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Temporary Changes in Populations of Soil Organisms after Field Application of Entomopathogenic Nematodes

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Abstract: To assess the effect of an inundative release of entomopathogenic nematodes on soil organisms, population densities of soil-dwelling organisms were monitored before and after an application of an aqueous suspension of *Heterorhabditis megidis* to field plots in mown grassland (Exp. I) at a level of 0.38 million/m^2 and to plots (Exp. II) situated in a forested area, a grass sports field and an orchard at a level of 1.5 million/m^2. At the forested site, heat-killed *H. megidis* (1.5 million/m^2) also were applied to two plots to compare the impact on soil organisms of a large introduction of living and dead nematodes. Post-treatment, temporary changes in natural population densities of several nematode genera and other organisms were detected in *H. megidis*-treated plots in both experiments. Temporary changes in the nematode trophic structure occurred in the percentages of nematode omnivores, herbivores and predators in both experiments. Evidence from all sites suggests that the changes were temporary and that the presence of decaying *H. megidis* following treatment contributed to nutrient enrichment of the soil and to direct and indirect effects on the nematode community.

Key words: Biological control, ecology, entomopathogenic nematode, field, *Heterorhabditis megidis*, impact, interaction, soil.

Entomopathogenic nematodes (EPN) are used as biopesticidal control agents of insect pests of various crops. The spraying of millions of EPN for insect biocontrol leads to an unnaturally high nematode population density in the soil for the first few days after release. As well, it provides a relatively homogeneous distribution of EPN in treated areas that leads to multiple contacts between these nematodes and a variety of soil-inhabiting arthropods and other soil-dwelling organisms (Bathon, 1996).

Various direct or indirect effects of EPN applications may occur within the soil community. For example, EPN may be fed upon directly by nematophagous mesoarthropods such as mites and collembolans (Epsky et al., 1988; Wilson and Gaugler, 2004), nematode-trapping fungi (e.g., Koppenhoffer et al., 1996; Kaya and Koppenhoffer, 1999), or predacious nematodes. These interactions could potentially indirectly influence the population densities of other species of prey or competing predators. Moreover, the subsequent death and decomposition of large numbers of EPN may indirectly affect population densities of bacterivorous and fungivorous soil organisms. Grewal et al. (1999), for example, observed direct repellency and allelopathic suppression of plant-parasitic nematode juveniles by metabolites of the EPN symbiotic bacteria *Xenorhabdus* sp., released upon decay of the respective nematode-infected cadavers. However, these secondary metabolites produced by the EPN bacterial symbionts and released into the soil from the insect cadaver have a very short-term anti-microbial activity (Chen et al., 1994).

Nematode communities have been used to study the impact of management practices on agroecosystems (e.g., Freckman and Ettema, 1993; Porazinska et al., 1998) and forest soils (e.g., Panesar et al., 2000), and to determine the impact of other ecosystem components, such as soil surface patrolling macroarthropods (Wasilewska, 2000). Changes in nematode community structure after application of steinernematids to nonsterilized pots of soil were detected by Ishibashi and Kondo (1986). These changes included increases in rhabditids and predatory nematodes and decreases in some other major orders of nematodes, including plant-parasitic ones. Decreased populations of plant-parasitic nematodes have been observed under laboratory conditions (Bird and Bird, 1986; Grewal et al., 1999) and in a turfgrass field site (Smitley et al., 1992) following EPN applications. However, after a field EPN treatment of turfgrass, Wang et al. (2001) found no changes in the nematode community compared with that from other treatments (e.g., insect pathogen *Beauvaria bassiana* or pesticide Chlorpyrifos), although they observed some variation in the number of a miscellaneous grouping of less common nematode orders.

Georgis et al. (1991) and Campbell et al. (1995) did not detect any adverse impacts of an EPN treatment on the surface abundance of mobile arthropods in different turfgrass and agrosystem habitats. Similarly, Ropek and Jaworska (1994) did not detect any effect of the EPN *Steinernema carpocapsae* on adult carabid beetles in field trials. In a detailed field study monitoring impacts of EPN on nontarget, soil-inhabiting insect larvae, no impact was detected on the population densities of broad taxonomic groups of arthropods (Coleoptera, Diptera, Hymenoptera), although population densities of a few coleopteran and dipteran species within those taxonomic groups were reduced, and some species showed greater abundance in the presence of EPN (Buck and Bathon, 1993; Koch and Bathon, 1993). Importantly, it appears that the application did not lead to the extinction of local populations of any pest insect species or nontarget organism.

