Multitrophic plant insect interactions in dependence of belowground processes

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Introduction

Traditionally, below-ground and above-ground communities have been investigated separately. However, it is important to take into account that all terrestrial ecosystems are divided in a belowground and an aboveground subsystem, which show high interdependence (Wardle 1999, Masters et al. 2001, van der Putten et al. 2001, Brown and Gange 2002, Scheu and Setälä 2002). These subsystems are dependent upon one another, since above the ground primary producers are the main source of organic carbon for the system, whereas below the ground organisms are in charge of the recycling of organic matter and the mineralization of the nutrients therein (Scheu and Setälä 2002, Wardle 2002). Therefore, in order to understand community and ecosystem-level processes it is necessary to study the interactions that occur within and between these subsystems. The interactions between above- and belowground organisms have only recently become a major field of study. Many of these studies focus on the effects of decomposers on plant growth (Scheu and Parkinson 1994, Bonkowski et al. 2000) and on above-ground plant-herbivore interactions (Scheu et al. 1999, Bonkowski et al. 2001), as on the effects of below-ground herbivores on plants, above-ground herbivores and their natural enemies (Brown et al. 1987, Brown and Gange 1989, Gange and Brown 1989, Moran and Whitham 1990, Masters and Brown 1992, Masters et al. 1993, Nötzold et al. 1998, Masters et al. 2001). Although the effects of below-ground organisms on plant growth have been investigated, their effect on plant reproduction is hardly studied (but see Brown et al 1987, Nötzold et al 1998, Maron 1998, Masters et al. 2001). Further, studies on the interactions of ecological processes, such as decomposition, above and belowground herbivory, parasitism and pollination, are needed to improve our knowledge on the role of plants as mediators between the belowground and the aboveground subsystems. The main idea of this thesis was to analyze the single and combined effects of decomposition, root herbivory and leaf herbivory on aboveground plant-insect interactions.

Decomposers are responsible for the breakdown of organic matter and the release and cycling of nutrients (Haimi and Einbork 1992, Wardle 2002). The activity of decomposers often results in increased plant growth and plant nitrogen content (Scheu and Parkinson 1994, Bonkowski et al. 2000, 2001, Wardle 2002) which in turn enhances plant herbivory (White 1993, Bonkowski et al. 2001).

Root herbivory is known to reduce plant growth and to increase mortality of plants (Brown and Gange 1990, Wardle 2002). Further, it may affect the flowering period, the number of flowers and the size of inflorescences (Nötzold et al. 1998, Masters et al. 2001), which could be expected to change attractiveness to flower visitors. The effect of root herbivores on aboveground herbivores is debated. One hypothesis predicts that root herbivory enhances aboveground herbivores. This is assumed to result from an accumulation of carbohydrates and nitrogen in shoots as a stress response of the plant to root herbivory (Gange and Brown 1989, Masters and Brown 1992, Masters et al. 1993, 2001). Another hypothesis suggests negative effects caused by the induction of secondary plant compounds which are stored in the foliage and act as deterrents to aboveground herbivores (Bezemer et al. 2002).

Foliar herbivory may affect plant fitness directly by reducing the photosynthetic area (Strauss 1991, Marquis 1992) and indirectly by altering patterns of pollinator visitation of damaged plants (Lehtilä and Strauss 1997, 1999, Mothershead and Marquis 2000, Strauss 1997). Seed production may be reduced substantially by herbivory, due to reduced resources available for flower, pollen or seed production or because of low pollination rates of damaged plants. Changes in the amount or chemistry of nectar (Hambäck 2001), the number or morphology of flowers (Karban and Strauss 1993, Strauss et al 1996, Lehtilä and Strauss 1997, 1999) or the height of the flowers (Strauss 1997, Mothershed and Marquis 2000, Hambäck 2001) may reduce the attractiveness to pollinators.

The experimental system

The main experimental system used for this research (chapters 2-5) focused on the insects associated with wild mustard plants (*Sinapis arvensis*, Brassicaceae) (Figure 1.1). *S. arvensis* is an annual plant that measures approximately 60 cm. The aboveground entomofauna of *S. arvensis* consists of 9 herbivores and 9 parasitoids (Tommes 1998). The most prominent herbivore in our experiments was the cabbage aphid (*Brevicoryne brassicae*). Flower visitors were composed of a series of solitary and social bees, but honey bess (*Apis mellifera*) were the most abundant flower visitor in our study. In the experiments where the soil biota was manipulated (chapter 2-5) we used as decomposers earthworms of the species *Octolasion tyrtaeum* (Lumbricidae). The density chosen (50 earthworms/m^2), is in the lower range of densities in the field (9-239 ind/m^2, Pizl 1999). As root herbivores we used wireworms, these are larvae of the click beetle *Agriotes* sp. (Coleoptera: Elateridae). The wireworms are patchily distributed in soil and its densities may vary from zero up to several hundreds per

square meter (Poveda et al. 2003). As leaf herbivores we used larvae of the cabbageworms (*Pieris rapae*) in the experiment presented in chapter 3 and larvae of *Spodoptera littoralis* in the experiments presented in chapters 2, 4 and 5. For the leaf herbivory treatment two larvae were put on the seedlings, which had their first four true leaves. We let the larvae consume 30 - 50% of the leaves, which took approximately 2 days. This level of leaf damage is well within the range recorded in the field (Strauss et al. 1996). Any other leaves subsequently produced were left undamaged.
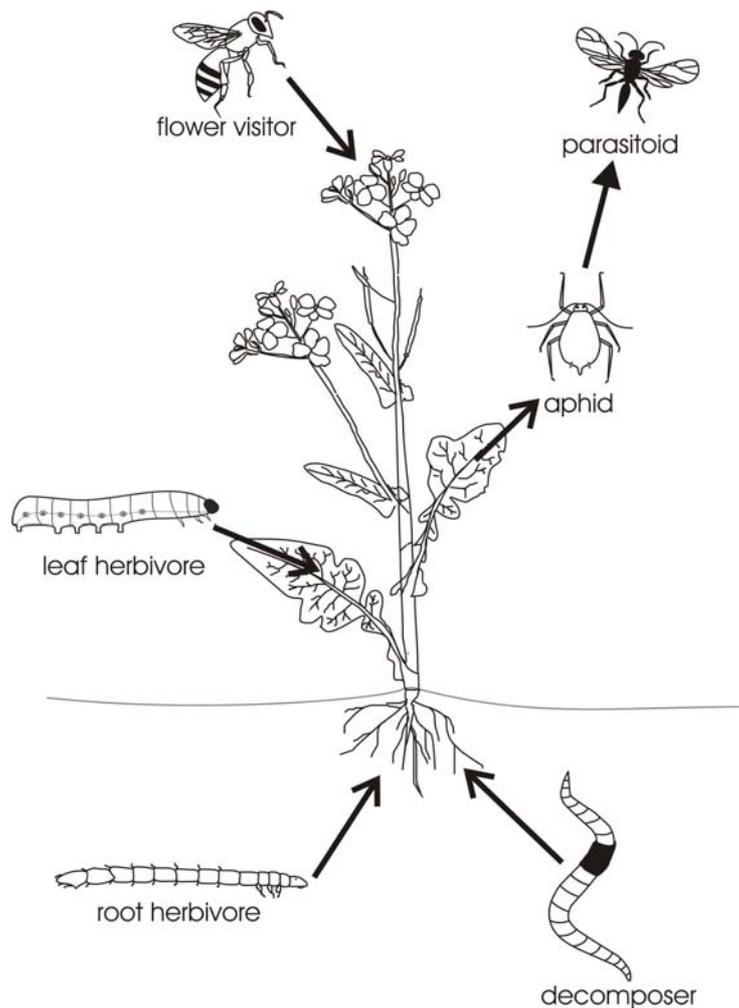
Fig. 1.1 The experimental system studied in chapters 2-5. The plant species was wild mustard (*Sinapis arvensis*). In our experimental design we manipulated the presence/absence of (1) decomposers, which were earthworms of the genus *Octolasion*, (2) root herbivores which were click beetle larvae from the genus *Agriotes* (also known as wireworms), and (3) leaf herbivores, which were in the experiment presented in chapter 3 cabbageworms (*Pieris rapae*) and in the other experiments *Spodoptera* larvae. The effect of these organisms was measured on (1) plant growth, (2) the cabbage aphid (*Brevicoryne brassicae*), (3) their parasitoids, and (4) flower visitors, mainly honey bees (*Apis mellifera*).

In the last experiment (chapter 6) we defaunated soils found on wheat fields, comparing soils from organic vs. conventional farms on wheat plant (*Triticum aestivum*) growth, the abundance of aphids and the infection by *Septoria* fungi. In this experiment the most common species of aphid was *Sitobion avenae*, with 93.5 % of the individuals followed by *Rhopalosiphum padi* with 5.6% of the individuals. *Metopolophium dirhodum* and two other species comprised each less than 0.5 % of the individuals. Plants were also left to be naturally infected by *Septoria* (mainly *S. tritici*) fungi during the experiment.

Research objectives

The present work focuses on elucidating the effects of below- and aboveground plant-animal interactions on plant performance and how these effects translated in the abundance and behavior of aboveground organisms like herbivores, pathogens, parasitoids and pollinators. The main objectives were to:

- review other studies that have investigated how belowground processes, such as decomposition and root herbivory, affect the aboveground biota (chapter 2).
- study the effects of root herbivores and leaf herbivores on plant fitness, and flower visitation (chapter 3).
- investigate how exposing a plant to decomposers, root herbivores and leaf herbivores affected both plant performance and the aboveground antagonistic and mutualistic plant-insect interactions (chapter 4).
- study single and combined effects of main ecological processes such as decomposition, root- and leaf herbivory on plant growth, flowering phenology and on floral traits, as indicators of plant attractiveness to pollinators (chapter 5).
- compare the effect of defaunation in soils from organic farms and conventional farms on the growth of wheat plants and the development of herbivores and pathogens (chapter 6).

Outline of chapters

Chapter 2 gives a general overview of the literature on the interactions between belowground and aboveground processes. First, we review patterns of how belowground processes, such as decomposition and root herbivory, are linked to the aboveground biota. Second, we discuss in more detail the way in which decomposers and root herbivores alone and in combination

cause aboveground responses. Finally, there is a general discussion where it gets clear that decomposition and root herbivory are only one part of the diverse plant-animal and plant-fungi interactions linking aboveground and belowground subsystems.

In chapter 3 we analyzed the effects of leaf herbivores and root herbivores on plant growth, flower visitation and seed set. Leaf herbivory reduced plant height, shoot mass, and delayed the onset of flowering. Root herbivory increased the amount of flower visitors on plants with root herbivores, compared to plants without root herbivores. Combined leaf and root herbivory affected flowering period, number of fruits per plant and number of seeds per fruit. Plants attacked by leaf and root herbivores had a shorter flowering period and less fruits per plant than plants with only root herbivores. The results of this chapter show that mustard plants responded strongly to root and leaf herbivores, but in the end, plant reproduction was not significantly affected by any of the treatments highlighting the flexibility and compensatory power of *S. arvensis* to herbivore damage.

In chapter 4 data are presented on how exposing *Sinapis arvensis* to wireworms, caterpillars and earthworms affected both plant performance and the aboveground associated insect community. We found that the individual presence of either root herbivores or decomposers resulted in increased aphid abundance, demonstrating that the same aboveground plant-insect interaction can be released by different belowground processes. Furthermore, decomposers increased plant growth and plant fitness (measured as the number of seeds produced), indicating that mustard may benefit from nutrients provided by decomposers, regardless whether plants are attacked by root herbivores or leaf herbivores, or both simultaneously. More flower visits were observed in plants attacked by root herbivores but without leaf herbivores than in plants with both herbivores, suggesting that root herbivory can modify flower attractivity to pollinators, thereby confirming the data presented in chapter 3.

In chapter 5 we present a study performed to investigate single and combined effects of decomposition, root- and leaf herbivory on (1) flowering phenology and floral trait, as indicators of plant attractiveness to pollinators and on (2) seed set, after hand pollinating all the plants. Leaf herbivory negatively affected flowering traits indicating that it could significantly affect plant attractiveness to pollinators. Decomposers increased total plant biomass and seed mass indicating that plants use the nutrients liberated by decomposers to increase seed production. From the data we suggest that *S. arvensis* faced no strong selection pressure from pollen limitation, for two reasons. First, reduced nutrient availability through leaf herbivory affected primarily floral traits that could be important for pollinator attraction. Second, improved nutrient supply through decomposer activity was invested in seed

production and not in floral traits. This study indicates the importance of considering multiple plant-animal interactions simultaneously to understand selection pressures underlying plant traits and fitness.

In chapter 6 an experiment was set up to compare the effect of defaunation in soils from organic farms and conventional farms on the growth of wheat plants and the development of aphids and fungi on them. Two main questions were addressed: (1) How important are soil organisms for plant growth and the development of herbivores and fungi on these plants? and (2) How does agricultural management affect interactions between soil organisms, plants and their herbivores and pathogens? Plant biomass in soils from conventional farms exceeded that of soils from organic farms, presumably due to the higher nutrient input in the conventional farming system. Soil defaunation likely mobilized nutrients that increased plant growth. Aphid abundance and *Septoria* infection was reduced by defaunation but only in organic soils. This suggests that soil organisms in organic farming systems are more important for the augmentation of aphids and the infection rate by *Septoria* than in conventional systems. Hence, farming practice appears to modify the effect of soil organisms on aboveground organisms, and soil organisms played a major role in organic farming systems. Further, the results indicate that soil organisms may modify higher trophic levels (aphid and pathogen infection) without significantly affecting lower trophic levels (plant growth).

Plant-mediated interactions between below- and aboveground processes: decomposition, herbivory, parasitism and pollination

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Introduction

All terrestrial ecosystems constitute of a belowground and an aboveground subsystem. These subsystems depend on each other, since above the ground primary producers are the main source of organic carbon for the system, whereas below the ground soil organisms are responsible for the breakdown and recycling of organic matter and the mineralization of the nutrients therein (Scheu and Setälä 2002, Wardle 2002, Porazinska et al. 2003). To understand community and ecosystem-level processes it is necessary to study the interactions within and between these subsystems. However, most ecologists have investigated belowground and aboveground communities separately, leaving the "between subsystems" interactions unstudied (but see Wardle 1999, 2002, Bonkowski et al. 2001, Masters et al. 2001, van der Putten et al. 2001, Brown and Gange 2002) . Moreover, studies of different types of plant-animal interactions, such as pollination, herbivory and seed dispersal, have traditionally progressed separately, focusing on just one kind of interaction and ignoring the possible interference with others (Herrera et al. 2002). This leads to an oversimplification of our understanding of plant-animal interactions, as most plants interact simultaneously with a broad spectrum of animals. Aboveground communities are affected by both direct and indirect effects of soil organisms on plants. Soil biota exert direct effects on plants by feeding on roots and forming antagonistic or mutualistic relationships with their host plants (Wardle et al. 2004). Such direct interactions with plants influence not only the performance of the host plants themselves, but also that of the herbivores and their predators. With regard to indirect pathways, feeding activities in the detrital food web stimulate nutrient turnover, plant nutrient acquisition and plant performance and thereby should indirectly influence the aboveground animal community (Scheu 2001, Wardle et al. 2004).

The aim of this chapter is to evaluate plant mediated interactions between aboveground and belowground processes. First, we review patterns of how belowground processes, such as decomposition and root herbivory, are linked to the aboveground biota. Second, we discuss in more detail the way in which decomposers and root herbivores alone and in combination cause aboveground responses. Finally, we stress that decomposition and root herbivory are only one part of the diverse plant-animal and plant-fungi interactions linking aboveground and belowground subsystems.

The belowground biota

Soil organisms are densely packed: underneath one footprint of forest soil there may be billions of protozoa, hundreds of thousands of nematodes, thousands of Collembola and mites and a large number of isopods, spiders, beetles, and other invertebrates (Scheu and Setälä 2002). The soil biota has been divided in four main groups depending on size: (1) the microflora, consisting mainly of bacteria and fungi with body width less than 50 μm; (2) the microfauna, composed primarily of nematodes and protozoa with body widths of less than 0.1 mm; (3) the mesofauna, including microarthropods such as mites and springtails as well as enchytraeids, with a body width between 0.1 and 2.0 mm; and (4) the macrofauna, with body widths greater than 2 mm, which is composed of earthworms, termites, millipedes and other arthropods that live in and above the soil (Lavelle and Spain 2001). The soil biota can also be divided into two main groups depending on the way they interact with plants. The first group are the root-associated organisms, i.e., root herbivores and their consumers, which influence the plant directly, for example, by feeding on roots. The second is the decomposer community, which breaks down dead plant material and indirectly regulates plant growth and community composition by determining the supply of available soil nutrients (Scheu and Setälä 2002, Wardle et al. 2004).

Effects of root herbivores …

Animals feeding on belowground plant parts include both vertebrates, mainly rodents, and invertebrates, such as herbivorous insects and nematodes. The role of nematode herbivory has been reviewed earlier (Stanton 1988, Mortimer et al 1999) and will not be considered in this chapter. The main functions of the root systems are anchorage and the acquisition of water and nutrients in order to support rapid growth and reproduction of the plant. The loss of roots to herbivores affects the vegetative growth of the plant not only through diminished nutrient and water uptake, but also through the loss of storage tissue (Mortimer et al. 1999).

… on plant growth

Root herbivory reduces plant growth and increases plant mortality (Brown and Gange 1990, Wardle 2002). For example, a study of Nötzold et al. (1998) on the effects of the weevil *Hylobius transvittatus* on purple loosestrife (*Lytrum salicaria*) showed that root herbivory

reduced plant height in the first year and plant biomass in the second year. A review of the effect of mechanical root pruning showed increases in growth of root tissue and a reduction in shoot growth for many plant species (Andersen 1987). Mortimer et al. (1999) stated that differences in responses of plants to belowground herbivory can be related to characteristics of their life histories, such as levels of stored resources. Long-lived clonal species appear to be able to respond to herbivory by compensatory regrowth, whilst annual and monocarpic species are usually more susceptible and respond with reduced growth or fecundity. It has also been noted that damage to belowground plant parts leads to altered source-sink relationships within the plant, with compensatory root growth occurring at the expense of shoot growth (Mortimer et al. 1999).

Further, effects of root herbivores may be altered by belowground interactions in the soil food web. For example ghost moth (*Hepialus californicus*) larvae are known to attack bush lupine (*Lupinus arboreus*), occasionally eradicating entire stands (Maron 2001). Similar to above the ground root damage by herbivores may be counteracted by predators as is the case in the ghost moth being attacked by nematodes (*Heterorhabditis marelatus*) (Strong et al. 1999, Preisser 2003).

… on floral traits and pollination

In animal-pollinated plants fitness may be influenced by floral traits that function as advertisement and reward for pollinators. In order to attain outcrossing, plants with flowers offer an extraordinary range of attractants to increase pollinator visitation. Floral advertisements include olfactory cues, short-range tactile cues and visual cues, such as size, shape and color of inflorescences and flowers. Floral rewards include nectar and pollen that are highly attractive for flower visitors (Dafni 1992, Pellmyr 2002). Floral advertisement and seed set can be affected by a range of organisms interacting with the plant. Leaf and floral herbivory reduce pollinator visitation in damaged plants through changes in floral traits (Strauss 1997, Lehtilä and Strauss 1997, 1999, Mothershead and Marquis 2000). Effects of aboveground herbivory on pollination have been considered in detail (see Bronstein et al., chapter 4) but also belowground herbivores have been shown to affect floral traits. For example, root herbivory by the weevil *Hylobius transvittatus* on purple loosestrife (*Lytrum salicaria*) delays the flowering period and decreases the number of flowers and the size of the inflorescences (Nötzold et al. 1998), although it is unknown if this translates into effects on plant fitness or visitation by pollinators. In addition, reduction in root herbivory resulted in larger flowerheads and an earlier onset of flowering in thistles (*Cirsium palustre*) (Masters et

al. 2001). In contrast to these findings, *Sinapis arvensis* attacked by root herbivores did not suffer from changes in floral traits, but had an increased number of flower visitors in comparison to control plants (Poveda et al. 2003), suggesting that root herbivory may somehow enhance the attractiveness of plants to their pollinators.

… on aboveground herbivores and their parasitism

A series of studies have shown that root herbivory increases the susceptibility of plants to the attack of aboveground herbivores. For example, Gange and Brown (1989) assessed the effects of the chafer larva *Phyllopertha horticola* (Coleoptera: Scarabaeidae) on the performance of the black bean aphid (*Aphis fabae*), mediated via a common annual host plant, *Capsella bursa pastoris*. Root feeding caused an increase in the weight, growth rate, fecundity and adult longevity of the aphid. They also reported a positive effect of root herbivory on the growth and performance of a foliar feeder, but this effect was mitigated at high soil moisture levels.

Masters (1995b) tested the effect of insect root herbivory on aphid performance under field conditions and in an experiment with controlled environmental conditions. In both experiments, root feeding by insects affected the performance of foliar-feeding aphids beneficially. Plants subjected to below-ground insect herbivory in the field supported greater numbers of aphids, and root feeding in the laboratory increased adult aphid weight and growth rate, thereby increasing fecundity. Similarly, Masters et al. (2001) showed that the abundance of tephritid flies, which induce galls in the flower heads of *Cirsium palustre*, increased as a result of root herbivory. Seed predation and parasitism also increased, the latter indicating an indirect effect of root herbivory on higher trophic levels.

