Effects of earthworms on plant and herbivore performance

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Susanne Wurst

aus Heilbronn

Referent: Prof. Dr. Stefan Scheu

Koreferent: Prof. Dr. Alfred Buschinger

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Contents

Zusammenfassung

In der vorliegenden Arbeit wurde der Einfluss von Regenwürmern in Kombination mit Bodenheterogenität und anderen Bodenorganismen (Mykorrhiza, phytophage Nematoden) auf Pflanzen und oberirdische Herbivore in mehreren Experimenten im Gewächshaus untersucht. Ziel der Arbeit war es, die Bedeutung von Regenwürmern in Ökosystemen hinsichtlich ihrer Effekte auf Pflanzenwachstum, Konkurrenz zwischen Pflanzen, und pflanzliche Abwehrmechanismen gegen Herbivore zu erforschen.

Die untersuchten Pflanzenarten waren ein Gras (*Lolium perenne*), ein Kraut (*Plantago lanceolata*) und eine Leguminose (*Trifolium repens*), die sich unter anderem in ihrer Wurzelmorphologie und Stickstoffaufnahme unterscheiden. Es wurde angenommen, dass Regenwürmer und die räumliche Verteilung von Streu die Pflanzenarten unterschiedlich beeinflussen und die Konkurrenz zwischen den Pflanzen verändern. Die Grasstreu wurde mit dem stabilen Isotop ^{15}N markiert, um den Stofffluss aus der Streu zu den Pflanzen zu verfolgen. Um die Frage zu klären, warum Regenwürmer die Reproduktion von Blattläusen (*Myzus persicae*) auf *P. lanceolata* reduzierten, wurden Abwehrstoffe in *P. lanceolata* untersucht. Da sowohl Regenwürmer als auch andere Bodenorganismen Wachstum und Konkurrenz von Pflanzen beeinflussen, wurden Interaktionen zwischen Regenwürmern und Mykorrhiza bzw. phytophagen Nematoden untersucht.

Bodenheterogenität (die räumliche Verteilung von Streu) und Regenwürmer beeinflussten die Pflanzenarten unterschiedlich. Auf Grund der Symbiose mit N-fixierenden Bakterien, war *T. repens* generell unabhängiger von Bodenfaktoren als *L. perenne* und *P. lanceolata*. Das Spross- und Wurzelwachstum von *L. perenne* und *P. lanceolata* wurde durch Regenwürmer gefördert, wohingegen das Wachstum von *T. repens* nicht beeinflusst wurde. Regenwürmer erhöhten die Stickstoffaufnahme bei allen Pflanzenarten. Die konzentrierte Streuverteilung als so genanntes „patch“ förderte vor allem das Wachstum von *L. perenne*. Wenn die Pflanzen in interspezifischer Konkurrenz wuchsen, führten Regenwürmer zu einem Konkurrenzvorteil von *L. perenne* gegenüber *T. repens*, wohingegen die Streuverteilung keinen Einfluss auf das Wachstum und die Konkurrenz der Pflanzen hatte.

Regenwürmer reduzierten die Reproduktion von Blattläusen (*M. persicae*) auf *P. lanceolata* und beeinflussten Inhaltsstoffe in *P. lanceoata*, die für die Abwehr gegen

Herbivore von Bedeutung sind. In Abhängigkeit von der Streuverteilung, erhöhten Regenwürmer die Konzentration an Stickstoff (N) und Phytosterolen in den Blättern. Die Konzentration an Phytosterolen korrelierte positiv mit der N-Konzentration. Die konzentrierte Streuverteilung („patch“) führte zu einem Anstieg an Aucubin in den Blättern. In einem weiteren Versuch erniedrigten Regenwürmer die Konzentration an Catalpol in den Blättern von *P. lanceolata*. Die Effekte der Regenwürmer auf pflanzliche Abwehrstoffe und Herbivore hingen von den Bodenbedingungen ab.

Es wurde angenommen, dass Regenwürmer die Symbiose zwischen Mykorrhiza und *P. lanceolata* beeinflussen. Regenwürmer hatten jedoch keinen signifikanten Effekt auf die Symbiose zwischen Mykorrhiza (*Glomus intraradices*) und *P. lanceolata*. Regenwürmer förderten das Sprosswachstum, wohingegen *G. intraradices* das Wurzelwachstum reduzierte. Die mykorrhizierten Pflanzen nahmen mehr Phosphor (P) auf, wohingegen der Gehalt an N in den Blättern der mykorrizierten Pflanzen geringer war als bei den nicht-mykorrhizierten Pflanzen. Regenwürmer und Mykorrhiza zusammen verkürzten die Entwicklungsdauer von *M. persicae* auf *P. lanceolata*, möglicherweise durch eine Erhöhung der Nahrungsqualität der Pflanzen für Herbivore.

Phytophage Nematoden (*Meloidogyne incognita*) beeinflussten die N-Aufnahme aller Pflanzen aus der Streu. Der geringe Befall durch *M. incognita* führte wahrscheinlich zu einer Zunahme an Wurzelexsudaten, die die mikrobielle Zersetzung der Streu förderten.

Die Ergebnisse der Arbeit zeigen, dass Regenwürmer das Pflanzenwachstum, die Konkurrenz zwischen Pflanzen, pflanzliche Abwehrstoffe und oberirdische Herbivore beeinflussen können. Diese Effekte sind jedoch von abiotischen Bodenbedingungen (Streuverteilung, Nährstoffverfügbarkeit) abhängig.

Summary

Effects of earthworms in combination with soil heterogeneity and other soil organisms (mycorrhiza, plant feeding nematodes) on plant and herbivore performance were investigated in greenhouse experiments. The aim of the studies was to understand the role of earthworms in terrestrial ecosystems concerning their effects on growth, competition and defensive chemistry of plants, and above-ground herbivore performance.

The plant species investigated were a grass (*Lolium perenne*), a forb (*Plantago lanceolata*) and a legume (*Trifolium repens*) that differ among other things in root morphology and N acquisition. I hypothesized that effects of earthworms and litter distribution affect plant species differently and change plant competition. The grass litter was labelled with the stable isotope ^{15}N to follow the N flow from the litter to the plants. To understand why earthworms reduced the reproduction of aphids (*Myzus persicae*) on *P. lanceolata*, I analysed defensive compounds in *P. lanceolata*. Since other soil organisms also affect growth and competition of plants, interactions between earthworms and mycorrhiza or plant-feeding nematodes were investigated.

As predicted, soil heterogeneity (i.e. litter distribution) and earthworms affected plant species differently. Because of its symbiosis with N fixing bacteria, *T. repens* was generally more independent of soil conditions than *L. perenne* and *P. lanceolata*. Shoot and root biomass of *L. perenne* and *P. lanceolata* were increased by earthworms, while growth of *T. repens* was not affected. Earthworms enhanced N uptake in all plant species. Litter concentrated in a patch enhanced mainly growth of *L. perenne*. When the plants were growing in interspecific competition, earthworms increased the competitive ability of *L. perenne* against *T. repens*, while the spatial distribution of litter had no effect on plant growth and competition.

Earthworms reduced aphid reproduction on *P. lanceolata*, and affected the defensive chemistry of *P. lanceolata*. Earthworms increased the concentrations of N and phytosterols in the leaves, but the effect depended on the litter distribution. The concentration of phytosterols increased with increasing N concentration in the leaves. Litter concentrated in a patch led to an increase in the concentration of aucubin in the leaves. In another experiment, earthworms reduced the concentration of catalpol in

P. lanceolata shoots. The effects of earthworms on plant defensive chemistry and herbivores depended on soil conditions.

It has been assumed that earthworms affect the symbiosis between mycorrhiza and plants. However, no significant effects of earthworms on the symbiosis between mycorrhiza (*Glomus intraradices*) and *P. lanceolata* were found. Earthworms enhanced shoot biomass, while *G. intraradices* reduced root biomass of *P. lanceolata*. The mycorrhizal plants took up more P, but the N content in the leaves of mycorrhizal plants was lower compared to non-mycorrhizal plants. Earthworms and mycorrhiza combined accelerated the development of aphids (*M. persicae*), probably by increasing plant host quality for herbivores.

Plant-feeding nematodes (*Meloidogyne incognita*) increased plant uptake of litter N. The low root infestation by *M. incognita* has probably enhanced root exudation that stimulated the microbial decomposition of the litter.

The results of the present study document that earthworms affect growth, competition, defensive chemistry of plants, and above-ground herbivores. However, the effects depend on abiotic soil conditions such as soil heterogeneity and nutrient availability.

1 General Introduction

1.1 Below-ground and above-ground compartments of terrestrial ecosystems

Terrestrial ecosystems consist of interdependent below-ground and above-ground compartments (Wardle 2002). Traditionally these compartments were investigated in isolation, without considering the interactions between below-ground and above-ground organisms (Scheu 2001; van der Putten 2001). Plants play a key role in terrestrial ecosystems. As primary producers they provide higher trophic levels with food and shelter (Price 2002). Since plants connect the below-ground with the above-ground compartment, changes in plant performance may influence ecosystems in both directions.

Much is known about the interactions between above-ground organisms and plants. Knowledge on the interactions between soil organisms and plants is scarcer; studies mainly focus on economically important species including pests (e.g. plant-feeding nematodes) and beneficial organisms (e.g. mycorrhiza, earthworms).

Below-ground organisms may interact with plant roots either directly or indirectly. Direct interactions are the consequence of actual trophic links between roots and soil organisms (e.g. root herbivory, mycorrhizal symbiosis), while indirect interactions are mediated through soil nutrients, soil structure or other soil biota (Wurst and Jones 2003). Both direct and indirect interactions of soil organisms affect plant growth and chemistry (Fig. 1.1).

The effects of soil organisms on plant performance may cascade upwards to affect above-ground herbivore performance. Most of the studies investigated effects of root herbivores (Gange and Brown 1989; Moran and Whitham 1990; Masters and Brown 1992; Masters 1995a, b), vesicular-arbuscular mycorrhiza (Gange and West 1994; Gange and Nice 1997; Gange et al. 1999; Gange 2001) and decomposers (Scheu et al. 1999; Wurst and Jones 2003) on above-ground invertebrates. The plant-mediated interactions between soil organisms and above-ground herbivores are likely driven by changes in water uptake, nutrient uptake and/or defensive compounds.

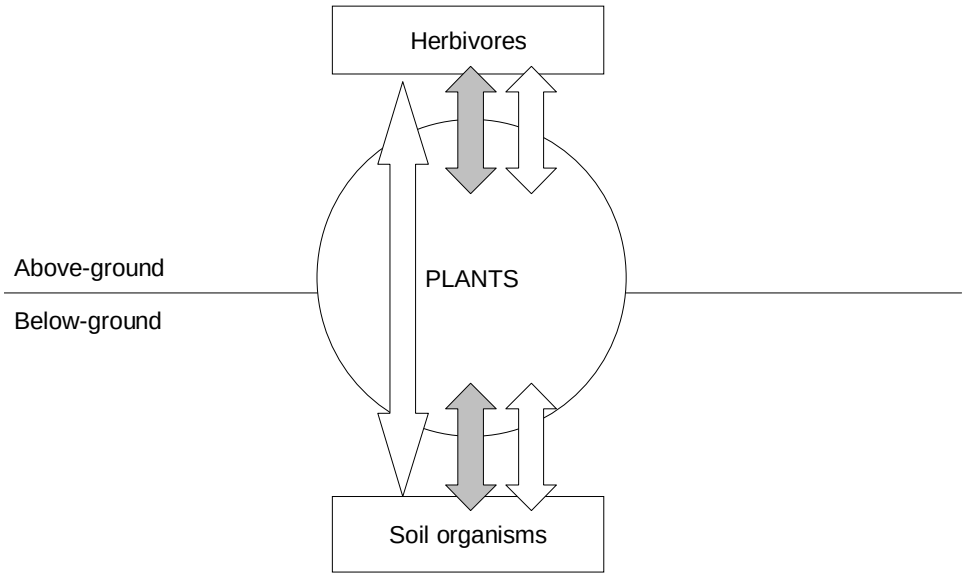

Fig. 1.1 Basic scheme of interactions between soil organisms and above-ground herbivores. Grey lines are direct trophic links, while white lines are indirect effects mediated through changes in soil conditions, plant performance or other organisms.

Until now, studies that follow the effects of soil organisms to higher trophic levels (i.e. predators or parasitoids) above the ground are scarce (Masters et al. 2001; Wurst and Jones 2003; Gange et al. 2003). Masters et al. (2001) documented that root herbivores can increase the abundance of both seed herbivores and their parasitoids on *Cirsium palustre*. Wurst and Jones (2003) found no effects of earthworms on the aphid parasitoid *Aphidius colemani*, but the number of parasitized aphids and the size of the emerging parasitoids were increased by soil fertility. Gange et al. (2003) reported lower parasitism of leaf-miners (*Chromatomyia syngenesiae*) by parasitoids (*Diglyphus isaea*) on mycorrhizal *Leucanthemum vulgare* in the field.

1.2 Earthworms as components of terrestrial ecosystems

Since the early work of Hensen (1877) and Darwin (1881), earthworms are known to be beneficial for soil fertility. Since then, effects of earthworms on plant performance have been intensely investigated, but most of the studies focussed on single crop plant species (Scheu 2003). Beside the strong effects of earthworms on soil processes, such as mineralization and aeration, and plant growth (Edwards and Bohlen 1996), the influence of earthworms on terrestrial ecosystems is widely unknown. As ecosystem engineers (Lawton 1994) earthworms affect the composition and function of terrestrial ecosystems. There is evidence that earthworms change the composition of plant communities by promoting individual plant species (Hopp and Slater 1948; Thompson et al. 1993; Schmidt and Curry 1999). Earthworm effects may also cascade up to herbivores (Scheu et al. 1999; Wurst and Jones 2003) and have the potential to affect above-ground multitrophic interactions (Wurst and Jones 2003). Surprisingly few studies investigate the effects of earthworms in a broader ecological context.

Earthworms and soil heterogeneity

Resources are not uniformly distributed in soil (Hutchings and de Kroon 1994; Hutchings and Wijesinghe 1997); rather there are nutrient-rich patches. The spatial distribution of nutrients is known to affect nutrient allocation and plant growth (reviewed by Robinson 1994) and may change plant competition (Cahill and Casper 1999; Fransen et al. 2001; Hodge 2003). Although interactions between plants and earthworms likely depend on resource distribution, few studies (Kreuzer 2000) have investigated their combined effects on plant growth and competition.

Earthworms and vesicular-arbuscular mycorrhiza (VAM)

Earthworms are known to be vectors for the dispersal of VAM propagules (Rabatin and Stinner 1988, 1989; Reddell and Spain 1991; Gange 1993). Pattinson et al. (1997) reported that an increased density of earthworms decreases root colonisation by VAM in *Trifolium subterraneum*. Since earthworms process great amounts of soil by burrowing and casting (Scheu 1987) and selectively feed on fungal mycelia (Bonkowski et al. 2000a), they may decrease the infectivity of VAM by disruption of the hyphal network. Mechanical soil disturbance has been documented to reduce the infectivity of VAM hyphae (Jasper et al. 1989). By enhancing nutrient availability in soil, earthworms

may also alleviate the mycorrhizal dependency of the plants. Only the study of Tuffen et al. (2002) has investigated the combined effects of earthworms and VAM on plant growth. Earthworms, but not VAM, enhanced plant growth, and both increased transfer of ^{32}P between *Allium porrum* plants. No significant interaction between earthworms and VAM on plant growth or ^{32}P dynamics was detected.

Earthworms and plant-feeding nematodes

Earthworms have been documented to increase the number of plant-feeding nematodes (Ilieva-Makulec and Makulec 2002). Generally, the number of plant-feeding nematodes increases with N fertilization (Sohlenius and Boström 1986; Yeates 1987; Todd 1996; Verschoor 2001). Since earthworms increase N availability for plants, they likely improve the host quality for plant-feeding nematodes. Plant-feeding nematodes affect plant growth and may change plant competition (Cook et al. 1992; Chen et al. 1995; Pantone 1995; van der Putten and Peters 1997; Verschoor et al. 2002). Thus, interactions between earthworms and plant-feeding nematodes likely modify effects of nematodes on plant growth and community structure.

1.3 Objectives

I investigated the effects of earthworms in combination with abiotic (soil heterogeneity) and biotic (mycorrhiza, nematodes) factors on growth and chemistry of plants, and herbivore performance. The experiments are presented chronically, because new questions and ideas arose during the interpretation of the results, and were followed up in later experiments.

I started with an experiment on the effects of earthworms and litter distribution (homogeneous vs. patch) on a grass (*Lolium perenne* L.), a forb (*Plantago lanceolata* L.) and a legume (*Trifolium repens* L.). Since the three plant species differ in root morphology and presumably root foraging strategies, I hypothesized that effects of earthworms and litter distribution depend on plant species. Additionally, the reproduction of aphids (*Myzus persicae* Sulzer) on the different plant species was assessed to document possible plant-mediated effects of earthworms or litter distribution on a higher trophic level above the ground (Chapter 2).

The fact that earthworms reduced the reproduction of *M. persicae* on *P. lanceolata* led to the second experiment. I hypothesized that plant defensive chemistry was affected by earthworms, and analysed concentrations of phytosterols and iridoid glycosides in *P. lanceolata* shoot samples from the first experiment (Chapter 3).

In the third experiment I investigated the interactions between earthworms and vesicular-arbuscular mycorrhiza (VAM) and their combined effects on plant and aphid performance. Since both earthworms and VAM have strong effects on plants and herbivores, possible interactions may change the separate influence of one of them (Chapter 4).

In the last experiment I used a similar set-up as in the first experiment to test the effects of earthworms, litter distribution and plant-feeding nematodes on the plants in interspecific competition. Considering the results of the first experiment, I interpreted the reactions of the same plant species in competition (Chapter 5).

The results of the experiments are synthesized and discussed in the general discussion, and an outline is given for future research (Chapter 6).

2 Effects of earthworms and organic litter distribution on plant performance and aphid reproduction

2.1 Abstract

Human management practices and large detritivores such as earthworms incorporate aboveground plant litter into the soil thereby forming a heterogeneous soil environment from which plant roots extract nutrients. In a greenhouse experiment we investigated effects of earthworms and spatial distribution of ^{15}N-labelled grass litter on plants of different functional groups (*Lolium perenne* grass/ *Plantago lanceolata* forb/ *Trifolium repens* legume). Earthworms enhanced shoot and root growth in *L. perenne* and *P. lanceolata* and N uptake from organic litter and soil in all plant species. Litter concentrated in a patch (compared with litter mixed homogeneously into the soil) increased shoot biomass and ^{15}N uptake from the litter in *L. perenne* and enhanced root proliferation in *P. lanceolata* when earthworms were present. Growth of clover (*T. repens*) was rather independent of the presence of earthworms and organic litter distribution: nevertheless, clover took up more nitrogen in the presence of earthworms. The magnitude of the effects of earthworms and organic litter distribution differed between the plant species, indicating different responses of plants with contrasting root morphology. Aphid (*Myzus persicae*) reproduction was reduced on *P. lanceolata* in the presence of earthworms. We suggest that earthworm activity may indirectly alter plant chemistry and hence defence mechanisms against herbivores.

2.2 Introduction

Nitrogen in soil is mainly bound in organic forms (e.g. litter) and the amount of nitrogen available for plant growth depends on complex interactions between roots, microorganisms and soil animals (Bonkowski et al. 2000b). Organic matter is not distributed uniformly (Hutchings and de Kroon 1994*;* Hutchings and Wijesinghe 1997): rather, there are patches with high concentrations of organic residues in soil. Several studies have investigated the influence of organic matter distribution on root foraging (e.g. Wijesinghe and Hutchings 1997; Hodge et al. 1998, 1999), but few studies have included the effects of soil animals (Bonkowski et al. 2000b). Earthworms, for example, affect soil parameters such as litter distribution, soil structure and mineralisation that subsequently influence plant growth. The formation of litter patches by earthworms differs from that by human activities (e.g. ploughing). Earthworms form small patches of organic matter by casting, whereas ploughing results in single layer patches of litter in soil. This is likely to affect plant growth differently. Furthermore, the effects of earthworms may vary with organic residue management. If there are differences in plant growth due to litter distribution or earthworm activity, how do these two factors interact and affect plants of contrasting root morphology?

