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Melanie Mira Maraun

Compartmentalization and energy channels within the soil animal food web investigated by stable isotope (13C/15N) and fatty acid analyses

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der
Georg-August-Universität Göttingen
vorgelegt von Melanie Mira Maraun (geb. Pollierer)

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– CENTRE OF BIODIVERSITY AND SUSTAINABLE LAND USE –
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vorgelegt von

Diplom Biologin

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aus
Langen

Göttingen, Januar 2012

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Contents

Summary

Forest soil food webs are complex and heterogeneous systems. Trophic relationships are hidden from direct observation due to small size of soil animals, cryptic habitat and complex mixtures of basal resources that are not easily separable. Despite the fundamental role of aboveground-belowground feedbacks and the major importance of decomposition processes for ecosystem functioning, the decomposer subsystem has become only recently the subject of ongoing research.

In this thesis, we investigated the trophic compartmentalization of the decomposer food web and traced energy fluxes to different compartments within the food web using stable isotope analyses of $\delta^{15}N$ and $\delta^{13}C$ and compound specific ^{13}C fatty acid analyses. To improve the applicability of fatty acid analyses for field studies we additionally investigated whether marker fatty acids for specific food sources are transferred to higher trophic levels including predators, and we studied the time span required to detect marker fatty acids in consumers after consumption of a specific food source as well as the time that marker fatty acids of the previous diet can be detected after switching to a different food source.

In our first study (Chapter 2) we depicted the trophic compartmentalization of the soil animal food web using the natural variation of $\delta^{15}N$ and $\delta^{13}C$ in basal resources and soil animals. We showed that the trophic compartment of primary decomposers utilizing leaf litter directly is comparatively small and hypothesized that it is of minor importance for the decomposer food web. The largest compartment comprised secondary decomposers presumably feeding on ectomycorrhizal fungi and predators. Due to similar $\delta^{13}C$ signatures of primary decomposers and ectomycorrhizal fungi, we were not able to separate predators preying on primary decomposers from ectomycorrhizal fungal feeders and therefore could not further resolve feeding strategies within this largest compartment of the soil animal food web.

By supplying specific and relative markers for bacteria, fungi and plants, fatty acid analysis was potentially applicable to obtain a finer resolution of feeding strategies within trophic compartments. To verify trophic transfer of marker fatty acids from basal resources to higher trophic levels including predators, we conducted a laboratory experiment (Chapter 3) in which we fed two major predators, the centipede *Lithobius forficatus* and the spider *Pardosa lugubris*, with the collembolan *Heteromurus nitidus* kept on different diets, including fungi, bacteria and tree leaves. Marker fatty acids for the respective diets were transferred over three trophic levels to predators; and predators could reliably be assigned to specific basal resources according to their fatty acid profiles, suggesting that fatty acid analysis is adequate for the analysis of whole food webs.

In another laboratory experiment (Chapter 4) we investigated physiological parameters of fatty acid metabolism, such as the chronological change of fatty acid biomarkers in

collembolans when switched between different food sources and the change of fatty cid biomarkers during starvation. Fatty acids typical for a specific diet were already present in the neutral lipids of consumers after one day, and were still detectable 14 days after switching to a different diet. During starvation, there were only minor changes in fatty acid composition, with marker fatty acids being still detectable in sufficient amounts after 14 days of food deprivation. Hence, fatty acid analyses provide a reliable and integrative measure of dietary composition, even for short and intermediate time intervals.

After verifying the applicability of fatty acid analyses for food web analysis, we conducted a field study (Chapter 5) in the frame work of the Swiss Canopy Crane Project, where the tree crowns of a mature temperate forest are labeled with CO_2 depleted in ^{13}C. By employing a leaf litter exchange experiment, we were able to separate carbon fluxes originating from aboveground via leaf litter and from belowground via roots/root exudates. Compound specific ^{13}C fatty acid analyses of leaf litter, soil, roots and soil animals in combination with the application of marker fatty acids for specific food sources allowed to separate energy fluxes through major channels of the decomposer food web, such as the ectomycorrhizal vs. saprotrophic fungi channel and the bacterial channel based on leaf litter or root exudates. Our findings suggest that root derived carbon is of major importance for the soil animal food web and that it mainly enters the food web via feeding on ectomycorrhizal fungi. In contrast to previous assumptions that forest soil food webs are mainly supported by the fungal energy channel, we also found considerable fluxes of energy through the bacterial channel, with all investigated predators containing significant amounts of bacterial marker fatty acids. Since systems based on multiple pathways of energy fluxes are assumed to be more stable, the partitioning between the fungal and bacterial channel presumably contributes to food web stability.

By identifying trophic compartments and by tracing energy fluxes via different energy channels, results of this thesis represent major advances in the understanding of soil animal food web structure and functioning.

Chapter 1

General Introduction

1. Forest soil animal food webs

Forest soil food webs are complex and heterogeneous systems. They have become a focus of recent research because the importance of the decomposer subsystem in terrestrial ecosystems is being increasingly appreciated. However, progress is hampered since direct observations of trophic relationships are difficult due to the opaqueness of the habitat. Additionally, ingested food materials are not necessarily also digested. Often, food resources of soil animals comprise a mixture of microorganisms and plant material that cannot easily be separated.

Recently, it has been shown that carbon enters the soil animal food web to a large extent from belowground via roots, with leaf litter playing only a minor role as food source (Albers et al., 2006; Ruf et al., 2006; Pollierer et al., 2007). This contrasts the previous view that plant litter input constitutes the main resource of energy and matter for soil organisms (Cadisch and Giller, 1997; Hättenschwiler et al., 2005) and poses the question on the exact way by which root derived carbon enters the soil animal food web.

Soil animal food webs are assumed to be compartmentalized (Moore et al., 2005), with energy being processed in different ways, including slow and fast cycling pathways (Coleman et al., 1983). These pathways were ascribed to the bacterial and fungal energy channel by Moore and Hunt (1988). The relative contribution of the bacterial vs. the fungal energy channel to the soil animal food web presumably influences food web stability, with food webs being more stable when energy is distributed more evenly between the two channels (Moore et al., 2005). However, for soil animal food webs, quantification of relative energy fluxes through these channels is difficult to accomplish.