Reviewing studies on the effect of belowground herbivores on aboveground herbivores Bezemer et al. (2002) documented that 7 out of 8 studies showed a positive effect of root herbivory on aboveground herbivory. Masters et al. (1993) put forward a conceptual model explaining the positive effects of root herbivores on aboveground herbivores (termed "stress response hypothesis" by Bezemer et al. 1993). They suggested that root feeding limits the ability of the plant to take up water and nutrients, and leads to a reduction in the relative water content of the foliage, increasing levels of soluble nitrogen (especially amino acids) and carbohydrates. The higher-quality food resource leads to increased insect growth, fecundity and population size of foliar feeding insects. Bezemer et al. (2002) proposed as an alternative the "defence induction hypothesis". It predicts that root herbivores detrimentally affect aboveground insect performance through the induction of secondary plant compounds in the

foliage. Suppporting this hypothesis they showed that root herbivory by wireworms (*Agriotes lineatus*) induced cotton plants (*Gossypium herbaceum*) to increase the concentration of terpenoids in the leaves, resulting in a reduced performance of *Spodoptera exigua* larvae on these plants.

Root and leaf feeders can also interact via the host plant in a plus-minus fashion, when belowground herbivory facilitates aboveground herbivory, but aboveground herbivory inhibits belowground hebivory. Moran and Whitham (1990) reported that root-feeding aphids (*Pemphigus batae*) affected neither their host plant *Chenopodium album* nor an aboveground leaf-galling aphid (*Hayhurstia atriplicis*). In contrast, aboveground aphids reduced overall plant biomass by more than 50%, seed set by 60% and the number of belowground aphids by 91%. Similarly, root feeding by chafer larvae (*P. horticola*) increased the fecundity of a leaf miner (*Chromatamyia syngensiae*, Diptera: Agromyzidae), whereas leaf feeding decreased the growth rate of the belowground insect herbivore (Masters and Brown 1992, Masters 1995a). Subsequent laboratory experiments were performed to test the effects of chafer larvae on different foliar-feeding guilds, namely the leaf chewer *Mamestra brassicae* (Lepidoptera: Noctuidae), the phloem feeder *Myzus persicae* (Homoptera: Aphididae) and the leaf miner *Chromatamyia syngensiae* (Masters and Brown 1997). In each case, the growth rate of the root feeders decreased when foliar feeders were present. On the other hand, root herbivory significantly increased the performance of phloem feeding aphids and leaf miners but there was no significant effect on any performance-related parameter of the leaf chewer. In this case, root herbivory reduced the consumption rate of leaf material. However, caterpillars still maintained their growth rate and developed normally.

In summary, aboveground responses appear to depend on the level of root herbivory, with low levels but not high levels being compensated for by the plant. Root herbivory presumably increases aboveground nutrient quality via soluble amino acids, but so far the mechanism is not clear. Depending on the plant species, this effect may be masked by the induction of secondary compounds. Further experimentation is necessary to generalize the importance of each of these mechanisms.

Effects of decomposers …

The primary consumers within the decomposer food web, bacteria and fungi, are directly responsible for most of the mineralization of nutrients in the soil, and are therefore the primary biotic regulators of nutrient supply for plants. This is due to their unique capacity to

directly break down complex carbohydrates and mineralize the nutrients contained therein (Wardle 2002). The nutrient mineralization process driven by the soil microflora is in turn influenced by the soil food webs in which micro- and mesofauna, including microarthropods, nematodes and protozoa, feed upon the microflora and on each other (Ruess et al. 2004), releasing nutrients that are locked up in the bacterial biomass (Bonkowski 2004). The importance of soil fauna is considerable given their influence on the growth and activity of the microflora (Wardle et al. 2004). The largest scale of the decomposer subsystem includes earthworms and termites and is characterized by their ability to build physical structures that create habitats for smaller organisms and to function as litter transformers. These large, structure-forming invertebrates process a major part of the detritus available and function as ecosystem engineers (Jones et al. 1994). Earthworms have been shown to transform soil systems from moder (medium humified humus) to mull (well humified organic matter) type humus due to physical action, i.e., by engineering (Bohlen et al. 2004). Given that decomposers are responsible for the breakdown of organic matter and the release and cycling of nutrients (Haimi and Einbork 1992, Wardle 2002), they could be expected to stimulate plant growth and herbivore performance.

… on plant growth

The activity of decomposers often results in increased plant growth and plant nitrogen content (Scheu and Parkinson 1994, Bonkowski et al. 2000, 2001, Wardle 2002). Scheu (2003) reviewed the response of plants to the presence of earthworms. He found that in 79% of all studies, shoot biomass of plants significantly increased in the presence of earthworms, in 9% it declined and in 12% no significant effect was found. Root biomass increased in 50% of the cases and decreased in 38%. For example, the presence of the earthworm *Dendrobaena octaedra* enhanced the shoot biomass of the grass *Agropyron trachycaulum* and increased the shoot-to- root ratio during early plant growth (Scheu and Parkinson 1994). Spain et al. (1992) showed that transfer of ^{15}N from microbial biomass to plants was enhanced by the addition of earthworms and that ^{15}N incorporated into both microbial biomass and earthworms served as a source of nutrients to plants. On the other hand, Newington et al. (2004) showed that earthworms increase soil nitrate and foliar nitrogen concentrations in *Veronica persica* and *Cardamine hirsuta*, but that there was no associated increase in plant biomass. More details on earthworm-plant interactions are given by Scheu (2003) and Brown et al. (2004).

In contrast to this scenario of decomposer effects, Scheu et al. (1999) found collembolans (*Heteromurus nitidus* and *Onychiurus scotarius*) to cause a reduction in plant biomass of *Poa annua* mainly in roots, while plant tissue nitrogen concentration was increased.

Generally, results from microcosm studies suggest that larger soil fauna can have strong positive effects on plant growth and nutrient acquisition, presumably through promoting microbial activity and therefore nutrient mineralization (e.g. Setälä and Huhta 1991, Haimi et al. 1992, Alphei et al. 1996, Bardgett et al. 1997, 1998). An important mechanism responsible for these processes is known as the "microbial loop" in the soil (Clarholm 1985, Coleman 1994, Moore et al 2003). It is triggered by the release of root exudates from plants that increase bacterial growth in the rhizosphere. Plant nutrients may be sequestered during microbial growth and remain locked up in bacterial biomass. Grazing by decomposer invertebrates re-mobilizes these nutrients, making them available for plant uptake. Due to the relatively small differences in the C:N ratios between decomposers and bacterial prey and the relatively low assimilation efficiency of the decomposers, only a small percentage of the consumed nitrogen is used for biomass production. The excess nitrogen is excreted as ammonia and hence is readily available for plant roots (see Bonkowski 2004, Scheu et al. 2004).

… on herbivores

As mentioned above, earthworms enhance nitrogen uptake from litter and soil into the plant (Wurst et al. 2003, Wurst et al. 2004a) leading to an enhanced nutrient concentration in plant tissue (Alphei et al. 1996, Callaham and Hendrix 1998, Schmidt and Curry 1999, Bonkowski et al. 2001). Since herbivore performance is known to depend strongly on plant tissue nitrogen concentration (White 1993), the effects of earthworms on plant growth likely propagate into the herbivore community.

Effects of earthworms on aboveground herbivores likely vary with soil type, litter distribution and plant species. Earthworm presence has been shown to increase the reproduction of aphids (*Myzus persicae*) on *Poa annua* and *Trifolium repens* (Scheu et al. 1999) and *Cardamine hirsuta* (Wurst and Jones 2003). However, earthworms increased aphid reproduction on *Trifolium repens* only when the litter was concentrated in patches in the soil and not when litter was mixed homogeneously into the soil (Wurst et al 2003). Also, Bonkowski et al. (2001) report no changes in aphid reproduction on wheat in the presence of earthworms. Wurst et al. (2003) even found aphid reproduction on *Plantago lanceolata* to be reduced in presence of earthworms. The reduction of aphid reproduction in the presence of earthworms

was associated with changes in the phytosterol content in leaves of *P. lanceolata*, indicating that not only belowground herbivores but also decomposers increase plant defense against herbivores (Wurst et al. 2004b). Newington et al. (2004) investigated the effect of earthworms on the development of *Mamestra brassicae* larvae on *Cardamine hirsuta* and *Veronica persica* plants. They found no effect of earthworms on the biomass of the larvae. However, in feeding trials the consumption rate of *V. persica* foliage by *M. brassicae* was higher when plants were grown in the presence of earthworms. Highest larval mortality occurred in microcosms without earthworms, suggesting that studying only larval biomass gives a misleading picture.

Although decomposers generally affect plant growth and herbivore development beneficially, this appears to be not always true; in fact, they may affect herbivores detrimentally. Secondary compounds in plants induced by decomposers have been ignored so far, but may significantly affect plant-herbivore interactions (Wurst et al. 2004a). Effects of decomposers on higher trophic levels and plant-pollinator interactions are unknown and need to be addressed in order to complete our understanding on the interrelationships between decomposers and aboveground food webs.

Multitrophic belowground - aboveground interactions: a case study

The interactions between above- and belowground organisms have only recently become a major field of study. Many of these studies focus on the effects of decomposers on plant growth (Scheu and Parkinson 1994, Bonkowski et al. 2000) and on aboveground plant-herbivore interactions (Scheu et al. 1999, Bonkowski et al. 2001), as well as on the effects of belowground and aboveground herbivores on plants and their natural enemies (Brown and Gange 1989, Gange and Brown 1989, Moran and Whitham 1990, Masters and Brown 1992, Masters et al. 1993, Nötzold et al. 1998, Masters et al. 2001). However, there are several limitations in the way that the effects of belowground organisms on aboveground organisms have been studied:

1. Most experiments have been done in microcosms.
2. Plants were not naturally colonized by herbivores.
3. Studies have been performed on plant-herbivore interactions, but not on plant-pollinator interactions.
4. None of the studies linked the effects of the decomposer and the root feeding fauna on aboveground processes.

We established an experiment to address some of these deficiencies. We examined the single and combined effects of root herbivores and decomposers on plant growth, floral traits, flower visitation and herbivore-parasitoid interactions of the annual herb *Sinapis arvensis* (Brassicaceae). By exposing plants to root herbivores (five wireworms, larvae of the click beetle *Agriotes* sp.) and decomposers (two earthworms of the species *Octolasion tyrtaeum*) in a full factorial design, we investigated how direct and indirect belowground plant-animal interactions affect plant performance and the insect community associated aboveground. The decomposer density chosen (50 earthworms/m^2), is in the lower range of densities in the field (9-239 ind/m^2, Pizl 1999). Wireworms are patchily distributed in soil and its densities may vary from zero up to several hundreds per square meter (Poveda et al. 2003). Plants were transferred to a fallow field, and pots were buried into the soil to simulate field conditions. In a first experiment, plants were exposed to natural herbivore colonization and pollination, whereas in a second experiment, plants were covered with a gauze tent to exclude effects of other organisms such as herbivores, seed predators and/or pollinators on plant growth and floral traits.

Table 2.1. ANOVA table of F and p -values on the effect of root herbivores (R) and decomposers (D) on plant biomass, seed production, flowering period, aphid abundance, parasitation rate and number of visited flowers per flower of *Sinapis arvensis* (n=40). Data for the first experiment. P-values are presented in brackets.

	plant biomass	seeds/plant	flowering period	aphid abundance	parasitism rate	visited flowers (%)
R	3.39 (n.s.)	2.11 (n.s.)	0.55 (n.s.)	0.59 (n.s.)	0.89 (n.s.)	8.92 (0.003)
D	4.19 (0.047)	6.88 (0.012)	4.19 (0.047)	0.81 (n.s.)	0.77 (n.s.)	0.37 (n.s.)
R x D	4.72 (0.036)	3.81 (0.058)	0.40 (n.s.)	7.56 (0.009)	0.55 (n.s.)	1.75 (n.s.)

In the first experiment total plant biomass (Fig. 2.1A) was reduced when root herbivores were present, but decomposers counteracted this negative effect when they co-ocurred with root herbivores (Table 2.1). This suggests that decomposers play an important role in counteracting detrimental effects of root herbivores on plant growth and reproduction. The marginally significant interaction between root herbivores and decomposers on the production of seeds per plant (Table 2.1) suggests that the presence of earthworms mitigated the negative effects of root herbivores (Fig. 2.1B). Root herbivores and decomposers affected the total number of aphids on the plants (Table 2.1). In treatments with only earthworms or with only root herbivores, the number of aphids was higher than control plants (Fig. 2.1C). In contrast,

aphid numbers were not significantly different from the control when both organisms were present. The enhanced numbers of aphids on plants with earthworms or root herbivores suggest an increased quality or quantity of assimilates transferred in the phloem. Nitrogen availability is one of the main factors limiting herbivore development (White 1993).
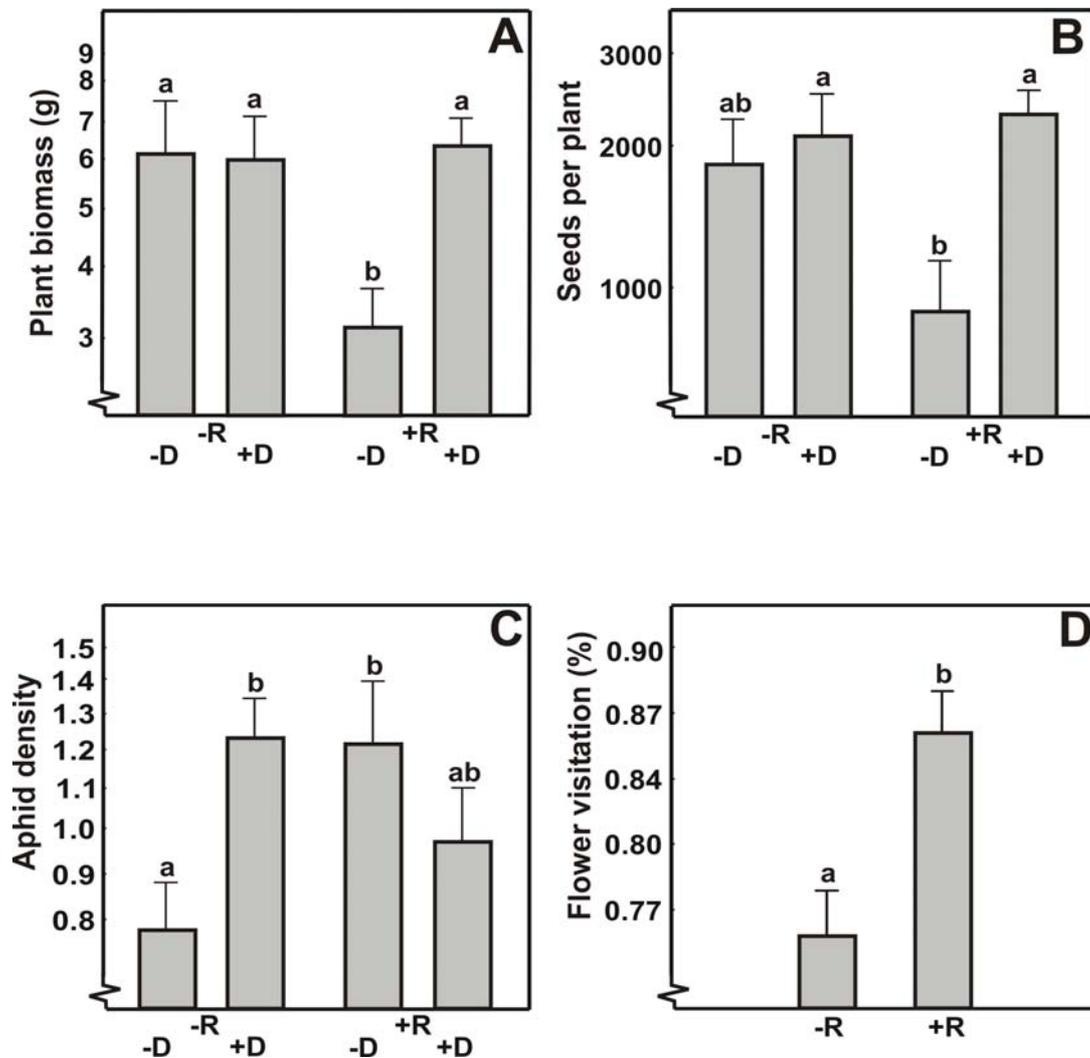
Fig. 2.1. Effects of decomposers (D) and root herbivores (R) on plant biomass (**A**), number of seeds per plant (**B**), on the number of aphids on a plant (**C**) and on the flower visitation rate (**D**) (mean ± one SE). Treatments with different letters are significantly different (Tukey's honestly significant difference test, $p < 0.05$). Note log-scale of x-axis.

Both decomposers and root herbivores likely increased nitrogen availability to aphids. By stimulating nitrogen mineralization, earthworms enhance plant nitrogen uptake and increase nitrogen concentration in plant tissues (see above). By feeding on roots, wireworms likely

decreased the water uptake by the plant and consequently increased the nitrogen concentration in the phloem (Gange and Brown 1989, Masters and Brown 1992, Masters et al. 1993, 2001). There was no effect of herbivores or decomposers on the rate of parasitism of the aphids (Table 2.1). The number of parasitoids was only affected by the number of aphids per plant (simple correlation: $r = 0.74$, $n = 40$, $p < 0.001$). Flower visitation rate was higher in plants with root herbivores (Table 2.1, Fig. 2.1D); the most abundant flower visitor was the honey bee, *Apis mellifera*, comprising 61% of the visits. This suggests that root herbivory increase plant attractiveness to flower visitors. In a previous study, lower quantities of root herbivores (2 wireworms of the genus *Agriotes*) also enhanced the flower visitation rate (Poveda et al. 2003). In the present experiment, the density of wireworms was more than twice as high as in our previous study (5 instead of 2 wireworms per pot), but effects on flower visitation were similar. This suggests that root herbivores, even at relatively high densities, may stimulate the attractiveness of flowers to flower visitors.

In the second experiment we measured floral traits, such as the amount of nectar, pollen production and size of the petals, because these parameters may reveal the mechanisms underlying the enhanced attractiveness of flowering plants in the herbivore treatment. In contrast to our expectations, none of the parameters of floral traits measured was affected by the presence of root herbivores or decomposers (Table 2.2). Potentially, changes in nectar concentration may have been responsible for the observed changes in flower visitation. Masters et al. (1993) shows that water stress caused by root herbivory on plants led to the accumulation of soluble amino acids and carbohydrates in the foliage. It is possible that the carbohydrate concentration of nectar also increased in the presence of root herbivores, thereby augmenting the attractiveness to flower visitors, but this was not measured in our study.

In both experiments, decomposers enhanced plant biomass and fruit and seed set, but did not affect floral traits. This suggests that decomposers increase the plants' fitness. It is challenging to investigate the evolutionary forces that resulted in the observed variation of responses of *S. arvensis* to a complex of plant-associated invertebrate guilds, and to relate these to other plant species of different functional groups and with different life histories. Such studies may lead to a more detailed understanding of how belowground and aboveground plant-animal interactions shaped the evolution of plant traits.

Table 2.2. ANOVA table of F and p -values on the effect of root herbivores (R) and decomposers (D) on plant biomass, seed production, anther length, nectar production and petal size of *Sinapis arvensis* (n=24). Data for the second experiment. P-values are presented in brackets.

	plant biomass	seeds/plant	anther length	nectar	petal size
R	0.34 (n.s.)	0.03 (n.s.)	2.25 (n.s.)	0.0006 (n.s.)	1.83 (n.s.)
D	8.3 (0.009)	6.47 (0.02)	1.05 (n.s.)	0.09 (n.s.)	0.31 (n.s.)
R x D	2.86 (n.s.)	0.51 (n.s.)	1.37 (n.s.)	0.002 (n.s.)	0.06 (n.s.)

Conclusions and future research

Belowground communities affect not only plant growth but also aboveground processes, including plant-herbivore-parasitoid interactions and even plant-pollinator interactions. Effects of indirect interactions mediated through the decomposer subsystem presumably are as important as direct interactions caused by root herbivory, leading in some cases to the same aboveground response. The mechanisms responsible for these effects are still poorly known, and further research is needed to understand the physiological processes in the plants. Our case studies shed some light on the effects of decomposers and root herbivores on aboveground plant-animal interactions (Fig. 2.2), but integrating these effects into the whole soil food web remains to be done.

