Ecology Letters, (2005) **8**: xxx–xxx doi: 10.1111/j.1461-0248.2005.00762.x

LETTER

Soil community composition drives aboveground plant–herbivore–parasitoid interactions

T. M. Bezemer,[1,2,3,4]* G. B. De Deyn,[1] T. M. Bossinga,[1] N. M. van Dam,[1] J. A. Harvey[1] and W. H. Van der Putten[1,3]

[1]Netherlands Institute of Ecology (NIOO-KNAW), Centre for terrestrial Ecology, PO Box 40, 6666 ZG Heteren, The Netherlands

[2]Nature Conservation and Plant Ecology Group, Wageningen University and Research Centre, Bornsesteeg 69, 6708 PD Wageningen, The Netherlands

[3]Laboratory of Nematology, Wageningen University and Research Centre, PO Box 8123, 6700 ES Wageningen, The Netherlands

[4]Laboratory of Entomology, Wageningen University and Research Centre, PO Box 8031, 6700 EH Wageningen, The Netherlands

**Correspondence: E-mail: martijn.bezemer@wur.nl*

Abstract

Soil organisms can influence higher trophic level aboveground organisms, but only very few studies have considered such effects. We manipulated soil community composition of model grassland ecosystems by introducing nematode communities, microorganisms, neither or both groups. Above ground, aphids (*Rhopalosiphum padi*) and parasitoids (*Aphidius colemani*) were introduced, and we measured individual performance and population dynamics of plants, aphids and parasitoids. In microcosms with nematode inoculations either with or without microorganism inoculation, aphids offspring production was significantly reduced by 31%. Aphid populations on both host plants *Agrostis capillaris* and *Anthoxanthum odoratum* were lowest in microcosms with combined nematode and microorganism inoculations. Opposite results were found for parasitoids. While the number of emerged parasitoids did not differ between treatments, parasitoid mortality and the proportion of males were significantly lower in microcosms with nematode and microorganism inoculations. Parasitized aphids were significantly larger in microcosms with nematodes inoculated. Plant biomass did not differ, but in the preferred host plant *A. odoratum*, foliar phenolic content was reduced in the presence of nematodes, and also the concentration of amino acids in the phloem. This study shows that the composition of the soil community matters for aboveground multitrophic interactions.

Keywords

Aboveground–belowground interactions, aphid, *Aphidius colemani*, fitness, herbivory, microcosm, nematode, parasitoid, *Rhopalosiphum padi*.

Ecology Letters (2005) 8: xxx–xxx

INTRODUCTION

Soil organisms influence the structure, composition and functioning of plant communities (Wardle 2002; De Deyn *et al.* 2003), the performance of aboveground herbivores (Masters *et al.* 1993) and have the potential to influence third and higher trophic level aboveground organisms (Van der Putten *et al.* 2001; Wäckers & Bezemer 2003; Wardle *et al.* 2004). Experimental studies show that soil organisms directly associated with plant roots (e.g. root feeding insects, arbuscular mycorrhizal fungi), as well as organisms belonging to the decomposer subsystem (collembola, protozoa and earthworms), can either increase or decrease aboveground insect herbivore performance via the shared host plant (Masters *et al.* 1993; Gange & West 1994; Goverde *et al.* 2000; Bonkowski *et al.* 2001; Masters *et al.* 2001; Scheu *et al.* 1999; Bezemer *et al.* 2003; Blossey & Hunt-Joshi 2003; Newington *et al.* 2004; Wurst *et al.* 2004). The variation in results of these studies suggest that aboveground–belowground interactions are highly dependent on the organisms studied and may be constrained by the many indirect effects (Wardle *et al.* 2004; Van Ruijven *et al.* 2005).

To date, most above–belowground interactions have focused on soil herbivore (or decomposer)–plant–aboveground herbivore systems, while only very few studies have considered influences of soil organisms on higher trophic level interactions above ground. One of the rare above–belowground studies that has included natural enemies of herbivores showed that root feeding insects

can increase the parasitism rate of seed feeding insects (Masters *et al.* 2001). Furthermore, arbuscular mycorrhizal infection can also affect aboveground parasitism rates but results are strongly influenced by the identity of the mycorrhizal species (Gange *et al.* 2003). However, parasitism of plants by aboveground aphids was unaffected by the presence or absence of earthworms below ground (Wurst & Jones 2003). Therefore, when adding trophic complexity to these above–belowground studies, the observed patterns appear to be highly species-dependent.

Experimental testing of the consequences of all possible species combinations would require endless numbers of experiments. As an alternative to adding individual species of phytophages, mutualists or decomposer organisms to study their influences on aboveground multitrophic interactions, studies may need to include different levels of complexity of species mixtures (e.g. Bradford *et al.* 2002). These studies can include effects of, for example, root-feeding nematodes which may substantially influence plant growth and aboveground plant nutritional quality (Hanounik & Osborne 1977; Bardgett *et al.* 1999) and of bacterivorous and fungivorous nematodes, microorganisms and decomposers which may influence plant nutrition (Wardle 2002) and plant defence (Van Loon *et al.* 1998). Changes in plant quality may lead to changes in aboveground herbivorous insect quality (Harvey *et al.* 2003), which, in turn, is known to have significant effects on the development of individual parasitoids, including fitness-related parameters such as adult size, development time, offspring sex ratio and mortality (Godfray 1994). The only published study that has measured the indirect effects of a soil organism on parasitoid quality revealed that parasitoid size was unaffected by the presence of earthworms (Wurst & Jones 2003). This result, however, may have been the result of either the relatively short period of the study (2 days) or because of a relatively limited influence of earthworms on aboveground interactions between second and higher trophic level organisms.

Here we report the results of a 1-year experiment in which we manipulated soil community composition of a model grassland ecosystem, by introducing nematode communities, microorganisms, neither or both groups. To test the hypothesis that soil community composition influences interactions between higher trophic level organisms above ground (Van der Putten *et al.* 2001), aphids and parasitoids were introduced, and we measured individual performance and population dynamics of plants, aphids and parasitoids. We show that soil community composition affects plant nutritional quality for aphids and thereby the fitness of an aphid parasitoid, as well as that soil-dwelling nematodes have a positive effect on the performance of aboveground parasitoids.