2. Methods for food web analysis

Methods to investigate trophic relationships include e.g., molecular gut content analyses, stable isotope analyses and fatty acid analyses. Molecular gut content analyses allows to detect individual prey species, but the method only provides a snapshot picture of an animal's diet and it is not quantitative (King et al. 2008). In contrast, stable isotope analyses provide a time-integrated measure of the food assimilated by consumers.

For the investigation of soil animal food webs, isotopic ratios of nitrogen ($^{15}N/^{14}N$) and carbon ($^{13}C/^{12}C$) have mainly been applied (Scheu and Falca, 2000; Ponsard and Arditi, 2000; Schmidt et al., 2004). For $\delta^{15}N$, the mean enrichment is about 3.4 ‰ per trophic level, whereas the carbon isotopic composition changes little from one trophic level to the other (Post 2002). Therefore, $\delta^{15}N$ is used to estimate the trophic position in the food web, while $\delta^{13}C$ can be used to evaluate carbon sources, such as carbon originating from C_3 or C_4 plants. For the identification and quantification of trophic links, mixing models have been applied (Phillips et al., 2005; Wolf et al., 2009). However, in soil animal food webs

there are many potential resources and their relative contribution to an animal's diet cannot be resolved using isotopes of only two different elements. Another shortcoming of stable isotope analyses is that there often are missing signatures of basal resources, such as bacteria and fungi. Moreover, different rates of isotopic fractionation hamper the interpretation of results (Gannes et al., 2005; Caut et al., 2009).

Similar to stable isotopes, the analysis of fatty acids initially has been used for food web analysis in aquatic systems (Bottino, 1974; Fraser et al., 1989), however, in recent years it has been successfully applied to terrestrial systems including soil animal food webs (Ruess et al., 2004, Chamberlain et al., 2005; Ruess and Chamberlain, 2010). The method takes advantage of the fact that it is energetically more efficient for an organism to incorporate ingested fatty acids directly without modification into its own fat body. This direct incorporation has been termed "dietary routing" (Ruess et al., 2004). Some fatty acids are specific to certain food sources, or occur in much greater amounts in some food sources than in others. The former are used as absolute the latter as relative markers for the diet of consumers. For example, membranes of bacteria comprise branched-chain and cyclic fatty acids, which animals are unable to synthesize themselves. These are used as absolute markers, i.e. if they are found in the fatty acid profile of soil animals, one can conclude that these animals fed directly on bacteria or on consumers of bacteria. Relative markers exist for fungi and plants, with linoleic acid (18:2ω6) occurring in higher abundance in fungi, and oleic acid (18:1ω9) in higher abundance in plants. The ratio between oleic and linoleic acid therefore has been used to differentiate between plant- and fungal feeding in soil animals (Ruess et al., 2007).

A further advancement of fatty acid analysis is the measurement of the carbon isotopic composition of individual fatty acids, which has been termed "compound specific ^{13}C fatty acid analysis" (Stott et al., 1997). Similar to conventional stable isotope analyses, this method can be applied to distinguish between carbon sources with different $\delta^{13}C$ signatures. However, using the combination of marker fatty acids for specific diets such as fungi or bacteria, and their respective $\delta^{13}C$ signatures, one can not only trace carbon fluxes, but additionally depict the pathway (bacterial vs. fungal) by which carbon enters the food web. This method has been successfully applied in a field experiment using ^{13}C pulse labeling (Elfstrand et al., 2008) and in an arable field with carbon originating from maize (C_4 plant) and soybean (C_3 plant; Haubert et al., 2009).

3. Study site

Our field experiments were conducted at the study site of the Swiss Canopy Crane (SCC) CO_2 enrichment project which is located in a mature temperate forest near Basel, Switzerland (47°28´ N, 7°30´ E, 550 m a.s.l.). The plant community is dominated by beech

(*Fagus sylvatica* L.), oak (*Quercus petraea* Mattuschka) and hornbeam (*Carpinus betulus* L.), but also includes lime (*Tilia platyphylla* Scop.), wild cherry (*Prunus avium* L.) and maple (*Acer campestre* L.). Trees are 80 to 120 years old and 30-35 m high and have a basal area of about 46 m^2 ha^{-1}. The climate is temperate with an annual precipitation of 990 mm and the soil is a mesophilic Rendzina (pH 5.8; Pepin and Körner, 2002).

For CO_2 enrichment, a new technique called "web-Face" was installed in the tree crowns. Laser-punctured irrigation tubes released CO_2 into the canopy. The enrichment was restricted to a height of at least 20 m above ground and a concentration of 530 ppm was maintained during the growing season. CO_2 originated from combustion of fossil fuel and was depleted in ^{13}C (-30‰ compared to -8‰ in ambient air). For details on the experimental site and the enrichment system see Pepin and Körner (2002) and Steinmann et al. (2004).

Our first field experiment (Chapter 2) was conducted in the non-enriched control area, whereas the second field experiment (Chapter 5) took advantage of the CO_2 labelling in the enriched area.

4. Objectives and chapter outline

This thesis focuses on the compartmentalization of the soil animal food web and on energy fluxes through the bacterial, fungal and plant litter channel. Chapters 3 and 4 are laboratory experiments aiming to further improve the applicability of fatty acid analysis for the investigation of soil animal food webs, whereas Chapters 2 and 5 are field experiments that were conducted to shed light into the "black box" (Scheu, 2002) of the soil animal food web.

We examined the following main hypotheses:

(1) The soil animal food web is divided into trophic compartments relying on different energy resources, i.e. detritus and fungi (Chapter 2).

(2) The different fatty acid composition of Gram-positive and Gram-negative bacteria, fungi and plant leaves will be transferred to higher trophic levels i.e., will be detectable in Collembola and their predators, such as centipedes and lycosid spiders (Chapter 3).

(3) Fatty acids are routed directly to an animal's fat body; therefore, analyses of fatty acid patterns reveal changes in diet more quickly and more specifically than analyses of stable isotope signatures (Chapter 4).

(4) Root derived carbon forms the main carbon source for the soil animal food web and enters the food web mainly via feeding on ectomycorrhizal fungi (Chapter 5).

