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The soil food web of temperate deciduous forests: litter and root resources as driving factors, and soil fauna effects on ecosystem processes

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Summary

Above- and belowground communities have been investigated independently for long, but there is increasing evidence that both are intimately linked and depend on each other. Plants provide energy and nutrients for the belowground consumer community, both via litter and root exudates, thereby affecting soil biota. Simultaneous investigations of both litter and root exudate based pathways are scarce and therefore, the relative importance of roots as compared to litter as food resource for soil organisms remains unknown. Soil organisms, in turn, contribute to ecosystem processes like litter decomposition and nitrogen cycling that are essential for nutrient supply of plants and primary production. Their individual effects may be facilitated or reduced by co-occurring species. The underlying mechanisms and the reasons for the variability of soil fauna interactions are little understood, but complementarity effects are likely to be related to dissimilarity of traits.

The present thesis focuses on litter- and root-derived resources for soil communities and analyzes feedbacks of detritivores to ecosystem processes and hence to plant nutrition.

In order to disentangle the relative importance of identity and diversity of root-derived as compared to litter-derived resources for soil microorganisms (Chapter 2) and mesofauna invertebrates (Chapter 3) a field experiment was performed in which aboveground litter and root species were manipulated independently. Saplings of four deciduous tree species, differing in litter quality and associated mycorrhizal fungi were planted in a full-factorial design in a 150 year old mountain oak forest. The response of soil microorganisms (Chapter 2) to variations in resources was measured by substrate-induced respiration (SIR) and phospholipid fatty acid (PLFA) analyses, while the response of mesofauna invertebrates (Chapter 3) was measured by changes in community composition and abundance of groups or species. Generally, neither soil microorganisms nor mesofauna invertebrates responded to the type of mycorrhizal fungi or root identity and diversity. In contrast, aboveground litter affected basal respiration and community composition of soil biota. However, there was no beneficial leaf litter mixture effect; rather soil microorganisms and mesofauna invertebrates responded to the quality and identity of litter, with the effects varying between microarthropod groups and species. Overall, the results point to a stand age dependent importance of aboveground and belowground resources for decomposer food webs of

forest ecosystems, with effects of litter quality and identity on mineralization processes and feedbacks to plants being most significant for young trees, i.e. during stand regeneration.

In Chapter 4 I investigated interactions between four detritivores species differing in body size or habitat association, i.e. two species of earthworms (*Lumbricus terrestris*, *Aporrectodea caliginosa*) and two species of collembolans (*Heteromurus nitidus* and *Protaphorura armata*). I tested if species with similar traits exert negative effects on their respective performance or their effects on ecosystem processes, i.e. leaf litter mass loss and ^{15}N cycling. Litter-associated species had more pronounced effects on litter decomposition and N cycling, but their effects depended on the presence of other soil invertebrates. Detrimental but also facilitative interactions between soil animal species occurred, independent of trait similarity, indicating other factors than dissimilarity of traits to be important for complementarity effects of soil organisms. Interactions in part were mutually dissimilar with one species benefitting and the second being detrimentally affected in presence of the other. Furthermore, some effects needed more than two species to occur, suggesting that the identity of soil animal species and the composition of the soil animal community override the importance of diversity for ecosystem processes. This suggests that soil fauna interactions are complex and difficult to predict, with predictions of their effects requiring knowledge on the identity of soil animal species that interact.

Overall, the results of this thesis indicate aboveground and belowground communities to be intimately linked and to closely depend on each other. Effects of plants on decomposer systems of deciduous forests vary with tree species identity and thereby tree species may drive feedbacks of soil detritivores to plants. Combining our approaches with compound-specific stable isotope analysis, molecular gut content analysis and real time PCR ultimately may allow the understanding of trophic relationships in soil food webs. It also may help to predict the relevance of individual species and community composition on ecosystem processes and hence on aboveground - belowground interactions in forest ecosystems.

Chapter 1

General Introduction

Aboveground - belowground interactions and ecosystem functioning

Biodiversity is considered to be a major determinant of ecosystem functioning and stability (Hooper et al., 2005; Balvanera et al., 2006; Bardgett and van der Putten, 2014). A considerable fraction of global biodiversity and species from virtually all taxonomic groups of microorganisms and invertebrates are living in soil (Wardle, 2002). Although aboveground and belowground communities have been investigated separately for long, there is mounting evidence that both are intimately linked and closely dependent on each other (Scheu, 2001; Wardle et al., 2004).

The belowground consumer community relies on plant-derived carbon and nutrients entering the soil (Beare et al., 1992; Bardgett et al., 2005). The majority of energy and nutrients obtained by plants, up to 90% in forest ecosystems (Gessner et al., 2010), enters the soil either aboveground as litter and woody debris or belowground in the form of root exudates or dead roots. The amount, availability and composition of nutrients entering the soil food web affects biomass, activity and abundance as well as community composition of soil microorganisms and soil invertebrates (Wardle et al., 2004; Bardgett and Wardle, 2010; Pollierer et al., 2012). Aboveground litter has been assumed to be the main source of energy and nutrients for soil organisms (Swift et al., 1979; Berg and McClaugherty, 2008). The diversity of litter resources has been considered to significantly affect soil invertebrates, but recent studies indicate that not diversity or mixture effects per se affect soil invertebrates, but rather the quality and identity of litter species contributing to the respective mixture (Wardle et al., 2006; Jacob et al., 2009; Eissfeller et al., 2013). Litter species differ in nutrient and metabolite composition (Bardgett, 2005) and therefore the role of litter for soil organisms also varies with litter quality and identity (Saetre and Baath, 2000). In addition to aboveground resources, root-derived resources are fuelling soil food webs, and this is receiving increasing interest (Albers et al., 2006; Ruf et al., 2006; Pollierer et al., 2007). Exudation by roots is an active process that enables plants to e.g., attract specific microorganisms that mobilize nutrients for uptake by plants (Bais et al., 2006). Living roots typically are associated with mycorrhizal fungi which may channel root resources to higher trophic levels of soil food webs (Smith and Read, 2008; Pollierer et al., 2012; Eissfeller et al., 2013a), but also function as food resources for fungal feeding soil organisms. In temperate forests ectomycorrhizal fungi (EMF), with hyphae forming a complex network between root cortical cells, and arbuscular mycorrhizal fungi (AMF), which from a highly branched arbuscule within root cortical cells (Bardgett, 2005), are the most abundant mycorrhizal types. Each mycorrhizal type also forms hyphae that extend into the soil, with the extrametrical mycelium of EMF being more intensively dispersed than that of AMF (Bardgett, 2005; Smith and Read, 2008; Cairney, 2012). Therefore, the transfer of carbon from plant roots to hyphae and nutrient transport from hyphae to roots in EMF likely exceeds that in AMF. A number of studies investigated the importance of either aboveground

litter (Wardle et al., 1997; Hättenschwiler and Gasser, 2005; Sayer, 2006) or of root-derived nutrients (Pollierer et al., 2007; Broeckling et al., 2008; Eissfeller et al., 2013a) for soil food webs although both pathways are included in energy and nutrient cycling (Moore et al., 2005). A simultaneous investigation of both pathways has rarely been conducted. Therefore, the relative importance of the identity and diversity of roots as compared to litter as food resource for soil organisms remains unknown.

Soil organisms affect life above the ground in a multitude of ways (de Deyn and van der Putten, 2005). Soil animals drive important ecosystem processes such as decomposition and nutrient turnover (Bardgett and van der Putten, 2014), thereby, e.g. contributing to the mineralization of nutrients entering the soil system and making them available for uptake by plants (Scheu, 2001; Porazinska et al., 2003). Nitrogen (N) together with phosphorus and potassium, is the main element that limits plant productivity in terrestrial ecosystems (Chapin, 1980; Vitousek and Howarth, 1991; LeBauer and Treseder, 2008). Therefore, decomposition of litter material and the release and cycling of N bound in detritus are important for the continuous nutrient supply of plants (Seastedt, 1984), and thereby for the productivity of terrestrial ecosystems (Vitousek, 1982). Due to different effects of soil organisms on ecosystem processes and interactions between them, the availability of nutrients changes with the composition of the soil fauna community, thereby affecting the productivity as well as the community composition of plants (Wardle et al., 2004).

Stable isotopes

Different versions of one element distinguishing only in the number of their neutrons, whereas the number of protons is equal, are referred to as isotopes (Fry, 2008). Besides radioisotopes that decay with time and may be used for age analyses of fossils there are stable isotopes that are used e.g., for the analysis of food webs or energy and nutrient fluxes (Ponsard and Arditi, 2000; Scheu and Falca, 2000; Schmidt et al., 2004). For food web analysis natural variations in stable isotope ratios are used, since they change during biological processes due to enzyme kinetics (Fry, 2008). The change is expressed by the delta notation that gives the difference between an international standard and the respective sample in per mill [‰] (Fry, 2008). Whereas, $\delta^{13}C$ that is not significantly enriched with trophic level and hence allows tracing the food resource of a given organism, $\delta^{15}N$ on average is enriched by 3.4 ‰ per trophic level, thereby indicating the trophic level of the analyzed species (Minagawa and Wada, 1984; Gannes et al., 1998; Post, 2002). Energy and nutrient fluxes may be investigated using resources artificially high enriched (or depleted) in $\delta^{15}N$ or $\delta^{13}C$ and follow their signal through the soil food web (Pollierer et al., 2007; Pausch et al., 2015).

Soil biota

Microorganisms

The majority of soil living organisms and the primary consumers of the soil food web are microorganisms (Bardgett, 2005; van der Heijden et al., 2008). The two most abundant and diverse groups of microorganisms are fungi and bacteria, that are responsible for the brake down of organic material entering the soil (Lavelle and Spain, 2001). The great majority of fungi produce filamentous hyphae that explore microhabitats in the soil (Bardgett, 2005). Their enzymes are able to degrade cell wall compounds including cellulose and lignin; therefore, saprotrophic fungi may process aboveground litter (Aira et al., 2006; Osono, 2007). Furthermore, they are able to transport nutrients from patches of high nutritional quality to zones where nutrients are limited in supply (Lummer et al., 2012), thereby facilitating substrate exploitation. Furthermore, as discussed above mycorrhizal fungi associated with living roots enhance nutrient exchange and communication between plants and their environment. In contrast to fungi the highly diverse soil bacteria (Torsvik et al., 1990) are prokaryotic and unicellular (Bardgett, 2005). Bacteria often depend on passive transport, since they are rather immobile, thus, they rely on local resources, that are likely to be depleted by them (Bardgett, 2005). Resource depletion is followed by a phase of inactivity of bacteria until resources become available again (Bardgett, 2005). Most soil bacteria are not able to degrade recalcitrant compounds such as cellulose and lignin and therefore rely on easily available, soluble components such as root exudates (Paterson et al., 2008; Cesarz et al., 2013; Baetz and Martinoia, 2014).

Besides their role as primary decomposers, soil microorganisms also act as plant pathogens (Lartey et al., 1994), bind soil particles to form aggregates, thereby enhancing soil structural stability (Rillig and Mummey, 2006) and function as food resource for microbial-feeding organisms.

Microarthropods

Microarthropods belong to the mesofauna with body width between ~ 100 µm and ~ 2 mm and are among the most abundant soil invertebrates in terrestrial ecosystems with densities of hundreds of thousands individuals per square meter (Coleman et al., 2004). They carry out important functions in soil, e.g. they change the structure of litter and soil, increase litter surface area for microbial attack, release nutrients from dead organic plant or animal material and control fungal and bacterial biomass (Seastedt, 1984; Addison et al., 2003; Cole et al., 2006). Due to their high abundance often one or only few microarthropod groups are studied at the level of species (Bardgett et al., 2005).

Collembolans are among the most abundant and well studied microarthropod groups (Fjellberg, 1998; Hopkin, 1997, 2002). They feed on a variety of food materials including fungi, but also plants, algae and detritus, bacteria and even other soil animals (Petersen, 2002; Chahartaghi et al., 2005; Heidemann et al., 2014). According to their habitat association, collembolans can be categorized into three ecological groups, epedaphic taxa that live in and under the litter layer, euedaphic taxa living in the mineral soil and hemiedaphic taxa that show intermediate distributions between litter and the upper soil layers (Scheu and Falca, 2000; Hopkin, 2007). Due to the broad variety of collembolan species, effects of the identity and diversity of aboveground and belowground resources on collembolan communities are difficult to predict, as is their effect on ecosystem processes.

Earthworms

Earthworms, as ecosystem engineers, are important macrofauna decomposers with body width typically > 2 mm (Swift et al., 1979). They modify the soil compactness, soil humidity and soil aeration via their borrowing activity (Boyle et al., 1997). They feed on and incorporate litter into the soil, and mingle organic material and mineral soil (Lavelle and Spain, 2001) thereby affecting microbial community composition, biomass and activity (Brown, 1995; Scheu, 2002), other soil invertebrates, decomposition processes and nutrient cycling (Scheu, 1987; Edwards and Bohlen, 1996; Butenschoen et al., 2009), as well as plant performance (Thompson et al., 1993; Scheu, 2003). According to their habitat association, earthworms are categorized into three ecological groups, epigeic, anecic and endogeic (Bouché, 1977; Brown, 1995; Eisenhauer, 2010). Epigeic species live in the upper organic layers of the soil and feed on litter material, thereby contributing to litter decomposition. They have limited effects on mixing of mineral and organic soil layers. In contrast, anecic earthworm species incorporate litter material from the soil surface, into their permanent vertical burrows in the mineral soil but also transport mineral soil materials to the surface by casting (Bouché, 1977; Edwards and Bohlen, 1996). Hence, these moderate to large earthworms strongly affect the mixing of organic and mineral soil layers. Endogeic earthworm species also live in the mineral soil, but they form non-permanent horizontal burrows and feed on mineral soil materials that are already mixed with organic matter (Eisenhauer, 2010).

Soil fauna interactions

Since soil organisms are embedded in a complex community their performance as well as their effect on ecosystem processes such as litter decomposition and nutrient cycling may be influenced by trophic and non-trophic interactions (Strong et al., 1996; Scheu, 2002; Adejuyigbe et al., 2006). Their

effects on each other and their effects on ecosystem processes vary from detrimental, over neutral to facilitative, but the reasons for this variability are little understood.

Eisenhauer (2010) suggested the effects of earthworms on mesofauna organisms to vary between the ecological groups of earthworms. While e.g. endogeic species exert negative effects on microarthropods, most likely due to competition between both groups for habitat and food resources (Milcu et al., 2006; Eisenhauer et al., 2007; Ke and Scheu, 2008), anecic earthworms primarily have positive effects on microarthropods. Positive effects may be due to the formation of stable microhabitats rich in nutrients and microorganisms (Wickenbrock and Heisler, 1997; Maraun et al., 1999; Salmon and Ponge, 1999). In contrast, Heemsbergen et al. (2004) suggested not only the traits of one group to be important for soil fauna interactions, but rather assumed trait similarity between different soil animals to be the main factor influencing soil animal interactions.

Besides habitat association or resource use, body size is thought to be an important trait affecting soil fauna interactions or soil animal effects on ecosystem processes (Bradford et al., 2002; Eisenhauer, 2010). Larger soil animals, such as anecic earthworms, are more mobile and thus have stronger direct effects on e.g., decomposition (Jones et al., 1994), while smaller soil animals rather have indirect effects on ecosystem processes e.g., due to selective microbial feeding (Newell, 1984a, 1984b; Klironomos and Kendrick, 1995).

Study site

The field studies (Chapter 2 and 3) were carried out in the framework of the "SPLIDRHEX" (**Sp**ecies **l**itter **i**dentity and **di**versity effects on the **rh**izosphere of trees **ex**periment) in a 150 year old deciduous forest in the vicinity of Göttingen (51°26‘27‘‘N, 10°01‘03‘‘O, 340 m a.s.l., Lower Saxony, Germany). Long-term mean annual temperature is 8.7°C and the mean annual precipitation is 644 mm. The forest is dominated by oak (*Quercus petraea*) and beech (*Fagus sylvatica*) and has a species rich understory, dominated by jewelweed (*Impatiens* spp.). The soil is an oligotrophic brown earth from bunter composed of mull humus and mineral matter and partly also served as experimental soil in the mesocosm experiment (Chapter 4). The mesocosm study (Chapter 4) took place in a greenhouse under controlled conditions.

Study objectives and chapter outline

This thesis focused on the importance of identity and diversity of aboveground as compared to belowground resources on soil microorganisms (Chapter 2) and mesofauna invertebrates, i.e. collembolans (Chapter 3) under field conditions. Furthermore, the importance of mesofauna and macrofauna invertebrates and their interactions for ecosystem processes, i.e. leaf litter decomposition and cycling of N was investigated in a mesocosm experiment (Chapter 4).

Main hypotheses

(1) The response of soil organisms to the presence of leaf litter is less pronounced as compared to their response to the presence of roots (root exudates), with litter mixtures decreasing and root mixtures increasing the difference.

(2) The response of soil organisms is more pronounced in EMF as compared to AMF roots, with the effect of mycorrhizal type varying between root (tree) species.

(3) The response of soil organisms is more pronounced in high as compared to low quality litter, with the effect of litter quality varying between litter species.

(4) Litter-associated soil invertebrates exert stronger effects on ecosystem processes, i.e. litter decomposition and ^{15}N cycling, than soil-associated species.

(5) Soil animals with similar traits, i.e. body size (macrofauna vs. mesofauna) and habitat association (litter vs. mineral soil), hamper the performance of each other, while species dissimilar in their traits complement each other and their effects on ecosystem processes.

(6) The effects of single soil animal species on litter decomposition and ^{15}N cycling are modified by interactions with other soil animals, with similar species reducing their effects on litter decomposition and ^{15}N cycling.

In the following, the content of the chapters is summarized.

Chapter 2

Using basal respiration and substrate induced respiration as well as phospholipid fatty acid (PLFA) analysis the relative importance of identity and diversity of aboveground as compared to belowground resources on biomass, activity and community composition of soil microorganisms was investigated eight month after establishment of the experiment. Soil microorganisms generally did not respond to the presence of roots, type of mycorrhizal fungi or root identity and diversity. In contrast, basal respiration and community composition of soil microorganisms varied with litter quality and identity, while litter mixture had no effect. Overall, the results suggest aboveground rather than belowground resources to affect mineralization processes and feedbacks to plants, with a high importance of resource quality and identity, at least for young trees, i.e. during stand regeneration.

Chapter 3

This study aimed at disentangling the relative importance of root-derived as compared to litter-derived resources for soil microarthropods, considering in particular the role of root and litter identity and mixtures eight month after establishment of the experiment. Generally, the analyzed soil microarthropods did not respond to the presence of roots, single or in mixtures, type of mycorrhizal fungi or root identity. Only the density of one collembolan species was increased in the four as compared to the one root species treatment. In contrast, the density of mites, proturans and of three out of ten collembolan species studied was significantly affected by aboveground litter. This supports the classical view that soil food webs are fuelled in large by litter-derived resources at least in the short term. However, there was no beneficial leaf litter mixture effect; rather soil microarthropods responded to the quality and identity of litter, with the effects varying between microarthropod groups and species.

Chapter 4

In Chapter 4 I investigated interactions between four species of detritivores differing in body size and habitat association, i.e. two species of earthworms (*Lumbricus terrestris*, *Aporrectodea caliginosa*) and two species of collembolans (*Heteromurus nitidus* and *Protaphorura armata*). I tested if similar species exert negative effects on their respective performance or their effects on ecosystem processes, i.e. leaf litter mass loss and ^{15}N cycling in a mesocosm experiment. Detrimental but also facilitative interactions between soil animal species occurred, independent of trait similarity. In

contrast, species identity was important for soil animal interactions both in regard to ecosystem processes and their effects on the performance of each other. Although species usually hampered their individual effects on ^{15}N incorporation into beech seedlings in two species treatments, facilitative effects occurred if *L. terrestris*, *A. caliginosa* and *P. armata* were present together. Therefore, the prediction of soil fauna effects on ecosystem processes in complex communities remains difficult. Overall, the results emphasize the importance of species identity and community composition for interactions of detritivores and for their effects on litter decomposition and N cycling.

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Chapter 2

The importance of aboveground and belowground resources for the microbial community of temperate deciduous forests

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Abstract

The classical view that aboveground litter is the main resource for the soil food web is challenged by recent studies, indicating the importance of belowground resources, such as root exudates to be underestimated. Our study aimed at disentangling the relative importance of root-derived resources for soil food webs and to quantify the relative contribution of aboveground litter and root-derived substances for soil microorganisms. Further, the study aimed at evaluating the role of identity and diversity of root and litter species for soil microorganisms. Therefore, two year old tree saplings of four deciduous tree species (*Fagus sylvatica*, *Acer pseudoplatanus*, *Fraxinus excelsior*, *Tilia cordata*) differing in the associated mycorrhizal fungi (EMF or AMF) and litter quality (high and low), were planted in a 150 year old mountain oak forest (*Quercus petraea*). For the first time aboveground litter and root species were manipulated independently. The response of soil microorganisms to variations in resources was measured by substrate-induced respiration (SIR) and phospholipid fatty acid (PLFA) analysis. Soil microorganisms generally did not respond to the presence of roots, type of mycorrhizal fungi or root identity and diversity. In contrast, aboveground litter affected basal respiration and community composition of soil microorganisms, suggesting litter to be of higher importance for the soil food web as compared to root-derived resources, at least in consideration of young trees. Unexpectedly, there was no beneficial leaf litter mixture effect; rather, microbial community significantly varied with litter quality and identity, with contrasting effects on different soil microorganisms. Overall, the results point to a stand age dependent importance of aboveground and belowground resources for decomposer food webs of forest ecosystems, with effects of litter quality and identity on mineralization processes and feedbacks to plants being most significant for young trees, i.e. during stand regeneration.

