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Effects of soil organisms on aboveground multitrophic interactions are consistent between plant genotypes mediating the interaction

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Abstract

Belowground communities can affect interactions between plants and aboveground insect communities. Such belowground–aboveground interactions are known to depend on the composition of belowground communities, as well as on the plant species that mediates these interactions. However, it is largely unknown whether the effect of belowground communities on aboveground plant–insect interactions also depends on genotypic variation within the plant species that mediates the interaction. To assess whether the outcome of belowground–aboveground interactions can be affected by plant genotype, we selected two white cabbage cultivars [*Brassica oleracea* L. var. *capitata* (Brassicaceae)]. From previous studies, it is known that these cultivars differ in their chemistry and belowground and aboveground multitrophic interactions. Belowground, we inoculated soils of the cultivars with either nematodes or microorganisms and included a sterilized soil as a control treatment. Aboveground, we quantified aphid [*Brevicoryne brassicae* (L.) (Hemiptera: Aphididae)] population development and parasitoid [*Diaeretiella rapae* (McIntosh) (Hymenoptera: Braconidae)] fitness parameters. The cultivar that sustained highest aphid numbers also had the best parasitoid performance. Soil treatment affected aphid population sizes: microorganisms increased aphid population growth. Soil treatments did not affect parasitoid performance. Cultivars differed in their amino acid concentration, leaf relative growth rate, and root, shoot, and phloem glucosinolate composition but showed similar responses of these traits to soil treatments. Consistent with this observation, no interactions were found between cultivar and soil treatment for aphid population growth or parasitoid performance. Overall, the aboveground community was more affected by cultivar, which was associated with glucosinolate profiles, than by soil community.

Introduction

Recently, it has become widely acknowledged that belowground communities can have profound effects on aboveground insect communities through plant-mediated interactions (Wardle et al., 2004; Bardgett & Wardle, 2010). Aboveground herbivore performance can be either stimulated or reduced by root-associated communities. Several studies recorded that root-associated communities

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increased (Gange & Brown, 1989; Poveda et al., 2003; Bezemer et al., 2005) or decreased aboveground herbivore fitness parameters (Sell & Kuosell, 1990). This paradox may be understood if we consider the various groups of belowground organisms that were used in these studies and the nature of their interactions with plants.

Soil organisms can be either beneficial or detrimental to the plant and the associated aboveground herbivores. By mobilizing nutrients, soil organisms can increase plant quality and the fitness of aboveground herbivores (Eisenhauer et al., 2010; Orwin et al., 2010). However, soil organisms that inflict damage to the plant can also upregulate defensive compounds in the plant and thereby influence aboveground herbivores in a negative way (Wurst et al., 2006). Interactions that underlie linkages between belowground and aboveground communities via the plant may also be less direct (Megias & Muller, 2010). For instance, carnivorous soil organisms potentially suppress the abundance of soil organisms mobilizing nutrients, thus influencing plant quality and aboveground communities. Consequently, the strength and direction of belowground–aboveground interactions are context-specific; therefore, the outcome of the interactions is likely to be less predictable when recorded at the community level than at the individual level (Wardle, 2002).

An additional layer of complexity affecting the outcome of belowground–aboveground interactions comes from variation at the plant level. Responses of plants, when exposed to belowground communities, often differ between species (van Dam & Raaijmakers, 2006). Such differences may result in differential effects on higher trophic levels aboveground. For instance, the effect of soil community on aphid performance differed among grass species, and these differential effects cascaded up to *Aphidius colemani* Viereck parasitizing the aphid *Rhopalosiphum padi* (L.) (Bezemer et al., 2005). Hence, both the soil community and the plant species mediating the interaction can significantly influence the response of aboveground insect communities.

Plant genotypic variation is well known to affect plant–insect interactions (Crutsinger et al., 2006), and recent studies suggest that the outcome of belowground–aboveground interactions may also depend on which genotype mediates the interactions. For example, in *Plantago lanceolata* L. chemotypes with different iridoid glycoside concentrations, exposure to wireworms changed the defensive compounds in a chemotype-specific way (Wurst et al., 2008). On the other hand, chemical profiles of two *Barbarea vulgaris* R. Br. chemotypes responded similarly to root feeding by *Delia radicum* L. (van Leur et al., 2008). However, both studies used relatively simple belowground communities, with only one root herbivore instead of a more complex community. Additionally, both studies did not examine effects on higher trophic levels aboveground, so that biological implications of intra-specific variation on higher trophic levels in belowground–aboveground interactions remain unknown.