Plant performance is known to be affected by earthworms and the distribution of organic residues. Earthworm activity enhances nitrogen availability in soil (Haimi et al. 1992; Alphei et al. 1996) and thus stimulates plant performance (e.g. Atlavinyté et al. 1968; Haimi et al. 1992). Patchy distribution of nutrients leads to enhanced root proliferation and nutrient uptake in many plant species (reviewed by Robinson 1994). From a more holistic, multitrophic point of view the activity of below-ground biota and organic matter distribution may affect not only plant nutrient status, but also, indirectly, plant herbivores. It has been shown that, by changing plant performance, earthworms may indirectly influence above-ground aphid reproduction (Scheu et al. 1999; Wurst and Jones 2003). As far as we are aware, the indirect effects of organic residue distribution in soil on herbivore performance have not been investigated, though the spatial distribution of organic residues by tilling is current practice in agriculture.

In the present study we investigated effects of endogeic earthworms, in combination with litter distribution (homogeneous/patch), on plants of different functional groups (grass/forb/legume) and performance of aphids on those plants. The experimental plants differed in root morphology, since effects of below-ground conditions on plant

performance may depend on root morphology and thus plant foraging strategies. *Lolium perenne*, the grass, has fine roots with high plasticity and was expected to respond to resource heterogeneity by root proliferation. *Plantago lanceolata*, the forb, has a hierarchic and less flexible root system with a main root ("tap root") and was expected to be less sensitive to resource heterogeneity than the grass. Earthworms were expected to promote growth of both non-legumes. *Trifolium repens*, the legume, possesses root symbionts (*Rhizobium* spec.) that fix N_2 from the air and was therefore expected to respond little to earthworms and resource distribution. Reproduction of aphids on the plants was expected to increase with increasing nitrogen uptake by the plants.

2.3 Materials and Methods

A nutrient-poor loamy soil was collected (10-40 cm below the surface) from a cultivated meadow (Roßberg near Darmstadt, Germany) on 1 August 2001 and sieved through a 1 cm mesh. The soil contained approximately 0.087% nitrogen and 1.58% carbon (C/N ratio 18.2) and had a pH (KCl) of 6.9 and a water content of 15.1% of dry weight.

Seeds of *Lolium perenne* L. (Poaceae) (Conrad Appel, Darmstadt, Germany) were sown in 10 pots (95x11x14 cm) in sandy soil (Flughafen Darmstadt/Griesheim, Germany) in a greenhouse (16 h light, 18°/20°C night/day temperature). Twenty days after sowing three pots were labelled by watering with $^{15}NH_4{}^{15}NO_3$ [142.8 mg 98 atom% $^{15}NH_4{}^{15}NO_3$ (Isotec Inc., Miamisburg, USA) in 1 l H_2O dest. per day] for 21 days. Plants in two other pots were labelled by spraying with 142.8 ml $^{15}N_2$ urea solution [250 mg 99 atom% $^{15}N_2$ urea (Isotec Inc., Miamisburg, USA) in 500 ml distilled H_2O plus 1 ml 30% Brij 35 (Skalar Chemical, Breda, Netherlands) per day] for 21 days (Schmidt and Scrimgeour 2001). The plants of the five remaining pots were watered with tap water. Two months after sowing the plants were harvested and the shoots and roots were dried at 100°C for 72 h. For the experiment 21.25 g shoot litter labelled with $^{15}N_2$ urea solution (73.97 atom% ^{15}N) and 101.16 g unlabelled shoot litter were cut into pieces (<1 cm) and mixed carefully. To each experimental container 1.43 g of this litter was added (13.14 atom% ^{15}N). Green rather than senescent leaf material was used to simulate mulching with green biomass which is frequently done prior to sowing pastures or meadows.

On 6 July, seeds of *Trifolium repens* L. (Fabaceae) and on 10 July seeds of *Plantago lanceolata* L. (Plantaginaceae) and *L. perenne* were sown on wet paper in Petri dishes and placed in a refrigerator (2°C). Seven days after sowing the seeds were placed in a climate chamber (14 h light, 20°C). Germinated plants of each species were transplanted to seedling trays filled with soil [2:1, compost (ASB Grünland, Ludwigsburg, Germany): sandy soil (Flughafen Darmstadt/Griesheim, Germany)] 14 days after sowing.

Myzus persicae Sulzer (Aphididae) were cultured on *Brassica oleracea* L. (Brassicaceae) before they were transferred to the experimental plants. All individuals belonged to one clone (from Brooms Barn, UK) reared on *B. oleracea.* The aphid culture was kept in a climate chamber (14 h light, 20°C).

Experimental set-up

Experimental containers consisted of PVC tubes (height 25 cm, diameter 10 cm) closed at the bottom by lids. The containers were equipped with ceramic cup lysimeters to allow drainage under semi-natural conditions. A pump maintained a vacuum of -200 to -500 hPa to drain the soil via a hose system.

On 18 August 84 experimental containers were set up in the greenhouse. Half of the containers (N = 42) were filled with 600 g soil, then 1.43 g ^{15}N-labelled *L. perenne* litter was put as a patch in the middle of the experimental container and another 600 g soil was added. The other 42 containers were filled with 1200 g soil mixed homogeneously with 1.43 g ^{15}N-labelled *L. perenne* litter.

Plants with approximately five leaves were planted from the seedling trays into the containers. Two plants of each species were planted separately in containers within 2 cm distance from the edge establishing containers with either *L. perenne, P. lanceolata* or *T. repens*. Then 50 g soil was added on the surface and each container was watered with 100 ml H_2O (dest.). On 22 August two individuals of *Aporrectodea caliginosa* Savigny (Lumbricidae) and one individual of *Octolasion tyrtaeum* Savigny (Lumbricidae) were placed in half of the containers with homogeneously or patchy distributed litter of each of the plant species treatments. The earthworms were kept in the experimental soil prior to the start of the experiment to prevent introduction of microorganisms that are not present in the control soil (without earthworms). These earthworm species are among the most abundant earthworms in arable systems.

The experimental containers were watered with 50 ml H_2O (dest.) every second day during the first week, then daily. They were placed in seven blocks on the greenhouse bench, each block contained one replicate of the 12 treatments. The blocks were redistributed randomly within the greenhouse every two weeks (16 h light, 18°/20°C night/day temperature, 60-70% humidity).

Parameters measured

To investigate whether aphid performance on the plants is influenced by the presence of earthworms or the litter distribution single aphids were enclosed in small clip cages (made from ventilated Petri dishes (diameter 3.5 cm) attached to either side of a hair clip) onto the leaves and their reproduction documented. On 11 September (Week 3) one adult aphid of *M. persicae* from the aphid culture was placed in a clip cage on an intermediately aged leaf of each *P. lanceolata* plant. On 12 and 13 September (Week 3) the same procedure was used with *L. perenne* and *T. repens*. Seven days later (Week 4) the numbers of offspring were counted.

On 6 November (Week 10) the plants were harvested. They were cut at ground level, freeze dried and weighed. The soil column in the experimental containers was cut with a knife into three layers of equal depth (top, centre, bottom), the roots in the layers were washed separately, dried at 100°C for 72 h and weighed. During the root washing procedure earthworms were collected, counted and weighed.

Freeze dried shoot samples were ground to a powder and approximately 1 mg was weighed into tin capsules. Isotope ratio $^{15}N/^{14}N$ was measured by an elemental analyser (NA 1500, Carlo Erba, Milan, Italy) coupled with a trapping box (type CN, Finnigan, Bremen, Germany) and a mass spectrometer (Finnigan, MAT 251). Atmospheric nitrogen served as base for $\delta^{15}N$ calculation and acetanilide (C_8H_9NO, Merck, Darmstadt, Germany) as internal standard (Reineking et al. 1993).

Statistical analyses

Data were analysed by factorial analysis of variance (ANOVA) in a general linear model (Statistica, Statsoft 2001). The plant species were tested separately, and the factors were litter distribution ("litter") and presence of earthworms ("earthworms"). Differences between the plant species were analysed by single factor ANOVAs including all the data. The distribution of errors was tested (Kolmogorov-Smirnov one-sample test) and if it was not normal, the data were log-transformed. In the earthworm

treatment there was one replicate where no earthworm survived, but given that the soil was affected by earthworm activity (burrowing, casting) this replicate was not excluded from the statistical analyses. In the aphid experiment those replicates were excluded from the statistical analysis where adults died in the clip cages and therefore did not reproduce.

2.4 Results

Earthworms

At harvest 70% of the 126 earthworms added were recovered. Total earthworm biomass added per experimental container decreased on average by 51% during the course of the experiment. The decrease in earthworm biomass was independent of plant species ($F_{[2,36]} = 3.02$, $P > 0.05$) and litter distribution ($F_{[1,36]} = 1.20$, $P > 0.05$).

Plant performance

At the end of the experiment (Week 10) the shoot biomass of *T. repens* was more then 3 times greater than the shoot biomass of *L. perenne* and *P. lanceolata* ($F_{[2,81]} = 178.87$, $P < 0.001$). The presence of the litter patch only increased shoot biomass of *L. perenne* (+23%). Earthworms caused an increase in shoot biomass of *L. perenne* and *P. lanceolata* by 49% and 20%, respectively, but did not affect shoot biomass of *T. repens*. In contrast to the shoot biomass, the average root biomass of *T. repens* was smaller (-42%) compared with *L. perenne* and *P. lanceolata* ($F_{[2,81]} = 12.62$, $P < 0.001$). The presence of the litter patch enhanced root biomass of *P. lanceolata* when earthworms were present (+89%). As for shoots the presence of earthworms increased the root biomass of *L. perenne* and *P. lanceolata* by 113% and 68%, respectively, but did not affect that of *T. repens* (Fig. 2.1, Table 2.1). In *T. repens* neither earthworm presence nor litter distribution affected plant growth except a local increase in root biomass (+128%) due to the litter patch in the central layer of the soil column ($F_{[1,24]} = 11.73$, $P < 0.01$; Fig. 2.2).

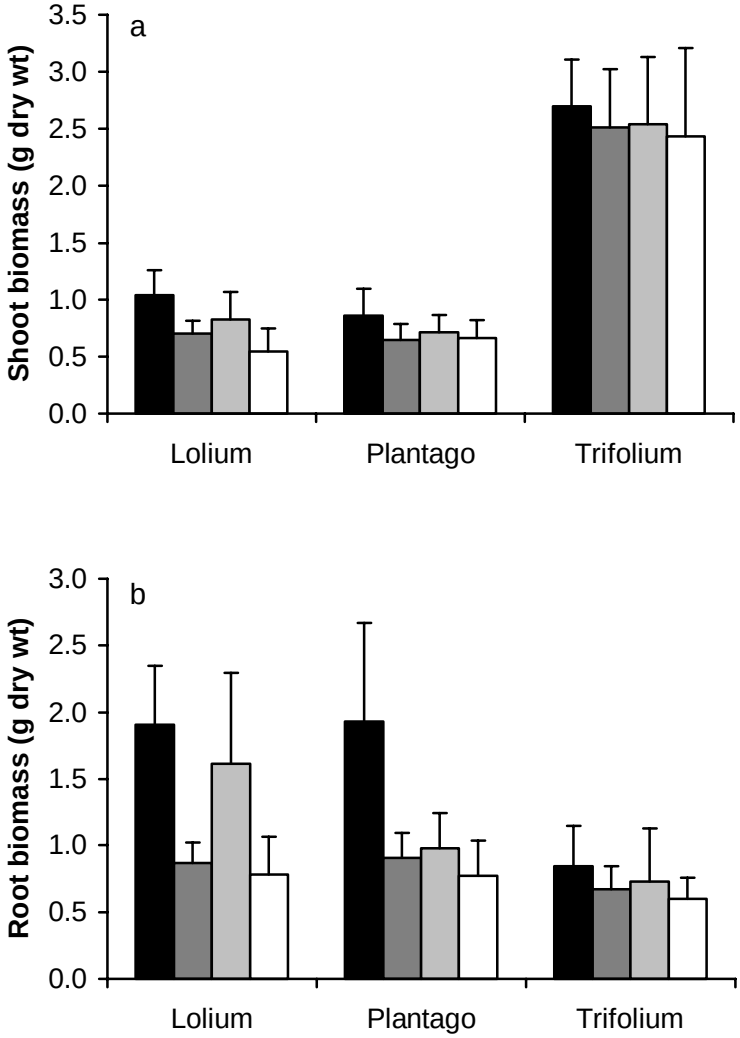

Fig. 2.1 Effect of litter distribution and earthworms (■ patch with earthworms ■ patch without earthworms ■ homogeneous with earthworms □ homogeneous without earthworms) on the (a) shoot and (b) root biomass of *L. perenne*, *P. lanceolata* and *T. repens* (mean + SD)

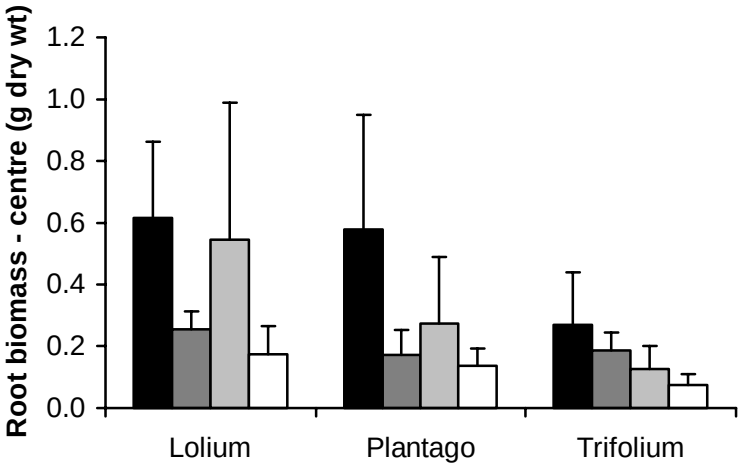

Fig. 2.2 Effect of litter distribution and earthworms (■ patch with earthworms ■ patch without earthworms ■ homogeneous with earthworms □ homogeneous without earthworms) on root biomass of *L. perenne*, *P. lanceolata* and *T. repens* in the centre of the soil column (mean + SD)

The total nitrogen content of *T. rep*ens shoots was more than twelve times greater than that of *L. perenne* and *P. lanceolata* ($F_{[2,81]} = 398.46$, $P < 0.001$). Earthworm presence increased shoot nitrogen content of *P. lanceolata* and *L. perenne* by 107% and 91%, but that of *T. rep*ens by only 31% (Fig. 2.3, Table 2.1).

^{15}N contents of shoots followed a similar pattern to that of total N content. In shoots of *T. rep*ens ^{15}N content was ten times greater than in *L. perenne* and *P. lanceolata* ($F_{[2,81]} = 126.39$, $P < 0.001$). Earthworm presence increased ^{15}N content in shoots of *P. lanceolata* and *L. perenne* by 239% and 195%, respectively, but that of *T. rep*ens by only 40%. Only in *L. perenne* the ^{15}N content of shoots was increased in presence of the litter patch (Fig. 2.3, Table 2.1).

The proportion of ^{15}N to total shoot N was on average 0.74 atom% and 0.61 atom% in *L. perenne* and *P. lanceolata*, respectively, but only 0.39 atom% in *T. rep*ens ($F_{[2,81]} = 61.08$, $P < 0.001$). Earthworm activity increased the proportion of ^{15}N in *L. perenne* and *P. lanceolata* by 83% and 64%, respectively, but that of *T. rep*ens by only 7% (Fig. 2.3, Table 2.1). In *L. perenne* the proportion of ^{15}N increased by 13% when the litter was concentrated in a patch, in *P. lanceolata* no litter effect was detected, and in *T. repens* the effect of earthworms was slightly stronger when the litter was homogeneously distributed in the soil.

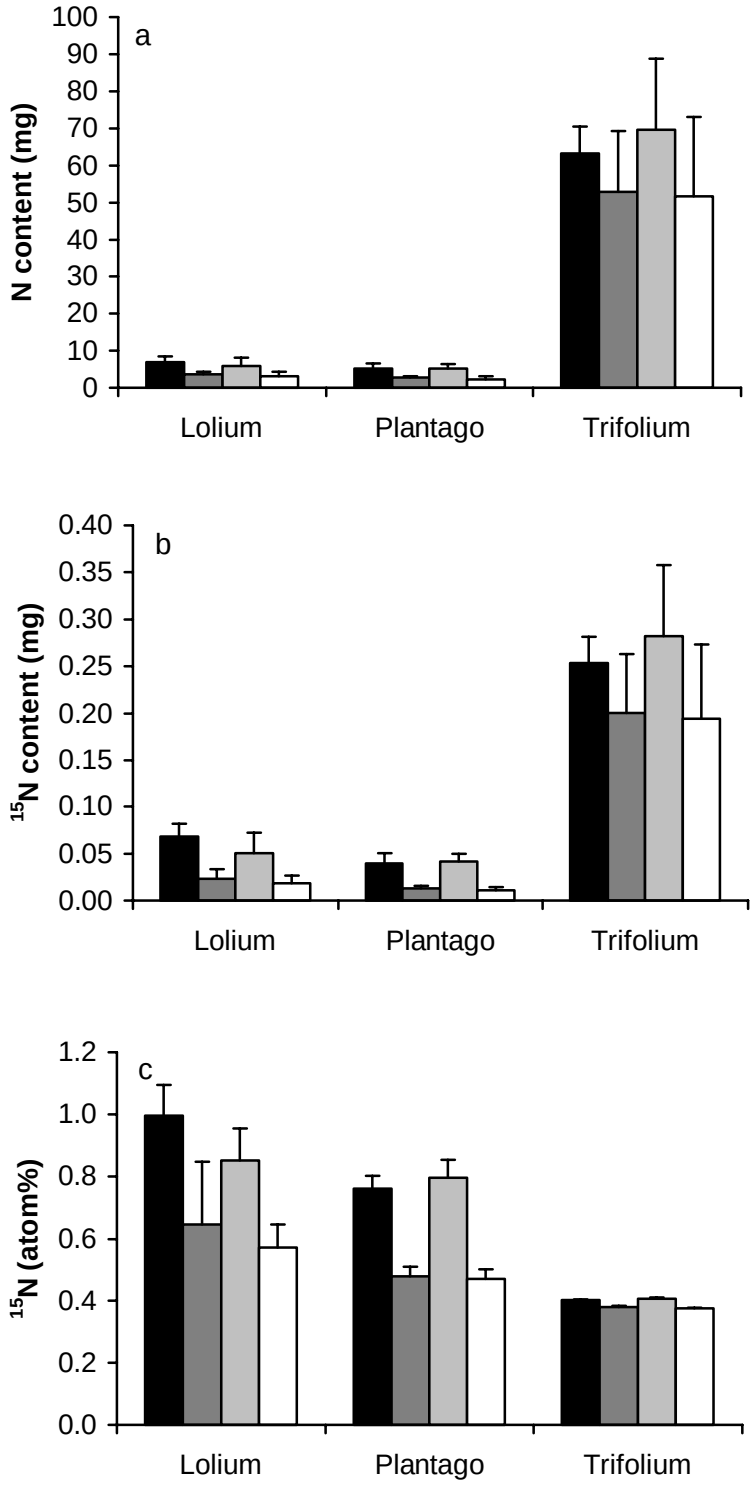

Fig. 2.3 Effect of litter distribution and earthworms (■ patch with earthworms ■ patch without earthworms ■ homogeneous with earthworms □ homogeneous without earthworms) on (a) total nitrogen content, (b) ^{15}N content and (c) proportion of ^{15}N (atom%) of shoots of *L. perenne*, *P. lanceolata* and *T. repens* (mean + SD)

Aphid performance

The plant species affected the reproduction of *M. persicae* ($F_{[2,59]} = 5.29$, $P < 0.01$). Aphids feeding on *L. perenne* produced fewer offspring than those on *P. lanceolata* and *T. repens.* Earthworm presence decreased the number of offspring on *P. lanceolata* by 39% (Fig. 2.4, Table 2.1).