The links between belowground and aboveground biotic interactions are much more complex than what we have presented here. Future research on the mechanism responsible for aboveground – belowground interrelationships are essential for a better understanding of the ecology and evolution of plant-animal interactions. Results of the studies presented suggest that the effects highly depend on plant species. Field studies are needed to understand more clearly the role of belowground organisms on aboveground biotic interactions in the context of natural plant communities. Feedbacks between the aboveground and the belowground systems should also be taken into account, since it is known that the amount of carbon translocated into roots and into the rhizosphere may significantly increase if plants are subjected to aboveground herbivory. This may strongly influence the rhizosphere food web (Bardgett and Wardle 2003, Mikola et al. 2001), which in turn likely affects aboveground interactions.
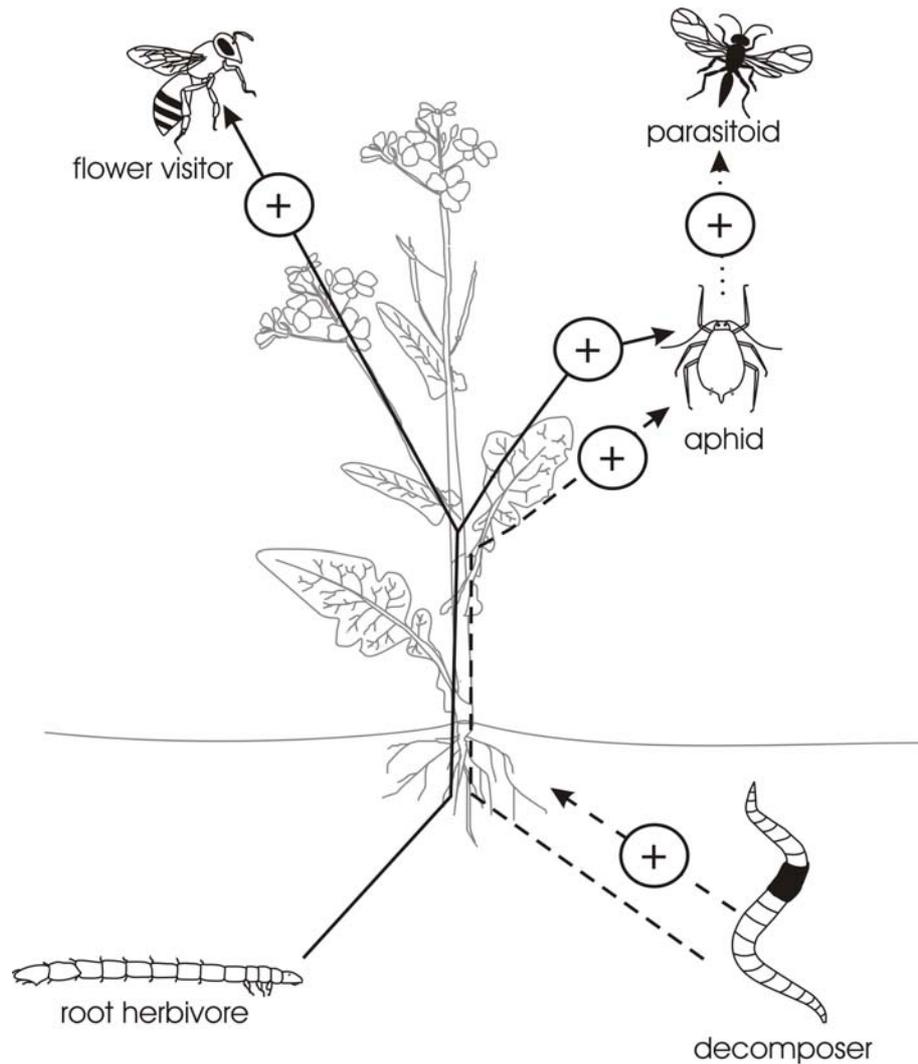
Fig. 2.2. Ways by which root herbivores and decomposers affect flower visitors, aphids and their parasitoids through *Sinapis arvensis* plants. Feeding activities by root herbivores (continuous line) cause an indirect positive effect on aphids and flower visitors. Decomposers (dashed line) indirectly increase plant growth and the number of aphids. An increase in the number of aphids cause an indirect increase in the number of parasitoids (dotted line).

Until recently the role of soil biota in modifying the attractiveness of plants to herbivores and pollinators has been largely ignored. Particularly in natural habitats where plant species interact with each other, soil biota may play a key role in modifying the insect community associated with plants. Future work needs to focus upon more complex systems consisting of plants and soil biota in different spatial and temporal contexts to better understand the functioning of terrestrial systems.

Effects of below- and above-ground herbivores on plant growth, flower visitation and seed set

K. Poveda, I. Steffan-Dewenter, S. Scheu & T. Tscharntke

Abstract. The relative importance and combined effects of root and leaf herbivores on plant growth, flower visitation and seed set were tested in a factorial experiment using potted mustard, *Sinapis arvensis*, on an old fallow field. Leaf herbivory with 50% leaf removal when the seedlings had their first four leaves (by cabbageworms, *Pieris rapae*) reduced plant height, shoot mass, and delayed the onset of flowering. Root herbivory (by two wireworms, *Agriotes* sp. over the whole experiment) changed the amount of flower visitors. The number of flower visitors per plant was higher in plants with root herbivores, than in plants without root herbivores. Combined leaf and root herbivory affected flowering period, number of fruits per plant and number of seeds per fruit. Plants attacked by leaf and root herbivores had a shorter flowering period and less fruits per plant than plants with root herbivores only. Although the experimental plants faced major herbivore-induced growth changes, plant reproduction (seed set and weight per plant) was similar in all treatments, documenting their ability to compensate for leaf and root herbivory. Accordingly, reduced size, delayed start of flowering, reduced flowering period or reduced flower visitation could not be used to predict plant fitness in this annual, cross-pollinated herb.

Keywords: Cabbageworms, Compensatory plant growth, Herbivory, *Sinapis arvensis*, Wireworms

Introduction

Traditionally, below-ground and above-ground communities have been investigated separately even though both systems show high interdependence (Wardle 1999, Masters et al. 2001, van der Putten et al. 2001, Brown and Gange 2002, Scheu and Setälä 2002). The interactions between above- and belowground organisms have only recently become a major field of study. Many of these studies focus on the effects of decomposers on plant growth (Scheu and Parkinson 1994, Bonkowski et al. 2000) and on above-ground plant-herbivore interactions (Scheu et al. 1999, Bonkowski et al. 2001), as on the effects of below-ground herbivores on plants, above-ground herbivores and their natural enemies (Brown et al. 1987, Brown and Gange 1989, Gange and Brown 1989, Moran and Whitham 1990, Masters and Brown 1992, Masters et al. 1993, Nötzold et al. 1998, Masters et al. 2001). Although the effects of below-ground organisms on plant growth have been investigated, their effect on plant reproduction is hardly studied (but see Brown et al 1987, Nötzold et al 1998, Maron 1998, Masters et al. 2001). Further, the relative importance and the combined effects of below-ground and above-ground herbivores on plant performance, fitness and on flower visitation are not known.

Plant fitness is affected by foliar herbivory through changes in plant traits or by altered pollinator visitation patterns of damaged plants (Lethilä and Strauss 1997, 1999, Mothershead and Marquis 2000, Strauss 1997). Seed production may be substantially reduced by herbivory, even when herbivores do not feed directly on reproductive tissue. This may be due to reduced resources available for flower, pollen or seed production or because pollination rates are lowered in damaged plants. Changes in floral characters may reduce attractiveness to pollinators. Foliar herbivory may change the amount or chemistry of nectar (Hambäck 2001), the number or morphology of flowers (Karban and Strauss 1993, Strauss et al 1996, Lethilä and Strauss 1997, 1999), the flowering phenology (Hambäck 2001) or the plant height, i.e. the height of flowers (Strauss 1997, Mothershed and Marquis 2000, Hambäck 2001). Root herbivores may affect the flowering period, the number of flowers and the size of the flowerheads (Brown et al 1987, Nötzold et al 1998, Masters et al 2001). Accordingly, leaf and root herbivores (separately and in combination) may be expected to reduce the number and quality of flowers and fruits, and thereby, overall plant fitness. The objective of this study was to analyze the effect of leaf herbivores (caterpillars, *Pieris rapae*) and root herbivores (wireworms, *Agriotes* sp.) on plant growth, flower visitation and seed set, using an annual, cross-pollinated plant (*Sinapis arvensis*).

Materials and methods

Experimental set-up

The experiment was carried out in a greenhouse and an adjacent fallow field in Göttingen (Lower Saxony, Germany) in 2001. The experiment was set up in a two factorial design. The effects of root herbivores (R) and leaf herbivores (L) on plant growth and reproductive parameters of the mustard (*S. arvensis*) were investigated. The plants were subjected to 4 treatments (Control, R, L, RL). Six replicates of each treatment were established. We used ten-liter pots that were filled with soil taken from a 10-year-old fallow field located on a limestone plateau east of Göttingen. The soil was defaunated by heating to 75-80°C for 2 hours. Two wireworms (larvae of the click beetle *Agriotes* sp.) were added to each pot as the root herbivore treatment, which is equivalent to a density of ca. 30 ind. m². Wireworms are patchily distributed and may reach densities of up to several hundred per square meter. The density chosen corresponds to that of patches frequently found in the field (K. Poveda, pers. obs.). Third-instar larvae of *P. rapae* (cabbageworms) were reared from eggs of butterflies collected around Göttingen and used as leaf herbivores.

Seeds of mustard from a regional wild population (provided by the botanical garden, Göttingen) were sown on 9 July 2001 in the greenhouse. Young seedlings (with 2 true leaves) were transplanted into small pots. On July 21 2001 they were transplanted into the experimental pots, to which the wireworms had been added. Six days after transplantation, two cabbageworms were put on the seedlings, which had their first four true leaves. We let the larvae consume 30 - 50% of the leaves, which took approximately 2 days. This level of leaf damage is well within the range recorded in the field (Strauss et al. 1996). Any other leaves subsequently produced were left undamaged. On July 30 all the pots were set in random order in the field.

Sampling and statistical analyses

Data on plant height, shoot mass, beginning and end of the flowering period were collected for each plant. When fruits turned yellow and the plant began to die (mid October 2001) fruits and shoots were collected and oven dried for 3 days at 60°C. The number of fruits (pods) of each plant was counted. Twenty fruits were randomly selected, and seeds of these fruits counted and weighed. These data were used to calculate the number of seeds and seed weight per plant. Total number of flowers produced by each plant was estimated by counting the pedicels left on the inflorescences.

Insects visiting the flowers were observed during the flowering period, August 1 to September 20, 2001. Each plant was observed for a total of 45 minutes, divided in three observation periods of 15 minutes. These observation periods were distributed randomly on three different days and at different hours each day, between 10:00 and 16:00 h. For each plant the number of open flowers was counted. The number of flower visitors per flower and per 15 min interval was then calculated by dividing the total number of flower visitors per plant by the number of open flowers.

Data were analyzed by two-way analysis of variance (ANOVA). Differences between means were inspected using Tukey's honestly significant difference test. We correlated the number of fruits per plant with the number of seeds per fruit. Statistical analyses were performed using Statistica 5 (StatSoft 1995).

Results

Leaf herbivory changed plant height, beginning and length of the flowering period (Table 3.1). Shoot mass was reduced by leaf herbivores with marginal significance (6.5 g $\pm$ 0.58 and 8.14 g $\pm$ 0.63, mean $\pm$ SE for plants with and without leaf herbivory, respectively; Table 3.1). Plants attacked by leaf herbivores were smaller (Fig. 3.1A) and began to flower later (Fig. 3.1B) than plants without leaf herbivores. The flowering period also depended on the combination of root and leaf herbivory treatments (Table 3.1).

Table 3.1. ANOVA table of F-values on the effect of root herbivores (R) and leaf herbivores (L) on plant height, shoot biomass, flowering onset, flowering period, total number of flowers, fruits per flower, number of fruits, number of seeds per fruit, number of seeds per plant, seed weight per plant and number of flower visitors per flower

	Plant height	Shoot mass	Flower onset	Flower period	Total flowers	Fruits per plant	Seeds per fruit	Seed per plant	Seed weight	Visitor per flower
L	4.6*	3.3$^{(*)}$	27.4***	8.9**	<0.1	0.6	1.9	0.6	1.7	1
R	0.5	<0.1	2.8	0.6	<0.1	<0.1	0.2	<0.1	0.1	4.5*
RxL	4.2$^{(*)}$	0.7	0.4	7.0*	1.6	5.1*	21.1***	<0.1	<0.1	<0.1

$^{(*)}$ $P < 0.1$; * $P < 0.05$; ** $P < 0.01$, *** $p < 0.001$

Plants attacked by both herbivores had a shorter flowering period than plants with root herbivores only (Fig 3.1C). Although the total number of flowers produced per plant was similar in all treatments, there was an interaction between the effects of root and leaf

herbivores on the number of fruits per plant (Table 3.1). Plants with root herbivores produced more fruits than plants with both herbivores (Fig. 3.2A).
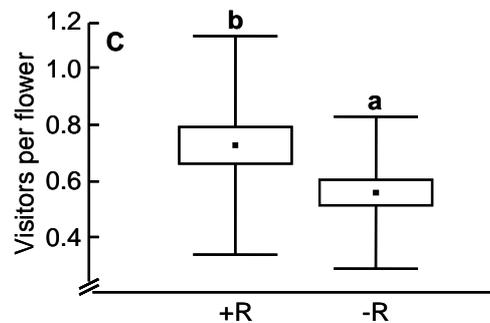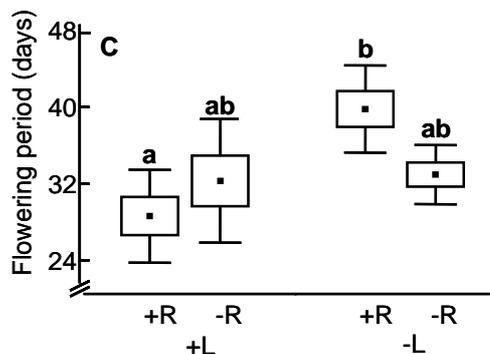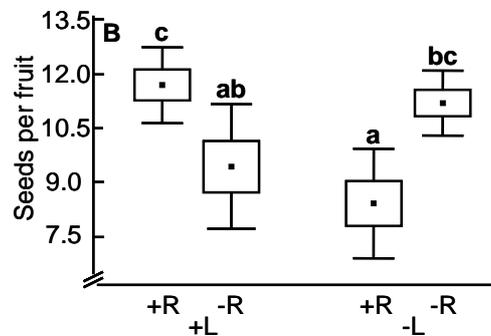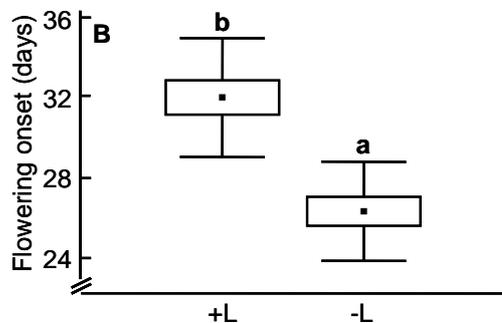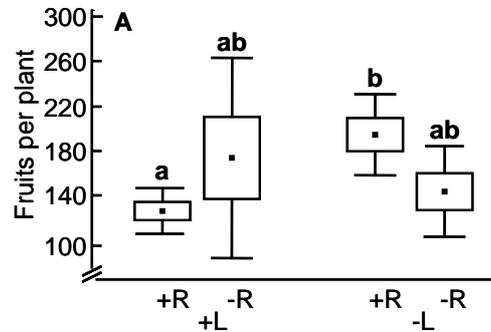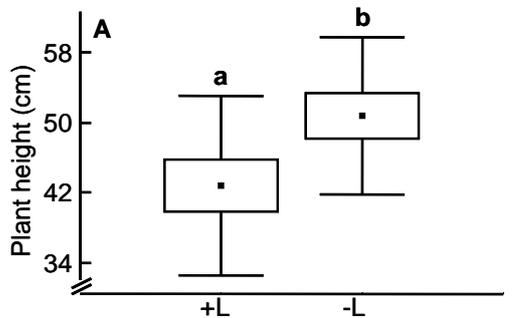

Fig. 3.1 Effect of root herbivores (R) and leaf herbivores (L) on plant height (**A**), flowering onset (days to the beginning of the flowering period) (**B**) and the length of the flowering period (**C**) of *Sinapis arvensis* (mean ± 1SE and 1SD). Treatments with different letters are significantly different (Tukey's test, $p < 0.05$).

Fig. 3.2 Effect of root herbivores (R) and leaf herbivores (L) on the number of fruits per plant (**A**), the number of seeds per fruit (**B**) and the number of visitors per flower (**C**) of *Sinapis arvensis* (mean ± 1SE and 1SD). Treatments with different letters are significantly different (Tukey's test, $p < 0.05$).

Plants with many fruits had fewer seeds per fruit than plants with few fruits (r=-0.53, n=24, p=0.007). Accordingly, the number of seeds per fruit depended on both root and leaf herbivory (Table 3.1). Plants with both herbivores had a similar number of seeds per fruit as control plants. In contrast, plants with either root or leaf herbivores had less seeds per fruit than plants with both herbivores, and plants with root herbivores had less seeds per fruit than control plants (Fig. 3.2B).

There was no significant effect of root herbivores, leaf herbivores or both on the number of flowers (range of arithmetic means: 231-275, n=6) and on the total number (1395-1579) and weight of seeds per plant (2.01-2.43 g; Table 3.1), i.e., overall plant reproduction was not affected despite the changes in growth parameters.

Altogether 859 flower visitors were observed. The most abundant visitor was the honeybee (*Apis mellifera*) with 486 visits, followed by hover flies (Syrphidae) with 265 visits (mainly of the species *Eristalis tenax*). The remaining visitors were predominantly bumblebees (*Bombus* spp.) and flies (Diptera). The number of visitors per flower was affected by root herbivores (Table 3.1) and was higher in plants with root herbivores than in plants without root herbivores (Fig. 3.2C).

Discussion

Although interest in interactions between below- and above-ground organisms has increased in recent years, no studies have considered the relative importance and combined effects of root herbivores and leaf herbivores on flowering, flower visitation and seed set of plants (but see Masters et al. 2001). The results of this paper show that mustard plants responded strongly to root and leaf herbivores, but in the end, plant reproduction was not significantly affected by any of the treatments highlighting the flexibility and compensatory power of *S. arvensis* to herbivore damage.

Plant performance

Plants that were attacked by leaf herbivores at an early stage had a smaller size, reduced shoot mass, a delayed and shorter flowering period. The reduction in size and shoot mass found in our experiment may have been caused by a reduced photosynthetic area (Crawley 1983, Kinsmann and Platt 1984, Marquis 1984, Strauss 1991), since a 50% removal of the leaf area at an early stage has been documented to translate into a reduction in total leaf area by 25% over the plant's lifetime (Strauss et al. 1996). Flowering phenology depends on the resources

plants allocate for reproduction, as documented for *Raphanus raphanistrum* (Strauss et al. 1996). The delayed flowering onset in *S. arvensis* in response to leaf herbivory in part was caused by abortion of the first flowers (K. Poveda pers. obs.) which presumably resulted from a reduction in resources allocated to inflorescences. Flower abortion is known to strongly depend on the resources available (Stephenson 1981, Stirling et al 2002). In plants attacked by leaf herbivores the photosynthetic tissue was reduced strongly. Consequently, fewer resources were available for flower production, including the production of pollen and nectar, and also for fruit and seed development.

Plants attacked by root herbivores had a longer flowering period, and a higher number of fruits than plants attacked by both herbivores. At the density used in this experiment root herbivores adversely affected reproductive components only in combination with leaf herbivores. In contrast, root herbivores in most studies detrimentally affected plant growth (Powell and Myers 1988, Gange and Brown 1989, Nötzold et al 1998) and plant reproductive parameters (Masters et al 2001, Maron 1998, Brown and Gange 1990, Powell and Myers 1988). Parts of the root system when removed by feeding may be replaced rapidly (Simberloff et al. 1978, Andersen 1987, Riedell 1989). Plants may respond by increased lateral root proliferation to compensate for damage (Brown and Gange 1990). This may benefit the plant, since lateral roots enhance the uptake of nutrients and water. Ridsdill-Smith (1977) reported foliage yield of ryegrass to be unaffected by feeding of scarabaeid larvae. Riedell (1989) even found the yield in corn to be increased at low corn rootworm (*Diabrotica* spp.) density. Since the number of wireworms used in our experiment was low (two per pot), mustard compensated root damage.

In plants with both root and leaf herbivores the flowering period was shortened and less fruits per plant were produced compared to plants with root herbivores only. Obviously, these plants suffered most because of the loss of photosynthetic tissue and roots, both important for nutrient acquisition. Plants are known to respond to root herbivory by mobilizing carbohydrate reserves and directing them to the attacked area which is termed re-growth potential (Davidson et al. 1970). Usually plant re-growth potential increases with plant size, so damage by root feeders should be less important in large plants (Davidson et al. 1970). In our experiment plants damaged by leaf herbivores were smaller, which likely reduced the re-growth potential of plants additionally attacked by root herbivores.

Plant reproduction

Plants strongly responded to the treatments, but in contrast to our expectations, the differences in plant height, flowering onset, flowering period, and number of fruits did not result in significant differences in the number of seeds produced. Interestingly, all plants had the same number of total flowers, but the number of fruits differed between treatments and plants with many fruits had fewer seeds per fruit than plants with many fruits. Obviously, the mustard plants compensated for reduced fruit numbers with an increase in seed number per fruit. Different compensatory mechanisms may have contributed to the ultimately similar plant fitness. Plants often produce more ovules within flowers than they turn into seeds (Stephenson 1981). This allows adjusting plant reproduction to changes in resources available. In our experiment plants attacked by both herbivores produced far less fruits than plants attacked by root herbivores only. Presumably, this was due to early flower and fruit abortion. It is well documented that defoliation results in a reduced number of fruits due to increased fruit abortion, however this may not be associated with a decrease in the number of seeds (reviewed by McNaughton 1982). In our study, mustard plants attacked by leaf herbivores aborted flowers at an early stage, leaving only the pedicels on the inflorescence (K. Poveda, pers. obs.). Since the number of pedicels was taken to estimate the number of flowers this may have resulted in an overestimation of the number of flowers produced by a plant. However, the mustard plants compensated for the reduced number of fruits by producing more seeds per fruit.

Herbivory significantly affected the number of visiting pollinators per flower but flower visitation did not affect reproduction; all plants produced a similar number of seeds. Plants attacked by root herbivores had more flower visitors than plants without root herbivores. Attack by leaf herbivores is known to affect flower morphology and, thereby flower visitation (Conner 1996, Lethilä and Strauss 1997, 1999, Mothershed and Marquis 2000). Surprisingly, root herbivores increased the number of flower visitors possibly due to an enhanced nectar production.

In conclusion above- and below-ground herbivores affected plant height, shoot mass, flowering phenology, fruit set and even the number of flower visitors. Ultimately, however, neither the growth parameters nor the flower visitation could be used to predict the fitness in this annual, cross-pollinated herb. Both leaf and root herbivory did not affect final plant reproduction (number of seeds per plant), presumably due to compensatory responses of *S. arvensis*.

