



Interactions between root and shoot herbivores of *Ammophila arenaria* in the laboratory do not translate into correlated abundances in the field

Martijn L. Vandegehuchte, Eduardo de la Peña and Dries Bonte

M. L. Vandegehuchte (martijn.vandegehuchte@ugent.be), E. de la Peña and D. Bonte, Dept Biology, Terrestrial Ecology Unit, Ghent Univ., K. L. Ledeganckstraat 35, BE-9000 Gent, Belgium.

Over the past decades a growing body of literature has presented proof of the possible interactions between foliar and root herbivores. These effects can be positive, negative or neutral in either direction, depending on the species and the involved mechanism. Most of these studies however concern experiments under controlled conditions. Whether these interactions affect the distribution of herbivores under natural conditions still largely remains an open question. This study examined interactions between root feeding nematodes and shoot feeding aphids on *Ammophila arenaria* in the laboratory. We subsequently addressed the question whether expectations from this experiment are reflected in correlations between plant related variables and the abundance of both herbivores in the field. We demonstrated that nematodes and aphids can negatively affect each other in a controlled microcosm. In the field however no significant correlations between nematode and aphid abundances could be detected. There, shorter plants with a more vital leaf set and a higher root density supported the highest numbers of aphids. Plants with a lower root density and higher root vitality held more migratory endoparasitic nematodes, while more nematode cysts were found among roots with a low vitality. A certain plant property can furthermore affect above- and belowground herbivores in the opposite direction, such as root density in this case. This study suggests that effects of root herbivores on foliar herbivores or vice versa seem to be blurred in a field situation where other variables related to plant vitality and water content structure the herbivore populations. Therefore, caution should be used in generalising the prevalence of these interactions between the above- and belowground fauna, based solely on laboratory experiments.

Over the past decades it has become clear amongst ecologists that plants in terrestrial ecosystems enable different types of interactions and feedbacks between the above- and belowground community. These interactions can furthermore affect biodiversity and ecosystem processes (reviewed by van der Putten et al. 2001). Root feeding organisms, pathogens or root mutualists such as mycorrhizae can have direct effects on the performance of plants and as a consequence on that of foliar herbivores and potentially their predators (Wardle et al. 2004). Aboveground herbivores have furthermore been proven beneficial as well as adverse for their belowground counterparts (Masters et al. 1993, Tindall and Stout 2001, van Dam et al. 2003, Soler et al. 2007, Kaplan et al. 2008). While the mechanisms behind the interactions between foliar and root herbivores were explained in terms of water stress, primary chemistry and available biomass in early studies (Masters et al. 1993), recent studies highlighted the importance of plant secondary metabolism as an explanation of both positive and negative feedbacks (reviewed by van Dam et al. 2003).

Most of the above mentioned studies are based on laboratory experiments with potted plants. Since a wide variety of at first sight unrelated environmental actors can cause the same metabolic response in plants (White 1984), it seems

hard to predict whether the effect – if any – of herbivores on other herbivores at the opposite side of the soil surface will be relevant or detectable compared to that of other environmental agents. Only a few studies have investigated whether predictions from controlled experiments on above- and belowground herbivore interactions hold in field situations. These either involved artificial field manipulations (Moran and Whitham 1990, Kaplan et al. 2009) or were conducted in agricultural systems (Kaplan et al. 2009, but see Moran and Whitham 1990). Because agricultural systems have artificially high levels of herbivory due to additional nitrogen fertilisation and unnatural densities of host plants (White 1978), it is unlikely that these findings reflect patterns from more natural systems.

In this study Marram grass *Ammophila arenaria* is used as a model species both in a laboratory experiment and a large field survey. This is a dominant grass species of dynamic sand dunes and it is distributed along all European coasts south of latitude 63°N (Huiskes 1979). The plant grows most vigorously in the open habitat of mobile dunes and tolerates extreme wind and sun exposure and wide fluctuations of soil temperature (Huiskes 1979). *Ammophila arenaria* displays strong growth as long as the plant is regularly buried with windblown sand. As soon as regular sand burial ceases, the

plant starts to degenerate and decreases in shoot weight per plant and abundance per unit area (Eldred and Maun 1982).

