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After-life effects: living and dead invertebrates differentially affect plants and their associated above- and belowground multitrophic communities

Martine Kos, Jingying Jing, Ivor Keesmaat, Steven A. J. Declerck, Roel Wagenaar and T. Martijn Bezemer

M. Kos (http://orcid.org/0000-0001-5505-7379) (m.kos@nioo.knaw.nl), J. Jing, I. Keesmaat, R. Wagenaar and T. M. Bezemer, Netherlands Inst. of Ecology (NIOO-KNAW), Dept of Terrestrial Ecology, PO Box 50, NL-6700 AB, Wageningen, the Netherlands. – S. A. J. Declerck, Netherlands Inst. of Ecology (NIOO-KNAW), Dept of Aquatic Ecology, Wageningen, the Netherlands.

Above–belowground (AG–BG) studies typically focus on plant-mediated effects inflicted by living organisms. However, animal cadavers may also play an important role in AG–BG interactions. Here, we explore whether living and dead foliar-feeding and soil-dwelling invertebrates differentially affect plants and their associated AG and BG multitrophic communities.

In a mesocosm study we separated effects of living and dead locusts (AG herbivores) and earthworms (BG detritivores) on experimental multitrophic communities consisting of eight plant species, an AG aphid and parasitoid community and a BG nematode community. We measured root and shoot biomass and determined plant community composition and densities of aphids, parasitoids and nematodes.

Living locusts decreased total shoot and root biomass in the mesocosms, whereas living earthworms enhanced total root biomass. Cadavers of both invertebrates strongly increased total root and shoot biomass, and changed the plant community composition mainly via enhanced growth of grasses. Earthworm cadavers affected plant biomass and community composition more strongly than their living counterparts, while this was reversed for locusts. Structural equation models showed that aphids and parasitoids were influenced via changes in plant community composition. Nematode densities in the soil, especially those of bacterivorous and entomopathogenic nematodes, were strongly increased by dead invertebrates, but unaffected by living ones.

We conclude that effects of invertebrates on plant growth and densities of AG and BG organisms strongly depend on whether the invertebrates are dead or alive. Remarkably, invertebrate cadavers may inflict even stronger effects than their living counterparts. Hence, our study reveals an important, but often neglected, role of animal cadavers in AG–BG studies.

Aboveground and soil-dwelling organisms live in separate domains. However, a rapidly increasing body of literature is showing that aboveground (AG) and belowground (BG) organisms can influence each other via their effects on the growth and chemistry of the shared host plant (van der Putten et al. 2001, Wardle et al. 2004, Bezemer and van Dam 2005, Johnson et al. 2012). The field of AG–BG interactions has quickly developed into one of the hottest topics in ecological research (Bezemer and van Dam 2005, Bardgett and Wardle 2010, Soler et al. 2013).

Most AG–BG studies have focused on organisms with direct trophic links to the plant, such as herbivores, pathogens and arbuscular mycorrhizal fungi, which directly influence plant growth and chemistry and thereby the performance of spatially separated organisms that feed on the same host plant (reviewed by Bezemer and van Dam 2005, Koricheva et al. 2009, van Dam and Heil 2011, Johnson et al. 2012). For example, shoot herbivory may indirectly reduce root biomass, resulting from e.g. limiting photosynthesis and allocation of more dry matter to leaves instead of roots to compensate for defoliation (Vranjic and Gullan 1990, van Dam and Heil 2011, Johnson et al. 2012), thereby adversely influencing root-feeding herbivores (Masters et al. 1993, van Dam and Heil 2011, Johnson et al. 2012). Organisms without direct trophic links to the plant, such as detritivores, can also exert strong effects on plant and herbivore performance (Wardle et al. 2004, Wurst 2013). Detritivores can change the structure of the soil and are responsible for several important soil processes, such as litter incorporation and fragmentation and nutrient immobilization and mineralization, and thereby influence the availability of nutrients to the plant and plant growth (Scheu and Setälä 2001, Wardle et al. 2004, Frund et al. 2010, Wurst 2013). Via their effects on plant performance, detritivores

can influence the performance of AG herbivores (Scheu 2003, Wurst 2013). Earthworms are among the best-studied detritivores in AG–BG studies. Several studies have shown that they can enhance plant growth and plant quality, and can thereby enhance the performance of phloem-feeding and leaf-chewing AG herbivores and their parasitoids (Scheu et al. 1999, Wurst and Jones 2003, Newington et al. 2004, Poveda et al. 2005, Eisenhauer et al. 2010, Johnson et al. 2011). By influencing soil nutrient levels, earthworms can also change the composition of plant communities. For example, increased soil nutrient levels likely enhance the competitive ability of grasses against legumes (Thornley et al. 1995, Schwinning and Parsons 1996), and by increasing soil nitrogen availability, earthworms have been found to selectively promote the growth of grasses at the cost of legumes (Wurst et al. 2005, 2008, Eisenhauer and Scheu 2008).

AG–BG studies typically focus on plant-mediated effects inflicted by living organisms. However, animal cadavers and animal waste may also play an important role in AG–BG interactions. The decomposition of insect cadavers, for example, can produce a pulse of nitrogen in the soil, which may be readily available for uptake by plants (Fielding et al. 2013). Nutrient subsidies with a relatively low C:N content, such as nitrogen fertilizer, urea but also insect cadavers, may promote microbial mineralization of nutrients, and thereby enhance the availability of nitrogen for the plant (Vince et al. 1981, Denno et al. 2002, Hines et al. 2006). Such effects on the availability of nutrients for plants can increase plant growth or increase the nutritional quality (e.g. % nitrogen) of plant tissues, and can thereby enhance the performance and abundance of herbivores feeding on the plant and their natural enemies, both above and below the ground (Gratton and Denno 2003, Huberty and Denno 2006, Sauge et al. 2010, Staley et al. 2011, Dreyer et al. 2012, Bultman et al. 2014). Increased soil nutrient availability resulting from the decomposition of animal cadavers may also change plant community composition due to changes in the competitive ability of certain plant species or functional groups (Thornley et al. 1995, Schwinning and Parsons 1996). However, the decomposition of animal waste may also cause immobilization of nitrogen in the soil when it stimulates growth of the soil microbial community (Lovett and Ruesink 1995).

Nematodes are tiny unsegmented worms that occur in a wide range of environments and that are parasitic in animals or plants or free living in soil or water. In soil, they inhabit a range of trophic levels of the food web (Yeates et al. 1993). The composition and abundance of nematodes can be influenced by organisms such as herbivorous insects or earthworms. For example, AG herbivory may negatively affect root growth and quality, thereby limiting food availability for root-feeders (Masters et al. 1993). Furthermore, the activity of earthworms, but also the presence of cadavers on or in the soil, can influence fungivorous and bacterivorous nematodes that consume the microorganisms that decompose these cadavers or earthworm casts (Senapati 1992, Villenave et al. 2010). Entomopathogenic nematodes (EPNs) are natural enemies of arthropods that live in or close to the soil surface. Juvenile EPNs enter the arthropod host and subsequently release bacterial symbionts that kill the host and convert its tissue into a suitable nutrient substrate. The EPNs then feed on the partly decomposed insect tissue and the bacteria (Kaya and Gaugler 1993).