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In order to clarify our understanding of the effects of field applications of EPN on other soil organisms, field experiments were done to address three objectives, as follows:

(i) Assess the impact over time of an inundative release of *Heterorhabditis megidis* in aqueous sus pension on a grassland site by recording changes in population density of soil macrofauna (earthworms and insect larvae), mesofauna (microarthropods), microfauna (nematodes), microflora (nematode-trapping fungi) and changes in nematode community structure.
(ii) Assess the idiosyncrasy of the impact of inundative releases of *H. megidis* on soil organisms in different habitats, namely field plots in an alder stand, an apple orchard and a sports field. These widely different habitats were selected because populations of different types of organism in different soils may respond differently to an EPN application.
(iii) Determine the possible nutrient effect of EPN treatments on the numbers of soil organisms by comparing the effect of inundative releases of dead and living *H. megidis* at an alder stand site.

Materials and Methods

Commercial *Heterorhabditis megidis* (NLH-E87.3), reared in vivo in late instar Greater Wax Moth, *Galleria mellonella*, and marketed as the biocontrol agent LARVANEM®, were obtained from Koppert B.V. (Berkel en Rodenrijs, The Netherlands). Late-instar larvae of *G. mellonella* were used in bioassays to determine *H. megidis* population density in the soil and for the in vivo culture of *H. megidis* (Kaya and Stock, 1997) for nematode-trapping fungi bioassays.

Experimental sites: Experiment I. To address objective (i), the field experiment was carried out at Simon Fraser University on a grassland site dominated by a perennial grass (likely Kentucky Blue Grass, *Poa pratensis*) with scattered patches of at least 10 plant species growing in a loamy sand (61% sand, 30% silt, 9% clay). The site was not amended with fertilizers or biocides and was not irrigated. Within the site, 10 plots, each 5 m × 5 m with a minimal interplot distance of 10 m, were established. On 19 May 2000, five plots were randomly chosen for treatment and sprayed, using a back-pack hand-sprayer, with an aqueous suspension of 0.38 million infective juveniles (IJ) of *H. megidis*/m^2. The remaining five plots received a water spray as controls.

Experiment II: To address objectives (ii) and (iii), three sites were chosen based on their dominant species of plant cover: an intensely managed sports field and a natural alder stand located at Simon Fraser University and an apple orchard (Annie's Orchard) located in Langley, BC, about 30 km east of the University. The Sports Field site was a Kentucky Blue Grass (*Poa pratensis*) high-input monoculture with underground drainage and sprinkler irrigation systems growing on sand (81% sand, 19% silt). The Alder Stand site was a natural, unmanaged stand of red alder trees (*Alnus rubra*) shading an understory of two unidentified grass species (*Poa spp.*), salmonberry (*Rubus ursinus*), aster (*Hypochaetus radicata*) and an unidentified fern growing on sand (79% sand, 21% silt). The Orchard site consisted of rows of apple trees (*Malus* sp.) growing on sandy silt (43% sand, 57% silt) without undercanopy vegetation. Grasses and weeds in the inter-row areas were removed manually or with herbicide (Round-up®) and the soil surface left bare.

Within each site, two plots (each 2 m × 2 m) with a minimum inter-plot distance of 5 m were sprayed on 9 September 2000 with an aqueous suspension of 1.5 million IJ/m^2, and two were sprayed with water as controls. At the Alder Stand site, two additional plots (each 2 m × 2 m) received an application of heat-killed EPN at a dose of 1.5 million IJ/m^2.

There was no visible evidence of inter-plot differences in vegetation composition among plots at the same site. All EPN applications were made after sunset on rainy days.

Sampling: Experiment I. Every 2 wk throughout a 1.5 mon pre-treatment period (April and May 2000) and post-treatment until August 2000, then every mon until November 2000, soil samples were taken randomly from each plot to assess the occurrence and population densities of nematodes and other soil organisms. Each of the five treated and five control plots was divided into 400 subplots of 25 × 25 cm^2, and 10 subplots within a plot were randomly sampled twice at each time interval. At each sampling time, two sets of 10 soil cores were taken at random in each plot with a soil auger (2-cm-diam.) to a depth of 7.5 cm, the high density of stones impeding auger penetration of the soil below 8 cm.

Experiment II: From September to November 2000, 3 d, 25 d and 50 d post-treatment, soil samples (2-cm-diam. × 15-cm-deep) were taken randomly from each of the two treated and two control plots at each site and from the dead-*H. megidis*-treated plots at the Alder Stand site.