The interactions occurring in the rhizosphere of dune grasses, and of *A. arenaria* in particular, form one of the best documented illustrations of the influence of root-feeders on plant community development (Zoon et al. 1993, van der Putten et al. 2001). The soil and rhizosphere of plants in coastal dunes contain an ample variety of organisms, including root-feeding nematodes, that contribute to the plant–soil feedbacks that modify the fitness of competing plant species over the course of succession (van der Stoel et al. 2002). Therefore, the successional dynamics of the plant community, in particular the decline of species in favour of later successional species, are linked to the net effect of different soil-borne organisms. Since investigating the whole soil community was not practically feasible, we focused on the occurrence of root feeding nematodes that are known from literature to be specific and abundant on *A. arenaria* (van der Stoel and van der Putten 2006, de la Peña et al. 2007). Previous studies in the sampling area (de la Peña et al. 2007) revealed that most abundant root feeding nematodes inside *A. arenaria* roots belonged to the genus *Pratylenchus*, which is a group of migratory endoparasitic nematodes that penetrate into the roots of the plant and move through and feed on the cortex cells. The cyst forming nematode *Heterodera arenaria* is also present in abundant numbers in this area. This is a sedentary endoparasitic species which penetrates the root, establishes a feeding site and produces persistent forms (cysts) that protect the eggs. After one growing season these cysts usually get detached from the roots and accumulate in the soil. In contrast to *Pratylenchus* species (Seliskar and Huettel 1993, de la Peña et al. 2008), *H. arenaria* is shown to have little negative effect on the growth of its host plant (van der Stoel and van der Putten 2006). In addition to *Pratylenchus* spp., juvenile stages of *H. arenaria* and of root knot nematodes of the genus *Meloidogyne* can be found within *A. arenaria* roots.

Schizaphis rufula is an aphid species known to live specifically on leaves of *A. arenaria* and *Elymus arenarius* in sand dune areas. The leaves of the plant can turn yellowish brown as a result of the infestation (Heie 1992).

We conducted a fully crossed laboratory experiment to assess the potential for interactions between nematodes and aphids under controlled circumstances. We subsequently addressed the question whether expectations based on this manipulation experiment would be reflected in the abundances of both herbivore groups in the field. If so, we hypothesised that herbivore–plant feedbacks would result in correlated abundances of both below- and aboveground herbivores. The foliar herbivore could attain higher numbers on growing plants with a more vital leaf and root set (plant vigour hypothesis, Price 1991, Dhileepan 2004) or on plants with less vital senescing leaves (plant stress hypothesis, White 1978), depending on whether it is a “flush feeder” or “senescence feeder” respectively (White 1978, 2009). Both types of plant tissue are characterised by an increased mobilisation of nitrogen, either towards the growing shoot, or out of the senescing leaf, that is beneficial to herbivores. Root feeding nematodes are expected to colonise the plant in early spring (van der Stoel et al. 2002), before the first appearance of aphids in June (unpubl.). If nematodes are causing stress

to the plant, then we expect a negative correlation between below- and aboveground herbivores under the plant vigour hypothesis, a positive one under the plant stress hypothesis. In case other stressors have a stronger impact on plant vitality, independent and uncorrelated responses to plant quality are expected. Aphids might positively affect nematodes by increasing the transport of nutrients or minerals to the root system (Kaplan et al. 2008, Johnson et al. 2009), or have a negative impact by decreasing plant productivity (Moran and Whitham 1990, Masters et al. 1993). Negative effects in both directions could be expected if one herbivore elicits some plant defence response that spreads to the other components of the plant (Bezemer and van Dam 2005). Analogously, the destruction of production sites of such defences in one plant part can lead to lowered defence of other plant parts, leading to positive interactions (Kaplan et al. 2008).

Material and methods

Controlled experiment

Seeds of *A. arenaria* were surface sterilised by submersing in 4% household bleach solution, rinsing 10 times with demineralised water, submersing in 10% ethanol and rinsing another 10 times with demineralised water. This sterilisation method effectively eliminates endophytic fungi that otherwise could colonise the young seedling. Seeds were subsequently germinated at a light regime of 9/15 hours dark/light in plastic 1-l pots filled with 190 cm³ of sterile dune sand that was autoclaved for 1 h at 120°C and 1 atm. The sand was saturated with demineralised water. Plastic foil that covered the pots was perforated to allow of enough ventilation. Moisture level was reset to near saturation daily.

Nematodes were extracted using a modification of the Baermann technique (Whitehead and Hemming 1965). Roots of vigorous *A. arenaria* plants were collected in the Westhoek nature reserve (Belgium) and placed on trays. The water was removed from the trays daily and poured into glass columns, after which trays were refilled with fresh water. Columns were left overnight so that the nematodes could sink, and then decanted leaving 200 ml of liquid in the columns. Nematodes were harvested daily for one week according to this method. Nematodes were kept in a fridge at 8°C. Nematodes were counted in five 10 ml subsamples of the final solution, and averaged over those subsamples to calculate the number of nematodes per ml. The nematode community at the field site where the roots were harvested consisted of 53.75% *Pratylenchus brzeskii*, 15.62% *Tylenchorhynchus ventralis*, 15.62% *Meloidogyne* sp., 12.5% *Heterodera* sp., 4.25% *Paratylenchus* sp. 3.125% *Rotylenchus* sp., 3.125% *Filenchus* sp. and 2% other species.