Keywords: soil microorganisms, field experiment, SIR, PLFAs, resource identity, resource diversity

Introduction

In forest ecosystems up to 90% of the annual biomass production enters the soil as dead organic matter (Gessner et al., 2010). Aboveground litter has been assumed to be the main source of energy and nutrients for soil organisms (Swift et al., 1979; Berg and McClaugherty, 2008). However, the role of litter for soil food webs varies with litter quality and litter identity (Saetre and Baath, 2000), since nutrient and metabolite composition are species specific (Bardgett, 2005). For example the attractiveness of litter for decomposers increases with increasing N concentration (Jacob et al., 2009) and with decreasing lignin concentration (Bardgett, 2005) and hence with litter quality (Cornwell et al., 2008). High quality litter is more attractive for soil organisms (McClaugherty et al., 1985; Jacob et al., 2009); thus, high quality litter will be more intensively decomposed as compared to low quality litter in the same duration of time. Therefore, the decomposition rate may serve as indicator for litter quality.

In addition to aboveground resources, root-derived resources are fuelling soil food webs, and this is receiving increasing interest (Albers et al., 2006; Pollierer et al., 2007). Living roots typically are associated with mycorrhizal fungi which may channel root resources to higher trophic levels of soil food webs (Smith and Read, 2008; Pollierer et al., 2012; Eissfeller et al., 2013a). The most abundant mycorrhizal types in temperate forests are ectomycorrhizal fungi (EMF; e.g. on beech and lime roots) with hyphae forming a complex intercellular network between root cortical cells, and arbuscular mycorrhizal fungi (AMF; e.g. on ash and maple roots) which form a highly branched arbuscule within the root cortical cells (Bardgett, 2005; Smith and Read, 2008). Each mycorrhizal type also forms hyphae that extend into the soil for capturing nutrients to be transported to plant roots. Since extrametrical mycelium of EMF is more intensively dispersed than that of AMF (Bardgett, 2005; Smith and Read, 2008; Cairney, 2012), the transfer of carbon and nutrients from the plant to the rhizosphere also may be more pronounced in EMF.

Microorganisms, such as fungi and bacteria are responsible for decomposition of most of the organic material entering the soil, both via aboveground litter and roots (Lavelle and Spain, 2001). Since the hyphal network of saprotrophic fungi may penetrate dead plant cells, and their enzymes are able to degrade cell wall compounds including lignin, saprotrophic fungi may process aboveground litter. Although bacteria also degrade litter material, soil bacteria predominantly rely on easily available, soluble nutrients such as root exudates (Bardgett, 2005; Paterson et al., 2008; Cesarz et al., 2013).

The diversity of root species and litter species increases spatial heterogeneity, habitat structure and nutrient resources, thereby affecting soil microorganisms (Bardgett et al., 2005). With increasing diversity and heterogeneity of habitat and nutrient resources competition between decomposers decreases (Hutchinson, 1957; Schneider et al., 2004). Therefore, mixtures of root or litter species

likely increase microbial diversity and biomass. Further, combining different root species or litter species, also likely results in changes in community composition of soil microorganisms by increasing the diversity of nutrients available as compared to single species treatments.

Various studies investigated the importance of either aboveground litter (Wardle et al., 1997; Hättenschwiler and Gasser, 2005; Sayer, 2006) or of root-derived nutrients (Pollierer et al., 2007; Broeckling et al., 2008; Eissfeller et al., 2013a) for soil food webs. To the best of our knowledge the importance of both root and litter of different species and their combination for soil food webs has not been investigated simultaneously under field conditions, which is surprising as typically multiple tree species grow together. Due to the lack of such studies the relative importance of identity as well as of mixing effects of roots as compared to litter for soil food webs remains unknown.

In the present study aboveground litter and root species were manipulated independently. The study aimed at disentangling the relative importance of root- vs. litter-derived resources for soil food webs. Further, the study aimed at evaluating the role of identity and diversity of root and litter species for soil microorganisms. Plant species differ in the mycorrhizal fungi associated with roots, i.e. EMF or AMF, and in the quality of litter as indicated by decomposition rate. The response of microorganisms was analyzed by measuring microbial respiration, biomass and phospholipid fatty acids (PLFAs) as indicator of microbial community composition.

We hypothesized that (1) the response of microorganisms to the presence of leaf litter is less pronounced as compared to its response to the presence of roots (root exudates), with litter mixtures decreasing and root mixtures increasing the difference; (2) the response of microorganisms is more pronounced in EMF as compared to AMF roots, with the effect of mycorrhizal type varying between root (tree) species; (3) the response of microorganisms is more pronounced in high as compared to low quality litter, with the effect of litter quality varying between litter species.

Material and methods

Study site

The study was conducted in a 150 year old deciduous forest in the vicinity of Göttingen (51°26‘27‘‘N, 10°01‘03‘‘O, 340 m a.s.l., Lower Saxony, Germany). The region has a continental climate with a long-term mean annual temperature of 8.7°C and a mean annual precipitation of 644 mm. The forest is dominated by oak (*Quercus petraea*) and beech (*Fagus sylvatica*) (95%) interspersed by larch (*Larix decidua*), spruce (*Picea abies*), pine (*Pinus sylvestris*), willow (*Salix* spp.) and birch (*Betula* spp.), representing 5% of all tree individuals at the study site. The understory is species rich and dominated by jewelweed (*Impatiens* spp.), stinging-nettle (*Urtica dioica*) and fern (*Athyrium filix-femina*). The

soil is an oligotrophic brown earth from bunter composed of mull humus and mineral matter with pH ($CaCl_2$) of 5.01 ± 0.07 and soil moisture of 26.74 ± 0.72% of soil fresh weight. Soil carbon and nitrogen concentrations are 2.22 ± 0.05% and 0.14 ± 0.003%, respectively.

Experimental set up

Approximately three month before establishing the experiment some of the inferior trees were cut down and removed from the study area by the forester to improve growing conditions for the experimental trees, e.g. due to reduce canopy cover. In November 2010 a total of 144 experimental plots (each 180 x 210 cm), 36 in one of four blocks, respectively, were established at the study site. Experimental plots were arranged among the mature trees in a way to prevent the presence of visible roots of mature trees in the plot area. Plots had a minimum distance of 50 cm from one another. The original litter layer was removed and 800 g (dry weight = dry wt) of air-dried leaf litter (water content between 6.7 – 9.8%) of four different deciduous tree species [beech (*Fagus sylvatica*), maple (*Acer pseudoplatanus*), ash (*Fraxinus excelsior*), lime (*Tilia cordata*)] was placed on the Of-horizon of the mull humus. Litter species differed in their quality as indicated by decomposition rate; with high quality litter being decomposed more than 75% of initial after 12 month and low quality litter being decomposed less than 50%. Leaf litter was added to the plots either in monocultures or as four species combination including equal amounts of each leaf litter species. Plots were covered by nets to prevent litter losses by wind.

In spring 2011 two year old saplings of the selected tree species were obtained from a local forest nursery (Billen Forst GmbH, Bösinghausen; Germany). Saplings differed in regard to their associated mycorrhizal fungi. Beech and lime roots are colonized by EMF, maple and ash roots are colonized by AMF. On each plot 30 bare root, unfertilized saplings were planted in a square 5 x 6 design with a distance of 25 cm forming a core area with 12 trees and a plot margin area with 18 trees. In a full factorial design, saplings of every tree species were planted in monocultures or in mixtures of all four tree species into experimental plots of every litter treatment. Additionally, plots with only tree or litter treatments or without trees and without litter were established. The experiment was set up in a complete randomized block design with four blocks, i.e. four replicates per treatment (Table 1).

Table 1: Study design: Roots were present single (ash and maple with AMF, beech and lime with EMF), in mixtures of all four species (AMBL with AMF and EMF) or absent from the system (no). Litter was present as single species (ash and lime of high quality, beech and maple of low quality), in mixtures of all four species (ALBM with high and low quality) or absent from the system (no). Every combined root-litter treatment was replicated 4 times.

		Roots					
		ab		ec		ab x ec	Ctrl
Litter		ash	maple	beech	lime	AMBL	no
high	ash	4	4	4	4	4	4
	lime	4	4	4	4	4	4
low	beech	4	4	4	4	4	4
	maple	4	4	4	4	4	4
high x low	ALBM	4	4	4	4	4	4
Ctrl	no	4	4	4	4	4	4

Sampling and analytical procedure

In November 2011 soil cores were taken in the core area of each experimental plot using a steel corer (Ø 5 cm). Leaf litter was removed, dried at 60°C for six days and weighed for information on decomposition rate and differences between the different species. The amount of decomposed litter increased from beech litter (26.4 ± 9.15% of initial) over maple (45.46 ± 10.68% of initial) and lime (78.04 ± 6.89% of initial) to ash litter (79.62 ± 4.58% of initial) indicating beech and maple litter to be of low quality and lime and ash litter to be of high quality, as defined earlier.

Soil samples (0-5 cm depth) were homogenized by passing through a 2 mm sieve to remove stones and larger plant material. Soil samples were analyzed for soil moisture (a subsample of soil from every core was weighed before and after drying at 105°C for 72h) and soil pH (2 g of soil from every core in 20 ml 0.01M $CaCl_2$). Microbial basal respiration (BR; µl O_2 g^{-1} soil dry wt h^{-1}) and therefore microbial activity as well as microbial biomass (Cmic; µg C g^{-1} soil dry wt) in the bulk soil were determined using an automated oxygen (O_2) microcompensation system (Scheu, 1992). The average oxygen consumption rate without addition of substrate within 10- 30 h after attachment of the samples to the analysis system was taken as microbial basal respiration (BR). Microbial biomass (C_{mic}) was measured by substrate-induced respiration (SIR) i.e., the respiratory response of microorganisms to glucose (Anderson and Domsch, 1978). Eight mg of glucose were added as aqueous solution to fresh soil equivalent to one gram dry weight. The maximum initial respiratory response (MIRR) was calculated as the mean of the three lowest hourly measurements within the first 10 h after glucose

addition. Microbial biomass (C_{mic}; µg C g^{-1} soil) was calculated as 38 × MIRR (µl O_2 g^{-1} soil dry wt h^{-1}) according to Beck et al. (1997).

To investigate the community composition of soil microorganisms, i.e. the relative contribution of saprotrophic fungi and soil bacteria, phospholipid fatty acids (PLFAs) were extracted according to Frostegard and Baath (1996). Fatty acids were analyzed by gas chromatography using Clarus 500 (Perkin Elmer, Waltham, USA). PLFAs a15:0, i15:0, i16:0 and i17:0 were used as marker fatty acids for $Gram^+$ bacteria; cy17:0 and cy19:0 as marker FAs for $Gram^-$ bacteria; 18:2ω6 as a saprotrophic fungal marker and 16:1ω7 as an unspecific bacterial marker (Ruess and Chamberlain, 2010). Furthermore, PLFAs were used to calculate fungal-to-bacteria ratio and $Gram^-$-to-$Gram^+$ bacteria ratio. Identification of fatty acids was confirmed by GCeMS using a Varian CP-3800 chromatograph coupled to a 1200 L mass spectrometer fused to a silica column (Phenomenex Zebron ZB-5MS, 30 m, 0.25 mm film thickness, ID 0.32 mm) with helium as carrier gas. For statistical analyses values of all markers for $Gram^+$ bacteria, $Gram^-$ bacteria or fungi, respectively (Supplement T1) were summarized and converted in percent of total PLFA measured; thus, changes in community composition of soil microorganisms due to the treatments may be discovered.

Calculations and statistical analyses

The effect of root presence and litter presence including mixture effects (0, 1, 4) on microbial activity (BR), microbial biomass (C_{mic}), fatty acid percentage of $Gram^-$ and $Gram^+$ bacteria or fungi on total PLFA measured as well as fungal-to-bacteria ratio and $Gram^-$-to-$Gram^+$ ratio were analyzed by two factorial General Linear Model (GLM; type III sum of squares). In a multivariate, hierarchical GLM (type I sum of squares (Schmid et al., 2002)) the effect of the co-variables soil pH (overall mean 5.01 ± 0.07) and soil moisture (overall mean 26.74 ± 0.72 % of soil fresh wt) fitted before the effect of mycorrhizal type (EMF, AMF), litter quality (high, low), root identity and litter identity (beech, maple, ash, lime) on soil microorganisms (BR, C_{mic} and PLFAs) were analyzed. Co-variables are continuous while treatment variables are categorical. Prior to statistical analyses, data were inspected for homogeneity of variance (Levene-Test) and logit (y'=ln(y/(1-y))) or log transformed (log10(y+1)) if required. Means were compared using Tukey's HSD test ($P < 0.05$). Statistical analyses were performed using SAS (Statistical Analysis System, Version 9.3; SAS Institute Inc., Cary, USA). Means presented in text and tables are based on non-transformed data and given with the corresponding standard error of the mean (SEM).

Results

Response of soil microorganisms to roots and litter

Microbial basal respiration (BR) did not significantly respond to the presence and/or diversity of roots ($F_{2,112}$ = 0.17, P = 0.85), but to the presence and/or diversity of litter ($F_{2,112}$ = 3.62, P = 0.03). It was at a maximum in treatments with one litter species (23.50 ± 7.24 µl O_2 h^{-1} g^{-1} soil dry wt) as compared to treatments with four litter species (7.08 ± 1.14 µl O_2 h^{-1} g^{-1} soil dry wt) or without litter (8.17 ± 0.61 µl O_2 h^{-1} g^{-1} soil dry wt). Microbial biomass neither was affected by the presence and/or diversity of roots ($F_{2,112}$ = 0.06, P = 0.94) nor by the presence and/or diversity of litter ($F_{2,112}$ = 0.99, P = 0.37). There was no significant interaction between roots and litter, neither in BR ($F_{4,112}$ = 0.06, P = 0.99) nor in C_{mic} ($F_{4,112}$ = 0.02, P = 1.00). According to percentage of the respective PLFAs, soil microbial community was dominated by $Gram^+$ bacteria (50.87 ± 0.44% of total PLFA), followed by $Gram^-$ bacteria (22.08 ± 0.75% of total PLFA), unspecific bacteria (21.51 ± 0.40) and saprotrophic fungi (5.54 ± 0.21% of total PLFA as expressed by the values for 18:2ω6,9) and did not vary significantly with the presence of roots or litter, no matter of single species or mixtures of roots and litter were considered (Table 2).

Table 2: Two factorial General Linear Model (type III sum of squares) table of F- values on the effect of root presence (Root), litter presence (Litter) and interaction between roots and litter (Root x Litter) on the concentrations of $Gram^+$ bacterial, $Gram^-$ bacterial and fungal fatty acids (percentages of total) as well as fungi/bacteria ratio and $Gram^-/Gram^+$ ratio.
*p < 0.05; **p < 0.01; ***p < 0.001

	Root	**Litter**	**Root x Litter**
	$F_{2,128}$	$F_{2,128}$	$F_{4,128}$
$logitGram^+$	0.91	2.28	1.27
$logitGram^-plus1$	0.35	1.47	1.08
logitFungi	1.01	0.37	0.91
log10FBRatio	1.01	0.37	0.91
$log10G^-/G^+Ratio$	1.21	1.59	1.1

Response of soil microorganisms to mycorrhizal type and identity of roots

Neither microbial basal respiration nor microbial biomass were affected by mycorrhizal type ($F_{1,44}$ = 3.24, P = 0.08 and $F_{1,44}$ = 0.67, P = 0.42, respectively) or root species ($F_{2,44}$ = 0.31, P = 0.73 and $F_{2,44}$ =

0.38, P = 0.69, respectively). Also, community composition of soil microorganisms was not significantly affected by mycorrhizal type and root species (Table 3).

Table 3: Multivariate General linear model (type 1 sum of squares) table of F- values on the effect of soil moisture (H2O), soil pH (Co-Variables), mycorrhizal type (MT), litter quality (LQ), MTxLQ interactions, root identity (RI), litter identity (LI) and RIxLI interactions on $Gram^+$ bacterial, $Gram^-$ bacterial and fungal fatty acid (percentages of total) as well as fungi/bacteria ratio and $Gram^-/Gram^+$ ratio. *p < 0.05; **p < 0.01; ***p < 0.001

	H2O	**soilpH**	**MT**	**LQ**	**MTxLQ**	**RI**	**LI**	**RIxLI**
	$F_{1,46}$	$F_{1,46}$	$F_{1,46}$	$F_{1,46}$	$F_{1,46}$	$F_{2,46}$	$F_{2,46}$	$F_{8,46}$
$logitGram^+$	3.81	0.18	1.33	4.36*	0.14	0.38	2.82	1.53
$logitGram^-$	0.11	0	1.29	6.48**	0.05	2.12	1.5	2.1
logitFungi	0.21	0.48	0.83	4.31*	1.56	0.55	9.43***	0.8
log10FBRatio	0.21	0.48	0.83	4.31*	1.56	0.55	9.43***	0.8
$log10G^-/G^+Ratio$	0.74	2.3	1.11	5.27*	1.78	0.72	1.39	1.43

Response of soil microorganisms to quality and identity of leaf litter

Microbial basal respiration and microbial biomass neither were affected by litter quality ($F_{1,44}$ = 0.35, P = 0.55 and $F_{1,44}$ = 0.04, P = 0.85, respectively) nor by litter identity ($F_{2,44}$ = 0.32, P = 0.73 and $F_{2,44}$ = 0.77, P = 0.47, respectively). In contrast, microbial community composition significantly varied with litter quality. The percentage of $Gram^+$ bacteria ($F_{1,46}$ = 4.36, P = 0.04) was reduced in high quality litter (49.4 ± 0.7%) as compared to low quality litter treatments (51.5 ± 0.8%), while the percentage of $Gram^-$ bacteria ($F_{1,46}$ = 6.48, P = 0.01) was increased in high quality litter (24.4 ± 1.1%) as compared to low quality litter treatments (19.8 ± 1.5%). Hence, $Gram^-$-to-$Gram^+$ ratio was significantly ($F_{1,46}$ = 5.27, P = 0.03) lower in low quality (0.4 ± 0.03) as compared to high quality litter treatments (0.5 ± 0.03). The percentage of saprotrophic fungal PLFA was higher in low quality (5.8 ± 0.4%) as compared to high quality litter treatments (5.0 ± 0.4%; $F_{1,46}$ = 4.31, P = 0.04). Consequently, fungal-to-bacteria ratio was also higher in low quality litter (0.06 ± 0.004) as compared to high quality litter treatments (0.05 ± 0.004; $F_{1,46}$ = 4.31, P = 0.04).

Microbial community composition also significantly varied with litter identity. The percentage of saprotrophic fungal PLFA was significantly higher in beech litter ($F_{2,46}$ = 9.89, P = 0.0003) as compared

to maple and lime litter treatments, and the percentage of fungal PLFA in ash exceeded those in lime litter treatments (Figure 1).

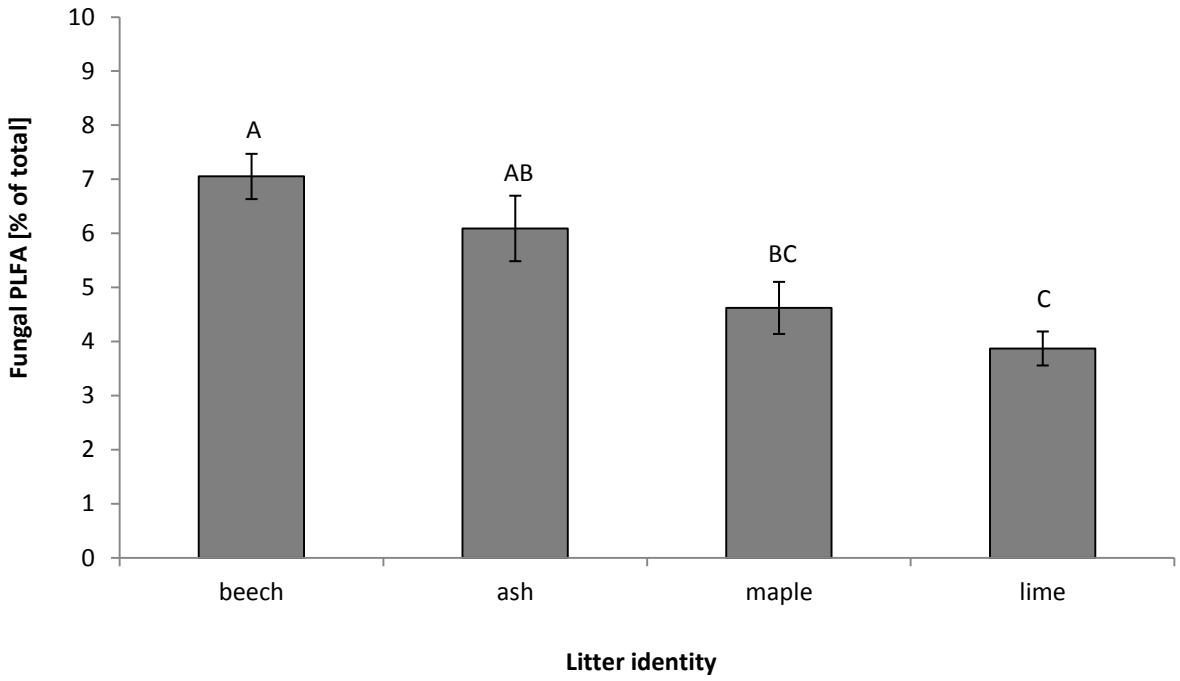

Figure 1: Percentages of fungal PLFA of total as affected by litter identity. Bars sharing the same letter do not differ significantly (Tukey's HSD test; $P < 0.05$). For statistical analysis see Table 3.

Litter identity did not significantly affect PLFAs of $Gram^-$ and $Gram^+$ bacteria ($F_{2,46} = 1.5$, $P = 0.23$ and $F_{2,46} = 2.85$, $P = 0.07$, respectively). Consequently, fungal-to-bacteria ratio was higher ($F_{2,46} = 9.71$, $P = 0.0003$) in beech (0.076 ± 0.005) as compared to maple (0.049 ± 0.005) and lime (0.040 ± 0.003) litter treatments and higher in ash (0.066 ± 0.007) as compared to lime litter treatments; hence following the same pattern as fungal PLFA. In contrast, the $Gram^-$-to-$Gram^+$ ratio was not significantly affected by litter identity ($F_{2,46} = 1.47$, $P = 0.24$).