MATERIAL AND METHODS

The experiment was carried out in a climate controlled greenhouse at 60% RH, 16 L : 8 D, and 20 ± 1 °C at day and 14 ± 1 °C at night. Natural daylight was supplemented with 400 W metal halide bulbs (1 per 1.5 m^2). Throughout the experiment, light intensities were at least 300 PAR during daytime. Thirty six 8 L pots (20 cm height, 26 cm diameter) were filled with gamma irradiated (25 Kgray) sandy loam soil (particle size distribution: <2 μm 3%; 2–63 μm 17%; >63 μm 80%). After filling the pots, soil was saturated, and then flushed with 2 L of water to remove nutrients released as a result of the soil sterilization (Troelstra *et al.* 2001). Into each pot, one 2-week-old seedling of each of 12 species that typically co-occur in Dutch mid-succession grasslands was planted. The position of each species within the pot was randomized. Seedlings were germinated on glass beads. Three grasses (*Agrostis capillaris*, *Anthoxanthum odoratum*, *Festuca ovina*), one legume (*Lotus corniculatus*) and eight other forbs (*Achillea millefolium*, *Campanula rotundifolia*, *Cerastrium fontana*, *Plantago lanceolata*, *Prunella vulgare*, *Rumex acetocella*, *Senecio jacobaea*, *Tripleurospermum matricariae*) were planted into each pot. Two weeks after transplanting, pots were randomly divided over four treatments (nine replicates per treatment): control without soil inoculum (C), inoculum with soil microorganisms (MO), nematodes (N), or microorganisms and nematode addition (MO + N). The soil microorganism inoculum was adapted from a procedure described in Jones *et al.* (1998), by mixing 5 kg mid-succession grassland soil with 5 L tap water. The mixture was left for 4 h to enable large soil particles to become settled and then the supernatant was sieved through 75 μm followed by two sieves of 45 μm. These sieves omitted the nematodes but let most of the microorganisms in the suspension pass through. However, spores of most species of arbuscular mycorrhiza may not have passed through the sieves. Nematodes were extracted from 36 kg of the same grassland soil as used for the microorganism inoculum using Cobbs' decantation and sieving method (1 × 180 μm, followed by 1 × 75 μm, and 3 × 45 μm). Nematodes were collected from the 75 and 45 μm sieves and incubated for 48 h on two filters (Hygia favorit, 220 mm; NIPA Instruments, Leeuwarden, The Netherlands). Pots with nematode inoculation received the following nematode community: 1350 plant associates (98% Tylenchidae), 1660 plant feeders (48% Paratylenchidae, 28% Pratylenchidae, 9% Dolichodoridae), 6500 bacterial feeders (51% Rhabditidae, 33% Cephalobidae, 11% Plectus), 600 fungivores (90% Aphelenchoidae, 9% Tylolaimophorus) and 300 omni-carnivores (95% Dorylaimida). Inoculated nematode densities were not untypical for semi-natural grasslands. Into each pot (MO, N, or both) 6 mL inoculum was injected into the soil with a pipette adjacent to each of the 12 plant positions (72 mL total). Control pots received the same amount of water.

Pots were randomly rearranged within the greenhouse once a week. After 2, 4 and 6 months, aboveground biomass was clipped at 4 cm above the soil surface (De Deyn *et al.* 2003). Ten weeks after the third clipping, all microcosms were individually placed inside cylindrical meshed cages equipped with a zip to allow entrance into the cage (height 1.2 m, diameter 35 cm). Into each microcosm 25 late instar bird cherry-oat aphids (*Rhopalosiphum padi* L.) were then introduced. The aphids, which exclusively feed on grasses, were obtained from the Laboratory of Entomology (Wageningen, The Netherlands), and had been reared on barley (*Hordeum vulgare*). Eight weeks later, after aphid populations had established, 10 female and 10 male parasitoids (*Aphidius colemani* Vierek, Hymenoptera: Braconidae) were introduced into each microcosm. The parasitoids were obtained from Koppert Biological Systems (Berkel en Rodenrijs, The Netherlands).

Aphid measurements

Once every 7 days, starting 12 days after introduction, all aphids that occurred on the grasses *A. capillaris* and *A. odoratum* were counted in each microcosm, by carefully checking each grass leaf and recording the aphid density on it. Aphids were rarely found on *F. ovina*, these data were therefore not included in the study. Aphid population dynamics were analysed using two-way repeated measures analysis of variance (RANOVA). As populations followed a clear nonlinear pattern over time (see Results), data were log-transformed to obtain normality and homogeneity of variance, and analysed using second order polynomials with microorganisms and nematodes as fixed factors. To obtain an indication of individual aphid performance, 6 weeks after introduction, within each microcosm fifth instar aphid nymphs were selected and placed individually within clip-on cages of 2 cm diameter (Bezemer *et al.* 1999) on fully mature leaves of *A. capillaris* or *A. odoratum*. Once a day, each clip-on cage was checked for offspring, and from the first day of reproduction onwards, number of daily offspring was recorded for at least five successive days. Settling time was defined as the number of days between introduction of the aphid into the clip-on cage and day of first reproduction. Mean daily offspring per microcosm and settling time were analysed using two-way ANOVA with MO and N as fixed factors.

Parasitoid measurements

Seven days after introduction of the parasitoids the first aphid mummies (parasitoid cocoons) appeared. From the day of first aphid mummy appearance until after all mummies had emerged (8 days after first appearance) all microcosms were checked twice a day for mummies. Mummies were carefully removed from the leaf, placed individually in plastic vials, and these vials were then checked three times a day for parasitoid emergence. After emergence, parasitoids were anaesthetized with CO_2, sexed, weighed using a microbalance and released back into the same microcosm. The length and width of the empty mummy was recorded. For each microcosm, mortality was calculated as the percentage of mummies failing to emerge. Length and width of dead mummies were also recorded, so that we could compare size of emerged and dead mummies. Seven days after the last parasitoid emerged from the first generation, second generation emergence started.

Levels of parasitism were much higher during the second generation, and unfortunately it was not possible to collect all mummies. Instead, all adult parasitoids that emerged within a microcosm were collected individually in glass vials three to four times per day for a period of 7 days. At each collection, each microcosm was inspected for parasitoids for 5 min. Parasitoids were sexed, and weighed but not released back into the microcosms. Sex ratios in each microcosm were calculated for both generations. Mortality and sex ratio data were arcsine transformed and analysed using a three-way ANOVA with MO, N and generation as main factors. Cumulative parasitoid emergences were analysed using RANOVA. As more than one individual emerged from each microcosm (up to 132 during the second generation) adult weight was analysed with a mixed model using residual analysis (REML) in Genstat 7. The analysis calculates Wald-type *F*-statistics and can process different sources of variation, enabling the use of all individuals rather than using averages per microcosm (Piepho *et al.* 2003). Data were analysed using sex, generation, MO and N as fixed factors and microcosm as random factor.