Wild and cultivated *Brassica* species (Brassicaceae) are widely used as model species for belowground–aboveground interactions (Poveda et al., 2003; van Dam & Raaijmakers, 2006; Soler et al., 2010). Brassicaceae are characterized by a structurally diverse group of defensive compounds called glucosinolates, which upon herbivory hydrolyse to (iso)thiocyanates and nitriles, that are toxic to a variety of insects (Mithen, 2001). Glucosinolates in aboveground tissues of *Brassica* species are known to respond to belowground herbivory. For example, *D. radicum* increases glucosinolate concentrations in shoots of *Brassica nigra* L. (van Dam & Raaijmakers, 2006), whereas nematodes reduce glucosinolate concentrations in *Brassica oleracea* L. (Wurst et al., 2006). Differences between genotypes in their response of these defensive compounds to belowground organisms could thus potentially result in differences in the outcome of belowground–aboveground interactions.

The objective of this study was therefore to examine whether plant intra-specific variation can affect the outcome of belowground–aboveground plant-mediated interactions up to the third trophic level. Making use of previous studies (Poelman et al., 2009; Kabouw et al., 2010a), we chose to examine belowground–aboveground interactions in two white cabbage cultivars that were known to differ among others in their root and shoot glucosinolate profiles and in their interactions with nematodes belowground and herbivores aboveground. We assessed whether soil treatments (nematodes or microorganisms) affect aboveground aphid populations of the specialist aphid *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae). The performance of this aphid is known to be negatively affected by specific glucosinolates in plants (Kissen et al., 2009). Therefore, we examined soil-treatment-mediated changes in total leaf and phloem glucosinolate concentrations as well as leaf and phloem glucosinolate profiles. In addition, we examined whether higher trophic level interactions, between the aphid and its parasitoid *Diaeretiella rapae* (McIntosh) (Hymenoptera: Braconidae), differed among cultivars or soil treatments. *Brevicoryne brassicae* is known to sequester glucosinolates for its own defence (Francis et al., 2001; Jones et al., 2001). We expected that if soil organisms are able to modify the glucosinolate profiles in the *Brassica* cultivars, it could be reflected in changes in glucosinolate profiles of the aphid as well. Such changes in herbivore defensive chemistry have previously been shown to affect higher trophic levels (Müller, 2009).

In addition to glucosinolates, we measured primary metabolites, amino acids, as well as plant biomass parameters (relative growth rate, root and shoot biomass), as a measure for plant quality which might increase or decrease for instance owing to the ability of soil organisms to mobilize nutrients (Eisenhauer et al., 2010; Orwin et al., 2010).

Materials and methods

Experimental set-up

Seeds of two white cabbage cultivars – Rivera (breeder and seed source: Bejo Zaden, Warmenhuizen, The Netherlands) and Badger Shipper (breeder: University of Wisconsin, Madison, WI, USA; seed source: Centre of Genetic Resources, Wageningen, The Netherlands) – were germinated for 10 days in a growth cabinet, at L16 (25 °C):D8 (15 °C) hours. A total of 90 seedlings per cultivar were transferred to pots filled with 2 kg of a loamy, sandy mineral soil (0.13% N, 2.1% C, C/N = 16.7; particle size distribution: 3% <2 μm; 17% 2–63 μm; 80% >63 μm), which had been sieved (mesh size 5 mm) and gamma-sterilized at 25 kGrey (Isotron, Ede, The Netherlands). Pots were relocated to a greenhouse with L16 (21 °C):D8 (16 °C) hours, regulated by sodium lamps to maintain a minimum photoactive radiation of 225 μmoles m^{-2} s^{-1}. Plants were randomly divided over three treatments (30 replicates per cultivar per treatment). The treatments per cultivar consisted of (1) a control group of 30 plants without a soil inoculum, (2) 30 plants supplied with a nematode inoculum, and (3) 30 plants with only a microorganism inoculum.

To obtain a microorganism solution, 11 kg of the same unsterilized soil in which the plants were potted was suspended with 11 l of deionized water and incubated for 24 h at room temperature. The supernatant was passed over five sieves (1 × mesh size 75 μm, 3 × 45 μm, and 1 × 10 μm), which retained nematodes but allowed passing of most microorganisms (Bezemer et al., 2005). Aliquots of 70 ml were added to each pot of the microorganism treatment. Nematodes were extracted from the same soil as described earlier (but unsterilized) by filtering it over four sieves (1 × 75 μm, 3 × 45 μm), which retained the nematodes. The residue with the nematodes was purified by incubating for 24 h at room temperature on two milk filters suspended between clamps. This allowed nematodes to migrate into tap water; it was not possible to use deionized water because the osmotic potential could kill the nematodes. The resulting solution, inevitably containing microorganisms, was pooled, and nematodes were counted in 10 subsamples to determine the density. To add 2 000 nematodes per kg of soil, which is an average density for the soil type used in this experiment (Bezemer et al., 2004), 40 ml of the solution was required and added to the pots assigned for nematode treatment. We added 30 ml of tap water to supply the same amount of liquid as in the control and microorganism treatments. Control plants were supplied with 70 ml of demineralized water.