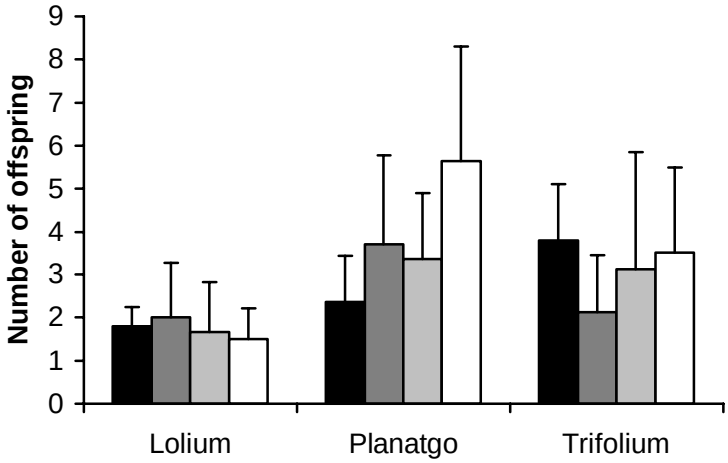

Fig. 2.4 Effect of litter distribution and earthworms (■ patch with earthworms ■ patch without earthworms ■ homogeneous with earthworms □ homogeneous without earthworms) on the number of offspring of *M. persicae* on *L. perenne, P. lanceolata* and *T. repens* 7 days after inoculation (mean + SD)

Table 2.1 ANOVA table for the three plant species studied on the effect of litter distribution and earthworms on shoot and root biomass, nitrogen content and ^{15}N content of shoots at Week 10 and aphid reproduction at Week 5

			Shoot biomass		**Root biomass**		**N (mg)**		**^{15}N (mg)**		**^{15}N (atom%)**			**Aphid reproduction**	
	Treatments	*df*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*df*	*F*	*P*
Lolium perenne	Litter (L)	1	4.91	0.036	1.31	0.263	1.71	0.203	4.36	0.048	4.95	0.036	1	0.34	0.573
	Earthworms (E)	1	17.21	< 0.001	32.52	< 0.001	28.01	< 0.001	50.19	< 0.001	41.88	< 0.001	1	< 0.01	0.976
	Lx E	1	0.19	0.667	0.40	0.532	0.21	0.653	1.30	0.265	0.50	0.487	1	0.11	0.743
	Error	24											12		
Plantago lanceolata	Litter (L)	1	1.20	0.284	11.48	0.002	0.20	0.656	< 0.01	0.987	0.76	0.391	1	4.05	0.056
	Earthworms (E)	1	4.34	0.048	14.77	< 0.001	49.44	< 0.001	104.74	< 0.001	373.55	< 0.001	1	6.26	0.020
	L x E	1	1.83	0.188	6.55	0.017	0.30	0.591	0.50	0.487	1.83	0.189	1	0.41	0.530
	Error	24											24		
Trifolium repens	Litter (L)	1	0.09	0.772	0.80	0.380	0.18	0.678	0.20	0.659	0.02	0.884	1	0.15	0.703
	Earthworms (E)	1	0.26	0.613	2.12	0.159	4.93	0.036	8.31	0.008	303.87	< 0.001	1	0.52	0.481
	L x E	1	0.05	0.821	0.03	0.855	0.35	0.560	0.52	0.479	7.94	0.010	1	1.30	0.273
	Error	24											14		

2.5 Discussion

The two plant species relying exclusively on nitrogen resources from soil were strongly affected by earthworms and litter distribution. Earthworm presence enhanced root and shoot biomass of *L. perenne* and *P. lanceolata*, but did not affect the biomass of *T. repens*. A similar increase in wheat biomass by earthworms, but no effect on *T. repens* was reported in a wheat-clover intercropping model system (Schmidt and Curry 1999). Organic residues concentrated in a patch led to an enhanced shoot growth in *L. perenne* and root growth in *P. lanceolata*; in *T. repens* root biomass was only increased nearby the litter patch. Obviously, *T. repens* is more independent of biotic (earthworms) and abiotic (litter distribution) soil conditions due to its symbiosis with nitrogen fixing bacteria. Nodules were abundant on *T. repens* roots in the present study and N_2-fixation must have been intense. The proportion of ^{15}N in *T. repens* (^{15}N atom% = 0.39) was closest to that of atmospheric air (^{15}N atom% = 0.3663033), also indicating that *T. repens* took up mainly atmospheric nitrogen with the aid of N_2 fixing rhizobia. The independence of clover from soil nitrogen acquisition was also reflected by the smaller root system but a more than tenfold greater biomass and nitrogen content of shoots compared to the other species.

Though, earthworms and the concentration of organic litter material in a patch enhanced plant growth in *L. perenne* and *P. lanceolata*, there were differences in responses between the two species. At harvest root proliferation was increased in presence of the litter patch in *P. lanceolata* but not in *L. perenne*. In a recent study *P. lanceolata* responded to soil heterogeneity by producing a more densely branched root system in presence of a nutrient enriched patch (Šmilauerová and Šmilauer 2002). In contrast, above-ground biomass of *L. perenne* was enhanced in presence of the litter patch, however, shoot biomass of *P. lanceolata* was not affected. Presumably, exploitation of the litter patch was more efficient in *L. perenne* since shoot biomass and ^{15}N uptake from the litter were increased in presence of the patch without investing more resources in root biomass. Similar increases in shoot biomass and ^{15}N uptake of *L. perenne* in presence of one layer of organic matter compared with organic matter mixed into the soil were observed by Bonkowski et al. (2000b), though in this study root biomass was also increased. However, in the study of Hodge et al. (2000b) growth and ^{15}N uptake of *L. perenne* was not affected by organic matter placement. They concluded that the plants respond to patches by proliferation, increased N capture and growth only when

the uptake of N from the residues is high (>10% of plant N). Their prediction that plants do not respond when the uptake is low (ca. 1% of plant N) was not supported by the present study. However, the plants in the present study were N-deficient and this might have increased their responsiveness to the litter patch.

In *P. lanceolata* the increase in root biomass in presence of the litter patch depended on earthworms. Root biomass more than doubled in presence of both earthworms and a litter patch, but was only slightly affected by earthworms when the litter was homogeneously distributed in the soil. Since the patch was concentrated in the middle of the experimental container, locally high concentrations of litter nutrients presumably were made available by earthworms which stimulated root growth of *P. lanceolata*. It is known that the production of lateral roots is stimulated by locally high nutrient concentrations in the soil (Drew 1975; Fitter 1976). In *Arabidopsis* a nitrate-inducible gene (*ANR 1*) was detected that regulates lateral root proliferation and hence root plasticity (Zhang and Forde 1998).

Earthworm activity enhanced nitrogen mobilization from litter and from soil. However, the earthworm-mediated increase in nitrogen uptake depended on the plant species. *P. lanceolata* profited most, followed by *L. perenne*; in *T. repens* the effect of earthworms on nitrogen uptake was less pronounced. In clover the positive effect of earthworms on nitrogen uptake from litter was slightly stronger when the litter was homogeneously distributed, while in the other plant species the effect of earthworms was independent of litter distribution.

Generally, nitrogen uptake and biomass of plants tended to be low when the litter was mixed in the soil and no earthworms were present, and high when the litter was concentrated in a patch and earthworms were present. This indicates that artificial mixing of organic residues into the soil differs strongly from mixing by earthworms. Since earthworms do not only mix organic residues, but increase mineralisation, this was not unexpected. Nevertheless, our results document the importance of earthworms for plant nitrogen uptake.

Earthworm activity resulted in decreased reproduction of *M. persicae* on *P. lanceolata*. As far as we are aware, no study has reported previously an earthworm-mediated reduction in aphid reproduction. Scheu et al. (1999) found the numbers of *M. persicae* offspring on *Poa annua* and *T. repens* to be increased in presence of earthworms. Similar results were reported for *Cardamine hirsuta* by Wurst and Jones (2003). In the present experiment aphid reproduction on *T. repens* was not affected by earthworms,

but tended to be increased by earthworm activity when the litter was concentrated in a patch. In the study of Scheu et al. (1999) grass litter was placed on top of the soil and earthworms increased reproduction of *M. persicae*. This indicates that effects of earthworms on aphid reproduction on clover might depend on the distribution of litter. Reproduction of *M. persicae* on *L. perenne* was low, presumably it was a non-preferred host, and no effects of earthworms or litter distribution could be detected.

Effects of earthworms on aphid reproduction have been explained by changes in plant nitrogen content, since aphids are highly susceptible to changes in host plant quality (Dixon 1985). However, changes in aphid reproduction in presence of earthworms were not correlated with plant tissue nitrogen concentration in the studies of Scheu et al. (1999) and Wurst and Jones (2003). It has been assumed that plant tissue nitrogen concentration does not reflect nitrogen availability for aphids, since aphids feed on phloem sap. In the present study aphid reproduction on *P. lanceolata* was reduced in presence of earthworms, despite the nitrogen content in above-ground plant tissue was increased. This indicates that earthworms may affect other plant compounds such as phytosterols or iridoid glycosides in *P. lanceolata* influencing aphid performance. Changes in plant chemistry might be indirectly driven by increased N availability (Bryant et al. 1983) resulting in increased production of secondary compounds.

The present study documented that earthworms and litter distribution strongly affect plant performance. Earthworms increased biomass in *L. perenne* and *P. lanceolata* and N uptake in all plant species studied. Compared to earthworms the effect of litter distribution was less pronounced and less general, but shoot biomass and ^{15}N uptake in *L. perenne* increased when the litter was concentrated in a patch and root biomass in *P. lanceolata* was enhanced when earthworms were also present. Clover was rather independent of soil conditions because of its symbiosis with N_2-fixing bacteria. We propose that earthworms and litter distribution may alter plant communities by affecting nitrogen uptake, plant growth and thereby competition. For the first time we detected a reduction in aphid reproduction by earthworms but this only occurred on *P. lanceoleta*. We suggest that earthworms not only affect N availability and subsequently plant growth, but may also indirectly change plant secondary compounds. Altering plant chemistry by earthworms may affect plant defence mechanisms against herbivores adding to the spectrum of indirect interactions between below- and above-ground communities.

3 Earthworms and litter distribution affect plant defensive chemistry

3.1 Abstract

Studies on plant defensive chemistry have mainly focused on plants in direct interaction with above-ground and occasionally below-ground herbivores and pathogens. Here we investigate whether decomposers and the spatial distribution of organic residues in soil affect plant defensive chemistry.

Litter concentrated in a patch (vs. homogeneously mixed into the soil) led to an increase in the aucubin content in shoots of *Plantago lanceolata*. Earthworms increased total phytosterol content of shoots, but only when the litter was mixed homogeneously into the soil. The phytosterol content increased and aphid reproduction decreased with increasing N concentration of the shoots.

The present study documents for the first time that earthworms and the spatial distribution of litter may change plant defensive chemistry against herbivores.

3.2 Introduction

Few studies have addressed the potential effects of soil biota on plant defensive chemistry (reviewed by van Dam et al. 2003) and all of these studies deal with below-ground organisms that directly interact with living plants (e.g. root herbivores). However, large decomposers, such as earthworms, that interact indirectly with plants via nutrients or soil structure have strong effects on plant growth and may also change plant chemistry (reviewed by Brussaard 1999; Scheu 2003). Furthermore, soil heterogeneity affects nutrient uptake, growth, and chemistry of plants (Robinson 1994), since plant roots proliferate in nutrient-rich patches and "forage" for nutrients (Hutchings and de Kroon 1994).

In a study investigating the effects of earthworms and litter distribution on plants of different functional groups (grass/forb/legume) and the reproduction of aphids (*Myzus persicae*) (Wurst et al. 2003), we found that the presence of earthworms reduced aphid reproduction on *Plantago lanceolata*. This was unexpected as other studies had suggested that earthworm activity increased aphid reproduction (Scheu et al. 1999; Wurst and Jones 2003). We speculated that earthworms may have modified the defensive chemistry of *P. lanceolata* (Wurst et al. 2003) and decided to conduct further analyses of iridoid glycosides and phytosterols in *P. lanceolata* that are presented in this publication.

Iridoid glycosides (aucubin and catalpol) are important secondary metabolites in *P. lanceolata*. Jarzomski et al. (2000) documented that nutrient availability determined iridoid glycoside concentration to a greater extent than herbivory. Since earthworms and litter distribution affect nutrient availability to plants, it might also change the concentration of iridoid glycosides. Deterrent effects of iridoid glycosides on generalist insect herbivores are well documented (Bowers and Puttick 1988; Puttick and Bowers 1988).

Primary plant metabolites also affect herbivore performance (Karban and Baldwin 1997) and may be important for plant defence. As well as being structural constituents of plant membranes, phytosterols are precursors of insect hormones and must be taken up with diet by insects that are unable to biosynthesise them directly (Svoboda et al. 1994). Since phytosterols are transported in the phloem sap (Lehrer et al. 2000) aphid performance may be affected by their concentration. Bodnaryk et al. (1997) reported reduced aphid reproduction on *Brassica napus* treated with a systemic fungicide that led

to a depletion of sitosterol, campesterol, and stigmasterol. However, only one study has shown that aphids (*Schizaphis graminum*) can convert dietary phytosterols into cholesterol, a precursor to moulting hormones, the ecdysteroids (Campell and Nes 1983). Although many studies have suggested that aphids rely on their endocytobionts to synthesise sterols (reviewed by Ishikawa 1989), there is strong evidence that endocytobiotic bacteria (*Buchnera* spec.) are not involved in the sterol nutrition of aphids (Douglas 1998).

Phytosterol composition in leaves is affected by soil organisms such as VA-mycorrhiza (Dugassa-Gobena et al. 1996) and root-born endophytic fungi (Vidal and Dugassa-Gobena 1999). In the latter study, phytosterols of tomato (*Lycopersicon esculentum*) and cabbage (*Brassica oleracea*) were affected by inoculation with endophytic fungi (*Acremonium* spec.) leading to changes in performance of diamondback moth (*Plutella xylostella*) on cabbage.

Here we investigate whether the presence of soil macrofauna (endogeic earthworms) and the spatial distribution of organic residues (homogeneous vs. patch) affect defensive chemistry (iridoid glycosides and phytosterols) of *P. lanceolata*.

3.3 Materials and Methods

Analyses of iridoid glycoside and phytosterol contents were carried out with freeze-dried *Plantago lanceolata* L. (Plantaginaceae) plants from a greenhouse experiment. Since the plants were part of a more extensive study, further data on biomass, total N and ^{15}N content (mg) of the plants, and aphid reproduction were provided in a former publication (Wurst et al. 2003).

Greenhouse studies

A total of 28 experimental containers (height 25 cm, diameter 10 cm) were set up in the greenhouse (16 h light, night/day temperature 18/20°C, humidity 60-70%). Half of the containers were filled with 600 g of a nutrient-poor mineral soil, and 1.43 g 15N-labelled *Lolium perenne* L. (Poaceae) shoot litter (13.14 atom% 15N) was placed as a "patch" in the middle of the container before a further 600 g of soil was added. The remaining 14 containers were filled with 1200 g of the same soil mixed homogeneously with 1.43 g 15N-labelled *L. perenne* shoot litter.

Two individuals of *P. lanceolata* with approximately 5 leaves were transplanted from seedling trays into the containers. Four days later, two individuals of the endogeic earthworm *Aporrectodea caliginosa* Savigny (Lumbricidae) and one individual of *Octolasion tyrtaeum* Savigny (Lumbricidae) were placed into half of the containers of each litter distribution type ("earthworm treatment"). The experimental containers were watered with 50 ml distilled H_2O daily and redistributed randomly within the greenhouse every 2 weeks. In Week 3 of the experiment, one adult aphid of *Myzus persicae* Sulzer (Aphididae) was placed into a clip cage (height 2 cm; diameter 4 cm) on an intermediately-aged leaf of each *P. lanceolata* individual. Seven days later, the number of offspring was counted. In Week 10, all plants were harvested. Plants were cut at ground level, freeze-dried, and weighed. The two individuals of *P. lanceolata* per pot were combined into a single sample.

Nitrogen

Freeze-dried shoot samples were ground into a powder and approximately 1 mg was weighed into tin capsules. Isotope ratio $^{15}N/^{14}N$ was measured by an elemental analyser (NA 1500, Carlo Erba, Milan, Italy) coupled with a trapping box (type CN, Finnigan, Bremen, Germany) and a mass spectrometer (MAT 251, Finnigan, Bremen, Germany).

Atmospheric nitrogen served as base for $\delta^{15}N$ calculation and acetanilide (C_8H_9NO, Merck, Darmstadt, Germany) as internal standard (Reineking et al. 1993).

Iridoid glycosides

From each ground, freeze-dried shoot sample (three replicates per treatment; N = 12), 25 mg were extracted overnight in methanol (95%). The supernatant was filtered and discarded. Phenyl-beta-d-glucopyranoside solution (200 µl; 2.5 mg/ml in 95% methanol) was added as internal standard, and the extract was evaporated to dryness. After partitioning, the ether layer was discarded and the water layer (that contains mainly iridoid glycosides and sugars) was evaporated to dryness. An aliquot was derivatized with Tri-Sil Z (Pierce Chemical Company, Rockford, IL, USA) and analyzed by gas chromatography (Gardner and Stermitz 1988; Stamp and Bowers 2000).

Phytosterols

Each freeze-dried sample (500 mg; five replicates per treatment; N = 20) was dissolved with 20 ml of solvent (10M KOH, 96% ethanol (1:5; v/v%) and 0.3% pyrogallol as antioxidant) in a water bath (80°C) for 2.5 h. Cholesterol solution (50 µl; 5 mg/ml in chloroform) was then added as an internal standard. Phytosterols were extracted by washing twice with 10 ml hexane and evaporating the hexane solution to dryness. After dissolving in 1.5 ml hexane, the extracts were transferred into auto-sampler vials and dried overnight in a thermo-block (50°C). The residual was dissolved with 240 µl N´-N´-dimethylformamide, and 60 µl bistrimethylsilyltrifluoracetamide (BSTFA) were added for methylation (70°C for 10 min). Samples were then injected into a gas chromatograph (modified after Newton 1989; Dugassa et al. 1996).

Statistical analyses

Data were analysed by analysis of variance (ANOVA) in a general linear model, and regression analyses (Statistica, Statsoft 2001). The explanatory variables were litter distribution ("litter") and presence of earthworms ("earthworms"). The distribution of errors (Kolmogorov-Smirnov one-sample test) and the homogeneity of variances (Levene test) were tested. The data were log-transformed if appropriate.

3.4 Results

Iridoid glycosides

Iridoid glycoside (aucubin and catalpol) content in shoots of P. lanceolata increased when the litter was concentrated in a patch ($F_{[1,6]} = 10.93$, $P = 0.016$). This was due to an increase of aucubin by 54% ($F_{[1,6]} = 15.02$, $P = 0.008$; Fig. 3.1), as catalpol was not affected by the litter distribution ($F_{[1,6]} = 0.89$, $P = 0.383$; Fig. 3.1). Earthworm presence did not affect iridoid glycoside content in shoots ($F_{[1,6]} = 1.44$, $P = 0.276$).

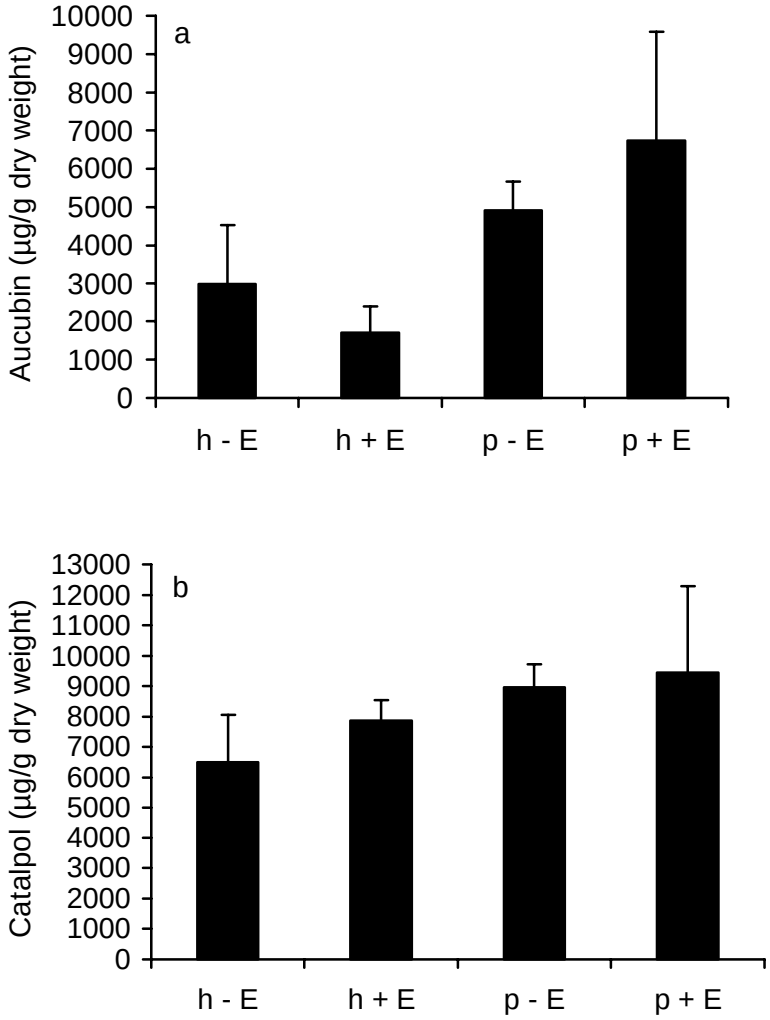

Fig. 3.1 Effect of litter distribution and earthworms [homogeneous without earthworms (h – E), homogeneous with earthworms (h + E), patch without earthworms (p – E), patch with earthworms (p + E)] on (a) the aucubin and (b) the catalpol content (µg/g dry weight) of *Plantago lanceolata* shoots (means + SD)

Phytosterols

When the litter was mixed homogeneously into the soil, total phytosterol, sitosterol, and campesterol content in shoots increased in the presence of earthworms by 75% ($F_{[1,15]}$ = 14.38, P = 0.002), 81% ($F_{[1,15]}$ = 15.76, P = 0.001) and 63% ($F_{[1,15]}$ = 12.33, P = 0.003), respectively (Fig. 3.2). No differences were observed when the litter was concentrated into a patch. Stigmasterol was not affected by earthworm presence ($F_{[1,15]}$ = 1.13, P = 0.305) or litter distribution ($F_{[1,15]}$ = 0.01, P = 0.906; Fig. 3.2).