Determination of soil macrofauna densities: Within 24 hr of sampling for each plot, one bag of 10 mixed soil cores was hand-sorted by eye in a glass tray to extract large arthropods and other invertebrates. Collected organisms were placed in glass vials of 70% ethanol for subsequent identification and counting. The second bag of soil from each plot was later used for the extraction of microscopic organisms and for the determination of abiotic parameters.

Estimation of entomopathogenic nematode density: Prior to field sampling, a baiting-rebaiting method (Koppenhoffer et al., 1998) was conducted in the lab with three replicates of eight different quantities (0, 5, 10, 20, 40, 80, 160, 320) of *H. megidis* applied to sterilized soil from the grassland site to obtain a correlation between larval

G. mellonela bait insect mortality and the number of nematodes present in the soil. After every field sample was taken, 50 cm^3 of mixed soil from each plot was placed in a petri dish (100 × 25 mm), and 10 wax moth larvae were added as bait every 3 d until no nematode-infected larvae were recovered from two rounds of bioassay baiting. Total bait mortality was recorded for each plot, and EPN densities per 50 cm^3 were derived from the correlation obtained a priori in the lab.

Determination of densities of nematophagous fungi and parasites: Dilution plating and the most probable number methodology were used (Eren and Pramer, 1965; Alexander, 1982), as modified by Koppenhoffer et al. (1996). Fungi were identified to genus using Cooke and Godfrey (1964).

Determination of population densities of free-living nematodes and enchytraeids: A wet extraction process using a modified Baermann funnel followed by sucrose centrifugal flotation (Jenkins, 1964) was utilized to extract nematodes and enchytraeids from a 100 cm^3 mixed soil sample from each plot. The extracted organisms were preserved (Seinhorst, 1959, 1966), and the nematodes were counted and identified to genus based on the classifications of Goodey (1963), Anderson and Mulvey (1979), and Jairajpuri and Ahmad (1992).

Determination of population densities of microarthropods: Modified Tullgren funnels were used to extract mites and collembolans from soil samples. Extracted organisms from the 100 cm^3 samples were preserved for storage in 70% ethanol. Collembolans were identified to family based on Borror et al. (1989) and counted. Mites were sorted into broad morphological groups and counted.

Estimation of soil nematode community structure: Nematodes were identified to genus and then assigned to one of six trophic groups after Yeates et al. (1993a), namely bacterivores (B), fungivores (F), obligate herbivores (OPF), facultative herbivores (FPF), omnivores (Om) and predators (P). Ratios of trophic groups, B/F, (B+F+FPF)/OPF, and B+F/(OPF+FPF), indicative of differences in the contribution of nematodes to the mineralization occurring in the detritus and grazing food web of the soil (Wasilewska, 1997). The number of taxa per sample (richness), the Simpson (1-D) diversity index (Simpson, 1949), giving weight to more abundant taxa, and the Shannon-Weaver H′ diversity index (Shannon and Weaver, 1949), giving weight to rare taxa, were calculated to assess effects on the community structure.

Nematode genera were assigned colonizers-persisters (c-p) scores based on their life strategies, after Bongers (1990). The following maturity indices were then calculated as the weighted mean of the c-p values of the different nematode families evaluated in each index: Maturity Index for free-living nematodes (MI) and Maturity Index for plant-parasitic nematodes (PPI), as described by Bongers (1990); Maturity Index for total nematodes (ΣMI), as suggested by Yeates et al. (1993b); Maturity Index excluding enrichment opportunists (c-p 1) (MINO) and Maturity Index for total nematodes excluding opportunists (ΣMINO), as suggested by Bongers and Ferris (1999); and the Maturity index for bacterivores (Ba MI) (Wasilewska, 1998).

Soil abiotic conditions: Soil temperature was measured and recorded on each sampling day by inserting a soil thermometer 5 cm into the soil in each plot. Relative soil moisture content in each plot was quantified in the laboratory by weighing 50 g of soil before and after an overnight drying process in an oven at 105°C. Soil pH was measured for each sample in the laboratory with a pH meter (Corning pH Meter model 320).