Effects of decomposers and herbivores on plant performance and aboveground plant-insect interactions

K. Poveda, I. Steffan-Dewenter, S. Scheu & T. Tscharntke

Abstract. Most ecologists acknowledge that plants are subject to complex interactions between both below- and aboveground dwelling animals. However, these complex interactions are seldomly investigated simultaneously. In a factorial common-garden experiment we tested single and combined effects of decomposers, root herbivores and leaf herbivores on the growth, flower visitation, and abundance of naturally colonizing aphids and parasitoids on wild mustard (*Sinapis arvensis*). We found that the individual presence of either root herbivores or decomposers resulted in increased aphid abundance, demonstrating that the same aboveground plant-insect interaction can be released by different belowground processes. Enhanced aphid densities caused higher numbers of parasitoids. Furthermore, decomposers increased plant growth and plant fitness (measured as the number of seeds produced), indicating that mustard may benefit from nutrients provided by decomposers, regardless whether plants are attacked by root herbivores or leaf herbivores, or both simultaneously. More flower visits were observed in plants attacked by root herbivores but without leaf herbivores than in plants with both herbivores, suggesting that root herbivory can modify flower attractivity to pollinators. Our results suggest that patterns in plant-insect interactions above the ground are not only affected by aboveground factors but also by a wealth of different belowground processes mediated by the plant.

Introduction

All terrestrial ecosystems are divided in a belowground and an aboveground subsystem. These subsystems are obligately dependent upon one another, since above the ground primary producers are the main source of organic carbon for the system, whereas below the ground organisms are in charge of the recycling of organic matter and the mineralization of the nutrients therein (Scheu and Setälä 2002, Wardle 2002). Therefore, in order to understand community and ecosystem-level processes it is necessary to study the interactions that occur within and between these subsystems. Most ecologist have investigated belowground and aboveground communities separately, leaving the "between subsystems" interactions mostly unstudied (but see Bonkowski et al. 2001, van der Putten et al. 2001, Brown and Gange 2002). Studies on the interactions of ecological processes, such as decomposition, herbivory, parasitism and pollination, are needed to improve our knowledge on the role of plants as mediators between the belowground and the aboveground subsystems.

It is known that decomposers are responsible for the breakdown of organic matter and the release and cycling of nutrients (Haimi and Einbork 1992, Wardle 2002). The activity of decomposers often results in increased plant growth and plant nitrogen content (Scheu and Parkinson 1994, Bonkowski et al. 2000, 2001, Wardle 2002) which in turn enhances plant herbivory (White 1993, Bonkowski et al. 2001).

Root herbivory is known to reduce plant growth and to increase mortality of plants (Brown and Gange 1990, Wardle 2002). Further, it may affect the flowering period, the number of flowers and the size of inflorescences (Nötzold et al. 1998, Masters et al. 2001), which could be expected to change attractiveness to flower visitors. The effect of root herbivores on aboveground herbivores is debated. One hypothesis predicts that root herbivory enhances aboveground herbivores. This is assumed to result from an accumulation of carbohydrates and nitrogen in shoots as a stress response of the plant to root herbivory (Gange and Brown 1989, Masters and Brown 1992, Masters et al. 1993, 2001). Another hypothesis suggests negative effects caused by the induction of secondary plant compounds which are stored in the foliage and act as deterrents to aboveground herbivores (Bezemer et al. 2002).

Foliar herbivory may affect plant fitness directly by reducing the photosynthetic area (Strauss 1991, Marquis 1992) and indirectly by altering patterns of pollinator visitation of damaged plants (Lehtilä and Strauss 1997, 1999, Mothershead and Marquis 2000, Strauss 1997). Seed production may be reduced substantially by herbivory, due to reduced resources available for flower, pollen or seed production or because of low pollination rates of damaged plants.

Changes in the amount or chemistry of nectar (Hambäck 2001), the number or morphology of flowers (Karban and Strauss 1993, Strauss et al 1996, Lehtilä and Strauss 1997, 1999) or the height of the flowers (Strauss 1997, Mothershed and Marquis 2000, Hambäck 2001) may reduce the attractiveness to pollinators. Studies on the combined effects of root herbivores, leaf herbivores and decomposers on plants are, however, lacking, and there is little information on the effects of these organisms on plant mutualists like pollinators.

Exposing *Sinapis arvensis* to wireworms, caterpillars and earthworms in a full factorial design we investigated how below- and aboveground plant-animal interactions affect both plant performance and the aboveground associated insect community. In a previous investigation we found that root and leaf herbivores modify plant growth and that root herbivores increase flower visitation (Poveda et al. 2003). Based on these results the present study investiges how decomposers and root herbivores and also leaf herbivores, separately and in combination, affect plant performance and aboveground antagonistic and mutualistic plant-insect interactions. We expected negative effects of root and leaf herbivores and positive effects of decomposers on plant growth, plant reproduction, flower visitation and the abundance of aphids and their parasitoids. We also expected that below- and aboveground herbivores in combination most strongly affect plant fitness and plant-insect interactions, and that the detrimental effects of herbivores are counteracted by decomposers.

Methods

Experimental set-up

The experiment was carried out on a fallow field in the vicinity of Göttingen (Lower Saxony, Germany) in 2002. Two-liter pots were lined inside with a gauze-bag (1 mm mesh) in order to prevent the escape of soil organisms in the pots and colonization by soil macrofauna from outside through the drain holes. This permitted to manipulate the larger decomposers and root herbivores but allowed colonization of the pots by meso- and microfauna. After lining the bottom of each pot was filled with 200 mL gravel to facilitate drainage. Pots were filled with defaunated soil, taken from a 10-year old fallow located on a limestone plateau east of Göttingen. The soil was defaunated by freezing at -20°C for 3 days. This method has been shown to effectively eliminate macrofauna in the soil (Huhta et al. 1989). Five grams of litter material consisting mainly of grass leaves were placed on top of the soil. The litter material was collected on the field margin, dried at 60°C and cut into pieces of about 2 cm in length.

The experiment was set up in a three factorial design. The effects of root herbivores (R), decomposers (D) and leaf herbivores (L) on plant growth and reproductive parameters of mustard (*Sinapis arvensis*) were investigated. The full complement of combinations was set up (Control, D, R, DR, L, DL, LR, DLR) with ten replicates each. Two earthworms of the species *Octolasion tyrtaeum* were added to each pot of the decomposer treatment. The density chosen (50 earthworms/m^2), is in the lower range of densities in the field (9-239 ind/m^2, Pizl 1999). Earthworms were weighed prior to placement in the pots (average fresh weight of the two individuals: 6.18 ± 0.8 g, mean $\pm$ SE). Five wireworms (larvae of the click beetle *Agriotes* sp.) were added to each pot of the root herbivore treatments, which is equivalent to a density of ca 125 individuals/m^2 (average fresh weight 0.4 ± 0.03, mean $\pm$ SE). Wireworms are patchily distributed in soil and may reach densities of up to several hundred per square meter (Poveda et al. 2003).

Seeds of *S. arvensis* from a regional wild population (provided by the Botanical Garden, Göttingen) were sown in the greenhouse on 29 April 2002. On May 20 young seedlings were transplanted into the experimental pots, to which the soil organisms had been added previously. The seedlings had developed four to six true leaves. One day after transplantation two third instar larvae of *Spodoptera litoralis* (obtained from Syngenta Corp. Protection Münchwilen AG) were put on the seedlings. The larvae were left on the plants until they had consumed ca. 50% of the leaves, which took approximately two days. Leaf herbivory was restricted to a short period early in the experiment to allow investigation of its effect on herbivores (aphids) attacking the plant later.

Any other subsequently produced leaves were left undamaged. On May 28 all pots were transferred in random order to the field. The pots were buried into the soil leaving only 3 cm of the upper margin above the soil surface. At periods of low precipitation the pots were watered with tap water.

Sampling and statistical analyses

The beginning and the end of the flowering period was recorded for each plant. When the plants began to flower the number of aborted flowers was counted. We considered aborted flowers as the flower buds that never opened.

Observations of flower-visiting insects were made during the flowering period from 14–20 June 2002. Each plant was observed for 45 min, divided in three observation periods of 15 min. These observation periods were distributed randomly on three different days between 10 a.m. and 4 p.m. For each plant the number of open flowers was counted to compare plants

with different numbers of flowers. In order to compare flower visitation rates we calculated the number of flower visits per open flower within a 15 min interval.

Plants were naturally colonized by aphids during the experiment in the field. Each week (beginning on June 12) the aphids were identified and number of aphids on each plant was counted as well as the number of mummified aphids until plants started dying off (July 16). Mummies (i.e. parasitised aphids) were collected and stored in test tubes closed with cotton in order to let parasitoids hatch for further identification. Percent parasitism was calculated as the percent mummified aphids.

The experiment was finished when fruits turned yellow (July 2002). Individual plants were collected and oven dried for three days at 60°C to determine dry weight. While washing the roots, earthworms and wireworms were collected and counted. At the end of the experiment 83% of the earthworms and 90% of the wireworms were present and no differences were found in the number of earthworms or wireworms between treatments ($p>0.1$).

Number of fruits (=pods) of each plant was counted. Twenty fruits were randomly selected and seeds were counted and weighed. These data were used to calculate the number of seeds per plant. Data on plant biomass, shoot/root ratio, flowering period, total number of flowers and number of seeds per plant were analyzed using a multivariate analysis of variance (MANOVA). If significant, protected ANOVA's were performed to locate which of the dependent variables responded most to the factors studied (Scheiner and Gurevitch 2001). This procedure was preferred to Bonferroni corrections to avoid increasing the probability of type I errors (Legendre and Legendre 1998, Moran 2003). There were significantly more differences than expected by chance (Moran 2003; Table 1; the probability that 6 out of 35 possible tests are significant by chance is 0.0057 according to Bernoulli equation). Data on flower visitation, aphid abundance and parasitism rates of aphids were also analyzed by three-factor analysis of variance. Differences between means were inspected using Tukey's honestly significant difference test. Since we were not interested in measuring differences in aphid numbers between weeks but in differences between treatments the data were standardized prior to ANOVA. For this the relative abundance of aphids was calculated by dividing aphid abundance per pot by the mean abundance of all pots in each week. From the relative abundance of aphids for each week the mean of all weeks was calculated and used for the ANOVA. Data on plant biomass, shoot/root ratio, number of flowers and seeds per plant and flower visitation rate were log transformed prior to ANOVA to adjust to normal distribution and to increase homogeneity of variance. Correlations between the number of aborted flowers and the length of the flowering period and between the number of visited

flowers and the number of flowers were analyzed with Spearman's rank correlations. Statistical analyses were performed using Statistica 5 (StatSoft 1995).

Results

Plant performance

The plant growth parameters measured varied between plants with and without decomposers (MANOVA; $F_{5,68} = 2.95$, $p = 0.018$) and between plants with and without leaf herbivores (MANOVA; $F_{5,68} = 10.70$, $p < 0.0001$) but they didn't vary due to root herbivory or the interaction between the factors (MANOVA, $P > 0.1$). Separate ANOVA's indicated that decomposers increased plant biomass (Fig 4.1A), number of flowers (Fig. 4.1B) and number of seeds (Fig. 4.1C; Table 4.1). Decomposers also increased the shoot/root ratio, but only in absence of leaf herbivores (Fig. 4.1D). Plants subjected to leaf herbivory had a shorter flowering period than plants without leaf herbivory (Fig. 4.1E). Flowering period was negatively correlated with the number of aborted flowers at the beginning of the flowering period ($r = -0.54$, $n = 80$, $p < 0.001$).

Table 4.1. ANOVA table of F-values on the effect of decomposers (D), leaf herbivores (L) and root herbivores (R) on plant biomass, shoot/root relationship, number of flowers, flowering period and seeds per plant.

Treatment	df	Plant biomass	Shoot/ Root	Flowers	Flowering period	Seeds per plant
D	1, 72	8.44**	0.66	5.75*	3.93	12.17***
L	1, 72	2.518	2.25	1.12	34.17***	2.677
R	1, 72	4.83*	0.34	1.83	0.03	2.33
DL	1, 72	0.142	6.63*	0.33	1.13	0.6
DR	1, 72	2.52	0.87	3.5	0.18	3.04
LR	1, 72	0.57	0.005	1.58	0.82	0.69
DLR	1, 72	3.59	0.56	1.24	0.26	2.04

* $P < 0.05$; ** $P < 0.01$, *** $P < 0.001$
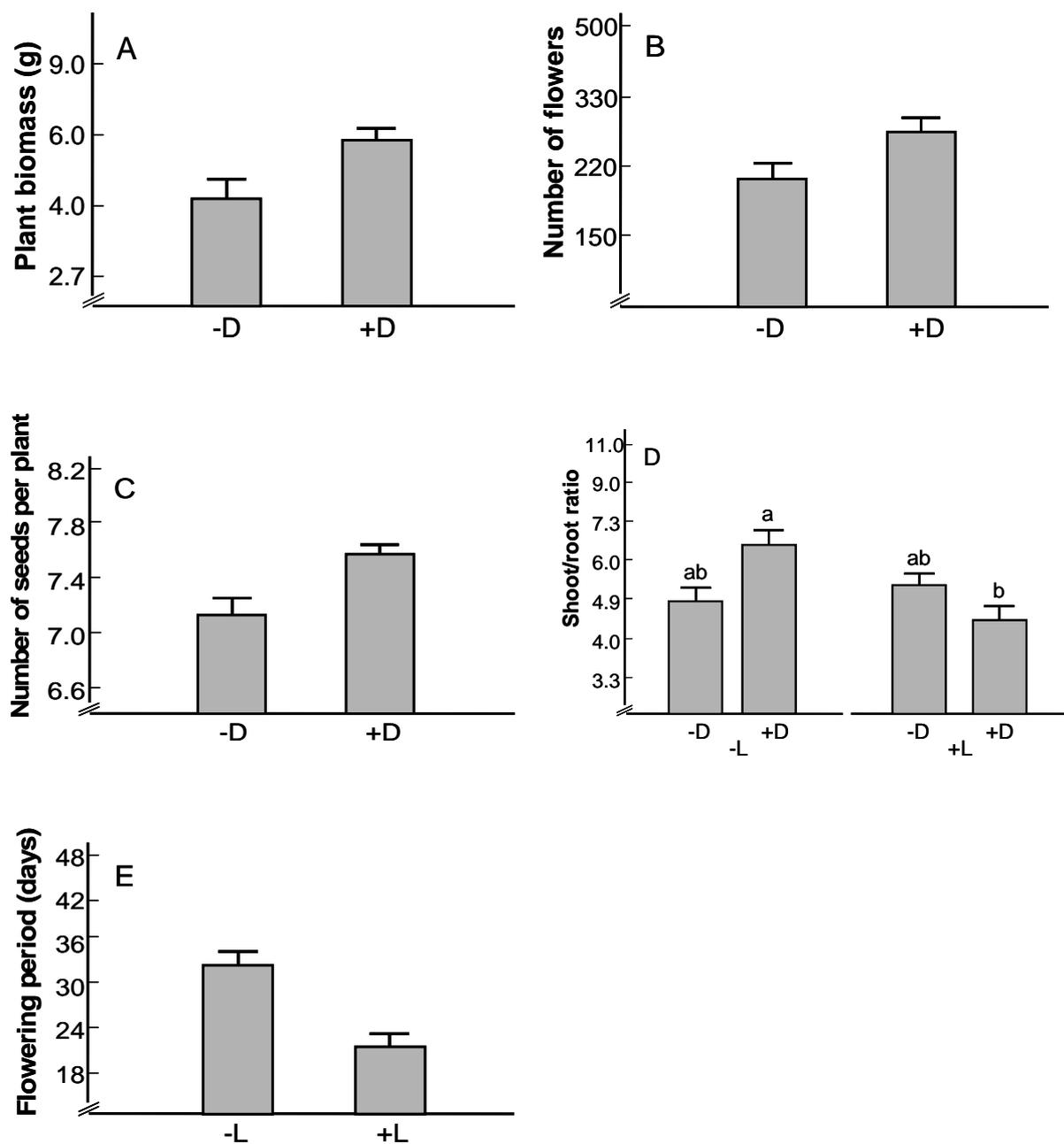
Fig. 4.1 Effects of decomposers (D), leaf herbivores (L) and root herbivores (R) on plant biomass (**A**), number of flowers (**B**), number of seeds per plant (**C**), shoot-root ratio (**D**) and flowering period (**E**) of *Sinapis arvensis* (mean ± one SE). In Fig. **4.1D** treatments with different letters are significantly different (Tukey's test, p<0.05).

Flower visitation

We observed 860 flower visitors. The most abundant species was the honeybee (*Apis mellifera*) with 531 visits. The second most important group were wasps with 169 visits. The remaining visitors were bumblebees (predominantly *Bombus pascuorum*), hover flies (predominantly *Eristalis tenax*) and other flies (Diptera). The number of flower visits increased with the number of flowers per plant ($r = 0.64$, $n = 80$, $p < 0.001$). There were more flower visits per open flower (i.e. the proportion of all flowers per plant that were visited during one observation period) in plants with root herbivores than without them but only when leaf herbivores were absent (Fig. 4.2A; Table 4.2).

Table 4.2. ANOVA table of F-values on the effect of decomposers (D), leaf herbivores (L) and root herbivores (R) on the flower visitation rate, the relative abundance of aphids and the number of parasitoids per aphid.

Treatment	d.f.	Flower visits/ flower	Aphids	Parasitism (%)
D	1, 72	2.02	0.56	0.82
L	1, 72	0.95	37.99***	0.27
R	1, 72	2.54	0.31	0.15
DL	1, 72	0.02	0.21	2.84
DR	1, 72	0.21	5.83*	0.34
LR	1, 72	4.45*	0.23	2.99
DLR	1, 72	2.12	1.59	1.53

* $P < 0.05$; ** $P < 0.01$, *** $P < 0.001$

Herbivores and parasitoids

Aphids on the plants were dominated by *Brevicoryne brassicae* (77% of all aphids present). Other species included *Lipaphis erysimi* (14.4%) and *Aphis* sp., *Myzus persicae* and *Macrosiphon euphorbiae* (8.6%). Plants which had been attacked by leaf herbivores at an early stage in their development had less aphids than plants without leaf herbivory pre-treatment (Table 4.2, Fig. 4.2B). Root herbivores and decomposers also affected the total number of aphids on the plants (Table 4.2). In treatments with only earthworms or with only root herbivores the number of aphids was increased compared to control plants (Fig. 4.2C). The number of parasitoids increased as the number of aphids on a plant increased ($r = 0.6739$, $n = 80$, $p < 0.001$) but there was no effect of herbivores or decomposers on the rate of parasitism of the aphids (Table 4.2).
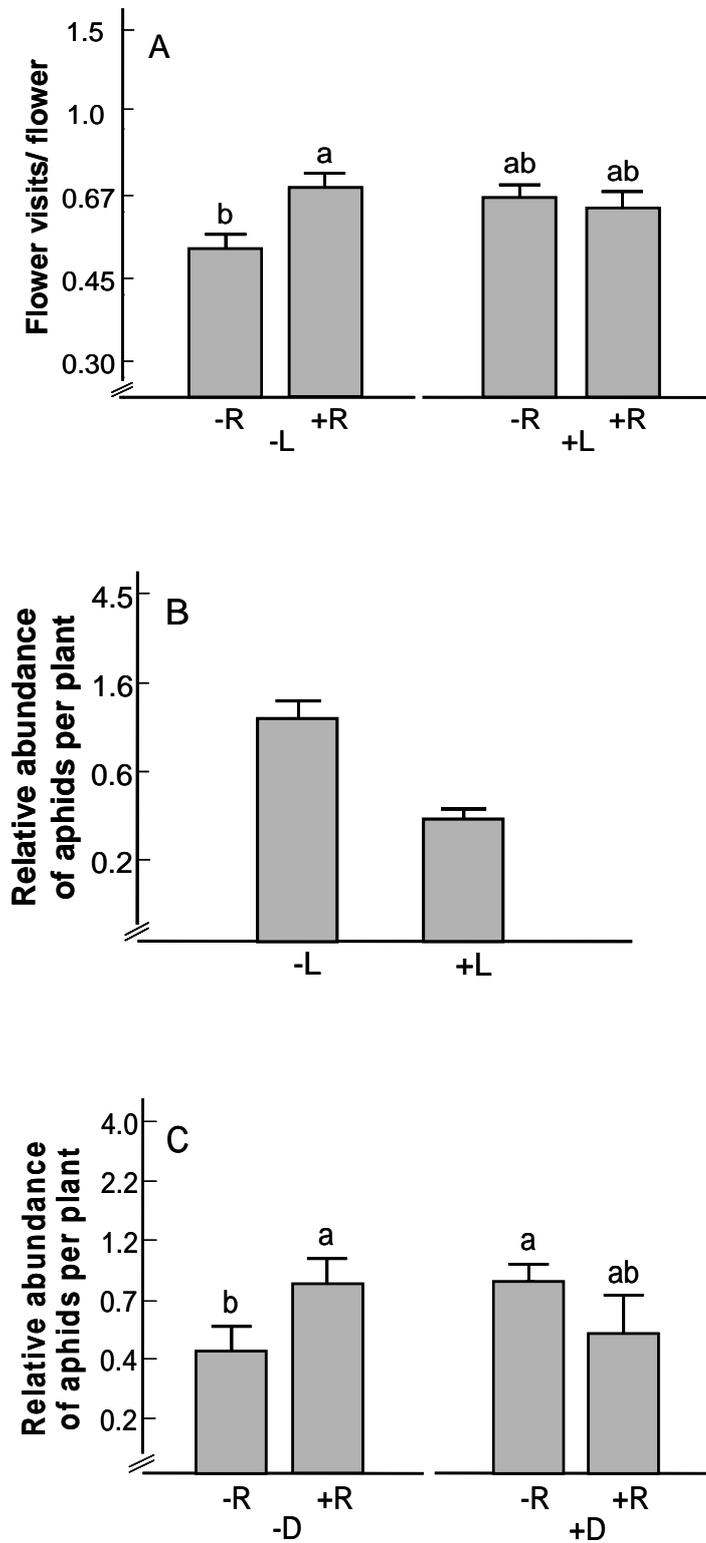
Fig. 4.2 Effects of decomposers (D), leaf herbivores (L) and root herbivores (R) on the flower visitation rate (**A**) and the number of aphids (**B, C**) (mean ± one SE). In Figs. **4.2A** and **4.2C** treatments with different letters are significantly different (Tukey's test, p<0.05).