Nitrogen

Earthworms increased shoot N concentration (% w/w) by 112% when the litter was mixed homogeneously into the soil, but only by 40% in treatments with a litter patch ($F_{[1,24]}$ = 7.96, P = 0.009 for the interaction litter x earthworms; Fig. 3.3).

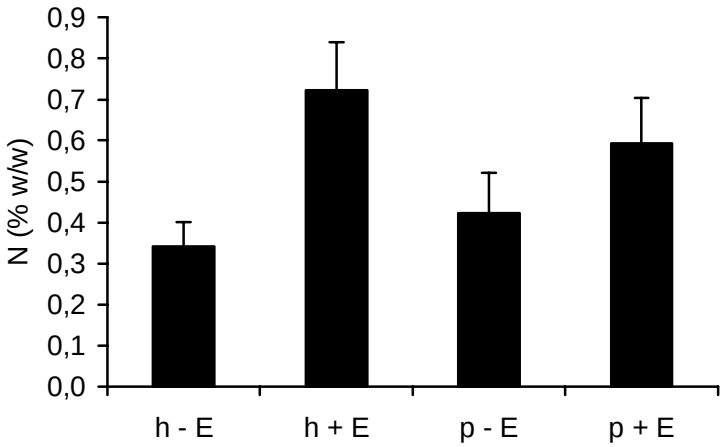

Fig. 3.3 Effect of litter distribution and earthworms [homogeneous without earthworms (h – E), homogeneous with earthworms (h + E), patch without earthworms (p – E), patch with earthworms (p + E)] on the nitrogen concentration (% w/w) of *Plantago lanceolata* shoots (means + SD)

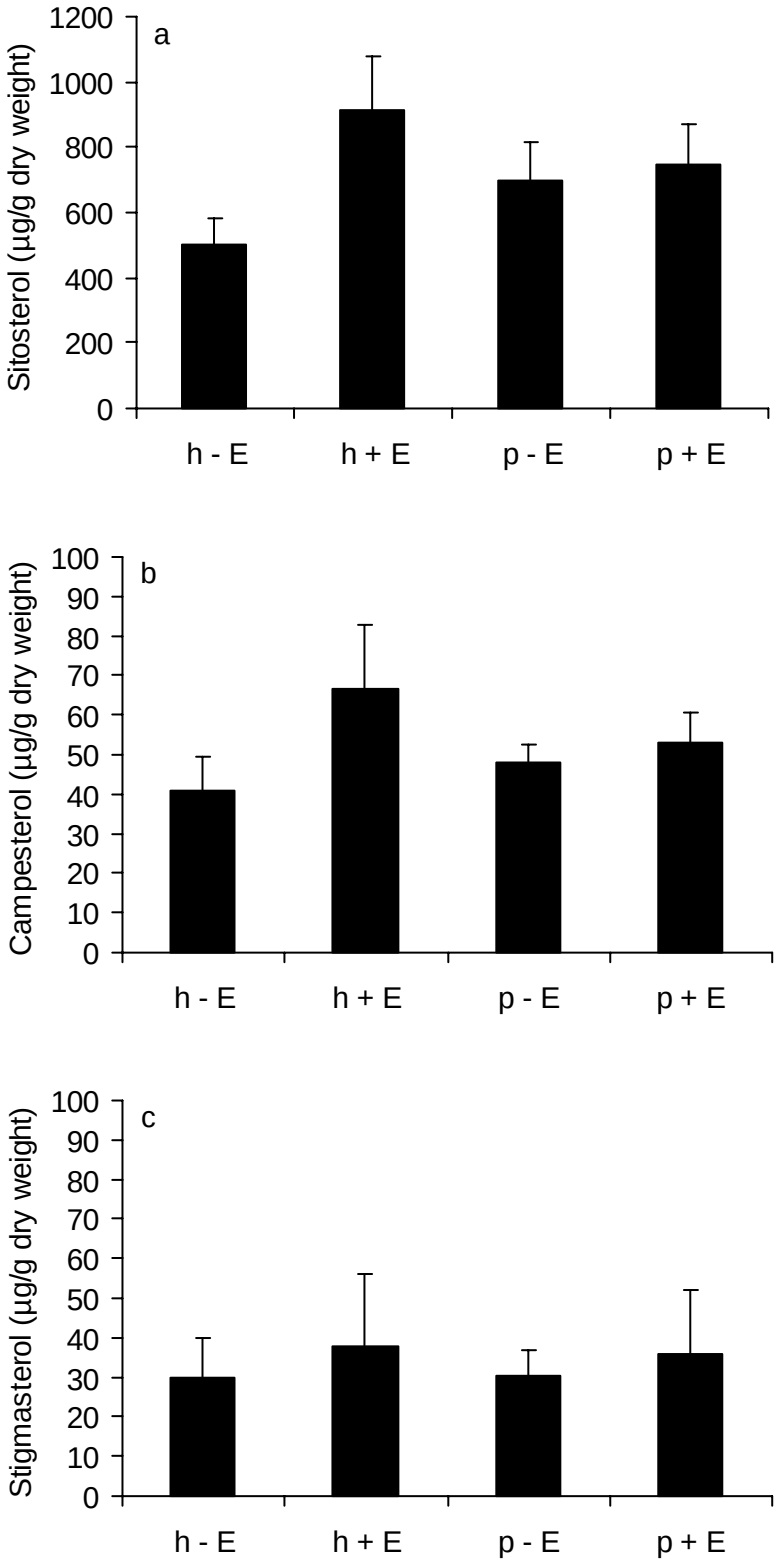

Fig. 3.2 Effect of litter distribution and earthworms [homogeneous without earthworms (h – E), homogeneous with earthworms (h + E), patch without earthworms (p – E), patch with earthworms (p + E)] on (a) sitosterol, (b) campesterol and (c) stigmasterol content (µg/g dry weight) of *Plantago lanceolata* shoots (means + SD)

Iridoid glycosides, phytosterols and nitrogen

Iridoid glycosides were not correlated with shoot N concentration ($F_{[1,8]}$ = 0.14, P = 0.723, R^2 = 0.13 for aucubin; $F_{[1,8]}$ = 0.46, P = 0.513, R^2 = 0.06 for catalpol). Total phytosterol, sitosterol, and campesterol content increased with the concentration of N in shoots ($F_{[1,17]}$ = 33.97, P < 0.001, R^2 = 0.67 for total phytosterols; $F_{[1,17]}$ = 32.83, P < 0.001, R^2 = 0.66 for sitosterol; $F_{[1,17]}$ = 20.60, P < 0.001, R^2 = 0.55 for campesterol; Fig. 3.4). Stigmasterol was not correlated with the N concentration in shoots ($F_{[1,17]}$ = 1.93, P = 0.183, R^2 = 0.10).

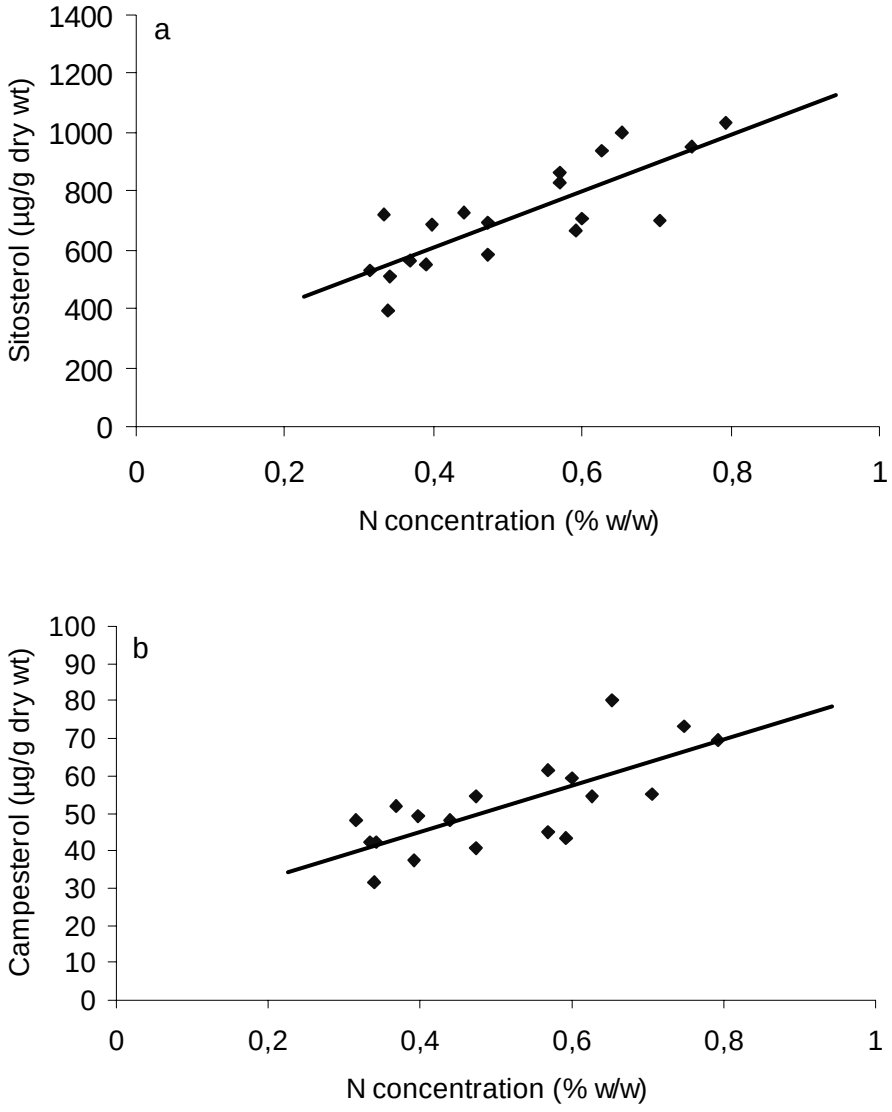

Fig. 3.4 Regression between N concentration (% w/w) and (a) sitosterol content, and (b) campesterol content (µg/g dry weight) in shoots of *Plantago lanceolata*

Correlations between aphid reproduction and plant chemistry

The number of offspring produced by *M. persicae* decreased with increasing shoot N concentration ($F_{[1,26]} = 7.68$, $P = 0.010$, $R^2 = 0.23$; Fig. 3.5), but was not correlated with the contents of aucubin ($F_{[1,8]} = 0.31$, $P = 0.596$, $R^2 = 0.04$), catalpol ($F_{[1,8]} = 3.14$, $P = 0.114$, $R^2 = 0.282$), sitosterol ($F_{[1,17]} = 0.30$, $P = 0.590$, $R^2 = 0.02$), campesterol ($F_{[1,17]} = 0.01$, $P = 0.938$, $R^2 < 0.001$) or stigmasterol ($F_{[1,17]} = 2.18$, $P = 0.158$, $R^2 = 0.11$).

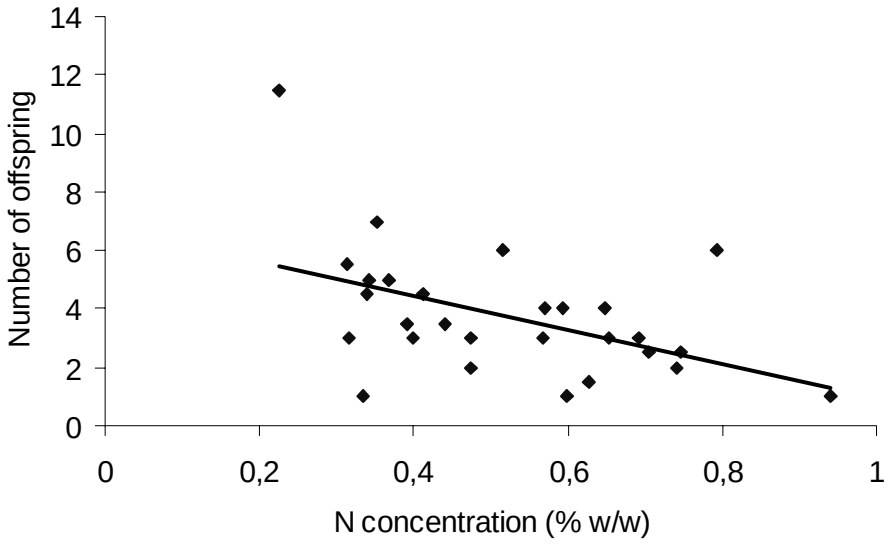

Fig. 3.5 Regression between N concentration (% w/w) in shoots of *Plantago lanceolata* and the number of offspring produced by *Myzus persicae* during 7 days

3.5 Discussion

Earthworms and the spatial distribution of organic residues significantly affected the plant chemistry of *P. lanceolata*. Aucubin content of shoots increased in the presence of a litter patch. Earlier studies documented that the aucubin concentration of *P. lanceolata* decreased with increasing fertilization, i.e., plant N concentration (Fajer et al. 1992; Jarzomski et al. 2000). Jarzomski et al. (2000) assumed that the iridoid glycoside concentrations reflected a physiological response to nutrient availability. In the present study, plants were not fertilized, and shoot N concentration and aucubin content were not correlated. The presence of a litter patch led to increased aucubin concentrations without influencing the N concentration of shoots. The mechanisms by which the spatial distribution of organic residues in soil affects plant secondary compounds are unknown and need further investigation.

The changed aucubin content did not affect reproduction of *M. persicae*. Gange and West (1994) reported increased levels of aucubin and catalpol in *P. lanceolata* in symbiosis with arbuscular mycorrhizal fungi (AMF), and improved growth and reproduction of *M. persicae* on the mycorrhizal plants. However, they suggested that plant morphology rather than chemistry was responsible for improved aphid performance on mycorrhizal plants. Increased levels of aucubin and catalpol in mycorrhizal plants presumably reduced growth, food consumption, and frass production of the generalist *Arctia caja* (Gange and West 1994). Consistent with these findings, iridoid glycosides are known to deter generalist insect herbivores (Bowers and Puttick 1988; Puttick and Bowers 1988). Variation in iridoid glycoside content due to soil conditions (e.g. litter distribution, presence of AMF, fertilization) may influence above-ground plant-herbivore interactions. Darrow and Bowers (1999) documented higher levels of aucubin in *P. lanceolata* roots grown under nutrient limitation. However, nutrient availability did not change the phytochemical response of plants to herbivore damage by larvae of *Junonia coenia*.

Total phytosterol, sitosterol, and campesterol content in shoots increased with N concentration. Earthworm presence increased the N concentration and the phytosterol content of *P. lanceolata* shoots when litter was mixed homogeneously into soil. The increase in phytosterols with increasing N concentration in shoots suggests that the availability of N affects the biosynthesis of phytosterols. By influencing N availability to plants, earthworms may indirectly affect the content of phytosterols in shoots. Since

insects rely on phytosterols to build up ecdysteroids as moulting hormones (Svoboda et al. 1994), earthworms may affect herbivore development and reproduction by indirectly changing the phytosterol content of plants. In the present study, the reproduction of *M. persicae* decreased with increasing shoot N concentration, but did not correlate with the phytosterols. Since aphids feed on phloem sap, changes in other chemical compounds transported in the phloem (e.g. amino acids) might have affected their performance.

The present study documents that decomposers and the spatial distribution of litter may alter the defensive chemistry of plants. Therefore, earthworms may not only increase plant nutrient contents (Wolters and Stickan 1991; Haimi et al. 1992; Alphei et al. 1996; Wurst et al. 2003), but also alter the defence mechanisms of plants. So far the majority of studies on plant defensive chemistry have concentrated on the above-ground world. It is time to broaden this perspective and include the effects of below-ground biota that directly (e.g. root herbivores, VAM) and indirectly (decomposers) interact with the roots.

4 Combined effects of earthworms and vesicular-arbuscular mycorrhiza on plant and aphid performance

4.1 Abstract

Vesicular-arbuscular mycorrhiza (VAM) and earthworms are known to affect plant and herbivore performance. Surprisingly few studies investigated their interactions.

In a greenhouse experiment we investigated the effects of earthworms (*Aporrectodea caliginosa*) and VAM (*Glomus intraradices*) on the growth and chemistry of *Plantago lanceolata* and the performance of aphids (*Myzus persicae*).

Earthworms did not affect VAM root colonisation. Earthworms enhanced shoot biomass, and VAM reduced root biomass. VAM increased plant phosphorus content, but reduced the total amount of nitrogen in leaves. Earthworms led to a preferential uptake of soil N compared to ^{15}N from the added grass residues in the absence of VAM. Earthworm presence reduced the concentration of catalpol. Earthworms and VAM combined accelerated the development of *M. persicae*, while the development tended to be delayed when only VAM or earthworms were present.

We suggest that earthworms promoted plant growth by enhancing soil N availability and may affect herbivores by influencing concentrations of secondary metabolites. VAM enhanced the P uptake of plants, but presumably competed with plant roots for N.

4.2 Introduction

Vesicular-arbuscular mycorrhizal fungi (VAM) form symbioses with 80% of all plant genera (Smith and Read 1997). The hyphal network of the fungal symbiont serves as extension of the plant root system and often increases nutrient uptake and plant growth. Generally the uptake of phosphorus is increased due to VAM root colonisation; for nitrogen no consistent effects of VAM have been documented (Goverde et al. 2000). In addition to plant P and N content, other plant compounds, such as secondary metabolites (Gange and West 1994) and phytosterols (Dugassa-Gobena et al. 1996), are affected by VAM root colonisation. Changes in foliar chemistry may influence plant herbivore interactions. Herbivore performance has been reported to be affected positively (Gange and West 1994; Borowicz 1997; Gange et al. 1999) or negatively (Rabin and Pacovsky 1985; Gange and West 1994; Gange et al. 1994; Gange 2001) by VAM colonisation, depending on both herbivore and fungal species present.

Other soil organisms, such as earthworms, enhance nutrient mineralisation in soil (Scheu 1994) and increase N uptake and plant growth (Haimi et al. 1992). Earthworm-mediated changes in plant chemistry may affect plant interactions with herbivores. Earthworm presence has been documented 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), but to reduce aphid reproduction on *Plantago lanceolata* (Wurst et al. 2003). Recently, earthworms also have been found to affect the concentration of phytosterols in leaves of *P. lanceolata* (Wurst et al. 2004a).

Studies investigating the combined effects of earthworms and VAM concentrated on earthworms as vectors for the dispersal of VAM propagules (Rabatin and Stinner 1988, 1989; Reddell and Spain 1991; Gange 1993). Pattinson et al. (1997) reported that an increased density of earthworms results in a transient decrease of root colonisation by VAM in *Trifolium subterraneum*. Since earthworms process large amounts of soil by burrowing and casting (Scheu 1987) and selectively feed on fungal mycelia (Bonkowski et al. 2000a), they might decrease the infectivity of VAM by disruption of the hyphal network. Mechanical soil disturbance has been documented to reduce the infectivity of VAM hyphae (Jasper et al. 1989). By enhancing nutrient availability in soil, earthworms might also alleviate the mycorrhizal dependency of the plants. Surprisingly only one study (Tuffen et al. 2002) has investigated the combined effects of earthworms and VAM on plant growth. Earthworms, but not VAM, enhanced plant growth, and both

increased ^{32}P transfer between *Allium porrum* plants. No significant interaction between earthworms and VAM on plant growth or ^{32}P dynamics was detected. Since the earthworms were separated from the root zone, potential direct effects of earthworms on root colonisation by VAM were precluded.