Aphid population growth and parasitoid performance

Brevicoryne brassicae were obtained from cultures maintained in a greenhouse on Brussels sprouts (*B. oleracea*). Parental aphids from the stock rearing were exposed to additional plants of the respective treatment and allowed to larviposit for 24 h on these plants, after which they were removed. When the newborn nymphs reached the second instar, 10 of them were put on two young leaves of 10 plants per treatment and cultivar. Plants were placed individually in gauze nets. Aphid population sizes on these plants were monitored twice a week for 29 days, starting 8 days after introduction of the aphids. After 29 days of infestation, all aphids were collected from the plant for glucosinolate analysis and stored at −20 °C until freeze drying. The extraction of the glucosinolates is described below.

To monitor the effect on the third trophic level, 10 plants per treatment and cultivar were infested with 10 s instars that had been parasitized by the solitary endoparasitoid *D. rapae*. These aphids were reared on plants of the respective treatment, parasitized and then again transferred to plants of the respective treatment. An aphid was considered parasitized when the female parasitoid inserted her ovipositor in the aphid. From the day the first mummy (aphid remains containing a parasitoid pupa) appeared, plants were surveyed daily for new mummies. Mummies were then transferred into glass vials and from 07:00 hours until 23:00 hours checked every 2 h to record survival rate, egg-to-adult development time, adult dry mass, and sex ratio of the adult parasitoids. Since their collection from a Brussels sprouts field near Wageningen (The Netherlands), the parasitoids were reared in a climate room on *B. brassicae*.

Collecting plant and soil samples

From the start of the experiment until aphid infestation, the first and second leaves were photographed weekly and the size of the leaves was calculated using a standard reference area, which was included in the photograph. From these photographs, the leaf area was determined using WinFolia (Regent Instrument, Ottawa, ON, Canada). Based on these measurements, the relative growth rate was estimated as $(\ln \text{area2} - \ln \text{area1})/(t_2 - t_1)$.

Synchronized with the aphid infestation, so 4 weeks after the initiation of soil treatments, 10 plants per treat-

ment and cultivar were harvested. The first, second, and fourth youngest leaf of each plant were pooled and stored at −20 °C for glucosinolate analysis. Additionally, we collected phloem of the third leaf. For this, we followed the procedure outlined in Bezemer et al. (2005) with the modification that we used 2 ml 8 mM EDTA solution, left the leaf 2 h instead of 1 h in this solution, and initially put the leaf for 5 min in an extra EDTA solution to remove any plant chemicals from the wound resulting from detaching the leaf from the plant. The remaining aboveground plant material, including the third leaf, was dried at 70 °C before measuring its dry mass. All plant material for glucosinolate extraction was freeze-dried and weighed, and its dry mass was included in total shoot biomass.

Half of the roots of each plant was cleared from the soil and washed to remove soil particles, dried with filter paper, and stored at −20 °C for glucosinolate analysis. The other half of the roots was removed from the soil, shaken, and stored for 3 days at 4 °C in a dark climate chamber before nematode extraction. Afterwards, the roots were dried at 70 °C and they were weighed. The soil not directly adhering to these roots was bagged and stored in the dark for 3 days at 4 °C before nematode extraction. A subsample of the soil adhering to the roots (rhizosphere soil) was frozen at −80 °C for polymerase chain reaction (PCR) analysis and denaturing gradient gel electrophoresis (DGGE).

After 29 days of aphid infestation, the remaining plants were harvested and the third leaf was, similar to the control plants, used for phloem collection as described earlier. Plant roots and shoots were separated, dried, and weighed.

Soil community analysis

To classify differences in microbial biodiversity, DNA was isolated from the rhizosphere soil using a PowerSoil DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA, USA) following the producer's instructions. Per treatment and cultivar, we analysed four replicates. PCR-DGGE was performed as described in Kabouw et al. (2010b). We analysed all treatments within a cultivar on the same gel, because it is generally difficult to compare gels.

