 6 days after they had been added. The nematodes were counted by microscope (×200 magnification).

Experiment 2: choice of T. ventralis in Y-tubes with dune soil

Ninety Y-shape vertical plastic tubes were constructed. The lower part was 15 cm long and both arms 10 cm. The arms were closed at the bottom with a 0.5-mm mesh to prevent root growth into the lower part of the Y-tube, while still enabling the nematodes to migrate through the mesh towards the plant roots. The bottom of the Y-tube was closed by a plastic cap. All Y-tubes were filled with sterilized dune soil and *A. arenaria* seedlings were planted in sixty of them. Thirty tubes contained no plants. Three weeks after the seedlings were planted and they were about 10 cm long, 500 nematodes were inoculated in the same way as done in experiment 2a. Sixty Y-tubes with *A. arenaria* were divided randomly into six groups and the arms received following treatment combinations: Control (sterilized tap water)↔Control; Control↔Microorganisms; Control↔Filtrate; Microorganisms↔Filtrate; Microorganisms↔Microorganisms; Filtrate↔Filtrate. The Y-tubes without plants were randomly divided into three groups and the arms were inoculated with the treatment combinations: Control↔Control; Control↔Microorganisms; Microorganisms↔Microorganisms.

Five days after inoculation with nematodes and microorganisms, the arms and the lower part of Y-tubes were dissembled and the soil from each of those parts was collected separately. *T. ventralis* from each soil portion were collected by Cobbs' decantation method (Oostenbrink 1960) and counted by microscope (×200 magnification).

Statistical analyses

Normal distribution of data and homogeneity of variance were checked by inspection of the residuals after model fit (using the package Statistica 7). In case of experiment 1, the numbers of nematodes counted on both halves of Petri dishes were compared by *T*-test. First, we tested if the numbers of migrated nematodes did not differ when the treatments of both halves of Petri dishes were the same. Then, we tested if the numbers of nematodes differed between both halves of Petri dishes when having received different treatments. Finally, using one-way ANOVA (three levels: Control↔Control; Control↔Microorganisms; Microorganisms↔Microorganisms) we tested if total migration of the nematodes away from the inoculation point was dependent on the microorganism addition.

In case of the experiment 2, *T*-tests were used to test if the migrated nematodes differed between both arms of the Y-tube. Then, by one-way ANOVA, we checked if the total migration of the nematodes was influenced by microorganism addition into the Y-tubes.

Results

Experiment 1: choice of *T. ventralis* in Petri dishes

In the Petri dishes without *A. arenaria* seedlings, the nematodes clustered in the centre close to the inoculation point and did not move more than 0.5 cm (data not shown).

In the Petri dishes with *A. arenaria* seedlings (Fig. 1), the only effect that came close to significance (P=0.06) was that after 1 day, more nematodes had moved towards seedlings without microorganisms than to seedlings with microorganisms (Fig. 1 c). On Petri dishes with seedlings and no inoculated microorganisms, 61% of the inoculated nematodes migrated. When the microorganisms were inoculated on only one side, 53% of the inoculated nematodes migrated. On the dishes with microorganisms on both sides, 41% of nematodes had moved away from the centre. After 4–7 days, the nematodes started to die and the Petri dishes became covered by microorganisms that most likely colonized the dishes by being attached to the nematode cuticle.

Experiment 2: choice of *T. ventralis* in Y-tubes with dune soil

The nematode choice effects depended on the actual presence of both plant roots and inoculated microorganisms. If no *A. arenaria* seedlings were present, the majority (95%) of the nematodes stayed in the lower part of Y-tubes (Fig. 2). One-way ANOVA showed that there were no differences between treatments in the total number of migrated nematodes. There was only a trend of more nematode migration when both arms were not inoculated (migration 42% of total number of nematodes inoculated) than when both arms had contained microorganisms (migration 24% of total inoculated). There was more nematode migration to *A. arenaria* seedlings without than to seedlings with microorganisms added (P=0.01) (Fig. 3). Other treatment combinations did not result in significant differences (P>0.05).