Discussion

The results suggest that our first hypothesis that belowground resources (root exudates) are more important for soil microorganisms than aboveground resources (leaf litter) has to be rejected. Soil microorganisms generally did not respond to the presence of roots (root exudates); whereas microbial activity was influenced by the presence of litter. The missing effect of roots is in contrast to Pollierer et al. (2007) and Scheunemann et al. (2015), who showed root-derived resources to be the main driver for soil food webs. However, they studied the effect of root exudates and leaf litter input on soil invertebrates in mature tree stands and in agricultural systems, whereas we investigated the effect of both resources on soil microorganisms in presence of two year old tree saplings. The

response of microorganisms to root-derived resources varies between soil types and is affected by management practices (Paterson et al., 2007, 2008). Therefore, microbial communities in forest soils rich in soil organic matter may differ from those in arable soils with lower organic matter content (Frostegard and Baath, 1996), and may also differ between mature and young tree stands (Leuschner et al., 2009). Root exudation varies between different plant groups (Jones et al., 2004) and between mature trees and tree saplings, with tree saplings e.g. exuding less amino acids as compared to mature trees (Smith, 1969, 1990). Moreover, different biota of the soil food web likely react differently to root exudates, e.g. effects on microarthropods likely differ from those on microorganisms. In a recent study of Eissfeller et al. (2013a) rhizosphere C was of limited importance for primary decomposers. Notably, in our experiment effects of roots on the soil food web were not only less pronounced as compared to effects of leaf litter, but were missing entirely. This is likely due to the short experimental duration of our study of only eight months, i.e. one vegetation period. Presumably, this duration was too short for trees to establish rooting systems well colonized by mycorrhizal fungi, which likely form a major prerequisite for channeling root-derived resources into decomposer food webs (Smith and Read, 2008; Pollierer et al., 2012; Cesarz et al., 2013).

Unexpectedly, there was no beneficial mixture effect of root or litter species on soil microorganisms; rather, microbial basal respiration was significantly higher in soil covered by single litter species as compared to soil with a litter layer of four species and soil microbial biomass remained unaffected by mixtures of roots or litter. This is in contrast to the expectation that complexity of habitat and nutrient resources beneficially affects soil microorganisms (Bardgett, 2005; Hättenschwiler et al., 2005). Further, our findings are in contrast to Eisenhauer et al. (2010) who showed plant species richness to significantly affect soil microorganisms. However, Eisenhauer et al. (2010; 2012) documented that in grassland diversity effects of plants on soil microorganisms only established after a lag phase of 3-4 years; therefore, the lack of root effects on soil microorganisms in our experiment likely was due to the short duration of the experiment and due to the different ecosystem observed.

Our results show that litter mixing effects on soil microorganisms may range from negative to positive to neutral, depending on the time scale investigated as well as on the relative contribution of different species and their characteristic litter traits (Hättenschwiler et al., 2005; Wardle et al., 2006; Nilsson et al., 2008). While some litter species may stimulate microbial activity in providing easily available resources, others may contribute less due to the dominance of recalcitrant carbon compounds or due to low nitrogen concentration (Saetre and Baath, 2000; Bardgett and Wardle, 2010; Bell et al., 2015). Hence, the effect of single species may vanish in litter mixtures due to mixing of litter species with antagonistic effects on soil biota (Pan et al., 2015).

Our second hypothesis that the response of microorganisms is more pronounced in EMF as compared to AMF roots, with the effect of mycorrhizal type varying between root (tree) species also

has to be rejected. The effect of EMF on soil microorganisms was not more pronounced than that of AMF. The missing difference of effects between EMF and AMF on microorganisms may have been due to increased bacterial activity in the rhizosphere of AMF roots compensating the more pronounced effect of EMF roots via providing more hyphal resources for rhizosphere consumers (Cesarz et al., 2013; Eissfeller et al., 2013a) or due to the short experimental duration. Eight months may have been too short for mycorrhizal fungi to successfully infest the roots of the tree saplings; therefore, differences between EMF and AMF fungi may not have established yet. Further, mycorrhizal type and root identity neither affected microbial biomass and activity nor community composition of soil microorganisms. Effects of mycorrhizal type and root identity of the planted trees may have been overlain by root exudates of the 150 year old mountain oak trees of the forest the experiment was established in.

In contrast to our third hypothesis the effect of high quality litter on soil microorganisms was not generally more pronounced than that of low quality litter. Unexpectedly, neither microbial activity nor microbial biomass was significantly affected by litter quality or identity. This is in contrast to Nilsson et al. (2008), who found litter of e.g., *Populus tremula* to significantly stimulate basal respiration and therefore microbial activity in Swedish boreal forest soils. The difference to our findings is likely due to the fact that in boreal forests nutrients from litter are rather limited as compared to temperate forest soils and hence litter addition of broad leaf litter has a pronounced effect, since it is more easily decomposable than coniferous needles (Osono, 2007). Further, in previous studies the response of microorganisms to the addition of plant residues was investigated shortly after application and varied with composition, complexity and degradability as well as the amount of the added litter (Mondini et al., 2006; Paterson et al., 2008). Therefore, the analysis of microbial biomass and activity in our experiment possibly missed changes early after litter addition.

In contrast to microbial biomass and activity, microbial community composition varied significantly with litter quality, although variations were small and $Gram^+$ bacteria dominated over $Gram^-$ bacteria and soil fungi in each of the treatments. The decrease in $Gram^+$ bacteria in high quality litter may have been due to faster decomposition of this resource and therefore less litter layer remaining in these treatments as compared to treatments with low quality litter after eight months of exposure in the field or impaired abiotic soil conditions such as reduced soil moisture (Jacob et al., 2009). Further, $Gram^+$ bacteria may use other nutrient resources such as exudates of young growing roots or residues of other microorganisms (Ruf et al., 2006; Cesarz et al., 2013; Lemanski and Scheu, 2014). The percentage of $Gram^-$ bacteria increased in presence of high quality litter, presumably due to high nutrient supply and reduced abundance of $Gram^+$ bacteria and therefore reduced competition for easily available resources. Paterson et al. (2008) also found $Gram^-$ bacteria to be favored if soluble and easily available carbon resources were abundant. The increased abundance of fungal PLFA in low

quality litter as compared to high quality litter likely was due to their ability to digest complex plant compounds such as cellulose and lignin (Neely et al., 1991; Cox et al., 2001; Paterson et al., 2008), thereby avoiding competition with soil bacteria. Our findings are in agreement with previous studies showing that fungi are the most effective decomposers of recalcitrant plant residues (Neely et al., 1991; Lundquist et al., 1999) while easily available, soluble nutrients are mostly degraded by soil bacteria (Paterson et al., 2008).

According to the analysis of litter identity on microbial community composition soil bacteria were not affected by litter species. This likely was due to bacteria using other resources than leaf litter or at least not to be specialized on specific litter species (Paterson et al., 2007; Cesarz et al., 2013). In contrast to bacteria, fungi varied significantly with litter species identity. The percentage of fungal PLFA was highest in beech, followed by ash and maple and lowest in lime litter. As a consequence, fungal-to-bacteria ratio was higher in low as compared to high quality litter, with highest values in beech followed by ash and maple and lowest in lime litter. The difference in litter species effects on soil fungi is likely due to species specific litter traits (Hättenschwiler et al., 2005; Jacob et al., 2009). Further, saprotrophic fungi in soil of the deciduous forest composed of mountain oak and beech trees may be better adapted to the colonization of stand specific litter as compared to litter species uncommon or not present in the system (Ayres et al., 2009; Jacob et al., 2010). Therefore, fungal PLFA contributes more to the sum of extracted and assigned PLFAs of the microbial community in presence of beech litter as compared to the presence of other litter species.

Neither the diversity of aboveground nor of belowground resources affected soil microbial community composition as indicated by PLFA analysis. Similarly, earlier studies showed that litter diversity and decomposer organisms are only weakly connected, but plant community structure to be of significant importance (Salamon et al., 2004; Scheu, 2005). Different litter species vary in species specific traits, such as litter nitrogen, plant nutrient and plant polyphenol concentration (Swift et al., 1979; Hättenschwiler and Vitousek, 2000; Knops et al., 2001). Mixing of different nutrient resources such as different litter species therefore only affects soil microbial communities if mixtures include litter species with different, but not antogonistic traits (Hättenschwiler and Vitousek, 2000; Jacob et al., 2010; Eissfeller et al., 2013b).

The results of the present study suggest that identity rather than diversity of resources affects soil food webs. Further, the comparison of our results to previous studies highlights aboveground litter to be the main source of soil microbial nutrition in young, i.e. regenerating forest stands, while belowground resources are more important in grasslands or mature forest ecosystems, where the rooting system and associated mycorrhizal fungi are well established. Hence, the importance of aboveground and belowground resources for decomposer food webs of forest ecosystems likely varies with stand age, with effects of litter on mineralization processes and feedbacks to plants being

most significant for young trees at least in short term. Further samplings and analyses should be conducted in the frame of this experiment to verify or differentiate our findings arisen from data of tree and litter effects on soil microorganisms within the first vegetation period.

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Supplement T1: Absolute values (Mean ± standard error of the mean) of analyzed markers (c nmol/gDW) for Gram$^+$ bacteria (Gram$^+$ = i15:0; a15:0; i16:0; i17:0), Gram$^-$ bacteria (Gram$^-$ = cy17:0; cy19:0), other bacteria (Bacteria = 16:1w7) and saprotrophic fungi (Fungi = 18:2w6) according to root and litter presence and absence as well as diversity (Root Mix = 0;1;4, Litter Mix = 0;1;4), Mycorrhizatype (MT = AMF; EMF), Litter quality (LQ = high; low), root identity (RI = ash; beech; lime; maple) and litter identity (LI = ash; beech; lime; maple).

		Gram$^+$				**Gram$^-$**		**Bacteria**	**Fungi**
		i15:0	a15:0	i16:0	i 17:0	cy 17:0	cy 19:0	16:1w7	18:2w6
Root Mix	0	10.62 ± 0.94	5.15 ± 0.42	3.41 ± 0.35	1.58 ± 0.17	2.79 ± 0.35	8.25 ± 1.03	8.36 ± 0.72	2.16 ± 0.30
	1	9.77 ± 0.39	5.11 ± 0.20	3.01 ± 0.17	1.54 ± 0.08	2.89 ± 0.16	5.48 ± 0.46	8.20 ± 0.32	2.20 ± 0.13
	4	11.12 ± 1.03	5.81 ± 0.61	3.27 ± 0.35	1.99 ± 0.21	3.66 ± 0.49	5.94 ± 1.08	9.03 ± 0.95	2.60 ± 0.42
Litter Mix	0	9.24 ± 0.59	4.81 ± 0.33	2.90 ± 0.28	1.57 ± 0.11	2.54 ± 0.23	5.34 ± 0.84	8.00 ± 0.59	2.16 ± 0.32
	1	10.35 ± 0.47	5.44 ± 0.24	3.24 ± 0.18	1.63 ± 0.09	3.11 ± 0.18	6.61 ± 0.51	8.69 ± 0.38	2.31 ± 0.15
	4	10.14 ± 0.75	4.88 ± 0.37	2.85 ± 0.32	1.64 ± 0.19	3.08 ± 0.43	4.22 ± 0.89	7.41 ± 0.54	2.15 ± 0.27
MT	AMF	10.10 ± 0.56	5.25 ± 0.28	3.12 ± 0.23	1.51 ± 0.12	3.00 ± 0.23	5.68 ± 0.66	8.20 ± 0.41	2.32 ± 0.18
	EMF	9.43 ± 0.55	4.98 ± 0.29	2.90 ± 0.25	1.58 ± 0.10	2.79 ± 0.22	5.27 ± 0.65	8.19 ± 0.50	2.07 ± 0.20
LQ	high	10.88 ± 0.67	5.62 ± 0.36	3.60 ± 0.28	1.73 ± 0.14	3.39 ± 0.27	7.84 ± 0.76	9.13 ± 0.59	2.37 ± 0.23
	low	9.84 ± 0.65	5.27 ± 0.33	2.90 ± 0.23	1.54 ± 0.12	2.84 ± 0.24	5.43 ± 0.64	8.18 ± 0.47	2.22 ± 0.21
RI	ash	9.89 ± 0.67	5.04 ± 0.32	2.98 ± 0.34	1.32 ± 0.16	2.96 ± 0.30	6.26 ± 1.01	8.14 ± 0.56	2.19 ± 0.23
	beech	9.31 ± 0.90	4.85 ± 0.48	2.97 ± 0.40	1.52 ± 0.15	2.68 ± 0.27	5.89 ± 0.97	7.89 ± 0.84	1.94 ± 0.30
	lime	9.54 ± 0.67	5.10 ± 0.34	2.82 ± 0.31	1.63 ± 0.13	2.90 ± 0.34	4.68 ± 0.86	8.48 ± 0.57	2.19 ± 0.27
	maple	10.30 ± 0.90	5.45 ± 0.46	3.26 ± 0.32	1.69 ± 0.18	3.03 ± 0.36	5.13 ± 0.86	8.26 ± 0.60	2.45 ± 0.27
LI	ash	11.16 ± 0.71	5.51 ± 0.29	3.88 ± 0.39	1.86 ± 0.14	3.28 ± 0.23	9.41 ± 1.05	9.47 ± 0.68	2.72 ± 0.30
	beech	8.92 ± 0.79	4.80 ± 0.44	2.69 ± 0.25	1.45 ± 0.13	2.29 ± 0.31	4.86 ± 0.77	7.88 ± 0.74	2.51 ± 0.29
	lime	10.59 ± 1.18	5.73 ± 0.68	3.30 ± 0.40	1.59 ± 0.24	3.50 ± 0.51	6.20 ± 1.01	8.78 ± 0.99	2.01 ± 0.35
	maple	10.81 ± 1.02	5.76 ± 0.48	3.12 ± 0.38	1.64 ± 0.20	3.43 ± 0.34	6.02 ± 1.04	8.67 ± 0.65	1.98 ± 0.29

Chapter 3

The importance of aboveground and belowground resources for soil invertebrates of temperate deciduous forests

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Abstract

Biodiversity in soil essentially contributes to the maintenance of ecosystem services, such as nutrient cycling and litter decomposition. Soil invertebrates and their ecosystem services crucially rely on both aboveground and belowground resources. Due to species specific traits not only the amount but also the composition of these resources is important. A number of studies investigated the importance of either aboveground or belowground resources on soil organisms, but their relative importance has rarely been taken in account. Our study aimed at disentangling the relative importance of root-derived as compared to litter-derived resources for soil microarthropods, considering in particular the role of root and litter mixtures and identity. Two year old tree saplings of four deciduous tree species (*Fagus sylvatica*, *Acer pseudoplatanus*, *Fraxinus excelsior*, *Tilia cordata*) differing in the associated mycorrhizal fungi (EMF or AMF) and litter quality (high and low) were planted in a 150 year old mountain oak forest (*Quercus petraea*). For the first time aboveground litter and root species were manipulated independently. After one year we extracted soil microarthropods from soil cores (5 cm diameter and 5 cm depth) and sorted them into collembolans, mites, proturans, pauropodes and symphylans with collembolans identified to species level. There was no uniform response of soil microarthropods to aboveground or belowground resources. The analyzed soil microarthropods generally did not respond to the presence of roots, single or in mixtures, type of mycorrhizal fungi or root identity. Only the density of one collembolan species was increased in the four as compared to the one root species treatment. In contrast, aboveground litter significantly affected total density of microarthropods, the density of mites, proturans and of three out of ten collembolan species. However, there was no beneficial leaf litter mixture effect; rather soil microarthropods responded to the quality and identity of litter, with the effects varying between microarthropod groups and species. Our results suggest that soil microarthropods are little affected by root and litter mixtures, but rather by the identity of these resources with the importance of aboveground exceeding that of belowground resources in short term experiments.

Keywords: biodiversity, roots, litter, resource identity, resource mixtures, soil microarthropods, collembolans, field experiment

Introduction

Biodiversity essentially affects ecosystem functioning (Naeem et al., 1999; Hooper et al., 2005; Bardgett and van der Putten, 2014), but studies on the effect of the diversity of resources for soil food webs and therefore for ecosystem processes, such as nutrient cycling and litter decomposition, are sparse. The diversity of aboveground and belowground resources, e.g. in mixtures, increases spatial heterogeneity, habitat structure and nutrient resources (Bardgett et al., 2005), thereby changing the community composition of soil invertebrates, e.g. due to reduced competition in presence of more abundant and more variable resources.

In forest ecosystems up to 90% of the annual biomass production enters the soil and therefore the decomposer community as dead organic matter (Gessner et al., 2010), predominantly as aboveground litter (Swift, Heal, & Anderson, 1979; Berg & McClaugherty, 2008). The diversity of litter resources has been considered to significantly affect soil invertebrates, but recent studies indicate that not diversity or mixture effects per se affect soil invertebrates, but rather the quality and identity of litter species contributing to the respective mixture (Jacob et al., 2009; Eissfeller et al., 2013b). Litter species differ in specific traits (Bardgett, 2005), such as concentrations of nitrogen (N), lignin and polyphenols (Swift et al., 1979; Hättenschwiler & Vitousek, 2000; Knops et al., 2001). The higher the N concentration of litter in comparison to the carbon (C) or lignin concentration the higher its attractiveness for decomposers (Bardgett, 2005), the rate of its decomposition and hence its litter quality (Cornwell et al., 2008). Therefore, we took leaf litter mass loss to express litter quality, with high losses in high quality litter and low losses in low quality litter.

Besides with leaf litter, high amounts of C enter the belowground food web via roots (Bardgett et al., 2005; Leake et al., 2006), but the importance of belowground resources has been underestimated for long (Albers et al., 2006; Pollierer et al., 2007). Living roots typically are associated with mycorrhizal fungi which channel root resources to higher trophic levels of soil food webs (Smith and Read, 2008; Pollierer et al., 2012; Eissfeller et al., 2013b). In temperate forests ectomycorrhizal fungi (EMF), with hyphae forming a complex network between root cortical cells, and arbuscular mycorrhizal fungi (AMF), which from a highly branched arbuscule within root cortical cells (Bardgett, 2005), are the most abundant mycorrhizal types. From roots mycorrhizal hyphae extend into the soil, but these extramatrical hyphae typically are more pronounced in EMF than in AMF (Högberg et al., 2007; Smith and Read, 2008; Cairney, 2012). Therefore, in EMF carbon transport from plant roots to hyphae and nutrient transport from hyphae to roots likely exceeds that in AMF.

Microarthropods are among the most abundant soil invertebrates carrying out important functions in soil, e.g. they change the structure of litter and soil, increase litter surface area for microbial attack, release nutrients from dead organic plant or animal material and control fungal and bacterial

biomass (Seastedt, 1984; Addison et al., 2003; Cole et al., 2006). Due to their high abundance often only one or a few microarthropod groups are studied at the level of species with collembolans receiving most attention (Bardgett et al., 2005).

Collembolans are among the most abundant and well described microarthropod groups (Fjellberg, 1998; Hopkin, 1997, 2002). Although, classically viewed as typical fungivores, collembolans also feed on plants, algae and detritus (Hopkin, 1997; Petersen, 2002; Chahartaghi et al., 2005). Some have been also reported to feed on soil bacteria or other soil animals (Heidemann et al., 2014; Ferlian et al., 2015). Due to the broad variety of feeding types, effects of the identity and diversity of aboveground and belowground resources on collembolan communities are difficult to predict. Presumably, collembolans respond directly or indirectly to both belowground and aboveground resources as both may serve as food or control bacteria, fungi and nematodes serving as prey of collembolans, with the effect varying between collembolan species.

Despite the investigation of the importance of either leaf litter (Wardle et al., 1997; Hättenschwiler & Gasser, 2005; Sayer, 2006) or root-derived resources for soil food webs (Pollierer et al., 2007; Broeckling et al., 2008; Eissfeller et al., 2013a), to the best of our knowledge the combined investigation of the influence of both above- and belowground resources on soil microarthropods under field condition is missing. Due to the lack of such studies the relative importance of the identity as well as of mixing effects of roots as compared to litter for soil food webs remains unknown.

In the present study aboveground litter and roots were manipulated for the first time independently under field conditions. The study aimed at disentangling the relative importance of root- vs. litter-derived resources for microarthropods and at evaluating the role of identity and mixtures of root and litter species for soil invertebrates. Plant species differed in the mycorrhizal fungi associated with roots, i.e. EMF or AMF, and in the quality of litter as indicated by decomposition rate.

We hypothesized that (1) the response of microarthropods to the presence of aboveground litter is less pronounced as compared to their response to the presence of roots (root exudates), with litter mixtures decreasing and root mixtures increasing the difference; (2) the response of microarthropods is more pronounced in presence of EMF as compared to AMF roots, with the effect of mycorrhizal type varying between root (tree) species; (3) the response of microarthropods is more pronounced in presence of high as compared to low quality litter, with the effect of litter quality varying between litter species.

Material and methods

Study site

Our study was conducted in a 150 year old deciduous forest in the vicinity of Göttingen (51°26‘27‘‘N, 10°01‘03‘‘O, 340 m a.s.l., Lower Saxony, Germany). The region has a continental climate with a long-term mean annual temperature of 8.7°C and a mean annual precipitation of 644 mm. The forest is dominated by oak (*Quercus petraea*) with an admixture of beech (*Fagus sylvatica*) representing 95% of the trees at the study site, interspersed by single individuals of larch (*Larix decidua*), spruce (*Picea abies*), pine (*Pinus sylvestris*), willow (*Salix* spec.) and birch (*Betula* spec.).The understory is species rich and dominated by jewelweed (*Impatiens* spec.), stinging-nettle (*Urtica dioica*) and fern (*Athyrium filix-femina*). The soil is an oligotrophic brown earth from bunter composed of mull to mull like moder humus and mineral soil (Ah horizon) with a pH ($CaCl_2$) of 5.01 ± 0.07 and soil moisture of 26.74 ± 0.72% of soil fresh weight. Soil carbon and nitrogen concentrations are 2.22 ± 0.05% and 0.14 ± 0.003%, respectively.