The nematodes from the roots were extracted with a mistifier according to van Bezooijen (2006). Numbers of root-inhabiting nematodes are expressed per gram root dry mass. Free-dwelling nematodes were extracted from the soil as described in the experimental set-up. The nematodes that passed through the milk filters were conserved with 4% formaldehyde. We counted all nematodes present in the samples, both free living and root inhabiting. A subset of 150 nematodes was identified to family and genus level with the help of an inverted microscope. Free-living nematode numbers are expressed per 100 g dry mass of soil.

Extraction and analysis of amino acids and glucosinolates

The freeze-dried samples of roots, shoots, and aphids were ground with a Ball mill. Approximately 100 mg of the finely ground material was used for glucosinolate extraction. For further purification of glucosinolates and amino acids, see the study of van Dam & Raaijmakers (2006). For the extraction of glucosinolates and amino acids from the phloem, we used a modified protocol. From the initial 2 ml of EDTA solution, 1.5 ml was used for glucosinolate extraction. The solution was boiled in a water bath and subjected to an ultrasonic bath for 15 min to inactivate myrosinase activity and brought directly on the Sephadex column. After elution, the freeze-dried elute was resuspended in 100 µl MilliQ to concentrate the samples. Dilution factors for high-performance liquid chromatography (HPLC) were adjusted accordingly. Amino acids were analysed as in the study of van Dam & Raaijmakers (2006), by taking 20 µl of the EDTA phloem solution. HPLC analyses were performed as described in the study of Kabouw et al. (2010b).

Statistical analysis

Nematode numbers were analysed by two-way ANOVA. DGGE gel banding patterns were analysed by distance-based redundancy analysis (db-RDA) as described by Kabouw et al. (2010b). However, data were visualized by correspondence analysis.

Aphid numbers were log-transformed and analysed by repeated measures two-way ANOVA. Development time and adult dry mass of the parasitoids were averaged per plant. Generalized linear models (GLM) were used to analyse parasitoid survival and sex ratio. Both survival and sex ratio were analysed as a binomially distributed dependent variable per plant (for survival: Y adult parasitoids surviving out of N recollected aphids on that plant; for sex ratio: Y females out of N adult parasitoids). Two-way ANOVAs were used to assess differences on egg-to-adult development time and adult dry mass of parasitoids. Prior to these analyses, Student's t-tests were used to analyse differences between females and males in egg-to-adult development time and adult dry mass of the parasitoids. As male and female wasps did not differ in development time ($t = 0.58$, $P>0.5$) or adult weight ($t = 0.28$, $P>0.5$) and no interaction was recorded between sex and cultivar, nor between sex and soil treatment, data for male and female wasps were pooled in the analyses.

Total concentrations of glucosinolate and amino acids in plants and aphids were analysed by two-way ANOVA. Aphid and plant glucosinolate profiles and plant phloem amino acid profiles were analysed by Monte Carlo permutation (MCP) tests in a redundancy analysis (RDA) with cultivar or treatment as environmental variable and gluco-

sinolates as species data. Multivariate interactions of glucosinolate profiles were analysed by MCP tests, i.e., to see whether soil treatments are differently ranked within the cultivars, with cultivar and treatment as environmental factors. GLM analyses were performed in GenStat (11th edition; VSN International, Hemel Hempstead, UK), RDA models and MCP tests were performed using CANOCO (version 5; Biometris, Wageningen, The Netherlands), whereas all other statistical tests were performed using STATISTICA (version 8; StatSoft, Tulsa, OK, USA).

Results

Aphid population growth

Aphid population development differed both between the two cultivars and between the soil treatments. Aphid populations on plants from microorganism-inoculated soils increased significantly faster than those on plants from nematode-inoculated or control soils (repeated-measures ANOVA: $F_{1,53} = 8.62$, $P<0.05$). By contrast, aphid populations tended to develop slower on plants from nematode-treated soils than on plants from control soils, but this difference was not statistically significant on either of the cultivars (Figure 1). Overall, the cultivar effect was stronger, with two-fold differences in aphid numbers, than the soil treatment effect (Figure 1). On Rivera, the aphid population growth was considerably slower than on Badger Shipper (repeated-measures ANOVA: $F_{2,57} = 21.70$, $P<0.001$). Aphid population growth rates responded similarly to soil treatments on both cultivars (repeated-measures ANOVA, soil treatment*cultivar: $F_{2,57} = 0.87$, $P>0.05$).

