Plant Soil (2010) 336:509–518
DOI 10.1007/s11104-010-0507-y

REGULAR ARTICLE

Effects of intraspecific variation in white cabbage (*Brassica oleracea* var. *capitata*) on soil organisms

Patrick Kabouw · Wim H. van der Putten · Nicole M. van Dam · Arjen Biere

Received: 2 June 2010 / Accepted: 20 July 2010 / Published online: 31 July 2010
© The Author(s) 2010. This article is published with open access at Springerlink.com

Abstract Intraspecific variation in plants can affect soil organisms. However, little is known about whether the magnitude of the effect depends on the degree of interaction with the roots. We analyzed effects of plant intraspecific variation on root herbivores and other soil organisms that interact directly with living plant roots, as well as on decomposer organisms that interact more indirectly with roots. We used four different white cabbage (*Brassica oleracea* var. *capitata*) cultivars exhibiting a high degree of intraspecific variation in root glucosinolate profiles. Intraspecific variation affected root-feeding nematodes, whereas decomposer organisms such as earthworms and Collembola were not affected. Root-feeding nematodes were most abundant in one of the cultivars, Badger Shipper, which lacked the glucosinolate gluconasturtiin. The effect of the intraspecific variation in glucosinolate composition may have been restricted to root-feeding nematodes due to the rapid degradation of glucosinolates and their breakdown products in the soil. Additionally, the low biomass of root-feeding nematodes, relative to other soil organisms, limits the possibility to affect higher trophic level organisms. Our results show that variation in root chemistry predominantly affects belowground herbivores and that these effects do not extend into the soil food web.

Keyword Glucosinolates · Trophic level · 2-Phenylethyl · *Pratylenchus*

Abbreviations

RDA	Redundancy Analysis
PCR	Polymerase Chain Reaction
DGGE	Denaturing Gradient Gel Electrophoresis
CCA	Canonical Correspondence Analysis
MCP	Monte Carlo Permutations
ANCOVA	Analysis of Covariance

Responsible Editor: Harry Olde Venterink.

Electronic supplementary material The online version of this article (doi:10.1007/s11104-010-0507-y) contains supplementary material, which is available to authorized users.

P. Kabouw (✉) · W. H. van der Putten · N. M. van Dam · A. Biere
Department of Terrestrial Ecology,
Netherlands Institute of Ecology (NIOO-KNAW),
Boterhoeksestraat 48,
6666 GA Heteren, the Netherlands
e-mail: p.kabouw@nioo.knaw.nl

W. H. van der Putten
Laboratory of Nematology, Wageningen University,
Droevendaalsesteeg 1,
6708 PB Wageningen, the Netherlands

N. M. van Dam
Institute for Water and Wetland Research,
Radboud University,
Heyendaalseweg 135,
6500 GL Nijmegen, the Netherlands

Introduction

Soil food webs include a wide variety of organisms feeding on a broad range of different resources. Their basal resources are of plant origin, such as living roots, recalcitrant and labile plant litter, and root exudates. Plant roots are important factors in determining the composition of soil food webs (Bezemer et al. 2010; Witt and Setälä 2010). Depending on the trophic level in the soil food web, the level of interaction with the plant differs. Root feeders directly interact with living plant roots, whereas detritivores and predacious soil organisms feed on plant litter or on second and higher trophic level organisms (Berg and Bengtsson 2007; de Ruiter et al. 1995; Holtkamp et al. 2008; Mulder and Lotz 2009; Mulder et al. 2003), leading to a more indirect interaction with living plant roots (Wardle et al. 2004). Due to their direct exposure to the roots, root-associated and lower trophic level organisms in the soil food web are more likely to be affected by variation in plant quality than other soil organisms (Wardle 2006). Nevertheless, decomposer organisms and higher trophic level organisms can potentially be influenced via changes in the quality and quantity of litter, in root-exudates, or via differences in the quality or quantity of prey or host species (Mulder and Lotz 2009). In the present study, we examine how intra-specific variation among plant cultivars may affect these different components of the soil food web.

The effects of intraspecific plant variation have been well studied for aboveground food webs (Broekgaarden et al. 2007; Crutsinger et al. 2006; Poelman et al. 2009). Soil organisms could also be affected by intraspecific variation in plants, for example via chemical variation between plant roots, e.g. differences in the production of toxic compounds that affect soil organisms. However, belowground effects of intraspecific plant variation on plant-animal interactions have only been studied to a limited extent and mostly within single organism groups (Andersen et al. 2007; Griffiths et al. 2005; Wardle 2006; Wurst et al. 2008).