Plant, soil and nematode measurements

For *A. odoratum* the concentration and composition of amino acids was determined 10 weeks after aphid introduction. From one plant in each microcosm, one fully mature but not senesced leaf was cut off with a sharp scalpel at the base and placed immediately in a 0.5 mL Eppendorf tube containing 200 μL 8 mM EDTA solution pH = 7.0 (Merck, Darmstadt, Germany). The leaf-containing tubes were then placed in the dark at 20 °C (Sandström *et al.* 2000). After 2 h, the leaves were removed and the tubes with EDTA and phloem sap were frozen at −20 °C until analysis. Two tubes containing only EDTA solution were treated similarly to determine the background of amino acids in the EDTA solution. Free amino acids were analysed on reversed phase HPLC, following derivatization with *o*-phtaldialdehyde and *N*-isobutyrylcysteine as in Fitznar *et al.* (1999), using a Waters Alliance

2690 separation module (Waters Corporation, Milford, MA, USA), equipped with a Nova-Pak C18 3.9 × 150 mm column (Waters Corporation) with an Alltech Allsphere guard column (Alltech Associates, Deerfield, IL, USA) and a Water fluorescence detector 474 (Waters Corporation). Amino acid concentrations in the extract were calculated based on a series of standard amino acid solutions (Pierce, Rockford, IL, USA) supplemented with asparagine, glutamine and the derivate of glutamate, γ-amino butyric acid (GABA). Amino acid composition was analysed using two-way MANOVA with MO and N as fixed factors followed by canonical correlation analysis. Standardized canonical coefficients were used to determine the contribution of individual amino acids to the observed effects.

One week after final parasitoid collection, soil samples were taken (4 cores, 2 cm diameter), and all aboveground plant material was clipped, sorted to species and oven-dried at 70 °C. After weighing, *A. capillaris* and *A. odoratum* leaf material from each microcosm was ground and analysed for total phenolic content [following extraction method described in Bezemer *et al.* (2000), see also Waterman & Mole (1994)], and carbon and nitrogen content using total combustion. Soil samples were analysed using a $CaCl_2$-extraction for available nitrogen (NO_3^-, NH_4^+) phosphorous (PO_4^-) and potassium (K^+). All chemistry data were arcsine transformed prior to analysis and data were analysed using two-way ANOVA with MO and N as fixed factors.

Nematode densities in each microcosm were determined at the end of the experiment by taking four soil cores (2 cm diameter) and extracting nematodes from 100 g of soil using an Oostenbrink elutriator (Oostenbrink 1960). Nematodes were identified to genus or family level, and allocated to feeding groups according to Yeates *et al.* (1993). Nematode densities were log-transformed and analysed using two-way MANOVA with MO and N as fixed factors, followed by canonical correlation analysis.

RESULTS

Aphids

Independent of plant species, daily aphid offspring was significantly reduced in the presence of nematodes regardless of whether microorganisms were inoculated (effect of N: $F = 7.52$, $P = 0.008$). While the effect was qualitatively similar for both grass species, the reduction was significant for *A. capillaris* ($F = 7.21$, $P = 0.012$) but not for *A. odoratum* ($F = 1.77$, $P = 0.19$; Fig. 1a). Aphid settling time, defined as the time from introduction on the leaf until reproduction, was not significantly affected by soil inoculum treatments, but was generally longer on *A. capillaris* (mean ± SE: 4.0 ± 0.5 days) than on *A. odoratum* (mean ± SE: 2.3 ± 0.3 days). Aphid populations were, on average, six times higher on *A. odoratum* than on *A. capillaris* (Fig. 1b). Overall, aphid populations were significantly lower in the presence of nematodes, independent of microorganisms ($F = 4.33$, $P = 0.03$), but when both grass species were analysed separately, this was only significant for *A. capillaris* ($F = 5.53$, $P = 0.025$). On both plant species, aphid populations were, on average, lowest in the combined MO × N treatment.

Parasitoids

While aphids were negatively affected by nematodes, opposite results were found for parasitoids. First generation

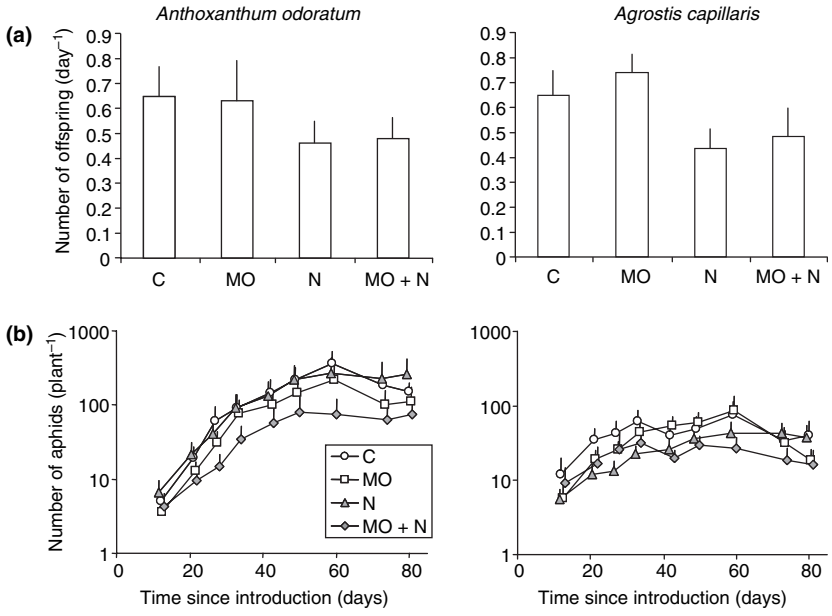

Figure 1 Aphid performance. Mean (+SE) (a) number of offspring of individual aphids and (b) population dynamics on *A. capillaris* and *A. odoratum* in microcosms with different soil faunal compostions. C, control; MO, microorganism inoculation; N, nematode inoculation; MO + N, microorganism and nematode inoculation.