In the present study we investigated the effects of earthworms (*Aporrectodea caliginosa*) and VAM (*Glomus intraradices*) on both P and N uptake from soil and organic residues by *P. lanceolata* and the subsequent changes in plant growth, plant chemistry (primary and secondary metabolites), and host quality for aphid herbivores (*M. persicae*). Phytosterols (primary metabolites) and iridoid glycosides (secondary metabolites) were studied as potential determinants of aphid performance. Iridoid glycosides are important secondary metabolites in *P. lanceolata*; their deterrent effects on generalist herbivores are well documented (Bowers and Puttick 1988; Puttick and Bowers 1988). Phytosterols are precursors of insect hormones and must be taken up with diet by insects that are unable to biosynthesize them directly (Svoboda et al. 1994).

4.3 Materials and Methods

Greenhouse experiment

Experimental containers consisted of PVC tubes (height 25 cm, diameter 10 cm) closed at the bottom by lids. The containers were equipped with ceramic lysimeters which were connected via a hose system to a vacuum pump to allow drainage of the soil under semi-natural conditions (-200 to -500 hPa).

A nutrient-poor mineral soil from a cultivated meadow (Rossberg near Darmstadt, Germany) was used in the experiment (Wurst et al. 2003). The soil was sieved through a 1 cm mesh and autoclaved (121°C; 2 h).

Seeds of *Lolium perenne* L. (Poaceae) (Conrad Appel, Darmstadt, Germany) were sown in pots (95 x 11 x 14 cm) in a sandy soil (Flughafen Griesheim, Hessen, Germany) in a greenhouse (16 h light, night/day temperature 18/20°C). Twenty days after sowing the plants were labelled by spraying with 143 ml $^{15}N_2$ urea solution [250 mg 99 atom% $^{15}N_2$ urea (Isotec Inc., Miamisburg, USA) in 500 ml distilled H_2O plus 1 ml 30% Brij 35 (Skalar Chemical, Breda, Netherlands)] per day for 21 days (Schmidt and Scrimgeour 2001; Wurst et al. 2003).

The VAM inoculum consisted of culture substrate mixed with *Glomus intraradices* Schenck and Smith (Glomaceae) hyphae and spores (Isolat 150, Dr. C. Grotkass, Institut für Pflanzenkultur, Schnega, Germany).

Endogeic earthworms, *Aporrectodea caliginosa* Savigny (Lumbricidae), collected at the Jägersburger Wald (Darmstadt, Germany), were transferred three times into fresh autoclaved soil to minimise contamination of the experimental soil with naturally-occurring mycorrhizal propagules. This earthworm species is among the most abundant earthworm species of agriculture systems and gardens (Edwards and Bohlen 1996).

Myzus persicae Sulzer (Aphididae) were cultured on *Brassica oleracea* L. (Brassicaceae) before they were transferred to the experimental plants. All individuals belonged to one clone (from Brooms Barn, UK) reared on *B. oleracea.* The aphid culture was kept in a climate chamber (14 h light, 20°C).

Seeds of *Plantago lanceolata* L. (Plantaginaceae) (Conrad Appel, Darmstadt, Germany) were sown on wet filter paper in Petri dishes, watered with distilled H_2O and placed in the greenhouse. Germinated seedlings were transplanted into seedling trays filled with the autoclaved soil seven days after sowing.

On 13 March 2002, 28 experimental containers were set up in the greenhouse. The containers were filled with 1000 g (fresh weight) autoclaved soil. Then, 50 ml soil suspension [280 g fresh soil dispensed in 280 ml distilled H_2O from which 100 ml was filtered through a 25 µm mesh with 1400 ml distilled H_2O] was added to each container to inoculate the autoclaved soil with microorganisms. Four weeks later 400 g autoclaved soil mixed with 0.4 g autoclaved *L. perenne* roots (unlabelled), 0.1 g autoclaved *L. perenne* roots (11 atom% ^{15}N) and 60 g autoclaved VAM inoculum were added to half of the containers. The other half of the containers was treated in the same way except that the VAM inoculum was not autoclaved ("mycorrhiza treatment"). One *P. lanceolata* plant with 2-3 leaves (except the cotyledons) was planted in each container. All containers received 50 ml VAM washing filtrate [140 g VAM inoculum suspended in 280 ml distilled H_2O from which 100 ml was filtered through a 25 µm mesh with 1400 ml distilled H_2O] to correct for differences in microbial communities (bacteria, fungi, protozoa) between the "mycorrhiza" and the "non-mycorrhiza" treatment. One week later two specimens of *A. caliginosa* were placed in half of the containers ("earthworm treatment"). The experimental containers were watered with 50 ml distilled H_2O every second day during the first week, then daily. The containers were rearranged randomly every two weeks.

The performance of the aphid herbivores were tested in a "clip cage experiment". In Week 3 of the experiment two adult aphids from the *M. persicae* culture were placed each in one clip cage (height 2 cm; diameter 4 cm) on two intermediately aged leaves of each *P. lanceolata* plant. Seven days later (Week 4) the number of offspring was counted. The oldest nymph remained in the clip cage and the time until it started to reproduce was reported.

On 8 July (Week 10), the plants were harvested. Plants were cut at ground level, separated into inflorescences and leaves, freeze dried and weighed. Roots were washed, dried at 100°C for 72 h and weighed. During the root washing procedure earthworms were collected, counted and weighed.

For the assessment of VAM colonisation approximately 1.5 g fresh root samples were cleared with 20 ml 1N KOH (water bath, 80°C, over night). The KOH was decanted and the samples were washed twice with distilled H_2O. Then, 10 ml 3.7% HCl and 1-2 drops of ink (Quink, Parker Permanent Blue, Germany) were added. After two hours the root samples were transferred into Petri dishes and discoloured with lactic acid:H_2O (bidest.) (1:1). The VAM colonisation was estimated using the gridline intersection method (Giovannetti and Mosse 1980).

Chemical analyses

Nitrogen and carbon

Freeze dried leaf samples were ground to a powder and approximately 1 mg was weighed into tin capsules. Isotope ratio $^{15}N/^{14}N$ and ^{12}C were measured by an elemental analyser (NA 1500, Carlo Erba, Milan, Italy) coupled with a trapping box (type CN, Finnigan, Bremen, Germany) and a mass spectrometer (MAT 251, Finnigan, Bremen, Germany). Atmospheric nitrogen served as base for $\delta^{15}N$ calculation and acetanilide (C_8H_9NO, Merck, Darmstadt, Germany) as internal standard (Reineking et al. 1993). The ^{15}N atom% excess was calculated by subtracting the natural background (0.365) from the measured ^{15}N atom% in the plants.

Phosphorus

After chemical pulping, the phosphorus concentration of the leaves was determined photometrically by a molybdate blue method (Chapman and Pratt 1961).

Iridoid glycosides

Of each ground, freeze-dried green leaf sample (four replicates per treatment; N = 16), 100 mg were extracted overnight in methanol (95%). Phenyl-beta-d-glucopyranoside solution (25 µl; 10 µg/µl in 95% methanol) was added as internal standard, then the supernatant was filtered out and discarded and the extract was evaporated to dryness. After partitioning between water and ether, the ether layer was discarded and the water layer (that contains mainly iridoid glycosides and sugars) evaporated to dryness. An aliquot was derivatized with Tri-Sil Z (Pierce Chemical Company, Rockford, Illinois, USA) and injected into a gas chromatograph (Gardner and Stermitz 1988; Stamp and Bowers 2000).

Phytosterols

Green leaves were ground to a powder and 0.5 g of each sample (four replicates per treatment; N = 16) were dissolved in 20 ml of solvent (10M KOH, 96% ethanol (1:5; v/v%) and 0.3% pyrogallol as antioxidant) in a water bath (80°C) for 2.5 h. Cholesterol solution (50 µl; 5 mg/ml in chloroform) was added as an internal standard. Phytosterols were extracted by washing twice with 10 ml hexane and evaporated to dryness. After dissolving in 1.5 ml hexane, the extracts were transferred into auto-sampler vials and dried overnight in a thermo-block (50°C). The residual was dissolved with 240 µl N´-N´-dimethylformamide, and 60 µl bistrimethylsilyltrifluoracetamide (BSTFA) was added for methylation (70°C for 10 min). Samples were then injected into a gas chromatograph (Dugassa-Gobena et al. 1996).

Statistical analyses

Data were analysed by factorial analysis of variance (ANOVA) in a general linear model (Statistica, Statsoft 2001). The factors were mycorrhiza (“mycorrhiza”) and presence of earthworms (“earthworms”). The distribution of errors (Kolmogorov-Smirnov one-sample test) and the homogeneity of variances (Levene test) were inspected, and the data were log-transformed if necessary to match the prerequisites for ANOVA.

4.4 Results

Mycorrhiza

At the end of the experiment 39% of the roots of plants inoculated with *G. intraradices* were colonised by mycorrhiza. No mycorrhizal colonisation was detected in roots of the control plants ("without mycorrhiza"). Earthworms did not affect the colonisation of roots by *G. intraradices* ($F_{[1,12]} = 1.72$, $P = 0.214$).

Earthworms

At harvest 26 of the initially added 28 earthworms were recovered. Total earthworm biomass decreased on average by 34% during the course of the experiment. This decrease was independent of VAM presence ($F_{[1,12]} = 2.46$, $P = 0.142$).

Plant performance

Inoculation with *G. intraradices* caused a decrease in root biomass of *P. lanceolata* by 16% ($F_{[1,24]} = 6.86$, $P = 0.015$; Fig. 4.1). The amount of total nitrogen and ^{15}N excess (i.e. ^{15}N uptake from the added litter) in the roots were not affected, but root nitrogen concentration (% w/w) increased from 0.65% to 0.72% in the presence of mycorrhiza. VAM increased root carbon concentration (% w/w) from 39.28% to 40.81%. VAM increased total amount of phosphorus in the roots by 35% and the phosphorus concentration (% w/w) from 1.04% to 1.67% (Fig. 4.2; Table 4.1).

VAM did not affect the shoot biomass of *P. lanceolata*, but reduced the amount of total nitrogen in the leaves by 15% (Fig. 4.3; Table 4.1). ^{15}N excess was not affected by VAM. Leaf N concentration was on average 1.05% (w/w) and not affected by VAM. Leaf carbon concentration decreased from 42.15% to 41.00% when only VAM was present, and tended to decrease to 41.59% when only earthworms were present (significant mycorrhiza x earthworm interaction; Table 4.1). Inoculation with *G. intraradices* increased the total amount of P in the leaves by 75% (Fig. 4.2; Table 4.1) and the P concentration (% w/w) from 0.92% to 1.80%. The phytosterol concentration in the leaves was not affected by VAM ($F_{[1,12]} = 0.06$, $P = 0.811$), but correlated positively with leaf nitrogen concentration ($F_{[1,14]} = 14.32$, $P = 0.002$; $R^2 = 0.506$; Fig. 4.5).

Presence of earthworms neither affected root biomass (Fig. 4.1), nor the amount of total N, ^{15}N excess and the concentration of N in the roots. However, earthworms decreased the proportion of ^{15}N (atom% excess) in roots by 20%, but only in the absence of mycorrhiza (significant mycorrhiza x earthworm interaction; Fig. 4.4; Table 4.1). Carbon concentration, total amount of phosphorus and P concentration in roots were not affected by earthworms. In contrast to the roots, earthworms increased total shoot biomass by 15% ($F_{[1,24]} = 4.69$, $P = 0.041$; Fig. 4.1). In the leaves, earthworm presence decreased the proportion of ^{15}N (atom% excess) by 14%, and this effect tended to be stronger in the absence of mycorrhiza (-24%; Fig. 4.4; Table 4.1). In the presence of earthworms the concentration of catalpol in the leaves was reduced ($F_{[1,12]} = 33.15$, $P < 0.001$; Fig. 4.6). The phytosterol concentration in the leaves was not affected by earthworms ($F_{[1,12]} = 1.85$, $P = 0.199$).

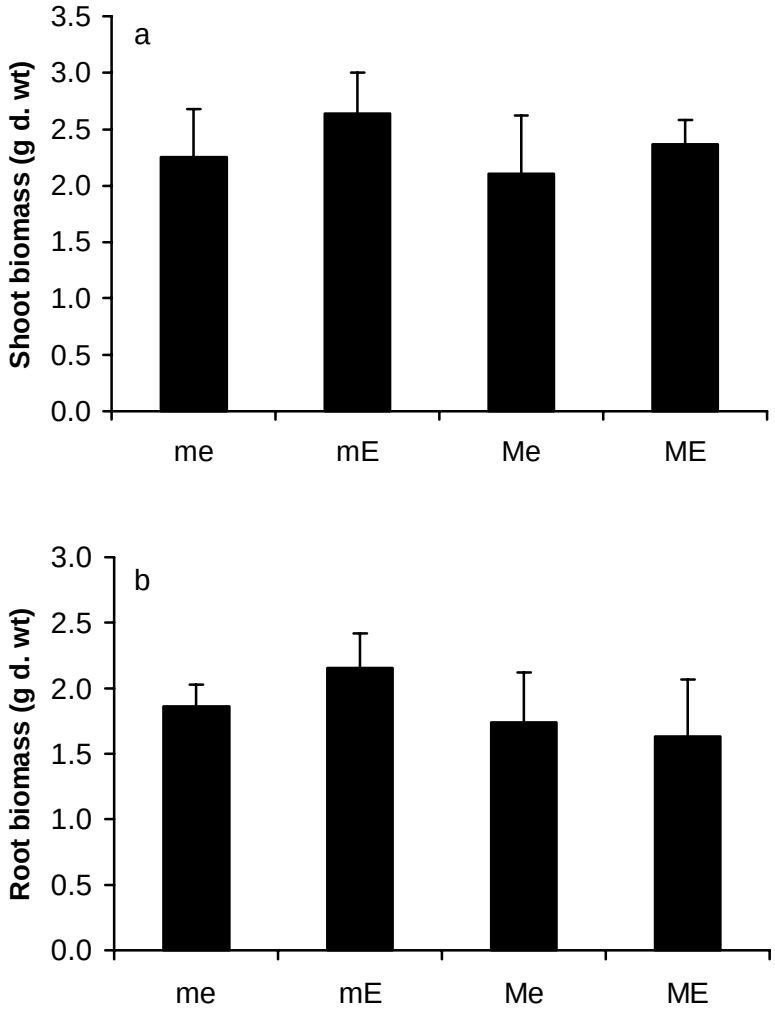

Fig. 4.1 Effect of mycorrhiza (m = without mycorrhiza; M = with mycorrhiza) and earthworms (e = without earthworms; E = with earthworms) on (a) shoot and (b) root biomass of *Plantago lanceolata* (mean + SD)

Table 4.1 ANOVA table of F-values on the effects of mycorrhiza and earthworms on total amounts and concentrations of P, N, ^{15}N excess and C in leaves and roots of *Plantago lanceolata* (*P < 0.05; **P < 0.01; ***P < 0.001)

		Total P	P (%)		Total N	N (%)	Total ^{15}N excess	^{15}N atom% excess	C (%)
	df			*df*					
Leaves									
Mycorrhiza (M)	1	22.99***	44.95***	1	6.35*	0.94	1.73	0.82	1.58
Earthworms (E)	1	0.13	0.26	1	0.64	0.88	2.03	4.78*	1.36
M x E	1	0.60	0.43	1	0.02	0.03	2.17	3.08	12.40**
Error	24			24					
Roots									
Mycorrhiza (M)	1	7.54*	19.36***	1	2.25	4.65*	1.38	0.32	12.43**
Earthworms (E)	1	1.09	1.29	1	0.01	0.96	0.55	1.70	1.42
M x E	1	1.05	<0.01	1	3.23	0.09	1.12	4.51*	0.07
Error	23			24					

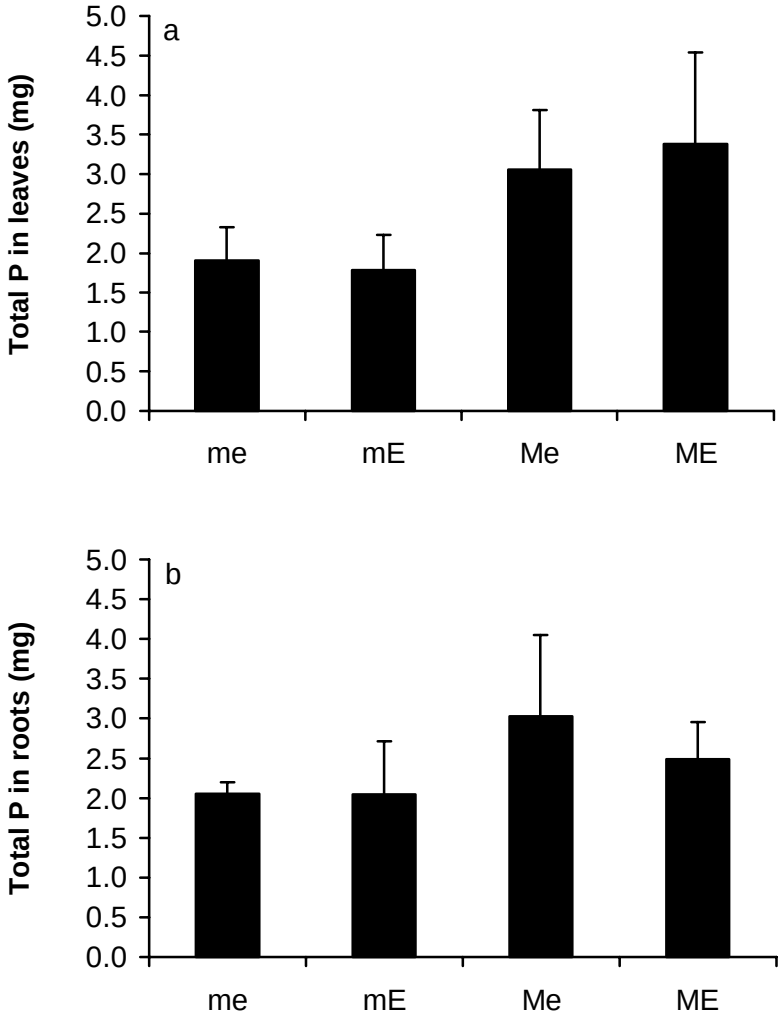

Fig. 4.2 Effect of mycorrhiza (m = without mycorrhiza; M = with mycorrhiza) and earthworms (e = without earthworms; E = with earthworms) on the total amount of phosphorus in (a) leaves and (b) roots of *Plantago lanceolata* (mean + SD)

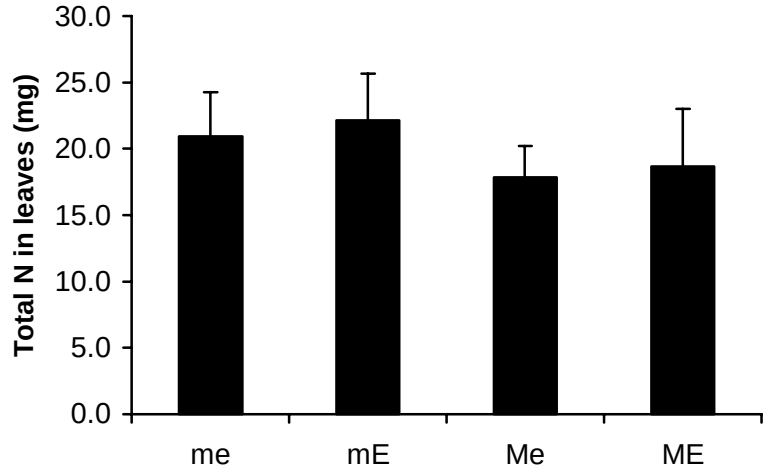

Fig. 4.3 Effect of mycorrhiza (m = without mycorrhiza; M = with mycorrhiza) and earthworms (e = without earthworms; E = with earthworms) on the total amount of nitrogen in leaves of *Plantago lanceolata* (mean + SD)