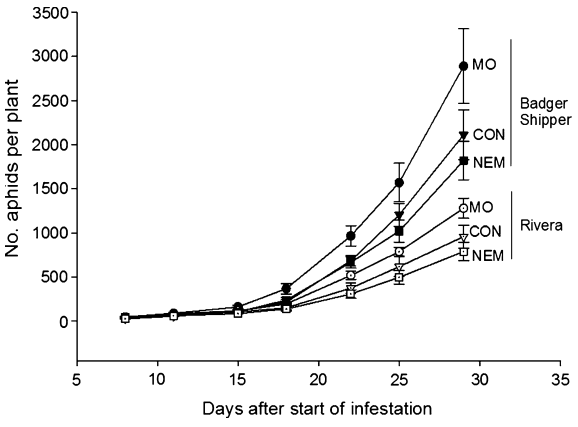

Figure 1 Aphid population development (mean ± SE number of aphids per plant). Aphids were reared on soils treated with microorganisms (○), untreated control soils (▽), or nematode-treated soils (□). White symbols represent aphids reared on Rivera, and black symbols represent aphids reared on Badger Shipper.

Parasitoid performance

Soil treatment did not affect parasitoid developmental time (two-way ANOVA: $F_{2,54} = 0.29$, $P>0.5$), adult dry mass (two-way ANOVA: $F_{2,54} = 0.35$, $P = 0.5$), sex ratio (GLM: deviance = 0.68, $P>0.5$), or survival (GLM: deviance = 0.595, $P>0.5$).

By contrast, parasitoid development was significantly affected by cultivar. Similar to the aphids, parasitoids developed faster on Badger Shipper (13.11 ± 0.24 days) than on Rivera (13.61 ± 0.29 days; two-way ANOVA: $F_{1,54} = 4.80$, $P<0.05$). Also, adult mass was higher on Badger Shipper (0.090 ± 0.003 mg) than on Rivera (0.080 ± 0.002 mg; two-way ANOVA: $F_{1,54} = 10.28$, $P>0.01$). The survival of the parasitoids was similar on both cultivars (GLM: deviance = 2.163, $P>0.2$; Badger Shipper: 92 ± 4%, Rivera: 97 ± 3%). However, wasps emerging from aphids reared on Rivera had a higher fraction of females than those on Badger Shipper (GLM: deviance = 5.205, $P<0.05$; 43 ± 7 vs. 25 ± 8%, respectively). No significant interaction between cultivar and soil treatment was observed for parasitoid developmental time, adult mass, percentage survival, or the fraction of females that emerged.

DGGE fingerprints and nematode numbers

For both Rivera and Badger Shipper, DGGE patterns of bacteria and fungi differed significantly between the treated soils on both the first and second axes of the db-RDA (MCP tests, Figure S1). Before aphid exposure, total nematode abundances were significantly higher in nematode-treated soils, both in the bulk and in the rhizosphere soil, as was intended (Table 1). The number of phytophagous nematodes also differed between treatments and was several magnitudes higher in nematode-treated soils than in microorganism-treated and control soils (Table 1). The dominant nematodes belonged to the bacterivorous families Cephalobidae and Rhabditidae. Cultivar affected neither total nematode abundance nor number of plant–parasitic nematodes (Table 1). There were also no interaction effects between the cultivars and soil treatments on nematode abundances (Table 1).

Plant chemistry

Glucosinolates. Glucosinolate concentrations in the phloem, leaves, and roots were not significantly affected by soil treatment (see Table S1). Also, the glucosinolate profiles were not significantly affected by soil treatment, in leaves (MCP: $P>0.5$; Figure 2A), roots (MCP: $P>0.1$), and in the phloem, before (MCP: $P>0.1$) or after aphid infestation (MCP: $P>0.1$).

Analogous to the aphid population growth data, differences in glucosinolate concentrations and profiles were

Table 1 Average number (± SD) of nematodes per treatment expressed per 100 g of dry soil or g dry root

Treatment	Total no. nematodes in the bulk soil	No. plant–parasitic nematodes in the bulk soil	Total no. nematodes per gram dry root	No. plant–parasitic nematodes per gram dry root
Rivera microorganisms	56 ± 40a	0.6 ± 0.5a	21.0 ± 15.1a	0.1 ± 0.1a
Rivera control	4 ± 1b	0.1 ± 0.1a	1.6 ± 0.5b	1.0 ± 0.8a
Rivera nematodes	1189 ± 182c	11.2 ± 3.7b	585.2 ± 152.3c	25.8 ± 20.1b
Badger Shipper microorganisms	68 ± 61a	0.2 ± 0.2a	11.5 ± 10.1a	0.2 ± 0.2a
Badger Shipper control	33 ± 23a	0.0 ± 0.0a	26.5 ± 25.8a	0.2 ± 0.2a
Badger Shipper nematodes	848 ± 101b	5.7 ± 2.4b	728.9 ± 248.2b	42.5 ± 20.1b
F cultivar	1.8	1.8	0.3	0.5
F treatment	73.8*	13.6*	19.2*	8.8*
F interaction	2.9	1.3	0.2	0.6