Discussion

Previous choice experiments in Petri dishes have shown that the roots of *A. arenaria* are attractive to *T. ventralis* (Brinkman et al. 2008). In field soil the root zone of *A. arenaria* contains soil microorganisms which are directly attacking *T. ventralis* although local inductions can not be excluded (Piśkiewicz et al. 2008). Here, we examined whether these soil microorganisms may reduce the attractiveness of plant roots to the nematodes. Whereas an experiment in Petri

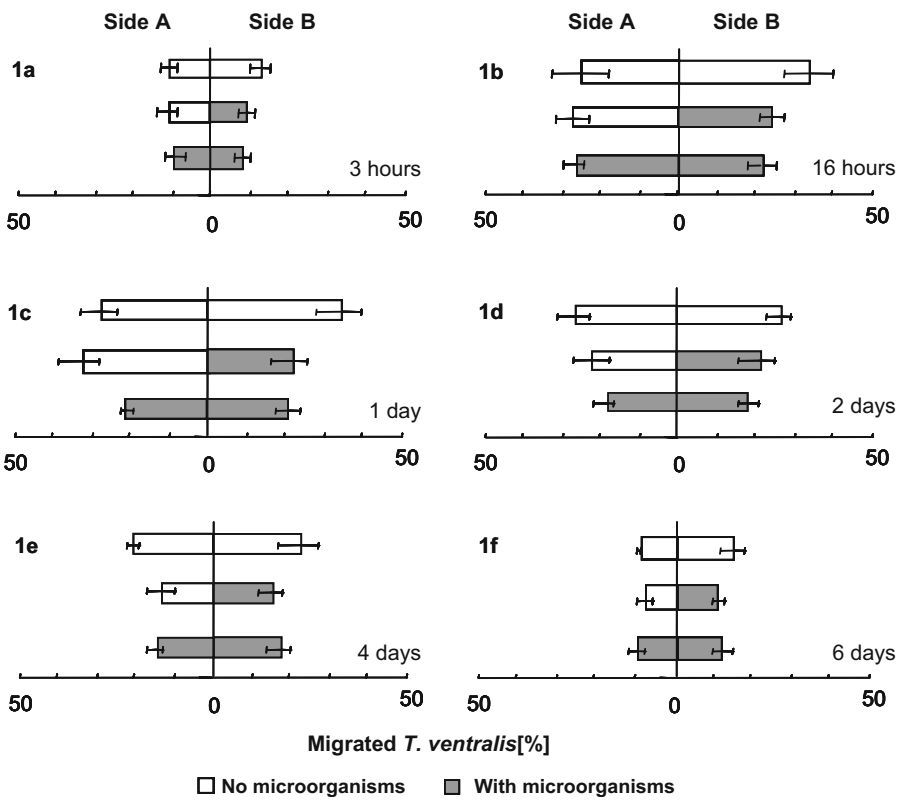

Fig. 1 Experiment 1. Percentages of nematodes migrated towards *A. arenaria* seedlings on two halves of agar Petri dishes inoculated with or without microorganisms. The nematodes were scored 3 h (*1a*), 16 h (*1b*), 1 day (*1c*), 2 days (*1d*), 4 days (*1e*) and 6 days (*1f*) after inoculation with nematodes. *Error bars* indicate standard errors

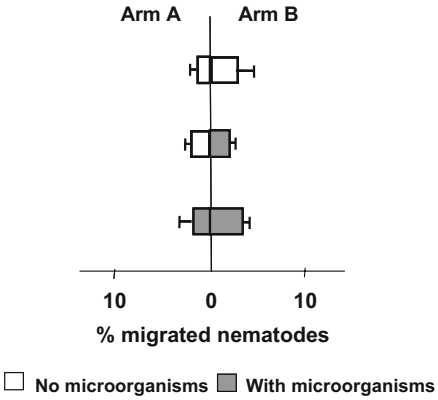

Fig. 2 Experiment 2. Percentages of nematodes migrated upwards in two arms of Y-tubes filled with sterilized dune soil and inoculated with microorganisms or without microorganism addition (no plant seedlings present in the arms). *Error bars* indicate standard errors

dishes did not show significant effects, testing the nematode choice in Y-tubes revealed a stronger preference of *T. ventralis* for plant roots without than with microbial suspension. The microbial effects on nematode choice depend on the combined presence of both plant roots and microorganisms. Microorganisms alone or roots with microbial filtrate did not influence nematode choice significantly.