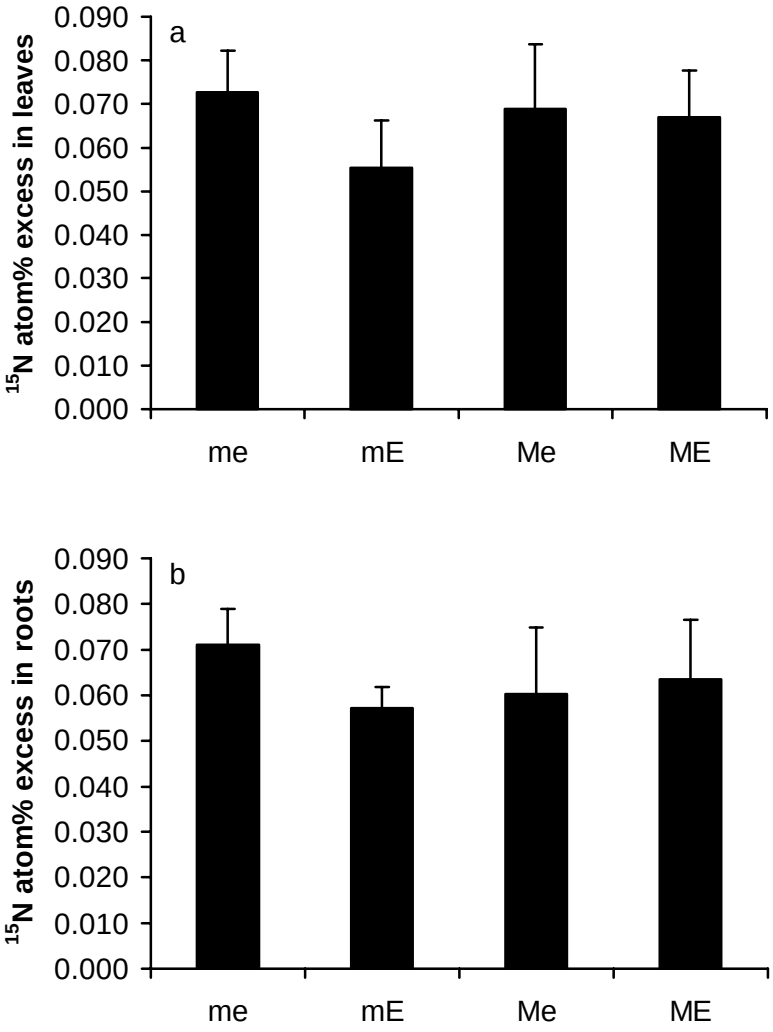

Fig. 4.4 Effect of mycorrhiza (m = without mycorrhiza; M = with mycorrhiza) and earthworms (e = without earthworms; E = with earthworms) on ^{15}N atom% excess in (a) leaves and (b) roots of *Plantago lanceolata* (mean + SD)

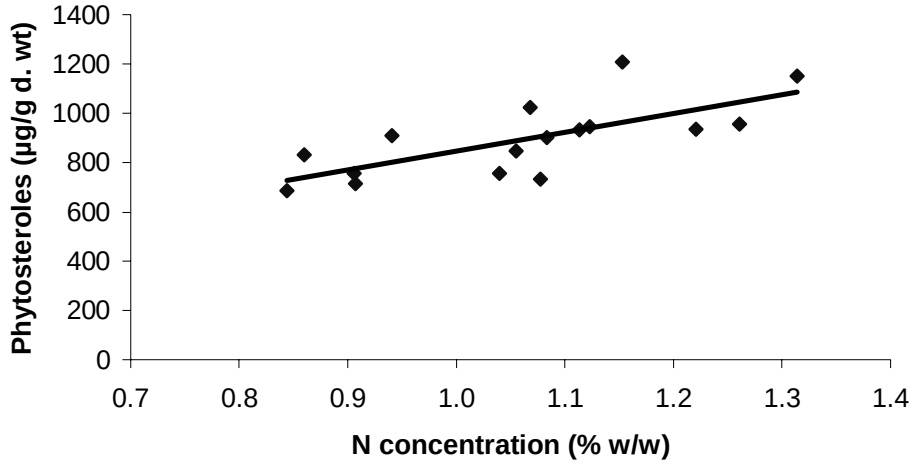

Fig. 4.5 Regression between N concentration (% w/w) and phytosterol concentration (µg/g d. wt) in *Plantago lanceolata* leaves

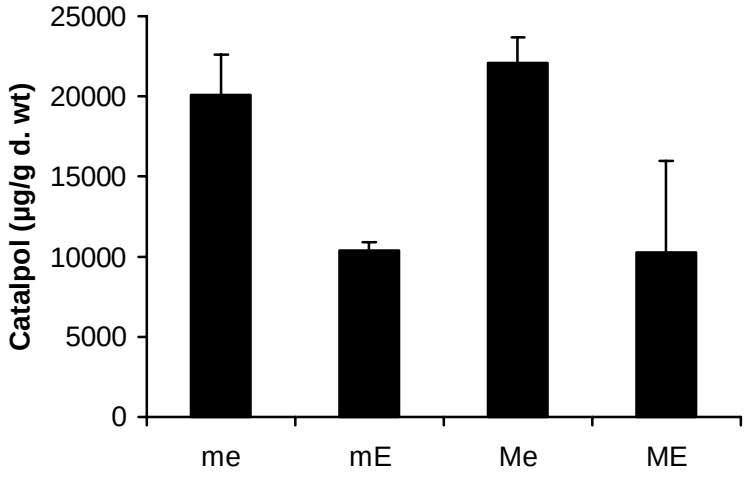

Fig. 4.6 Effect of mycorrhiza (m = without mycorrhiza; M = with mycorrhiza) and earthworms (e = without earthworms; E = with earthworms) on the catalpol concentration of *Plantago lanceolata* leaves (mean + SD)

Aphid performance

The reproduction of *M. persicae* was not affected by VAM ($F_{[1,24]}$ = 0.55, P = 0.467) or earthworms ($F_{[1,24]}$ = 0.55, P = 0.467). The development time of the nymphs tended to be delayed when only VAM or earthworms were present, but was accelerated in the presence of both VAM and earthworms ($F_{[1,21]}$ = 6.84, P = 0.016 for the interaction mycorrhiza x earthworms; Fig. 4.7). The development time of aphids decreased with increasing carbon concentration in the leaves ($F_{[1,23]}$ = 4.63, P = 0.042; R^2 =0.167).

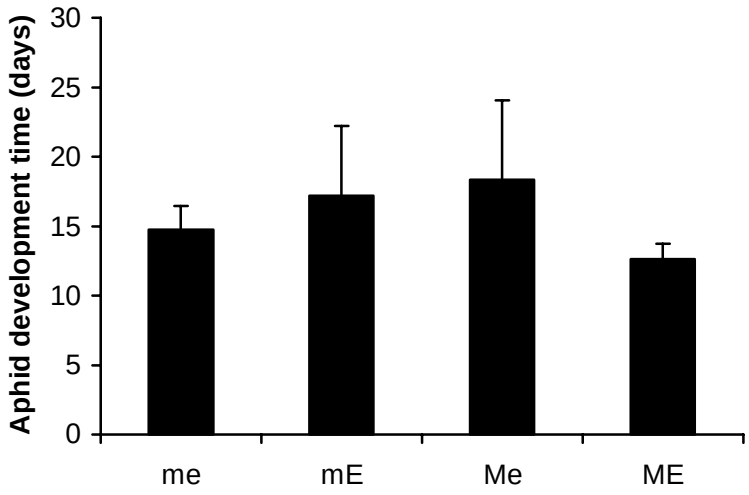

Fig. 4.7 Effect of mycorrhiza (m = without mycorrhiza; M = with mycorrhiza) and earthworms (e = without earthworms; E = with earthworms) on the developmental time of *Myzus persicae* (mean + SD)

4.5 Discussion

No direct interaction between earthworms and VAM was found. Earthworm activity did not reduce VAM root colonisation. Since the effects of earthworms on VAM root colonisation were reported to be density-dependent (Pattinson et al. 1997), a higher number of earthworms might have decreased the infectivity of VAM.

Similar to the study of Tuffen et al. (2002) earthworms, but not VAM, enhanced plant growth. Earthworms increased shoot biomass of *P. lanceolata* and led to a preferential plant uptake of soil N compared to litter N in the absence of VAM. Scheu (1994) proposed that there is an earthworm mobilizable pool of N in soil. Earthworms break up soil aggregates during the gut passage and thus physically protected N is mobilized. The results of the present study indicate that *A. caliginosa* promoted plant growth mainly via mobilisation of soil N. However, the mycorrhizal plants took up less of the earthworm-mobilized N and thus their $^{15}N/^{14}N$ isotope ratio remained unchanged. The total amount of N in the leaves was also decreased in the mycorrhizal plants. In contrast, plant phosphorus concentration was doubled in mycorrhizal plants compared to non-mycorrhizal plants.

VAM are known to increase plant phosphorus content (Smith and Read 1997; Goverde et al. 2000), and decreased plant N concentration in association with VAM has been documented (Gange and West 1994; Gange and Nice 1997). In the present study leaf N concentration remained unchanged, but the total amount of N in the leaves decreased in symbiosis with *G. intraradices*. The smaller root system of the mycorrhizal plants presumably hampered plant N uptake. Although *G. intraradices* has been reported to absorb N and effectively deplete soil for inorganic N, it transports only part of it to the host plant (Johansen et al. 1992). Generally, uptake of N by VAM hyphae does not lead to an increased plant N content (Smith and Read 1997). In many studies on N uptake by VAM, roots were separated physically from the hyphal compartment where the ^{15}N-labelled source was supplied (Ames et al. 1983; Johansen et al. 1992; Frey and Schüepp 1992; Tobar et al. 1994; Hodge et al. 2001) and thus potential competition between roots and VAM hyphae for the ^{15}N was precluded. When both roots and VAM hyphae had access to the ^{15}N source (Hodge et al. 2000a), the mycorrhizal symbiosis did not result in an increase of plant ^{15}N uptake. Since the mycorrhizal plants were more depleted in N than the non-mycorrhizal plants in the present study, we assume that *G. intraradices* competed with the roots for N. Consistently, Hawkins et al. (2000)

documented that hyphae of *G. intraradices* took up less ^{15}N when carrot roots were present than in the hyphal compartment without roots.

Earthworm presence reduced the catalpol concentration in *P. lanceolata* leaves. As far as we are aware, this is the first documentation that decomposers can affect plant secondary metabolites. Increasing fertilizer has been reported to decrease concentrations of iridoid glycosides in *P. lanceolata* (Fajer et al. 1992; Jarzomski et al. 2000). By enhancing N availability and plant growth, earthworms might have produced physiological responses comparable to fertilization. However, in a former study earthworms did not affect iridoid glycoside concentrations in *P. lanceolata*, despite increasing plant growth and N uptake (Wurst et al. 2003; Wurst et al. 2004a). In the present study, inoculation with *G. intraradices* did not affect the iridoid glycosides, although higher aucubin and catalpol levels in mycorrhizal compared to fungicide-treated *P. lanceolata* plants have been documented (Gange and West 1994). The inconsistency of the results indicates that effects of earthworms and VAM on plant chemistry depend on soil characteristics and experimental organisms. The sterilisation of the soil in the present study changed soil characteristics (Alphei and Scheu 1993), and likely affected interactions between soil organisms and the plants. Since mycorrhizal effects depend on the genotypes of the organisms involved (Klironomos 2003), it is not surprising that effects of a single VAM isolate differ from the effects of a whole mycorrhizal community. Both earthworms and VAM have the potential to change plant defensive chemistry (Gange and West 1994; Dugassa-Gobena et al. 1996; Wurst et al. 2004a) and herbivore performance. However, more investigations are necessary to uncover the mechanisms responsible for these effects and the environmental conditions under which they occur.

The phytosterol concentration in *P. lanceolata* was not affected by earthworms or VAM, but increased with increasing N concentration of the leaves. This correlation has been documented before (Wurst et al. 2004a) though in the latter study earthworms increased the phytosterol concentration of *P. lanceolata* when grass residues were distributed homogeneously into the soil. Note that we only investigated effects of earthworms and VAM on plants with aphids (there were no control plants without an aphid treatment). Possible combined effects of soil organisms and aphids on plant performance can thus not be excluded. However, since a maximum of two aphids were clip-caged per plant, the effect of aphids was probably small.

Earthworms and VAM combined accelerated aphid development. Nymphs of *M. persicae* reached earlier maturity when both earthworms and VAM were present, but the development time tended to be delayed when only VAM or earthworms were present. Since the phytosterol concentration was not affected by earthworms or VAM in the present study, it is unlikely that the accelerated development of *M. persicae* was caused by changes in plant phytosterol concentration. Presumably, the earthworm-mediated increase in plant growth and N uptake and the VAM-mediated increase in plant P uptake synergistically increased host quality for the aphids. Gange et al. (1999) reported a reduced development time and increased fecundity of *M. persicae* on *P. lanceolata* in symbiosis with *G. intraradices* at low P levels. In the present study the reproduction of *M. persicae* was not affected by the presence of earthworms or VAM. This is in contrast to former studies where earthworms (Scheu et al. 1999; Wurst and Jones 2003; Wurst et al. 2003) and VAM (Gange and West 1994; Gange et al. 1999) affected the reproduction of *M. persicae.* Since the soil was autoclaved in the present study which is known to mobilize nutrients (Alphei and Scheu 1993), nutrients may not have limited plant growth. Consequently, the effects of earthworms and mycorrhiza on plant and herbivore performance may have been less pronounced than in non-autoclaved soil.

In conclusion, earthworms and VAM significantly affected the performance of *P. lanceolata*, but these effects were mainly independent of each other. Earthworms increased shoot biomass, and VAM reduced root biomass. As expected, the content of P in leaves and roots was enhanced in the presence of mycorrhiza. However, the mycorrhizal association reduced the amount of N in the leaves suggesting that *G. intraradices* competed with the roots for available N. Earthworms reduced the catalpol concentration of *P. lanceolata* documenting the potential of decomposers to influence concentrations of plant secondary metabolites. Earthworms and mycorrhiza combined accelerated the development of *M. persicae.* Further investigations are necessary to understand under which soil conditions earthworms and VAM change plant chemistry (primary and secondary metabolites) and affect herbivore performance.

5 Do earthworms change plant competition?

5.1 Abstract

Plants compete for limited resources. Although nutrient availability for plants is affected by resource distribution and soil organisms, surprisingly few studies investigate their combined effects on plant competition.

Effects of earthworms (*Aporrectodea jassyensis*), the spatial distribution of ^{15}N labelled grass litter and root-knot nematodes (*Meloidogyne incognita*) on the competition between a grass (*Lolium perenne*), a forb (*Plantago lanceolata*) and a legume (*Trifolium repens*) were investigated in the greenhouse. Earthworms promoted N uptake and growth of *L. perenne* and enhanced its competitive ability. Consequently, *T. repens* was suppressed by *L. perenne* in the presence of earthworms and its shoot biomass and N uptake decreased. *P. lanceolata* was not affected by the earthworms. Litter distribution (homogeneous vs. patch) did not affect the biomass of any plant species. However, *P. lanceolata* took up more ^{15}N, when the litter was homogeneously mixed into the soil. Nematodes increased the proportion of litter N in each of the plant species.

The results indicate that earthworms change plant competition by promoting single plant species. Litter distribution did not affect plant competition. Nematodes presumably affected root exudation which stimulated litter decomposition and plant N uptake from the litter. More studies including soil organisms are necessary to understand their role in determining plant community structure.

5.2 Introduction

Resource competition is a major factor in determining plant communities (Tilman 1982). Since nutrient availability for plants is determined by below-ground biotic interactions, soil organisms play an important role in plant community dynamics and may contribute to the coexistence of plant species (Bever 2003). By burrowing, mixing and casting, earthworms enhance nutrient availability in soil and stimulate plant growth (Edwards and Bohlen 1996). Despite the strong effects of earthworms on the performance of single plant species (Scheu 2003), only few studies have investigated effects of earthworms on plant communities (Hopp and Slater 1948; Stockdill 1982; Thompson et al. 1993; Schmidt and Curry 1999; Zaller and Arnone 1999). Earthworms were documented to increase above-ground plant biomass production (Hopp and Slater 1948; Stockdill 1982) and affect plant community structure by influencing the contribution of single plant species to the community (Hopp and Slater 1948; Thompson et al. 1993; Schmidt and Curry 1999).

Nutrient availability for plants is also determined by below-ground abiotic interactions. Resources are not uniformly distributed in soil (Hutchings and de Kroon 1994; Hutchings and Wijesinghe 1997), rather there are nutrient-rich patches in soil. The spatial distribution of nutrients is known to affect nutrient allocation and plant growth (reviewed by Robinson 1994), and may change plant competition (Cahill and Casper 1999; Fransen et al. 2001; Hodge 2003). Although below-ground biotic interactions likely depend on resource distribution, few studies have investigated their combined effects on plant growth (Bonkowski et al. 2000b; Wurst et al. 2003) and plant competition (Hodge et al. 1999; Kreuzer 2000; Hodge 2003). Recently we conducted a study on the effects of earthworms and litter distribution on a grass (*Lolium perenne*), a forb (*Plantago lanceolata*) and a legume (*Trifolium repens*) that were growing separately in microcosms (Wurst et al. 2003). Earthworms enhanced growth of the grass and the forb, but did not affect the biomass of the legume. Litter concentrated in a patch led to an increased shoot biomass of *L. perenne* and root biomass of *P. lanceolata* in the presence of earthworms. We speculated that earthworms and litter distribution may change plant community structure by promoting single plant species and altering their competitive ability. The present study builds on our previous work investigating the effects of earthworms and the spatial distribution of ^{15}N labelled grass litter on the same plant species in competition. We use the same soil and added approximately the amount of ^{15}N labelled grass litter as in the previous study (Wurst et al. 2003) to compare plant growth in monoculture and under interspecific competition. Since other soil organisms

such as nematodes affect plant competition (Cook et al. 1992; Chen et al. 1995; Pantone 1995; van der Putten & Peters 1997; Verschoor et al. 2002) we also investigate effects of the root-knot nematode *Meloidogyne incognita* on the plant species in competition. Generally, the number of plant-feeding nematodes increases with N fertilization (Sohlenius and Boström 1986, Yeates 1987; Todd 1996; Verschoor 2001). Earthworms have been documented to increase the number of plant-feeding nematodes (Ilieva-Makulec and Makulec 2002). Since earthworms affect N availability of plants, they likely improve the host quality for plant-feeding nematodes and modify their effects on plant competition.

5.3 Materials and Methods

On 1 November 2002, seeds of *Lolium perenne* L. (Poaceae), *Plantago lanceolata* L. (Plantaginaceae) and *Trifolium repens* L. (Fabaceae) were sown on wet paper in Petri dishes and placed in a climate chamber (14 h light, 20°C). Germinated plants of each species were transplanted into seedling trays filled with the experimental soil 6 days after sowing. The experimental soil was a nutrient-poor loamy mineral soil (0.087% N; 1.58% C; C/N ratio 18.2) from a cultivated meadow, sieved through a 1 cm mesh.

Experimental set-up

Experimental containers consisted of PVC tubes (height 25 cm, diameter 10 cm) closed at the bottom by lids. The containers were equipped with ceramic cup lysimeters to allow drainage under semi-natural conditions. A pump maintained a vacuum of -200 to -500 hPa to drain the soil via a hose system.

On 26 November 2002, 56 experimental containers were set up in the greenhouse (16 h light, 18°/20°C night/day temperature, 70-80% humidity). Half of the containers were filled with 600 g soil, then 1250 mg litter [250 mg ^{15}N-labelled *L. perenne* shoot litter (73.97 atom% ^{15}N) mixed with 1000 mg unlabelled *L. perenne* shoot litter] was put as a patch in the middle of the experimental container and another 600 g soil was added. The other half of the containers were filled with 1200 g soil mixed homogeneously with 1250 mg litter prepared as above (litter treatment).

Plants with 2-4 leaves were planted from the seedling trays into the containers on 28 November. One individual of each species was planted within 2 cm distance from the edge and the same distance to the neighbouring plants into each container, establishing containers

with *L. perenne*, *P. lanceolata* and *T. repens*. Five days later two individuals of the endogeic earthworm species *Aporrectodea jassyensis* Michaelsen (Lumbricidae) were placed in half of the containers with homogeneously or patchy distributed litter (earthworm treatment). The earthworms had been sampled in a garden in the suburbs of Darmstadt (Germany). Two weeks later 2 ml of a suspension with approximately 250 *Meloidogyne incognita* (Kofoid and White) Chitwood (Heteroderidae) eggs (from tomato plants) was added in the middle of the three plants in half of the experimental containers (nematode treatment). The experimental containers (with 7 replicates of each treatment combination) were watered with 50 ml distilled H_2O every second day, and redistributed randomly within the greenhouse every two weeks.

On 2 January (Week 10) the plants were harvested. They were cut at ground level, freeze dried and weighed. Roots were separated from soil by washing; during the washing procedure earthworms were collected, counted and weighed.

Fresh root samples were stained with aniline-blue (Sarathchandra et al. 1995), and examined with a stereo-microscope for nematode infection.