Experimental set up

In November 2010 a total of 144 plots (180 x 210 m) were established at the study site, after some of the inferior trees had been cut down and removed, to enhance space and light conditions for the experiment. Experimental plots were arranged in between mature trees in a way to prevent presence of visible roots of mature trees. Plots had a minimum distance of 50 cm from each other. Original litter was removed from the plots and replaced by 800 g of previously collected, dried and hand sorted litter of four different deciduous tree species, beech (*Fagus sylvatica*), maple (*Acer pseudoplatanus*), ash (*Fraxinus excelsior*) and lime (*Tilia cordata*). The litter was placed in the field single or as four litter species mixtures according to study design (Chapter 2, Table 1). Litter species differed in their quality; with high quality litter decomposing faster than low quality litter (Cornwell et al., 2008). According to Jacob et al. (2010) ash and lime litter lost more than 70%, while beech and maple litter lost less than 60% of their initial mass after seven month of incubation. Therefore, ash and lime litter were taken as high quality litter species, whereas beech and maple litter were taken as low quality litter. Plots were covered by nets to prevent litter displacement by wind.

In April 2011 two year old, bare root, unfertilized saplings of the selected tree species were planted in a square with 5 samplings per row and 6 per column with the distance between adjacent saplings (in rows and columns) of 25 cm forming a core area comprising 12 saplings and a plot margin area comprising 18 saplings not used for taking samples. The saplings were obtained from a local nursery

(Billen Forst GmbH, Bösinghausen, Germany). Saplings of every tree species were planted in monocultures and in mixtures of all four tree species into experimental plots of every litter treatment. Saplings differed in regard to the associated mycorrhizal fungi. While beech and lime roots are colonized by EMF, maple and ash roots are colonized by AMF (Lang et al., 2011). Additionally, control plots with only tree (root) or leaf litter treatments or without trees and without litter were established. The experiment was set up in a complete randomized block design with four blocks, i.e. four replicates per combined tree and litter treatment (Chapter 2).

Sampling and analytical procedure

In November 2011 two soil cores were taken in the core area of each plot using a steel corer of a diameter of 5 cm. One soil core (0-5 cm depth) of every plot was used for the extraction of soil microarthropods in a heat gradient extractor modified according to Kempson et al. (1963). During extraction microarthropods were collected in glycerol - water solution (1/1 v/v). After the extraction, soil microarthropods were transferred to ethanol (70%) for storage. Then, soil microarthropods were sorted to groups including mites, collembolans, proturans, pauropods and symphylans. Collembolans were identified to species level according to Hopkin (2007). The second soil core was used for analysis of soil parameters such as soil moisture (a subsample of soil from every core was weighed before and after drying at 105°C for 72 h) and soil pH (2 g of soil from every core in 20 ml 0.01 M $CaCl_2$). Leaf litter was removed from the top of every soil core, dried at 60°C for six days and weighed for information on leaf litter mass loss as affected by litter identity. The amount of decomposed litter increased from beech litter (26.4 ± 9.15% of initial) over maple (45.46 ± 10.68% of initial) and lime (78.04 ± 6.89% of initial) to ash litter (79.62 ± 4.58% of initial) supporting the classification of beech and maple litter as low quality litter and of lime and ash litter as high quality litter.

Calculations and statistical analyses

The effect of root presence and litter presence including mixture effects (0, 1, 4) on the total density of microarthropods, the number of microarthropod groups and the density of mites, collembolans, proturans, pauropodes and symphylans, was analyzed by two factorial General Linear Model (GLM; type III sum of squares). Furthermore, a two factorial GLM (type III sum of squares) was conducted to analyze the effects of root presence and litter presence including mixture effects (0, 1, 4) on the number of collembolan species as well as on the density of collembolan species. In a multivariate hierarchical GLM (type I sum of squares; Schmid et al., 2002) the effect of the co-variables soil pH (overall mean 5.01 ± 0.07) and soil moisture (overall mean 26.74 ± 0.72 % of soil fresh weight) fitted

before the effect of mycorrhizal type (EMF, AMF), litter quality (high, low), root identity and litter identity (beech, maple, ash, lime) on total density of microarthropods, number of microarthropod groups, the total density of mites, collembolans, proturans, pauropodes and symphylans, the number of collembolan species and the density of individuals within collembolan species were analyzed. Prior to statistical analyses, data were inspected for homogeneity of variance (Levene-Test) and log transformed (log10(y+1)) if required. Means were compared using Tukey's HSD test ($P < 0.05$). Linear regressions were done, to identify how soil pH and soil moisture affected soil microarthropods.

Due to statistical feasibility only collembolan species that were found in more than three plots and that were represented by at least 100 individuals (sum of all samples) were included in the statistical analyses. Juveniles, determined only to family level, also were excluded from statistical analyses. Statistical analyses were performed using SAS (Statistical Analysis System, Version 9.3; SAS Institute Inc., Cary, USA). Regressions were done using Excel (Microsoft Office 2007). Means presented in text and tables are based on non-transformed data and given with the corresponding standard error of the mean (SEM).

Results

The microarthropod community was dominated by collembolans (71.8 ± 0.14% of total), followed by mites (25.48 ± 1.13%) and minor contributions by proturans (1.2 ± 0.2%), pauropodes (0.8 ± 0.1%) and symphylans (0.7 ± 0.1%). In total 11254 collembolans of 30 species and 6 different collembolan families had been identified (Supplement T1). Ten species occurred at higher total numbers than 100 individuals and in more than three plots (*Folsomia quadrioculata*, *Protaphorura armata*, *Parisotoma notabilis*, *Isotomiella minor*, *Sminthurinus aureus*, *Ceratophysella denticulata*, *Mesaphorura macrochaeta*, *Megalothorax minimus*, *Paratullbergia callipygos*, *Frisea mirabilis*); they accounted for more than 85% of all collembolans (incl. juveniles).

Response of microarthropods to soil pH and soil moisture

As indicated by fitting as co-variable, the number of microarthropod groups and the density of pauropods varied with soil pH; both decreasing with increasing pH (Figure 1; Supplement T2).

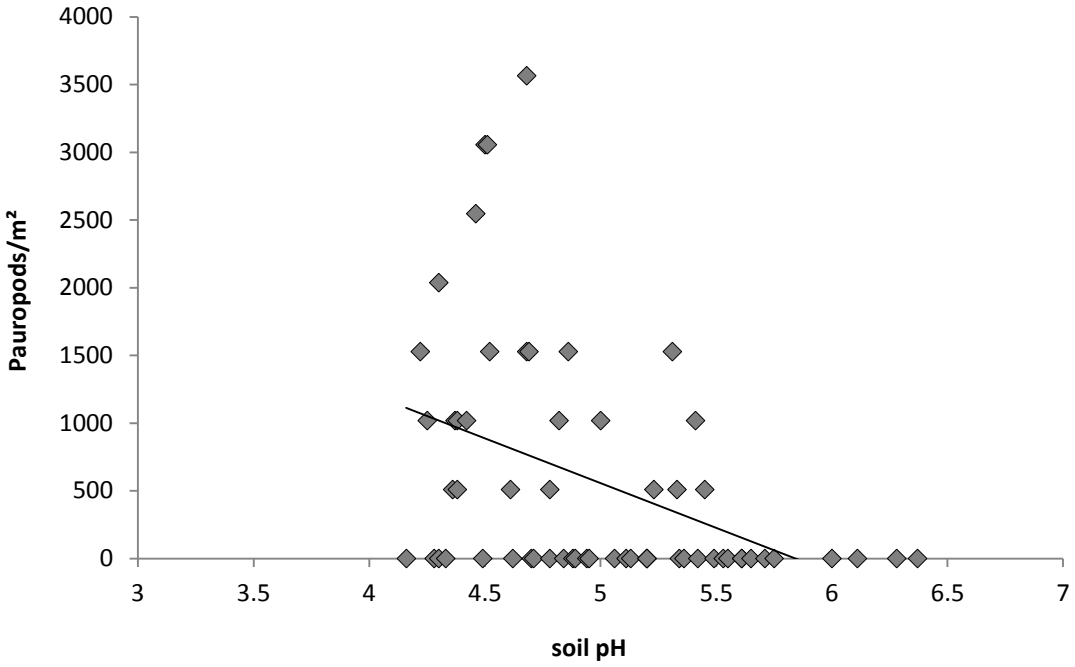

Figure 1: Density of pauropods as affected by soil pH (R^2 = 0.1723).

In contrast, the total density of microarthropods, the number of microarthropod groups, the density of mites, collembolans, proturans and the density of symphylans were not affected (Supplement T2). Of the species of collembolans, only the density of *P. armata* and *I. minor* varied with soil pH; it decreased with increasing soil pH (Figure 2a, b; Supplement T3). Soil moisture did not significantly affect the total density of microarthropods, the number of microarthropod groups, the density of mites, pauropods, proturans, symphylans (Supplement T2) and most collembolan species (Supplement T3). However, the density of the collembolan species *M. minimus* increased with increasing soil water content, from 0 ind./m² at soil moisture between 10-20% to 287 ± 152 ind./m² and 946 ± 3.84 ind./m² at soil moisture contents between 20-30% and 30-40%, respectively.

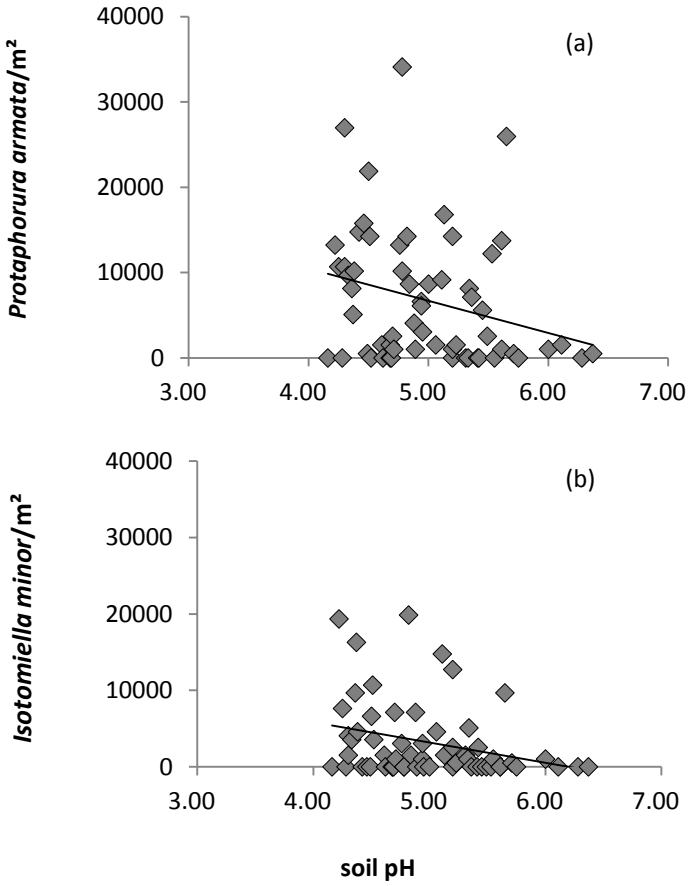

Figure 2: Density of (a) *Protaphorura armata* and (b) *Isotomiella minor* as affected by soil pH; with R^2 = 0.0713 and R^2 = 0.0846 for (a) and (b), respectively.

Response of microarthropods to presence of litter and roots

The total density of microarthropods did not significantly respond to root presence ($F_{2, 130}$ = 0.85, P = 0.43), but increased on average by 75% in presence of litter ($F_{2, 130}$ = 3.76, P = 0.03) as compared to treatments without litter, irrespective if single species or litter mixtures (Figure 3).

The number of microarthropod groups was not significantly affected by the presence of single root species or root mixtures ($F_{2, 130}$ = 1.66, P = 0.19), but significantly varied with the presence and mixture of litter ($F_{2, 130}$ = 5.14, P = 0.007); increasing from treatments without litter (2.4 ± 0.2 groups) to four (2.9 ± 0.2) to one litter species treatments (3.1 ± 0.1). The density of collembolans was not significantly affected by root presence or mixture ($F_{2, 130}$ = 1.07, P = 0.35) as well as litter presence or litter mixture ($F_{2, 130}$ = 1.48, P = 0.23).

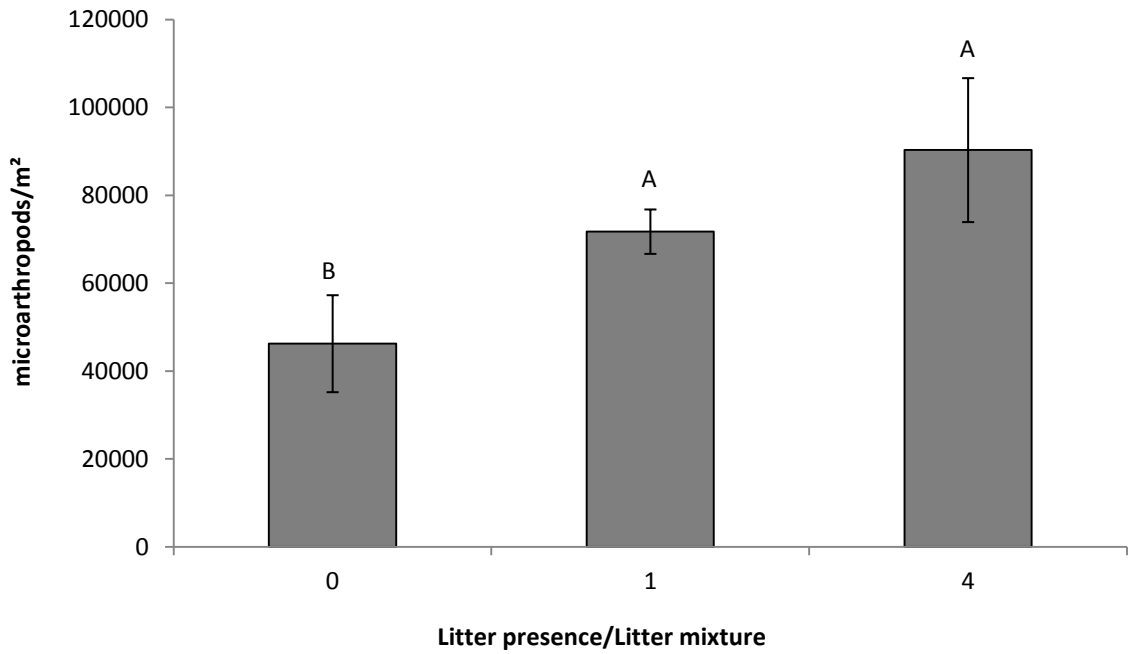

Figure 3: Variations in total density of microarthropods with absence (0) as compared to presence of litter. Litter was present as single litter species (1) or as a mixture of four litter species (4). Significant differences between means are indicated by different letters (Tukey's HSD test; $P < 0.05$).

Furthermore, the density of mites was not significantly affected by root presence and mixture ($F_{2, 130} = 0.05$, $P = 0.96$), but in presence of one and four litter species it exceeded that of treatments without litter ($F_{2, 130} = 9.88$, $P = 0.0001$; Figure 4). The density of proturans did not significantly respond to root presence and mixture, although in trend ($F_{2, 130} = 2.86$, $P = 0.06$) it was higher in treatments without roots (trees) as compared to treatments with roots. In contrast, the density of proturans was significantly ($F_{2, 130} = 3.06$, $P = 0.05$) higher in presence of one litter species (954.3 ± 178.9 individuals/m²) as compared to treatments without litter (169.8 ± 84.9 ind./m²), and intermediate in four litter species treatments (597.9 ± 259.4 ind./m²). The density of pauropods and symphylans was not significantly affected by root presence and mixture ($F_{2, 130} = 0.35$, $P = 0.71$ and $F_{2, 130} = 0.04$, $P = 0.96$, respectively) as well as by litter presence and mixture ($F_{2, 130} = 1.40$, $P = 0.25$ and $F_{2, 130} = 1.08$, $P = 0.34$).

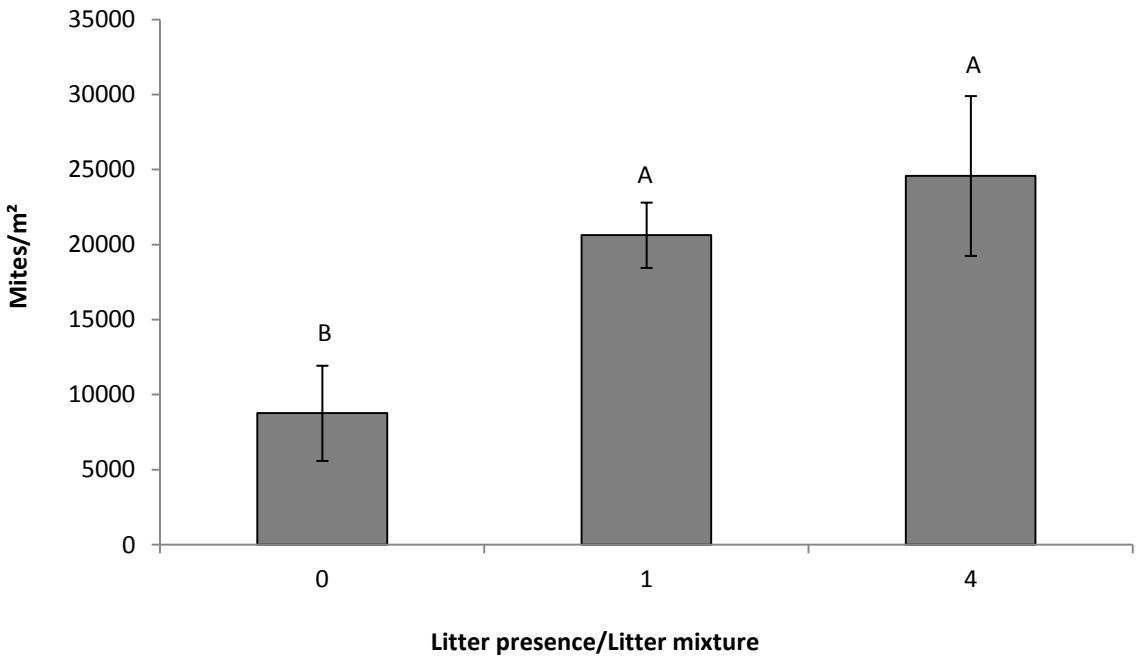

Figure 4: Variations in the density of mites with absence (0) as compared to presence of litter. Litter was present as single litter species (1) or as a mixture of four litter species (4). Significant differences between means are indicated by different letters (Tukey's HSD test; $P < 0.05$).

The number of collembolan species varied significantly with the presence and mixture of litter, but this depended on presence and mixture of roots ($F_{4,129} = 3.91$, $P = 0.005$ for the interaction between litter and root presence and mixture) increasing from no litter to four litter species treatments except for treatments with four species of roots (Figure 5).The density of certain collembolan species was neither affected by root presence or mixture nor litter presence or mixture, except for that of *M. minimus* which responded to root diversity (Table 5). The density of *M. minimus* was significantly ($F_{2,129} = 3.84$, $P = 0.0002$) higher in treatments with four root species (1018.6 ± 519.3 ind./m²) as compared to treatments with only one root species (503.9 ± 117.5 ind./m²) and intermediate in treatments without roots (594.2 ± 329.7 ind./m²).

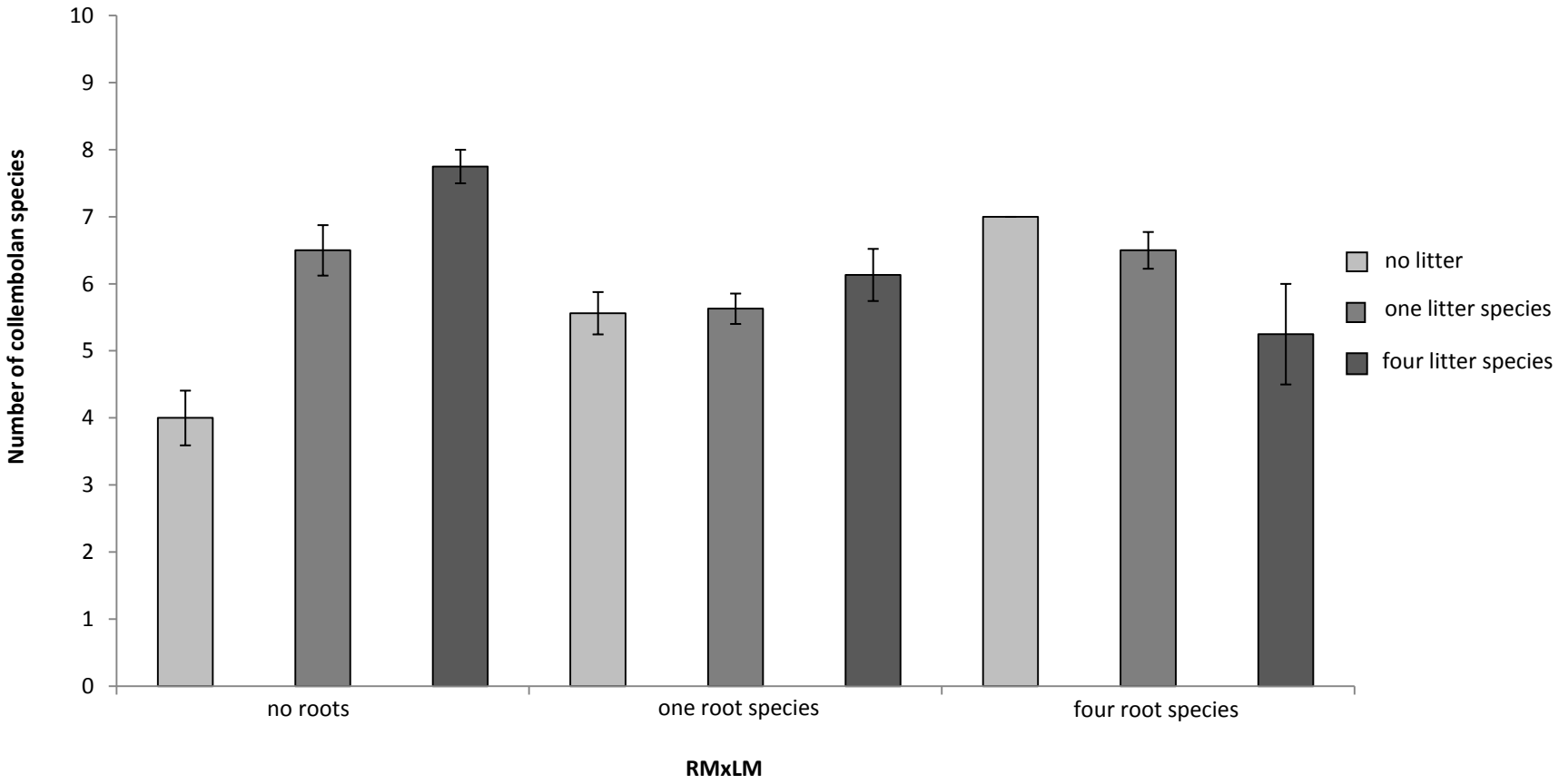

Figure 5: Number of collembolan species as affected by the interaction of root and litter presence and diversity (RM x LM). For statistical analysis see Table 5.