There are several possibilities to explain our results. The first is that the microorganisms, when feeding on root exudates, mask the attractive effects of cues produced by the plant roots, for example by utilizing these cues for their own growth. The second possibility is that the microorganisms themselves, or easily decomposable products, produce compounds that actively repel the nematodes. This latter possibility is less likely, because soil microorganisms without plant roots did not influence nematode choice. Nevertheless, it could be that the microbial community was starved in the absence of plant roots. A third possibility is that in the sites inoculated with microorganisms, the nematodes were suppressed by parasitism, predation or antagonism. The duration of the experiments, however, probably was so short that direct predatory activities observed in experiments running much longer (Piśkiewicz et al. 2007) most likely did not reduce nematode numbers. Therefore, we think to be able to exclude such 'apparent choice' effects, leaving reduced attraction or distraction as possible explanations for the choice pattern. However, we can not exclude the "arrestment effect". It means that if nematodes would migrate randomly and reach a desirable resource, they would rather stay there. If the nematodes would arrive in a less suitable environment they would probably move to find a better spot to feed. It is also possible that the added microorganisms had negative effects on plant quality and nematodes simply avoided host plants of poorer quality

The trials using the artificial agar medium did not show any differences in choice behavior of the nematodes, whereas the Y-tubes did. This may stress the importance of performing such choice experiments, especially when including rhizosphere communities in soil (Rasmann et al. 2005). Probably, on artificial agar medium the microorganisms may not develop as in a natural rhizosphere in soil. Also signaling between plane, microorganisms and nematodes is different in these two systems. Previous studies showed that the ectoparasitic nematodes *T. ventralis* are parasitized mostly by *Pasteuria* sp., *Catenaria* sp. and *Paenibacillus*-like organisms and other unidentified bacteria (Piśkiewicz et al. 2007). Until now, there are no proper methods to successfully culture *Pasteuria* sp. on agar medium (Davies 2005).

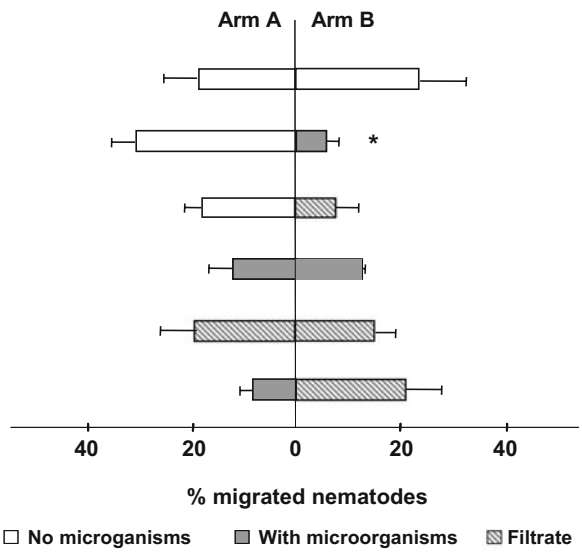

Fig. 3 Experiment 2. Percentages of nematodes migrated towards *A. arenaria* seedlings growing in Y-tubes filled with sterilized dune soil and inoculated with microorganisms, inoculated with microbial filtrate or clean (no microorganisms, no filtrate). *Error bars* indicate standard errors. The asterisks show significance at $P<0.05$

Interactions between roots, nematodes and microbial communities, can have effects on the distribution of soil organisms on or near the plant roots. In our experiments, we have used a whole microbial community, but in the rhizosphere the microbial community is known to vary in composition along plant roots (Duineveld, van Veen 1999). Therefore, it will be of interest to unravel whether the signals received by plant and/or by nematodes are due to the microbial community as a whole, or to specific components of the microbial community. For example, in theory hot spots of microbial repellents might be found near root tips where active growth takes place or a bit further up where active uptake of nutrients occurs. These hot spots could be less likely at the basal part of the root, where exudation and uptake activity are less intensive. In this case, theoretically, plants could be actively defending the most valuable parts of their root system against ectoparasitic nematodes in a tri-trophic way. Tri-trophic indirect defense is commonly known for aboveground plant defense, but such phenomena in soil have received limited attention (thus far focusing exclusively on entomopathogenic nematodes) (Rasmann et al. 2005; van Tol et al. 2001).