Organisms such as earthworms or foliar feeding insects that are often used in AG–BG studies will not always survive the entire experimental period, and mortality of earthworms in pot experiments can be as high as 30–50% (Wurst et al. 2003, 2008). Hence, the effects of AG and BG organisms on the plant and its multitrophic AG and BG communities, can be inflicted when being alive but also after they have died. An important open challenge is to determine the relative contribution of the living and dead effects of these organisms.

In this mesocosm study, we examined how addition of an AG herbivore (the locust *Locusta migratoria*) and a BG detritivore (the earthworm *Lumbricus rubellus*) influenced experimental multitrophic communities consisting of eight plant species belonging to different functional (taxonomic) groups (forbs, grasses and legumes), an AG aphid and parasitoid community and a BG nematode community. We specifically tested whether effects of adding these AG and BG invertebrates depended on whether they were alive or dead (cadavers) (Fig. 1). By using a multifactorial design, we could test for interactions between adding AG and BG organisms, and the status of these organisms (dead or alive). We measured total root and shoot biomass in each mesocosm and determined the composition of the plant community and densities of aphids, parasitoids and nematodes. We tested five hypotheses: 1) addition of foliar-feeding locusts will decrease total shoot biomass and, due to drained resources and allocation of more dry matter to leaves instead of roots to compensate for defoliation, total root biomass. As they preferentially feed on grasses, addition of living locusts will change plant community composition to the advantage of forbs and legumes; 2) due to their positive effects on soil nutrient availability for plants, addition of living earthworms will increase total root and shoot biomass. Furthermore, plant community composition will change to the advantage of grasses due to their enhanced competitive strength in nutrient-richer soils; 3) resulting from nutrient release from the decomposing cadavers into the soil, locust and earthworm cadavers will increase total root and shoot biomass and change plant community composition to the advantage of grasses; 4) a decrease in total plant biomass (as expected with addition of living locusts) will lead to decreased densities of the organisms at higher trophic levels that (indirectly) depend on these plants for food, i.e. aphids, parasitoids and nematodes. Similarly, an increase in total plant biomass (as expected with addition of living earthworms and addition of invertebrate cadavers) will lead to increased densities of aphids, parasitoids and nematodes; 5) addition of living locusts and earthworms, which continuously remove foliar tissue of the plants (locusts) or change the structure and quality of the soil by burrowing, mixing and casting (earthworms), will have stronger effect sizes than addition of dead ones.

Material and methods

The experiment was performed in a greenhouse compartment (60% relative humidity; 16 h light (20°C) and 8 h dark (16°C) photo regime). Natural day-light was supplemented by 400 W metal halide lamps (225 μmol m^{-2} s^{-1} PAR, 1 lamp per 1.5 m^2). 126 containers (18 × 18 × 19 cm) were filled

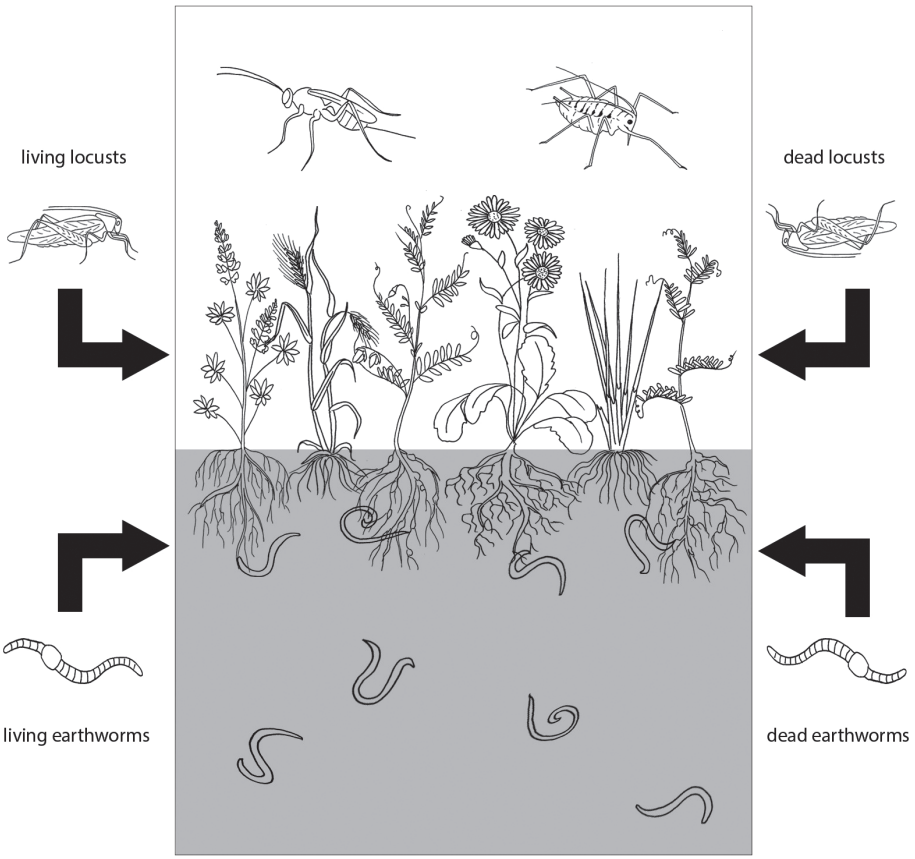

Figure 1. Schematic diagram of the experimental set-up. We set-up mesocosms containing multitrophic communities consisting of eight plant species, an aboveground aphid and parasitoid community and a belowground nematode community. To each mesocosm, either living or dead locusts, earthworms or both locusts and earthworms were added. After several weeks, total root and shoot biomass in each mesocosm were measured, and the composition of the plant community and densities of aphids, parasitoids and nematodes were determined. Drawings by Cindy ten Broeke from *Cindy's art*.

with 4.3 kg soil (based on fresh weight, 9% soil moisture). Containers were filled with a homogenised mixture of live (non-sterilised) and sterilised field soil (1:1 ratio). A layer of sterilised field soil (0.7 kg) was added on the surface of each container to reduce possible germination of the seeds that were present in the field-collected soil (total 5 kg soil per container). The soil was a sandy loam with particle size distribution: 3% < 2 μm, 17% 2–63 μm, 80% > 63 μm, with 4.5% organic matter and was collected from a restoration grassland at Planken Wambuis (Ede, the Netherlands) at 0–20 cm depth. The soil was sieved (0.5 cm mesh size) to remove coarse fragments and root pieces and homogenized, and all macro-arthropods were manually removed. To obtain sterilised soil, a part of the field soil was sterilised by gamma irradiation (mean dose 25 KGray). Details on soil chemistry are presented in the Supplementary material Appendix 1 Method A1 and Table A1. Into each container, two one-week-old seedlings of each of eight plant species that typically co-occur in Dutch mid-succession grasslands were transplanted. The eight plant species consisted of four forbs: *Achillea millefolium, Hypochaeris radicata*, *Leucanthemum vulgare* and *Tripleurospermum maritimum*, two grasses: *Anthoxanthum odoratum* and *Holcus lanatus* and two legumes: *Lotus corniculatus* and *Trifolium repens*. The position of each plant species within the container was fixed (Supplementary material Appendix 1 Fig. A1). Seeds from these grassland species were obtained from a specialized wild plant seed supplier. Seeds were surface sterilised (1 min in 2.5% sodium hypochlorite solution and rinsed with water afterwards) and germinated on glass beads in a climate chamber at 20°C. Plants were watered three times per week throughout the experiment and the soil was kept at 15% moisture by weighing each container. Containers were randomly rearranged within the greenhouse once a week.