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

Chapter 2 Using natural abundances of ^{15}N and ^{13}C, we comprehensively analyzed the soil animal food web of a temperate forest near Basel (see above). The combined measurement of $\delta^{15}N$ and $\delta^{13}C$ provided insights into the compartmentalization of the soil animal food web. A comparatively small group of litter feeders was separated from a large group of animals consisting of secondary decomposers, presumably feeding on ectomycorrhizal fungi, and their predators. Another group of predators was separated by high $\delta^{13}C$ values that likely fed on enchytraeids and nematodes. Litter feeders, such as some oribatid mite and diplopod species, as well as earthworms appear to be trophic dead ends in the soil animal food web i.e., to live in enemy free space, presumably due to large size and/or strong sclerotization.

Chapter 3: A shortcoming of the study presented in Chapter 2 was the difficulty to separate fungal feeders from predators feeding on primary decomposers due to similar $\delta^{13}C$ signatures of ectomycorrhizal fungi and primary decomposers. Fatty acid analysis has the potential to overcome this problem by providing markers for fungi, bacteria and plants. However, for the analysis of whole food webs it had to be verified that marker fatty acids can be transferred not only from resource to consumer, but also to higher trophic levels, i.e. predators. Therefore, we conducted a laboratory experiment in which we fed two species of common top predators of soil animal food webs, *Lithobius forficatus* (Chilopoda) and *Pardosa lugubris* (Arachnida), with *Heteromurus nitidus*, representing Collembola as widespread prey. The Collembola were kept on different diets including fungi (*Chaetomium globosum*), Gram-positive (*Bacillus amyloliquefaciens)* and Gram-negative bacteria (*Stenotrophomonas maltophilia)*, and freshly fallen tree leaves (*Tilia europaea)*. Fatty acid profiles of predators fed Collembola that were kept on different basal resources differed significantly and marker fatty acids of these resources were detected in predators, suggesting that fatty acid analysis allows separating different trophic channels of soil food webs. The results of this experiment formed the basis for the analysis of complete food webs in the field using fatty acid analysis.

Chapter 4: Another important precondition for field studies is the knowledge of physiological parameters of fatty acid metabolism. While the influence of food quality on fatty acid composition had been studied (Haubert et al., 2004), the detection time (time

until fatty acids can be detected in the neutral lipids of an animal after consumtion of a specific food source) and storage period of marker fatty acids in soil animals was unknown. Therefore, we performed a laboratory experiment investigating the chronological change of marker fatty acids in the Collembola species *Heteromurus nitidus* when switched from one to another diet. Additionally, we investigated changes of bacterial marker fatty acids in the fat body of Collembola during starvation. Marker fatty acids were already present one day after switching the diet and were still detectable after 14 days of feeding on a different food source, suggesting that fatty acid analyses provide a time integrated measure of animal nutrition. The short detection time of newly acquired marker fatty acids allows investigating dietary changes at short time intervals, which is especially useful in short term pulse labeling experiments.

Chapter 5: This field study was conducted at the study site of the Swiss Canopy Crane Project, where a mature temperate forest was labeled with CO_2 depleted in ^{13}C allowing to trace carbon fluxes from trees into the soil animal food web. By exchanging leaf litter between the labeled canopy crane area and an unlabeled control area, we were able to separate carbon fluxes originating from leaf litter and roots, respectively. We analyzed fatty acid $\delta^{13}C$ signatures of basal resources (leaf litter, soil, roots) and soil animals, including decomposers and taxa from higher trophic levels. By analyzing marker fatty acids and their individual $\delta^{13}C$ signatures, for the first time we were able to separate energy fluxes through major channels of decomposer food webs, such as the ectomycorrhizal vs. saprotrophic fungi channel and the bacterial channel based on leaf litter or root exudates. Our findings suggest that root derived carbon is of major importance for soil animal food webs of temperate forests. In addition to energy fluxes via ectomycorrhizal fungi, we found considerable fluxes of energy through the bacterial channel to higher trophic levels. The partitioning of energy between these channels presumably contributes to food web stability.

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Chapter 2

Compartmentalization of the soil animal food web as indicated by dual analysis of stable isotope ratios ($^{15}N/^{14}N$ and $^{13}C/^{12}C$)

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Abstract

The soil animal food web has become a focus of recent ecological research but trophic relationships still remain enigmatic for many taxa. Analysis of stable isotope ratios of N and C provides a powerful tool for disentangling food web structure. In this study, animals, roots, soil and litter material from a temperate deciduous forest were analyzed. The combined measurements of $\delta^{15}N$ and $\delta^{13}C$ provided insights into the compartmentalization of the soil animal food web. Leaf litter feeders were separated from animals relying mainly on recent belowground carbon resources and from animals feeding on older carbon. The trophic pathway of leaf litter feeding species appears to be a dead end, presumably because leaf litter feeders (mainly diplopods and oribatid mites) are unavailable to predators due to large size and/or strong sclerotization. Endogeic earthworms that rely on older carbon also appear to exist in predator-free space. The data suggest that the largest trophic compartment constitutes of ectomycorrhizal feeders and their predators. Additionally, there is a smaller trophic compartment comprised of predators likely feeding on enchytraeids and potentially nematodes.

Keywords: soil animal food web, stable isotopes, ^{15}N, ^{13}C, fungi, ectomycorrhiza, beech forest, decomposers, compartmentalization, trophic channels

1. Introduction

Forest soil food webs are complex and heterogeneous systems. Studying the trophic interactions of soil animals and microorganisms is difficult since the soil matrix hampers direct observation. The analysis of gut contents is also problematic since ingested material is not necessarily digested and incorporated into consumer tissue. Laboratory food choice experiments may also be of limited use since they may not reflect feeding preferences in the field.