We conclude that plant ectoparasitic nematodes of the species *T. ventralis* have the capacity to sense and avoid roots colonized by a microbial community, when having roots without such microorganisms as an alternative. This may be due to reduced attraction to the cues produced by the roots without dune soil microorganisms, or by repellence of roots, which contain these microorganisms. The precise mechanism of this choice is yet unknown. The outcome of the microbial filtrate addition suggested that the repelling effects are not caused by microbial products, or their interactions with plant roots. Therefore, our results suggest that the possible repellence is due to direct interactions between plant roots and rhizosphere microorganisms. The questions whether in the field plants may direct the generalist root feeding nematodes to less sensitive parts of their root system, and which components of the microbial community are involved in these tri-trophic interactions require further studies.

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References

Abou-Setta MM, Duncan LW (1998) Attraction of *Tylenchulus semipenetrans* and *Meloidogyne javanica* to salts in vitro. Nematropica 28:49–59

Alabouvette C, Olivain C, Steinberg C (2006) Biological control of plant diseases: the European situation. Eur J Plant Pathol 114:329–341 doi:10.1007/s10658-005-0233-0

Bezemer TM, de Deyn GB, Bossinga TM, van Dam NM, Harvey JA, van der Putten WH (2005) Soil community composition drives aboveground plant-herbivore-parasitoid interactions. Ecol Lett 8:652–661 doi:10.1111/j.1461-0248.2005.00762.x

Bird AF, Bird J (1991) The structure of nematodes. Academic, San Diego

Bird DM, Kaloshian I (2003) Are roots special? Nematodes have their say. Physiol Mol Plant Pathol 62:115–123 doi:10.1016/S0885-5765(03)00045-6

Boff MIC, Zoon FC, Smits PH (2001) Orientation of *Heterorhabditis megidis* to insect hosts and plant roots in a Y-tube sand olfactometer. Entomol Exp Appl 98:329–337 doi:10.1023/A:1018907812376

Brinkman EP, Duyts H, van der Putten WH (2005) Consequences of variation in species diversity in a community of root-feeding herbivores for nematode dynamics and host plant biomass. Oikos 110:417–427 doi:10.1111/j.0030-1299.2005.13659.x

Brinkman EP, Duyts H, van der Putten WH (2008) Interactions between root-feeding nematodes depend on plant species identity. Soil Biol Biochem 40:2186–2193

Davies KG (2005) Interactions between nematodes and microorganisms: bridging ecological and molecular approaches. Adv Appl Microbiol 57:53–78 doi:10.1016/S0065-2164(05)57002-3

de Deyn GB, Raaijmakers CE, van der Putten WH (2004) Plant community development is affected by nutrients and soil biota. J Ecol 92:824–834 doi:10.1111/j.0022-0477.2004.00924.x

de Deyn GB, van Ruijven J, Raaijmakers CE, de Ruiter PC, van der Putten WH (2007) Above- and belowground insect herbivores differentially affect soil nematode communities in species-rich plant communities. Oikos 116:923–930 doi:10.1111/j.0030-1299.2007.15761.x

de la Peña E, Rodriguez-Echeverria S, van der Putten WH, Freitas H, Moens M (2006) Mechanism of control of root-feeding nematodes by mycorrhizal fungi in the dune grass *Ammophila arenaria*. New Phytol 169:829–840 doi:10.1111/j.1469-8137.2005.01602.x

de Rooij van der Goes PCEM (1995a) The role of plant parasitic nematodes and soil borne fungi in the decline of *Ammophila arenaria* (L) Link. New Phytol 129:661–669 doi:10.1111/j.1469-8137.1995.tb03035.x

de Rooij van der Goes PCEM, van der Putten WH, van Dijk C (1995b) Analysis of nematodes and soil borne fungi from *Ammophila arenaria* (Marram grass) in Dutch coastal foredunes by multivariate techniques. Eur J Plant Pathol 101:149–162 doi:10.1007/BF01874761

Doran JW, Sarrantonio M, Liebig MA (1996) Soil health and sustainability. Adv Agron 56:1–54 doi:10.1016/S0065-2113(08)60178-9

Duineveld BM, van Veen JA (1999) The number of bacteria in the rhizosphere during plant development: relating colony-forming units to different reference units. Biol Fertil Soils 28:285–291 doi:10.1007/s003740050495

Fortuner R, Luc M (1987) A reappraisal of *Tylenchina* (*Nemata*). 6. The family *Belonolaimidae* Whitehead, 1960. Rev Nematol 10:183–202