Two freshly germinated seedlings were transplanted to each of 40 plastic pots (1 l) filled with 550 g of autoclaved dune sand. Half of the pots were inoculated with 15 ml of nematode inoculum, containing 552 nematodes on average. This corresponds with the higher densities observed in the field. The other half of the pots received 15 ml of tap water as a control. Pots were sealed with a piece of fine mesh cloth and placed under a 9/15 hours dark/light regime. Seventy-five ml half-strength Hoagland's nutrient solution was added

four days after transplantation. From then onwards all pots alternately received 50 ml of tap water and 50 ml of half-strength Hoagland's nutrient solution at three-day intervals. Twenty-four days after nematode inoculation, all pots were trimmed down to one seedling. Half of the nematode inoculated plants and half of the other plants received a single newborn first instar of *S. rufula*. The four treatment combinations thus were control (C), nematodes (N), aphids (A) and both aphids and nematodes (N+A), with ten replicate plants per combination. Aphids were obtained from a parthenogenetic lab culture that was propagated from one wild individual from the Westhoek nature reserve. Aphids were counted daily to determine a population growth curve for each replicate. Previous experiments with this system have shown that populations usually grow to a peak density after which aphid numbers rapidly decline. As soon as the last aphid of a population died, the plant was uprooted and root and shoot fresh weight were obtained. Roots were stored at 4°C until the end of the experiment. At day 40 the experiment was ended and all remaining aphid populations were collected and plants were uprooted. Root and shoot fresh weight were determined, after which all roots were stained with acid fuchsin, a dye that stains animal and fungal tissue (Baker and Gowen 1996). Because of the relatively small size of the roots, stained nematodes could be counted in situ in the complete root system, using large microscope slides (76 × 40 mm). Of the 20 first instar aphids that were added to the plants, four died before becoming adult. These data points were omitted from all analyses. Of the 16 plants with aphids, six contained nematodes in their root system, and 10 were nematode free.

The effects of nematode and aphid addition on both root and shoot fresh weight were analysed by means of a general linear model, after which the assumptions about residuals were confirmed. The effect of aphid addition on the number of nematodes in the roots was assessed through a generalised linear mixed model with aphid treatment as categorical predictor and root fresh weight as a covariate. The effect of nematode inoculation on the population dynamics of aphids through time was tested by means of a repeated measures analysis with individual plant as subject (generalised estimating equation, GEE). We included nematode treatment, time (days), higher order power terms of time (up to sixth order) and all interactions as predictors. This allowed modelling the different maxima and minima in aphid populations that were observed. Shoot fresh weight was included as a covariate. A stepwise backward deletion of non-significant terms at the $p = 0.05$ level was conducted. All generalised models used a Poisson distribution and contained a correction for the observed overdispersion.

Field survey

From August until September 2007, a sampling campaign was held in six different sites in Westhoek nature reserve, three of which are situated in the fore dunes close to the coast line and three within a large inland dynamic dune area. At each site, 20 stands of *A. arenaria* were chosen, covering the spectrum from vital to partly degenerated and from large to small tussocks. Each selected stand was then swept five times with an insect net to catch aphids. The diameter and perimeter of the tussock and the length of the highest

leaf were measured and a circular part of the tussock with a diameter of 20 cm was cut out at ground level. If the plant was small and had a diameter smaller than 20 cm, the entire plant was cut off. Since the number of aphids remaining in these entire tussocks after sweep netting was negligible compared to the numbers in the net, sweep netting seems to be an adequate method to quantify aphids in this particular system. The aboveground diameter of the tussock (cm) was used as a measure of plant size. Plant height was equated with the length of the highest leaf. Within a radius of 2.5 m around each sampled stand, the number of *A. arenaria* stands and the perimeter of each stand were determined. The surface of each stand (cm²) within this radius was derived from its perimeter that was assumed to approximate a circle. The surfaces of all stands were summed and this sum was divided by the surface of the circle, to calculate the proportion of the area within the radius occupied by *A. arenaria*. This proportion serves as an estimate of the relative amount of suitable habitat cover in the local environment of the sampled plant. For each sampled stand, a soil sample of 1 l including roots was taken at the point where the leaves were cut. Leaf and soil samples were transferred to the lab. Dead leaves were separated from living leaves of *A. arenaria* and living leaves of other plant species and the different types of leaves were weighed. Dead leaves were pooled because they were often decomposed too far to identify the species. An aboveground vitality measure was calculated as the weight of living leaves of *A. arenaria* divided by the summed weight of living leaves of *A. arenaria* and dead leaves. The soil samples were sieved over a 4 mm mesh sieve with 4 l of tap water. Roots were collected from the sieve and if necessary roots from *A. arenaria* were separated from roots of other plant species. The root density was determined as the fresh weight of the roots in grams per litre of soil. The fresh roots were then cut into pieces of approximately 1 cm length and stained according to the above described method. For each sample, 30 randomly chosen root fragments were inspected under a microscope. The length of each fragment was measured in millimeters, the fragment was categorised as vital or degenerate, with a fragment that had lost more than half of its cortex being denoted as degenerate, and the number of nematodes in the fragment was recorded. A vitality measure for the root system was calculated as the proportion of vital fragments in each sample. The suspension of 1 l of soil in 4 l of tap water was decanted over a 180 µm mesh sieve. The sieved fraction was dried at room temperature and inspected with a stereomicroscope to determine the number of cysts of *Heterodera* sp. within the sample. Insect nets were emptied in the lab and aphids were transferred to ethanol. Aphids were cleared and mounted according to the method of Hille Ris Lambers (1950) and were identified with the identification key by Heie (1980, 1982, 1986, 1992, 1994, 1995). The 120 samples contained a total of 1803 aphids. Of these, 1797 belonged to the species *S. rufula* and the other six individuals were excluded from further analyses.