Statistical Analyses: To obtain a relationship between larval *G. mellonella* insect bait mortality and *H. megidis* density, the number of nematode-infected cadavers and the number of nematodes inoculated per dish was correlated on a log:log scale and fitted to zero. To assess the effect of *H. megidis* applications on the density of soil organisms and on nematode community structure indices, a random effects-mixed model analysis was conducted using the JMP IN® Start Statistics program to take into account the repeated measures design (Sall et al., 2001). Plots were nested within treatment types and considered as random effects. Tukey's HSD, accounting for Bonferroni effects occurring when using multiple Student *t*-tests, was used to assess the significance of differences among sample dates (interaction between treatment and time). Comparisons of the relative density of different nematode trophic groups were done using χ^2 tests. Correlation analyses were conducted between population densities of different groups of soil organisms, *H. megidis* densities and abiotic factors.

For Experiment I, different time frames were tested to assess different types of treatment effects between treatment (n = 5) and control plots (n = 5). Comparisons between or within treatments were made either (a) after the treatment as a whole (Treatment effect), (b) for short periods of time (grouping of three consecutive sampling times) after treatment (Shortly after: 22 May, 5 Jun, 19 Jun; Mid-way after: 3 Jul, 17 Jul, 31 Jul; Long after: 5 Sep, 3 Oct, 7 Nov), and (c) between individual sampling dates (Time × Treatment effects).

For Experiment II, comparisons between control plots (n = 2) and treated plots (n = 2) were made (a) overall (Treatment effect) (average of the three sampling dates), and (b) between individual sampling dates (Time × Treatment effect).

Results

Experiment I: From April 2001 to November 2001, a total of 52,157 soil organisms were recovered from soil samples, of which an estimated 30,108 were from two genera of nematode-trapping fungi and 22,049 were counted directly as other types of organisms. The latter

TABLE 1. Summary of *Heterorhabditis megidis*-treatment effects on the populations of soil organisms at the Grassland (n = 120), Alder Stand (n = 12), Orchard (n = 12) and Sports Field (n = 12) sites ($P \leq 0.05$). Dashes indicate nonsignificant difference between EPN-treated and control plots.

Soil organism (c-p value[b])	Site[a]	Mean number in treated plots	Treatment effect % Increase (↑) or % Decrease (↓) (Prob > F)	Time X treatment effect number of temporary Increase (↑) or Decrease (↓)	
				For periods (Prob > F)	For individual dates (Prob > F)
NEMATODA	G	179	—	—	1 ↓ (0.0086)
Maturity Index	SF	2.68	18% ↑ (0.0273)	—	—
MINO Maturity Index	SF	2.73	18% ↑ (0.0219)	—	—
Plant-Parasitic Index	AS	0.13	53% ↓ (0.0338)	—	—
Omnivores	SF	226	—	—	2 ↑, 1 ↓ (0.0011)
Aporcelaimellus sp. (5)	SF	58	61% ↓ (0.0219)	—	2 ↓ (0.0057)
	G	103	—	1 ↓ (0.0335)	1 ↓ (0.0024)
Eudorylaimus sp. (4)	G	5	—	1 ↓ (0.0058)	3 ↓ (0.0046)
Mesodorylaimus sp. (4)	SF	31	—	—	2 ↑ (0.0011)
Predators	All sites		—	—	—
Chrysonema sp. (5)	G	1	—	—	2 ↑ (0.044)
Bacterivores	All sites		—	—	—
Acrobeloides sp. (2)	G	1	—	1 ↑ (0.0322)	—
Cephalobus sp. (2)	SF	7	—	1 ↓ (0.0454)	—
Chiloplacus sp. (2)	SF	12	205% ↑ (0.0483)	—	1 ↑ (0.0455)
Eucephalobus sp. (2)	G	4	—	1 ↑ (0.0296)	—
Prismatolaimus sp. (2)	G	4	200% ↑ (0.0112)	1 ↑ (<0.0001)	3 ↑ (0.0037)
Rhabditis sp. (1)	O	1	—	—	1 ↑ (0.0494)
Facultative herbivores	G	8	—	1 ↑ (0.002)	2 ↑ (0.0125)
Anguina sp. (2)	AS	1	294% ↑ (0.0333)	—	1 ↑ (0.0041)
Aphelenchus sp. (2)	G	3	—	2 ↑ (<0.0001)	2 ↑ (0.0023)
Boleodorus sp. (2)	SF	6	338% ↑ (0.0174)	—	2 ↑ (0.0054)
Ottolenchus sp. (2)	G	1	—	1 ↑ (0.0391)	—
Tylenchus sp. (2)	G	1	—	1 ↑ (0.0023)	—
	AS	1	96% ↓ (0.0142)	—	3 ↓ (0.0066)
COLLEMBOLA	O	4	28% ↓ (0.0486)	—	—
Nematode-trapping FUNGI	All sites		—	—	—
Arthrobotrys sp.	G	1 312	—	↑, ↓ (0.0112)	—
ACARI	All sites		—	—	—
Soil mites (nonpigmented)	G	3	—	↑, ↓ (0.0159)	—