To examine the effect of intraspecific variation we used a model system consisting of White Cabbage (*Brassica oleracea* var. *capitata*) cultivars which exhibit a high degree of intraspecific variation, both aboveground and belowground (Broekgaarden et al. 2007; Poelman et al. 2009). The roots of these cabbage cultivars are known to differ in their glucosinolate composition (Kabouw et al. 2010). Glucosinolates are a group of secondary plant compounds prominently present in the Brassicaceae which could potentially influence the soil food web (Yang et al. 2009). Upon tissue damage, glucosinolates are degraded by the enzyme myrosinase, thereby forming toxic breakdown products such as (iso)thiocyanates (Wang et al. 2009). Belowground, glucosinolates and their breakdown products are known to reduce the abundance of phytophagous organisms such as root-feeding nematodes (Lazzeri et al. 2004; Potter et al. 1998; Potter et al. 2000), fungi (Bressan et al. 2009; Rumberger and Marschner 2003; Snapp et al. 2007), and bacteria (Aires et al. 2009; Bressan et al. 2009; Rumberger and Marschner 2003). Aromatic glucosinolates, particularly gluconasturtiin and its breakdown product 2-phenylethyl isothiocyanate, are generally considered as the most toxic glucosinolates in plant roots (Potter et al. 1998; Potter et al. 2000; Rumberger and Marschner 2003; van Dam et al. 2009; Vierheilig et al. 2000).

Besides exerting negative effects on root feeding organisms, glucosinolates can potentially influence decomposer organisms and higher trophic levels in the soil food web. Indeed, it has been shown in laboratory experiments that glucosinolates and their hydrolysis products affect Collembola (Jensen et al. 2010) and entomopathogenic nematodes (Ramirez et al. 2009), although these taxa are not directly feeding on living plant roots.

The objective of the present study is to examine the effects of intraspecific variation among plants on components of the soil food web which differ in their degree of exposure to variation in plant chemistry. Therefore we analyzed the fate of organisms that feed on living plant roots and organisms belonging to the detritus-based food web. These soil organisms differ in their nutrient requirements, body size, and growth/reproductive rates (Mulder and Lotz 2009). We tested the hypothesis that the intraspecific chemical variation in our plants will affect root-feeding nematodes due to their close contact with the roots and we determined the extent to which this variation influences other important groups such as mites, Collembola, earthworms, and enchytraeids in the soil food web.

Material and methods

Cultivar and field experiment

To test our hypothesis, we performed a field experiment with four white cabbage cultivars. The cultivars (with seed supplier and if known the breeder) used in this study were Badger Shipper (Centre for Genetic Resources NL, University of Wisconsin US), Christmas Drumhead (Centre for Genetic Resources, NL), Lennox, and Rivera (both breeder and source Bejo seeds, NL). The four cultivars were selected based on their divergent glucosinolate profiles (Kabouw et al. 2010). Badger Shipper had relatively low concentrations of aromatic glucosinolates (gluconasturtiin) whereas Rivera had relatively high concentrations; the two other cultivars had intermediate concentrations. The setup of the experiment is described in detail in Kabouw et al. (2010). In short the field, comely used for cultivation of crops, was located near Wageningen, 51.95° north 5.64° east, The Netherlands, with the following soil characteristics N=0.14%, C=1.7%, C/N= 12, particle size distribution: <2 μm 5%, 2–63 μm 79%, >63 μm 19% . The field consisted of 32 plots (eight plots per cultivar), separated by 6 m wide grassland strips. On each plot one of the four cultivars was randomly assigned. The plots consisted of 49 plants in 7×7 grids separated by at 75 cm distance. Plants were planted as three-week old plantlets pre-grown in a greenhouse on Lentse-potgrond (Horticoop, the Netherlands). The trial started on April the 28th, 2008. The field was weeded on a monthly basis and fertilized twice. On September 8th the roots of two plants per plot were harvested. These roots were pooled and half of the root material was used for glucosinolate analyses by HPLC as previously described by van Dam and Oomen (2008). The other pooled half was used for extraction of nematodes as described below.

Soil organism sampling

Soil organisms were sampled at two time points. The initial sampling took place on April 27, 1 day prior to planting, and represents the initial abundance of soil organisms in the field plots. The final sampling took place on September 8, 133 days after planting of the cultivars, at the same time as root samples for glucosinolate analysis were collected.