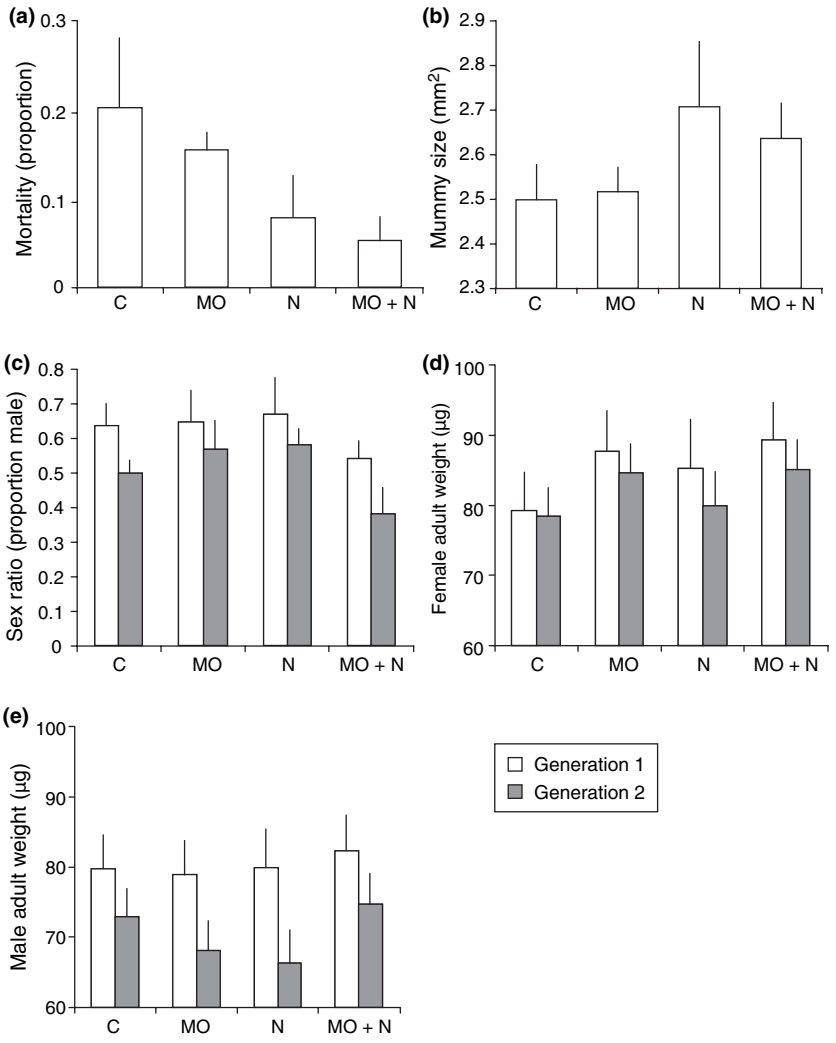

Figure 2 Parasitoid performance. Mean (a) mortality, (b) mummy size, (c) sex ratio and (d) female and (e) male weight during two successive generations in microcosms with different soil faunal compositions. Vertical bars indicate 1 standard error. C, control; MO, microorganism inoculation; N, nematode inoculation; MO + N, microorganism and nematode inoculation.

mortality of parasitoids was significantly reduced by 50–70% in the presence of nematodes (effect of N: $F = 10.38$, $P = 0.003$, Fig. 2a). Mummies from parasitoids that did not emerge were on average 13% smaller than from those that did emerge ($F = 10.99$, $P = 0.002$) and this was independent of treatments. There was a highly significant positive relationship between parasitoid size and mummy size ($F = 428.1$, $P < 0.0001$, $R^2 = 0.69$), indicating that parasitoid mortality was the result of aphid size. Mummy size was significantly larger in microcosms with nematodes (MO: $F = 0.11$, $P = 0.74$; N: $F = 4.1$, $P = 0.05$, MO × N: $F = 0.29$, $P = 0.60$; Fig. 2b). A total of 443 parasitoids emerged during the first and 1919 during the second generation. There was no difference in the number of parasitoids that emerged or the number of mummies collected from the different inoculum treatments, and parasitoid density was not related to aphid density (data not shown).

Sex ratios of parasitoids (proportion of males) were lower during the second generation than the first generation ($F = 4.88$, $P = 0.03$), but during both generations they were lowest in the combined treatment resulting in a significant MO × N interaction ($F = 4.93$, $P = 0.03$, Fig. 2c). Adult parasitoids were small in all treatments, indicating probable low aphid host quality. Females and males weighed about 85 and 75 µg, respectively, far less than introduced adults that weighed between 150 and 200 µg. Males were smaller than females (Wald = 103.6, $P < 0.001$), and adults from the second generation were significantly smaller than those from the first generation (Wald = 24.6, $P < 0.001$). Independent of treatments, male parasitoid weight was far more reduced between generations (on average 13%) than that of females (on average 4%); this resulted in a significant interaction between sex and generation (Wald = 5.1, $P = 0.02$, Fig. 2d,e). For both parasitoid sexes weight was, on average, highest in the combined MO + N treatment during both generations, but while male weight was strongly reduced in the separate inoculation treatments, this was not so for females. This resulted in a significant sex × MO × N

interaction (Wald = 11.64, $P < 0.001$). The largest females were found in the MO treatments (sex × MO: Wald = 5.6, $P = 0.02$).

Plants

Total amino acid concentrations in *A. odoratum* phloem sap tended to be lower in microcosms with nematodes added although this was not significant (MO: $F = 0.07$, $P = 0.79$, N: $F = 3.43$; $P = 0.07$; MO × N: $F = 0.90$, $P = 0.35$). A total of 18 amino acids were detected, and the composition of amino acids varied significantly between treatments (Wilk's lambda: MO: $F = 2.54$, $P = 0.04$; N: $F = 4.61$, $P = 0.002$, MO × N: $F = 2.27$, $P = 0.06$). Inspection of the standardized canonical coefficients revealed that the amino acids aspartate, glutamine, isoleucine and threonine contributed most to the observed effects (Table 1). For the majority of amino acids lowest concentrations were found in the combined MO and N treatment, while two essential amino acids arginine and methionine appeared absent in the MO + N treatment (Table 1).