[a] G = Grassland; AS = Alder stand; O = Orchard; SF = Sports field.
[b] Colonizers-persisters values for nematodes, ranging from 1 to 5, after Bongers (1990).

were classified as 53 different genera of nematodes, three morphologically broad groups of mites, two families of earthworms, four families of collembolans and nine families of insects.

Among the common soil-dwelling organisms recovered from the soil samples, mature insects, molluscs, crustaceans, protozoans, non-nematophagous fungi and the bacterial flora were not counted in this experiment.

Overall, the densities of 14 taxa or functional groups in *H. megidis*-treated plots differed from the controls for single sampling dates, short-term periods or for the entire post-application period (Table 1).

To assess *H. megidis* densities in field samples, the derived log:log relationship between numbers of EPN-infected *G. mellonella* and *H. megidis* inoculated per dish was forced through zero ($y = 10^{[0+1.85*\mathrm{Log}(x)]}$, df = 19, SS = 318.23, $P < 0.0001$). The estimated density of *H. megidis* in treated plots declined rapidly and then alternated between detectable and nondetectable levels (Fig. 1). The IJ were not present in the top layer of soil at any time period when the relative soil moisture was lower than 18%. Over time, the population density of *H. megidis* was positively correlated (df = 3, $P < 0.05$) with soil moisture ($R^2 = 0.69$), relative density of nematode bacterivores ($R^2 = 0.80$), numbers of nematode enrichment opportunists (c-p 1) ($R^2 = 0.62$), and hypogasturid collembollans ($R^2 = 0.60$) and had a negative

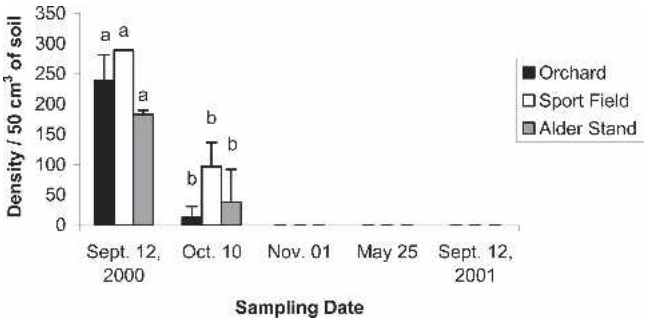

FIG. 1. Population density of *Heterorhabditis megidis* (histograms) and soil moisture (lined curve) in treated (19 May) Grassland plots (n = 45) of Experiment I from May 2000 to May 2001.

relationship with soil temperature (R^2 = –0.75), numbers of earthworms (R^2 = –0.64), the Shannon-Weaver diversity index (R^2 = –0.60), and the total nematode (R^2 = –0.19) and MINO Maturity Indices (R^2 = –0.62). No *H. megidis* were found one yr after application.

Only 38 specimens of insect larvae, potential hosts of EPN, were collected over all the plots and sampling periods. The relative density of insect larvae was not affected by the *H. megidis* treatment. The probability of encountering an insect larva in control (21.7%) and in treated plots (26.7 %) was not different. The only larva encountered simultaneously in the same plot as *H. megidis* was a member of the coleopteran family Elateridae. In treated plots, there was a marginally higher probability of encountering any other insect larva than elaterids when *H. megidis* was absent (27.7%) than when the nematodes were present (0%) (LR: df = 4, χ^2 = 7.286, $P < 0.075$).