Recently, analyses of stable isotope ratios ($^{13}C/^{12}C$, $^{15}N/^{14}N$) have been shown to be a powerful tool for the study of terrestrial and aquatic food webs and their use for investigating trophic interactions is increasing rapidly (Schmidt et al., 2004; Kupfer et al., 2006; Albers et al., 2006; Hobbie et al., 2006; Tiunov, 2007). Stable isotopes provide a time-integrated measure of the trophic position of animals and allow the detection of complex interactions such as intraguild predation or cannibalism (Post, 2002; Schmidt et al., 2004). To estimate the trophic position of animals the isotopic baseline as well as mean trophic fractionation of isotopes per trophic level need to be known (Post, 2002). The baseline for decomposer food webs has been calibrated using the isotopic signature of litter (Scheu and Falca, 2000; Schneider et al., 2004; Halaj et al., 2005). However, there is increasing evidence that the use of litter as the only baseline in decomposer food webs is inadequate (Tiunov, 2007). Carbon enters the decomposer food web to a large extent via roots (Pollierer et al., 2007); litter derived carbon may only be used by few decomposer animals, indicating that there are at least two different trophic pathways within the soil food web. Coleman et al. (1983) found slow and fast cycling pathways which Moore and Hunt (1988) ascribed to the fungal and bacterial channel. Moore et al. (2005) assumed that soil communities are compartmentalized with the energy being processed in parallel by these two channels. Their models were based on functional groups which in belowground food webs are hard to define. Moore et al. (2004) stated that there is need for new techniques to identify the exact fraction of detritus consumed and the contribution of microbes to the diet of animals. Stable isotope ratios provide the unique opportunity to test for different trophic pathways since the combined use of $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios of soil animals has the potential to relate soil animals to different basal resources.

The isotopic signature of $\delta^{15}N$ is used to assign animals to trophic levels or feeding guilds. Mean trophic fractionation of $\delta^{15}N$ has been assumed to be 3.4‰ (SD = 1‰; Post, 2002), but fractionation may vary between consumers of plant and animal prey (Vanderklift and Ponsard, 2003). Especially organisms consuming detritus were found to be less enriched in ^{15}N than aboveground invertebrates (Vanderklift and Ponsard, 2003; Tiunov, 2007).

In contrast to ^{15}N, ^{13}C is only slightly enriched per trophic level. Trophic fractionation of ^{13}C has been found to vary between -0.5‰ (Spence and Rosenheim, 2005) and 1‰ (DeNiro and Epstein, 1978). However, for detritivores enrichment in ^{13}C has been shown to be substantially higher than expected (Ponsard and Arditi, 2000; Halaj et al., 2005). For food webs, Ponsard and Arditi (2000) stated that values of $\delta^{13}C$ are of little use because they do not reflect a distinct trophic structure. However, Post (2002) found that $\delta^{13}C$ signatures are appropriate to evaluate the ultimate sources of carbon for an organism when the isotopic signatures of the sources are different, i.e. organisms feeding on C3 plants can be distinguished from those feeding on C4 plants (Peterson and Fry, 1987), or a diet containing mostly mosses can be traced since their $\delta^{13}C$ signatures differ from those of kormophytes (Erdmann et al., 2007). Schmidt et al. (2004) showed for earthworms, enchytraeids and slugs that the dual analysis of stable isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) provides a way of separating litter and soil feeding taxa.

We hypothesized that the soil animal food web is divided into trophic compartments relying on different energy resources, i.e. detritus and fungi (Rooney et al., 2006). Our aim was to uncover the compartmentalization of the soil animal food web in a temperate forest and to separate the detrital and fungal food chains using the combined analysis of the natural variations of $\delta^{15}N$ and $\delta^{13}C$.

2. Materials and methods

2.1 Site description

A mature temperate forest in Hofstetten near Basel, Switzerland (47°28´N, 7° 30´E, 550 m a.s.l.) was investigated; the site forms part of the Swiss Canopy Crane Project (Steinmann et al., 2004; Körner et al., 2005). The tree layer consisted mainly of beech (*Fagus sylvatica* L.) and oak (*Quercus petraea* (Mattuschka) and *Quercus robur* L.), but also included hornbeam (*Carpinus betulus* L.), lime (*Tilia platyphylla* Scop.), wild cherry (*Prunus avium* L.) and maple (*Acer campestre* L.). Abundant herbaceous plants in the understory were *Anemone nemorosa* L., *Mercurialis perennis* L. and *Galium odoratum* L. The forest was 80 to 120 years old and 30-38 m high, with a basal area of about 46 m^2 ha^{-1}. The climate was humid temperate with precipitation averaging 800 to 1,000 mm per year. The soil was a mesophilic Rendzina with a pH of 5.8. More details of the site can be found in Steinmann et al. (2004) and Keel et al. (2006).

2.2 Sampling and processing of soil animals, leaf litter, roots and soil

Within an area of about 2000 m^2 six randomly distributed samples of litter and mineral soil (0-5 cm) were taken in May 2005 using a soil corer (Ø 20 cm). Animals were extracted using a high gradient heat extractor and collected in salt (NaCl) solution. The animals

were stored in saturated salt solution at -10°C until identification and further processing. Leaf litter, fine roots and soil were taken from samples after extraction of the animals, dried at 60°C for 24 h and ground to powder. Fine roots (< 1 mm diameter) were picked by hand and washed before drying.

For analysis of plant components, approximately 100 mg of ground leaf litter and root material were extracted with a methanol:chloroform:water (MCW; 2:2:1) mix (modified after Dickson, 1979). The chloroform fraction (lipids and pigments) and the water-methanol fraction (sugars and proteins) were kept for analysis. For the hot water extraction, the pellets from the MCW extraction were suspended in 5 ml deionized water and shaken in a water bath at 60°C for 24 h. After centrifugation, the supernatant was stored for analysis. Half of the remaining pellet was used for holocellulose extraction, the other half was used for lignin extraction as described in Allen (1974). Holocellulose was subsequently separated into α-cellulose and hemicellulose (Allen, 1974). Cold water-soluble carbon was extracted from 50 mg of ground and dried plant material by shaking with 1 ml of deionized water at 20°C for 24 h. After centrifugation, the supernatant was stored for analysis. To prevent contamination of plant components by extractant residues with different isotopic signatures, only volatile extractants such as chloroform and methanol (for lipids, pigments, sugars and proteins) or carbon- and nitrogen-free extractants such as sulphuric acid were used.