Discussion

Although attention on complex food web interactions has increased in recent years, we are not aware of any study that has considered the relative importance and combined effects of decomposers and above- and belowground herbivores on the aboveground herbivore-parasitoid and plant-pollinator interactions. Having a standardized experimental approach, this study permitted new insights in the complex world of belowground and aboveground plant-animal interactions, including important indirect effects of root herbivores on pollinators and also of decomposers and root and leaf herbivores on aphid performance (Fig. 4.3).
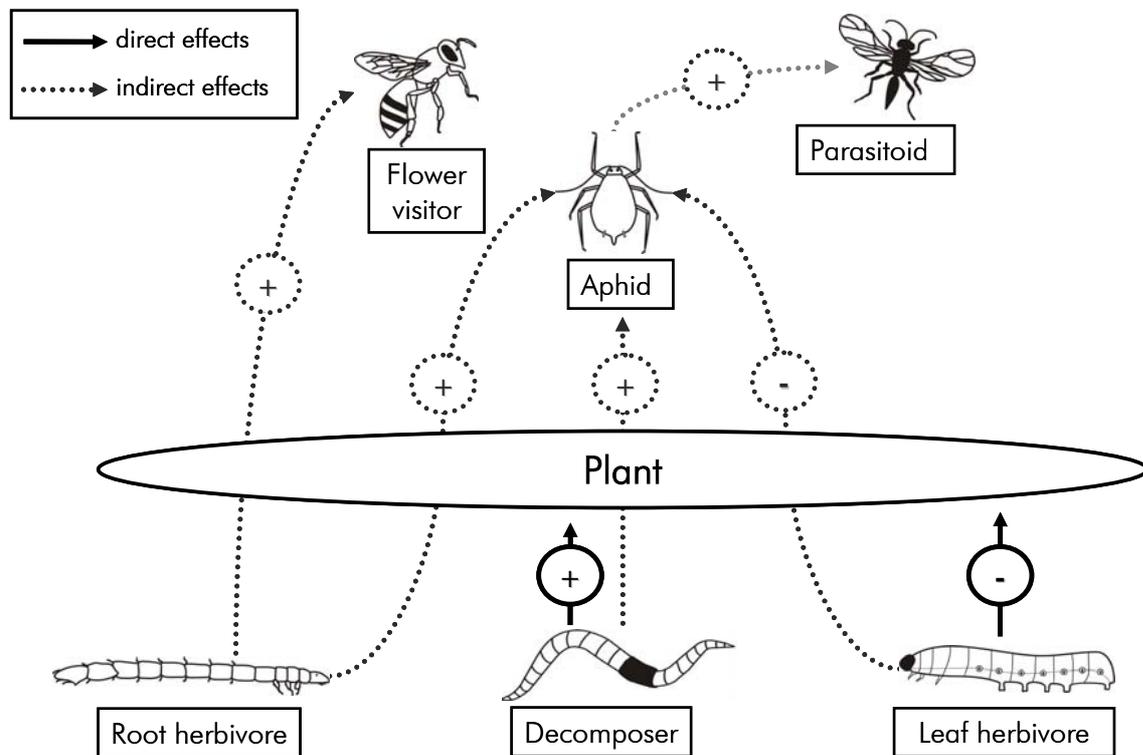

Fig. 4.3 Conceptual model of the direct and indirect effects of root herbivores, decomposers and leaf herbivores on *S. arvensis*, flower visitors, aphids and their parasitoids

Plant performance

Plant biomass was enhanced by the presence of earthworms, regardless of the presence of herbivores (below- and aboveground). Presumably, earthworms increased the amount of nutrients in soil which subsequently was taken up by the plant leading to an increase in plant biomass, number of flowers and seed production. Earthworms are known to increase nutrient cycling in soil through bioturbation, acceleration of decomposition of organic materials and an increase in microbial activity (Haimi and Einbork 1992, Wardle 2002). The enhanced nutrient mineralization has been shown to increase plant nutrient uptake and subsequently the growth of plants (Brussaard 1999, Bonkowski et al. 2001).

In the present study earthworms also increased the shoot/root ratio but only in plants that were not attacked by leaf herbivores. This indicates that *S. arvensis* was able to increase the shoot biomass in the presence of earthworms only when there were no leaf herbivores. Most likely this was caused by an increase in nutrient supply to the plants. Similar changes in resource allocation by plants due to increased nutrient mobilization by earthworms were observed by Scheu and Parkinson (1994) and Alphei et al. (1996). The lack of increase in the shoot/root ratio in presence of both decomposers and leaf herbivores suggests that nutrients mobilized by earthworms could not compensate the damage caused by leaf herbivores.

Although root herbivory is known to reduce plant growth and to increase the mortality of plants (Brown and Gange 1990, Wardle 2002) in the present study, as in our previous experiment (Poveda et al. 2003) wireworms did not affect plant biomass. Leaf herbivory caused a shortening of the flowering period as a consequence of early flower abortion. The latter was presumably caused by a reduction of photosynthetic tissue, as suggested previously (Poveda et al. 2003). Again, this early flower abortion and reduced flowering period did not affect female plant fitness.

Flower visitation

The rate of flowers visited was higher in plants with root herbivores but without leaf herbivores. This suggests that root herbivores increase plant attraction to flower visitors, as long as leaf herbivory did not counteract this effect. Low quantities of root herbivores have been shown to have a positive effect on flower visitors (Poveda et al. 2003). In the present experiment the density of wireworms was more than twice as high as in our previous study, but effects on flower visitation were similar. This suggests that root herbivores even at high density may stimulate the attractiveness of flowers to flower visitors. Masters et al. (1993)

report that water stress, caused by root herbivory, leads to the accumulation of soluble amino acids and carbohydrates in the foliage. Possibly the carbohydrate concentration of nectar increased in presence of root herbivores augmenting the attractivity to flower visitors.

Aphid performance

Brevicoryne brassicae and *Lipaphis erysimi*, the most abundant species on *Sinapis arvensis*, are specialist herbivores on Brassicaceae (Blackman and Eastop 1984). They mainly colonized the flower stems of mustard. The stem forms a bottleneck for assimilates transported from the leaves to the buds, flowers and fruits (Stephenson 1981). Earthworms and root herbivores alone, but not in combination, increased the number of aphids. Aphids are highly susceptible to changes in host-plant quality (Way and Cammell 1970). Therefore the enhanced number of aphids on plants with earthworms or root herbivores may indicate an increased quality or quantity of assimilates transferred in the phloem. Nitrogen availability is one of the main factors limiting herbivore development (Mattson 1980, White 1993). Both decomposers and root herbivores likely increased nitrogen availability to aphids. By stimulating nitrogen mineralization earthworms enhance the plant's nitrogen uptake and increase nitrogen concentration in plant tissues (Alphei et al. 1996). By feeding on roots wireworms decrease the water uptake by the plant and consequently increased the nitrogen concentration in the phloem (Gange and Brown 1989, Masters and Brown 1992, Masters et al. 1993, 2001).

In our study root herbivory did not reduce the colonization of aphids in contrast to results of Bezemer et al. (2002) where root herbivores had a negative effect on foliar feeding herbivores, as a result of enhanced concentration of secondary plant compounds in the foliage. Since *S. arvensis* is a Brassicaceae, known for its glucosinolate content, the defense of *S. arvensis* against root herbivores may have been associated with an increase in glucosinolate concentrations. The reduced aphid colonization on plants with leaf herbivores suggest that the production of secondary plant compounds in *S. arvensis* may have been increased in response to leaf herbivory but not to root herbivory, although both below- and aboveground herbivores can induce the production of secondary compounds (reviewed by van Dam et al. 2003).

Reduced colonization of aphids on plants damaged by leaf herbivores might also have been caused by the delayed flowering of the plants affected by leaf herbivory. Since the inflorescence stem, where aphids feed, developed later on plants damaged by leaf herbivory, they could have been less attractive for colonizing aphids.

Herbivores in our study did not directly affect the third trophic level, aphid parasitism, which is concordant with the study of Masters et al. (2001). The number of parasitized aphids only depended on the number of aphids per plant, the response of parasitoids therefore was indirect and density dependent.

Results of this experiment documented that belowground organisms have much more effects on aboveground processes than assumed previously. The findings (1) that root herbivory affects pollinators and (2) that aphids respond equally to decomposers and root herbivores add to existing knowledge on indirect effects of belowground processes on aboveground plant-animal interactions. Further studies and "in situ" experiments are necessary to evaluate the importance of these interactions in the field. Although relatively little experimental work has been done, the indirect interactions shown in this study are in line with mechanisms described in other investigations (Gange and Brown 1989, Masters et al. 1993, 2000, Alphei et al. 1996) suggesting that the interactions found in the present study are significant drivers of plant-animal interactions above the ground in a variety of ecosystems.

Floral trait expression and plant fitness in response to below- and aboveground plant-animal interactions

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Abstract. Although plant-animal interactions like pollination and herbivory are obviously interdependent, ecological investigations focus mainly on one kind of interaction ignoring the possible significance of the others. Plants with flowers offer an extraordinary possibility to study such mutualistic and antagonistic interactions since it is possible to measure changes in floral traits and fitness components in response to different organisms or combinations of them. In a three factorial common garden experiment we investigated single and combined effects of root herbivores, leaf herbivores and decomposers on flowering traits and plant fitness of *Sinapis arvensis*. Leaf herbivory negatively affected flowering traits indicating that it could significantly affect plant attractiveness to pollinators. Decomposers increased total plant biomass and seed mass indicating that plants use the nutrients liberated by decomposers to increase seed production. We suggest that *S. arvensis* faced no strong selection pressure from pollen limitation, for two reasons. First, reduced nutrient availability through leaf herbivory affected primarily floral traits that could be important for pollinator attraction: Second, improved nutrient supply through decomposer activity was invested in seed production and not in floral traits. This study indicates the importance of considering multiple plant-animal interactions simultaneously to understand selection pressures underlying plant traits and fitness.

Key words: decomposers, floral characters, leaf herbivores, root herbivores, seed set, *Sinapis arvensis*

Introduction

In animal-pollinated plants, fitness is influenced by floral traits that function as an advertisement and reward for pollinators. In order to attain outcrossing, plants with flowers offer an extraordinary range of attractants to increase pollinator visitation. Floral advertisement includes olfactory cues, short-range tactile cues and visual cues, such as size, shape and color of inflorescences and flowers. Floral rewards include nectar and pollen that are highly looked for by flower visitors (Dafni, 1992, Pellmyr, 2002). Floral advertisement and seed set can be affected by a whole range of organisms interacting with the plant. Investigations on the effect of leaf and floral herbivory documented that pollinator visitation is reduced in damaged plants through changes in floral traits (Lehtilä and Strauss, 1997, 1999, Mothershead and Marquis, 2000, Strauss, 1997). However, plant attraction to pollinators may also be affected by plant – animal interactions below the ground. For example, *Sinapis arvensis* plants attacked by root herbivores had an increased flower visitation (Poveda et al., 2003). It is increasingly recognized that belowground and aboveground communities are highly interdependent (Gange and Brown, 1989, Moran and Whitham, 1990, Masters and Brown, 1992, Masters et al., 1993, Scheu and Parkinson, 1994, Nötzold et al., 1998, Bonkowski et al., 2001, Masters et al., 2001, Poveda et al., 2003). Understanding community and ecosystem level processes needs more information on the interactions between both subsystems. Studies on how belowground organisms affect flowering traits and seed set may lead to a more integrative view of the functioning of terrestrial ecosystems.

Belowground plant parts are attacked by root herbivores, and thereby, plant organs for nutrient and water uptake are damaged. Root herbivory is known to reduce flowerbud size and to delay the flowering period (Masters et al., 2001) but also to increase flower visitation rates (Poveda et al., 2003)

A second important belowground process is decomposition of organic material. The effects of decomposers on flowering traits have not been studied yet. Since decomposers are responsible for the breakdown of organic matter and the release and cycling of nutrients (Haimi and Einbork, 1992, Wardle, 2002), plants likely benefit from decomposer activity by the production of more attractive flowers for pollination, i.e. more and bigger flowers where nectar and pollen production is increased. Since in self-incompatible plants enhanced visitation of flowers by pollinators leads to an enhanced seed production, analyzing the mechanisms how decomposition and root herbivory influence floral traits and seed set is an important step in understanding below- and aboveground plant-insect interactions.

Important floral characters that have been shown to affect pollinator visits are the number of anthers (Krupnick et al., 1999), the amount and chemistry of nectar (Krupnick et al., 1999), the number and morphology of flowers (Karban and Strauss, 1993, Ohara and Higashi, 1994, Strauss et al., 1996, Lehtilä and Strauss, 1997, 1999), the flowering phenology (Strauss et al., 1996) and the height of the flowers (Strauss, 1997, Mothershed and Marquis, 2000, Hambäck, 2001).

This study was performed to investigate single and combined effects of main ecological processes such as decomposition, root- and leaf herbivory on plant growth, flowering phenology and on floral traits, as indicators of plant attractiveness to pollinators. However it was not our objective to measure these effects on flower visitors, since we already did it in a previous experiment (Poveda et al., 2003). Additionally, we analyzed the effects of these three functional groups of plant-associated animals on seed production using *Sinapis arvenis*, a self-incompatible annual crucifer (Kunin, 1993). We expected negative effects of leaf herbivores and positive effects of decomposers on floral traits and seed production. Although root herbivory may negatively affect floral traits (see above), our previous experiments showed that *Sinapis arvensis* plants attacked by wireworms had an enhanced flower visitation (Poveda et al., 2003). Given these results, we expect positive effects of root herbivory on flowering traits which would mediate between the plant and the flower visitors. We also expect that below- and aboveground herbivores in combination more strongly affect floral traits and seed set. Furthermore, we expect that potential detrimental effects of herbivores on plant performance are counteracted by decomposers.

Materials and methods

Experimental set-up
The experiment was carried out under a gauze tent in an old fallow field close to Göttingen (Lower Saxony, Germany) in 2002. Two-liter pots were filled with defaunated soil, taken from a 10-year old fallow located on a limestone plateau east of Göttingen. The soil was defaunated by freezing at -20°C for 3 days. The pots were lined with a gauze-bag (1 mm mesh size) in order to prevent the escape of soil organisms from the pots and colonization by soil macrofauna from outside the pots. The bottom of each pot was filled with 200 mL gravel to facilitate drainage. Five grams of litter material consisting mainly of grass leaves were placed on top of the soil. The litter material was collected on the field margin, dried at 60°C and cut into pieces of about 2 cm in length. The experiment was set up in a three-way factorial design.

The effects of root herbivores (R), decomposers (D) and leaf herbivores (L) on floral traits and components of plant fitness of mustard (*Sinapis arvensis*) were investigated. The full complement of possible combinations was set up (Control, D, R, DR, L, DL, LR, DLR) with six replicates each (n=48 plants). Two earthworms of the species *Octolasion tyrtaeum* were added to each pot of the decomposer treatment. The density chosen (50 earthworms/m^2) is in the lower range of densities in the field (9-239 ind/m^2; Pizl, 1999). Earthworms were weighed prior to placement in the pots (average fresh mass of the two individuals 6.19 ± 0.7 g). Five wireworms (larvae of the click beetle *Agriotes* sp.) were added to each pot of the root herbivore treatments, which is equivalent to a density of approx. 125 individuals/m^2 (average fresh mass 0.4 ± 0.03). Wireworms are patchily distributed in soil and may reach densities of up to several hundred per square meter (Poveda et al., 2003).

Seeds of *S. arvensis* from a regional wild population (provided by the Botanical Garden, Göttingen) were sown in the greenhouse on 29 April 2002. On 20 May young seedlings that had four to six leaves were transplanted into the experimental pots, to which the soil organisms had been added five days before. One day after transplantating two third instar larvae of *Spodoptera litoralis* (obtained from Syngenta Crop. Protection Münchwilen AG, Stein, Switzerland) were put on the seedlings. The larvae were left on the plants until they had consumed ca. 50% of the leaves, which took approximately two days. All other subsequently produced leaves were left undamaged, to obtain an equal and reproducible amount of herbivory per individual. On May 28 all pots were set randomly in the tent. The gauze-tent (mesh size 0.1 mm) was constructed in order to prevent other insects from attacking the plants, but using almost natural conditions of light, temperature and precipitation. The pots were buried into the soil to simulate natural temperature and moisture conditions for root herbivores and decomposers. During the flowering period plants where hand-pollinated using a mixture of pollen from other plants in order to secure cross-pollination between plants. Each day all open flowers were pollinated once, using a tooth-pick to deposit the pollen on the stigma. Rain was let to water all the pots naturally and during dryer weeks pots were watered with tap water.

Sampling and statistical analyses

For each plant the beginning and the end of the flowering period was noted. When plants began to flower the number of aborted flowers (the flower buds that never opened) was counted. Nectar production, anther length and size of the petals were measured for ten flowers of each plant. Nectar production and petal size was measured in the first opened flower

(flower number 1) on the central stalk and then on all flowers with an uneven number, to flower 19. Anther length was measured on the second flower and on all even numbered flowers to number 20. We could not measure the same parameters on the same flowers since anther size was measured in flowers that were about to open and petals had to be removed to reach anthers. Nectar production and petal size were measured the day after flower opening. In order to have a standardized estimate of nectar production, each morning from 7:00 to 8:00 all the liquid contained in the flowers was taken out with help of disposable capillary pipettes. After one hour the quantity of nectar produced was extracted with a new capillary pipette for a volume of 1 μL and the height of the nectar column was measured with help of a caliper. For each flower we measured the lengths and widths of the outer, showy parts (the limb) of the petals. Afterwards the area of each limb was calculated, as if it was an oval (length * width * π) and the sum of all four limbs would give the complete petal area. Anther length was measured as an estimate of pollen production (method proposed by Kearns and Inouye, 1993). When flowers were about to open we measured the length of 3 out of the 6 anthers with help of a caliper. Since *S. arvensis* has 4 anthers on a long filament and 2 on a short one, we measured two anthers on a long filament and one anther on a short one. There was no difference in the length of the anthers on long filaments and on short filaments (t-test for matched pairs: t= -1.17, p = 0.24, d.f.= 479). At the end of the experiment, when fruits turned yellow and the plant began to die (mid of July) plant height was measured. Also, fruits, roots and shoots were collected and dried for 3 days at 60°C to determine dry mass. The number of fruits (=pods) of each plant was counted. Twenty fruits were randomly selected, and seeds per fruit were counted and weighed. These data were used to calculate the number of seeds and seed mass per plant. While washing the roots, earthworms and wireworms were collected and counted. At the end of the experiment 86% of the earthworms and 87% of the wireworms were present.

Data on plant biomass, number of flowers, flowering period and petal area were log transformed prior to statistical analysis to adjust to normal distribution and to increase homogeneity of variance. The data were analyzed using a multivariate analysis of variance (MANOVA). Protected ANOVA's were performed to locate which of the dependent variables responded most to the factors studied (Scheiner and Gurevitch, 2001). This procedure was preferred to Bonferroni corrections to avoid increased probability of type I error (Legendre and Legendre, 1998, Moran, 2003). There were significantly more differences than expected by chance (Moran, 2003; Table 1: the probability that 8 out of 63 possible tests are significant by chance is 0.009 according to Bernoulli equation).

Differences between means were inspected using Tukey's honestly significant difference test. Correlations between the number of aborted flowers, flowering onset and the length of the flowering period were analyzed with Spearman's rank correlations. Statistical analyses were performed using Statistica 5 (StatSoft, 1995).

Results

Leaf herbivory had a significant effect on the parameters studied (MANOVA; $F_{8,33} = 8.87$, p < 0.001); the effect of decomposers was marginally significant (MANOVA; $F_{8,33} = 2.15$, p $= 0.058$) whereas neither root herbivores nor any of the interactions between the factors studied significantly affected plant traits (MANOVA, p > 0.1). Separate ANOVA's indicated that leaf herbivores shortened the flowering period (Fig. 5.1A) and the length of the anthers (Fig. 5.1B) and reduced the petal area (Fig. 5.1C, Table 5.1). A shorter flowering period was correlated with a higher number of aborted flowers at the beginning of the flowering period (r= - 0.43, p = 0.002) and a delay in the flowering onset (r= - 0.37, p = 0.009). There was no single or combined effect of root herbivores or decomposers on floral traits (Table 5.1).