Experiment II: The experimental sites where Experiment II was conducted were chosen based on the potential soil community differences associated with their vegetation management. Whereas soil temperature and soil texture were similar at the three sites, there were differences among sites in soil moisture (df = 2, F = 77.1942, $P < 0.0001$) and pH (df = 2, F = 60.3064, $P < 0.0001$). Although, as regards to nematodes, all sites had similar genus richness and no difference in the average number of genera per sample, the Sports Field site had a higher total nematode population density (df = 2, F = 31.9869, $P < 0.0001$), but a lower Shannon-Weaver nematode diversity index (df = 2, F = 5.0575, $P = 0.0209$). The nematode community functional composition was different at each site (LR: df = 2, χ^2 = 60.3064, $P < 0.0001$). The unmanaged Alder Stand site had a higher proportion of predatory nematodes (11%) and a lower proportion of herbivores (8%) than either of the other sites. The Orchard site had the highest proportion of bacterivores (49%). The Sports Field site had the highest proportion of omnivores (32%) and herbivores (18%), but a relatively low proportion of fungivores (0%), facultative herbivores (5%), and predators (3%). The plant-parasitic nematode index (PPI) was highest in the Sports Field (df = 2, F = 4.8096, $P = 0.0243$). The orchard site had the highest population density of mites (df = 2, F = 31.8148, $P < 0.0001$), while the Sports Field had a greater number of earthworms than the two other sites (df = 2, F = 133.67, $P < 0.0001$).

The same correlation as in Experiment I ($y = 10^{[0+1.85*\mathrm{Log}(x)]}$, df = 19, SS = 318.23, $P < 0.0001$) was used to assess *H. megidis* population densities after treatment of the Experiment II sites. Numbers of *H. megidis* in the soil (Fig. 2) continued to be relatively high in all three sites 3 d after application, but had decreased by at least 67% in all sites after 25 d. No difference was found in *H. megidis* population densities between sites. No *H. megidis* were found at any of the sites after 50 d, 6 mon or 1 yr after application. There was no difference in the mean population density of insect larvae between treated and control plots at any site.

From 12 September to 1 November 2001, a total of about 26,209 soil organisms was recovered from the three sites, of which 10,183 were estimated densities of nematode-trapping fungi, and 16,026 other organisms were counted individually.

At the Orchard site, the total number of Collembola and one nematode genus, the bacterivore *Rhabditis* sp., had different population densities between treatment plots (Table 1). The nematode community trophic structure was not different between *H. megidis* treatments and controls in general, but treatment effects were found 50 d after application. There were more facultative herbivores and omnivores and fewer bacterivores and herbivores in *H. megidis*-treated plots (LR: df = 11, χ^2 = 20.114, $P = 0.0012$).

At the Sports Field site, three nematode genera, the bacterivore *Chiloplacus* sp., the facultative herbivore *Boleodorus* sp., and the omnivore *Aporcelaimellus* sp., had different population densities in treatment versus control plots overall while three more groups, the bacteri-

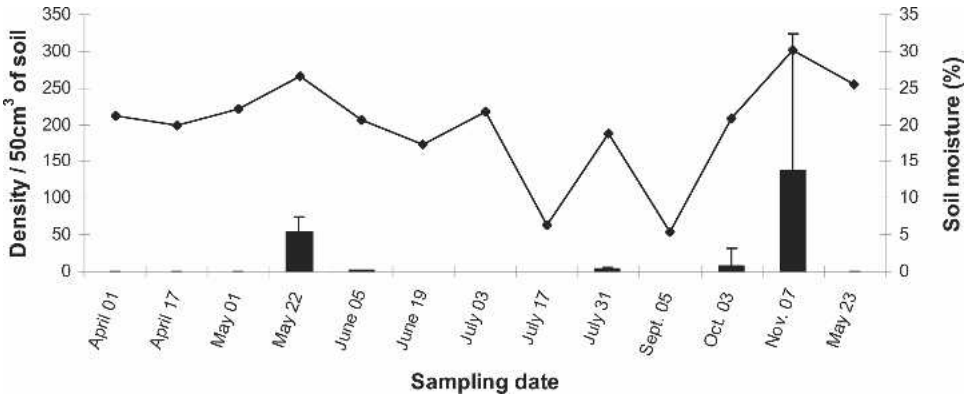

FIG. 2. Post-application population density of *Heterorhabditis megidis* in plots (n = 2) at the Alder Stand, Orchard and Sports Field experimental sites 3 d (12 Sep), 25 d (10 Oct) and 50 d (1 Nov) after treatment. Different letters indicate significant differences between columns ($P < 0.0001$).

vore *Cephalobus* sp., total omnivores and the omnivore *Mesodorylamus* sp., showed temporary differences between treatments (Table 1). *Heterorhabditis megidis*-treated plots overall had lower relative densities of herbivores (7%) and relatively more omnivores (39%) than did the control plots (18% and 32%, respectively) (LR: df = 11, χ^2 = 43.637, $P < 0.0001$). Temporary changes in the relative population densities of nematode trophic groups were recorded for bacetrivores, predators, omnivores, and obligate herbivores (df = 11, $P < 0.0001$).