Freeze dried shoot samples were ground to a powder and approximately 1 mg was weighed into tin capsules. $^{15}N/^{14}N$ ioosotope ratio was measured by an elemental analyser (NA 1500, Carlo Erba, Milan, Italy) coupled with a trapping box (type CN, Finnigan, Bremen, Germany) and a mass spectrometer (MAT 251, Finnigan). Atmospheric nitrogen served as base for $\delta^{15}N$ calculation and acetanilide (C_8H_9NO, Merck, Darmstadt, Germany) as internal standard (Reineking et al. 1993).

The ^{15}N atom% excess in the plants was calculated by subtracting the atmospheric background from the measured ^{15}N atom%.

Statistical analyses

Data on the biomass and chemistry of the three plant species were analysed by a multivariate analysis of variance (MANOVA) with the factors “litter”, “earthworms” and “nematodes” in a general linear model, and regression analyses (Statistica, Statsoft 2001). When the MANOVA documented a significant treatment effect, data of the individual plant species were analysed by analyses of variance (ANOVA) to locate which of the plant species contributed to the significant MANOVA effect (Scheiner and Gurevitch 2001).

5.4 Results

Earthworms

All earthworms survived until the end of the experiment. Total earthworm biomass added per experimental container increased on average by 33.8%. Litter distribution and nematodes did not significantly affect earthworm biomass.

Nematodes

No galls or nematodes were detected in the root sub samples at the end of the experiment; presumably the infestation was low and local.

Plant growth

Earthworms increased the total root biomass in the experimental containers by 50.6% ($F_{[1,48]}$ = 48.04, P < 0.001; Fig. 5.1). Total shoot biomass in the experimental containers was not affected by earthworms ($F_{[1,44]}$ = 0.43, P = 0.515), but as indicated by MANOVA the plant species differentially responded to the presence of earthworms (Table 5.1). Earthworms increased shoot biomass of *L. perenne* by 62.9% ($F_{[1,53]}$ = 65.46, P < 0.001), but led to a decrease in shoot biomass of *T. repens* by 32.0% ($F_{[1,49]}$ = 11.97, P = 0.001). Shoot biomass of *P. lanceolata* was not affected by earthworms ($F_{[1,53]}$ = 0.10, P = 0.748; Fig. 5.1). The shoot biomass of *T. repens* decreased with increasing *L. perenne* shoot biomass ($F_{[1,49]}$ = 8.84, P = 0.005; R^2 = 0.15) and the ratio between *L. perenne* and *T. repens* shoot biomass was significantly increased by earthworms ($F_{[1,49]}$ = 36.75, P = 0.001; Fig. 5.3).

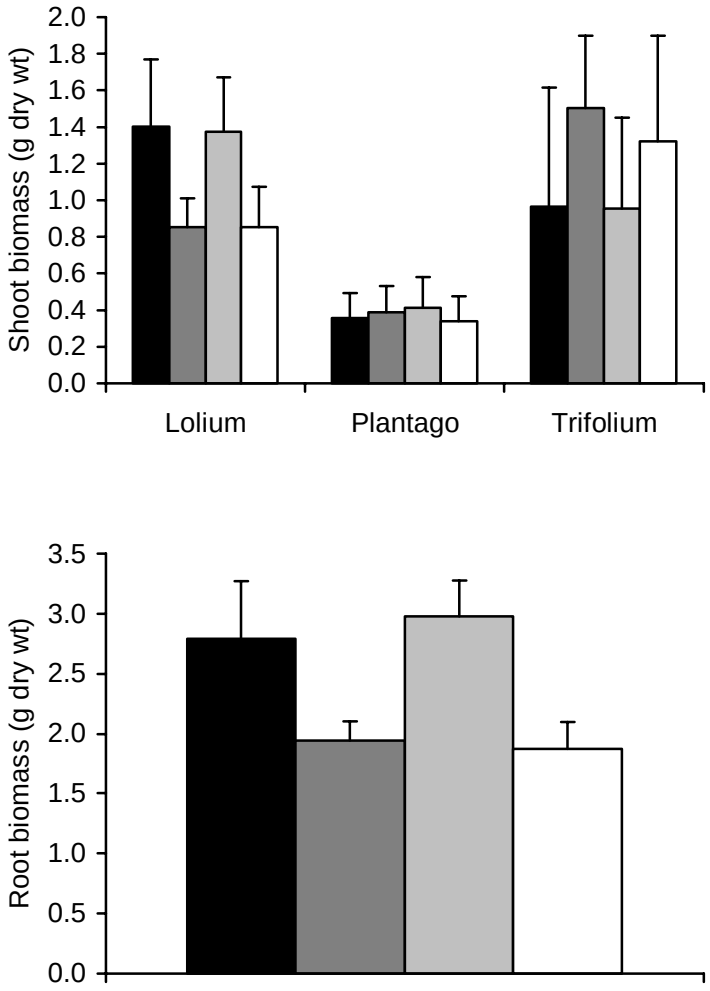

Fig. 5.1 Effect of litter distribution and earthworms (■ patch with earthworms ■ patch without earthworms ■ homogeneous with earthworms □ homogeneous without earthworms) on (a) the shoot biomass of *L. perenne*, *P. lanceolata* and *T. repens*, and (b) the total root biomass (plant species not separated) (mean + SD)

Plant elements

Plant elements (N, ^{15}N, C) were significantly affected by earthworms, litter distribution and nematodes (Table 5.1). Earthworm activity increased the amount of N in *L. perenne* shoots by 79.3% ($F_{[1,52]}$ = 61.66, P < 0.001), but decreased the amount of N in *T. repens* shoots by 35.2% ($F_{[1,50]}$ = 12.37, P < 0.001; Fig. 5.2). The N uptake of *T. repens* decreased with increasing N uptake of *L. perenne* ($F_{[1,47]}$ = 16.99, P < 0.001, R^2 = 0.27; Fig. 5.3). Earthworms decreased the amount of ^{15}N captured from the litter as proportion of total shoot N (^{15}N atom% excess) in *L. perenne* shoots ($F_{[1,46]}$ = 5.74, P = 0.021), but increased ^{15}N atom% excess in *T. repens* shoots ($F_{[1,44]}$ = 10.99, P = 0.002; Fig. 5.2). Neither N nor ^{15}N uptake by *P. lanceolata* was affected by earthworms.

When the litter was mixed homogeneously into the soil, *P. lanceolata* took up more ^{15}N from the added litter ($F_{[1,54]}$ = 10.20, P = 0.002), and ^{15}N atom% excess in shoots increased ($F_{[1,48]}$ = 19.06, P < 0.001; Fig. 5.2). By contrast, the concentration of C in *P. lanceolata* shoots was decreased from 44.56% to 43.85%, when the litter was mixed homogeneously into the soil ($F_{[1,54]}$ = 8.98, P = 0.004). ^{15}N atom% excess in *T. repens* shoots also increased, when the litter was mixed homogeneously into the soil ($F_{[1,44]}$ = 4.17, P = 0.047; Fig. 5.2), while *L. perenne* was not affected by the spatial distribution of litter.

Nematodes significantly increased ^{15}N atom% excess in *L. perenne*, *P. lanceolata* and *T. repens* by 38.0% ($F_{[1,46]}$ = 8.81, P = 0.005), 31.4% ($F_{[1,48]}$ = 5.57, P = 0.022) and 26.9% ($F_{[1,44]}$ = 6.55, P = 0.014), respectively.

Table 5.1 MANOVA table of F values on the effects of litter distribution, earthworms and nematodes on shoot biomass, amount and concentration of N, ^{15}N excess and C of the three experimental plant species (*P < 0.05; **P < 0.01; ***P < 0.001)

		Shoot		N (mg)	^{15}N (mg)	^{15}N (atom%)	N (%)	C (%)
	df	biomass	*df*		excess	excess		
Litter (L)	3	0.27	3	0.56	2.93*	9.29***	1.15	3.81*
Earthworms (E)	3	18.84***	3	15.33***	2.07	8.00***	1.77	1.04
Nematodes (N)	3	1.06	3	0.81	2.55	3.46*	0.23	1.37
L X E	3	0.27	3	0.66	0.67	0.85	0.37	0.25
L X N	3	0.84	3	2.40	1.45	1.10	0.51	0.16
E X N	3	0.43	3	0.56	0.27	1.74	0.51	0.54
L X E X N	3	1.66	3	0.87	0.74	0.84	0.37	0.64
Error	41		40					

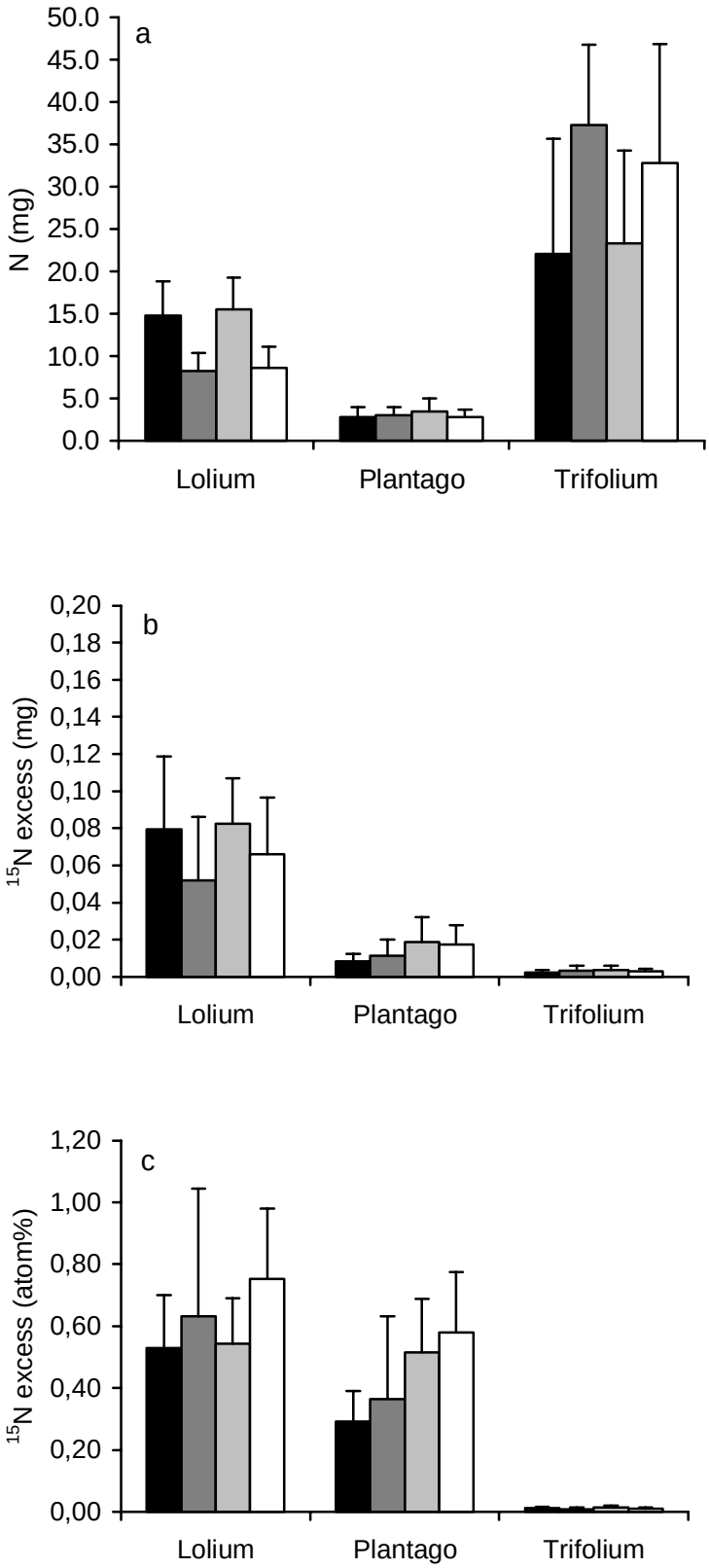

Fig. 5.2 Effect of litter distribution and earthworms (■ patch with earthworms ■ patch without earthworms ■ homogeneous with earthworms □ homogeneous without earthworms) on (a) the amount of N, (b) the amount of ^{15}N excess, and (c) ^{15}N atom% excess in shoots of *L. perenne*, *P. lanceolata* and *T. repens* (mean + SD)

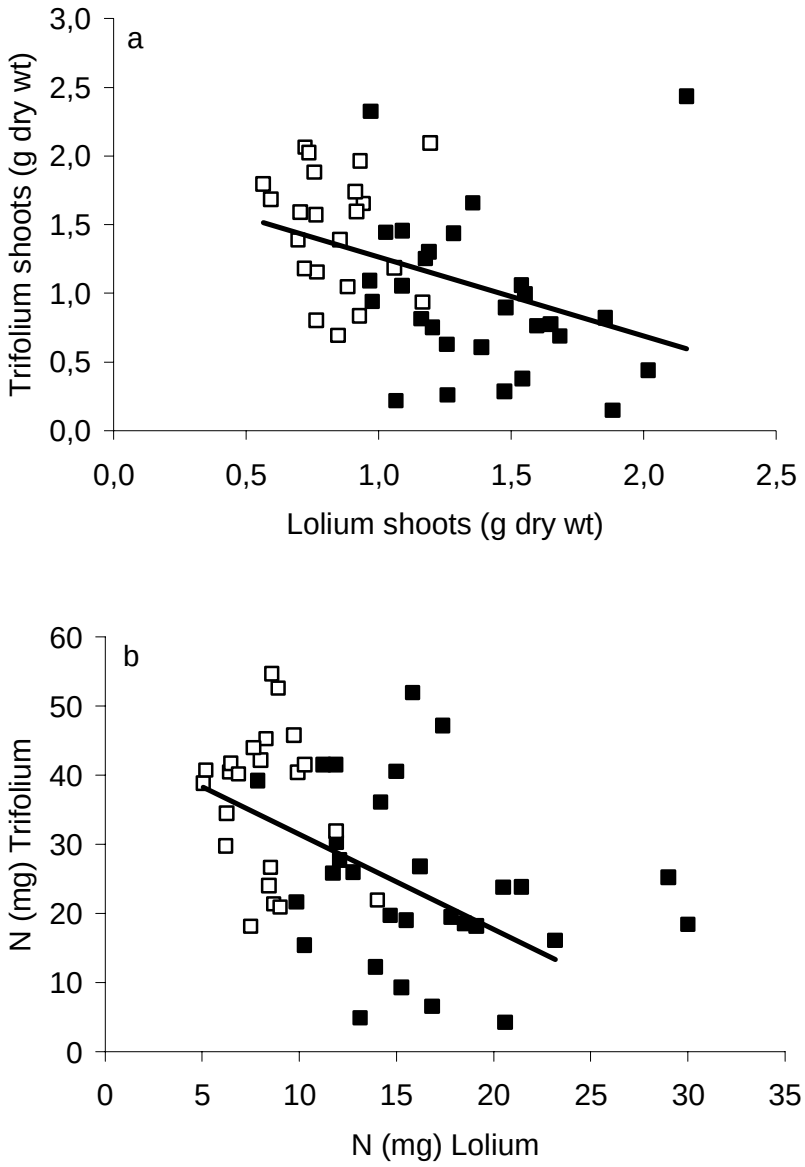

Fig. 5.3 Regression between (a) the shoot biomass of *L. perenne* and *T. repens,* and (b) the amount of N in shoots of *L. perenne* and *T. repens* (■ with earthworms □ without earthworms)

5.5 Discussion

Models predict a nitrogen-based competitive trade-off between grass and clover (Thornley et al. 1995; Schwinning and Parsons 1996): when soil N is low, the N-fixing clover has a greater relative growth rate than the grass, while when soil N is high, the relative growth rate of the grass is enhanced, because mineral N uptake is more efficient than the combination of N uptake and N fixation. Consistent with this prediction, the earthworm-mediated increase in N availability enhanced the competitive ability of *L. perenne* against *T. repens* in the present study.

In the presence of earthworms, the shoot biomass of *L. perenne* increased, while the shoot biomass of *T. repens* decreased. *P. lanceolata* shoot biomass was not affected by earthworms. In a former study (Wurst et al. 2003), when the three plant species were growing without interspecific competitors, earthworms enhanced the shoot and root biomass of *L. perenne* and *P. lanceolata*, while *T. repens* biomass was not affected. Since shoot biomass of *T. repens* decreased with increasing shoot biomass of *L. perenne* in the present study, we suggest that earthworms increased the competitive ability of *L. perenne* against *T. repens*. Other studies have also documented that earthworms selectively promoted grasses. Earthworms increased the proportion of *Triticum aestivum* (wheat) in a wheat-clover (*T. repens*) mixture (Schmidt and Curry 1999). Furthermore, earthworms increased the biomass of *L. perenne* when competing with *T. repens* (Kreuzer 2000). However, Hopp and Slater (1948) reported an earthworm-mediated increase in growth of clover in barrels seeded with a grass-legume mixture. Similarly, Thompson et al. (1993) documented that earthworms enhanced the growth and performance of *Trifolium dubium* in a three species plant community consisting of *Poa annua*, *Senecio vulgaris* and *T. dubium*. It is striking that the studies reporting a promotion of clover due to earthworms were longer (more than 9 months) than the studies that document a promotion of grasses (less than 3 months). Possibly, the effects of earthworms on plant community composition change with time. In the study of Thompson et al. (1993), *T. dubium* was suppressed by *S. vulgaris* and *P. annua* in the first 4 months of the experiment, but later earthworms enhanced its growth (in treatments without snails). Since earthworms enhanced phosphate availability (Thompson et al. 1993), they may have affected soil N:P ratio and thus changed plant species dominance (Tilman 1982). It has been assumed that legumes are most dominant in habitats with a low N:P ratio (Tilman 1982).

Total root biomass was increased in the presence of earthworms, but unfortunately we have no information on the root biomass of the individual plant species. When the plant species

were growing without interspecific competitors (Wurst et al. 2003), earthworms increased root and shoot biomass of *L. perenne* and *P. lanceolata*. Since shoot biomass of *L. perenne* was increased by earthworms in the present study, earthworms likely increased also root biomass of *L. perenne*. To elucidate below-ground plant competition, methods to separate roots of individual plant species are essential (Linder et al. 2000).

The uptake of N followed a similar pattern like the biomass. In the presence of earthworms the amount of N in *L. perenne* shoots increased, while the amount of N in *T. repens* shoots decreased. N uptake of *P. lanceolata* was not affected by earthworms. The proportion of litter N in *L. perenne* shoots decreased in the presence of earthworms, indicating that earthworms enhanced uptake of soil N to a greater extent than litter N. Consequently, less soil N was available for *T. repens* and thus the proportion of litter N in *T. repens* shoots increased in the presence of earthworms. This indicates that the plant species competed mainly for soil N (rather than litter N) made available by earthworms. Possibly, the amount of litter added was too small to play a significant role in the nutrition of the three plants in the pot.

The spatial distribution of organic matter in soil did not affect the biomass of the plant species in competition. When growing without interspecific competitors, shoot biomass of *L. perenne* was increased in the presence of a litter patch (Wurst et al. 2003). Consistently, Cahill and Casper (1999) documented that soil heterogeneity increased shoot biomass of individually grown *Phytolacca americana* plants, but did not affect their shoots in interspecific competition with *Ambrosia artemisiifolia*. The authors suggest that caution should be exercised in extrapolating effects of soil nutrient heterogeneity on isolated plants to plants in competition.

P. lanceolata took up more ^{15}N from the added grass litter, when the litter was mixed homogeneously into the soil. Generally, *P. lanceolata* was a subordinate competitor in the present study. Instead of competing for nutrients in the litter patch, *P. lanceolata* exploited small organic residues mixed homogeneously into the soil. In fact, high precision of root placement has been documented for *P. lanceolata* (Wijesinghe et al. 2001). The proportion of litter N increased in *P. lanceolata* and *T. repens* shoots, when the litter was mixed homogeneously into the soil. Studies on plant N capture from organic litter placed either homogeneously or in discrete patches into the soil reported contrasting results. Increased N capture from a discrete patch (Bonkowski et al. 2000b; Wurst et al. 2003), increased N uptake from homogeneously mixed residues (Hodge 2003) and no response to litter placement (Hodge et al. 2000b) were documented. Foraging responses appear to be plastic, rather than being genetically fixed (Wijesinghe et al. 2001), and likely depend on the strength of the

nutrient patch relative to the background soil fertility (Hodge 2004), and on the presence or absence of competitors.