Different letters within a column within a cultivar denote significant differences (Tukey's post-hoc analysis: $P<0.05$).
*Significant effect (two-way ANOVA: $P<0.05$).

much more pronounced between cultivars than between soil treatments. Phloem concentrations were significantly higher in Rivera than in Badger Shipper at the second sampling (Table S1) and also the glucosinolate profiles in the phloem differed between the cultivars, both before and after aphid infestation (MCP: both $P<0.01$). Indolic glucosinolates, mainly 4-hydroxyglucobrassicin and 4-methoxyglucobrassicin, dominated in the phloem of Badger Shipper, which had the highest aphid numbers, whereas in Rivera aliphatic glucosinolates, mainly gluconapin and sinigrin, dominated. No interaction between soil treatment and cultivar was recorded for glucosinolate profiles at either the initial (MCP: $P>0.9$) or the second (MCP: $P>0.05$) phloem sampling. In the roots and leaves, total glucosinolate concentrations did not differ between the cultivars, but their profiles did. Leaf glucosinolate profiles differed significantly between cultivars (MCP: $P<0.01$), with indolic glucosinolates dominating in Badger Shipper (Figure 2A), similar to what was observed for the phloem glucosinolates. Root profiles also differed significantly between cultivars (MCP: $P<0.01$). Gluconasturtiin, which was not recorded in Badger Shipper, contributed to this difference. No interaction between soil treatment and cultivar was recorded for glucosinolate profiles in either leaves (MCP: $P>0.9$) or roots (MCP: $P>0.05$).

Amino acids. At the initial sampling, before aphid infestation, soil treatment did not affect phloem amino acid concentrations ($P>0.8$, Table S1) or profiles (MCP: $P>0.05$). At the second sampling, i.e., after aphid feeding, there was a significant soil treatment effect within Rivera. Compared with control plants, amino acid phloem concentrations were higher in this cultivar on microorganism-treated soils, which also had the highest aphid numbers, and on nematode-treated soils, which in contrast had the lowest aphid numbers (Table S1). The differences in amino acid concentrations among treatments after aphid infestation were accompanied by significant differences in amino acid profiles (MCP: $P<0.01$).

Amino acid concentration in the phloem initially did not differ between the two cultivars. However, after aphid infestation, Rivera had considerably higher amino acid concentrations than Badger Shipper (Table S1). The profiles differed significantly between cultivars before aphid infestation (MCP: $P<0.001$). Rivera had higher levels of phenylalanine, whereas Badger Shipper had higher concentrations of all other amino acids. After aphid infestation, the profiles also differed between the cultivars (MCP: $P<0.001$), with all amino acids dominating in Rivera.

Aphid chemistry

Total glucosinolate concentrations (two-way ANOVA: $F_{2,58} = 0.67$, $P>0.1$) and glucosinolate profiles (MCP: $P>0.5$; Figure 2B) in the aphids were not affected by soil treatment. Total glucosinolate content also did not differ between aphids reared on the different cultivars (two-way ANOVA: $F_{1,58} = 0.48$, $P>0.1$; Rivera: 16.18 ± 1.20 μmol g^{-1} d.w., Badger Shipper: 17.89 ± 1.68 μmol g^{-1}). Glucosinolate profiles, however, varied between the aphids reared on the two cultivars (MCP: $P<0.01$). In contrast to what was observed in the plants, indolic glucosinolates dominated in aphids on Rivera and aliphatic glucosinolates dominated in aphids on Badger Shipper (Figure 2B). There was no significant interaction between soil treatment and cultivar with regard to aphid glucosinolate concentration (two-way ANOVA: $F_{2,54} = 1.07$, $P>0.1$) or profiles (MCP: $P>0.5$).