Microorganism community analysis

Initial samples of microorganisms, collected before planting of the cultivars, were obtained by pooling two soil cores per plot of 1.5 cm ø, which were collected from the top 10 cm of soil. Final samples, 133 days after planting of the cultivars, were obtained by sampling the soil directly adherent to the roots (rhizosphere soil) from the same two plants from which we collected root material for glucosinolate and nematode analysis. We analyzed the soil of six plots per cultivar. All samples were immediately put on ice, and transported to the laboratory before storage at -80°C. To analyze the microbial diversity we used PCR-DGGE. DNA was isolated using a PowerSoil DNA isolation kit (MO BIO Laboratories, CA, USA) following the producer's instructions, with the addition of an initial step of 10 min of bead beating. DNA was eluted in 100 μl MilliQ. PCR was performed with universal 968-GC and 1,378 primers for bacteria (Muyzer et al. 1993) and FR1-GC and FF390 primers for fungi (Vainio and Hantula 2000). For both PCR reactions 1 μl of total DNA was added to 24 μl PCR reaction (17.6 μl MilliQ water, 2.5 μl 10 × PCR buffer (Roche Diagnostics, Germany) 2.5 μl of 2 mM dNTP (Amersham Biosciences, US), 0.4 μl taq-Polymerase (Roche Diagnostics, Germany) and 0.5 μl of both primers). For DNA amplification we followed the protocol of Yergeau et al. (2007) for bacteria and Vainio and Hantula (2000) for fungi. Both amplifications were performed with a thermal cycler PTC-200 (Bio-Rad, US) and successful amplification was confirmed by electrophoresis of the PCR product (2 μl). Denaturing Gradient Gel Electrophoresis (DGGE) was performed as described previously (Yergeau et al. 2007) in a Universal Mutation Detection System (Bio-Rad, US). Gels were stained in ethidium bromide and photographed with an Imago apparatus (Gentaur, Belgium).

Nematode extraction and analysis

Nematodes were extracted from the roots only at the final sampling. For this we used half of the pooled root material (see glucosinolate analysis above). Root material was stored in a cooling box during transport to the laboratory before storing the samples for 18 hours in a climate chamber at 4°C. The nematodes were extracted with the help of a mistifier according

to van Bezooijen (2006). Numbers of root-inhabiting nematodes are expressed per g dry root. For nematodes from the soil we collected eight sub-samples per plot by removing soil cores (see above). All sub-samples were pooled to produce one replicate sample per plot. For the final sampling soil nematodes were collected, using similar soil cores, next to the main root. Nematodes were extracted from soil by Oostenbrink elutriator and conserved with 4% formaldehyde before identification and expressed as numbers per 100 g dry soil. For both the nematodes from the root and soil we counted all nematodes present in the samples. Subsets of 150 nematodes were identified according to Bongers (1988) with the help of an inverted microscope to estimate the distribution over the families/genera. Identified nematodes were grouped on the basis of their feeding guilds (Yeates et al. 1993). Additionally weight of the nematodes was calculated by the formula $W = (w^2 \cdot l) \cdot (1.6 \cdot 10^6)^{-1}$ (Freckman 1982), whereby W is the wet weight, w is the width of the nematodes at the widest point, and l is the length of the nematodes in μm. Lengths were taken from Bongers (1988).

Mites and Collembola extraction and analysis

Mites and Collembola were sampled and extracted simultaneously. Per plot we sampled one soil core of 10 cm depth and of 8.5 cm ø. This was done in the middle of the plot at the initial sampling and next to the root system of one of the cabbage plants at the final sampling. Extraction was performed following the protocol of Holtkamp et al. (2008). The Collembola were identified to the family level according to Fjellberg (1980) whereas for mites only total abundances were recorded. As microarthropods were not divided into functional groups we could not calculate the biomass (Holtkamp et al. 2008). Therefore we expressed the abundances as numbers and analyzed these. Numbers of mites and Collembola were counted using a stereomicroscope.

Earthworms and enchytraeids

For enchytraeids one sample per plot was taken, both at the initial and at the final sampling. Soil cores (10 cm depth and 5 cm ø) at final sampling were taken near the roots. Extraction was done using a Baermann funnel (O'Connor 1957). For earthworms one soil monolith of 20×20 cm and 10 cm depth was excavated per plot, both at the initial and final sampling. The earthworms were collected by hand-sorting.

Enchytraeids were counted using a stereomicroscope and their length, width, and surface area was measured with the program Axio vision (Carl Zeiss imaging solutions) and their weight was calculated by the formula $\log W = -0.6056 + 1.8546 \log L$ (Abrahamsen 1973b), where L is the length and W the wet weight. Earthworms were indentified to the species level according to Christian and Zicsi (1999), their length was measured, and their wet weight calculated by the formula $\log W = -2.255 + 2.071 \log L$ (Abrahamsen 1973a). Weights are expressed on the basis of 100 g dry weight of the soil.

Statistical analysis

Glucosinolate data

Glucosinolate profiles of the four cabbage cultivars, in each of the plots, previously described in Kabouw et al. (2010), were used in the current study to test whether variation in these profiles could explain variation in the abundance of microorganisms, nematodes in the roots, nematodes in the soil, microarthropods, and earthworms. Analyses were based on plot means for glucosinolate concentrations and the abundances of the different species per plot for soil organisms.

To analyze whether glucosinolates can explain differences in diversity we used a multivariate method, redundancy analysis (RDA). This model allows multiple variables, in our case the glucosinolates, to be tested for their explanatory power. In cases where glucosinolates could significantly explain the abundance data we used a forward selection procedure to identify the glucosinolates with the highest explanatory power within the profile. For the microorganisms we used canonical correspondence analysis (CCA) instead of a RDA because of the binarity of the data. Monte Carlo permutations (MCP) were used to test the significance of differences along the first axis and all other axes. All RDA/CCA analyses were performed in CANOCO 4.5 for Windows (Biometris, Wageningen, NL).