Table 5: Two factorial General Linear Model (type III sum of squares) table of F- values on the effect of root mixture (RM), litter presence (LM) and interaction between roots and litter (RM x LM) on log10 transformed densities of collembolan species, total density of collembolans and number of collembolan species. *p < 0.05; **p < 0.01; ***p < 0.001

	RM	LM	RMxLM
	$F_{2,129}$	$F_{2,130}$	$F_{4,131}$
Folsomia quadrioculata	0.43	0.93	1
Protaphorura armata	2.91	0.66	1.46
Parisotoma notabilis	0.28	2.47	0.3
Isotomiella minor	0.6	0.65	1.51
Sminthurinus aureus	0.11	0.74	0.19
Ceratophysella denticulata	0.01	0.66	1.96
Mesaphorura macrochaeta	0.42	0.28	0.51
Megalothorax minimus	**3.84***	0.39	2.37
Paratullbergia callipygos	0.81	2.62	0.95
Frisea mirabilis	0.28	0.49	1.02
Total density of collembolans	1.21	1.84	0.66
number of collembolan species	0.81	1.47	**3.91****

Response of microarthropods to mycorrhizal type and identity of roots

Neither mycorrhizal type nor root identity significantly affected the density of microarthropods, the number of microarthropod groups or the density of any of the investigated taxonomic groups (Supplement T2). Nevertheless, there was a trend for symphylans ($F_{1,44}$ = 3.64, P = 0.06) to occur at higher density in presence of EMF (716 ± 1.99 ind./m²) as compared to AMF (246 ± 115 ind./m²) and for pauropods ($F_{2,44}$ = 2.53, P = 0.09) to occur at higher density in presence of beech roots (1783 ± 651 ind./m²) as compared to ash roots (159 ± 111 ind./m²). Further, neither the number of collembolan species nor the density of individuals within the different collembolan species significantly varied with mycorrhizal type or root identity (Supplement T3).

Response of microarthropods to quality and identity of leaf litter

Neither the total density of microarthropods nor the number of microarthropod groups were significantly affected by litter quality ($F_{1,44}$ < 0.01, P = 0.96 and $F_{1,44}$ = 0.63, P = 0.43, respectively) or litter identity ($F_{2,44}$ = 1.24, P = 0.30 and $F_{2,44}$ = 1,95, P = 0.15, respectively). Also, the density of mites, collembolans, pauropods, proturans and symphylans did not significantly respond to litter quality or litter identity (Supplement T2).

Furthermore, litter quality did not significantly affect the number of collembolan species or the density of single collembolan species, except for *C. denticulata* (Supplement T3), which occurred in significantly higher densities in treatments with high (3407 ± 655 ind./m²) as compared to those with low quality litter (1314 ± 387 ind./m²). In contrast, litter identity did affect the number of collembolan species (Supplement T3) which was significantly higher in ash (6.3 ± 0.4) as compared to maple litter treatments (4.9 ± 0.5). The same was true for the density of *P. armata* (Figure 6a). In contrast, the density of *P. callipygos* was significantly higher in beech as compared to lime litter treatments and intermediate in ash and maple litter treatments (Figure 6b). Litter identity generally did not significantly affect the density of the other collembolan species (Supplement T3).

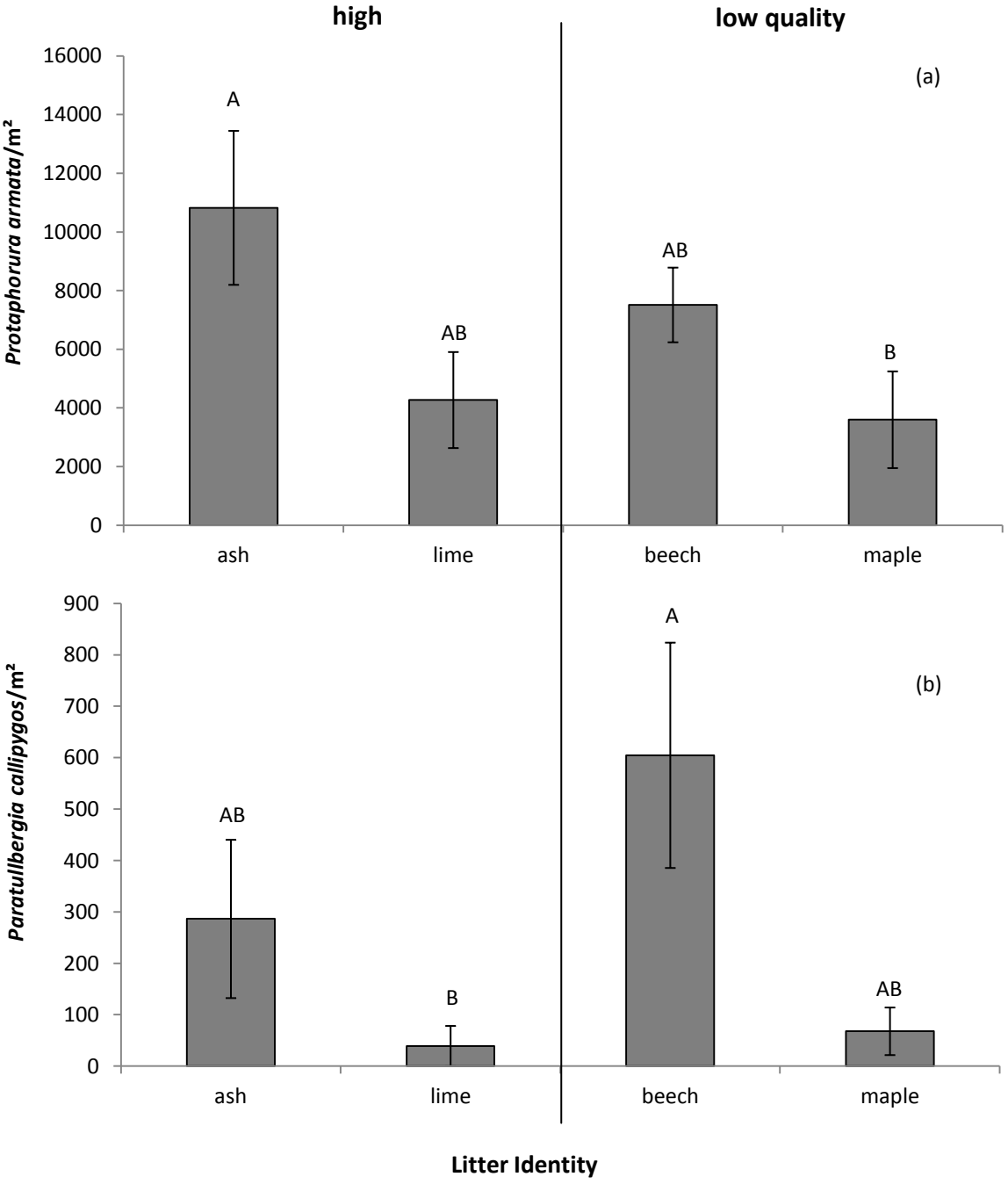

Figure 6: Density of (a) *Protaphorura armata* and (b) *Paratullbergia callipygos* as affected by litter identity (ash and lime litter of high quality; beech and maple litter of low quality). For statistical analysis see Supplement T3; significant differences between means are indicated by different letters (Tukey's HSD test; $P < 0.05$).

Discussion

Root presence did not significantly affect the total density of microarthropods, the number of microarthropod groups the density of mites, collembolans, pauropods, proturans, symphylans, the number of collembolan species as well as the density of nine out of ten collembolan species. Likewise, there was no mixture effect of root species on the density or community composition of microarthropods. Only the collembolan species *M. minimus* was significantly affected by belowground but not aboveground resources, with the density in the four exceeding that in the one root species treatment. The lack of effects of roots on soil microarthropods except for *M. minimus* is in contrast to Scheunemann et al. (2015) and Pollierer et al. (2007), who showed root-derived resources to be the main driver for soil animal food webs. The short duration of our experiment may be an important reason for the missing root effect in our study. Probably, the rooting system of the two year old tree saplings was not fully established or the colonization by mycorrhizal fungi was not completed after one vegetation period, what is a major prerequisite for channeling root-derived resources into decomposer food webs (Smith and Read, 2008; Pollierer et al., 2012; Cesarz et al., 2013). Furthermore, the lack of effects of roots in our study in contrast to that of the studies of Scheunemann et al. (2015) and Pollierer et al. (2007) may be due to the different systems investigated (Frostegard and Baath, 1996; Leuschner et al., 2009; Erdmann et al., 2012), as Scheunemann et al. (2015) and Pollierer et al. (2007) studied roots of agricultural plants and mature trees, respectively, whereas we investigated roots of two year old tree saplings. Root exudation, affecting soil microarthropods via soil microorganisms benefitting from soluble substances released by root tips (Bais et al., 2006; Bardgett and Wardle, 2010), varies between different plant groups (Jones et al., 2004) and between mature trees and tree saplings, with mature trees releasing greater diversity and abundance of e.g. amino acids (Smith, 1969). Therefore, the effect of mature tree roots on soil microarthropods may be more pronounced as compared to that of tree saplings, irrespective of the experimental duration.

In contrast to belowground resources, the presence of aboveground litter markedly affected soil microarthropods. However, the effect of litter presence and mixture on soil microarthropod groups was not uniform, with significant importance of litter presence for the density of mites and proturans affecting total density of microarthropods and the number of microarthropod groups, but no effect of litter on e.g. the density of total collembolans or collembolan species. The missing effect of litter on collembolans was unexpected, but indicates that collembolans use other resources than litter or litter-associated fungi. This is in agreement with Hopkin (1997) and Petersen (2002) arguing that collembolans feed on plants, algae and detritus, besides being fungal feeders. Furthermore, the analysis of fatty acids and stable isotopes by Ferlian et al. (2015) indicated poor correlations between

collembolans and litter, but distinct feeding niches of different collembolan species, which is in agreement with our findings. Further, few effects of litter presence on soil microarthropods and no positive mixture effect was found. This contrasts our expectation that the density of soil microarthropods increases with increasing complexity of habitat and nutrient resources (Bardgett, 2005; Hättenschwiler et al., 2005). Eisenhauer et al. (2010, 2012) showed plant species richness and therefore mixture effects to significantly affect soil biota only after a lag phase of 3 - 4 years. Hence, the missing positive mixture effect in our experiment may be due to the short experimental duration. Furthermore, the missing mixture effect on soil microarthropods may be due to the composition of litter species in this experiment, with different specific litter traits (Hättenschwiler et al., 2005; Wardle et al., 2006; Nilsson et al., 2008) that may have cancelled out species effects in mixture (Pan et al., 2015). This is in agreement with Milcu et al. (2006), Jacob et al. (2009) and Eissfeller et al. (2013b) who also found composition of litter mixtures to be more important than species number per se, with even higher effects of single key species than of mixtures. More litter mixtures including two species treatments need to be investigated to answer this question. Overall, however, microarthropods more intensively responded to the presence of aboveground litter than to the presence of roots suggesting that our first hypothesis needs to be rejected at least in the timeframe of the current experiment.

Further, in contrast to our second hypothesis the response of microarthropods was not more pronounced in presence of EMF as compared to AMF roots. Since many microarthropods are feeding on free living saprotrophic microorganisms the missing difference of effects between EMF and AMF may be due to the missing effect of mycorrhizal type on bacteria and fungi shown previously in the same experiment (Grubert, unpubl. data). Nevertheless, the missing difference in the effect of EMF and AMF on soil biota is surprising, since EMF have a more intensively dispersed extramatrical mycelium than AMF (Smith and Read, 2008) providing more food resources. Probably, the relatively short experimental duration is responsible for the missing EMF effects on soil microarthropods. One vegetation period may have been too short for mycorrhizal fungi to successfully infest the roots of the tree saplings or to establish a properly developed hyphal network; therefore, differences between EMF and AMF fungi may not have established yet. However, there was a trend that symphylans were more abundant in presence of EMF as compared to AMF. Pauropodes feeding or sucking on fungal hyphae (Shear and Selden, 2001) were also found in higher densities when beech roots (EMF) were present as compared to ash roots (AMF); although the difference was not significant. This trend may have been due to higher nutrient supply by beech as compared to ash roots, or due to a higher soil pH in presence of ash roots as compared to beech roots (Cesarz et al., 2013), since the density of pauropodes decreased with increasing pH values. Moreover, the higher density of pauropodes in presence of beech may be due to faster colonization of beech roots by

mycorrhizal fungi already present in the forest that comprised mainly of oak and beech trees, while mycorrhiza infesting the other three tree species studied may not have been present to the same extent. Since the weak or missing effects of mycorrhizal type and root identity on soil microarthropods may have been due to the young tree saplings and the short experimental duration, further samplings and analyses after at least two vegetation periods are needed to reliably answer if EMF or AMF is more important for soil microarthropods.

Concerning our third hypothesis that the response of soil microarthropods is more pronounced in presence of high as compared to low quality litter, only the epedaphic collembolan species *C. denticulata* (Hopkin, 1997) occurred in significantly higher density in high as compared to low quality litter treatments. The close relationship between the density of this collembolan species and litter quality suggests *C. denticulata* to feed on microorganisms such as bacteria and fungi benefitting from high quality litter. A litter identity effect was shown for *P. armata*, being significantly more abundant in ash as compared to maple litter treatments and *P. callipygos* preferring beech over lime litter. However, the effect of high quality litter on soil microarthropods varied with microarthropod groups/species. Some microarthropod groups, i.e. mites and proturans, responded to the presence of litter, without quality, identity or diversity of the resource being of significant importance. The correlation may be due to more stable conditions in soil under an intact litter layer, indicating abiotic factors rather than resource quality to affect these microarthropod groups. This is supported by the study of Eissfeller et al. (2013b), who suggested mites to occur in higher densities in presence of thick humus layers since it favors a more stable abiotic environment. Furthermore, the relatively low taxonomic resolution of the studied soil fauna groups except for collembolans may have been responsible for small effects of litter quality and litter identity on soil microarthropods. It is known that species of oribatid mites and collembolans use very different food resources including fungi, bacteria and even animal prey such as nematodes (Chahartaghi et al., 2005; Hopkin, 1997; Heidemann et al., 2014). Hence, some species of the same microarthropod group may reach high densities, whereas others may decrease under the same conditions. Thus, total density of the group may not significantly change, although single species do, as indeed was the case in collembolans. In particular mites are diverse comprising a large number of species; hence, quality and identity of litter are likely to affect community composition without affecting the total density. Therefore, detailed information on soil fauna community on species level is indispensable to understand the interaction between belowground and aboveground resources and soil food webs.

Conclusions

A number of studies investigated the importance of either aboveground or belowground resources on soil organisms arguing on their relative importance. For the first time in this study both aboveground and belowground resources were manipulated independently in one experiment. Our results suggest the importance of aboveground resources to exceed that of belowground resources after one vegetation period; thus, supporting the classical view that soil food webs are fuelled in large by leaf litter resources. Furthermore, soil organisms were affected by resource identity rather than by resource mixture. However, effects in the present study were relatively weak and restricted to certain groups or species. The comparison of our results to previous studies highlights aboveground litter to be the main source of nutrients for the soil food web in regenerating forest stands, while, as shown previously, belowground resources are more important in grasslands and mature forest ecosystems, where the rooting system and associated mycorrhizal fungi are well established. Further samplings and analyses in the context of this field study are needed to identify if the importance of aboveground and belowground resources changes with time, thus being stand age dependent, or rather depends on the respective system, with its specific abiotic conditions and its specific microarthropod community. To test for this not only collembolans but also other soil microarthropods groups should be studied at the level of species, since according to our results, rather than on group level the effects of resources are pronounced at the level of species.

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Supplement T1: Average density of collembolan species (Mean/m²; with standard error of the mean) and average percentage (Mean [% of all]; with standard error of the mean) of single species on collembolan community.

Collembolan species (adult individuals)	Mean/m²	SEM	Mean [% of all]	SEM
Folsomia quadrioculata	15089	1778.1	30.27	1.64
Protaphorura armata	6854	826.2	13.29	1.22
Parisotoma notabilis	5843	515.9	16.42	1.26
Isotomiella minor	3820	601.9	7.28	0.74
Sminthurinus aureus	2575	313.3	8.30	1.05
Ceratophysella denticulata	2267	269.9	6.31	0.86
Mesaphorura macrochaeta	1438	306.5	3.51	0.78
Megalothorax minimus	603	126.8	1.66	0.31
Paratullbergia callipygos	369	103	0.73	0.18
Frisea mirabilis	366	67.6	1.22	0.24
Isotoma viridis	280	147.3	0.94	0.50
Lepidocyrtus lanuginosus	247	51.1	0.66	0.14
Desoria violacea	172	85.8	0.58	0.30
Willemia aspinata	86	72.5	0.14	0.11
Desoria propinqua	72	61.2	0.23	0.18
Isotomurus palustris	61	57.5	0.14	0.13
Onychiurus jubilarius	61	21.3	0.15	0.05
Protaphorura furcifera	54	38	0.09	0.06
Lepidocyrtus cyaneus	25	10.6	0.05	0.02
Sphaeridia pumilis	25	9.3	0.16	0.07
Entomobrya nivalis	11	8.	0.02	0.02
Neanura muscorum	11	6.2	0.01	0.01
Pogonognathellus flavescens	7	5.1	0.01	0.01
Pseudosinella alba	7	7.2	0.01	0.01
Dicyrtomina minuta	4	3.6	0.01	0.01
Entomobrya muscorum	4	3.6	0.01	0.01
Lipothrix lubbocki	4	3.6	0.01	0.01
Sminthurides parvulus	4	3.6	0.01	0.01
Sminthurinus elegans	4	3.6	0.03	0.03
Tomocerus vulgaris	4	3.6	0.09	0.09
Collembolan juveniles	Mean/m²	SEM	Mean [% of all]	SEM
Tullbergiidae	2586	856.7	4.23	0.88
Isotomidae	653	230.2	1.54	0.49
Onychiuridae	546	123.9	1.40	0.28
Katiannidae	91	46.2	0.34	0.23
Entomobryidae	25	12.8	0.08	0.04
Hypogastruridae	18	12.9	0.05	0.03
Symphypleona	7	7.2	0.02	0.02

Supplement T2: Multivariate General Linear Model (type I sum of squares) table of F- values on the effect of soil pH, soil moisture (Co-Variables), mycorrhizal type (MT), litter quality (LQ), MT x LQ interactions, root identity (RI), litter identity (LI) and RI x LI interactions on log10 transformed densities of mites, collembolans, pauropods, proturans, symphylans as well as on the total density of microarthropods (TDM) and the number of microarthropod groups (NMG). *p < 0.05; **p < 0.01; ***p < 0.001

	soil pH	soil moisture	MT	LQ	MTxLQ	RI	LI	RIxLI
	$F_{1,44}$	$F_{1,44}$	$F_{1,44}$	$F_{1,44}$	$F_{1,44}$	$F_{2,44}$	$F_{2,44}$	$F_{8,44}$
Mites	2.55	0.01	0.74	1.01	0.33	0.27	0.01	1.68
log10Mites	3.38	0.49	1.05	0.34	0.50	0.05	0.69	1.17
Collembola	1.22	0.08	0.10	0.01	1.39	0.35	0.82	0.74
log10Collembola	0.41	0.00	0.19	0.06	0.39	0.09	0.72	0.88
Pauropods	**5.08***	0.03	2.57	0.14	0.09	2.79	1.39	1.05
log10Pauropods	**13.45*****	0.36	2.07	0.01	0.10	2.53	2.05	1.35
Proturans	0.85	2.07	0.10	0.44	2.21	0.10	1.68	1.40
log10Proturans	1.33	2.93	0.20	0.30	0.71	0.18	1.01	1.26
Symphylans	1.33	0.00	3.43	0.01	0.21	1.20	1.37	0.18
log10Symphylans	1.42	0.05	3.64	0.00	0.17	1.19	1.23	0.27
TDM	2.23	0.04	0.21	0.12	1.21	0.06	0.53	1.20
log10TDM	1.27	0.02	0.35	0.00	0.46	0.06	0.80	1.07
NMG	**9.18****	0.58	3.43	0.04	0.12	0.02	0.85	0.46
log10NMG	**9.22****	0.84	2.43	0.01	0.13	0.06	0.85	0.39

Supplement T3: Multivariate General Linear Model (type I sum of squares) table of F- values on the effect of soil pH, soil moisture (Co-Variables), mycorrhizal type (MT), litter quality (LQ), MT x LQ interactions, root identity (RI), litter identity (LI) and RI x LI interactions on log10 transformed densities of *Folsomia quadrioculata*, *Protaphorura armata*, *Parisotoma notabilis*, *Isotomiella minor*, *Sminthurinus aureus*, *Ceratophysella denticulata*, *Mesaphorura macrochaeta*, *Megalothorax minimus*, *Paratullbergia callipygos* and *Frisea mirabilis* as well as on total density of collembolans and number of collembolan species. *p < 0.05; **p < 0.01; ***p < 0.001

	soil pH	soil moisture	MT	LQ	MTxLQ	RI	LI	RIxLI
	$F_{1,39}$	$F_{1,39}$	$F_{1,39}$	$F_{1,39}$	$F_{1,39}$	$F_{2,39}$	$F_{2,39}$	$F_{8,39}$
Folsomia quadrioculata	0.10	2.41	0.16	0.02	0.28	0.60	1.23	1.42
Protaphorura armata	**6.7***	1.69	0.83	0.12	0.06	0.35	**3.32***	1.84
Parisotoma notabilis	2.36	0.28	0.01	1.50	0.77	0.33	0.76	2.18
Isotomiella minor	**4.4***	2.21	0.01	0.50	1.56	0.32	0.33	1.96
Sminthurinus aureus	0.33	0.94	2.22	0.41	0.49	0.01	1.00	0.95
Ceratophysella denticulata	0.05	0.05	0.76	**5.17***	0.62	0.21	0.38	0.30
Mesaphorura macrochaeta	0.28	2.68	0.21	0.70	0.07	0.79	0.92	0.32
Megalothorax minimus	0.26	**6.1***	0.83	2.18	1.23	0.42	0.86	1.69
Paratullbergia callipygos	0.00	0.06	0.90	2.07	0.08	0.64	**4.53***	0.59
Frisea mirabilis	0.10	0.28	0.70	0.04	0.42	0.41	0.51	1.06
Total density of collembolans	0.49	1.49	0.52	0.03	0.07	0.13	1.49	1.69
number of collembolan species	1.06	2.81	0.12	0.44	0.84	0.42	**3.89***	1.14

Chapter 4

Species identity and diversity rather than trait similarity affects macrofauna - mesofauna interactions and their importance for ecosystem processes

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Abstract

Biodiversity is considered to be a major determinant of ecosystem functioning and stability, with soil animals and their interactions exerting strong effects on ecosystem processes, such as leaf litter mass loss and ^{15}N cycling. The understanding of how and why certain species interact is important to predict the effect of a specific species combination on ecosystem processes. Complementarity has been proposed as predictor for soil animal interactions, at least for macrofauna detritivores. We investigated interactions between four detritivore species differing in body size and habitat association, i.e. two species of earthworms (*Lumbricus terrestris*, *Aporrectodea caliginosa*) and two species of collembolans (*Heteromurus nitidus* and *Protaphorura armata*). Mesocosms with natural forest floor containing one beech (*Fagus sylvatica*) seedling were set up and incubated in the laboratory for three months. ^{15}N labeled beech litter was used to follow the effect of detritivore animals on nitrogen (N) cycling and N uptake by beech seedlings. We assumed that similar species exert negative effects on the performance of each other and reduce their respective effects on leaf litter mass loss and ^{15}N cycling. We found detrimental but also facilitative interactions between soil animal species with similar and those with dissimilar traits. Furthermore, interactions were partly unbalanced with one species benefitting and the second being detrimentally affected in presence of the other. Additionally, ^{15}N incorporation into the species studied decreased in presence of *L. terrestris*, irrespective of trait similarity, while its own incorporation was unaffected by other species. Leaf litter mass loss and ^{15}N cycling were mainly affected by litter-associated species, with the effect of *L. terrestris* being most pronounced. Usually, species hampered their individual effects on ^{15}N incorporation into beech seedlings in two species treatments, while facilitative effects occurred if *L. terrestris*, *A. caliginosa* and *P. armata* were present together. The results suggest that soil fauna interactions mainly vary with the identity of species rather than with trait similarity, indicating that results found for interactions between macrofauna detritivore species cannot be generalized easily. This highlights the complexity of soil fauna interactions and the difficulty to predict their effects on ecosystem processes in complex communities. Overall, the results emphasize the importance of species identity for interactions of detritivores and for their effects on litter decomposition and N cycling.