2.3 Stable isotope analysis

For dual C and N stable isotope ratio analysis, appropriate amounts of animal tissue were transferred into tin capsules and dried at 60°C for 24 h, weighed and stored in a desiccator until analysis. Appropriate amounts of dried and ground leaf litter, fine roots and soil were weighed into tin capsules. For the plant component analysis, the chloroform fraction, the water-methanol fraction and the supernatants from the hot and cold water extractions were concentrated in a rotary evaporator, transferred to tin capsules, dried at 40°C, weighed and stored in a desiccator until analysis. Holocellulose, α-cellulose, hemicellulose and lignin were also weighed into tin capsules. Plant components were analysed for $\delta^{13}C$ only. Samples were analysed with a coupled system consisting of an elemental analyser (NA 1500, Carlo Erba, Mailand) and a mass spectrometer (MAT 251, Finnigan, Bremen). The computer controlled system allows on-line measurement of stable isotopes (^{13}C and ^{15}N). Their abundance (δX) is expressed using the δ notation with X (‰) = $(R_{sample}-R_{standard})/R_{standard}\times1000$. R_{sample} and $R_{standard}$ represent the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of samples and standard, respectively. For ^{13}C PD belemnite (PDB) and for ^{15}N atmospheric nitrogen served as the primary standard. Acetanilide (C_8H_9NO, Merck, Darmstadt) was used for internal calibration.

2.4 Statistical analysis

Differences in $\delta^{13}C$ and $\delta^{15}N$ signatures of potential resources were tested with single-factor analysis of variance (ANOVA) using SAS 9.1 (SAS Institute; Cary, NC, USA). Differences between the potential food resources were statistically compared using the post hoc Scheffe test.

Four trophic compartments were separated according to their $\delta^{13}C$ and $\delta^{15}N$ signatures. Differences between these compartments were analysed using discriminant function analysis (DFA; STATISTICA 7.1, StatSoft, Inc. 1984-2006).

3. Results

3.1 $\delta^{13}C$ and $\delta^{15}N$ of possible food resources

The mean $\delta^{13}C$ and $\delta^{15}N$ signatures of soil were -26.8‰ (SD=0.1) and 0.1‰ (SD=0.4), respectively. Fine roots had a mean $\delta^{13}C$ signature of -28.5‰ (SD=0.04) and a mean $\delta^{15}N$ signature of -0.9‰ (SD=0.4). The mean $\delta^{13}C$ signatures of beech and oak leaf litter collected in May 2005 were -29.5‰ (SD=0.7) and -29.2‰ (SD=0.5), respectively. $\delta^{15}N$ values of beech and oak leaf litter were -4.2‰ (SD=0.3) and -2.8‰ (SD=1.0), respectively (Fig. 1). $\delta^{13}C$ signatures of leaf litter, roots and soil differed significantly ($F_{3,17}$=42.2, p<0.0001) and increased in the order beech leaf litter ≈ oak leaf litter < roots < soil (Fig. 1). $\delta^{15}N$ signatures of roots, soil, beech and oak leaf litter also differed significantly ($F_{3,17}$=72.5, p<0.0001) and increased in the order beech leaf litter < oak leaf litter < roots < soil (Fig. 1).

Fine root components had $\delta^{13}C$ signatures spanning 4.1 delta units, with lipids being most depleted (-31.8‰; SD=1.2) and hot water-soluble carbon being least depleted (-27.7‰; SD=1.0). $\delta^{13}C$ signatures of fine root components differed significantly ($F_{7,21}$=8.2, p<0,0001; Fig. 2). The relative proportions of fine root components were 49.6% holocellulose, 36.4% lignin, 8.5% sugars/proteins, 3.4% hot water-soluble carbon and 2.1% lipids/pigments. $\delta^{13}C$ signatures of leaf litter components spanned 7.4 delta units for oak leaf litter and 5.5 delta units for beech leaf litter, with lipids/pigments being most depleted (-32.9‰; SD=0.8 and -32.1‰; SD=0.8, respectively) and α-cellulose being least depleted (-25.5‰ and -26.6‰; SD=1.1, respectively; Fig. 2). Oak and beech leaf litter components differed significantly ($F_{7,6}$=17.3, p=0.001 and $F_{7,28}$=20.9, p<0.0001, respectively; Fig. 2). Relative proportions of oak leaf litter components were 48.4%, 36.0%, 3.9%, 6.8% and 4.8% for holocellulose, lignin, sugars/proteins, hot water-soluble carbon and lipids/pigments, respectively. Relative proportions of beech leaf litter components were 48.5%, 42.1%, 2.4%, 3.7% and 3.3%.