DGGE data

DGGE gel banding patterns were analyzed using the Image Master 1D program (Amersham Biosciences,

the Netherlands). The resulting binary matrices were used as 'species' presence–absence matrices. To test for the effects of cultivar multivariate tests of significance were carried out using CCA in CANOCO. The significance of each treatment was tested by 999 permutations on both the first and second axis.

Nematode data

Analysis of Covariance (ANCOVA) was used to analyze plot and cultivar effects on nematode biomass. The biomass of the nematodes at the first sampling was used as a continuous predictor, cultivar as a categorical variable, and the biomass of the nematodes at the second sampling as the dependent variable. This analysis was performed to correct for possible spatial heterogeneity at the start of the experiment. Data was log transformed, if needed, to achieve normality or homogeneity of variances.

To assess whether our sampling scheme was sufficient at both the initial and final sampling to allow further analyses of the nematode community we estimated sampling success (*i.e.*, the number of sampled families out of the estimated real number of families). We applied a non-parametric first order jackknife approach using the program EstimateSWin820 (University of Connecticut, US).This analysis revealed that our sampling success was comparable between initial sampling, before the cabbages were planted, and final sampling (Table S1). Therefore we analyzed both samplings simultaneously. To analyze the differences in nematode abundances among cultivars we used a RDA model with MCP as test of significance. To correct for spatial heterogeneity, plot identity was additionally coded as a dummy variable and defined as co-factor in the analysis, an approach similar to linear polynomial contrasts (Lepš and Šmilauer 2003).

Mites, Collembola, enchytraeids, and earthworms

ANCOVA was used to analyze abundances for mites and Collembola, and enchytraeids, as well as earthworm biomass as described above. t-Tests or Wilcoxon rank-sum tests were used to compare initial and final samplings. RDA as described above was used for differences in diversity of Collembola or earthworms between cultivar, as these groups were identified at the family and species level, respectively.

Results

Nematodes in the roots

The majority of nematodes in the roots belonged to the genus *Pratylenchus*. The number of root nematodes differed vastly between cultivars. Badger Shipper had higher numbers of nematodes than the other cultivars (Table 1). Glucosinolate profiles of the cultivars significantly correlated with nematode abundance (RDA MCP $P<0.01$). Following forward selection to identify the most explanatory glucosinolates, the model only retained gluconasturtiin, which was negatively correlated ($P=0.004$, $F=6.8$), and glucoerucin, which was positively correlated ($P=0.002$, $F=13.8$) with *Pratylenchus* abundances (Fig. S1).

Nematodes in the soil

RDA did not reveal cultivar-specific effects on nematode communities in the soil (MCP $P>0.1$,). In addition, glucosinolates were not associated with nematode abundances (RDA MCP $P>0.5$). The *Pratylenchus* recorded in the soil tended to be negatively correlated with root concentrations of gluconasturtiin, as observed for nematodes recorded in the roots, but glucosinolate effects on the soil nematode community were not significant (Fig. 1, glucosinolates by nematode community RDA MCP $P=0.066$).

The cultivar Badger Shipper supported a higher biomass of plant-feeding nematodes in the soil (significant effect of Badger Shipper vs. Lennox and Rivera, Table 2). The biomass of other feeding guilds did not differ between cultivars (Table 2).

Table 1 Number of nematodes recovered from the roots of the cultivars

	Total nr.	*Pratylenchus*
Badger shipper	156±65a	151±64a
Christmas drumhead	10.0±3.6b	7.3±3.4b
Lennox	9.9±4.1b	8.9±4.2b
Rivera	2.9±0.6b	1.7±0.5b

Different letters denote significant differences by one-way ANOVA and Tukey's test at $P<0.05$

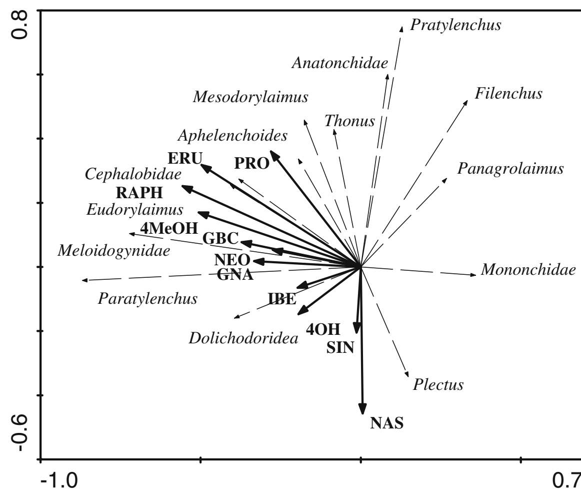

Fig. 1 Associations between glucosinolates and the nematode community in the soil, axis one explains 31.2% of the variation axis 2 explains 15.6%. Abbreviations: IBE; glucoiberin, NAS; gluconasturtiin, SIN; sinigrin, 4OH; 4-hydroxyglucobrassicin, GNA; gluconapin, NEO; neo-glucobrassicin, GBC; glucobrassicin, NEO; neo-glucobrassicin, 4MeOH; 4-methoxyglucobrassicin, RAPH; glucoraphanin, ERU; glucoerucin, PRO; progoitrin