So for each of the 120 sampled *A. arenaria* stands (20 plants in each of six sites) six plant related variables were recorded: leaf vitality, root vitality, root density, tussock diameter, local habitat cover and plant height. In addition three herbivore related variables were considered: the number of aphids, the number of nematodes per cm of root and the number of

cysts per litre of soil. This amounted to nine variables with 120 values each and the variable site with six levels.

To analyse the relationships between the different herbivores and their habitat, a double approach was followed. The general habitat-centered approach describes the variation in the available habitats and evaluates the position of the focal herbivores within that variation. The focal herbivore-centered approach attempts to predict abundances of the different herbivores from those habitat variables. These variables might be environmental factors as well as other herbivores.

The general habitat-centered approach consisted of an ordination by means of non-metric multidimensional scaling (NMS) of all variables, based on Sørensen distances. A random starting configuration was used and 50 runs were performed with real data for each number of dimensions, ranging from 1 to 6. A Monte Carlo test was performed with an additional 50 runs with randomised data for each dimensionality. Additional dimensions are considered useful if they reduce the final stress by 5 or more. The highest dimensionality that meets this criterion was chosen for the final solution. The stability criterion was set to 0.00001, with a maximum number of iterations of 500. Prior to the analysis, variables were relativised by variable totals. For an argumentation on the benefits of NMS compared to other ordination techniques, see McCune and Grace (2002).

The focal herbivore-centered approach consisted of multiple regression analyses. For each of the three herbivore related variables, all other variables were combined to a set of eight predictors, including abundances of other herbivores. These explanatory variables were included as fixed effects in a generalised linear mixed model with site as random effect, and the abundance of the herbivore in question as response variable. Poisson models with a correction for the observed overdispersion and Satterthwaite's approximation of the effective degrees of freedom were used in all cases. No interactions between variables were included. A backwards stepwise selection procedure was followed in which predictors with a p-value larger than 0.05 were successively deleted, to obtain a final model for each response variable. For nematodes, total numbers in a sample were used as response variable, with the combined length of the 30 investigated root fragments as an offset variable.

Results

Controlled experiment

There was a highly significant negative effect of aphid addition on root biomass ($F_{1,34} = 72.71$, $p < 0.0001$). Plants that received no aphids produced on average 0.042 g of roots, while plants with aphids had an average root weight of 0.014 g (a decrease of 65.2%). Aphids also significantly depressed shoot growth ($F_{1,34} = 89.95$, $p < 0.0001$), with shoots reaching an average weight of 0.049 g compared to 0.175 g for plants without aphids (a decrease of 72.1%). Nematodes did not significantly influence root or shoot biomass.

The development of aphid numbers through time differed significantly between plants with and without nematodes

(Fig. 5). After stepwise deletion of non-significant terms, the final model retained time and all higher order power terms of time up to the fifth order ($\chi^2 = 6.49$ or higher, $p < 0.01$), as well as nematode treatment ($\chi^2 = 4.9$, $p = 0.0268$). There were no significant interactions between nematode treatment and any of the time power terms. This implies that the position in time of different minima and maxima in aphid numbers was not statistically different between treatments. In other words, population dynamics of aphids on plants with or without nematodes were similar, but lower numbers of aphids were attained in the presence of nematodes. Numbers of aphids on nematode inoculated plants can be predicted by the equation " $A = \exp(-0.00000267 \times T^5 + 0.000299 \times T^4 - 0.01169 \times T^3 + 0.1839 \times T^2 - 0.9032 \times T + 0.6431)$ ", with A = number of aphids and T = number of days. For nematode free plants, the equation is identical except for the addition of the constant 0.6615.

The numbers of nematodes within the roots were significantly affected by root weight ($F_{5,48}$, $p = 0.0358$) and presence or absence of aphids ($F_{9,95}$, $p = 0.0076$). The interaction was not significant (Fig. 6). Within the different treatment groups, numbers of nematodes were higher on roots with lower biomass. Apart from this effect, nematodes reached higher numbers on plants without aphids.

Field survey: NMS

A three-dimensional solution was retained, which means that three gradients captured most of the variation in sampled *A. arenaria* stands. The three axes contained 49.5%, 32.6% and 10.2% of the total variance (92.3% cumulative). Further axes resulted only in a comparably small reduction of final stress. The final solution lowered the stress to 10.98646, and reached the desired instability of 0.00001 after 141 iterations. The Monte Carlo test yielded a p-value of 0.0196, i.e. the probability of obtaining an equal or lower stress than the observed stress by chance. Ecological datasets tend to produce solutions with stress between 10 and 20. Values in the lower half of this range are considered satisfactory (McCune and Grace 2002).