After 6 months, *A. odoratum* shoot biomass was significantly lower in microcosms with microorganisms added while *A. capillaris* biomass did not differ between treatments (Table 2). At the end of the experiment, and for both plant species, aboveground biomass did not differ between treatments but did within microcosms: *A. odoratum* had more aboveground biomass than *A. capillaris* (Table 2). In presence of nematodes, nitrogen levels were significantly reduced in *A. capillaris* ($F = 4.63$, $P = 0.03$) resulting in significantly higher C : N ratios ($F = 4.50$, $P = 0.04$). No significant treatment differences were detected for *A. odoratum* nitrogen levels but, for both species, lowest nitrogen levels were found in the treatment where nematodes alone were added. When analysed together there was a significant nematode effect for nitrogen ($F = 6.18$, $P = 0.015$) and C : N ratios ($F = 5.50$, $P = 0.02$). Nitrogen levels were significantly lower ($F = 10.61$, $P = 0.002$) in *A. odoratum* than *A. capillaris*.

Total phenolic concentration in *A. capillaris* was higher than in *A. odoratum* ($F = 54.14$, $P < 0.0001$). There was also a difference between treatments but the direction of the response differed between plant species (Table 2). In *A. odoratum* phenolic concentration was significantly reduced in the presence of nematodes, while in *A. capillaris* levels were lower in the MO and N treatments than in the control, but were highest in the combined MO + N treatment, resulting in a significant MO × N interaction for this species (Table 2).

At the end of the experiment levels of available phosphorous and potassium in the soil were very low in

Table 1 Concentration ($\mu M\ l^{-1}$) and percentage (±SE) of amino acids in the phloem sap of *Anthoxanthum odoratum* exposed to different soil inoculation treatments

	C		MO		N		MO + N		
	Concentration	%	Concentration	%	Concentration	%	Concentration	%	SCC
Ala	1.29 ± 0.74	15.2	0.72 ± 0.11	11.5	0.47 ± 0.11	12.6	0.48 ± 0.10	14.8	−0.46
Arg*	0.25 ± 0.17	2.2	0.06 ± 0.02	1.0	0.13 ± 0.08	2.1	0.00 ± 0.00	0.0	0.65
Asn	0.19 ± 0.08	2.4	0.09 ± 0.03	1.5	0.06 ± 0.02	1.2	0.02 ± 0.01	0.6	0.35
Asp	0.56 ± 0.13	13.0	0.79 ± 0.12	12.5	0.46 ± 0.08	12.9	0.45 ± 0.08	14.5	−0.91
GABA	0.28 ± 0.07	5.1	0.40 ± 0.09	6.3	0.17 ± 0.03	5.4	0.22 ± 0.05	6.8	−0.09
Gln	0.30 ± 0.08	5.6	0.33 ± 0.05	5.2	0.14 ± 0.02	4.0	0.19 ± 0.05	5.8	0.65
Glu	1.18 ± 0.35	22.1	1.77 ± 0.36	26.9	0.78 ± 0.12	22.1	0.69 ± 0.14	21.7	1.09
Gly	0.39 ± 0.10	6.8	0.42 ± 0.09	6.4	0.33 ± 0.12	7.5	0.17 ± 0.04	5.8	0.63
His*	0.10 ± 0.05	1.3	0.15 ± 0.03	2.5	0.07 ± 0.02	1.4	0.04 ± 0.02	1.2	−0.12
Ile*	0.13 ± 0.04	2.1	0.11 ± 0.01	2.0	0.11 ± 0.02	3.0	0.09 ± 0.01	3.4	−1.23
Leu*	0.18 ± 0.07	2.3	0.14 ± 0.02	2.3	0.12 ± 0.04	2.7	0.06 ± 0.01	1.9	−0.32
Lys*	0.10 ± 0.04	1.6	0.06 ± 0.02	0.8	0.08 ± 0.04	1.5	0.04 ± 0.01	1.0	−0.17
Met*	0.02 ± 0.01	0.1	0.05 ± 0.02	0.5	0.03 ± 0.02	0.5	0.00 ± 0.00	0.0	−0.06
Phe*	0.12 ± 0.05	1.6	0.12 ± 0.03	1.8	0.08 ± 0.02	2.1	0.04 ± 0.01	1.2	0.28
Ser	0.51 ± 0.15	9.0	0.65 ± 0.08	10.9	0.55 ± 0.24	12.1	0.35 ± 0.04	12.9	0.68
Thr*	0.19 ± 0.06	2.6	0.24 ± 0.06	3.4	0.18 ± 0.07	3.8	0.11 ± 0.03	3.4	−1.01
Tyr*	0.53 ± 0.44	3.4	0.09 ± 0.02	1.4	0.09 ± 0.03	1.9	0.06 ± 0.03	1.5	0.66
Val*	0.22 ± 0.08	3.5	0.19 ± 0.03	3.0	0.14 ± 0.04	3.4	0.11 ± 0.02	3.4	−0.23

Standardized canonical coefficients (SCC) for the first, and only significant canonical correlation are also presented.
Asterisks denote essential amino acids.
C, control; MO, microorganism communities; N, nematode communities; MO + N, microorganism and nematode communities; Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartate; GABA, γ-aminobutric acid; Gln, glutamine; Glu, glutamate; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Ser, serine; Thr, threonine; Tyr, tyrosine; Val, valine.

Table 2 Mean (±SE) aboveground biomass, foliar nitrogen concentration, C : N ratio and phenolic concentration in *A. capillaris* and *A. odoratum* growing in microcosms with different soil faunal compositions, nutrient availability in the soil after 12 months, and *F*-values of an ANOVA test