Microorganisms

At the initial sampling, bacterial communities did not show a separation according to plots assigned to different cultivars (1st CCA axis MCP $P>0.9$ 2nd axis MCP $P>0.9$). Thus, there were no plot differences prior to the experiment. At the final sampling, the bacterial composition did not show any response to cultivars (1st axis MCP $P>0.5$, 2nd axis MCP $P>0.2$). The community composition of fungi also did not differ among cultivars (initial sampling: 1st axis MCP $P>0.3$, 2nd axis MCP $P>0.1$; final sampling: 1st axis MCP $P>0.5$, 2nd axis MCP $P>0.2$). There was no correlation between glucosinolate profiles and DGGE patterns of bacteria (CCA MCP $P>0.1$) or of the fungi (CCA MCP $P>0.5$).

Soil microarthropods

Numbers of mites significantly increased between initial and final sampling (t-test $P<0.05$, 0.79±0.08 vs. 1.61±0.14 individuals per 100 g dry soil respectively). In contrast, numbers of Collembola did not differ between the first and second sampling (t-test

Table 2 Average wet weight or average number of individuals ± standard error, expressed per 100 g dry weight soil^{-1}. Different letters denote significant differences by ANCOVA analysis and Tukey's test at $P<0.05$

	Badger shipper	Christmas drumhead	Lennox	Rivera
Nematodes in the soil				
Total biomass in µg wet	1,815±302a	1,686±466a	1,018±144a	1,088±131a
Plant-feeding biomass in µg wet	255±41	186±33	161±13b	167±32b
Hyphal-feeding biomass in µg wet	2.55±0.64a	1.27±0.51a	2.31±0.76a	2.06±0.91a
Bacterial-feeding biomass in µg wet	1,298±223a	1,370±473a	772±111a	731±111a
Animal-predator biomass in µg wet	227±68a	99±43a	70±49a	170±59
Omnivores biomass in µg wet	30.7±5.1a	28.9±10.7a	13.6±6.7a	19.2±5.80a
Mites				
Number of total individuals	1.93±0.18a	1.82±0.27a	1.48±0.35a	1.18±0.25a
Springtails				
Number of total individuals	2.09±0.36a	2.20±0.45a	2.62±0.23a	3.01±0.71a
Number of Onychiuridae	0.53±0.15a	0.46±0.15a	0.35±0.12a	1.00±0.44a
Number ofPoduridae	0.08±0.08a	0.12±0.06a	0±0a	0.05±0.05a
Number of Entomobryidae	0.71±0.21	1.09±0.33a	0.78±0.14a	0. 96±0.68a
Number of Isotomidae	0.77±0.25a	0.53±0.20a	1.49±0.63a	1.00±0.21a
Earthworms and Enchytraeids				
Earthworm biomass in µg wet	15,004±3,509a	14,886±4,327a	6,505±1,116a	4,756±962a
Enchytraeids biomass in µg wet	724±329a	1,843±669a	696±338a	735±242a

$P>0.5$, 1.9±0.2 vs. 2.4±0.2 individuals per 100 g dry soil respectively).

The total numbers of Collembola and mites were not different between the four cultivars (Table 2). Neither the mites nor the Collembola showed any correlation with glucosinolate profiles (both mites and Collembola RDA MCP $P>0.1$) or cultivars (both RDA MCP $P>0.5$).

Enchytraeids and earthworms

The estimated biomass of enchytraeids and earthworms significantly increased between initial and final sampling dates (enchytraeids: t-test $P<0.05$, 92±36 µg vs. 1,000±221 µg per 100 g dry soil respectively; Earthworms: Wilcoxon rank-sum test $P<0.05$, 4845±642 µg vs. 10,288±1,549 µg per 100 g dry soil respectively). However, neither the magnitude of this increase, for both enchytraeids and earthworms, nor the diversity of earthworms was affected by cultivar (biomass Table 2, diversity RDA MCP $P>0.1$). Additionally, earthworms diversity was not correlated with glucosinolate profiles (RDA MCP $P>0.05$).