At the Alder Stand site (Table 2), the population densities of two nematode facultative herbivore genera, *Anguina* sp. and *Tylenchus* sp., were affected by treatment types (both live and dead EPN applications) (df = 17, $P < 0.0001$). The plant-parasitic nematode index (PPI) after both treatments was, in general, lower than in control plots ($P < 0.0338$). The nematode community trophic structure was different between types of treatment in general and for each sampling date individually.

Discussion

Response of soil organisms: This study demonstrated temporary changes in population density of some soil organisms, mostly nematodes, and changes in the nematode trophic structure after an inundative release of *H. megidis* IJ on plots in different habitats.

Increases in population density of some bacterivorous nematodes post-application of H. megidis: Specifically, increases were detected for *Prismatolaimus* sp. in Experiment I and for *Rhabditis* sp. in the Orchard site and *Chiloplacus* sp. in the Sports Field site in Experiment II. Temporary population density increases occurred for some bacterivorous nematode genera in the Grassland (*Acrobeloides* sp. and *Eucephalobus* sp.) and Sports Field (*Cephalobus* sp.) sites. All these bacterivores are opportunists (colonizers), with short life-cycles of only three days for some species. They respond rapidly to transient conditions in the soil and are known to increase following habitat disturbance (Bongers, 1990; Bongers and Bongers, 1998).

TABLE 2. Comparative table of increases and decreases of soil nematode parameters, measured in different ways, following an application of living or dead *Heterorhabditis megidis* to the soil at the Alder Stand forest site (df = 1.7, $P \leq 0.05$). Dashes indicate nonsignificant difference between EPN-treated and control plots.

	Treatment effect Increase (I) or Decrease (D)		Time × treatment effects temporary Increase (I) or Decrease (D)	
	Dead EPN treatment	Living EPN treatment	Dead EPN treatment	Living EPN treatment
Density of *Anguina*	I	—	I	I
Density of *Tylenchus*	D	D	D	—
Plant Parasitic Index	D	D	—	—
% Bacterivores	D	D	—	—
% Predators	I	I	—	—
% Fungivores	I	—	—	—
% Facultative herbivores	—	—	D	—
% Omnivores	—	—	I	I

The effect of the EPN application on the numbers of certain nematode bacterivores is consistent with previous studies (Yeates, 1976; Ishibashi and Kondo, 1986, Wasilewska, 2000; Wang et al., 2001), where a variety of disturbances has been shown to affect members of the soil community. The processing of organic matter derived from decaying *H. megidis*-infected insects and dead *H. megidis* cadavers likely favored bacterial growth and ameliorated the food supply for bacterivorous nematodes. Increases in the proportion of bacterivores in the community and in the numbers of opportunists (c-p 1) were positively correlated with the peaks in population density of *H. megidis* in the Grassland site. The possibility that the *H. megidis* treatment contributed to an enrichment of the soil that favored detritivorous microflora growth is also supported by the observed increases of hyphal-feeding, facultative herbivore genera of nematodes in the Grassland and Sports Field sites. Facultative herbivores are also general opportunists with a low c-p value (2) (Bongers, 1990) that would benefit from conditions favorable for fungal growth triggered by the addition of organic matter. Such increases in facultative plant feeders were not observed in the aforementioned studies by other authors, in which increases in bacterivorous nematode numbers were observed.

In a forest, the application of fertilizers depressed the abundance of fungivorous and omnivorous nematodes, while favoring bacterivores (Sohlenius and Wasilewska, 1984). The amount of inorganic fertilizers applied was 0.019 g/m^2 in total (0.009 g/m^2 N, 0.003 g/m^2 P, 0.007 g/m^2 K). Considering that the average weight of a nematode is around 4.4×10^{-8} g (based on Curry, 1969), the biomass of *H. megidis* IJ applied (smaller than the average nematode) was slightly less than 0.02 g/m^2 in Experiment I and 0.07 g/m^2 in Experiment II. In terms of weight, therefore, a standard treatment of EPN could effectively be comparable to a fertilizer application.