A nematode suspension was added to each container one week after transplanting of the seedlings. The nematodes were extracted from 25 kg of soil collected at a grassland adjacent to the Netherlands Inst. of Ecology (NIOO-KNAW) in Wageningen using Cobbs' decantation and sieving method (1 × 180 μm, followed by 1 × 75 μm, and 3 × 45 μm). We collected the nematodes from the 75 and 45 μm sieves and incubated them for 48 h on two filters (220 mm). Into each pot, 5 ml nematode inoculum was injected into the soil with a pipette at four positions (20 ml in total, Supplementary material Appendix 1 Fig. A1). Total nematode densities in two 1 ml samples were determined and 150 nematodes in each sample were identified to genus or family and grouped into feeding guilds according to Yeates et al. (1993). Each pot received, on average, 879 root feeders, 1078 bacterivores, 104 fungivores, 70 omnivores/carnivores

and 160 dauer larvae (EPN of the genus Steinernema and Heterorhabditis).

Three weeks after transplanting, each container was caged individually using fine meshed cylindrical cages (height 120 cm, diameter 35 cm). Four adult aphids (*Myzus persicae*; Hemiptera: Aphididae) were then added to each mesocosm. The aphids, which are generalist phloem-feeders, were obtained from the Laboratory of Entomology in Wageningen, and had been reared on Brussels sprouts (*Brassica oleracea* convar. *gemmifera* cv. Cyrus). Aphids were allowed to move freely between all plants in the mesocosm.

Six weeks after transplanting, mesocosms were randomly divided over seven treatments: 1) control; 2) addition of living locusts (AG–L); 3) addition of dead locusts (AG–D); 4) addition of living earthworms (BG–L); 5) addition of dead earthworms (BG–D); 6) addition of living locusts and earthworms (AG+BG–L) and 7) addition of dead locusts and earthworms (AG+BG–D). Because of the size and complexity of our mesocosm study, it was important to keep the replication as high as possible. Hence, it was not practically feasible to include all factorial combinations of all treatments (two treatments were not included: addition of dead locusts and live earthworms, and addition of live locusts and dead earthworms). In the BG and AG+BG treatments, we added 10 individuals of the earthworm *Lumbricus rubellus* (Haplotaxida: Lumbricidae), ensuring that the total weight of the 10 earthworms was between 7.8 and 8.2 g (based on fresh weight with gut content). Earthworms were extracted from a grassland at the NIOO-KNAW. Both living and dead earthworms were introduced into the soil in holes (2 cm diameter, 7 cm deep), which were covered afterwards. To ensure that all mesocosms in the experiment were treated equally, we made similar holes in the soil of the mesocosms in which no earthworms were introduced. In the AG and AG+BG treatments, we added 10 individuals of the migratory locust *Locusta migratoria* (Orthoptera: Acrididae), again ensuring that the total weight was between 7.8 and 8.2 g (based on fresh weight with gut content). This species is a herbivore of grasses. Both living and dead locusts were introduced by placing them on top of the soil. Due to time limitations, locusts were introduced one week later than the earthworms. Because they started to bite holes in the meshed cages, living locusts were removed one week after introduction. All introduced locusts were alive at the time of their removal, and they had done considerable damage to the grasses by then. Living earthworms remained in the soil until the end of the experiment, and 95% of them were recovered alive. To obtain cadavers, locusts and earthworms were killed by immediate freezing (–20°C). There were 14 replicates per treatment, and 42 for the Control (21 randomly allocated a priori to the Living treatment and 21 to the Dead treatment). Details on the chemical composition of the locusts and earthworms are presented in Supplementary material Appendix 1 Method A1 and Table A2.

Seven weeks after transplanting, and thus four weeks after introduction of the aphids, 10 female and 6 male parasitoid wasps *Aphidius matricariae* (Hymenoptera: Braconidae) were introduced into each mesocosm.

Plant, aphid, parasitoid and nematode measurements

Twelve weeks after transplanting, the experiment was harvested. First, all adult parasitoid wasps in each mesocosm were collected during a number of collection events over a period of seven days using aspirators. All collected parasitoids from each mesocosm were pooled and the total number of parasitoids per mesocosm was determined. Then, all AG plant material was clipped. All shoots as well as the cage of each mesocosm were rinsed in a container with water and all aphids (including aphid mummies containing a parasitoid pupa) were collected using a fine meshed sieve and oven-dried (40°C). The total aphid biomass was then determined for each mesocosm. Hereafter, from each mesocosm pot soil samples (100 g) were taken for nematode analysis, and the shoot biomass of each plant species was sorted to species and oven-dried (40°C). Roots were then carefully washed from the soil and oven-dried (60°C). Roots could not be separated per species and total root biomass was determined per mesocosm (i.e. pooled for all plant species). For 10 replicates of each treatment (20 for the Control; 10 for each of the two a priori determined control groups), nematodes were extracted 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).

Statistical analysis

Effects on total root biomass, total shoot biomass, aphid biomass and total nematode densities were tested using a three-way ANOVA with AG addition, BG addition, status (dead or alive) and their interactions as fixed factors. Control mesocosms had been a-priori allocated to the dead or the alive group. Effects on 1) biomass of each individual plant species and 2) density of each nematode feeding group was tested with a three-way MANOVA (multivariate ANOVA) with the same three-way model. MANOVA can be used to test differences among groups for multiple dependent variables (here: plant species or nematode feeding groups) simultaneously ('overall effect'), and also provides results from the univariate analysis for each individual parameter (i.e. plant species or nematode feeding group). Plant and aphid biomass and nematode densities were natural log-transformed to obtain normality. Treatment effects on parasitoid densities were tested with a generalized linear model with a negative binomial distribution and log-link function with the same three-way model. Pearson's correlation tests were used to test the relationships between 1) biomass of each plant species, 2) aphid biomass and 3) parasitoid density. Univariate analyses were performed in IBM SPSS Statistics for Windows (19th edn, SPSS Inc).

Multivariate analyses were used to compare 1) plant communities and 2) nematode communities among the seven treatments. Detrended correspondence analysis indicated that the longest gradient was < 3, hence data were analyzed using principal component analysis (PCA) and redundancy analysis (RDA) (Lepš and Šmilauer 2003). Significances in multivariate analyses were inferred by Monte Carlo permutation tests (999 permutations). Data were log-transformed before the RDA analysis. Multivariate analyses were performed in Canoco ver. 5.03 (Ter Braak and Šmilauer 2002).