Key words: litter decomposition, ^{15}N, N cycling, earthworms, collembolans, beech, soil fauna interactions, mesocosms

Introduction

Biodiversity is considered to be a major determinant of ecosystem functioning and stability (Naeem et al., 1999; Balvanera et al., 2006; Tilman et al., 2014). A considerable fraction of global biodiversity and species from virtually all taxonomic groups of microorganisms and invertebrates are found in soil (Wardle, 2002), and soil animals are driving important ecosystem processes such as decomposition and nutrient turnover (Bardgett and van der Putten, 2014). The majority of energy and nutrients obtained by plants is used for primary production and returned to soil as dead organic matter or detritus. In forest ecosystems up to 90% of the annual biomass production enters the soil and therefore the decomposer community as dead organic matter (Gessner et al., 2010), mainly as aboveground litter (Swift et al., 1979; Berg and McClaugherty, 2008), thereby returning nutrients to soil, most importantly nitrogen (N) and phosphorus (P). Most terrestrial ecosystems are limited by N (Vitousek and Howarth, 1991; LeBauer and Treseder, 2008) that is together with P and potassium (K), the main element that limits plant productivity (Chapin, 1980). Therefore, decomposition of litter material and the release and cycling of N bound in detritus are important for the continuous nutrient supply for soil animals, microorganisms and plants (Seastedt, 1984), and thereby for the productivity of terrestrial ecosystems (Vitousek, 1982).

Earthworms and collembolans are among the most important and abundant soil invertebrate taxa involved in decomposition and nutrient cycling (Edwards and Bohlen, 1996; Hopkin, 2002, 1997). Earthworms modify physical, chemical and biological properties of the soil, feed on or incorporate litter into the soil, and mingle organic material and mineral soil, thereby affecting decomposition processes and nutrient cycling (Scheu, 1987; Edwards and Bohlen, 1996; Butenschoen et al., 2009). Collembolans are among the most abundant (up to 10^5 individuals/m²) and best studied microarthropods (Hopkin, 1997, 2002; Fjellberg, 1998). Although classically viewed as typical fungivores, collembolans are trophically diverse and feed on a variety of food materials including fungi, but also plants, algae, detritus, bacteria and even other soil animals (Petersen, 2002; Chahartaghi et al., 2005; Heidemann et al., 2014). Since soil animals affecting decomposition and nutrient cycling are part of complex trophic and non-tropic networks, their influence on ecosystem processes is modified by interactions between them (Torsvik et al., 1990; Wardle, 2002). Previous studies on the effects of earthworms on collembolans (Wickenbrock and Heisler, 1997; Maraun et al., 1999; Eisenhauer et al., 2007) suggest those effects to vary from detrimental, over neutral to facilitative. The variability in the effects likely is due to differences in the traits of the investigated earthworm species such as body size, distribution within the soil and resource use (McLean and Parkinson, 2000; Eisenhauer, 2010). However, even earthworms with similar traits may exert

different effects on collembolans (Eisenhauer, 2010). This suggests that not only the traits of earthworms, but also those of collembolans are important for their interactions.

The similarity of traits is assumed to be the main factor influencing effects of soil animals on each other (Heemsbergen et al. 2004). This is supported by Uvarov (2009) who found competition between earthworm species to increase with increasing trait similarity. Moreover, Heemsbergen et al. (2004) found different macrofauna detritivores to decrease their respective performance as well as their effect on the rate of ecosystem processes with increasing similarity of traits. In contrast, animal species with dissimilar traits facilitated each other's influence on the rate of e.g., leaf litter mass loss (Heemsbergen et al., 2004). We investigated if these relationships also apply for interactions between macrofauna and mesofauna detritivores. We conducted a full factorial mesocosm experiment in the greenhouse with two earthworm species and two collembolan species, with one earthworm and one collembolan species being associated with soil and one earthworm and one collembolan species being associated with litter. We analysed earthworm biomass and collembolan abundance after three months to inspect their effects on each other. Beech saplings and ^{15}N labelled beech litter were introduced into the mesocosms to allow investigating the importance of soil fauna interactions for ecosystem processes, i.e. leaf litter mass loss and N cycling.

We hypothesized that (1) effects of litter-associated species (*Lumbricus terrestris* and *Heteromurus nitidus*) on ecosystem processes, i.e. litter decomposition and ^{15}N cycling, exceed those of soil-associated species (*Aporrectodea caliginosa* and *Protaphorura armata*). Further, we hypothesized that (2) soil animals with similar traits, i.e. body size (macrofauna vs. mesofauna) and habitat association (litter vs. mineral soil), hamper the performance of each other. Accordingly, we hypothesized that (3) the effects of single soil animal species on litter decomposition and ^{15}N cycling are modified by interactions with other soil animals, with similar species reducing their effects on litter decomposition and ^{15}N cycling.

Material and methods

Soil and plant material

Soil samples were taken in April 2012 in a 150 year old deciduous forest in the vicinity of Göttingen (51°26‘27‘‘N, 10°01‘03‘‘O, 340 m a.s.l., Lower Saxony, Germany). The region has a continental climate with a long-term mean annual temperature of 8.7°C and a mean annual precipitation of 644 mm. The forest is dominated by oak (*Quercus petraea*) and beech (*Fagus sylvatica*) interspersed by single individuals of larch (*Larix decidua*), spruce (*Picea abies*), pine (*Pinus sylvestris*), willow (*Salix* spec.) and birch (*Betula* spec.), presenting 5% of all tree individuals. The understory is species rich

and dominated by jewelweed (*Impatiens* spec.), stinging-nettle (*Urtica urens*) and fern (*Athyrium filix-femina*). The soil is an oligotrophic brown earth from bunter composed of mull to mull like moder humus and mineral soil (Ah horizon) with a pH ($CaCl_2$) of 5.01 ± 0.07 and soil moisture (November) of 26.7 ± 0.7% of soil fresh weight. Soil carbon (C) and N concentrations are 2.22 ± 0.05% and 0.14 ± 0.003%, respectively, with a $\delta^{15}N$ signature of 0.49 ± 0.24‰. The soil was taken from the upper 10 cm homogenized by passing through a 10 mm screen and defaunated by three freeze-thaw cycles switching between -30°C and +20°C every 72 h.

Two year old beech saplings (*Fagus sylvatica*) were obtained from a local forestry nursery (Billen Forst GmbH, Bösinghausen, Göttingen). Five trees were cut into roots and shoots, dried at 105°C for 72 h, milled to powder and analyzed for concentration of C, N and ^{15}N stable isotope signature. Initial concentration of C was 43.61 ± 1.49% and 48.15 ± 0.72%, initial concentration of N was 1.12 ± 0.03% and 0.76 ± 0.15% and initial $\delta^{15}N$ signature was 2.45 ± 0.42‰ and 1.69 ± 0.95‰ for roots and shoots, respectively.

To obtain ^{15}N labeled leaf litter, young beech trees were grown in PVC containers in a climate controlled greenhouse and watered with ^{15}N labeled ammonium ($^{15}NH_4^+$ 99 atom% ^{15}N; Campro Scientific, Berlin, Germany) over a period of two years. Senescent leaf litter was collected, air dried and stored at room temperature until set up of the experiment. C and N concentrations of the litter were 47.10 ± 0.80% and 1.09 ± 0.04%, respectively, with a C-to-N ratio of 43.22 and $\delta^{15}N$ signature of 2888.7 ± 791.3‰.

Earthworms and collembolans

Individuals of the soil-associated (endogeic) earthworm species *Aporrectodea caliginosa* (Savigny) were collected from forest sites in the vicinity of Göttingen using electrical octet and mustard extraction methods (Eisenhauer et al., 2008). Individuals of the litter-associated (anecic) earthworm species *Lumbricus terrestris* L. were bought from a commercial supplier (Schwarzangler GmbH, Göttingen, Germany). Prior to adding earthworms into the mesocosms they were kept in plastic boxes filled with experimental soil for two weeks. Unlabeled beech litter was added as nutrient resource and the soil was watered once per week.

Individuals of the litter-associated (epi- to hemiedaphic) collembolan species *Heteromurus nitidus* (Templeton) and of the soil-associated (euedaphic) collembolan species *Protaphorura armata* (Tullberg) were taken from laboratory cultures, where they were kept at constant temperature (18°C) and fed on baker's yeast.

Initial ^{15}N stable isotope signatures were measured in triplicate and averaged 5.26 ± 2.98‰, 5.42 ± 0.73‰, 4.42 ± 0.001‰ and 5.67 ± 0.38‰ for *A. caliginosa, L. terrestris, H. nitidus and P. armata*, respectively.

Experimental set up

The experiment started in May 2012. A total of 64 mesocosms was set up. Mesocosms consisted of PVC tubes (160 mm diameter and 350 mm height) sealed with 45 µm mesh at the bottom to allow water to pass. Mesocosms were filled with fresh soil equivalent to 3000 g dry weight. One two year old beech sapling (55.22 ± 18.00 g fresh weight) was planted in each mesocosm and 5 g dry weight ^{15}N labeled beech litter was placed on the soil surface. Two individuals of *A. caliginosa* (0.95 ± 0.25 g fresh weight in sum), one individual of *L. terrestris* (3.88 ± 0.93 g fresh weight), and 50 (treatments including only one collembolan species) or 25 individuals (treatments including both collembolan species) of *H. nitidus* and *P. armata*, corresponding to 2829 collembolans/m², were added to experimental mesocosms in a full factorial design. Therefore, 16 treatments were established: without soil animals, with *L. terrestris*, *A. caliginosa*, *H. nitidus* or *P. armata* only, with all possible two species and three species combinations and one treatment with all four species. Treatments were replicated four times and mesocosms were incubated under controlled conditions (18°C, 80% air humidity, long day conditions = 16 h, 200 µmol m^{-2} s^{-1} photosynthetic photon flux density) in a greenhouse for three month and watered at regular intervals.

Sampling and analytical procedure

After three months the saplings were cut at soil surface and dried at 105°C for 72 h. Earthworms were collected by hand, identified to species level, counted, washed to remove adherent soil, dried on filter paper for 1 min, weighed and preserved at -21°C until stable isotope analysis. Collembolans were extracted from soil cores using heat (Kempson et al., 1963) and transferred into ethanol (70%). Animals were counted and identified to species level (Hopkin, 2007). Approximately 40 g fresh weight soil of each mesocosm was dried at 105°C for 72 h, filled in plastic bags and stored for further analysis. Remaining beech litter was removed, dried at 105°C for 72 h and weighed. Roots were separated from soil by rinsing with tap water, collected on a screen (1 mm), dried at 105°C for 72 h and weighed.

Dried soil and plant material was ground using a ball mill (Retsch Mixer Mill MM200, Haan, Germany); approximately 30 mg soil and 2 mg plant material was transferred into tin capsules for ^{15}N analysis. For earthworms the anterior body part without gut content was used for ^{15}N analysis. Due

to low body weight collembolans had to be pooled (up to 40 individuals) to allow stable isotope analysis. Soil animals were transferred into tin capsules and dried at 65°C for 24 h before measurement of stable isotopes.

Incorporation of ^{15}N into soil animals, soil and plant compartments was determined using a coupled system of an elemental analyzer (NA1500 Fisons-Instruments, Rodano, Milano, Italy) and a mass spectrometer (Delta plus Finnigan MAT, Bremen, Germany MAT 251, Finnigan, Bremen, Germany) (Reineking et al., 1993). Atmospheric N_2 served as the primary standard and acetanilide (C_8H_9NO, Merck, Darmstadt) for internal calibration.

Calculations and statistical analyses

For statistical analyses the values of leaf litter mass [g], biomass of earthworms [g] and abundance of collembolans [ind./mesocosm] after the experiment were expressed as percentages of initial. Incorporation of ^{15}N in soil, soil animals and roots and shoots of beech saplings were calculated as $\Delta^{15}N_x = \delta^{15}N_{x\text{-harvest}} - \delta^{15}N_{x\text{-initial}}$ with x representing soil, soil animals, roots or shoots.

Data were inspected for homogeneity of variance (Levene-Test) and log transformed if required. Eight microcosms in which the introduced earthworms died or escaped were excluded from the analysis. Four-factorial General Linear Model (GLM; type III sum of squares) was used to analyze effects of soil animal species and interactions between them on leaf litter mass loss, ^{15}N excess in soil, roots and shoots with *L. terrestris* (0|1), *A. caliginosa* (0|1), *H. nitidus* (0|1) and *P. armata* (0|1) as factors. Three-factorial GLM (type III sum of squares) was used to analyze the effects of the presence of each soil animal species (0|1) on the biomass, abundance of as well as on ^{15}N incorporation into the other soil animal species.

The variance accounted for by the different soil animals and their interactions was calculated as Eta^2 [%] = SS_{treat}/SS_{total} x 100, with SS_{treat} and SS_{total} the treatment and total sum of squares, respectively (Brown, 2008). Analyses of variance were performed using SAS (Statistical Analysis System, Version 9.3; SAS Institute Inc., Cary, NC, USA). Means in text and tables are based on non-transformed data and given with the corresponding standard error of the means (SEM).

Results

Effects of soil animals and their interactions on leaf litter mass loss

Leaf litter mass loss averaged 22.1 ± 2.1% at the end of the experiment. In presence of *L. terrestris* leaf litter mass loss increased by 26.8% (Table 1). In contrast, *A. caliginosa*, *H. nitidus* and *P. armata* ($F_{1,38}$ = 0.27, P = 0.60) did not affect leaf litter mass loss, neither alone nor in combination.

Table 1: Four-factorial General Linear Model (type III sum of squares) table of F-values on the effect of *L. terrestris* (Lt), *A. caliginosa* (Ac), *H. nitidus* (Hn), *P. armata* (Pa) and their combinations on the amount of leaf litter remaining (percentages of initial; logit transformed data; Litter mass) and Δ^{15}N in soil, roots and shoots (log10 transformed data); *p < 0.05, **p < 0.01, ***p < 0.001.

		Litter mass	Δ^{15}N soil	Δ^{15}N roots	Δ^{15}N shoots
	df	F	F	F	F
Lt	1	**131.81*** **	1.66	**9.66** **	0.66
Ac	1	0.01	0.84	2.09	0.47
Hn	1	0.00	0.03	**4.74***	1.5
Pa	1	0.27	0.38	1.33	0.09
Lt × Ac	1	0.35	0.44	0.21	1.37
Lt × Hn	1	1.45	0.04	**14.92*** **	1.04
Lt × Pa	1	1.38	0.15	0.10	2.41
Ac × Hn	1	0.07	**4.97***	**5.12***	0.14
Ac × Pa	1	0.01	0.00	**7.59** **	0.16
Hn × Pa	1	0.65	0.05	0.00	0.05
Lt × Ac × Hn	1	1.00	3.36	2.92	0.20
Lt × Ac × Pa	1	1.26	0.00	**12.97*** **	**5.21***
Lt × Hn × Pa	1	0.04	0.91	0.03	0.20
Ac × Hn × Pa	1	0.28	0.13	0.31	0.36
Lt × Ac × Hn × Pa	1	0.12	2.07	1.45	2.10
Error	38				

Effects of soil animals and their interactions on ^{15}N cycling

On average, δ^{15}N in soil increased by 66.6 ± 6.9 ‰ during the experiment. None of the animal species significantly affected soil δ^{15}N if present as single species (Table 1). However, the increase in δ^{15}N was less pronounced if both *A. caliginosa* and *H. nitidus* were present (Fig. 1).

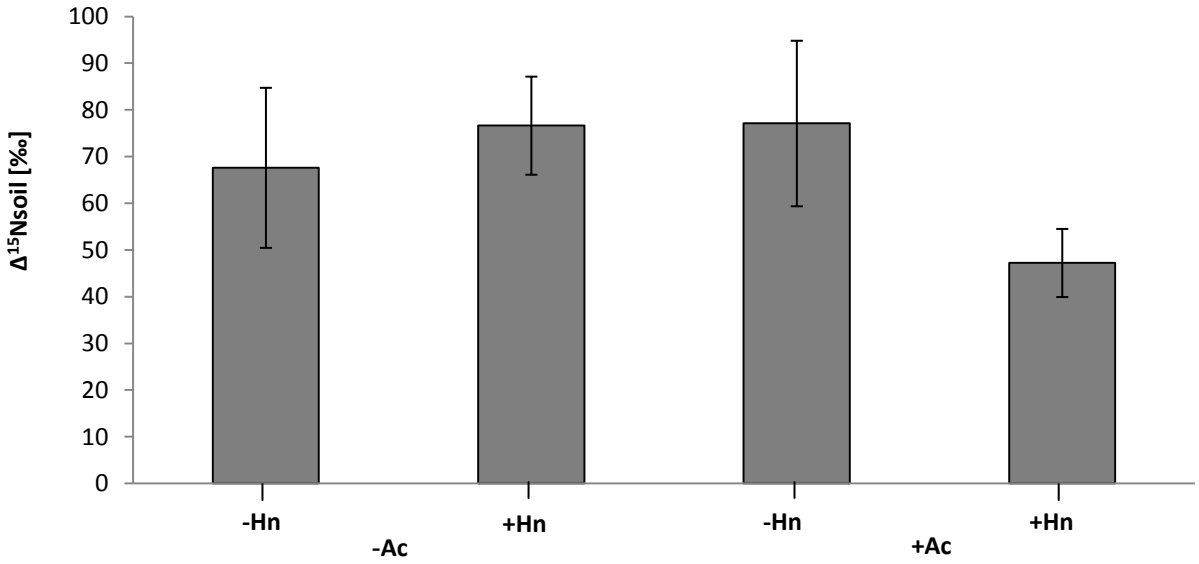

Figure 1: $\Delta^{15}N$ [‰] in soil as affected by *Aporrectodea caliginosa* (Ac) and *Heteromurus nitidus* (Hn); – and + indicate absence or presence of the respective soil animal species; means with SE; for statistical analysis see Table 1.

$\delta^{15}N$ in roots of beech saplings increased on average by 19.4 ± 3.5 ‰. Incorporation of ^{15}N into roots was significantly increased in presence of *L. terrestris* from 15.9 ± 4.9 to 23.3 ± 4.8 ‰, and in presence of *H. nitidus* from 19.0 ± 5.4 to 19.7 ± 4.4 ‰. However, the increase in $\delta^{15}N$ in roots was less pronounced if both *L. terrestris* and *H. nitidus* were present (significant *L. terrestris* x *H. nitidus* interaction; Fig. 2a). Furthermore, the positive effect of *H. nitidus* on $\delta^{15}N$ in roots was reduced in presence of *A. caliginosa* (significant *A. caliginosa* x *H. nitidus* interaction; Fig. 2b). Both *A. caliginosa* and *P. armata* slightly increased $\delta^{15}N$ in roots as single species, but this was less pronounced if both species were present (significant *A. caliginosa* x *P. armata* interaction). Furthermore, in presence of all three species, *L. terrestris*, *A. caliginosa* and *P. armata*, $\delta^{15}N$ in roots was even higher as compared to treatments with *L. terrestris* as single species (significant *L. terrestris* x *A. caliginosa* x *P. armata* interaction; Fig. 2c).