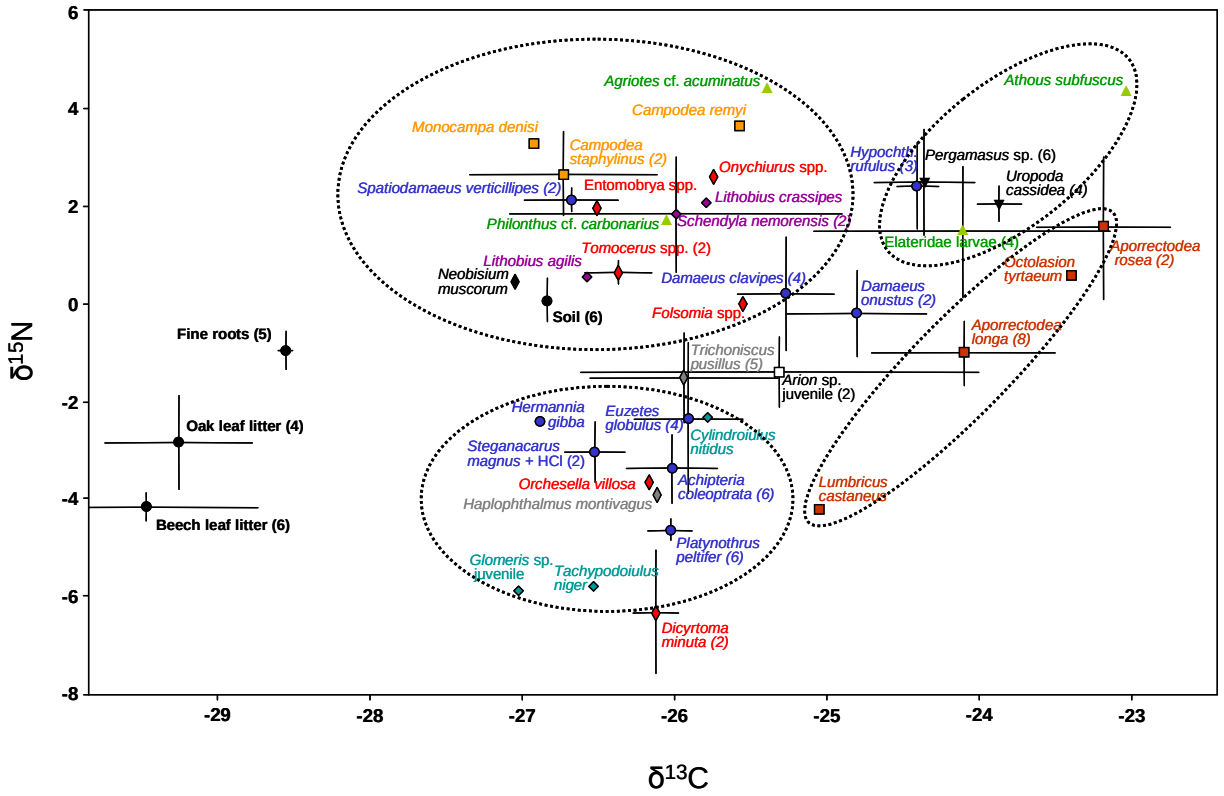

Fig. 1: Mean (± standard deviation) $\delta^{13}C$ and $\delta^{15}N$ values of beech and oak leaf litter, fine roots, soil (black circle), Oribatida (blue circles), Collembola (red diamonds), Diplopoda (turquoise tilted squares), Isopoda (grey diamonds), Chilopoda (purple tilted squares), Diplura (orange squares), Elateridae (green triangles), *Neobisium muscorum* (black diamond), *Arion* sp. juvenile (open square), Mesostigmata (black reversed triangles) and earthworms (brown squares). *Hypochthonius rufulus* is abbreviated as *Hypochth. rufulus*. Numbers of replicates are included in parentheses. Dots with no standard deviation represent single measurements. For visual clarification of the four food web compartments, dotted lines were included (drawn by eye).

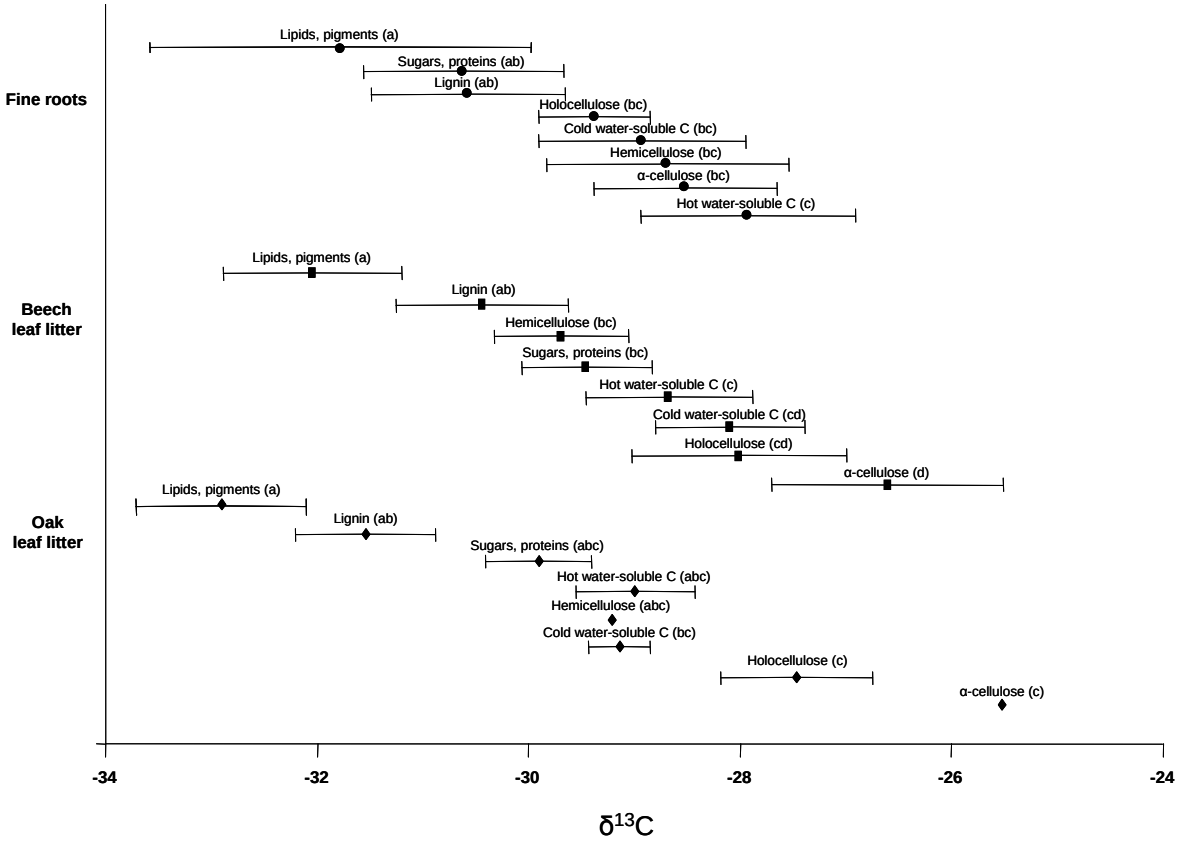

Fig. 2: Mean (± standard deviation) $\delta^{13}C$ values of components extracted from fine roots (circles), beech leaf litter (squares) and oak leaf litter (diamonds). Plant components with different letters (in brackets) are significantly different (Scheffe test; $p < 0.05$).

3.2 $\delta^{13}C$ and $\delta^{15}N$ of soil animal taxa

$\delta^{15}N$ signatures of animal taxa spanned 10.6 δ units, ranging from -6.3‰ for *Dicyrtoma minuta* to 4.3‰ for *Agriotes* cf. *acuminatus* (Fig. 1). $\delta^{13}C$ signatures of animal taxa spanned 4.0 δ units from -27.0‰ for *Neobisium muscorum* to -23.0‰ for *Athous subfuscus* (Fig. 1).