Johnson SN, Gregory PJ (2006) Chemically-mediated host-plant location and selection by root-feeding insects. Physiol Entomol 31:1–13 doi:10.1111/j.1365-3032.2005.00487.x

Kerry BR (2000) Rhizosphere interactions and the exploitation of microbial agents for the biological control of plant parasitic nematodes. Annu Rev Phytopathol 38:423–441 doi:10.1146/annurev.phyto.38.1.423

Kerry BR, Hominick WM (2002) Biological control. Taylor & Francis, London

Klironomos JN (2002) Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417:67–70 doi:10.1038/417067a

Le Saux R, Queneherve P (2002) Differential chemotactic responses of two plant-parasitic nematodes, *Meloidogyne incognita* and *Rotylenchulus reniformis*, to some inorganic ions. Nematology 4:99–105 doi:10.1163/156854102760082258

Oka Y, Koltai H, Bar-Eyal M, Mor M, Sharon E, Chet I et al (2000) New strategies for the control of plant–parasitic nematodes. Pest Manag Sci 56:983–988 doi:10.1002/1526-4998(200011)56:11<983::AID-PS233>3.0.CO;2-X

Oostenbrink M (1960) Estimating nematode populations by some selected methods. In: Sasser JN, Jenkins WR (eds) Nematology. The University of North Carolina Press, Chapel Hill, pp 85–102

Piśkiewicz AM, Duyts H, Costa SR, Berg MP, van der Putten WH (2007) Soil microorganisms control plant ectoparasitic nematodes in natural coastal foredunes. Oecologia 152:505–514 doi:10.1007/s00442-007-0678-2

Piśkiewicz AM, Duyts H, van der Putten WH (2008) Soil microorganisms in coastal foredunes control the ectoparasitic root-feeding nematode *Tylenchorhynchus ventralis* by local interactions. Funct Ecol (in press)

Rasmann S, Kollner TG, Degenhardt J, Hiltpold I, Toepfer S, Kuhlmann U et al (2005) Recruitment of entomopathogenic nematodes by insect-damaged maize roots. Nature 434:732–737 doi:10.1038/nature03451

Robinson AF (1995) Optimal release rates for attracting *Meloidogyne incognita*, *Rotylenchulus reniformis*, and other nematodes to carbon dioxide in sand. J Nematol 27:42–50

Stanton NL (1988) The underground in grasslands. Annu Rev Ecol Syst 19:573–589 doi:10.1146/annurev.es.19.110188.003041

van der Putten WH, van Dijk C, Troelstra SR (1988) Biotic soil factors affecting the growth and development of *Ammophila arenaria*. Oecologia 76:313–320 doi:10.1007/BF00379970

van der Putten WH, Cook R, Costa S, Davies KG, Fargette M, Freitas H et al (2006) Nematode interactions in nature: models for sustainable control of nematode pests of crop plants? Adv Agron 89:227–260 doi:10.1016/S0065-2113(05)89005-4

van der Stoel CD, van der Putten WH, Duyts H (2002) Development of a negative plant-soil feedback in the expansion zone of the clonal grass *Ammophila arenaria* following root formation and nematode colonization. J Ecol 90:978–988 doi:10.1046/j.1365-2745.2002.00727.x

van der Stoel CD, Duyts H, van der Putten WH (2006) Population dynamics of a host-specific root-feeding cyst nematode and resource quantity in the root zone of a clonal grass. Oikos 112:651–659 doi:10.1111/j.0030-1299.2006.13547.x

van Tol RWHM, van der Sommen ATC, Boff MIC, van Bezooijen J, Sabelis MW, Smits PH (2001) Plants protect their roots by alerting the enemies of grubs. Ecol Lett 4:292–294 doi:10.1046/j.1461-0248.2001.00227.x

Verschoor BC, de Goede RGM, Brussaard L (2002) Do plant parasitic nematodes have differential effects on the productivity of a fast- and a slow-growing grass species? Plant Soil 243:81–90 doi:10.1023/A:1019913417792

Zhang Y, Lu H, Bargmann CI (2005) Pathogenic bacteria induce aversive olfactory learning in *Caenorhabditis elegans*. Nature 438:179–184 doi:10.1038/nature04216