Structural equation modelling (SEM) was used to disentangle direct and indirect effects of living and dead AG and BG invertebrates on plant, aphid, parasitoid and nematode responses. SEM was performed with the 'sem' package in R (ver. 3.0.1, <www.r-project.org>). We performed two different SEM analyses, the first to disentangle treatment effects on the AG compartment (shoots–aphids–parasitoids), and the second to disentangle treatment effects on the BG compartment (roots–nematodes). The conceptual models considered both direct and indirect effects of addition of living and dead AG and BG invertebrates on 1) total shoot biomass, plant community composition, aphid biomass and parasitoid densities (analysis 1; AG compartment); or on 2) total root biomass, plant community composition, total nematode density and nematode community composition (analysis 2; BG compartment). Prior to the SEM analysis, data were natural log-transformed. For plant community composition, the sample scores on the first axis of a PCA on shoot biomass of each plant species was used. Because we did not determine root biomass separately for each plant species, we also used the sample scores from the PCA on shoot biomass for analysis 2 on the BG compartment, under the assumption that the relative composition of the plant community AG (shoots) and BG (roots) would be comparable. For nematode community composition, the sample scores on the first axis of a PCA on the density of each feeding group (herbivorous, bacterivores, fungivores, omnivores, carnivores and EPN), was used. We removed non-significant paths from our SEM model to select the model that best fitted our data. In SEM, the goodness of fit of the model is assessed by comparing the observed and model-predicted covariances with a χ^2-test. If the χ^2-values have an associated p-value > 0.05, the model is acceptable (there is reasonable fit between model and the data) (Grace 2006).

Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.7b354> (Kos et al. 2016).

Results

Plant biomass and community composition

For both total root and total shoot biomass (summed over all species), the effect of addition of AG and BG invertebrates depended on the status of the organism (dead or alive) (significant AG × Status and/or BG × Status interaction; Table 1). Overall, the addition of dead organisms (cadavers), whether AG, BG or both, had a positive effect on total root and shoot biomass, with a relatively stronger positive effect on shoot biomass than on root biomass (Fig. 2a–b). The addition of living locusts (AG) had a negative effect on total root and shoot biomass, with a relatively stronger negative effect on root biomass than on shoot biomass (Fig. 2a–b). The addition of living earthworms (BG) had a positive effect on total root biomass, but no effect on total shoot biomass (Fig. 2a–b). The two grass species, *Anthoxanthum odoratum* and *Holcus lanatus*, were most affected by the treatments (Fig. 2g–h; Supplementary material Appendix 1 Table A3). Dead locusts and earthworms strongly increased shoot biomass of both grass species, whereas living locusts strongly reduced their shoot biomass. Living earthworms had positive effects on shoot biomass of the two grasses, but the effects were weaker than those of dead earthworms (Fig. 2g–h). Of the forbs, *Hypochaeris radicata* was most affected by the treatments. Adding living locusts or dead earthworms increased *H. radicata* biomass (Fig. 2d). Biomass of *Achillea millefolium* was higher when dead invertebrates were added than when living invertebrates were added (Fig. 2c). The legume *Trifolium repens* was negatively affected by addition of earthworms (Fig. 2j). Biomass of the grass *A. odoratum* was positively correlated with biomass of the grass *H. lanatus* ($r = 0.58$, $p < 0.001$, $n = 126$), but negatively with biomass of the forb *H. radicata* ($r = -0.18$, $p = 0.040$, $n = 126$), whereas biomass of *H. lanatus* was negatively correlated with biomass of the legume *T. repens* ($r = 0.18$, $p = 0.045$, $n = 126$). Furthermore, biomass of the forb *A. millefolium* was positively correlated with biomass of the forb *H. radicata* ($r = 0.18$, $p = 0.044$, $n = 126$), but negatively with the forb *Tripleurospermum maritimum* ($r = 0.35$, $p < 0.001$, $n = 126$).

Multivariate analyses (RDA) of the plant community composition revealed a significant difference among the seven treatments (pseudo-$F = 17.5$, $p = 0.002$; 46.9% explained variation; Fig. 3a). Plant communities in mesocosms with addition of dead organisms separated most from those with addition of living organisms, with the exception of mesocosms to which living earthworms were added, which were located in the quadrant of the RDA with mesocosms to which dead organisms (locusts or earthworms) were added (Fig. 3a). The two grass species contributed most to the separation of plant communities among treatments, with higher grass biomass in mesocosms with addition of

Table 1. Statistical output of a three-way ANOVA on the effects of addition of locusts (AG) and earthworms (BG), the status of the organism (dead or alive) and the factorial interactions on total root and shoot biomass, aphid biomass and parasitoid and nematode densities in a mesocosm. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$. The absence of asterisks denote no significant effects.

	F-value						
	AG	BG	Status	AG × BG	AG × Status	BG × Status	AG × BG × Status
Total root biomass	**12.16****	**4.62***	**13.44*****	0.14	**13.06*****	1.47	0.29
Total shoot biomass	2.52	**41.49*****	**285.95*****	0.54	**99.37*****	**47.65*****	0.52
Aphid biomass	0.04	1.75	0.52	0.03	1.13	0.13	2.04
Parasitoid density[a]	0.17	0.13	**6.47***	0.57	0.25	0.10	0.01
Nematode density	**18.52*****	3.71	**38.32*****	0.01	**20.86*****	0.90	0.68

[a]Parasitoid densities were analysed with a generalized linear model with a negative binomial distribution and log-link function. The Wald χ^2-values are reported.