According to their $\delta^{13}C$ and $\delta^{15}N$ signatures, soil animals were assigned to four groups (Fig. 1). These four groups differed significantly from each other (DFA; Wilk's Lambda $F_{6,138}$=58.15; p<0.00001; for all Mahalanobis distances: $p < 0.0001$). The first group included animals with low $\delta^{13}C$ and low $\delta^{15}N$ signatures. $\delta^{13}C$ signatures of this group were close to those of least depleted components of leaf litter and roots and ranged from -27.2‰ for juvenile *Glomeris* sp. (Diplopoda) to -25.8‰ for *Cylindroiulus nitidus* (Diplopoda). $\delta^{15}N$ signatures ranged from -5.9‰ for juvenile *Glomeris* sp. to -2.4‰ for *Hermannia gibba* (Oribatida). The group comprised mainly typical decomposer taxa such as diplopods, isopods and some oribatid mite species.

The second group included animals with low $\delta^{13}C$ signatures and high $\delta^{15}N$ signatures. $\delta^{13}C$ signatures ranged from -26.7‰ for *Neobisium muscorum* (Pseudoscorpionida) to -25.3‰ for *Damaeus clavipes* (Oribatida). $\delta^{15}N$ signatures ranged from 0.0‰ for *Folsomia* spp. (Collembola) to 4.3‰ for *Agriotes* cf. *acuminatus* (Coleoptera). The group comprised

predatory taxa such as *Lithobius agilis*, *L. crassipes*, *Schendyla nemorensis* (all Chilopoda) and *N. muscorum*, but also putative fungal feeders such as *Monocampa denisi*, *Campodea staphylinus*, *Campodea remyi* (all Diplura), *Tomocerus* spp., *Onychiurus* spp. (both Collembola) and *Spatiodamaeus verticillipes* (Oribatida).

The third group included animals with high $\delta^{13}C$ and high $\delta^{15}N$ signatures. $\delta^{13}C$ signatures ranged from -24.4‰ for *Hypochthonius rufulus* (Oribatida) to -23.0‰ for *Athous subfuscus* (Elateridae, Coleoptera). $\delta^{15}N$ signatures ranged from 1.5‰ for elaterid larvae to 4.2‰ for *Athous subfuscus*. The group comprised known predatory taxa such as *Pergamasus* sp., *Uropoda cassidea* (both Mesostigmata), *H. rufulus* and *Athous subfuscus*.

The fourth group included animals with high $\delta^{13}C$ and intermediate $\delta^{15}N$ signatures. It included all investigated earthworm taxa. Their $\delta^{13}C$ signatures ranged from -25.0‰ for *Lumbricus castaneus* to -23.2‰ for *Aporrectodea rosea* and their $\delta^{15}N$ signatures ranged from -4.2‰ for *L. castaneus* to 1.6‰ for *A. rosea*.

Some taxa did not fall into any of the above groups due to intermediate values of $\delta^{15}N$ and $\delta^{13}C$, e.g. juveniles of the omnivorous slug *Arion* sp., the isopod *Trichoniscus pusillus* and the oribatid mite *Damaeus onustus* (Fig. 1). Further, due to very low $\delta^{15}N$ values, the collembola *Dicyrtoma minuta* was not included in any group.

4. Discussion

4.1 The soil food web

Overall, $\delta^{15}N$ signatures of animal taxa in the investigated food web spanned more than 10 δ units, which is similar to other temperate forests (Ponsard and Arditi, 2000; Scheu and Falca, 2000). Assuming enrichment in ^{15}N of about 3.4 delta units per trophic level the food web spans about three trophic levels.

$\delta^{13}C$ signatures of all animal taxa spanned 4 δ units. ^{13}C on average is only enriched by 0.5‰ per trophic level (Tiunov, 2007). The wide range of ^{13}C signatures therefore indicates that the food web is based on more than one primary resource. The enrichment of 3.4‰ and 0.5‰ per trophic level for ^{15}N and ^{13}C, respectively, can only be applied to animals that are trophically linked to the same resource, i.e. that constitute one trophic channel. This is unlikely to apply to forest soil food webs with decomposers feeding on bacteria, fungi and leaf litter. Schmidt et al. (2004) separated earthworms, slugs and enchytraeids into groups feeding mainly on litter or soil using stable isotopes (^{15}N, ^{13}C) and postulated that the dual analysis of ^{15}N and ^{13}C has the power to separate resource types if the spacing between corresponding ^{13}C signatures is wide enough.

4.2 The decomposer compartment

In our study, animals also formed groups with differing $\delta^{15}N$ and $\delta^{13}C$ signatures. There were several taxa with $\delta^{15}N$ signatures close to those of the litter layer, i.e. *Glomeris* spp. (Diplopoda), *Euzetes globulus*, *Steganacarus magnus*, *Achipteria coleoptrata*, *Platynothrus peltifer* (all Oribatida) and *Orchesella villosa* (Collembola), suggesting that they function as primary decomposers feeding on less enriched components of leaf litter, such as structural compounds (Scheu and Falca, 2000; Vanderklift and Ponsard, 2003). Animals feeding on low protein diets often use "protein sparing" (Castellini and Rea, 1992), a strategy in which dietary protein is reserved for body composition and maintenance rather than catabolizing it for energy. Since litter is low in protein (Swift et al., 1979) protein sparing may also account for the low $\delta^{15}N$ signatures of primary decomposers (Gannes et al., 1997).

Each of the species investigated, including the putative primary decomposers, were considerably enriched in ^{13}C compared to bulk litter materials. The species that were closest to the ^{13}C signature of bulk leaf litter were *Glomeris* spp. with a mean $\delta^{13}C$ signature of -27.2‰ which is still 2 delta units higher than bulk oak litter. Similar results have been reported by Ponsard and Arditi (2000) and Halaj et al. (2005). However, leaf litter and fine root components differed substantially in ^{13}C signatures, with the span being as high as 7.5 delta units for oak leaf litter. Some more enriched components, such as α-cellulose, had ^{13}C signatures close to those of putative primary decomposers and may therefore constitute the principal component of their diet. It has been proposed that detritivores preferentially feed on parts of litter that are enriched in ^{13}C (e.g. starch and cellulose; Gleixner et al., 1993), and that depleted parts, such as lignin (Benner et al., 1987), remain undigested. Indeed, faeces of Glomeridae, investigated by means of near infrared spectroscopy, contain significantly higher concentrations of lignin, but less structural compounds and nitrogen than the ingested material (Gillon and David, 2001).