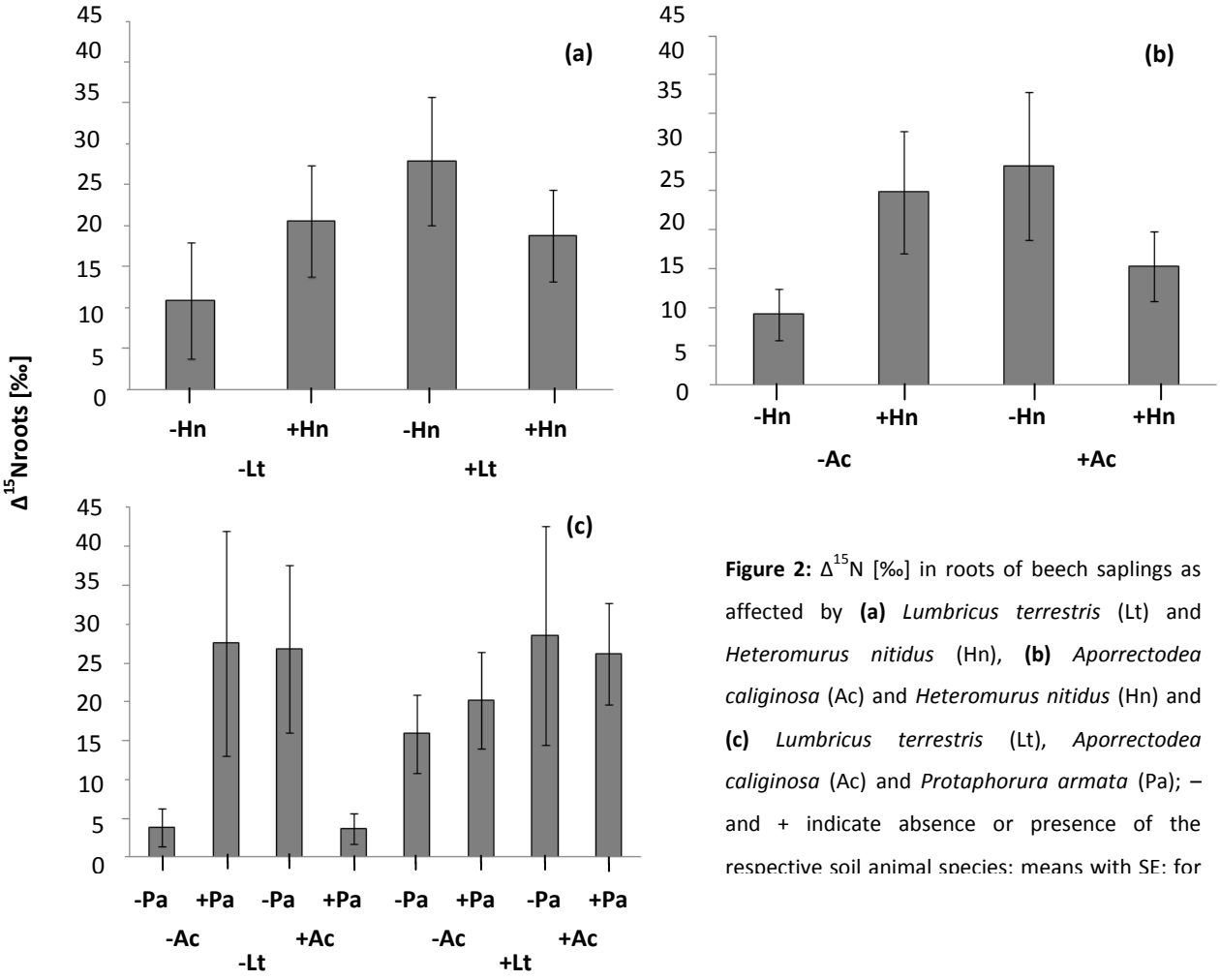

Figure 2: $\Delta^{15}N$ [‰] in roots of beech saplings as affected by **(a)** *Lumbricus terrestris* (Lt) and *Heteromurus nitidus* (Hn), **(b)** *Aporrectodea caliginosa* (Ac) and *Heteromurus nitidus* (Hn) and **(c)** *Lumbricus terrestris* (Lt), *Aporrectodea caliginosa* (Ac) and *Protaphorura armata* (Pa); – and + indicate absence or presence of the respective soil animal species; means with SE; for

$\delta\ ^{15}N$ in shoots varied little in single and two species treatments, but as compared to the control without animals it was increased by 5.9 ± 5.5 ‰ in the combined treatment with *L. terrestris*, *A. caliginosa* and *P. armata* (significant *L. terrestris* x *A. caliginosa* x *P. armata* interaction; Fig. 3).

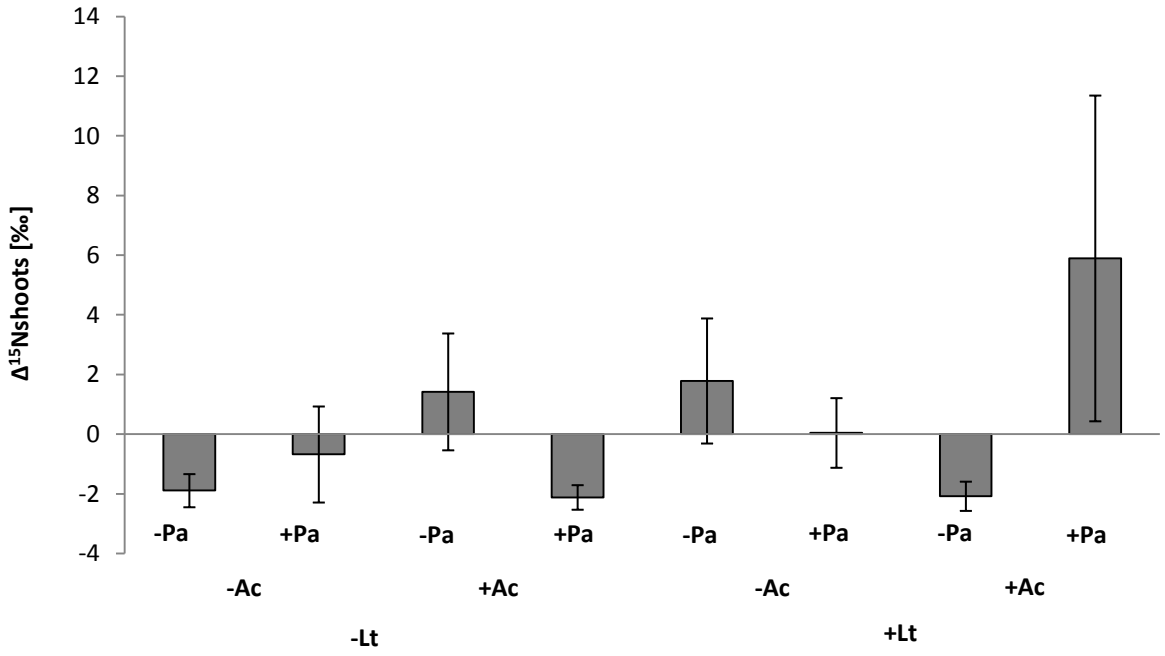

Figure 3: $\Delta^{15}N$ [‰] in shoots of beech saplings as affected by *Lumbricus terrestris* (Lt), *Aporrectodea caliginosa* (Ac) and *Protaphorura armata* (Pa); – and + indicate absence or presence of the respective soil animal species; means with SEM; for statistical analysis see Table 1.

Soil animal biomass and abundance

On average, the biomass of *L. terrestris* decreased by 19.1 ± 2.9%, whereas that of *A. caliginosa* increased by 74.4 ± 13.6%. Biomass loss of *L. terrestris* increased in presence of *A. caliginosa* (24.9 ± 3.6%), but this effect was counteracted by *P. armata*, with biomass loss of *L. terrestris* being only 19.5 ± 3.9% in presence of both species (significant *A. caliginosa* × *P. armata* interaction; Table 2; Fig. 4). Biomass gain of *A. caliginosa* increased in presence of *P. armata* (45.5 ± 14.3% and 107.7 ± 21.1% without and with *P. armata*, respectively; Table 3). Moreover, the biomass of *A. caliginosa* increased in presence of *L. terrestris* (49.1 ± 17.6% and 103.6 ± 18.5% without and with *L. terrestris*, respectively). On average, the abundance of *H. nitidus* increased by a factor of 3.99 ± 1.00 whereas that of *P. armata* increased by a factor of 11.32 ± 1.65. The abundance of both *H. nitidus* and *P. armata* was not significantly affected by the presence of other soil animals (Tables 4 and 5).

Table 2: Tree-factorial General Linear Model (type III sum of squares) table of F-values on the effect of *A. caliginosa* (Ac), *H. nitidus* (Hn), *P. armata* (Pa) and their interactions (Ac × Hn, Ac × Pa, Hn × Pa, Ac × Hn × Pa) on biomass of *L. terrestris* (percentages of initial; Lt biomass) and the change in $\delta^{15}N$ values in *L. terrestris* ($\Delta^{15}N$ Lt); *p < 0.05, **p < 0.01, ***p < 0.001.

	Ac	Hn	Pa	Ac × Hn	Ac × Pa	Hn × Pa	Ac × Hn × Pa
	$F_{1,17}$	$F_{1,17}$	$F_{1,17}$	$F_{1,17}$	$F_{1,17}$	$F_{1,17}$	$F_{1,17}$
Lt biomass [%]	**5.9***	0.26	0.46	0.21	**5.9***	0.38	1.02
$\Delta^{15}N$ Lt [‰]	0.16	0.31	0.58	0.01	0.53	0.49	0.58

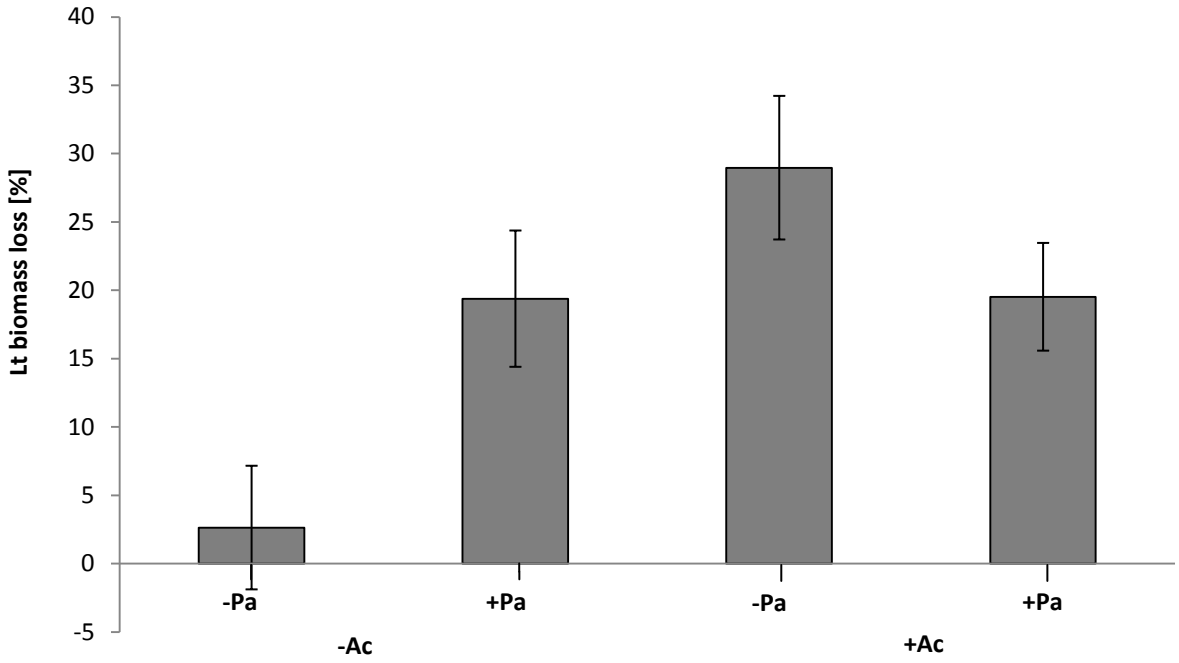

Figure 4: Biomass loss of *L. terrestris* (Lt) as affected by *A. caliginosa* (Ac) and *P. armata* (Pa); – and + indicate absence or presence of the respective soil animal species; means with SE; for statistical analysis see Table 2.

^{15}N incorporation into soil animals

On average, $\delta^{15}N$ of *L. terrestris* increased by 264.6 ± 26.3 ‰ during the experiment; it was not affected by experimental treatments (Table 2). $\delta^{15}N$ of *A. caliginosa* increased on average by 42.6 ± 9.8 ‰, but the increase was less pronounced in presence of *L. terrestris* (71.2 ± 14.4 ‰ and 7.4 ± 1.6 ‰ without and with *L. terrestris*, respectively; Table 3). Notably, the negative effect of *L. terrestris* on $\delta^{15}N$ of *A. caliginosa* was reduced by *H. nitidus* (significant Lt x Hn interaction; Fig. 5 a). Moreover, the increase in $\delta^{15}N$ of *A. caliginosa* was reduced if *H. nitidus* or *P. armata* or both were present, with the reduction in $\delta^{15}N$ of *A. caliginosa* being less pronounced in the combined treatment (significant *H. nitidus* x *P. armata* interaction; Fig. 5 b).

Table 3: Tree-factorial General Linear Model (type III sum of squares) table of F-values on the effect of *L. terrestris* (Lt), *H. nitidus* (Hn), *P. armata* (Pa) and their interactions (Lt × Hn, Lt × Pa, Hn × Pa, Lt × Hn × Pa) on biomass of *A. caliginosa* (percentages of initial; Ac biomass) and the change in $\delta^{15}N$ values in *A. caliginosa* ($\Delta^{15}N$ Ac); *p < 0.05, **p < 0.01, ***p < 0.001.

	Lt	Hn	Pa	Lt × Hn	Lt × Pa	Hn × Pa	Lt × Hn × Pa
	$F_{1,19}$	$F_{1,19}$	$F_{1,19}$	$F_{1,19}$	$F_{1,19}$	$F_{1,19}$	$F_{1,19}$
Ac biomass [%]	4.34	0.13	**6.25***	0.16	0.05	0.17	1.39
$\Delta^{15}N$ Ac [‰]	**25.9*****	3.22	2	**4.41***	2.2	**5.17***	3.61

On average, $\delta^{15}N$ of *H. nitidus* increased by 586.8 ± 79.8 ‰ during the experiment. Incorporation of ^{15}N into *H. nitidus* was less pronounced in presence of *L. terrestris* (900.8 ±84.2 ‰ and 224.5 ± 29.3 ‰ without and with *L. terrestris*, respectively; Table 4). Compared to *H. nitidus* the increase in $\delta^{15}N$ of *P. armata* was less pronounced averaging 365.0 ± 56.5 ‰; similar to *H. nitidus* incorporation of ^{15}N also was reduced in presence of *L. terrestris* (550.9 ± 73.0 ‰ and 148.1 ± 20.9 ‰ without and with *L. terrestris*, respectively; $F_{1,17}$ = 52.12; P < 0.001). Generally, the effect of *L. terrestris* was most pronounced on the litter-associated *H. nitidus* (Eta^2 = 171.0%) than on the soil-associated *A. caliginosa* (Eta^2 = 115.5%) and *P. armata* (Eta^2 = 137.9%).

Table 4: Tree-factorial General Linear Model (type III sum of squares) table of F-values on the effect of *L. terrestris* (Lt), *A. caliginosa* (Ac), *P. armata* (Pa) and their interactions (Lt × Ac, Lt × Pa, Ac × Pa, Lt × Ac × Pa) on the abundance of *H. nitidus* (percentages of initial; log10 transformed data; Hn abundance) and changes in $\delta^{15}N$ values in *H. nitidus* (log10 transformed data; $\Delta^{15}N$ Hn). *p < 0.05, **p < 0.01, ***p < 0.001

	Lt	Ac	Pa	Lt × Ac	Lt × Pa	Ac × Pa	Lt × Ac × Pa
	$F_{1,19}$	$F_{1,19}$	$F_{1,19}$	$F_{1,19}$	$F_{1,19}$	$F_{1,19}$	$F_{1,19}$
Hnabundance	0.27	0	1.17	0.01	1.9	0.02	1.52
$\Delta^{15}N$ Hn	**134.9*****	1.64	**5.42***	0.02	1.14	0.12	0.09

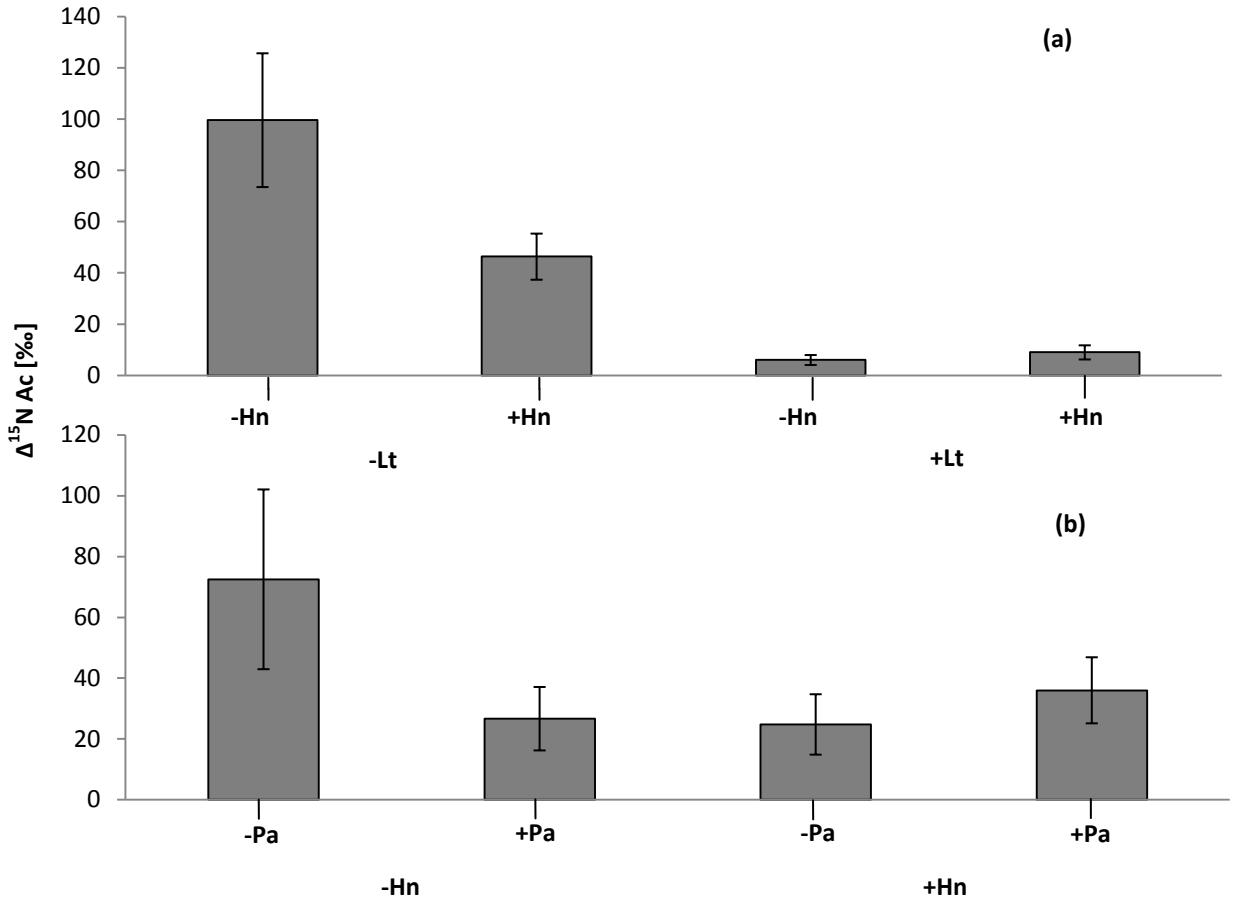

Figure 5: Δ^{15}N [‰] in *Aporrectodea caliginosa* (Δ^{15}N Ac) as affected by **(a)** *Lumbricus terrestris* (Lt) and *Heteromurus nitidus* (Hn) and **(b)** *Heteromurus nitidus* (Hn) and *Protaphorura armata* (Pa); – and + indicate absence or presence of the respective soil animal species; means with SE; for statistical analysis see Table 3.

Discussion

Macrofauna detritivores have been shown to hamper their performance as well as their effects on ecosystem processes with increasing similarity of their traits, while dissimilar species rather increase each other's performance (Heemsbergen et al., 2004). Building on these findings we explored interactions between earthworms (macrofauna) and collembolans (mesofauna), each of them represented by species associated with litter or soil. Body size and habitat association are major traits of soil invertebrates allowing to explore the role of trait similarity for ecosystem functioning.

Results of the study in part supported our first hypothesis that the effects of litter-associated species (*L. terrestris* and *H. nitidus*) on ecosystem processes exceed those of soil-associated species (*A. caliginosa* and *P. armata*). Decomposition of beech litter was increased by *L. terrestris*, while it remained unaffected by *H. nitidus*, and the soil-associated species *A. caliginosa* and *P. armata*. In contrast, mass loss of leaf litter of *Senna siamea*, a nitrogen fixing legume, was highest in presence of microarthropods and earthworms (Adejuyigbe et al., 2006). In comparison of both studies, the missing effect of *H. nitidus*, *A. caliginosa* and *P. armata* on leaf litter mass loss in our experiment

suggests that except for *L. terrestris* effects of both microarthropods and earthworms on litter decomposition are restricted to high quality resources. In contrast, *L. terrestris* is known to exert strong effects on leaf litter mass loss via feeding on litter including litter of low quality, which is accumulated in middens thereby enhancing microbial attack and litter decomposition (Edwards and Bohlen, 1996). Furthermore, ^{15}N cycling was affected by *L. terrestris* and *H. nitidus*; both significantly increasing ^{15}N incorporation into beech roots as single species. The positive effect of *L. terrestris* may be due to incorporation of litter material into the soil, where roots are abundant, and fragmenting as well as mixing them with mineral soil during gut passage, thereby enhancing mineralisation of organic matter and nutrient turnover (Butenschoen et al., 2009). The positive effect of *H. nitidus* on ^{15}N cycling likely was due to moderate grazing on saprotrophic fungi, thereby enhancing their effect on N release from labelled leaf litter (Ineson et al., 1982). Overall, litter-associated species exerted stronger effects on leaf litter mass loss and ^{15}N cycling as compared to soil-associated species; with stronger effects of the macrofauna species *L. terrestris*, as compared to the mesofauna species *H. nitidus*. These results confirm our hypothesis one but also emphasize the importance of body size.