					ANOVA results		
	C	MO	N	MO + N	MO	N	MO × N
A. capillaris							
Biomass (g) (6 months)	2.6 ± 0.3	1.9 ± 0.3	2.1 ± 0.2	1.8 ± 0.3	2.44	0.62	0.41
Biomass (g) (12 months)	4.1 ± 0.6	3.8 ± 0.6	4.7 ± 0.6	5.6 ± 1.0	0.46	0.27	0.08
% N	1.13 ± 0.07	1.11 ± 0.05	0.94 ± 0.05	1.05 ± 0.06	0.71	4.63*	1.38
C : N ratio	39.0 ± 2.3	39.7 ± 1.8	46.8 ± 2.6	41.8 ± 2.5	0.87	4.50*	1.48
Phenolics (mg g^{-1})	12.0 ± 1.0	10.7 ± 0.5	11.3 ± 0.5	12.7 ± 0.4	0.04	1.27	4.24*
A. odoratum							
Biomass (g) (6 months)	3.8 ± 0.3	3.3 ± 0.5	4.4 ± 0.4	3.0 ± 0.5	4.54*	0.01	0.65
Biomass (g) (12 months)	11.4 ± 1.3	12.1 ± 1.3	11.7 ± 1.7	10.8 ± 1.6	0.02	0.15	0.14
% N	0.97 ± 0.05	0.96 ± 0.05	0.84 ± 0.04	0.95 ± 0.05	0.88	1.79	1.66
C : N ratio	45.5 ± 2.6	46.2 ± 2.5	52.1 ± 2.8	45.9 ± 2.3	1.54	1.17	1.76
Phenolics (mg g^{-1})	9.7 ± 0.8	8.6 ± 0.7	8.0 ± 0.3	7.8 ± 0.4	1.13	4.63*	0.53
Soil							
NO_3^- (mg kg^{-1})	1.3 ± 0.4	15.5 ± 3.3	8.2 ± 1.3	10.8 ± 2.5	17.73***	4.93*	12.91**
NH_4^+ (mg kg^{-1})	13.5 ± 2.5	4.0 ± 0.7	7.2 ± 1.1	4.1 ± 0.3	19.50***	1.65	2.78
PO_4^- (mg kg^{-1})	0.72 ± 0.08	0.75 ± 0.05	0.64 ± 0.02	0.72 ± 0.05	1.30	0.92	0.09
K^+ (mg kg^{-1})	0.68 ± 0.42	0.54 ± 0.54	6.05 ± 3.02	0.09 ± 0.07	1.61	0.01	1.00

C, control; MO, microorganism communities; N, nematode communities; MO + N, microorganism and nematode communities.
Asterisks denote significant effects.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

all microcosms, but did not differ between treatments (Table 2). In presence of microorganisms the availability of ammonium was significantly reduced. Control microcosms had highest levels of ammonium and lowest levels of nitrate, while soil nitrate levels were highest in microcosms with MO inoculations (Table 2).

Nematodes

At the end of the experiment nematode abundance still differed considerably between treatments (Wilk's lambda: MO: $F = 3.49$, $P = 0.01$; N: $F = 64.84$, $P < 0.0001$, MO × N: $F = 4.11$, $P = 0.005$). This effect was not only caused by differences in nematode densities of plant feeders and plant associates but also by differences in bacterivores and carnivores (standardized canonical coefficients; Table 3). Plant feeders and plant associates were abundant in microcosms with nematode additions but not in the other microcosms. Abundance was lower in the MO + N treatment than when nematodes were added alone (N treatment; Table 3). Bacterial feeders were relatively abundant in all treatments, but densities were somewhat lower in N treatments. Carnivore densities were very low but appeared to be somewhat higher in treatments with microorganism inoculations (Table 3).

DISCUSSION

Soil community composition can influence the dynamics of an aboveground multitrophic system through influen-

Table 3 Mean (±SE) number (per 100 g soil) of nematodes in microcosms 12 months after inoculation with different soil communities

Functional group	C	MO	N	MO + N	SCC1	SCC2
Plant feeders	9 ± 8	104 ± 88	14169 ± 4150	4967 ± 2076	−0.42	0.07
Plant associates	1 ± 1	22 ± 15	1891 ± 632	494 ± 172	−0.21	−0.80
Bacterial feeders	1715 ± 480	1853 ± 439	1197 ± 338	979 ± 424	0.06	0.43
Fungal feeders	136 ± 73	90 ± 66	15 ± 6	19 ± 9	0.02	0.002
Carnivores	0 ± 0	68 ± 49	18 ± 10	148 ± 40	−0.22	1.21
Omnivores	0 ± 0	1 ± 1	94 ± 35	78 ± 16	−0.29	0.04

Standardized canonical coefficients (SCC) for the first, and second canonical correlation are also presented. C, control; MO, microorganism communities; N, nematode communities; MO + N, microorganism and nematode communities.

cing plant and aphid quality. In this study, addition of a mixture of nematode feeding groups to microcosms with multi-species plant communities had a profound negative influence on aboveground aphids. However, secondary consumers (the parasitoids) were positively influenced by the combined inoculation of nematodes and microorganisms, suggesting that the composition of the soil community matters for aboveground multitrophic interactions.

Effects of soil community composition on aphids

Nematode addition reduced foliar nitrogen and amino acid concentrations. This indicates that the nematode effects on aphids occur through a reduction in the nutritional quality of the aboveground plant parts. Plants exposed to root-feeding nematodes can have reduced nitrogen contents in foliage (Khan & Khan 1995), but we are not aware of any study that has shown that soil nematodes can alter foliar amino acid concentrations. By flushing the soil and removing aboveground biomass, we know from other experiments that all microcosms in this study were nutrient limited. The additional negative effects of the soil community on foliar nitrogen can, under conditions similar to what may be experienced in the field, significantly influence aboveground insect performance. Our results may have implications for our understanding of plant herbivore–parasitoid dynamics as nematodes are an important component of terrestrial foodwebs (De Ruiter *et al.* 1995).

Different mechanisms have been proposed to explain interactions between aboveground and belowground organisms mediated through a shared host plant. The induced stress hypothesis (Gange & Brown 1989; Masters *et al.* 1993) assumes that root herbivory induces a stress response within the host plant leading to the accumulation of amino acids and carbohydrates in the foliage which enhances the performance of foliar feeders. Alternatively, the induced defence hypothesis (Bezemer *et al.* 2003; Van Dam *et al.* 2003) assumes that root herbivory induces a defence response in the plant, leading to higher levels of plant defence compounds in the foliage, or to altered aboveground distribution of these compounds (Bezemer *et al.* 2004) reducing the performance of aboveground herbivores. The present study suggests that soil organisms may alter both primary and secondary plant compounds concurrently, and that they may not necessarily enhance concentrations of primary or secondary metabolites in the foliage. As nematode addition reduced the concentrations of both amino acids and phenolic compounds in the dominant host plant, *A. odoratum*, the reduced performance of the aphids in our study may have been the result of altered primary plant compounds.