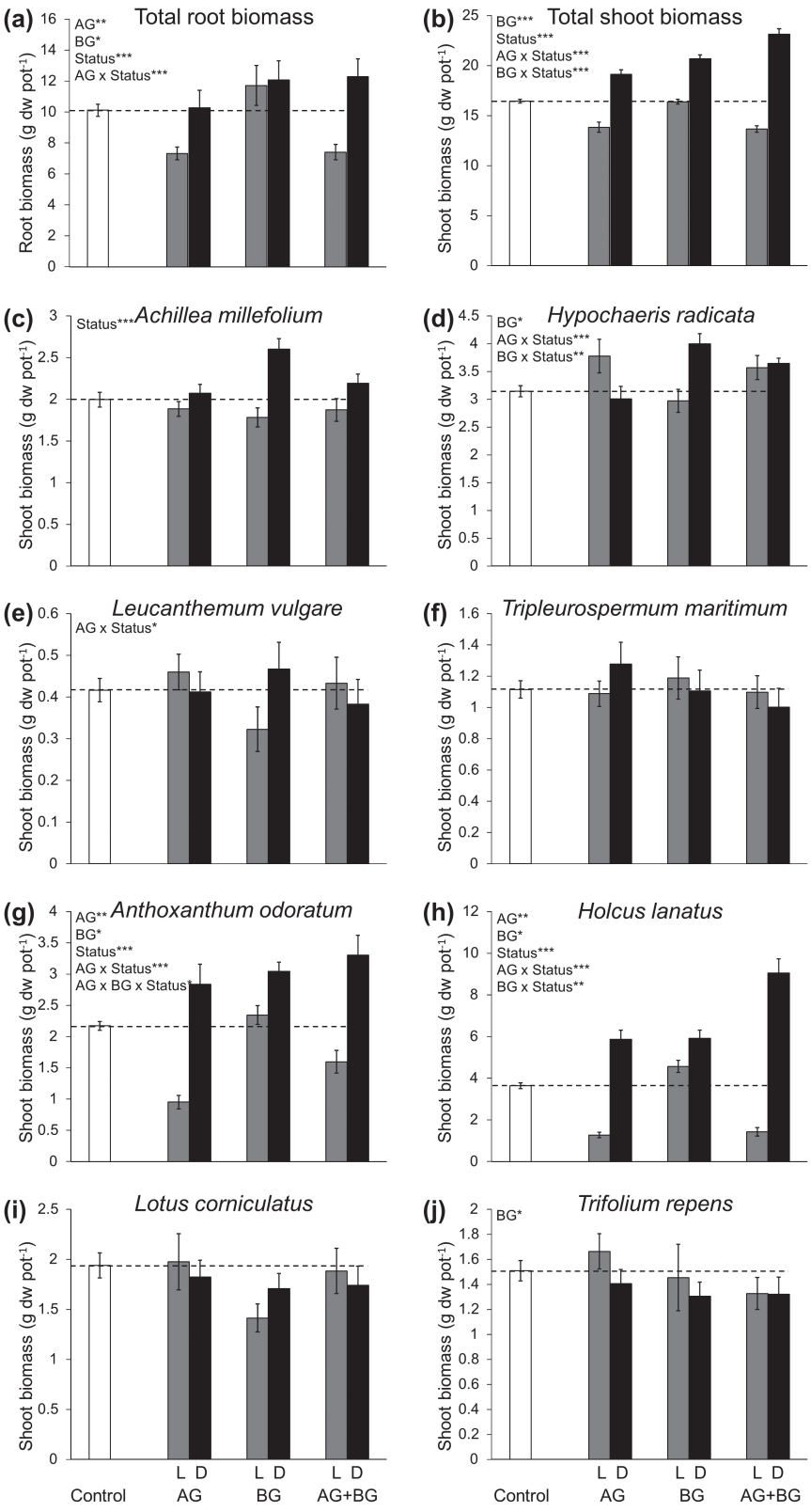

Figure 2. (a) Total (mean ± SE) root biomass, (b) total shoot biomass and shoot biomass of the forbs (c) *Achillea millefolium*, (d) *Hypochaeris radicata*, (e) *Leucanthemum vulgare* and (f) *Tripleurospermum maritimum*, the grasses (g) *Anthoxanthum odoratum* and (h) *Holcus lanatus* and the legumes (i) *Lotus corniculatus* and (j) *Trifolium repens* growing in mesocosms without invertebrate addition (Control; white bar) or with addition of living (L; grey bars) or dead (D; black bars) locusts (AG), earthworms (BG) or both locusts and earthworms (AG+BG). The main effect of adding AG organisms, BG organisms, the status of the organisms (dead or alive) and the interactions were tested with three-way ANOVA; ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$. The dashed line indicates the mean value for the Control treatment.

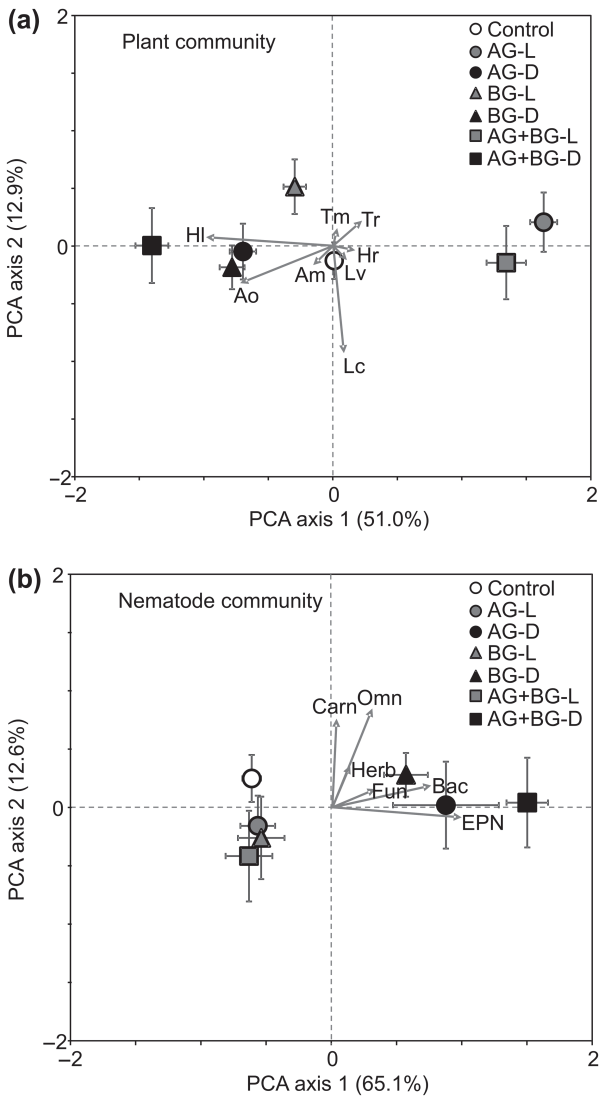

Figure 3. Ordination diagram of principal component analysis (PCA). Shown are mean sample scores (± SE) of (a) plant community composition and (b) nematode community composition in mesocosms without invertebrate addition (Control) or with addition of living (L) or dead (D) locusts (AG), earthworms (BG), or both locusts and earthworms (AG+BG). In (a), arrows represent plant species (forbs: Am = *Achillea millefolium*, Hr = *Hypochaeris radicata*, Lv = *Leucanthemum vulgare*, Tm = *Tripleurospermum maritimum*, grasses: Ao = *Anthoxanthum odoratum*, Hl = *Holcus lanatus*, legumes: Lc = *Lotus corniculatus*, Tr = *Trifolium repens*), in (b) arrows represent nematode feeding groups (Bac = bacterivores, Carn = carnivores, EPN = entomopathogenic nematodes, Fun = fungivores, Herb = herbivores, Omn = omnivores). Percentages of total explained variation by PCA axes are given in parentheses.