According to our second hypothesis similar species detrimentally affect the performance of each other. Supporting this assumption the biomass of *L. terrestris* was negatively affected by the other studied macrofauna species, *A. caliginosa*. The negative effect of *A. caliginosa* on *L. terrestris* likely is related to habitat competition. Presumably, the intensive burrowing activity of *A. caliginosa* detrimentally affected *L. terrestris* by disturbing its permanent vertical burrows and the food material gathered in middens. Notably, however, the biomass of *A. caliginosa* increased in presence of *L. terrestris*, suggesting that *A. caliginosa* benefited from the presence of *L. terrestris*, presumably due to exploitation of litter resources mixed into the soil by *L. terrestris*. Our findings are in agreement with Uvarov (2009) who showed *L. terrestris* to suffer in presence of other earthworm species and *A. caliginosa* to benefit from the presence of litter-associated earthworm species such as *L. terrestris*. Biomass of *A. caliginosa* also increased in presence of *P. armata*, suggesting facilitative effects of soil animals dissimilar in size on the performance of each other as indicated earlier (Milcu et al., 2006). Moderate grazing on roots or fungal hyphae by *P. armata* has been found to stimulate root (Lohmann et al., 2009) and fungal growth (Hanlon and Anderson, 1979; Ineson et al., 1982; Ruf et al., 2006), and this may have enhanced the availability of resources of *A. caliginosa*. As indicated by the incorporation of litter N into the soil animal species studied, *L. terrestris* reduced the availability of litter N to other soil animal detritivores, in particular the litter-associated species *H. nitidus*. Overall, the results provide mixed support for our second hypothesis; with interactions between species similar in size being detrimental for one of the interacting species, while in part being beneficial for the other species, and interactions between species dissimilar in size being beneficial or little pronounced. Hence, interactions of soil animals are not only based on competitive

but also on facilitative interactions. Notably, facilitative effects were only pronounced in *A. caliginosa* and were independent of trait similarity with the interacting species, emphasizing species identity rather than similarity of traits to be important for soil animal interactions. This argues against the validity of our second hypothesis.

According to our third hypothesis effects of similar species on leaf litter mass loss and ^{15}N cycling should be reduced in combined treatments. Contrasting this hypothesis, leaf litter mass loss was only affected by *L. terrestris* and the negative effect of *A. caliginosa* on the biomass of *L. terrestris* did not translate into reduced litter decomposition. Despite no other soil animal species affected litter decomposition as single species or in combined treatments, soil fauna interactions affected ^{15}N cycling. Supporting our third hypothesis the positive effect of *L. terrestris* on $\delta^{15}N$ of beech roots was reduced in presence of *H. nitidus*, the second litter-associated species. Moreover, the two soil-associated species, *A. caliginosa* and *P. armata*, increased $\delta^{15}N$ of beech roots, but only in single species treatments suggesting that they hampered the effect of each other in combined treatments. If both species feed on roots or root hairs their effects add to each other, resulting in increased root exudation or increased root damage rather than nutrient uptake (Bais et al., 2006; Baetz and Martinoia, 2014). Arguing against the validity of our hypothesis three, we found detrimental interactions between soil animals of dissimilar body size or habitat association. The positive effect of the small litter-associated species *H. nitidus* was reduced by the large soil-associated species *A. caliginosa*. The reduction of the positive effect of *H. nitidus* on $\delta^{15}N$ in beech roots by *A. caliginosa* may have been due to adverse effects of the endogeic earthworm on hyphae of saprotrophic fungi (Tuffen et al., 2002). Interestingly, the positive effects of the litter-associated *L. terrestris* on $\delta^{15}N$ of beech roots only occurred in the single species treatment or when both soil-associated species, *A. caliginosa* and *P. armata*, were present. Similarly, the incorporation of ^{15}N into beech shoots also was only significantly increased if *L. terrestris*, *A. caliginosa* and *P. armata* were present together, suggesting that some facilitative effects on ecosystem processes may only occur if three or more species interact. This indicates soil fauna interactions to be difficult to predict and to vary or only occur if a higher number of species is present in certain combinations. Overall, the species studied interacted in detrimental but also facilitative ways with many of the interactions being independent of trait similarity suggesting that species identity and diversity rather than trait similarity drive many soil fauna interactions.

Conclusion

Overall, the results of this study provide mixed support for our hypotheses and emphasize the high complexity of soil fauna interactions. In agreement with our first hypothesis, ecosystem processes,

i.e. leaf litter mass loss and ^{15}N cycling, were more heavily affected by litter as compared to soil-associated species, with the effect of *L. terrestris* being most pronounced. Contrary to litter decomposition, effects of litter-associated species on ^{15}N cycling were modified by other species. For two species combinations we found species with similar habitat association to hamper each other's effect. However, *A. caliginosa* reduced the effect of *H. nitidus* on ^{15}N cycling although both species are dissimilar in size and habitat association. Notably, species of similar size also benefited from each other as documented by facilitative effects of *L. terrestris* on the body mass of *A. caliginosa*, indicating other factors than trait dissimilarity being important for complementarity effects. Generally, the effect of *A. caliginosa* was detrimental for other soil animals and their effects on ecosystem processes, independent of the similarity of size or habitat association, whereas *A. caliginosa* benefited from presence of other soil animals. Some effects only occurred in presence of three soil animal species, indicating that soil fauna interactions are complex and sometimes need more than two species to occur. This hampers predictions on the effect of soil animal interactions on ecosystem processes. Overall, the results indicate that in addition to the similarity of body size and habitat association the composition of the soil animal community and the identity of the interacting species determine their effects on ecosystem processes, indicating that relationships found by Heemsbergen et al. (2004) for interactions between macrofauna detritivores cannot easily be applied to soil animal interactions in general.

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Chapter 5

General Discussion

This thesis has led to major advance in the understanding of driving factors for soil food webs, and soil fauna effects on ecosystem processes in deciduous forests. In particular, the importance of identity and diversity of aboveground as compared to belowground resources on soil microorganisms (Chapter 2) and soil mesofauna, i.e. collembolans (Chapter 3) was investigated. In general, I found aboveground litter to be the major factor influencing activity, biomass, abundance and community composition of soil biota, whereas belowground resources were of minor importance. This supports the classical view that soil food webs are mainly fuelled by aboveground litter (Swift et al., 1979; Berg and McClaugherty, 2008). Litter identity, rather than mixture effects, was important for soil organisms, with the effects of different litter species varying with soil animal species. Furthermore, the importance of mesofauna and macrofauna invertebrates and their interactions for ecosystem processes, i.e. leaf litter decomposition and cycling of N was investigated (Chapter 4). Ecosystem processes were found to be mainly affected by litter-associated soil animal species, but also by soil fauna interactions, with species identity and community composition being the most important factors determining the direction of these interactions. Soil fauna interactions were not only based on competitive but also on facilitative effects and in some cases only occurred in presence of at least three different species. Overall, results of this thesis emphasize the high complexity of aboveground - belowground interactions and the importance of specific species.

The role of aboveground and belowground resources for forest soil organisms

The results of this thesis suggest aboveground rather than belowground resources to be important for soil biota in deciduous forest ecosystems, confirming previous assumptions (Swift et al., 1979; Berg and McClaugherty, 2008). Neither microorganisms nor microarthropods responded to roots, but e.g. the density of microarthropods or the number of microarthropod groups increased in presence of litter. Although, the diversity of litter resources has been considered to significantly affect soil invertebrates, our results indicate, in agreement with Wardle et al. (2006), Jacob et al. (2009) and Eissfeller et al. (2013), that litter identity rather than the number of species is important for activity, biomass, abundance and community composition of soil biota. Litter species differ in their traits (Bardgett, 2005), such as concentrations of nitrogen (N), lignin and polyphenols (Swift et al., 1979; Hättenschwiler & Vitousek, 2000; Knops et al., 2001). The higher the N concentration of litter in comparison to the carbon (C) or lignin concentration the higher its attractiveness for decomposers (Bardgett, 2005). Furthermore, high molecular substances or insoluble polymers, such as lignin, need special enzymes and more time to be depolymerized and broken down than soluble substances (McClaugherty et al., 1985). Not all soil biota are able to decay those complex structures. Hence, litter with a low N concentration and high concentration of cellulose and lignin has a slower rate of

decomposition and is viewed as litter of low quality (Cornwell et al., 2008). Therefore, some litter species provide easily available high quality resources for soil microorganism and soil microarthropods, stimulating their activity or growth, while others contribute less to their nutrition (Saetre and Baath, 2000; Bardgett and Wardle, 2010; Bell et al., 2015). Therefore, competition for low quality resources is likely less pronounced promoting soil organisms able to degrade high molecular substances. In litter mixtures the effect of single species may vanish due to mixing of litter species with different traits and antagonistic effects on soil biota (Pan et al., 2015). Accordingly, in our experiments e.g., the number of microarthropod groups was lower in mixtures of four litter species as compared to single species treatments (Chapter 3). Nevertheless, litter effects varied with soil fauna group or species, presumably due to different feeding behavior, resource exploitation or habitat use of those species. For example, the percentage of $Gram^-$ bacteria increased in presence of high quality litter, presumably due to easily available litter components. This is in agreement with Paterson et al. (2008) who found $Gram^-$ bacteria to be favored if low molecular weight soluble resources were abundant. In contrast to most bacteria, fungi are able to decompose recalcitrant plant components, such as cellulose and lignin (Neely et al., 1991; Cox et al., 2001; Paterson et al., 2008); therefore, competition between bacteria and fungi for nutrients may be reduced in low quality litter resulting in increased fungal PLFAs. Indeed, we found soil fungi but not bacteria to benefit from the presence of low quality beech litter, resulting in an increased fungal-to-bacterial ratio (Chapter 2). Our findings are in agreement with previous studies showing that fungi are the most effective decomposers of recalcitrant plant residues (Neely et al., 1991; Lundquist et al., 1999) while easily available soluble litter components are mostly degraded by soil bacteria (Paterson et al., 2008). Furthermore, the collembolan species *P. callipygos* benefited from the presence of low quality beech litter (Chapter 3), presumably due to increased availability of fungi. In contrast, the density of *P. armata* was increased in presence of high quality ash litter (Chapter 3), suggesting this species to use other resources than *P. callipygos* potentially bacteria or high quality litter compounds. Moreover, some microarthropod taxa responded to the presence of litter irrespective of litter quality, identity or diversity. Beneficial effects of litter may be due to more stable conditions in soil under an intact litter layer, indicating abiotic factors rather than resource quality to affect those microarthropods. Indeed, we found soil conditions, such as soil pH and soil water content to affect soil biota, with the effect varying with soil animal species. The number of microarthropod groups and the densities of pauropods and the collembolan species *P. armata* and *I. minor* decreased with increasing soil pH, while the other studied species did not respond to soil pH. Furthermore, the density of the collembolan species *M. minimus* increased with increasing soil water content (Chapter 3).

In contrast to the complex effects of aboveground litter on soil biota, effects of roots were missing entirely, which is in contrast to Pollierer et al. (2007) and Scheunemann et al. (2015) who found root resources to be of significant importance. The short experimental duration of our study may be one reason for the missing root effect. One vegetation period probably was too short for the two year old tree saplings to fully establish their rooting systems or for mycorrhizal fungi to fully colonize the roots of the saplings, with mycorrhiza being assumed to be a major agent channeling root-derived resources into decomposer food webs (Smith and Read, 2008; Pollierer et al., 2012; Cesarz et al., 2013). Additionally, root exudation varies between different plant groups (Jones et al., 2004) and between mature trees and tree saplings, with tree saplings generally investing less in root exudation than mature trees (Smith, 1969, 1990). Hence, the different systems studied by Pollierer et al. (2007) and Scheunemann et al. (2015) and the ones studied in the present thesis may also be a reason for the differences found between their and my study (Frostegard and Baath, 1996; Leuschner et al., 2009; Erdmann et al., 2012).

It has to be stressed that not all species responded to plant-derived resources, suggesting other resources, e.g. old carbon (Scheunemann et al., 2010), other soil animals or soil organic matter to be used by them. Hence, our results indicate C and nutrient resources of the belowground consumer community to be diverse and not restricted to aboveground or belowground plant-derived resources (Beare et al., 1992; Bardgett et al., 2005). Overall, the results show soil conditions and aboveground rather than belowground resources to affect soil biota, with the effects varying with identity of the resource as well as with soil fauna group or species.

The importance of litter associated soil animals for ecosystem processes

The results of this thesis indicate litter-associated soil organisms to exert stronger effects on ecosystem processes, i.e. leaf litter decomposition and N cycling, than soil-associated organisms, with the effect varying with species identity and soil community composition (Chapter 4). Decomposition of beech litter was only affected by the litter-associated macrofauna species *L. terrestris*, known to feed on litter including litter of low quality or accumulating it in middens, thereby enhancing microbial attack and litter decomposition (Edwards and Bohlen, 1996). ^{15}N cycling was affected by *L. terrestris* and by *H. nitidus* with both litter-associated species significantly increasing the incorporation of ^{15}N derived from labelled litter into beech roots (Chapter 4). The positive effect of *L. terrestris* may have been due to incorporation of litter material into the soil, fragmenting and mixing it with mineral soil during gut passage, thereby enhancing mineralisation of organic matter and nutrient turnover (Butenschoen et al., 2009). The positive effect of *H. nitidus* on ^{15}N cycling likely was due to moderate grazing on saprotrophic fungi, thereby enhancing their effect

on N release from ^{15}N labelled litter (Ineson et al., 1982). The missing effects of soil-associated organisms on leaf litter decomposition and N cycling indicates that these species use other resources than those associated with aboveground litter, thereby exerting no or only limited indirect effects on those ecosystem processes, which is in agreement with Postma-Blaauw et al. (2006).

Species identity, community composition and soil fauna interactions

Soil animals drive important ecosystem processes such as decomposition and nutrient turnover (Bardgett and van der Putten, 2014), thereby, contributing to the mineralization of nutrients entering the soil system and making them available for uptake by plants (Scheu, 2001; Porazinska et al., 2003). Our results indicate effects of soil animals on ecosystem processes to vary with their identity and the composition of the community they are living in, since the performance of soil organisms and effects of single species on ecosystem processes such as litter decomposition and nutrient cycling was affected by other soil organisms (Chapter 3). Previously, mainly two species interactions were studied (Salmon and Ponge, 2001; Uvarov, 2009). Species with similar traits were often found to exert negative effects on each other, most likely due to competition, and thereby not complementing each other in affecting ecosystem processes (Heemsbergen et al., 2004). Accordingly, we found species with similar habitat association to hamper each other's performance and effects on ecosystem processes in two species combinations. However, negative effects between soil animals also occurred if species had dissimilar traits. This is in contrast to the common view that organisms with different traits improve or foster each other's effects, hence complementing each other (Heemsbergen et al., 2004). Further, even species with similar traits, e.g. similar body size, in part exerted facilitating effects on each other. This indicates that other factors than dissimilarity of traits are important for complementarity effects of soil organisms. The contradictions between previous and my findings were mostly due to *A. caliginosa* that benefited from other soil animals, whereas its own effects on those of others predominantly were antagonistic independent of similarity in body size or habitat association (Chapter 4). The results indicate that soil fauna interactions and their effects on ecosystem processes are not only based on competition but also on facilitation depending on the interacting species. Moreover, incorporation of litter N into beech roots and shoots was increased if *L. terrestris*, *A. caliginosa* and *P. armata* were present together, whereas neither single species effects (except for that of *L. terrestris* on ^{15}N in roots) nor effects of two species combinations occurred, suggesting that facilitative effects on ecosystem processes may in some cases only occur if three or more species interact. This suggests that it is not biodiversity of species that determines effects of species on ecosystem functioning and stability but rather the identity and composition of the species that interact determine their effects on ecosystem processes and aboveground -

belowground interrelationships (Díaz et al., 2005). Hence, soil fauna interactions are complex and difficult to predict, as they depend not only on abiotic factors and resource availability but also on aboveground - belowground interactions and the specific community composition of soil biota.

Conclusion

This thesis contributed to the understanding of the importance of aboveground vs. belowground resources for soil decomposers and the influence of soil fauna interactions for ecosystem processes. A number of studies investigated the importance of either aboveground or belowground resources on soil organisms arguing on their relative importance. For the first time both resources were manipulated independently in one field experiment. After one vegetation period, the results of this experiment supported the classical view that soil food webs are fuelled in large by leaf litter resources, since soil microorganisms as well as soil mesofauna were affected by aboveground but not by belowground resources. The comparison of our findings to previous studies (Pollierer et al., 2007; Eissfeller et al., 2013a; Scheunemann et al., 2015) indicates the importance of aboveground and belowground resources for decomposer food webs to change with the age of the respective system. Aboveground litter presumably is the main resource for soil biota in regenerating forest stands, while belowground resources are more important in mature forest ecosystems and agricultural- or grasslands where the rooting system including associated mycorrhizal fungi is fully established. Furthermore, identity rather than diversity of resources affected soil organisms. However, even effects of aboveground resources were relatively weak indicating that in addition to recently added resources the soil animal community heavily relies on resources accumulated in dead organic matter. Further, the exploitation of leaf litter resources was species specific, suggesting different feeding strategies within taxonomic and trophic groups of soil organisms as already suggested for soil fauna of arable fields (Scheunemann et al., 2015).

Additionally, the results of this thesis indicate the effects of soil animals on leaf litter mass loss and N cycling to vary with species identity and soil community composition, due to variable soil fauna interactions. Leaf litter mass loss was only affected by one, whereas N cycling was influenced by both litter-associated species used in the mesocosm experiment, with their effects being modified by other species. Species with similar traits, such as habitat association or body size, hampered each other's effects in two species combinations, likely due to competition for habitat space and resources. However, not all species did follow this pattern. For example, *A. caliginosa* detrimentally affected the performance of other soil animal species and their effects on ecosystem processes, whereas itself *A. caliginosa* benefited from the presence of other species irrespective of the similarity of traits. Furthermore, some effects were only pronounced in presence of three soil animal species,

indicating that soil fauna interactions are complex and sometimes need more than two species to occur. Therefore, in addition to the similarity of body size and habitat association the identity of soil animal species and the composition of the soil animal community determine their effects on ecosystem processes. This suggests that predictions on the effects of soil animals and their interactions on ecosystem processes are difficult and require knowledge on the identity and number of soil animal species that interact.

Overall, the results of this thesis indicate that aboveground and belowground communities are intimately linked and closely dependent on each other (Scheu, 2001; Wardle et al., 2004), with interactions between both systems varying with the age of the ecosystem, abiotic factors and identity and composition of aboveground and belowground communities.

Outlook

To prove whether the importance of aboveground and belowground resources changes with tree age or species identity and community composition further samplings and analyses are needed. In the present study soil cores were taken in autumn 2011, one vegetation period after tree plantation and one year after litter addition. Presumably, in the long term the importance of roots as driving factor for the composition and functioning of belowground communities will increase due to the more solid establishment of a complex rooting system including species specific mycorrhizal fungi. Nevertheless, results of the present study suggest that litter presence and identity are important agents affecting the composition and functioning of soil microorganisms and animals, with both also appearing to heavily rely on old resources accumulated in soil organic matter. Further experiments should be established at other field sites and using other forest ecosystems to test if our findings are valid in general for young and regenerating systems. Further, for more detailed information on the resources used by soil organisms and the nutrient and energy flow between the aboveground and belowground system, compound-specific stable isotope and molecular gut content analyses need to be employed. The basal resource and the trophic position of soil organisms may be detected by fatty acid or stable isotope analysis, however, compound-specific analysis of stable isotope ratios in neutral lipid fatty acids or amino acids of soil invertebrates are needed for precise information of the basal resource and for tracing the flux of C and N through soil food webs (Schmidt et al., 2004; Ferlian et al., 2014; Bowes and Thorp, 2015). Furthermore, molecular gut content analysis may allow identifying trophic links between soil organisms at the level of species (Eitzinger et al., 2013; Fiera, 2014; Heidemann et al., 2014). In addition real time PCR may help to disentangle the percentage of various resources for the nutrition of individual species. According to the results of this thesis, for understanding the role of soil invertebrates for ecosystem processes interactions need to be investigated at the level of

species. Combining the described methods ultimately may allow the understanding of trophic relationships in soil food webs, may help to predict the impact of individual species as well as their interactions on ecosystem processes and hence aboveground - belowground interactions in forest ecosystems.

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Thesis declarations

Declaration of the author's own contribution to manuscripts with multiple authors

I am the first author in all presented manuscripts. The study design of the experiments described in Chapters 2 and 3 were developed in the framework of the SPLIDRHEX (Species litter identity and diversity effects on the rhizosphere of trees experiment), which forms part of the Cluster of Excellence "Functional Biodiversity Research" (FBR). I significantly contributed to the study design and the establishment of all described experiments. All data were collected by me except for the data on study site and soil type (Chapter 2 and 3), that were provided by Lars Köhler, Christina Lödige and Dietrich Hertel. Collembolan identification to species level was in part realized by Jörg Salamon. I have analyzed the data, written the manuscripts, developed the main ideas and created tables, figures and appendices. All persons contributing to the manuscripts have been named so. All co-authors contributed to finalizing the manuscripts. Photos used are provided by Simone Cesarz, Lars Köhler, Patrick Pachl, Nicole Scheunemann, Sarah Zieger and me.

The experiments described in this thesis have been in part presented at different conferences and abstracts regarding these topics may have been published in conference books. Chapter 4 is in preparation of submission to the European Journal of Soil Biology.

Plagiarism declaration

I, Diana Grubert, herewith declare that this doctoral thesis was independently written by me the undersigned. All persons contributing to the manuscripts have been named so. I have not used any other than permitted reference sources or materials. All sentences or passages quoted from other people's work have been specifically acknowledged by clear cross-referencing. I have not submitted this thesis in any form for another degree at any university or institution.

Diana Grubert

Göttingen, 19.02.2016