Similar to the effects of fertilizers on the number and diversity of soil-living nematodes in forestland, temporary depressions of nematode omnivorous groups occurred in the Grassland and Sports Field sites, though not in the Alder and Orchard sites. Similar effects were observed also by Ishibashi and Kondo (1986) after an EPN application to pots of soil. Omnivorous dorylaims are probably more susceptible to the decomposition by-products produced by the detritivorous flora than are the colonizers (B and FPF), which thrive directly on such detritivorous material (Johnson et al., 1974; Bongers, 1990). The omnivores may also have been adversely affected by metabolites produced by

Photorhabdus sp., the symbiont of *H. megidis*, which were released from the disintegrating EPN-infected cadavers or by the EPN cadavers themselves, as was demonstrated against plant-parasitic nematodes by Grewal et al. (1999) following applications of heat-killed steinernematids in the laboratory. Part of the observed response of soil nematodes to an application of *H. megidis* could thus be a direct adverse impact on some susceptible genera, and this might explain partly the decrease in omnivorous nematodes caused by the EPN applications. The metabolite-killed organisms could themselves promote bacterial growth following decomposition, positively stimulating the detrital food web, including nematode bacterivores and fungivores. Although, Chen et al. (1994) showed that the negative effects of metabolites produced by EPN bacterial symbionts have a temporary effect on soil flora of only a few days. Considering the fluctuation in *H. megidis* population densities observed in the Grassland sites, which suggested at least some host cadaver decay, it is likely that this direct effect of the metabolites occurred many times post-treatment, not only for a few days following the initial application. As observed in previous studies under field conditions where insects were present (Smitley et al., 1992; Grewal et al., 1999) or in insect-free pots (Bird and Bird, 1986; Ishibashi and Kondo, 1986), reductions in the relative number of obligate herbivores were observed temporarily at all sites, including the Alder Stand site, where live and dead *H. megidis* were applied. Plots from both living and dead *H. megidis* treatments also had a lower plant-parasitic index (PPI) than did the controls, suggesting that disturbance resistant herbivorous genera were favored, or that the number of susceptible herbivorous nematode genera had decreased. Comparison of the application of the dead and live *H. megidis* treatments at the Alder Stand site shows that many similar changes in soil nematode numbers were initiated by these treatments, regardless of whether EPN were alive or dead at the time of application.

It is apparent from the changes recorded in the treated plots at all sites that the application of *H. megidis* produced, either directly or indirectly, a disturbance of the nematode community and that this was similar to a fertilizer-type enrichment, probably triggered by the decay of dead EPN. If repeated frequently, such disturbance could affect negatively the populations of susceptible nematodes such as some omnivorous dorylaimids, but in the absence of repeated applications the changes to this group would appear to be only temporary, as shown in this study.

The extent of the impact of a *H. megidis* application on soil organism population densities depends, in part, on the persistence of the nematodes that are applied to the soil. At the Grassland site, *H. megidis* population density fluctuations in the soil followed the typical, post-release population curve described by Smits (1996), in that a rapid decline in population density was followed by alternating increases and decreases in density. However, in contrast with the typical population curve, instead of reaching a constant background level of population density, *H. megidis* was not recovered for periods of several weeks and then reappeared. Two causes for *H. megidis* post-application absences appear possible. Their persistence in the soil for more than five months, at least from May to November, could indicate that *H. megidis* survived for many generations by cycling through and remaining inside certain soil-inhabiting hosts to survive adverse ambient soil conditions. Alternatively, *H. megidis* could have migrated deeper into the soil in the drier summer months, below the top 7.5 cm of soil sampled at the Grassland site. *Heterorhabditis megidis* densities were positively correlated with moisture content and negatively correlated with temperature at the Grassland site.

This study did not demonstrate an impact of the *H. megidis* application on any group of larval insects and in that regard can be said to support those previous investigations that show no adverse effects of EPN on nontarget arthropods in the field (Georgis et al., 1991; Campbell et al., 1995; Wang et al., 2001).

Since all the habitats studied were not spatially replicated, the results obtained at each site cannot be generalized to similar ecosystems. Despite the apparent differences in vegetation and management between the sites studied, the different sites seemed to show a similar response to the *H. megidis* applications as measured by the soil organisms monitored, as well as a similar level of persistence of the applied EPN.

In conclusion, this study is the first to demonstrate that an application of an entomopathogenic nematode under field conditions produces changes to the population densities of bacterivorous, facultative herbivorous, and omnivorous nematodes. The study provides evidence that the application of *H. megidis* had direct or indirect enrichment effects on soil organisms in the Grassland, Alder Stand, apple Orchard and Sports Field soils. No treatment impact on nontarget larval insect populations was detected. The nutrient enrichment-type impact of the *H. megidis* treatment was mostly temporary.

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