Effects of soil community composition on parasitoids

Parasitoid performance, in terms of fitness-related traits such as adult size, mortality and sex ratio, was highest in microcosms in which the most complex soil community was added. Interactions between aphids and their parasitoids are well studied (e.g. Sequeira & Mackauer 1992; Chau & Mackauer 2001) and the majority of these studies have reported that parasitoid size is strongly influenced by host (aphid) size at parasitism, with larger aphids producing larger (and presumably fitter) parasitoids (Sequeira & Mackauer 1992). Most of these studies were performed in a strictly bi-trophic framework, frequently ignoring effects of plant quality on herbivore and parasitoid development (Harvey *et al.* 2003). As far as we are aware, this is the first study to report that the development of higher trophic levels above ground can be affected indirectly by the composition of the community below ground through the effects of nematodes and other microorganisms on the quality of the shared plant.

Our results strongly suggest that the observed effects on parasitoids were the result of differences in host quality. Mummies from which no parasitoids emerged were significantly smaller than mummies from which the parasitoids had emerged. As emerging parasitoids were on average much smaller than the ones that were released, and which had been reared under optimal conditions, parasitoid mortality appears to have been the result of size or quality of the aphids. We believe that this also explains why the negative effect of soil community composition on aphids resulted in a positive effect on parasitoids. In the treatment with microorganisms and nematode inoculation, aphid populations were lowest, but during the second parasitoid generation individual aphids in those microcosms were larger than those in other microcosms (T.M. Bezemer, personal observation). During the first parasitoid generation mummy size, and thus aphid size, in microcosms with nematode introductions was also larger.

During the second generation, adult parasitoids were significantly smaller than during the first generation. It would appear that the larger aphids were preferred and parasitized first. Host selection and preference is well-studied in parasitoids, and is very often based on qualitative aspects of the host, such as size (Godfray 1994). Many empirical studies have also reported that female parasitoids assess host size before oviposition, and selectively allocate female offspring to large hosts and male offspring to small hosts. Large females are assumed to benefit more in terms of fitness than males of comparable size (Godfray 1994). Male weight fell more during the second generation than female weight, suggesting that larger hosts of higher quality were selected for female offspring.

Within the time span of our experiment, this variation in host selection behaviour did not result in differences at the population level. However, the results indicate that, in the longer term, soil community composition can determine the dynamics of the aboveground community, thereby potentially initiating an aboveground trophic cascade. A shortcoming of this study in which we introduced soil communities rather than individual organisms, is that we do not know which organisms or combination of organisms were responsible for the observed aboveground effects. While we were able to identify the nematode community that we introduced and established, we also do not know which microorganisms were introduced and established in the MO treatment. Most likely the MO inoculum would have contained a large variety of bacteria and fungi including decomposers, plant pathogens and mutualists. Further studies should address the role of specific groups of the microorganism community for influencing aboveground multitrophic interactions. In this study which is the first of its kind, using semi-natural grassland systems, we have shown that aboveground–belowground interactions not only occur with individual root herbivore–plant-aboveground herbivore combinations but also in more complex belowground-plant–aboveground communities. Therefore we conclude that interactions between plants and aboveground insect herbivores and parasitoids cannot be viewed independent of the interactions in the plant root zone.

ACKNOWLEDGEMENTS

The work described here was supported by the Framework V Programme of the European Community within the Energy, Environment and Sustainable Development Programme, Key Action Global Change, Climate and Biodiversity, TLinks EVK2-CT-2001-00123. We thank Sebastiaan Verkade, Ineke van Veen and Lotte Dijk for assistance, Marco Houtekamer en Jan Sinke from NIOO-CEME for amino acid analysis, and Hefin Jones, Ted Turlings and three anonymous referees for providing very valuable comments on an earlier version of the manuscript. NIOO publication 3554.

REFERENCES

Bardgett, R.D., Denton, C.S. & Cook, R. (1999). Below-ground herbivory promotes soil nutrient transfer and root growth in grassland. *Ecol. Lett.*, 2, 357–360.

Bezemer, T.M., Knight, K.J., Newington, J.E. & Jones, T.H. (1999). How general are aphid responses to elevated atmospheric CO_2? *Ann. Entomol. Soc. Am.*, 95, 724–730.

Bezemer, T.M., Newington, J.E. & Jones, T.H. (2000). Effects of carbon dioxide and nitrogen fertilization on phenolic content in Poa annua L. *Biochem. Syst. Ecol.*, 28, 839–846.

Bezemer, T.M., Wagenaar, R., Van Dam, N.M. & Wäckers, F.L. (2003). Interactions between above- and belowground insect herbivores as mediated by the plant defense system. *Oikos*, 101, 555–562.

Bezemer, T.M., Wagenaar, R., Van Dam, N.M., Van der Putten, W.H. & Wäckers, F.L. (2004). Above- and below-ground terpenoid aldehyde induction in cotton, *Gossypium herbaceum*, following root and leaf injury. *J. Chem. Ecol.*, 30, 53–67.

Blossey, B. & Hunt-Joshi, T. R. (2003). Belowground herbivory by insects: influence on plants and aboveground herbivores. *Annu. Rev. Entomol.*, 48, 521–547.

Bonkowski, M., Geoghegan, I.E., Birch, A.N.E. & Griffiths, B.S. (2001). Effects of soil decomposer invertebrates (protozoa and earthworms) on an above-ground phytophagous insect (cereal aphid) mediated through changes in the host plant. *Oikos*, 95, 441–450.

Bradford, M.A., Jones, T.H., Bardgett, R.D., Black, H.I., Boag, B., Bonkowski, M. *et al.* (2002). Impacts of soil faunal community composition on model grassland ecosystems. *Science*, 298, 615–618.

Chau, A. & Mackauer, M. (2001). Preference of the aphid parasitoid *Monoctonus paulensis* (Hymenoptera : Braconidae, Aphidiinae) for different aphid species: Female choice and offspring survival. *Biol. Control*, 20, 30–38.

De Deyn, G.B., Raaijmakers, C.E., Zoomer, H.R., Berg, M.P., De Ruiter, P.C., Verhoef, H.A. *et al.* (2003). Soil invertebrate fauna enhances grassland succession and diversity. *Nature*, 422, 711–713.

De Ruiter, P.C., Neutel, A.M. & Moore, J.C. (1995). Energetics, patterns of interaction strengths, and stability in real ecosystems. *Science*, 269, 1257–1260.