L. perenne shoots contained five times more litter ^{15}N than *P. lanceolata*, and 24 times more than *T. repens*. In *L. perenne* and *P. lanceolata* the ^{15}N out of the litter made more than 50% of the total amount of ^{15}N in shoots, in *T. repens* this proportion was less than 3%. The small proportion of litter ^{15}N indicates that clover took up mainly atmospheric N with the aid of N_2 fixing bacteria (Vinther and Jensen 2000*)*. In a former study (Wurst et al. 2003), plant ^{15}N atom% was not corrected for the atmospheric background, and thus the uptake of ^{15}N from the added litter was overestimated.

Nematodes increased the proportion of litter N to soil N in each of the plant species studied. Presumably, root-feeding by *M. incognita* led to an increase in root exudation that stimulated the microbial activity in soil (Yeates 1999). Consequently the litter was better mineralised and the plants took up a greater proportion of litter N.

In conclusion, earthworms promoted N uptake and growth of *L. perenne* and enhanced its competitive ability. *T. repens* was suppressed by *L. perenne* in the presence of earthworms. In the absence of earthworms *T. repens* was the dominant plant species in this three species plant community. *P. lanceolata* did not benefit from the presence of earthworms, but it took up more N from the added litter, when the litter was homogeneously mixed into the soil. Nematodes enhanced the proportion of litter N in all plant species. By enhancing the growth of individual plant species earthworms may alter plant competition.

6 General Discussion

Earthworms play a significant role in ecosystems. Besides engineering the below-ground world, earthworm activity influences growth, competition and defensive chemistry of plants, and herbivore performance above the ground.

Earthworms

Three major conclusions about earthworms can be drawn from this thesis. Earthworms affect plant species differently (Chapter 2 and 5), change the competitive ability of individual plant species (Chapter 5), and may affect plant defensive chemistry (Chapter 3 and 4).

Earthworms affected plant species differently. I concentrated on three plant species that belong to different functional groups: a grass (*Lolium perenne*), a forb (*Plantago lanceolata*) and a legume (*Trifolium repens*). Both non-legumes profited from earthworm activity, when growing without interspecific competition (Chapter 2). Growth of clover was independent of earthworms; nevertheless, it took up more N when earthworms were present. Earthworms also augmented the proportion of ^{15}N in the plants, indicating a greater earthworm-mediated increase of litter N uptake than soil N uptake.

In interspecific competition *L. perenne* still profited, while *P. lanceolata* did not benefit from the presence of earthworms (Chapter 5). The earthworm-mediated increase in biomass of *L. perenne* changed its competitive ability against *T. repens*. Thus, clover biomass decreased with increasing biomass of *L. perenne* in the presence of earthworms. In the absence of earthworms, clover was the dominant plant in the three species plant community. Earthworms also increased N uptake into *L. perenne* shoots, while the uptake of N into *T. repens* shoots decreased. The proportion of ^{15}N in *L. perenne* shoots decreased in the presence of earthworms, indicating a greater earthworm-mediated increase of soil N uptake than litter N uptake. Consequently, less soil N was available for *T. repens*, and thus the proportion of N from the litter increased in *T. repens* shoots in the presence of earthworms. This indicates that the plant species competed mainly for soil N (rather than litter N) made available by earthworms. Possibly, the amount of litter added was too small to play a significant role in the nutrition of the three plants in the pot.

Earthworms reduced the reproduction of *Myzus persicae* on *P. lanceolata* (Chapter 2). This was unexpected, because former studies documented an increase of aphid reproduction due to

earthworm activity (Scheu et al. 1999; Wurst and Jones 2003). I hypothesized that earthworms might affect plant defensive chemistry. Indeed, earthworms did not only change the uptake N, but affected other primary metabolites, such as phytosterols (Chapter 3) and secondary metabolites (Chapter 4) in *P. lanceolata*. Earthworms enhanced the concentration of phytosterols, when grass litter was mixed homogeneously into the soil (Chapter 3). The phytosterol concentration increased with increasing N concentration in the shoots (Chapter 3 and 4). Since many phytophagous insects rely on phytosterols to synthesize essential hormones, changes in phytosterol concentrations may affect herbivore performance. However, aphid reproduction and development was not significantly correlated with phytosterol concentrations (Chapter 3 and 4). Earthworms decreased the concentration of the iridoid glycoside catalpol in *P. lanceolata* (Chapter 4). It has been documented that the concentration of iridoid glycosides decreases with soil fertility (Fajer et al. 1992; Jarzomski et al. 2000). Since earthworms enhance nutrient availability for plants, they may produce physiological responses in the plant comparable to fertilization. However, the effects of earthworms on plant defensive compounds depend on soil conditions, and further work is necessary to elucidate the mechanisms and the conditions under which these effects occur. Aphid performance was not correlated with the concentrations of iridoid glycosides (Chapter 3 and 4). In conclusion, earthworms have the potential to alter plant primary and secondary metabolites that are important for plant defence. However, the mechanisms how earthworms affect aphid performance remain unclear.

Litter distribution

The spatial distribution of litter affected plant species differently, when the plants were growing without interspecific competitors (Chapter 2). However, litter distribution did not change plant competition (Chapter 5). Surprisingly, litter distribution affected plant defensive chemistry (Chapter 3).

The spatial distribution of grass litter affected plant biomass production, when the three plant species studied were growing without interspecific competition (Chapter 2). Litter concentrated in a patch (compared with litter mixed homogeneously into the soil) increased shoot biomass and N uptake from the litter in *L. perenne*, and enhanced root proliferation in *P. lanceolata* in the presence of earthworms. Clover responded to the litter patch with local root proliferation close to the patch. When growing in interspecific competition (Chapter 5),

plant biomass was not affected by litter distribution. *P. lanceolata* took up more litter N when the litter was mixed homogeneously into the soil. The proportion of N from the litter also increased in *P. lanceolata* and *T. repens* shoots, indicating that both species exploited more N from the litter mixed homogeneously into the soil, instead of competing for nutrients in the litter patch.

Litter distribution can affect secondary metabolites in plants. *P. lanceolata* shoots contained more aucubin when the litter was concentrated in a patch (Chapter 3). Since this study documents for the first time that litter distribution affects plant secondary compounds, the mechanisms need further investigation. Possibly, effects of litter distribution on plant defence are mediated by the microbial community in the rhizosphere. Non-pathogenic rhizobacteria that induce systemic resistance in plants (van Loon et al. 1998) might be affected by the spatial distribution of organic residues in soil.

Vesicular-arbuscular mycorrhiza

VAM (*Glomus intraradices*) was not affected by the activity of earthworms (Chapter 4). *G. intraradices* increased plant P uptake, but likely competed with roots for N (Chapter 4).

The effects of earthworms and VAM were mainly independent of each other. Thus, earthworms affect plant growth by different mechanisms than VAM. Earthworms enhance N availability for plants by influencing mineralisation processes in soil. VAM increase the availability of P for plants, but might be costly concerning C and N. Indeed, the amount of N in leaves of *P. lanceolata* was lower in the mycorrhizal compared to the non-mycorrhizal plants (Chapter 4). I proposed that *G. intraradices* provided P for the plants, but competed with plant roots for N. Mycorrhizal associations range between mutualism and parasitism (Johnson et al. 1997), and depend on environmental conditions (e.g. soil fertility) and the organisms involved (Klironomos 2003).

The concentration of iridoid glycosides in *P. lanceolata* was not affected by VAM (Chapter 4). Contrastingly, Gange and West (1994) reported higher aucubin and catalpol levels in mycorrhizal compared to fungicide-treated *P. lanceolata* plants. Since mycorrhizal effects depend on the genotypes of the organisms involved (Klironomos 2003), it is not surprising that effects of single VAM isolates (here: *G. intraradices*) differ from the effects of a whole mycorrhizal community. Furthermore, interactions between soil organisms and plants depend

on soil conditions, and the experimental soil treatment (e.g. sterilisation, fungicide treatment) likely affects the results (Alphei and Scheu 1993).

Plant-feeding nematodes

Plant-feeding nematodes (*Meloidogyne incognita*) increased the uptake of N from the litter into the plants (Chapter 5).

M. incognita increased the proportion of litter N in *L. perenne*, *P. lanceolata* and *T. repens* shoots. Presumably, root-feeding by *M. incognita* led to an increase in root exudation that stimulated the microbial activity in soil (Yeates 1999; Bardgett 1999). Consequently, litter was better mineralised and the plants took up a greater proportion of litter N. Effects of plant-feeding nematodes depend on the severity of the infestation. Low infestation promotes plant growth by stimulating root exudations and microbial activity (Denton et al. 1999), while high infestation has detrimental effects on plants.

Interactions

Few interactions between earthworms and litter distribution, VAM or nematodes were found.

When growing without interspecific competitors (Chapter 2), root biomass of *P. lanceolata* more than doubled in the presence of both earthworms and a litter patch. Earthworms enhanced the availability of nutrients, and high concentrations of nutrients concentrated in the litter patch might have stimulated the root growth of *P. lanceolata*. It is known that the production of lateral roots is stimulated by locally high nutrient concentrations in the soil (Drew 1975; Fitter 1976). When the litter was mixed homogeneously into the soil, earthworms enhanced the N concentration in the shoots. The results indicate that effects of earthworms on root foraging and N incorporation in *P. lanceolata* depend on the spatial distribution of organic residues in soil. The changes in N incorporation may also affect higher trophic levels. With increasing shoot N concentration, the phytosterol content in the shoots increased, and aphid reproduction decreased (Chapter 3).

Earthworms did not reduce root colonisation by VAM, and VAM did not affect earthworm biomass. Thus, no direct interactions between earthworms and VAM were found (Chapter 4). Only plant-mediated interactions of earthworms and VAM were detected. The presence of

both earthworms and VAM accelerated the development of aphids, while the development tended to be delayed when only one of them was present. Possibly, the earthworm-mediated promotion of plant growth and N uptake and the VAM-mediated increase in plant P uptake synergistically increased host quality for the aphids. In the presence of earthworms without VAM and VAM without earthworms, the nutrient status and thus the host quality of *P. lanceolata* probably decreased. Leaf C concentration also decreased when only VAM or earthworms were present, indicating physiological changes in the plants.

No assumptions about effects of earthworms on plant-feeding nematodes could be made, because the root infestation by *M. incognita* was too small or only local (Chapter 5). It has been documented that earthworms may increase the number of plant-feeding nematodes (Ilieva-Makulec and Makulec 2002).

6.1 Ecological significance of earthworms

It has been hypothesized that earthworms affect the composition and functioning of terrestrial ecosystems. Consistent with former studies (Schmidt and Curry 1999; Kreuzer 2000), I found that earthworms promoted the growth of *L. perenne*, and enhanced its competitive ability against *T. repens*. However, other studies reported that earthworms enhanced the competitive ability of clover (Hopp and Slater 1948; Thompson et al. 1993). Since the experiments that documented a promotion of clover were longer (more than 9 months) than the experiments showing a promotion of grasses (less than 3 months), I suggest that the effects of earthworms on plant competition change with time. Earthworms are known to increase the availability of N and P in soil (Edwards and Bohlen 1996). The mobilization of nutrients by earthworms, and thus the ratio between N and other nutrients in soil, might change with time. Since legumes are most dominant in habitats with a low N:P ratio (Tilman 1982), changes in soil N:P ratio may affect plant competition and community structure.

Beside the lack of direct interactions between plants and earthworms, plant defensive chemistry changed in the presence of earthworms. In a recent review on below- and above-ground induced plant responses, van Dam et al. (2003) proclaimed that decomposers are unlikely to induce responses, because they do not interact directly with living plants. Since all experimental plants were exposed to above-ground herbivores in the present study, a differentiation between effects of soil organisms and combined effects of soil organisms and above-ground herbivores on plant defensive chemistry is impossible. Despite the small number of aphids clip-caged on the plants, aphids or the clipping of the leaves (Stamp and Bowers 1994) might have induced the observed plant responses. Nevertheless, earthworms affected the magnitude of the plant responses. Earthworms changed the concentrations of phytosterols and catalpol in *P. lanceolata* (Chapter 3 and 4); however the effects depended on soil conditions. Since the phytosterol concentration increased with the N concentration of the shoots, earthworms likely affected phytosterol synthesis through changes in nutrient availability. The fact that iridoid glycosides decrease with increasing fertilization (Fajer et al. 1992; Jarzomski et al. 2000) also indicates that the catalpol concentration was affected by earthworm–mediated changes in nutrient availability.

Generally, an induction of defensive responses by beneficial soil organisms would not be advantageous for the plants. Indeed, infection with VAM does not lead to major changes in the expression of defence-related genes (Smith and Read 1997; Mohr et al. 1998; van Dam et al. 2003).

6.2 Prospects

Earthworms play an important role in the mobilization of nutrients. By incorporation litter into the soil, earthworms mix organic residues with soil and enhance microbial decomposition. The quality of litter is crucial for decomposition processes, since microorganisms and decomposers "feed" on it. Therefore, future experiments should investigate how litter of different quality alters the effects of earthworms on plant performance and higher trophic levels (Fig. 6.1). Furthermore, earthworms change the quality of plant litter entering the soil. Earthworm activity affects soil conditions directly by burrowing and casting, but also indirectly by plant-mediated changes in the quantity and quality of litter input into the soil. Therefore, long-term studies are necessary to understand the role of earthworms in terrestrial ecosystems.

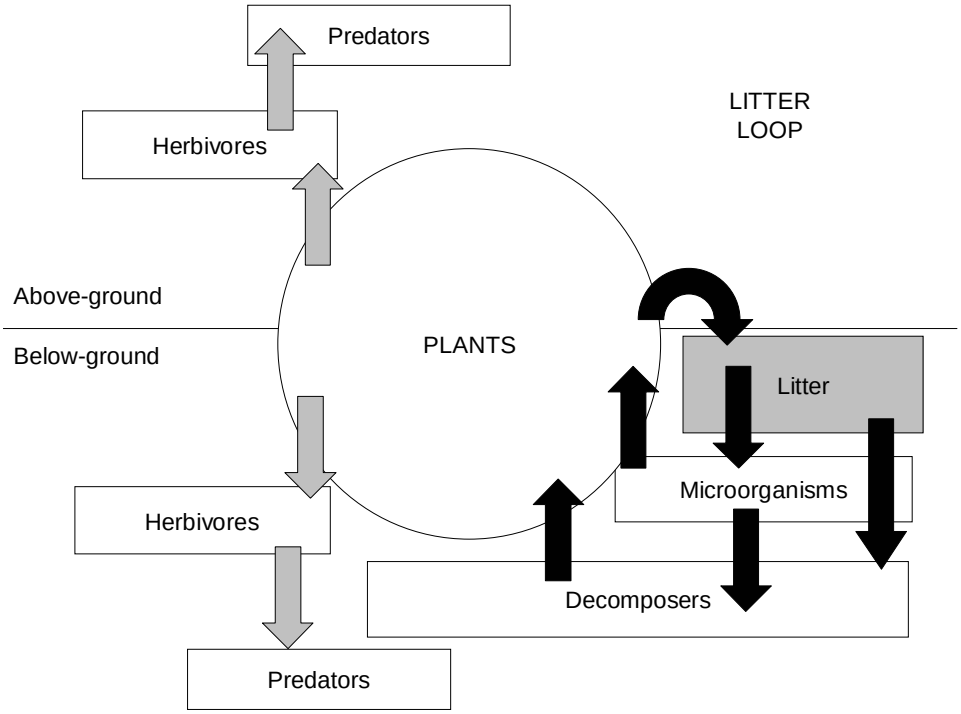

Fig. 6.1 Litter input affects microorganisms and decomposers, and subsequently plant performance and the higher trophic levels below and above the ground. Black arrows describe the way litter nutrients are incorporated into the soil, mineralized and taken up by the plants ("litter loop"). Grey arrows indicate the plant-mediated, indirect effects of litter input on higher trophic levels

As highlighted above, earthworms may alter plant competition. However, effects of earthworms on plant competition have mainly been studied in semi-natural conditions (i.e. microcosms, mesocosms, and barrels) for short time periods. Again, long-term studies under natural conditions are necessary. Future studies should also concentrate on the role of

earthworms in affecting plant communities during succession. Potential earthworm-mediated changes in soil nutrient ratios may affect plant competition and community structure.

To better understand multitrophic interactions in terrestrial ecosystem, the third trophic level (i.e. predators and parasitoids) below and above the ground should be included in future studies (van der Putten 2001). Recently, van Tol et al. (2001) found that roots damaged by weevil larvae attract entomopathogenic nematodes. Indirect defence, i.e. an attraction of predators or parasitoids by the plants to defend them against herbivores, might also affect beneficial soil organisms, such as decomposers. Thus, indirect defence below the ground might be confronted with a trade-off between getting rid of herbivores and loosing beneficial soil organisms. Another interesting question is whether direct plant defence below the ground affect non-target organisms, like decomposers and beneficial microorganisms in the rhizosphere (Fig. 6.2). Generally, plant defence mechanisms must be included in the research on multitrophic interactions above and below the ground. Recent studies (Gange and West 1994; Bezemer and Wäckers 2003; Bezemer et al. 2003) show that plant defence is crucial for understanding interactions between below- and above-ground organisms.

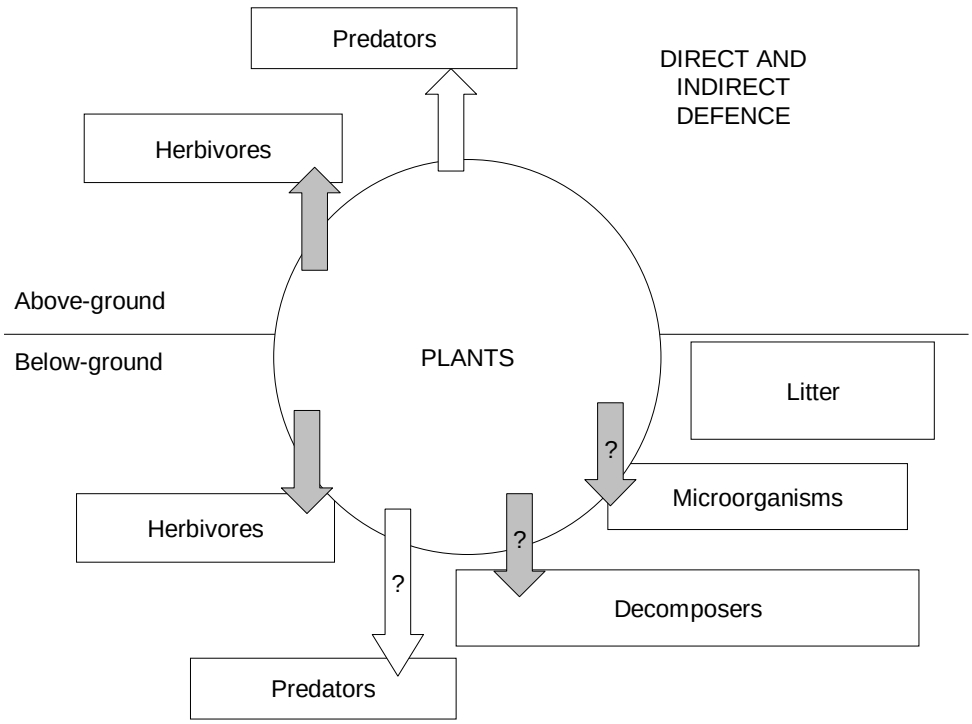

Fig. 6.2 Direct and indirect defence of plants, above and below the ground. Grey arrows are direct defences, white arrows are indirect defences, and arrows with question mark are possible, but largely unknown direct or indirect defences

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Lebenslauf

Name:	Susanne Wurst
Geburtsdatum:	16.07.1973
Geburtsort:	Heilbronn, Deutschland

Beruflicher Werdegang

10/1994-01/2001	Biologiestudium an der Georg-August Universität in Göttingen
08/1996-09/1997	Integriertes Auslandsstudium (IAS) an der Universidad Nacional in Heredia, Costa Rica gefördert durch den Deutschen Akademischen Austauschdienst (DAAD)
03/2000-06/2000	Forschungsprojekt am Centre of Population Biology (CPB), Imperial College at Silwood Park, Ascot, UK gefördert durch die Carl-Duisberg Gesellschaft (CDG)
01/2001	Diplom in Biologie, Georg-August Universität Göttingen Titel der Diplomarbeit: “Influence of earthworms and soil type on a tritrophic system: plant-aphid-parasitoid”
seit 04/2001	Doktorandin an der Technischen Universität Darmstadt gefördert durch das Hessische Ministerium für Wissenschaft und Kunst

Eidesstattliche Erklärung

Ich erkläre hiermit an Eides statt, dass ich die vorliegende Dissertation selbständig und nur mit den angegebenen Hilfsmitteln angefertigt habe. Ich habe noch keinen Promotionsversuch unternommen.

Darmstadt, den 14.04.2004