In the ordination diagram (Fig. 1) all sampled plants are plotted against the two axes that explain most of the variation. Variables are represented by lines that radiate from the centroid of the ordination scores. The r^2 value between a variable and an axis can be visualised by projecting the line perpendicularly onto the axis of interest. Plants from the six different sampling sites are spread evenly over the diagram, which means that the variation within sites is generally larger than between sites. All of the included variables correlate considerably with at least one of the axes. This implies that all of them contribute to the observed variation in sampled plants. The latter moreover form gradients along the axes rather than clusters, which confirms the sampling campaign succeeded in covering the complete spectrum of variation in plants. Aphid numbers correlate positively with leaf vitality and root density, and negatively with plant height and the strongly correlated variables vegetation cover and tussock diameter. These variables correlate with the first axis. Cyst number correlates negatively with root vitality and nematode abundance, along the second axis.

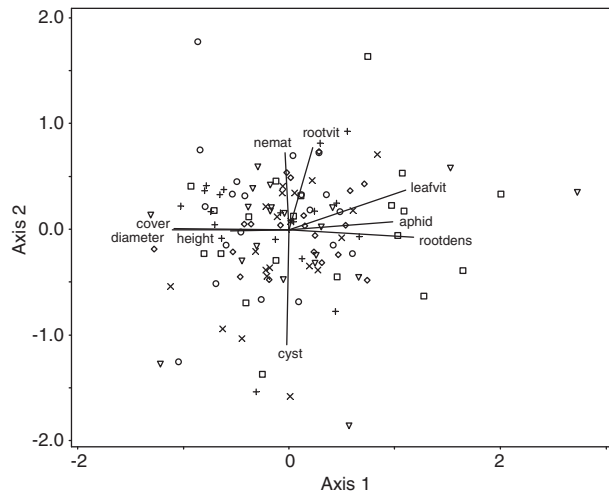


Figure 1. Non-metric multidimensional scaling (NMS) joint plot. Each data point represents a single sampled *A. arenaria* stand. Different sites are indicated by different symbols. Individual variables are represented by lines that radiate from the centroid of the point cloud. The projection of a line on an axis represents the r^2 value between that variable and the axis. Legend: nemat = number of endoparasitic nematodes per cm of investigated root, cyst = number of cysts per litre of soil, aphid = number of aphids, leafvit = vitality of the leaf set, rootdens = density of the root system (g l^{-1}), height = plant height (cm), diameter = tussock diameter (cm), cover = proportion of *A. arenaria* cover within a 2.5 m radius, rootvit = vitality of the root system.

Field survey: multiple regression analyses

Only root vitality was retained as a significant predictor ($F_{1,118} = 7.36$, $p = 0.0077$) of cyst abundance, with more cysts found on less vital roots (Fig. 2). A significant positive correlation of root vitality ($F_{1,116.7} = 4.20$, $p = 0.0428$)

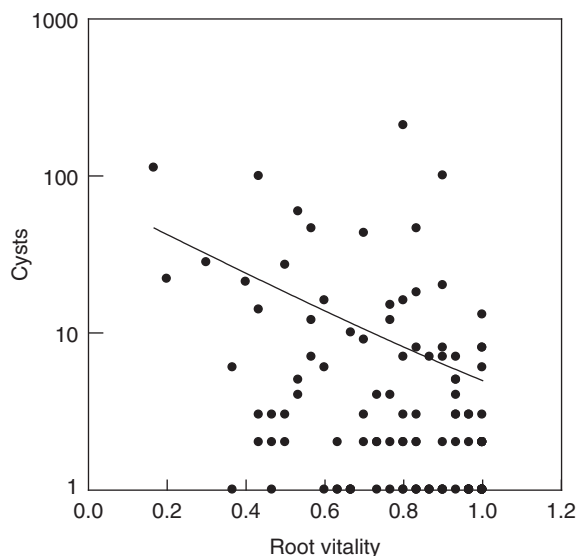


Figure 2. Correlation between root vitality of *A. arenaria* stand (proportion of vital root fragments within a random sample of 30 fragments) and number of cysts within a soil sample of 1 l. Note that the response variable was $y + 1$ transformed to visualise zeroes on a log scale. The line represents the final model.