Fitznar, H.P., Lobbes, J.M. & Kattner, G. (1999). Determination of enantiomeric amino acids with high-performance liquid chromatography and pre-column derivatisation with o-phthaldialdehyde and N-isobutyrylcysteine in seawater and fossil samples (mollusks). *J. Chromatogr. A*, 832, 123–132.

Gange, A.C. & Brown, V.K. (1989). Effects of root herbivory by an insect on a foliar-feeding species, mediated through changes in the host plant. *Oecologia*, 81, 38–42.

Gange, A.C. & West, H.M. (1994). Interactions between arbuscular mycorrhizal fungi and foliar-feeding insects in *Plantago lanceolata* L. *New Phytol.*, 128, 79–87.

Gange, A.C., Brown, V.K. & Aplin, D.M. (2003). Multitrophic links between arbuscular mycorrhizal fungi and insect parasitoids. *Ecol. Lett.*, 6, 1051–1055.

Godfray, H.C.J. (1994) *Parasitoids. Behavioral and Evolutionary Ecology*. Princeton University Press, Princeton.

Goverde, M., Van der Heijden, M.G.A., Sanders, I.R., Wiemken, A. & Erhard, A., (2000). Arbuscular mycorrhizal fungi influence life history traits of a lepidopteran herbivore. *Oecologia*, 125, 362–369.

Hanounik, S.B. & Osborne, W.W. (1977). The relationships between population density of *Meloidogyne incognita* and nicotine content of tobacco. *Nematologica*, 23, 147–152.

Harvey, J.A., Van Dam, N.M. & Gols, R. (2003). Interactions over four trophic levels: foodplant quality affects development of a hyperparasitoid as mediated through a herbivore and its primary parasitoid. *J. Anim. Ecol.*, 72, 520–531.

Jones, T.H., Thompson, L.J., Lawton, J.H., Bezemer, T.M., Bardgett, R.D., Blackburn, T.M. *et al.* (1998). Impacts of rising atmospheric carbon dioxide on model terrestrial ecosystems. *Science*, 280, 411–413.

Khan, M.R. & Khan, M.W. (1995). Effects of ammonia and root-knot nematode on tomato. *Agr. Ecosyst. Environ.*, 53, 71–81.

Masters, G.J., Brown, V.K. & Gange, A.C. (1993). Plant mediated interactions between above- and below-ground insect herbivores. *Oikos*, 66, 148–151.

Masters, G.J., Jones, T.H. & Rogers, M. (2001). Host-plant mediated effects of root herbivory on insect seed predators and their parasitoids. *Oecologia*, 127, 246–250.

Newington, J.E., Setälä, H., Bezemer, T.M. & Jones, T.H. (2004). Potential effects of earthworms on leaf-chewer performance. *Funct. Ecol.*, 18, 746–751.

Oostenbrink, M. (1960). Estimating nematode populations by some selected methods. In: *Nematology* (eds. Sasser, J.N. & Jenkins, W.R.). University of North Carolina Press, Chapel Hill, NC, pp. 85–102.

Piepho, H.P., Büchse, A. & Emrich, K. (2003). A hitchhiker's guide to mixed models for randomized experiments. *J. Agron. Crop Sc.*, 189, 310–322.

Sandström, J., Telang, A. & Moran, N.A. (2000). Nutritional enhancement of host plants by aphids – a comparison of three aphid species on grasses. *J. Insect Physiol.*, 46, 33–40.

Scheu, S., Theenhaus, A. & Jones, T.H. (1999). Links between the detrivore and herbivore system: effects of earthworms and Collembola on plant growth and aphid development. *Oecologia*, 119, 541–551.

Sequeira, R. & Mackauer, M. (1992). Covariance of adult size and development time in the parasitoid wasp *Aphidius ervi* in relation to the size of its host *Acyrthosiphon pisum*. *Evol. Ecol.*, 6, 34–44.

Troelstra, S.R., Wagenaar, R., Smant, W. & Peters, B.A.M. (2001). Interpretation of bioassays in the study of interactions between soil organisms and plants: involvement of nutrient factors. *New Phytol.*, 150, 697–706.

Van Dam, N.M., Harvey, J.A., Wäckers, F.L., Bezemer, T.M., Van der Putten, W.H. & Vet, L.E.M. (2003). Interactions between aboveground and belowground induced responses against phytophages. *Basic Appl. Ecol.*, 4, 63–77.

Van der Putten, W.H., Vet, L.E.M., Harvey, J.A. & Wäckers, F.L. (2001). Linking above- and below-ground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. *Trends Ecol. Evol.*, 16, 547–554.

Van Loon, L.C., Bakker, P.A.H.M. & Pieterse, C.M.J. (1998). Systemic resistance induced by rhizosphere bacteria. *Annu. Rev. Phytopathol.*, 36, 453–483.

Van Ruijven, J., De Deyn, G.B., Raaijmakers, C.E., Berendse, F. & Van der Putten, W.H. (2005). Interactions between spatially separated herbivores indirectly alter plant diversity. *Ecol. Lett.*, 8, 30–37.

Wäckers, F.L. & Bezemer, T.M. (2003). Root herbivory induces an above-ground indirect defence. *Ecol. Lett.*, 6, 9–12.

Wardle, D.A. (2002) *Communities and Ecosystems. Linking the Aboveground and Belowground Components*. Princeton University Press, Princeton.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., Van der Putten, W.H. & Wall, D.H. (2004). Ecological linkages between aboveground and belowground biota. *Science*, 304, 1629–1633.

Waterman, P.G. & Mole, S. (1994) *Analysis of Plant Metabolites. Methods in Ecology Series*. Blackwell Scientific Publications, Oxford.

Wurst, S. & Jones, T.H. (2003). Indirect effects of earthworms (*Aporrectodea caliginosa*) on an above-ground tritrophic interactions. *Pediobiologia*, 47, 91–97.

Wurst, S., Dugassa-Gobena, D., Langel, R., Bonkowski, M. & Scheu, S. (2004). Combined effects of earthworms and vesicular-arbuscular mycorrhizas on plant and aphid performance. *New Phytol.*, 163, 169–176.

Yeates, G.W., Bongers, T., De Goede, R.G.M., Freckman, D.W. & Georgieva, S.S. (1993). Feeding habits in soil nematode families and genera. An outline for soil ecologists. *J. Nematol.*, 25, 315–331.

Editor, Ted Turlings
Manuscript received 10 January 2005
First decision made 16 February 2005
Manuscript accepted 16 March 2005