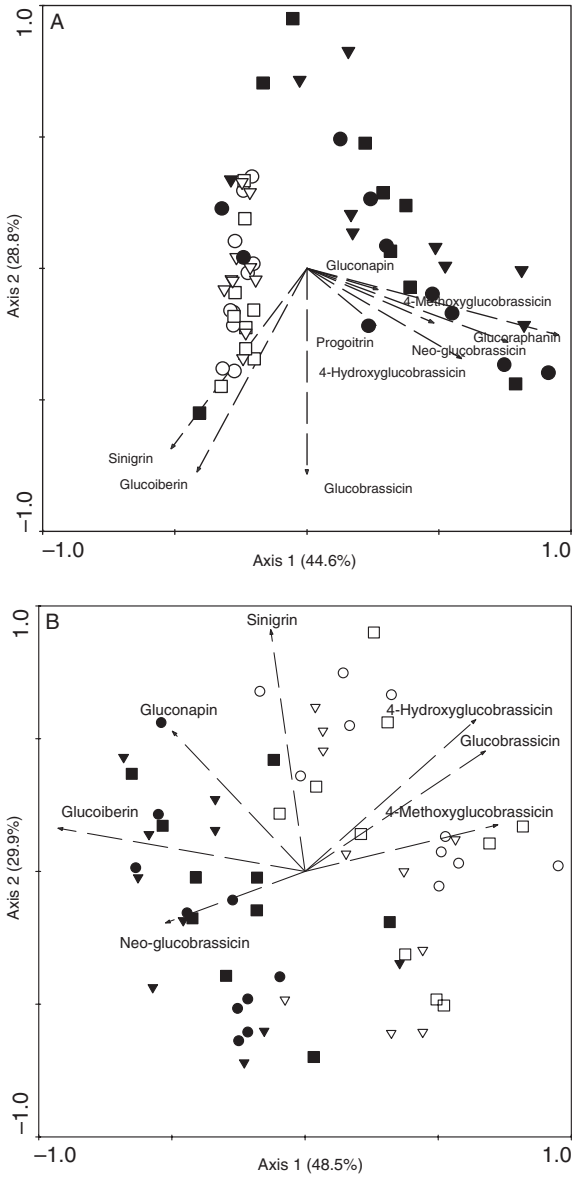

Figure 2 Principal component analysis plot for glucosinolate profiles of (A) leaf and (B) aphids. Plants or aphids were raised on soils treated with microorganisms (○), untreated control soils (▽), or nematode-treated soils (□). White symbols represent the cultivar Rivera, and black symbols represent Badger Shipper. Arrows indicate different glucosinolates.

Plant biomass and leaf relative growth rate

Root biomass was higher for control plants than for plants growing in nematode-treated soils, although this contrast was only significant within the Rivera cultivar (Table S1). The expansion of the leaves also differed significantly between soil treatments. The growth of the second leaf between weeks 3 and 4 of the experiment was faster in plants on the microorganism and control soils than in the nematode-treated soil, although the contrast was only significant for Badger Shipper.

Plant biomass did not significantly differ between the cultivars before aphid infestation. At the second sampling, after aphid infestation, both root and shoot mass differed significantly between the cultivars. Rivera had a higher root and shoot biomass than Badger Shipper. The relative growth rate of the first and second leaves between weeks 3 and 4 was significantly higher for Badger Shipper than for Rivera. There was no significant interaction between treatment and cultivar, indicating that cultivars responded similarly in relative growth rate and biomass to the treatments (Table S1).

Discussion

Both manipulation of soil communities and cultivar differences affected aphid population growth on white cabbage. Badger Shipper sustained more aphids than Rivera, irrespective of soil treatment. Microorganisms significantly enhanced aphid population development, whereas nematodes on average reduced aphid population development on both cultivars, although these differences were not statistically significant. Effects of soil treatment on plant parameters and aphid population growth were similar for both cultivars. This indicates that cultivar did not affect the direction (ranking of the soil treatments) or the strength (relative difference between the soil treatments) of the belowground–aboveground interaction. Cultivar also affected fitness parameters of the parasitoids. By contrast, the effects of soil treatment were not detected at the level of parasitoid performance. Overall, these results indicate that effects of belowground organisms on aboveground organisms are consistent across cultivars, i.e., independent of the identity of the plant that was mediating the interaction.

Our results are consistent with the observation that, despite the large differences in defensive chemistry between cultivars, their response to belowground organisms with respect to primary and secondary chemistry can be quite similar. In contrast to earlier studies (van Dam & Raaijmakers, 2006; Wurst et al., 2006), both cultivars failed to respond to belowground organisms with respect to glucosinolate concentrations or profiles, including those in the phloem that could have affected aphid performance. It will be interesting to see whether the same pattern holds for plants from wild *B. oleracea* populations. The cultivars used in this study are the result of artificial selection and the variation between these plants is well characterized both belowground and aboveground (Poelman et al., 2008, 2009; Kabouw et al., 2010a). Wild *B. oleracea* popu-

lations are also known to differ substantially at least in their aboveground glucosinolate composition (Gols et al., 2008; Newton et al., 2009) but potentially harbour additional variation in induced responses to belowground organisms that has not been retained in the cultivars. However, similar to cultivated brassicas, two wild populations of *B. vulgaris* showed no induced response in their glucosinolate concentrations to belowground feeding by *D. radicum* (van Leur et al., 2008), indicating that wild species do not necessarily have to respond differently from cultivated species.