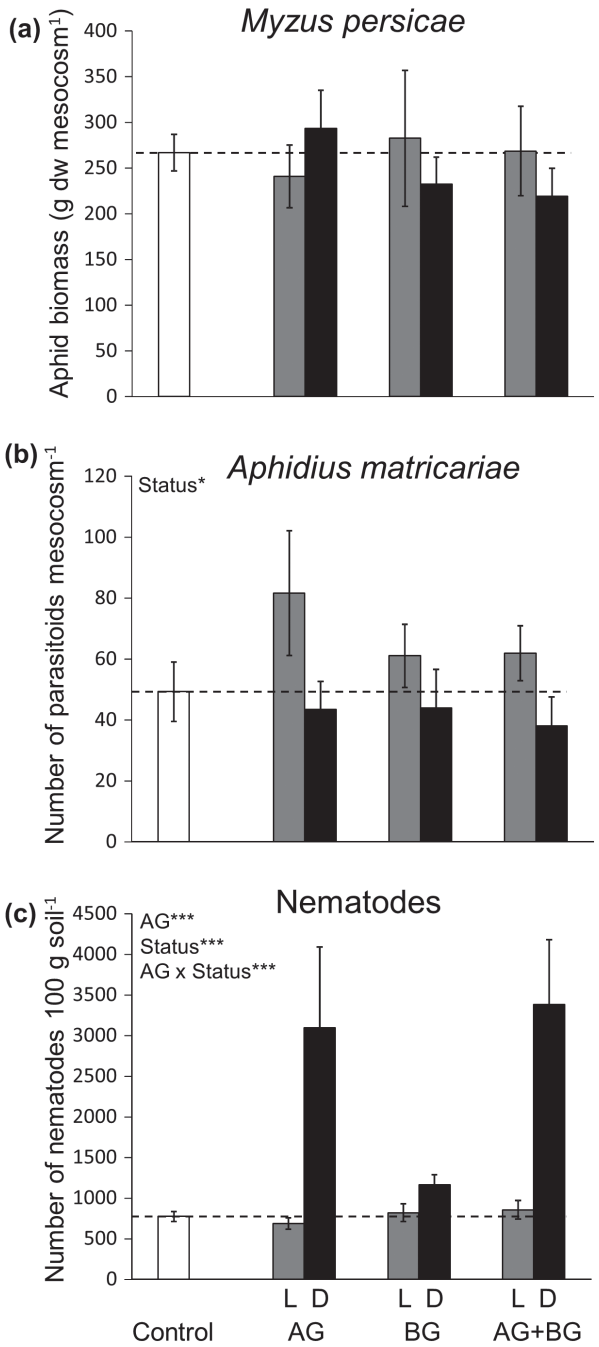

Figure 4. (a) Biomass (mean ± SE) of *M. persicae* aphids, (b) density of *A. matricariae* parasitoids and (c) total density of nematodes per 100 g soil in mesocosms without invertebrate addition (Control; white bar) or with addition of living (L; grey bars) or dead (D; black bars) locusts (AG), earthworms (BG) or both locusts and earthworms (AG+BG). The main effect of AG organisms, BG organisms, the status of the organisms (dead or alive) and the interactions were tested with three-way ANOVA for aphid biomass and nematode density or a generalized linear model with a negative binomial distribution and log-link function for parasitoid densities; *$p < 0.05$. The dashed line indicates the mean value for the Control treatment.

dead invertebrates (locusts and/or earthworms) or living earthworms (Fig. 3a, Supplementary material Appendix 1 Table A3).

Aphid biomass and parasitoid densities

There were no treatment effects on aphid biomass in the univariate analyses (Table 1, Fig. 4a). Parasitoid densities were higher when living invertebrates were added to a mesocosm than when dead invertebrates were added, but the effect was independent of type of organism (locust or earthworm) (Table 1, Fig. 4b). There was no relationship between aphid

biomass and parasitoid density, nor between aphid biomass and biomass of any of the eight plant species ($p > 0.05$ for all relationships). Parasitoid densities were positively related to biomass of the forb *H. radicata* ($r = 0.18$, $p = 0.043$, $n = 126$), but negatively to biomass of both grass species (*A. odoratum*: $r = -0.23$, $p = 0.009$, $n = 126$; *H. lanatus*: $r = -0.22$, $p = 0.014$, $n = 126$).

Nematode densities

For the total nematode density in soil of the mesocosms, the effect of invertebrate addition depended on whether the organisms were dead or alive (Table 1). Adding dead locusts and, to a lesser extent, dead earthworms to a mesocosm had strong positive effects on total nematode densities, whereas adding living locusts and earthworms did not have any effect (Fig. 4c). Densities of most nematode feeding groups were highest in mesocosms in which dead organisms were added, especially when dead locusts and earthworms were added simultaneously (Supplementary material Appendix 1 Fig. A2). Positive effects of adding invertebrate cadavers were particularly strong for the two most abundant feeding groups, bacterivorous and EPN (Supplementary material Appendix 1 Fig. A2, Table A3). EPN were almost absent in the soil of mesocosms in which no or living invertebrates were added (Supplementary material Appendix 1 Fig. A2). Overall, densities of fungivores and omnivores were increased by addition of dead invertebrates (whether locusts, earthworms or both), and decreased by addition of living earthworms. Densities of herbivores were slightly higher when dead locusts and earthworms were added simultaneously, than when living locusts and earthworms were added simultaneously. Densities of carnivores (non-EPN) were unaffected by any of the treatments (Supplementary material Appendix 1 Fig. A2, Table A3).

Multivariate analyses of the nematode community composition revealed a significant difference among the treatments (pseudo-$F = 10.5$, $p = 0.002$; 46.3% explained variation; Fig. 3b). Nematode communities in mesocosms with addition of dead organisms separated most from those with no addition or addition of living organisms (Fig. 3b). The EPN and bacterivorous nematodes contributed most to the separation of nematode communities among treatments (Fig. 3b, Supplementary material Appendix 1 Table A3). Overall, densities of the different nematode feeding groups were highest in mesocosms in which dead organisms were added, especially when dead locusts and earthworms were added simultaneously (Fig. 3b).

Structural equation modeling

We used SEM to disentangle the direct and indirect effect of addition of dead and alive invertebrates on responses of the AG compartment (shoots–aphids–parasitoids) and the BG compartment (roots–nematodes). The SEM on the AG compartment provided a good fit to the data ($\chi^2_{11} = 13.03$; $p = 0.29$). The model explained a large part of the variance for the plant responses (shoot biomass and community composition), but only a small part of the variance for aphid biomass and parasitoid density (Fig. 5a). For effects of locusts, living individuals had stronger effects on shoot biomass and plant community composition than dead individuals, and effects were in the opposite direction (negative effects of living locusts and positive effects of their cadavers on shoot biomass). In contrast, dead earthworms had a stronger effect on shoot biomass and plant community composition than living earthworms, and effects were in the same direction (positive effects on shoot biomass). Aphids and parasitoids were mostly indirectly affected by the treatments, via effects on plant community composition, although aphid biomass was also directly negatively affected by the presence of living locusts (Fig. 5a).

The SEM on the BG compartment provided a very good fit to the data ($\chi^2_{11} = 6.16$; $p = 0.86$), and the model explained a large part of the variance for both plant and nematode responses. Nematodes were affected directly by addition of AG and BG organisms, but not indirectly via effects on root biomass or plant community composition (Fig. 5b). Only addition of dead locusts and earthworms affected nematode densities and nematode community composition; addition of living organisms did not have any effect (Fig. 5b).

Discussion

Our study demonstrates that introducing AG and BG invertebrates significantly changes experimental multitrophic communities, both above and below the ground, and that these effects strongly depend on whether the introduced invertebrates were dead or alive. Classical AG–BG studies have typically focussed on plant-mediated effects inflicted by living organisms. Our results show that dead invertebrates may inflict even stronger effects on plants and their associated multitrophic communities than their living counterparts, revealing an important role of animal cadavers in AG–BG studies.