Discussion

Nematodes

Our results show that there is a clear cultivar effect on migratory endoparasitic nematodes of the genus *Pratylenchus* in the roots. The cultivar Badger Shipper supported more individuals of this genus than the other three cultivars. This positive effect is likely due to the lack of gluconasturtiin in roots of the Badger Shipper cultivar. The toxicity of gluconasturtiin on soil organisms, including plant parasitic nematodes, in Brassicaceae has been described earlier in greenhouse environments (Potter et al. 1998; Potter et al. 2000). *Pratylenchus* abundances in the present study were positively correlated with glucoerucin. This contradicts in vitro tests with root-knot nematodes in which their abundances were reduced when exposed to breakdown products of glucoerucin (Lazzeri et al. 2004). One possible explanation for these contrasting results is that *Pratylenchus*, in contrast to root-knot nematodes, might be resistant to this specific glucosinolate. Aboveground positive correlations between specific glucosinolates and particular insect species have been observed before (Moyes et al. 2000). To our knowledge, our result is the first report of such a positive correlation for a belowground herbivore. An additional explanation might be that there is a trade-off between the production of gluconasturtiin and other glucosinolates. Badger Shipper may have reallocated its resources from the production of gluconasturtiin to glucoerucin. This is supported by the observation that glucoerucin is occurring in twice as high concentrations in this cultivar when compared to the three other cultivars (Kabouw et al. 2010). The biomass of plant-feeding nematodes outside the roots was also higher under the Badger Shipper cultivar, which is a likely consequence of the high reproduction rates of *Pratylenchus* inside the roots of Badger Sipper, and subsequent migration into the rhizosphere.

Higher trophic levels and microorganisms

In principle, the higher numbers of nematodes associated with the Badger Shipper cultivar both outside and inside the roots could have resulted in higher abundances of other groups of organisms that feed on these nematodes in the soil of this cultivar as well. However we found no significant effects on higher trophic levels, as the numbers or the biomass of mites and carnivorous nematodes were not affected by cultivar. The lack of an effect on these higher trophic level organisms may be due to the relatively low biomass of root-feeding nematodes in and around the roots in relation to other soil organisms (Bezemer et al. 2010). In any case, cultivar effects on the genus *Pratylenchus* did not cascade up to higher trophic levels.

That belowground higher trophic levels were not affected by cultivars is in contrast to what has been previously recorded on the same cultivars for aboveground higher trophic levels (Poelman et al. 2009). In that study, the abundances of natural enemies aboveground were affected by differences in glucosinolate profiles. The difference in the effects that glucosinolates and their breakdown products have on aboveground and belowground communities might be explained by considering differences between these environments. First, belowground predators are less mobile than aboveground predators and parasitoids, so that the area at which belowground natural enemies can be affected may be much smaller. Second, volatile

breakdown products of glucosinolates are less mobile in the soil (Gimsing and Kirkegaard 2009). Defense against natural enemies by volatile breakdown products of glucosinolates may therefore be less effective belowground than aboveground. Therefore plants may, at least in our model system in which the roots exhibit a strong direct defense, mainly rely on constitutively direct instead of inducible indirect defenses belowground (van Dam et al. 2009).

The reduced exposure to glucosinolates or their breakdown products could also explain why detritus feeders were not affected. Decomposers, such as earthworms or enchytraeids, could potentially be influenced by exposure to glucosinolates either by the ingestion of senescent plant material or by exposure to exudation from roots. However, the biomass of organisms in the decomposer food web was not affected by cultivar, nor was their species composition correlated with glucosinolates. Even though microorganisms feed on a variety of food sources (i.e. living and dead plant material and root exudates) we still found no differences in the microorganism community between the cultivars or a response of the microorganisms to the glucosinolate profiles. This contrasts with previous studies recording that *B. napus* and *A. thaliana* chemotypes with different glucosinolate profiles influenced the micro-organism community (Bressan et al. 2009; Rumberger and Marschner 2003). Using identical bacterial primers as used in our study, Rumberger and Marschner (2003) found that 2-phenylethyl-isothiocyanate, the breakdown product of gluconasturtiin, influenced the bacterial community in roots and rhizosphere of *B. napus*. However, these effects only occurred at extremely high concentrations of 2-phenylethyl-isothiocyanate, above 1,300 pmol/g soil. These high concentrations are unlikely to occur in the roots or rhizosphere of white cabbage. Moreover, in *B. napus* gluconasturtiin is the most abundant glucosinolate (Sarwar et al. 1998) whereas gluconasturtiin is only the fourth most dominant glucosinolate in the cultivars used in this study (Kabouw et al. 2010).

In conclusion, we found that cultivar variation only affected plant-feeding nematodes. These soil organisms interact directly with living plant roots. No differences were recorded for soil microbes that potentially feed on dead plant materials and other trophic levels in the soil food web. This indicates that variation in chemical defense profiles causing differences in herbivore abundances do not necessarily trickle up to higher trophic levels in the soil food web.

Acknowledgements The authors would like to thank Martine Kos for field maintenance, Matty Berg for use of the Tullgren funnels, and the ERGO team for help during the experimental phase. Elke Vockenhuber is thanked for reading an earlier version of the manuscript. This research was funded by ERGO grant nr 83806012 of NWO. Publication 4831 Netherlands Institute of Ecology (NIOO-KNAW).