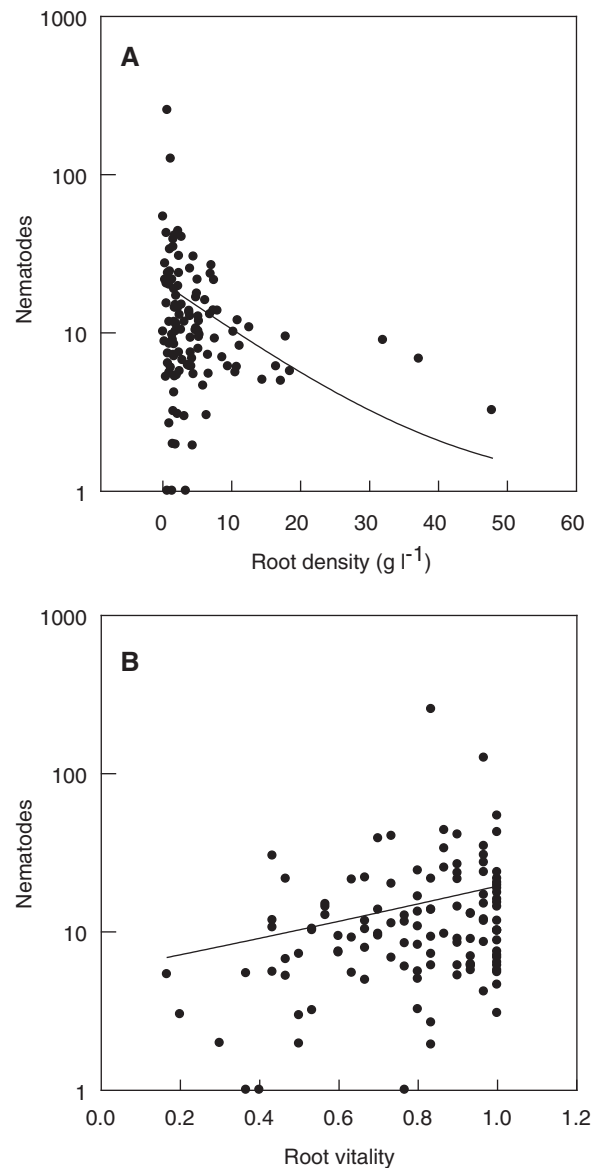


Figure 3. Correlation between (A) root density and (B) root vitality (proportion of vital root fragments within a random sample of 30 fragments) of *A. arenaria* stand and number of endoparasitic nematodes. Note that the response variable was $y + 1$ transformed to visualise zeroes on a log scale. The lines represent the final model with the other predictor variables substituted by their average value over all samples.

and negative correlation of root density ($F_{1,116.8} = 4.84$, $p = 0.0298$) with numbers of plant-parasitic nematodes were observed (Fig. 3). Root density ($F_{1,115.5} = 16.39$, $p < 0.0001$) and leaf vitality ($F_{1,113.9} = 20.02$, $p < 0.0001$) were retained as significant predictors of high aphid abundance, while aphid numbers responded negatively to plant height ($F_{1,111.9} = 23.28$, $p < 0.0001$, Fig. 4).

Note that in general, the results of the multiple regressions correspond well with the NMS ordination diagram. This means that both the general habitat-centered approach and the focal herbivore-centered approach lead to same conclusions. The main difference is that the multiple regressions allow determining which of the different correlated variables

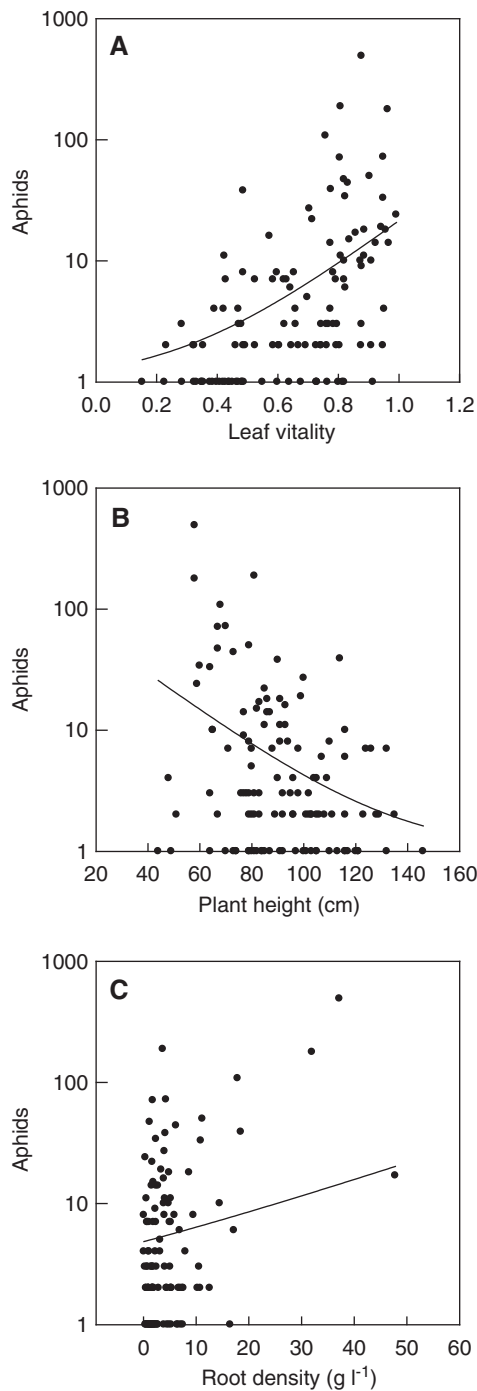


Figure 4. Correlation between (A) leaf vitality (weight of living leaves to total shoot weight ratio of subsample), (B) height and (C) root density of *A. arenaria* stand and number of aphids. Note that the response variable was $y + 1$ transformed to visualise zeroes on a log scale. The lines represent the final model with the other predictor variables substituted by their average value over all samples.

are actually able to predict the numbers of each herbivore. Although aphids correlate most with leaf vitality, root density, plant height, plant diameter and vegetation cover, the latter two are less suited to predict their numbers. Cysts, nematodes and root vitality all correlate with the second axis but only root vitality is a significant predictor of both their abundances.