Our first hypothesis predicted that living locusts would decrease not only total shoot biomass, but also total root biomass, and this was supported by our results. Interestingly, root biomass was relatively more affected by shoot herbivory than shoot biomass. This was also found in several other studies (Crawley 1984, Vranjic and Gullan 1990, Karban and Strauss 1993), and likely results from the allocation of more dry matter to leaves instead of roots to compensate for defoliation (Vranjic and Gullan 1990, van Dam and Heil 2011, Johnson et al. 2012). In our study, AG herbivory did not seem to lead to a reallocation of resources from the shoots to the roots, as often reported in the literature (Strauss and Agrawal 1999, Kaplan et al. 2008), although we cannot conclusively determine this as we did not collect root biomass for each individual plant species. As hypothesized, feeding by the locust, which feeds only on grasses, also changed plant community composition by strongly reducing grass biomass. Biomass of the forbs *Hypochaeris radicata* and *Leucanthemum vulgare* increased in the presence of living locusts. This probably resulted from reduced competition with grasses, as we found a negative relationship between biomass of the grass *Anthoxanthum odoratum* and the forb *H. radicata*. It is well-known that by feeding preferentially on certain plant species or functional groups, herbivores can strongly affect plant–plant competition (Crawley 1997, Carson and Root 1999, Agrawal 2004, Schadler et al. 2004).

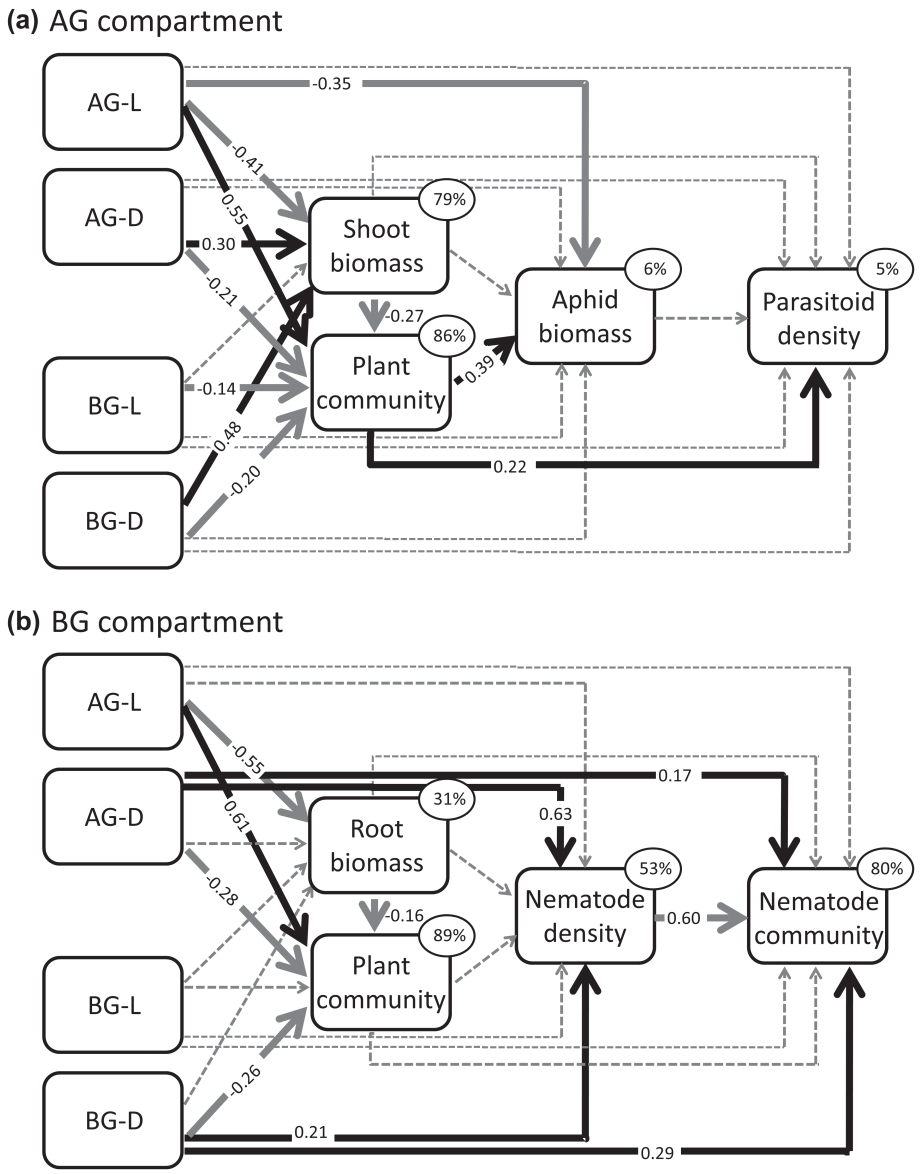

Figure 5. Structural equation models of the relationships between the addition of living (L) or dead (D) locusts (AG) and earthworms (BG) and (a) total shoot biomass, plant community composition, aphid biomass and parasitoid density (AG compartment) or (b) total root biomass, plant community composition, total nematode density and nematode community composition (BG compartment) in mesocosms. The plant or nematode community is represented by the sample scores on the first axis of a principle component analysis (PCA) on, respectively, plant or nematode community composition (respectively 51% and 65% explained variation). Solid arrows depict significant effects ($p < 0.05$), dashed arrows show non-significant effects. Standardized path coefficients are provided for significant paths (black = positive relationship, grey = negative relationship). Percentages indicate the variance explained by the model for each endogenous explanatory variable.

In line with our second hypothesis, addition of living earthworms positively affected total root biomass. This is in agreement with findings from several other studies (Edwards and Lofty 1980, Scheu et al. 1999, Wurst and Jones 2003, Eisenhauer et al. 2010). Earthworms can positively affect soil fertility and nutrient availability for plants because they redistribute soil organic matter, increase soil aeration and affect the activity of soil microorganisms (Edwards and Lofty 1980, Scheu 2003, Frund et al. 2010, Wurst 2013). Although addition of living earthworms increased total root biomass in our study, there were no effects on total shoot biomass. A similar finding was reported in several other studies (Wurst and Jones 2003, Wurst et al. 2008), although most other studies have reported that earthworms also increase shoot biomass (reviewed by Scheu 2003). As predicted, addition of living earthworms changed the plant community composition by particularly increasing grass biomass. Increased soil nutrient levels likely enhance the competitive ability of grasses against legumes (Thornley et al. 1995, Schwinning and Parsons 1996), and several studies showed that by increasing soil nitrogen availability, earthworms selectively promoted the growth of grasses at the cost of legumes (Wurst et al. 2005, 2008, Eisenhauer and Scheu 2008). In agreement with this, our results showed a negative relationship between biomass of the legume *Trifolium repens* and the grass *Holcus lanatus*, although overall, biomass of the two legume

species was hardly affected by any of the treatments in our study.

As predicted in our third hypothesis, addition of earthworm cadavers increased total root and shoot biomass in the mesocosms, although addition of locust cadavers increased shoot biomass only. The strong increase in plant growth after addition of invertebrate cadavers suggests that the nutrients from the decomposing invertebrate cadavers were rapidly mineralized by microorganisms and available for uptake by the plants (Hines et al. 2006). This confirms findings from another study in which much of the nitrogen in grasshopper cadavers was labile and rapidly available for uptake by plants (Fielding et al. 2013). Shoot biomass was relatively more affected by addition of cadavers than root biomass, in agreement with the general view that nutrients generally increase shoot biomass relatively to root biomass (Poorter and Nagel 2000). Similarly to what we found in the presence of living earthworms, adding invertebrate cadavers changed the plant community composition to the advantage of grasses, which was expected based on the predicted competitive advantage of grasses under increased nutrient availability in soils (Thornley et al. 1995, Schwinning and Parsons 1996).