Open Access This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

References

Abrahamsen G (1973a) Biomass and body-surface area of populations of enchytraeidae and Lumbricidae (Oligochaeta) in Norwegian coniferous forest soils. Pedobiologia 13:28–39

Abrahamsen G (1973b) Studies on body-volume, body-surface area density and live weight of enchytraeidae. Pedobiologia 13:6–15

Aires A, Mota VR, Saavedra MJ, Monteiro AA, Simoes M, Rosa EAS, Bennett RN (2009) Initial in vitro evaluations of the antibacterial activities of glucosinolate enzymatic hydrolysis products against plant pathogenic bacteria. J Appl Microbiol 106:2096–2105

Andersen M, Sausse C, Lacroix B, Caul S, Messéan A (2007) Agricultural studies of GM maize and the field experimental infrastructure of ECOGEN. Pedobiologia 51:175–184

Berg MP, Bengtsson J (2007) Temporal and spatial variability in soil food web structure. Oikos 116:1789–1804

Bezemer TM, Fountain MT, Barea JM, Christensen S, Dekker S, Duyts H, van Hal R, Harvey JA, Hedlund K, Maraun M, Mikola J, Mladenov AG, Robin C, de Ruiter P, Scheu S, Setälä H, Šmilauer P, van der Putten WH (2010) Divergent composition but similar function of soil food webs beneath individual plants: plant species and community effects. Ecology. doi:10.1890/09-2198

Bongers T (ed) (1988) De Nematoden van Nederland. Stichting uitgeverij van de Koninklijke Nederlandse Natuurhistorische Vereniging, Utrecht

Bressan M, Roncato M, Bellver F, Comte G, el Zahar Haichar F, Achouak W, Berge O (2009) Exogenous glucosinolate produced by *Arabidopsis thaliana* has an impact on microbes in the rhizosphere and plant roots. ISME J 2:1243–1125

Broekgaarden C, Poelman EH, Steenhuis G, Voorrips RE, Dicke M, Vosman B (2007) Genotypic variation in genome-wide transcription profiles induced by insect feeding: *Brassica oleracea-Pieris rapae* interactions. Bmc Genomics 8:239

Christian E, Zicsi A (1999) Ein synoptischer Bestimmungsschlüssel der Regenwürmer Österreichs (Oligochaeta: Lumbricidae). Die Bodenkultur 50:121–131

Crutsinger GM, Collins MD, Fordyce JA, Gompert Z, Nice CC, Sanders NJ (2006) Plant genotypic diversity predicts community structure and governs an ecosystem process. Science 313:966–968

de Ruiter PC, Neutel AM, Moore JC (1995) Energetics, patterns of interaction strengths, and stability in real ecosystems. Science 269:1257–1260

Fjellberg A (1980) Identification keys to Norwegian. Norsk Entomologisk Forening, Ås.

Freckman DW (1982) Parameters of the nematode contribution to ecosystems. University of Texas, Austin

Gimsing AL, Kirkegaard JA (2009) Glucosinolates and biofumigation: fate of glucosinolates and their hydrolysis products in soil. Phytochem Rev 8:299–310

Griffiths B, Caul S, Thompson J, Birch A, Scrimgeour C, Andersen M, Cortet J, Messean A, Sausse C, Lacroix B, Krogh P (2005) A comparison of soil microbial community structure, protozoa and nematodes in field plots of conventional and genetically modified maize expressing the *Bacillus thuringiens* is CryIAb toxin. Plant Soil 275:135–146

Holtkamp R, Kardol P, van der Wal A, Dekker SC, van der Putten WH, de Ruiter PC (2008) Soil food web structure during ecosystem development after land abandonment. Appl Soil Ecol 39:23–34

Jensen J, Styrishave B, Gimsing AL, Hansen HCB (2010) The toxic effects of benzyl glucosinolate and its hydrolysis product, the biofumigant benzyl isothiocyanate, to *Folsomia fimetaria*. Environ Toxicol Chem 29:359–364

Kabouw P, Biere A, van der Putten WH, van Dam NM (2010) Intra-specific differences in root and shoot glucosinolate profiles among white cabbage (*Brassica oleracea* var *capitata*) cultivars. J Agric Food Chem 58:411–417

Lazzeri L, Curto G, Leoni O, Dallavalle E (2004) Effects of glucosinolates and their enzymatic hydrolysis products via myrosinase on the root-knot nematode *Meloidogyne incognita* (Kofoid et White) Chitw. J Agric Food Chem 52:6703–6707

Lepš J, Šmilauer P (2003) Multivariate analysis of ecological data. Cambridge University Press, Cambridge

Moyes C, Hamish A, Collin A, Britton G, Raybould A (2000) Glucosinolates and differential herbivory in wild populations of *Brassica oleracea*. J Chem Ecol 26:1573–1561