Table 5.1. Single ANOVA table of F-values on the effect of root herbivores (R), decomposers (D) and leaf herbivores (L) on plant biomass, amount of flowers produced, length of the flowering period, anther length, petal area, amount of nectar produced, fruits produced per flower and seed biomass in each plant.

	d.f.	Biomass	Flowers	Flowering period	Anther length	Petal area	Nectar	Fruits/ flower	Seed mass
R	1, 40	2.08	0.56	0.44	0.39	0.30	0.01	0.01	0.37
D	1, 40	6.08*	1.72	0.43	1.76	0.35	0.68	4.82*	5.57*
L	1, 40	2.02	0.001	7.31**	25.43***	31.07***	3.47	1.03	1.90
RD	1, 40	1.08	1.44	1.00	1.86	0.30	0.95	1.12	0.22
RL	1, 40	0.44	0.02	0.49	1.99	2.38	0.19	0.82	0.13
DL	1, 40	1.95	0.66	0.00	0.04	0.067	1.79	5.31*	1.20
RDL	1, 40	1.51	1.54	0.02	0.05	0.02	1.32	0.28	0.25

* P < 0.05; ** P < 0.01, *** p < 0.001
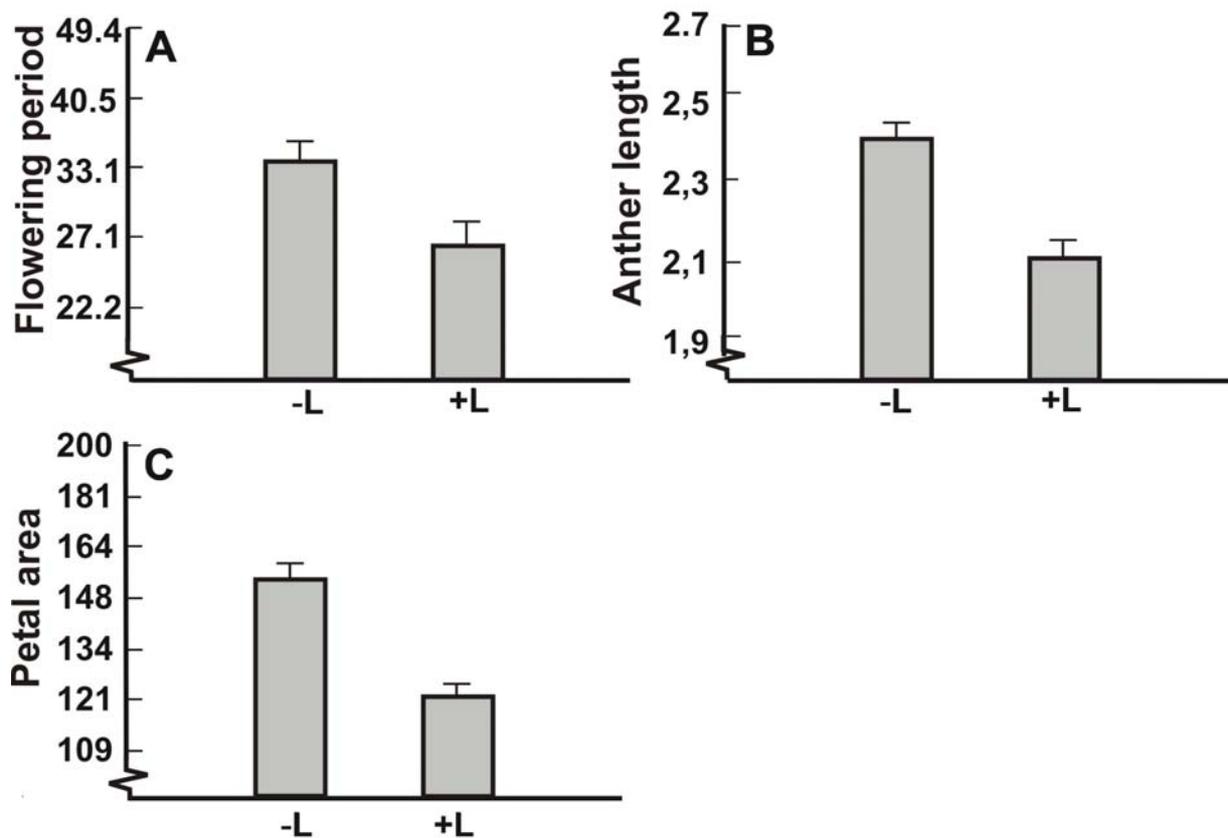
Fig. 5.1 Effects of leaf herbivores (L) on the flowering period (**A**), the petal area (**B**) and the length of the anther (**C**) of *Sinapis arvensis* (mean ± one SE and one SD).

Decomposers increased total biomass (Fig. 5.2A), seed mass per plant (Fig. 5.2B) and the number of fruits per flower (Fig. 5.2C, Table 5.1). Seed mass per plant was correlated with the number of seeds (r=0.93, p<0.001). The number of fruits per flower was higher when decomposers were present but leaf herbivores were absent compared to when decomposers and leaf herbivores were absent (Fig. 5.2D, Table 5.1). Root herbivores and interactions between root herbivores and other factors did not affect plant growth, floral or seed production traits (Table 5.1). There was no effect of any treatment on the amount of nectar and the number of flowers produced by the plants (Table 5.1).
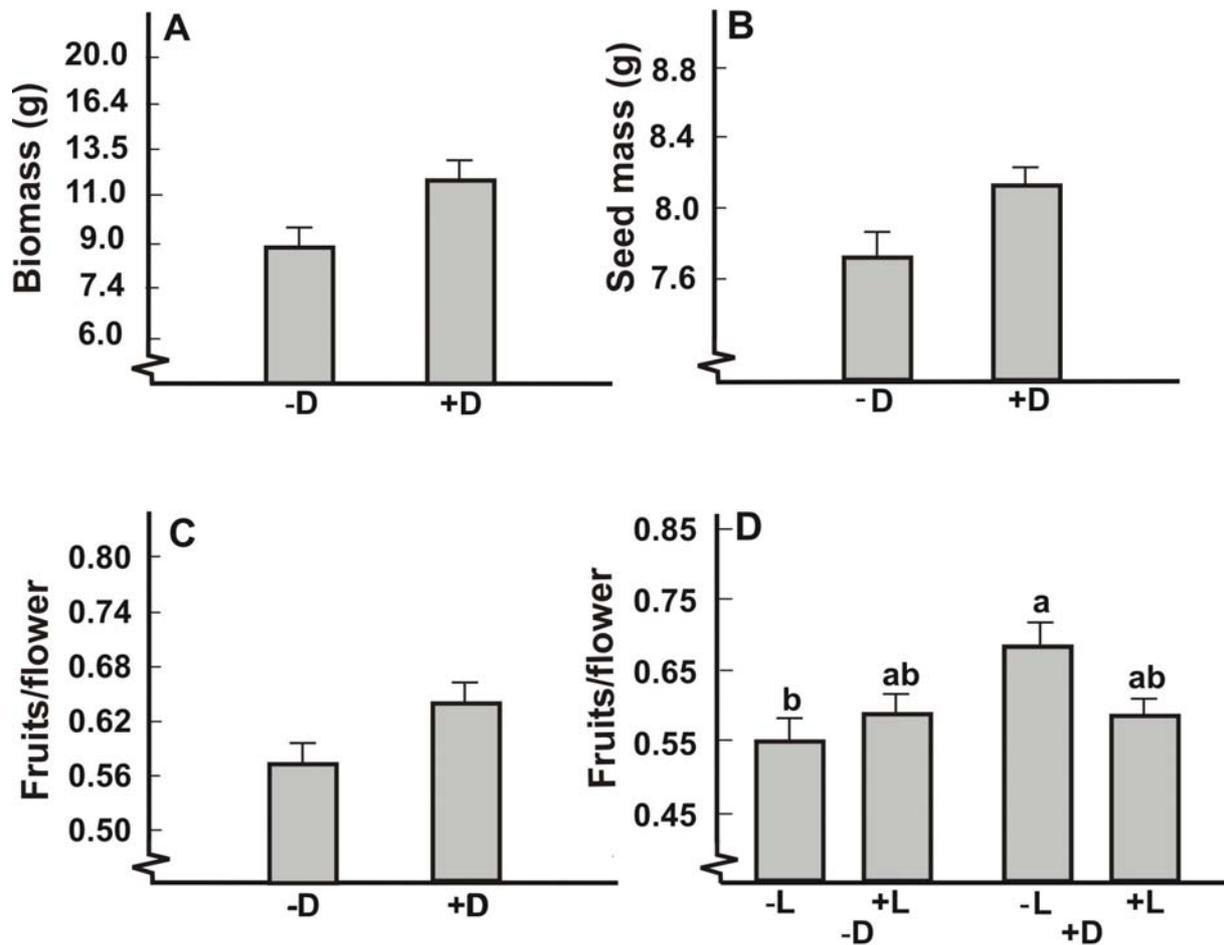
Fig. 5.2 Effects of decomposers (D) and leaf herbivores (L) on plants biomass (**A**) seed mass per plant (**B**) and the number of fruits per flower (**C**, **D**) of *Sinapis arvensis* (mean ± one SE and one SD). In Fig. **5.2D** treatments with different letters are significantly different (Tukey's test, $p < 0.05$).

Discussion

Flowering characters, such as the length of the flowering period, petal area and anther length, were detrimentally affected by leaf herbivores, while root herbivory and decomposers did not affect flowering phenology and flowering characters. Other characters relating to plant growth and seed production, such as plant biomass, seed mass per plant and number of fruits per flower, benefited from decomposers but not by above- and belowground herbivores.

The importance of leaf herbivory in modifying floral traits and thereby attractiveness and rewards for flower visitors has been reported previously (Quesada et al., 1995, Strauss et al., 1996, Lehtilä and Strauss, 1997). Herbivores reduce leaf area and photosynthetic surface which relates to a lower rate of net photosynthesis (Crawley, 1997). Plants may compensate this loss through a variety of mechanisms. Crawley (1997) proposed that plants redistribute

the photosynthate to the production of new leaves and away from roots, flowers, fruits or storage organs. By this, mustard may have compensated the damage caused by leaf herbivores by producing more leaves and aborting the first flowers, thereby delaying the onset of flowering and reducing petal size and pollen production. This would cause a reduction in flower attractiveness to pollinators, since it has been shown for other Brassicaceae, that plants with smaller flowers and a reduced amount of pollen are less visited than flowers with bigger petals and more pollen (Lehtilä and Strauss, 1997). It is surprising, however, that the effects of leaf herbivory on flowering characters remained unaffected by root herbivores and decomposers.

Root herbivory did not affect plant growth and seed production which is in contrast to previous studies (Brown and Gange, 1990, Masters et al., 2001). The results suggest that *S. arvensis* compensates for root damage. However, it has been reported that root herbivory may enhance flower visitation (Poveda et al., 2003), but this likely was caused by other cues than the ones measured in this study, e.g. changes in odour, nectar concentration and flower color (Dafni, 1992, Pellmyr, 2002).

Presence of decomposers (earthworms) enhanced plant and total seed biomass and fruit development per flower, but did not affect flowering traits. This suggests that *S. arvensis* does not invest extra nutrients for increasing floral attractiveness, but in increasing plant growth and seed development.

Seed production is determined by the number of flower buds, the rate of pollination, the rate of flower and fruit abortion, the number of seeds ripened per fruit, the amount of nutrients available and the rate of predispersal fruit predation (Stephenson, 1981, Crawley, 1983). Results of the present experiment document that all treatments had a similar number of flowers and under optimal pollination (hand pollination) plants with decomposers produced more fruits per flower than other treatments. This suggests that decomposer-mediated increase in nutrient supply benefits plants when pollination is not a limiting factor. Increased nutrient mobilization by earthworms has been reported frequently (Scheu and Parkinson, 1994, Alphei et al., 1996) and increased nutrient supply by decomposers likely was responsible for the increase in plant and seed biomass in this study.

Female fecundity is limited by pollen delivered to the stigmas and resources for maturation of fruits and seeds (Haigh and Westoby, 1988, Larson and Barrett, 2000). If pollen limits seed set, selection favors increased allocation to pollinator attraction at the expense of ovule investment, but in species receiving excess pollen, a shift from attractive investment to ovules is favored. The pattern found in *S. arvensis* suggests that this plant species faced no strong

selection pressure from pollen limitation, since reduced nutrient availability through leaf herbivory affected primarily floral traits important for pollinator attraction. Further, improved nutrient supply through decomposer activity was invested in other plant fitness traits, such as number and size of seeds and plant biomass and reduced fruit and seed abortion.

In conclusion, leaf herbivory strongly affected flowering phenology and flower development, suggesting that leaf herbivory significantly alters the attractivity of plants for pollinators. In contrast, floral traits and seed production appear to be little affected by root herbivores. Decomposers enhanced plant biomass and fruit and seed set, but did not affect floral traits. This suggests that decomposers increases female fitness. It is challenging to investigate the evolutionary forces which resulted in the observed plasticity of responses of *S. arvensis* to the complex of plant-associated invertebrate guilds and relate these to other plant species of different functional groups and with different life histories. Ultimately, these studies may lead to a more detailed understanding of how belowground and aboveground plant-animal interactions shaped the evolution of plant traits.

Belowground effects of organic and conventional farming on aboveground plant-herbivore and plant-pathogen interactions

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Abstract. Soil organisms may significantly affect the aboveground system. However, the influence of farming practices in modifying the effects of soil organisms on aboveground systems is poorly understood. The aim of our study was to investigate: (1) How important are soil organisms for plant growth and the development of herbivores and pathogens above the ground? (2) How do agricultural management practices affect interactions between soil organisms, plants and their aboveground herbivores and pathogens? To answer these questions we investigated the effect of experimental defaunation of soils from organic vs. conventional farms on growth of wheat, abundance of aphids and infection of wheat by *Septoria* fungi. Plant biomass in soil from conventional farms exceeded that of soils from organic farms, presumably due to the higher nutrient input in the conventional farming system. Soil defaunation likely mobilized nutrients that increased plant growth. Aphid abundance and *Septoria* infection was reduced by defaunation but only in organic soils. This suggests that soil organisms in organic farming systems are more important for aphid performance and the infection rate by *Septoria* than in conventional systems. Hence, changes in the soil animal food web caused by farming practice feed back on aboveground organisms, and this appears to be more pronounced in organic farming systems. Further, the results indicate that soil organisms may modify higher trophic levels (aphid and pathogen infection) without significantly affecting lower trophic levels (plant growth).

Key words: aphids, cereals, defaunation, farming systems, soil organisms, *Septoria* spp.

Introduction

Terrestrial ecosystems have a belowground and an aboveground subsystem. These subsystems depend on each other, since above the ground primary producers are the main source of organic carbon for the system, whereas below the ground soil organisms are responsible for the breakdown and recycling of organic matter and the mineralization of the nutrients therein (Scheu and Setälä 2002, Wardle 2002, Porazinska *et al.* 2003). To understand community and ecosystem level processes it is necessary to study the interactions within and between these subsystems. However, most ecologists have investigated belowground and aboveground communities separately, leaving the interactions between the subsystems unstudied (but see Wardle *et al.* 1999, 2002, Bonkowski *et al.* 2001, Masters *et al.* 2001, van der Putten *et al.* 2001, Brown and Gange 2002).

Current agricultural practices include the loss or degradation of non-crop habitats, the removal of weeds from within and around crops, enlargement of field sizes, tillage operations of varying degrees of intensity, and the use of chemicals to control pests and weeds in order to increase crop yield (reviewed by Gurr *et al*. 2003). These practices have caused serious ecological problems such as water contamination, habitat degradation and biodiversity loss (Matson *et al*. 1997, Krebs *et al*. 1999, Tilman *et al.* 2002), also affecting ecological functions such as pollination and biological control (Kruess and Tscharntke 1994, Matthies *et al.* 1995, Didham *et al.* 1996, Kruess and Tscharntke 2000). In the belief that low intensity farming systems are beneficial for the agricultural environment, the European Union is supporting organic farming systems as an alternative to conventional agriculture (EU 2002). In some European countries up to 8% of the agricultural area is managed organically (Mäder *et al.* 2002). Organic farming systems have a lower nutrient and pesticide input and an improved biological activity and biodiversity (Mäder *et al.* 2002, Hole *et al.* 2005).

Although there are several studies showing how belowground biota affect aboveground organisms (Gange and Brown 1989, Masters *et al.* 1993, Masters 1995b, Masters and Brown 1997, Masters *et al.* 2001, Bezemer *et al.* 2002, Poveda *et al.* 2003, 2005), studies comparing the importance of soil organisms in soils from different farming systems are missing. In order to study the importance of the soil biota on plant growth and the development of higher trophic levels and to analyse how farming systems affect the interactions between belowground and aboveground organisms we designed a replicated field experiment using wheat plants as model system. We investigated the effect of experimental defaunation in soils from organic farms and conventional farms on the growth of wheat plants and the

performance of aphids and fungi on them. Two main questions were addressed: (1) How important are soil organisms for plant growth and the development of herbivores and fungi on these plants? and (2) How does the agricultural management practice of the soil affect interactions between soil organisms, plants and their aboveground herbivores and pathogens? Our expectations were that soil organisms would increase plant growth in both soil types. However, since soils from the conventionally managed farms receive an increased input of fertilizers we expected that soil organisms would be more important for plant growth in soils from organic farms, and that this would also be reflected on the second trophic level, i.e. in the infestation rate of pathogens and herbivores.

Methods

The experiment was carried out in the vicinity of Göttingen in the summer of 2003. Effects of farming practice (conventional vs. organic) and the presence or absence of soil organisms (defaunated vs. control soil) on wheat growth, aphid development and *Septoria* spp. infection were investigated in five landscape sectors. The landscape sectors were separated by 17.27 km ± 8.26 km (mean ± SD). They were chosen to represent the regional landscape and used as blocks. In the centre of each landscape sector, we selected one conventionally managed winter wheat field. In the middle of these conventional fields a plot of 700 m^2 (20 m x 35 m) not treated with insecticides was established, where the experiment took place. Nearby (how much distance) we selected an organically farmed winter wheat field from which soil samples were taken. From each field (5 conventionally farmed and 5 organically farmed) 200 L of soil were collected and taken in plastic bags to a greenhouse. Half of the soil (100 L) were defaunated by freezing at -20°C for 3 days which significantly reduces the number of soil microarthropods and annelids (Huhta *et al.* 1989). The soil was put into 10 L pots that were covered at the bottom with a 200 μm mesh to prevent colonization by soil meso- and macrofauna. In each pot 80 wheat (*Triticum aestivum*) seeds were sown on April 30th 2003. After germinating, the wheat plants grew in the greenhouse until May 23rd, when pots were set out in the fields. The experiment was set up in a randomized complete two factorial design with the factors farming system (soil from organic farming system vs. soil from conventional farming system) and defaunation treatment (defaunated vs. control soil). The full complement of combinations was set up (defaunated organic, control organic, defaunated conventional and control conventional) with five replicates per treatment (soils from 5 different sites) in each landscape sector. Plants were left to be naturally colonized by aphids and infected by *Septoria*

(mainly *S. tritici*) fungi during the experiment. Starting on June 10, 2003, the aphids were identified and the number of aphids on 25 plants per pot was counted each week. The percentage infection of *Septoria* fungi was estimated by relating the amount of stems and leaves infected by *Septoria* to the total amount of stems and leaves in 25 plants in each pot. These measurements were repeated each week during 6 weeks, until the wheat was harvested on July 17 2002. Plants were collected and oven dried for three days at 60°C to determine dry weight of roots, shoots and ears. The number of ears per pot was counted.

To analyze the effects of defaunation and soil farming practice on plant growth we used a multiple analysis of variance (MANOVA) with farming practice and defaunation as categorical variables and the landscape sector as block. When significant, protected ANOVA's were performed to locate which of the dependent variables responded most to the factors studied (Scheiner and Gurevitch 2001). This procedure was preferred to Bonferroni corrections to avoid increasing the probability of type I errors (Legendre and Legendre 1998, Moran 2003). There were significantly more differences than expected by chance (Moran 2003; Table 1; the probability that 8 out of 15 possible tests are significant by chance is $0.175 \cdot 10^{-6}$ according to the Bernoulli equation). Differences between means were inspected using Tukey's honestly significant difference test. Data on aphid abundance was cumulated for all weeks and the number of aphids per unit plant biomass was calculated by dividing the number of aphids per pot by the total biomass of the plants. This was done to correct for the differences in aphid abundance due to differences in plant biomass. Data on plant biomass were square-root transformed, while data on the total number of aphids per biomass and on the percent damage by *Septoria* were log-transformed prior to the analysis to adjust to normal distribution and to increase homogeneity of variance. Correlations between the plant biomass and the number of aphids, as well as between the plant biomass and the percentage of *Septoria* infection were analyzed with Spearman's rank correlations. Statistical analyses were performed using Statistica 6.1 (StatSoft, Inc. 2003) and R (2003).

Results

Plant growth parameters measured varied between plants grown in defaunated vs. control soil (MANOVA; $F_{5, 88} = 5.78$, $p = 0.0001$) and between plants grown in soil from conventional vs. organic fields (MANOVA; $F_{5, 88} = 3.99$, $p = 0.002$); there was no significant interaction between these factors (MANOVA, $P > 0.1$). Separate ANOVAs indicated that total plant

biomass was affected by both the farming practice and the defaunation treatment (Table 6.1). Total biomass of plants grown in soil from conventional fields exceeded that of plants grown in soil from organic fields (Fig. 6.1A). This was true for the biomass of shoots and ears but not for that of roots (Table 6.1, Fig. 6.1A). Biomass of plants grown in defaunated soil exceeded that of plants grown in control soil and this was true for shoots, ears, roots and the number of ears per pot (Table 6.1, Fig. 6.1B).

Table 6.1. Effects of defaunation and farming practice (conventional vs. organic) on the total biomass, shoot mass, root mass, ear mass and number of ears. The landscape sector was used as block. F and P – values (after model simplification) are shown.

|	Total mass	Shoot mass	Root mass	Ear mass	Number of ears
defaunation	$F_{1, 93} = 11.85$	$F_{1, 93} = 5.21$	$F_{1, 94} = 10.58$	$F_{1, 93} = 20.13$	$F_{1, 94} = 4.63$
	$P < 0.001$	$P = 0.02$	$P = 0.0016$	$P < 0.0001$	$P = 0.034$
farming practice	$F_{1,93} = 6.35$	$F_{1,93} = 8.92$	not significant	$F_{1,93} = 7.63$	not significant
	$P = 0.013$	$P = 0.003$		$P = 0.007$	
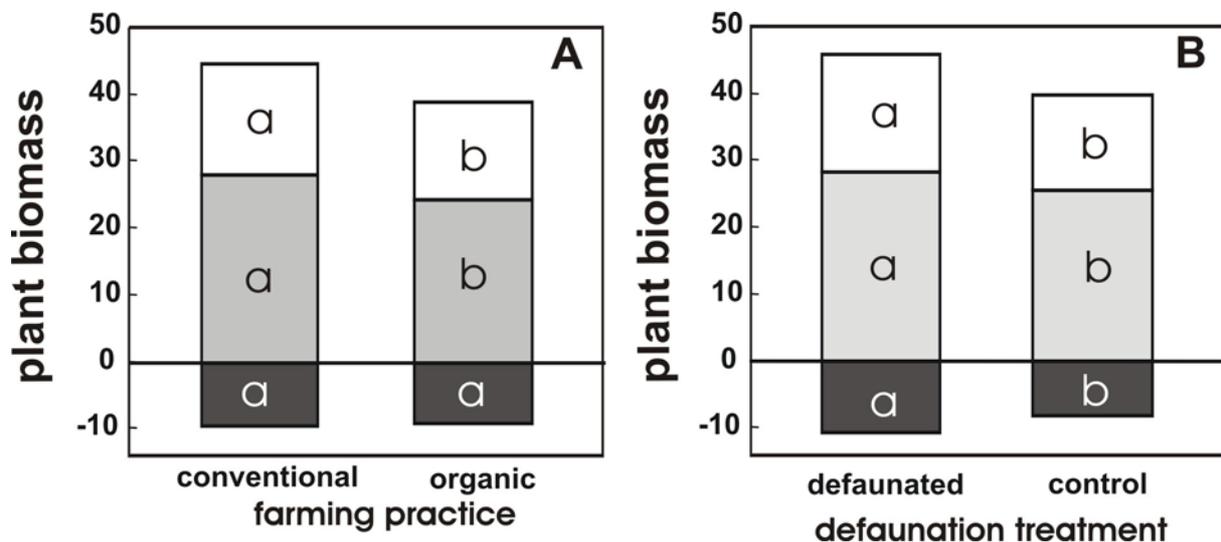
Fig. 6.1. Effects of **A**) conventional vs. organic farming system and **B**) defaunated soil vs. control soils on the biomass of wheat plants (▮ roots, ▯ shoots, and ☐ ears) at the end of the experiment. Means are presented. Different letters indicate significant differences (p<0.05).