In contrast to our fourth hypothesis, we found no treatment effects on aphid biomass in the univariate analysis, despite a strong treatment effect on total shoot biomass. Effects of earthworms on performance of AG aphids have been often studied, and although most studies report positive effects (Scheu et al. 1999, Wurst and Jones 2003, Poveda et al. 2005, Eisenhauer et al. 2010), others report neutral (Bonkowski et al. 2001) or even negative effects (Wurst et al. 2003, 2004, Wurst and Forstreuter 2010). In our study, *Myzus persicae* fed mainly on the forb *Tripleurospermum maritimum* (Keesmaat unpubl.), and as the growth of this plant species was unaffected by our treatments, this may explain why aphid biomass was also unaffected according to the univariate analysis. We did not measure plant quality for aphids, such as concentrations of primary and secondary metabolites in the phloem (Awmack and Leather 2002, Karley et al. 2002, Kos et al. 2012), although this is probably more important for aphid performance than plant biomass (Kos et al. 2015). It is important to note that we measured aphid biomass as a proxy for aphid density. Perhaps other aphid performance traits, such as development time, adult size or fecundity would have been more affected by our treatments. When we included all plant, aphid and parasitoid responses in the multivariate SEM analysis on the AG compartment, we did find an indirect effect of adding invertebrates, via changes in plant community composition, on aphid biomass. However, we could not trace this back to a specific plant species, as aphid biomass was not related to biomass of any of the eight plant species. Interestingly, SEM also showed a direct negative effect of living locusts on aphid biomass, which corresponds to our observation that the locusts were regularly feeding directly on the aphids themselves (Keesmaat unpubl.).

Parasitoid densities were higher with addition of living organisms than with addition of cadavers, irrespective of which organism was added (locusts or earthworms). This did not correspond to our fourth hypothesis, in which we predicted that parasitoid densities would be higher in treatments with higher total plant biomass (i.e. with addition of cadavers). Changes in plant quality or quantity can cascade up the food-web and, via effects on the performance of the herbivore host, affect the performance of third-trophic level parasitoids (Ode 2006, Gols and Harvey 2009). Aphid density (represented by aphid biomass) was not affected by any treatments in our study, and we did not measure aphid quality (e.g. body size), hence the mechanism behind the positive effect of living locusts and earthworms on the number of parasitoids remains unclear. Our finding of a positive effect of living earthworms on the number of parasitoids corresponds to the studies by Johnson et al. (2011) and Poveda et al. (2005). However, another study did not find any effects of earthworms on the number of parasitized aphids, despite positive effects on aphid performance (Wurst and Jones 2003), suggesting that effects of earthworms on aboveground multitrophic interactions are highly context-dependent and may be influenced by the experimental set-up. Like aphid biomass, the SEM showed an indirect effect of adding invertebrates on parasitoid densities via changes in plant community composition. More specifically, parasitoid densities were positively related to biomass of the forb *H. radicata* and negatively to biomass of the two grass species.

In line with our fourth hypothesis, addition of locust and earthworm cadavers strongly increased total nematode densities and changed the nematode community composition in mesocosms. However, in contrast to our expectations, addition of living invertebrates had no effects on total nematode densities or community composition, despite positive effects on plant biomass. Positive effects of invertebrate cadavers were particularly strong for bacterivorous and entomopathogenic nematodes, which have direct trophic links to the cadavers as they feed on bacteria and, for EPN, on the partly decomposed cadavers (Kaya and Gaugler 1993, Yeates et al. 1993, Puza and Mracek 2010). Although EPN have been considered to be obligate parasites of living invertebrates, they were recently found to be able to colonize and multiply in dead hosts as well, and several studies suggested that facultative scavenging can be considered an alternative survival strategy for these nematodes (San-Blas and Gowen 2008, Puza and Mracek 2010). In contrast to the bacterivorous nematodes and EPN, densities of herbivorous nematodes were hardly affected by adding invertebrates. This was unexpected, as we found strong effects on plant biomass, especially on biomass of grasses, and herbivorous nematodes often preferentially feed on grasses (De Deyn et al. 2004). Densities of carnivorous nematodes were not affected by any of the treatments, despite strong treatment effects on their potential prey (such as bacterivorous nematodes). Although unexpected, these findings corroborate the study by Wurst et al. (2008), in which no effects of earthworms on herbivorous and carnivorous non-EPN were found, despite strong effects on root biomass. SEM confirmed that addition of invertebrate cadavers affected the nematodes only directly, via direct feeding on the cadavers and the associated bacteria, and not indirectly via changes in plant biomass or community composition. Interestingly, densities of fungivores and omnivores did not only increase after addition of locust and earthworm cadavers, as was found for most nematode feeding groups, but also decreased by addition of living earthworms. Earthworms can directly suppress nematode densities through several mechanisms,

such as digestion of nematodes and stimulation of nematode-antagonistic soil microflora (Wurst 2010).

Our fifth hypothesis predicted that living invertebrates would have stronger effect sizes than dead ones, but our results showed that this was highly context-dependent, and differed depending on which invertebrate (locust or earthworm) was introduced. SEM showed that, as expected, addition of living locusts had stronger effects on plant biomass and plant community composition, and via plant community changes on aphid and parasitoid densities, than addition of dead ones. However, this was reversed for earthworms, which had much stronger effects on plant biomass and community composition, and thereby on aphid and parasitoid densities, when dead than when alive. For nematodes, SEM showed that addition of invertebrate cadavers had much stronger effect sizes than addition of living invertebrates, independent of whether locusts or earthworms were introduced. Earthworm mortality in pot experiments may be as high as 30–50% (Wurst et al. 2003, 2008). Hence, our finding may suggest that the positive effects of earthworms on plant performance that are often reported (reviewed in Scheu 2003, Wurst 2013) may actually result, at least to some extent, from nutrient release from the decomposing earthworm cadavers, rather than from the activity of the earthworms themselves. Future studies that disentangle the effects of living earthworms and their cadavers on plant performance should determine whether this is true.

We conclude that effects of adding invertebrates on plant growth and densities of AG and BG organisms strongly depend on whether the introduced invertebrates are dead or alive. Our results demonstrate that, depending on the species that was introduced, invertebrate cadavers can even have stronger effects on plant growth and densities of AG and BG organisms than their living counterparts. Mortality of invertebrates such as earthworms in pot experiments can be high (Wurst et al. 2003, 2008), and our findings demonstrate that this may strongly affect the outcome of such experiments. This may have far-reaching consequences for our understanding of AG–BG interactions in natural systems. Our study highlights that animal cadavers may play an important role in AG–BG studies, and we call for further studies that disentangle effects of living and dead invertebrates in other multitrophic communities.

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Supplementary material (available online as Appendix oik-03734 at <www.oikosjournal.org/appendix/oik-03734>). Appendix 1.