Mulder C, Lotz LAP (2009) Biotechnology, environmental forcing, and unintended trophic cascades. Arthropod-Plant Interact 3:131–139

Mulder C, Zwart DD, Wijnen HV, Schouten A, Breure A (2003) Observational and simulated evidence of ecological shifts within the soil nematode community of agroecosystems under conventional and organic farming. Funct Ecol 17:516–525

Muyzer G, Dewaal EC, Uitterlinden AG (1993) Profiling of complex microbial-populations by denaturing gradient gel-electrophoresis analysis of polymerase chain reaction-amplified genes-coding for 16s ribosomal-rna. Appl Environ Microb 59:695–700

O'Connor FB (1957) An ecological study of the enchytraeid worm population of a coniferous forest soil. Oikos 8:161–199

Poelman EH, van Dam NM, van Loon JJA, Vet LEM, Dicke M (2009) Chemical diversity in *Brassica oleracea* affects biodiversity of insect herbivores. Ecology 90:1863–1877

Potter MJ, Davies K, Rathjen AJ (1998) Suppressive impact of glucosinolates in Brassica vegetative tissues on root lesion nematode *Pratylenchus neglectus*. J Chem Ecol 24:67–80

Potter MJ, Vanstone VA, Davies KA, Rathjen AJ (2000) Breeding to increase the concentration of 2-phenylethyl glucosinolate in the roots of *Brassica napus*. J Chem Ecol 26:1811–1820

Ramirez RA, Henderson DR, Riga E, Lacey LA, Snyder WE (2009) Harmful effects of mustard bio-fumigants on entomopathogenic nematodes. Biol Control 48:147–154

Rumberger A, Marschner P (2003) 2-Phenylethylisothiocyanate concentration and microbial community composition in the rhizosphere of canola. Soil Biol Biochem 35:445–452

Sarwar M, Kirkegaard JA, Wong PTW, Desmarchelier JM (1998) Biofumigation potential of brassicas—III. In vitro toxicity of isothiocyanates to soil-borne fungal pathogens. Plant Soil 201:103–112

Snapp SS, Date KU, Kirk W, O'Neil K, Kremen A, Bird G (2007) Root, shoot tissues of *Brassica juncea* and Cereal secale promote potato health. Plant Soil 294:55–72

Vainio EJ, Hantula J (2000) Direct analysis of wood-inhabiting fungi using denaturing gradient gel electrophoresis of amplified ribosomal DNA. Mycol Res 104:927–936

van Bezooijen J (2006) Methods and techniques for nematology, Wageningen.

van Dam NM, Oomen MW (2008) Root and shoot jasmonic acid applications differentially affect leaf chemistry and herbivore growth. Plant Signal Behav 3:91–98

van Dam NM, Tytgat TOG, Kirkegaard JA (2009) Root and shoot glucosinolates: a comparison of their diversity, function and interactions in natural and managed ecosystems. Phytochem Rev 8:171–186

Vierheilig H, Bennett R, Kiddle G, Kaldorf M, Ludwig-Muller J (2000) Differences in glucosinolate patterns and arbuscular mycorrhizal status of glucosinolate-containing plant species. New Phytol 146:343–352

Wang D, Rosen C, Kinkel L, Cao A, Tharayil N, Gerik J (2009) Production of methyl sulfide and dimethyl disulfide from soil-incorporated plant materials and implications for controlling soilborne pathogens. Plant Soil 324:185–197

Wardle DA (2006) The influence of biotic interactions on soil biodiversity. Ecol Lett 9:870–886

Wardle DA, Bardgett RD, Klironomos JN, Setala H, van der Putten WH, Wall DH (2004) Ecological linkages between aboveground and belowground biota. Science 304:1629–1633

Witt C, Setälä H (2010) Do plant species of different resource qualities form dissimilar energy channels below-ground? Appl Soil Ecol 44:270–278

Wurst S, Van Dam NM, Monroy F, Biere A, Van der Putten WH (2008) Intraspecific variation in plant defense alters effects of root herbivores on leaf chemistry and aboveground herbivore damage. J Chem Ecol 34:1360–1367

Yang J, Zhu ZJ, Gerendas J (2009) Interactive effects of phosphorus supply and light intensity on glucosinolates in pakchoi (*Brassica campestris* L. ssp *chinensis* var. *communis*). Plant Soil 323:323–333

Yeates G, Bongers T, Goede RD, Freckman D, Georgieva S (1993) Feeding habits in soil nematode families and genera–an outline for soil ecologists. J Nematol 25:315–331

Yergeau E, Bokhorst S, Huiskes AHL, Boschker HTS, Aerts R, Kowalchuk GA (2007) Size and structure of bacterial, fungal and nematode communities along an Antarctic environmental gradient. FEMS Microbiol Ecol 59:436–451