In total we counted 33 985 aphids on the wheat plants. The most common species was *Sitobion avenae*, with 93.47 % of the individuals followed by *Rhopalosiphum padi* with 5.65% of the individuals. *Metopolophium dirhodum* and two other species comprised each less than 0.5 % of the individuals. Soil defaunation, but also the interaction between farming practice and defaunation, significantly affected the number of aphids per gram of plant biomass and the percentage of infection by *Septoria* (Table 6.2). The number of aphids per unit plant biomass in control soil exceeded that of defaunated soil but this was only true for organic soils and not for conventional soils (Fig. 6.2A). Defaunation of organic soil also reduced the infection of wheat by *Septoria*, but again this effect only occurred in organic soils (Fig. 6.2B). There was no significant correlation between the number of aphids and plant biomass per pot (r=0.159, p =0.113), nor between the percentage of *Septoria* infection and plant biomass (r=0.0029, p=0.977).

Table 6.2. Effects of defaunation and farming practice (conventional vs. organic) on the number of aphids per gram dry plant biomass and the maximal percentage of *Septoria* infection. The landscape sector was used as block. F – values and significance are shown.

|	df	Aphids/biomass	% Septoria
defaunation	1, 92	9.75**	5.89*
farming practice	1, 92	0.07	0.31
defaunation x farming practice	1, 92	6.26*	5.99*

*P < 0.05; ** P < 0.01
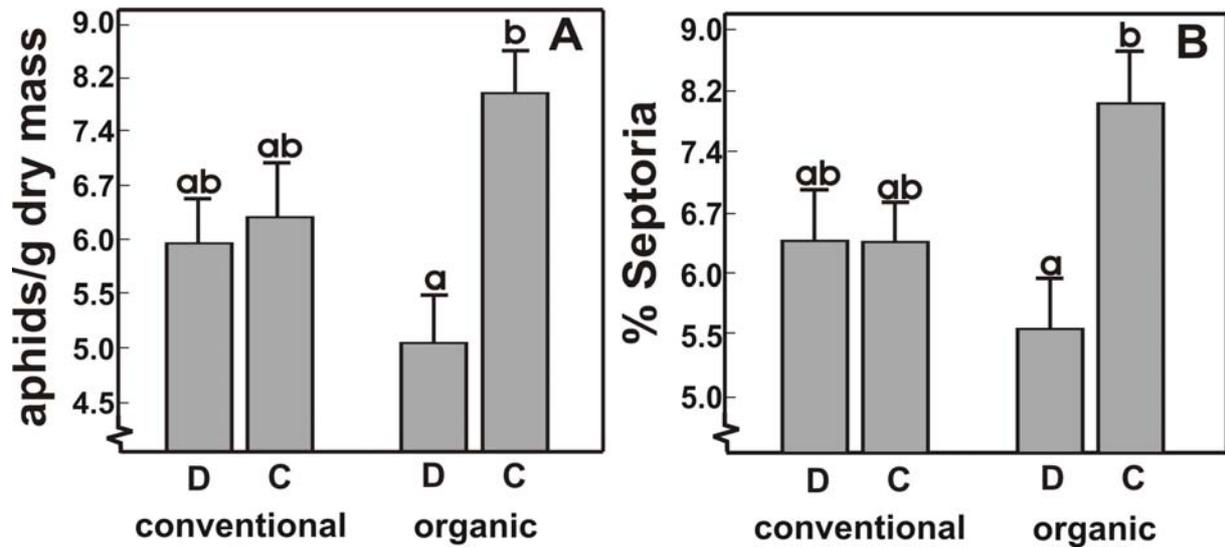
Fig. 6.2 Effects of defaunation treatment (D= defaunated soil, C = control soil) and farming practice (conventional vs. organic) on a) the number of aphids, b) the number of aphids per gram dry weight of total plant biomass and c) the maximal percentage of *Septoria* infected stems and leaves. Mean ± 1 SE are presented. Bars with different letters are significantly different (Tukey's test, p<0.05).

Discussion

Plants biomass was higher in soils from conventional fields than in soils from organic fields,. Soil defaunation increased plant biomass, irrespective of the farming system. In contrast, the number of aphids on wheat and the infection of wheat by *Septoria* were increased by defaunation in organic soil. This indicates that effects of soil organisms on plant–herbivore and plant–pathogen interactions depend on the farming system.

The higher plant biomass in the defaunated treatment compared to the control soil presumably was due to nutrients released by the defaunation procedure. Freezing is known to reduce the number of microarthropods and nematodes in soil and to increase the amount of mineral nitrogen, in particular that of ammonium (Huhta *et al.* 1989). Ammonium is easily absorbed by plants and results in increased plant growth. Similarly, differences in the growth of plants between organic and conventional farming systems might have been due to higher nutrient input (N, P, K) in the conventional system (Mäder *et al.* 2002). Organic farming systems typically receive less nutrient input resulting in reduced crop yield in comparison to conventional farms (Mäder *et al.* 2002).

Soil defaunation did not affect the number of aphids per plant biomass in soil of the conventional farming systems while it reduced aphid numbers on plants growing in soil of organic farming systems. Because aphid reproduction depends on the nutritional quality of the

host plant (Honek 1991) the decrease in the number of aphids likely were caused by lower nutritional quality of the plants, mainly that of ears, since this is were *Sitobion avenae* preferentially feeds on (Honek 1991). Soil organisms are known to affect the development of above ground organisms, such as herbivores, through indirect effects mediated by the plant (Scheu 2001, Bardgett and Wardle 2003, Wardle *et al.* 2004). The decomposition of organic matter and related processes, such as mineralization of nutrients, are regulated by the decomposer food web (Wardle *et al.* 1999, Ruess *et al.* 2004). Therefore, the presence of decomposer invertebrates in the soil may beneficially affect the reproduction of aphids, as has been shown previously (Scheu *et al.* 1999, Bonkowski *et al.* 2001, Wurst and Jones 2003). In our study soil defaunation was assumed to result in reduced decomposition processes. In fact, defaunation only caused a reduction in the aphid number in the organic soils but not in the conventionally managed soils suggesting that organically managed soils exhibit greater biological activity than conventionally managed soils, as has been shown previously (Mäder *et al*. 2002, Fließbach and Mäder 2000). Therefore, differences in the number of aphids presumably resulted from both an increased activity of soil organisms in organic soils and a higher nutrient input in conventional soils. It is important to realize that these results cannot be extrapolated easily to real agricultural environments. Although the results suggest that wheat plants in organic fields are more heavily attacked by aphids, it has to be considered that the plants were uniformly exposed in conventional fields. As has been shown by Mäder *et al.* (2002) and Hole *et al*. (2005) aboveground biodiversity and also predator abundance is increased by organic farming. This suggests that although aphid nutrition may be increased in organically managed fields predators may more vigorously control aphid populations.

The results from our study suggest that the attack of wheat by plant pathogens, such as *Septoria* fungi, is increased on control organically managed farming systems in comparison to defaunated organic soils. Presumably, soil nutrients mobilized due to the activity of decomposer invertebrates facilitated the attack of wheat by *Septoria* and aphids in organic soils, likely because of higher decomposer activity in organic soil than in conventional soil. The fact that soil invertebrates facilitated both the infestation of wheat with aphids and *Septoria* fungi in organic soils without increasing wheat biomass suggests that soil organisms may enhance the food quality of plants for herbivores and pathogens without affecting plant growth.

Contrary to our expectations soil from different farming systems and soil defaunation did not consistently affect plant growth and plant attack by herbivores and pathogens. Plant growth appeared to depend on nutrient resources already in the soil at the experimental set up which

was higher in conventional as compared to organic soils and in defaunated soils compared to control soils. In contrast, herbivores and pathogens relied on nutrients mobilized due to the activity of soil organisms in the organic soils during the course of the experiment. The results also suggest that soil organisms may increase the susceptibility of plants for attack by herbivores and pathogens without increasing plant biomass production. The effect of soil organisms therefore may propagate into higher trophic levels above the ground without visible effects on the basis of the aboveground food web, the plant. Improvement of biological activity and biodiversity belowground therefore likely results in a strengthening of food web interactions above the ground.

References

Alphei, J., M. Bonkowski, and S. Scheu. 1996. Protozoa, Nematoda, and Lumbricidae in the rhizosphere of *Hordelymus europaeus* (Poaceae): faunal interactions, response to microorganisms and effects on plant growth. *Oecologia* **106**: 111-126.

Andersen, D. 1987. Below-ground herbivory in natural communities: a review emphasizing fossorial animals. *The Quarterly Review of Biology* **62**: 261-286.

Bardgett, R.D., D.K. Leemans, R. Cook, P. Hobbs. 1997. Temporal and spatial variation in soil biota of grazed and ungrazed hill grassland in Snowdonia, North Wales. *Soil Biology and Biochemistry* **29**: 1286-1294.

Bardgett, R.D., S. Keiller, R. Cook, and A.S. Gilburn. 1998. Dynamic interactions between soil nimls ans microorganisms in upland grassland soils amended with sheep dung: a microcosm experiment. *Soil Biology and Biochemistry* **30**: 531-539.

Bardgett, R.D., and D.A. Wardle. 2003. Herbivore mediated linkages between aboveground and belowground communities. *Ecology* **84**: 2258-2268.

Bezemer, T. M., R. Wagenaar, N. M. van Dam, and F. L. Wäckers. 2002. Interactions between root and shoot feeding insects are mediated by primary and secondary plant compounds. *Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society (NEV)* **13**: 117-121.

Blackman, R. L. and Eastop, V. F. 1984. Aphids on the world's crops: an identification guide. John Wiley and Sons.

Bohlen, P., S. Scheu, C. Hale, M.A. McLean, S. Migge, P. Groffman and D. Parkinson. 2004. Non-native invasive earthworms as agents of change in northern temperate forests. *Frontiers in Ecology and the Environment* **2**: 427-435.

Bonkowski, M., W. Cheng, B. Griffiths, J. Alphei, and S. Scheu. 2000. Microbial-faunal interactions in the rhizosphere and effects on plant growth. *European Journal of Soil Biology* **36**:135-147.

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

Bonkowski, M. 2004. Protozoa and plant growth: the microbial loop in soil revisited. *New Phytologist* **162**: 617-631.

Bonkowski, M., I. Geoghegann, A. Nicholas, E. Birch, and B. Griffiths. 2001. Effects of soil decomposer invertebrates (protozoa and earthworms) on above-ground phytophagous insects (cereal aphid) mediated through changes in the host plant. *Oikos* **95**:441-450.

Bonkowski, M., W. Cheng, B. Griffiths, J. Alphei, and S. Scheu. 2000. Microbial-faunal interactions in the rhizosphere and effects on plant growth. *European Journal of Soil Biology* **36**:135-147.

Bronstein, J.L., T.E Huxman, and G. Davidowitz. in press. Plant-mediated effects linking herbivory and pollination. *in* T. Ohgushi, T. Craig, and P. Price, editors.Indirect interaction webs: nontrophic linkages through induced plant traits. Cambridge University Press.

Brown, V.K., A.C. Gange, I.M. Evans, and L. Storr. 1987. The effect of insect herbivory on the growth and reproduction of two annual *Vicia* species at different stages in plant succession. *Journal of Ecology*. **75**:1173-1189.

Brown, V.K., and A.C. Gange. 1989. Herbivory by soil-dwelling insects depresses plant species richness. *Functional Ecology* **3**:667-671.

Brown, V. K., and A.C. Gange. 1990. Insect herbivory below ground. *in* M. Begon, A. Fitter, A. Macfadyen, editors. *Advances in Ecological Research* **20**:1–58. New York: Academic Press.

Brown, V. K., and A. C. Gange. 2002. Tritrophic below-and above-ground interactions in succession. *in* T. Tscharntke, and B. Hawkins, editors. *Multitrophic Level Interactions.* pp 197-222. Cambridge. Cambridge University Press.

Brown, G.G., C.A. Edwards, and L. Brussaard. 2004. How earthworms affect plant growth: burrowing into mechanisms. In *Earthworm Ecology,* 2nd edition. C.A. Edwards, editor,. pp. 13-49. Boca Raton: CRC Press.

Brussaard, L. 1999. On the mechanisms of interactions between earthworms and plants. *Pedobiologia*. **43**: 880-885.

Callaham, M.A., and P.E. Hendrix. 1998. Impact of earthworms (Diplocardia, Megascolecidae) on cycling and uptake of nitrogen in coastal plain forest soils from northwest Florida, USA. *Applied Soil Ecology* **9**: 233-239.

Clarholm, M. 1985. Possible roles for roots, bacteria, protozoa and fungi in supplying nitrogen to plants. *in* A.H. Fitter, editor. *Ecological Interactions in Soils*,: Blackwell University Press. Oxford

Coleman, D.C. 1994. The microbial loop as used in terrestrial soil ecology. *Microbial Ecology* **28**:245-250.

Conner, J.K. and S. Rush. 1996. Effects of flower size and number on pollinator visitation to wild radish, *Raphanus raphanistrum. Oecologia* **105**:509-516.

Crawley, M.J. 1983. Herbivory: The dynamics of animal-plant interactions. Blackwell Science, Oxford. United Kingdom, pp 40-70.

Crawley, M.J. 1997. Plant ecology. 2nd Edition. Blackwell Science. Oxford.

Dafni, A. 1992. Pollination ecology: a practical approach. Oxford University Press. Oxford.

Davidson, R.L., R.J. Wensler, and V.J. Wolfe. 1970. Damage to ryegrass plants of different size by various densities of the pruinose scarab (Sericesthis geminata (Coleoptera)). *Australian Journal of Experimental Agriculture and Animal Husbandry* **10**:166-171.

Didham, R.K., J.M Ghazoul, E. Stork, and A.J. Davis. 1996. Insects in fragmented forests: a functional approach. *Trends in Ecology and Evolution* **11**: 255-260.

EU 2002. Organic farming in the EU: Facts and Figures. (http://europa.eu.int/comm/agriculture/qual/organic/facts_en.pdf).

Fließbach, A., and P. Mäder. 2000. Microbial biomass and size-density fractions differ between soils of organic and conventional agricultural systems. *Soil Biology and Biochemistry* **32**: 757-768.

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

Gurr, G.M., S.D. Wratten, and J.M. Luna. 2003. Multi-function agricultural biodiversity: pest management and other benefits. *Basic and Applied. Ecology* 4: 107-116.

Haigh, D. and M. Westoby. 1988. Inclusive fitness, seed resources, and maternal care, *in* J.L. Doust and L.L. Doust, editors. Plant reproductive ecology: patterns and strategies. Oxford University Press. Oxford.

Haimi, J., and M. Einbork. 1992. Effects of endogeic earthworms on soil processes and plant growth in coniferous forest soil. *Biology and Fertility of Soils* **13**: 6-10.

Haimi, J., V. Huhta, M. Boucelham. 1992. Growth increase of birch seedlings under the influence of earthworms - a laboratory study. *Soil Biology and Biogeochemistry* **24**:1525-1528.

Hambäck, P.A., 2001. Direct and indirect effects of herbivory: feeding by spittlebugs affects pollinator visitation rates and seed set of *Rudbeckia hirta. Ecoscience* **8**: 45-50.

Herrera, C. M., M. Medrano, P. J. Rey, A. M. Sánchez-LaFuente, M. B. García, J. Guitian, and A. J. Manzaneda. 2002. Interaction of pollinators and herbivores on plant fitness suggests a pathway for correlated evolution of mutualism- and antagonism-related traits. *Proceedings of the National Academy of Sciences* **99**: 16823-16828.

Hole, D.G., A.J. Perkins, J.D. Wilson, I.H. Alexander, P.V. Grice, and A.D. Evans. 2005. Does organic farming benefit biodiversity? *Biological Conservation* **122**: 113-130.

Honek, A. 1991. Nitrogen fertilization and abundance of the cereal aphids *Metapolophium dirhodum* and *Sitobion avenae* (Homoptera, Aphididae). *Journal of Plant Diseases and Protection* **98**: 655-660.

Huhta V., D.H. Wright, and D.C. Coleman. 1989. Characteristics of defaunated soil. I. A comparison of three techniques applied to two different forest soils. - *Pedobiologia* **33**: 415-424.

Jones, C.G., J.H. Lawton and M. Shachak. 1994. Organisms as ecosystem engineers. *Oikos* **69**: 373-386.

Karban, R. and S. Strauss. 1993. Effects of herbivores on growth and reproduction of their perennial host, *Erigeron glaucus*. *Ecology* **74**:39-46.

Kearns, C.A., and D.W. Inouye. 1993. Techniques for pollination biologists. University Press of Colorado. Colorado.

Kinsmann, S. and W.J. Platt. 1984. The impact of a herbivore upon *Mirabilis hirsuta*, a fugitive prairie plant. *Oecologia* **65**:2-6.

Krebs, J.R., J.D. Wilson, R.B. Bradbury and G.M. Siriwardena. 1999. The second silent spring? *Nature* **400**: 611-612.

Kruess, A., and T. Tscharntke. 1994. Habitat fragmentation, species loss and biological control. *Science* **264**: 1581-1584.

Kruess, A., and T. Tscharntke. 2000. Species richness and parasitism in a fragmented landscape: experiments and field studies with insects on *Vicia sepium*. *Oecologia* **122**: 129-137.

Krupnick, G.A., A.E. Weis, and D.R. Campbell. 1999. The consequences of floral herbivory for pollinator service to *Isomeris arborea*. *Ecology* **80**: 125-134.

Kunin, W.E. 1993. Sex and the single mustard: population density and pollinator behavior effects on seed set. *Ecology* **74**: 2145-2160.

Larsson, S. 1989. Stressful times for the plant stress-insect performance hypothesis. *Oikos* **56**:277-283.

Larsson, B.M.H., and S.C.H Barrett. 2000. A comparative analysis of pollen limitation in flowering plants. *Biological Journal of the Linnean Society* **69**: 503-520.

Lavelle, P., and A.V. Spain. 2001. Soil Ecology. Dordrecht: Kluwer Academic Publishers.

Legendre, P., and L. Legendre. 1998. Numerical Ecology: second english edition. Developments in Environmental Modelling 20. Elsevier Science B.V. The Netherlands.

Lethilä, K., and S. Strauss. 1997. Leaf damage by herbivores affects attractiveness to pollinators in wild radish, *Raphanus raphanistrum. Oecologia* **111**:396-403.

Lethilä, K., and S. Strauss. 1999. Effects of foliar herbivory on male and female reproductive traits of wild radish, *Raphanus raphanistrum. Ecology* **80**:116-124.

Mäder, P., A. Fließbach, D. Dubois, L. Gunst, P. Fried, and U. Niggli. 2002. Soil fertility and biodiversity in organic farming. *Science* **296**: 1694-1697.

Maron, J.L. 1998. Insect herbivory above- and belowground: individual and joint effects on plant fitness. *Ecology* **79**:1281-1293.

Maron, J.L. 2001. Intraspecific competition and subterranean herbivory: individual and interactive effects on bush lupine. *Oikos* **92**: 178-186.

Marquis, R.J. 1984. Leaf herbivores decrease fitness of a tropical plant. *Science* **226**:537-539.

Marquis, R.J. 1992. The selective impact of herbivores. *in* R.S. Fritz and E.L. Simms, editors. Plant resistance to herbivores and pathogens: ecology, evolution and genetics. University of Chicago Press.

Masters, G. J., and V. K. Brown. 1992. Plant mediated interactions between two spatially separated insects. *Functional Ecology* **6**:175-179.

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

Masters, G.J. 1995a. The effect of herbivore density on host plant mediated interactions between two insects. *Ecological Research* **10**:1225-133.

Masters, G.J. 1995b. The impact of root herbivory on aphid performance: field and laboratory evidence. *Acta Oecologica* **16**:135-142.

Masters, G. J., and V. K. Brown. 1997. Host-plant mediated interactions between spatially separated herbivores: effects on community structure. *in* A.C. Gange and V.K. Brown, editors. Multitrophic Interactions in Terrestrial Systems. pp.217-237. Oxford: Blackwell Science.

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

Matson, P.A., W.J. Parton, A.G. Power, and M.J. Swift. 1997. Agricultural intensification and ecosystem properties. *Science* **277**: 504-509.

Matthies, D., B. Schmid, and P. Schmid-Herpel. 1995. The importance of population processes for the maintenance of biological diversity. *Gaia* 4: 199-204.