Soil inoculum effect

Aphid population growth was enhanced on plants treated with microorganisms compared with control and nematode-treated plants. This contrasts with results from another study using grasses, in which no differences in aphid numbers were observed between microorganism-treated and control plants (Bezemer et al., 2005). Positive effects of microorganisms on aphid performance can be expected, as belowground (micro)organisms have the ability to influence aboveground insects by mobilizing nutrients and thus improving plant quality (Haase et al., 2008; Eisenhauer et al., 2010; Orwin et al., 2010; Wurst & Forstreuter, 2010). If the microorganism effect in our experiment was indeed attributable to improved plant quality, it does not seem to be mediated by consistently higher phloem amino acid concentrations or differences in the growth parameters 'relative growth rate' or 'total biomass'.

Effect of cultivar

It is generally known that intra-specific variation, also within the Brassicaceae, can affect aboveground herbivores and higher trophic level organisms (Crutsinger et al., 2008; Kissen et al., 2009; Newton et al., 2009; Lankau, 2010). Our observation that Rivera sustained fewer aphids than Badger Shipper is consistent with a previous study (Broekgaarden et al., 2008). Aphid population growth generally is dependent on plant traits, such as leaf relative growth rate (Hughes & Bazzaz, 2001), and leaf or phloem concentrations of defensive compounds, such as glucosinolates (Kim et al., 2008). Indeed, the leaf relative growth rate did differ between cultivars. Moreover, the glucosinolates 4-methoxyglucobrassicin and glucobrassicin dominated in the leaves and phloem of Badger Shipper, whereas gluconapin and sinigrin, which have been implicated to decrease aphid fitness (Cole, 1994), dominated in leaves and phloem of Rivera. Although phloem and leaf total glucosinolate levels were fluctuating (first higher in Badger shipper, later higher in Rivera), it might be the consistent differences in glucosinolate profiles that result in different aphid population growth rates. Replacing 4-methoxyglucobrassicin and glucobrassicin with gluconapin and sinigrin in *Arabidopsis thaliana* (L.) Heynh., analogous to the differences in glucosinolate profiles between Badger Shipper and Rivera in our study, reduced the population growth of *B. brassicae* on these plants (Kissen et al., 2009). Therefore, the higher concentrations of gluconapin and sinigrin could have been one of the factors responsible for the reduced aphid numbers on Rivera.

Soil treatment did not result in different glucosinolate profiles among the aphids, but the cultivars on which they had been reared greatly affected their glucosinolate profiles. Intriguingly, the glucosinolate profiles in the aphids were completely different from those in the phloem: 4-methoxyglucobrassicin and glucobrassicin were recorded in higher concentrations in aphids on Rivera, whereas these dominated in Badger Shipper plants. The incongruence between glucosinolates in the plants and aphids may be attributable to the ability of *B. brassicae* to sequester glucosinolates, thus regulating glucosinolate composition (Bridges et al., 2001; Kazana et al., 2007). Hence, aphid glucosinolates are not necessarily directly correlated with phloem and leaf glucosinolate levels.

The different aphid glucosinolate profiles might explain why *D. rapae* performed better on aphids reared on Badger Shipper. On Badger Shipper, adult mass of *D. rapae* was higher and its development time was shorter, two parameters usually associated with beneficial host quality (Bukovinszky et al., 2008). However, on Rivera, female sex ratio was higher, a parameter that is also usually associated with better host quality (Tanaka, 2009). This incongruence may be related to temporal shifts in host quality. Adult mass and developmental time are dependent on the conditions after egg laying, so during the main part of the development of the host, offspring sex ratio is determined by the female upon egg laying. Perhaps the effect of cultivar on host quality changes during the development of the host, therefore differentially affecting offspring sex ratio, determined by the female, and performance of the offspring.

In conclusion, our study has shown that aphid populations respond to both soil organisms and plant cultivar. However, belowground–aboveground interactions were independent of *Brassica* cultivar. The lack of an intra-specific plant effect on the belowground–aboveground interaction may be the result of rather similar responses of both cultivars in terms of defensive chemistry to the soil treatments. The stimulated aphid population development by microorganisms indicates that plant–animal interactions aboveground may change, depending on soil community composition. However, in our specific case, cultivar had a stronger effect on the aboveground interactions than the composition of the soil community.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Correspondence analysis ordination diagram of denaturing gradient gel electrophoresis (DGGE) patterns of the bacterial and fungal communities.

Table S1 Average values of several plant characteristics.

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