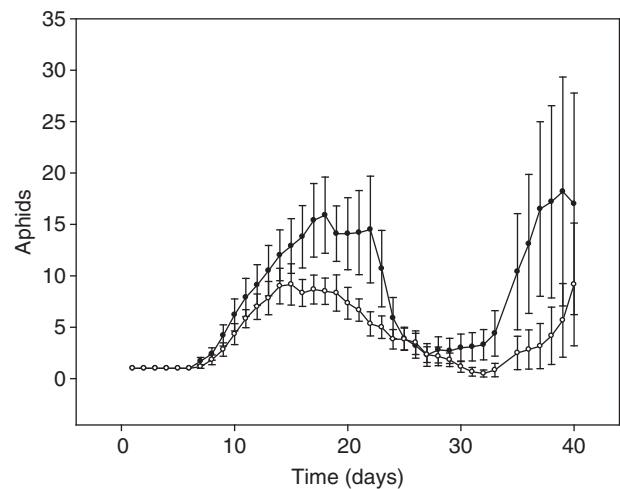


Figure 5. The growth of aphid populations over the course of time on plants with (open dots) or without (black dots) endoparasitic nematodes in the root system (average + SE).

Discussion

The laboratory experiment clearly demonstrated that root-feeding nematodes and aphids of Marram grass can negatively affect one another. Aphids reached higher population densities in the absence of nematodes. Nematode numbers in roots were highest in small root systems of plants without aphids. In the field survey however, of all the tested variables, only the strictly plant related variables were significantly related to both above- and belowground herbivore abundances. A negative correlation between root vitality and cyst numbers was found, while numbers of endoparasitic nematodes correlated positively with root vitality and negatively with root density of *A. arenaria*. The abundance of *S. rufula* aphids on the other hand showed a positive correlation with root density and leaf vitality and a negative correlation with plant height. The abundance of none of the studied herbivore groups was significantly related to that of any of the other herbivore groups, neither to the spatial context of the host plant. Above- and belowground herbivore abundances in the

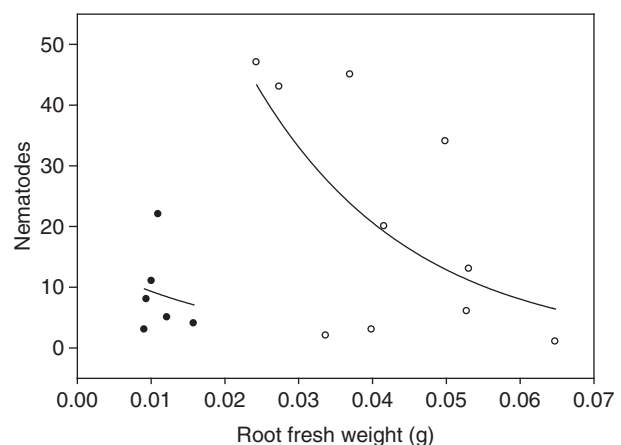


Figure 6. Relationship between root fresh weight (g) and number of endoparasitic nematodes in the root system for plants with (black dots) or without (open dots) aphids.

field are therefore determined by different characteristics of their mutual host plant rather than by each other.

Interactions between above- and belowground herbivores are highly diverse and hence hard to predict. The literature provides evidence of mutualism (Kaplan et al. 2008, Johnson et al. 2009) as well as competition (Tindall and Stout 2001, Staley et al. 2007). Furthermore ammensalism, the combination of a negative and neutral effect (Moran and Whitham 1990, Blossey and Hunt-Joshi 2003) and contramensalism, the combination of a positive and a negative effect (Masters et al. 1993, Blossey and Hunt-Joshi 2003, van Dam et al. 2003, De Deyn et al. 2007), have been reported for root and shoot herbivores in either direction. The underlying mechanisms are equally diverse. Positive effects of root herbivores on shoot herbivores were explained through the induction of water stress, causing an increase in the amount of soluble nitrogen in the leaves (Masters 1995), or by root herbivores damaging production sites of secondary metabolites in the roots (Kaplan et al. 2008). Positive effects on root herbivores were attributed to shoot herbivores causing an increase in sink strength of the roots (Kaplan et al. 2008) or an increase in root minerals (Johnson et al. 2009). Most negative effects, in either direction, are imputed to the induction of chemical defences (Bezemer and Van Dam 2005). Some other mechanisms of negative interactions have however been discovered. The lowering of amino acid levels in leaves caused by root-feeders can negatively affect shoot feeders (Bezemer et al. 2005) while the decrease of plant productivity by shoot feeders can have adverse effects on root feeders (Moran and Whitham 1990, Masters et al. 1993). It is furthermore noteworthy that in some of the tested systems above- and belowground herbivores did not affect each other (Staley et al. 2008), or only did so under particular levels of drought stress (Staley et al. 2007, 2008). The pulsed stress hypothesis (Huberty and Denno 2004), a modification of the plant stress hypothesis, states that sap-feeders can only benefit from water stress induced increases in available nitrogen when in between bouts of stress turgor is allowed to recover. In addition, specialist herbivores can be well adapted to cope with certain chemical defence traits, resulting in more pronounced effects of factors such as leaf age, water content and mechanical defences on their performance (Travers-Martin and Müller 2008).