Mattson, W.J. Jr. 1980. Herbivory in relation to plant nitrogen content. *Annual Review of Ecology and Systematics* **11**: 119-161.

McNaughton, S.J. 1983. Compensatory plant growth as a response to herbivory. *Oikos* **40**:329-336.

Mikola, J., G.W. Yeates, G.M. Barker, D.A. Wardle, and K.I. Bonner. 2001. Effects of defoliation intensity on soil food web properties in an experimental grassland community. *Oikos* **92**:333-343.

Moore, J.C., K. McCann, H. Setälä, and P.C. De Ruiter. 2003. Top-down is bottom-up: Does predation in the rhizosphere regulate aboveground dynamics? *Ecology* **84**:846-857.

Moran, M.D., 2003. Arguments for rejecting the sequential Bonferroni in ecological studies. *Oikos* **100**: 403-405.

Moran, N.A., and T.G. Whitham. 1990. Interspecific competition between root-feeding and leaf-galling aphids mediated by host-plant resistance. *Ecology* **71**: 1050-1058.

Mortimer, S.R., W. van der Putten, and V.K. Brown. 1999. Insect and nematode herbivory below ground: interactions and role in vegetation succession. *in* H. Olff, V.K. Brown and R.H. Drent. Herbivores: between Plants and Predators. Oxford: Blackwell University Press.

Mothershead, K., and R. Marquis. 2000. Fitness impacts of herbivory through indirect effects on plant-pollinator interactions in *Oenothera macrocarpa*. *Ecology* **81**:30-40.

Newington, J.E., H. Setälä, T.M. Bezemer, and T.H. Jones. 2004. Potential effects of earthworms on leaf-chewer performance. *Functional Ecology* **18**: 746-751

Nötzold, R., B. Blossey, and E. Newton. 1998. The influence of below ground herbivory and plant competition on growth and biomass allocation of purple loosestrife. *Oecologia* **113**:82-93.

Ohara, M., and S. Higashi. 1994. Effects of inflorescence size on visits from pollinators and seed set of *Corydalis ambigua* (Papaveraceae). *Oecologia* **98**: 25-30.

Pellmyr, O. 2002. Pollination by animals. *in* C.M. Herrera and O. Pellmyr, editors. Plant-Animal Interactions: an Evolutionary Approach. Oxford: Blackwell Science.

Pizl, V. 1999. Earthworm succession in abandoned fields- a comparison of deductive and sequential approaches to study. *Pedobiologia* **43**: 705-712.

Porazinska, D.L., R.D. Bardgett, M.B. Blaauw, H.W. Hunt, A.N. Parsons, T.R. Seastedt and D.H. Wall. 2003. Relationships at the aboveground-belowground interface: plants, soil biota and soil processes. *Ecological Monographs* **73**: 377-395.

Poveda, K., I. Steffan-Dewenter, S. Scheu, and T. Tscharntke. 2003. Effects of below- and above-ground herbivores on plant growth, flower visitation and seed set. *Oecologia* **135**: 601-605.

Poveda, K., I. Steffan-Dewenter, S. Scheu, T. Tscharntke. 2005. Below- and above-ground plant-animal interactions mediated through an annual plant. *Oikos* **108**: 503-510.

Powell, R.D. and J.H. Myers. 1988. The effect of *Sphenoptera jugoslavica* Obenb. (Col., Buprestidae) on its host plant *Centaurea diffusa* Lam. (Compositae). *Journal of Applied Entomology* **160**:25-45.

Preisser, E.L. 2003. Field evidence for a rapidly cascading underground food web. *Ecology* **84**: 869-874.

Quesada, M., K. Bollman and A. Stephenson. 1995. Leaf damage decreases pollen production and hinders pollen performance in *Cucurbita texana*. *Ecology* **76**: 437-443.

R 2003. The R Foundation for Statistical Computing. Version 1.8.1 (2003-11-21), ISBN 3-900051-00-3.

Ridsdill-Smith, T.J. 1977. Effects of root-feeding by scarabaeid larvae on growth of perennial ryegrass plants. *Journal of Applied Ecology* **14**:73-80.

Riedell, W. 1989. Western corn rootworm damage in maize: greenhouse technique and plant response. *Crop Science* **29**:412-415.

Ruess, L., M. Häggblom, R. Langel, and S. Scheu. 2004. Nitrogen isotope ratios and fatty acid composition as indicators of animal diets in belowground systems. *Oecologia* **139**: 336–346.

Scheiner, M.S. and J. Gurevitch. 2001. Design and Analysis of Ecological Experiments. Second Edition. Oxford University Press.

Scheu, S. 2001. Plants and generalist predators as links between the belowground and aboveground system. *Basic and Applied Ecology* **2**:3-13.

Scheu, S. 2003. Effects of earthworms on plant growth: patterns and perspectives. *Pedobiologia* **47**: 846-856.

Scheu, S., and D. Parkinson. 1994. Effects of invasion of an aspen forest (Canada) by *Dendrobaena octaedra* (Lumbricidae) on plant growth. *Ecology* **75**:2348-2361.

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

Scheu, S., and H. Setälä. 2002. Multitrophic interactions in decomposer food-webs. *In* T. Tscharntke, and B. Hawkins, editors. Multitrophic level interactions. Cambridge University Press, Cambridge, pp 223-264.

Scheu, S., L. Ruess, and M. Bonkowski. 2005. Interactions between micro-organisms and soil micro- and mesofauna. *in* F. Buscot, and A. Varma. Microorganisms in Soils: Roles in Genesis and Function. New York: Springer (in press).

Schmidt, O., and J.P. Curry. 1999. Effects of earthworms on biomass production, nitrogen allocation and nitrogen transfer in wheat-clover intercropping model systems. P*lant and Soil* **214**:187-198.

Setälä, H. and V. Huhta. 1991. Soil fauna increase *Betula pendula* growth: laboratory experiments with coniferous forest floor. *Ecology* **76**: 1844-1851.

Simberloff D, Brown BJ, Lowrie S (1978) Isopod and insect root borers may benefit Florida mangroves. Science 201:630-632

Spain, A.V., P. Lavelle, A. Mariotti. 1992. Stimulation of plant growth by tropical earthworms. *Soil Biology and Biochemistry* **26**:1179-1184.

Stanton, N. L. 1988. The underground in grasslands. *Annual Review in Ecology and Systematics* 19:573-89.

StatSoft Inc. 1995. Statistica Version 5 for Windows. Telsa. USA.

StatSoft, Inc. (2003). STATISTICA für Windows [Software-System für Datenanalyse] Version 6. www.statsoft.com

Stephenson, A.G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. *Annual Review in Ecology and Systematics* **12**:253-279.

Stirling, K.J., R.J. Clark, P.H. Brown, and S.J. Wilson. 2002. Effect of photoperiod on flower bud initiation and development in myoga (*Zingiber mioga* Roscoe.). *Scientia Horticulturae* **95**:261-268.

Strauss, S. 1991. Direct, indirect, and cumulative effects of three native herbivores on a shared host plant. *Ecology* 72:543-558.

Strauss, S. 1997. Floral characters link herbivores, pollinators, and plant fitness. *Ecology* **78**:1640-1645.

Strauss, S., J. Conner, S. Rush. 1996. Foliar herbivory affects floral characters and plant attractiveness to pollinators: implications for male and female plant fitness. *The American Naturalist* **147**:1098-1107.

Strong, D.R., A.V. Whipple, A.L. Child and B. Dennis. 1999. Model selection for a subterranean trophic cascade: root feeding caterpillars and entomopathogenic nematodes. *Ecology* **80**: 2750-2761.

Tilman, D., K.G. Casssman, P.A. Matson, R. Naylor, and S. Polasky. 2002. Agricultural Sustainability and intensive production practices. *Nature* **418**: 671-677.

Van Dam, N.M., J.A. Harvey, F.L. Wäckers, T.M. Bezemer, W.H. van der Putten, L. Vet. 2003. Interactions between aboveground and belowground induced responses against phytophages. *Basic and Applied Ecology* **4**: 63-77.

Van der Putten W, L. Vet, J. Harvey and F. Wäckers .2001. Linking above- and belowground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. *Trends in Ecology and Evolution* **16**:547-554.

Wardle, D. 1999. How soil food webs make plants grow. *Trends in Ecology and Evolution* **14**:418-420.

Wardle, D. 2002. Communities and Ecosystems: Linking the Aboveground and Belowground Components. Monographs in population biology Nr. 34. Princeton: Princeton University Press.

Wardle, D.A., R.D. Bardgett, J.N. Klironomos, H.Setälä, W.H. van der Putten, D.H. Wall. 2004. Ecological linkages between aboveground and belowground biota. *Science* **304**: 1629-1633.

Wardle, D.A., K.E.Giller, and G.M Barker. 1999. The regulation and functional significance of soil biodiversity in agroecosystems. *in* D. Wood, and J.M. Lenne, editors. Agrobiodiversity: characterization, utilization and management. CABI Publishing, Bristol, UK.

Way, M.J. and M. Cammell. 1970. Aggregation behaviour in relation to food utilization by aphids. *in* A.Watson, editor. Animal populations in relation to their food resources. Blackwell.

White, T. C. R. 1993. *The Inadequate Environment: Nitrogen and the Abundance of Animals.* Berlin: Springer Verlag.

Wurst, S., and T.H. Jones. 2003. Indirect effects of earthworms (*Aporrectodea caliginosa*) on an above-ground tritrophic interaction. *Pedobiologia* **47**: 91-97.

Wurst, S., D. Dugassa-Gobena, and S. Scheu. 2004a. Earthworms and litter distribution affect plant defence chemistry. *Journal of Chemical Ecology* **30**: 691-701.

Wurst, S., D. Dugassa-Gobena, R. Langel, M. Bonkowski, and S. Scheu. 2004b. Combined effects of earthworms and vesicular-arbuscular mycorrhizas on plant and aphid performance. 2004. *New Phytologist*. **163**:169-176.

Wurst, S., R. Langel, A. Reineking, M. Bonkowski, and S. Scheu. 2003. Effects of earthworms and organic litter distribution on plant performance and aphid reproduction. *Oecologia* **137**: 90-96.

Summary

Most ecologists acknowledge that plants are subject to complex interactions between both below- and aboveground dwelling animals. However, these complex interactions are seldomly investigated simultaneously. In a set of experiments we studied the effects of decomposers, root herbivores and leaf herbivores on plant growth, flower visitation, herbivores and their parasitoids. Our results show that belowground communities affect not only plant growth but also aboveground processes, including plant-pathogen, plant-pollinator and even plant-herbivore-parasitoid interactions. Leaf herbivory reduced plant height, shoot mass, and delayed the onset of flowering of *Sinapis arvensis*. Furthermore, it negatively affected flowering traits suggesting a decrease in plant attractiveness to pollinators. However, we could not identify any effect of leaf herbivory on flower visitors. Flower visitors were positively influenced by the presence of root herbivores in two independent experiments. This strongly suggests that root herbivores can have an impact on the pollination of *Sinapis arvensis.*However, we could not find any effect of root herbivory on the measured floral traits (anther size, nectar production, petal size, number of flowers). Although in most experiments the experimental plants faced major herbivore-induced growth changes, plant reproduction (seed set and weight per plant) was similar between herbivory treatments and control plants. These results show the ability of *Sinapis arvensis* to compensate for leaf and root herbivory. Accordingly, reduced size, delayed start of flowering, reduced flowering period or reduced flower visitation could not be used to predict plant fitness in this annual, cross-pollinated herb. In contrast, decomposers increased plant growth and plant fitness (measured as the number of seeds produced), indicating that mustard may benefit from nutrients provided by decomposers, regardless whether plants are attacked by root herbivores or leaf herbivores, or both simultaneously. We found that the individual presence of either root herbivores or decomposers resulted in increased aphid abundance, demonstrating that the same aboveground plant-insect interaction can be released by different belowground processes. Enhanced aphid densities caused higher numbers of parasitoids. Our results give an insight in the effects of belowground organisms on aboveground organisms and it gets clear that many aboveground patterns could be driven by belowground processes through the plant.

Using a different approach we investigated the significance of farming practices in modifying the effects of soil organisms on aboveground systems. Plant biomass in soil from conventional farms exceeded that of soils from organic farms, presumably due to the higher

nutrient input in the conventional farming system. Soil defaunation likely mobilized nutrients that increased plant growth. Aphid abundance and *Septoria* infection was reduced by defaunation but only in organic soils. This suggests that soil organisms in organic farming systems are more important for the augmentation of aphids and the infection rate by *Septoria* than in conventional systems. Hence, farming practice appears to modify the effect of soil organisms on aboveground organisms, and soil organisms played a major role in organic farming systems. Further, the results indicate that soil organisms may modify higher trophic levels (aphid and pathogen infection) without significantly affecting lower trophic levels (plant growth).

Until recently the role of soil biota in modifying the attractiveness of plants to herbivores, pathogens and pollinators has been largely ignored. Particularly in natural habitats where plant species interact with each other, soil biota may play a key role in modifying the insect community associated with plants. Future work needs to focus upon more complex systems consisting of plants and soil biota in different spatial and temporal contexts to better understand the functioning of terrestrial systems.

Zusammenfassung

Oberirdische und unterirdische Nahrungsnetze werden meist getrennt voneinander betrachtet. In den letzten Jahren wurde aber zunehmend deutlich, dass unterirdische Organismen einen starken Einfluss auf oberirdische biotische Interaktionen, wie die zwischen Pflanzen und Blütenbesuchern sowie Pflanze, Herbivoren und Parasitoiden, haben können. In dieser Arbeit wurden die Effekte von Zersetzern (Regenwürmern), unterirdischen Herbivoren (Drahtwürmern) und Blattherbivoren (Schmetterlingsraupen) auf das Pflanzenwachstum von Ackersenf (*Sinapis arvensis*) und auf Pflanze-Pathogen-, Pflanze-Herbivor-Parasitoid- und Pflanze-Bestäuber- Interaktion untersucht. Blattherbivorie verzögerte den Blühanfang und wirkte sich negativ auf die Pflanzenhöhe und Pflanzenbiomasse vom Ackersenf aus. Außerdem hatte sie einen negativen Effekt auf Blütenmerkmale, was darauf hindeutet, dass Blattherbivorie die Attraktivität der Pflanzen für Blütenbesucher beeinflussen kann. Trotzdem konnten wir in keinem unserer Experimente einen negativen Effekt von Blattherbivorie auf Blütenbesucher feststellen. In zwei unterschiedlichen Experimenten hatten Wurzelherbivore einen positiven Effekt auf Blütenbesucher, was darauf hindeutet, dass Blütenbesucher von Wurzelherbivoren mehr beeinflusst werden als von Blattherbivoren. Die Blütenmerkmale, die zu einem erhöhten Blütenbesuch führten, konnten nicht identifiziert werden, da Wurzelherbivorie keinen Einfluss auf Staubbeutellänge, Nektarproduktion, Blütenblattgröße und Anzahl der Blüten hatte.

Obwohl die Pflanzen durch den Herbivoriebefall (sowohl unterirdisch wie auch oberirdisch) ein verändertes Wachstum aufwiesen, konnten keine Unterschiede in den Pflanzen-Reproduktionsparametern (wie Samenzahl und -gewicht) zwischen den verschiedenen Behandlungen festgestellt werden. Diese Ergebnisse zeigen, dass *Sinapis arvenis* den Herbivorieschaden kompensieren kann. Im Gegensatz dazu führt die Anwesenheit von Zersetzern zu einem erhöhten Pflanzenwachstum und einer erhöhten Samenanzahl, unabhängig vom Vorkommen von Herbivoren. Pflanzen, in deren Substrat sich nur Wurzelherbivore oder Zersetzer befanden, hatten eine erhöhte Blattlausabundanz. Dies weist darauf hin, dass gleichartige oberirdische Muster durch unterschiedliche unterirdische Prozesse hervorgerufen werden können.

In einem weiteren Ansatz haben wir die Effekte der Defaunierung von unterschiedlich bewirtschafteten Böden auf oberirdische Systeme studiert. Die Pflanzenbiomasse in konventionell bewirtschafteten Böden war höher als in organisch bewirtschafteten Böden,

sicherlich durch die erhöhte Nährstoffzufuhr in konventionellen Böden. Pflanzen in defaunierten Böden hatten ebenfalls eine höhere Pflanzenbiomasse, vermutlich durch den Defaunierungsprozess, der beim Abtöten der Tiere zur Freisetzung von darin enthaltenen Nährstoffen führte. Sowohl die Blattlausabundanz wie auch die Infektion durch *Septoria* waren niedriger in defaunierten organischen Böden. Daraus kann man ableiten, dass Bodenorganismen in organisch bewirtschafteten Böden wichtiger für die Vermehrung von Blattläusen und die Infektionsrate von *Septoria* sind als in konventionell bewirtschafteten Böden. Dies deutet darauf hin, dass die Bewirtschaftungsweise den Effekt von Bodenorganismen auf oberirdische Prozesse modifiziert und dass Bodenorganismen in organisch bewirtschafteten Böden eine weit wichtigere Rolle spielen, als in konventionell bewirtschafteten. Die Ergebnisse deuten darauf, dass Bodenorganismen höhere trophische Ebenen, wie Herbivore und Pathogene, modifizieren können, ohne die unterste trophische Ebene (die Pflanze) zu beeinflussen.

Bis vor kurzem wurde die Rolle von unterirdischen Organismen auf oberirdische Herbivore, Pathogene und Bestäuber weitgehend ignoriert. Vor allem in natürlichen Habitaten, in denen zahlreiche Pflanzenarten miteinander interagieren, können Bodenorganismen eine Schlüsselrolle spielen indem sie die an eine Pflanze assoziierte Insektengemeinschaft, modifizieren. Um terrestrische Ökosysteme besser zu verstehen, sollten künftige Studien komplexere Systeme studieren, in denen Pflanzen mit Bodenorganismen in unterschiedlichen räumlichen und zeitlichen Kontexten interagieren.

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List of publications

Poveda K., I. Steffan-Dewenter, S. Scheu & T. Tscharntke. (submitted) Belowground effects of farming practice and soil organisms on aboveground plant-herbivore and plant-pathogen interactions.

Poveda K., I. Steffan-Dewenter, S. Scheu & T. Tscharntke. (2005c) Plant-mediated interactions between below- and aboveground processes: decomposition, herbivory, parasitism and pollination. In: Ohgushi T., T. Craig, P. Price. Indirect Interaction webs: nontrophic linkages through induced plant traits. Cambridge University Press. –inpress.

Poveda K., I. Steffan-Dewenter, S. Scheu & T. Tscharntke. (2005b) Floral trait expression in response to below- and aboveground plant-animal interactions. Perspectives in Plant Ecology, Evolution and Systematics – in press.

Poveda K., I. Steffan-Dewenter, S. Scheu & T. Tscharntke. (2005a) Effects of decomposers and herbivores on plant performance and aboveground plant-insect interactions. Oikos 108:503-510.

Poveda K., & P. Sánchez. (2004) Habitat use of *Saguinus leucopus*: a comparison between a forest-dwelling group and an urbanized group in Mariquita, Colombia. Neotropical Primates 12(1): 6-9

Morales-Jimenez A, . F. Sánchez, , K. Poveda & A. Cadena (2004). Mamíferos terrestres y voladores de Colombia : Guía de campo (Field guide on the mammals of Colombia). Editorial Ramos López. Bogotá, Colombia. ISBN 958-33-6773-7

Schröter D., L. Brussaard, G. De Deyn, K. Poveda, V.K. Brown, M. Berg, D. Wardle, J. Moore, D. Wall. (2004) Modelling above-and belowground interactions in a changing world. Basic and Applied Ecology 5:515-528.

Poveda K., I. Steffan-Dewenter, S. Scheu & T. Tscharntke. 2004. Floral attractiveness to pollinators and seed set in response to below- and aboveground plant-animal interactions. Verhandlungen der Gesellschaft für Ökologie Band 34.

Poveda K., I. Steffan-Dewenter, S. Scheu & T. Tscharntke. 2004. Above-ground plant-animal interactions in dependence of below-ground processes. Abstracts Volume of the 89th Annual Meeting of the Ecological Society of America (ESA) in Portland, Oregon, USA.

Poveda K., I. Steffan-Dewenter, S. Scheu & T. Tscharntke. 2003. Effects of decomposers, below and above-ground herbivores on plant growth and herbivore-parasitoid interactions. Verhandlungen der Gesellschaft für Ökologie Band 33.

Poveda K., I. Steffan-Dewenter, S. Scheu & T. Tscharntke. 2003. The influence of root and leaf herbivores on plant growth, flower visitation and seed set. Kurzfassung und Programm der DgaaE. Pag.54.

Poveda K., I. Steffan-Dewenter, S. Scheu & T. Tscharntke. 2003. Effects of below- and above-ground herbivores on plant growth, flower visitation and seed set. Oecologia 135:601-605.

Poveda K., I. Steffan-Dewenter, S. Scheu & T. Tscharntke. 2002. Effects of below- and above-ground herbivores on plant growth, flower visitation and seed set. Verhandlungen der Gesellschaft für Ökologie Band 32.

Poveda K. 2001. Habitat use of *Saguinus leucopus*: comparison between an urbanized group and a forest-dwelling group in Mariquita, Colombia. In: Müller A.E. & W. Scheffrahn (Eds.). Abstracts of the 7th Congress of the German Primate Society.

Poveda K. & P. Sanchez. 2000. Areas de acción de dos grupos del tití de pies blancos (*Saguinus leucopus*) que viven en hábitats diferentes en Mariquita, Colombia. pag. 71, en: Muñoz de Hoyos, P. & J. Aguirre C. (eds). Libro de Resúmenes del 1er Congreso Colombiano de Zoología - Año 2000, Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Sede Santafé de Bogotá.

Morales A. & K. Poveda. 1998. Santuario del Mono Aullador, Hojas de Yarumo, 1: 2-3

Morales A. & K. Poveda.1998. Los Monos aulladores, Intervalos 4:24-28

Curriculum Vitae

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