Competition between the above- and belowground herbivore could be observed under controlled conditions in the laboratory. Since aphids drastically reduced root biomass, this might explain their negative effect on the density of root-feeding nematodes. Nematodes on the other hand did not affect plant biomass, implying that their adverse effect on aphids is qualitative rather than quantitative. Interestingly, Bezemer et al. (2005) found that nematode inoculation of roots of the grasses *Agrostis capillaris* and *Anthoxanthum odoratum* reduced the performance of *Rhopalosiphum padi* aphids on the leaves. In this study the effect could be attributed to a reduction of foliar nitrogen and amino acid concentrations. Given the relatedness between their study organisms and ours, it is not unlikely that our result was caused by a similar change in primary plant compounds.

In a natural ecosystem however, plants differ in various characteristics like size, age, spatial context, root/shoot ratio and state of turgor. Moreover, if the interactions are driven

by a combination of water and nitrogen availability, it can be expected that these interactions will be hard to detect in a natural coastal dune system. The amount of water and nitrogen a plant can offer to a herbivore will be dependent on inherent features of that plant, such as root and shoot vitality, biomass, and plant age. These features will in turn be dependent on a plethora of interacting biotic and abiotic environmental factors, such as competition with other plants or position with respect to the ground water level. Of these factors, other herbivores might be of varying importance. We hypothesise that this caused the apparent lack of relationships between above- and belowground herbivores in the field and the divergent links with plant quality.

Evidently, our correlative field study does not allow the detection of plant-mediated mechanisms driving herbivore performance. The highly significant positive correlation of leaf vitality and negative correlation of plant height with numbers of aphids suggest that *S. rufula* has a strong preference for young growing shoots, which are very vital and have not reached mature size yet. In this sense, *S. rufula* is a flush feeder rather than a senescence feeder, potentially taking advantage of the increased flow of available nitrogen towards the inner expanding leaf (Coley et al. 2006). The highly significant positive correlation between root density and this species' abundance emphasises the potential additional role of water availability in a generally drought stressed environment such as sand dunes. The pulsed stress hypothesis was verified for sap-feeding insects (Huberty and Denno 2004), and our data on aphids suggest a similar dependency on both high turgor and increased levels of soluble nitrogen, albeit in young, growing plants rather than senescing ones. The positive correlation between root vitality and numbers of endoparasitic migratory nematodes suggests that these nematodes are also favoured by vital plants. In this case they could be regarded as flush feeders at the root scale. This could also explain the negative correlation with root density if nematodes choose plants with a less dense root system that is growing towards the maximum density and is allocating elevated amounts of soluble nitrogen towards expanding root tips. The negative correlation between nematode numbers and root weight in the controlled experiment corresponds with this observation. Another possible mechanism is that nematodes have an adverse effect on root development, which in the microcosm is subordinate to the negative effect of aphids on root growth. Since these nematodes are highly mobile, continuously moving within and between roots, it is not surprising that they are able to reach the desired vital parts. Finally, if cyst forming nematodes are structured bottom-up by the root system, and more cysts are found on less vital roots, this could provide a belowground extension of the plant stress hypothesis mentioned earlier (White 1969). However, cysts could also have accumulated in the soil through time, leading to higher local densities around the root system of degenerating *A. arenaria* stands at sites with decreased sand accumulation.

In the controlled experiment the used plants were to a high degree similar in terms of the above discussed characteristics such as vitality, size and water availability. This demonstrates that interactions between nematodes and aphids are possible when minimising the other sources of environmental variation. A lack of proof of those same interactions in the field

hence does not imply that these interactions are not existent, but rather that their result gets overruled by other factors with more pronounced effects on herbivore population dynamics.

A large body of literature documents on the various effects of aboveground herbivores on their belowground counterparts and vice versa. In the case of aphids and plant parasitic nematodes on *A. arenaria*, clear negative effects in both directions could be observed under standardised conditions. However, no significant correlations between abundances of these two groups of organisms could be detected in the field. The specialist aphid *S. rufula* seems to be a flush feeder on *A. arenaria*, choosing young vital shoots with a dense root system. Migratory endoparasitic nematodes reached their highest abundance on less dense and more vital roots, suggesting that they are flush feeders at the root scale. Cyst densities were highest on roots with a low vitality. It seems therefore that in this study system variables related to plant age, quality and water content are most important in structuring both above- and belowground herbivore populations in the field. Compared to other environmental factors, the effect of herbivores on these or other plant traits is not sufficient to induce a detectable effect on other herbivores at the other side of the soil surface.

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