Most taxa of the primary decomposer group are heavily sclerotized (Oribatida and Diplopoda) or large (Diplopoda), suggesting that they are well defended against predators. Indeed, the only predator taxa with $\delta^{15}N$ values about 3 delta units enriched compared to primary decomposers were the centipede *Lithobius agilis* and the pseudoscorpion *Neobisium muscorum* which likely feed on litter-feeding collembola. All other predators, including chilopods, mesostigmate mites and elaterid beetles, had higher $\delta^{15}N$ signatures and in part higher $\delta^{13}C$ signatures, suggesting that they are not trophically linked to litter feeders. Litter feeders may therefore represent a trophic dead end as proposed earlier (Scheu, 2002).

4.3 The fungal feeder/predator compartment

The group of soil animals with high $\delta^{15}N$ values and low $\delta^{13}C$ values included predatory centipedes such as *L*. *crassipes* and *Schendyla nemorensis*, that were more than 4 delta units enriched in ^{15}N compared to primary decomposers, suggesting that their diet consists mainly of secondary decomposers such as fungal feeding collembola. Most other taxa in this group, e.g. the collembola *Entomobrya* spp. and *Onychiurus* spp., the oribatid mite *Spatiodamaeus verticillipes* and *Monocampa denisi*, *Campodea staphylinus* and *C*. *remyi* (all Diplura), had equally high or even higher $\delta^{15}N$ signatures compared to the centipedes. These taxa are small sized animals, likely not capable of preying upon heavily sclerotized primary decomposers. We propose that they are not predators, but feed on fungi. There is increasing evidence that fungi constitute a major food resource for many soil animals (Ruf et al., 2006; Pollierer et al., 2007). Ectomycorrhizal fungi acquire carbon from trees resulting in similar $\delta^{13}C$ signatures, but obtain nitrogen from soil which is enriched in ^{15}N (Kohzu et al., 1999; Wallander et al., 2004), resulting in high signatures of $\delta^{15}N$ (Hobbie et al., 2001; Henn and Chapela, 2001) which may account for the high $\delta^{15}N$ signatures of these animals.

4.4 The earthworm compartment

Earthworms in this study formed a separate group with high $\delta^{13}C$ signatures. Compared to taxa with similar $\delta^{15}N$ signatures, they were more enriched in ^{13}C. Their $\delta^{13}C$ signatures were over 4‰ enriched compared to litter and more than 3.5‰ higher than those of roots. A similarly high enrichment compared to potential food sources was reported for other earthworm species by Martin et al. (1992a,b) and for *Lumbricus* species by Schmidt et al. (1997). Presumably, earthworms utilize old carbon that is enclosed in soil aggregates and is inaccessible to other species.

Values of $\delta^{13}C$ increase with soil depth (Ehleringer et al., 2000), and Boutton (1991) suggested that organic matter in deep soil is older and more resistant to decomposition than soil organic matter closer to the surface. Earthworms may constitute an exclusive trophic channel using this old carbon. Isotopic signatures of soil organic matter and microorganisms, including ectomycorrhiza, increase with the depth of soil layers (Wallander et al., 2004; Dijkstra et al., 2006). Dijkstra et al. (2006) found microbial biomass to be similarly enriched in ^{15}N and ^{13}C as total N and C in soils. They suggested that if this enrichment reflects the influence of fractionation processes, $\delta^{15}N$ and $\delta^{13}C$ of microbial products gradually increase with repeated organic matter processing and humification. Consequently, animals living in deeper soil layers, such as endogeic earthworms, may have higher $\delta^{15}N$ and $\delta^{13}C$ signatures. Indeed, Hyodo et al. (2008) found an increase in $\delta^{15}N$ along humification gradients of earthworm diets and a positive relationship between respective diet ages and $\delta^{15}N$ values of earthworms, with endogeic

earthworms living on the oldest diet (5-9 years).

In our study, the endogeic species *A. rosea* had the highest values of $\delta^{15}N$ and $\delta^{13}C$ of all investigated earthworms, and signatures of earthworms increased in the order *Lumbricus castaneus*, *A. longa* and *Octolasion tyrtaeum*, suggesting that *Aporrectodea longa*, *Octolasion tyrtaeum* and *A. rosea* feed on older and more humidified organic matter. Briones et al. (2005) found endogeic earthworms to assimilate 4-8 year old carbon, the anecic *A. longa* 4-6 year old carbon and epigeic species the most recently fixed carbon (0-3 years old). Schmidt et al. (1997) suggested that *A. longa* takes an intermediate position feeding on both soil organic matter and plant litter. However, in the study of Eisenhauer et al. (2008) *A. longa* only fed on mineral soil and did not incorporate litter into the soil. Considering $\delta^{15}N$ signatures, only *Lumbricus castaneus* functions as primary decomposer, and this species indeed has been found to consume little-decomposed plant remains by analysis of crop/gizzard contents (Judas, 1992).

4.5 The predator compartment

Species with high $\delta^{15}N$ and $\delta^{13}C$ signatures formed a fourth group consisting of predators such as mesostigmate mites (*Uropoda cassidea* and *Pergamasus* sp.), oribatid mites (*Hypochthonius rufulus*), but also larvae and adult elaterid beetles (*Athous subfuscus*). *A. subfuscus* is considered carnivorous according to Traugott et al. (2008). These taxa may prey on nematodes and/or enchytraeids. $\delta^{13}C$ signatures of enchytraeids have been shown to be high (Schmidt et al., 2004) and may therefore be responsible for the high ^{13}C signatures of this group of predators.

$\delta^{15}N$ and $\delta^{13}C$ signatures of some animal species did not fall into any of the four groups discussed above. *Arion* sp. is considered omnivorous and may feed on litter as well as on fungi. The same may apply to the isopod *Trichoniscus pusillus* which has been assumed to graze on litter-colonizing microbiota (Kautz et al., 2000). *Dicyrtoma minuta* (Collembola) had very low $\delta^{15}N$ values and has been assumed to feed on algae (Chahartaghi et al., 2005).

4.6 Conclusions

Overall, combined measurements of $\delta^{15}N$ and $\delta^{13}C$ suggest that there are different trophic compartments in the soil animal food web. Some of them likely represent trophic dead ends, e.g. primary decomposers, due to strong sclerotization and/or large body size. The soil food web therefore is strongly compartmentalized. The large number of taxa in the fungal feeder/predator compartment and the recent findings by Pollierer et al. (2007) suggest that the trophic pathway relying on mycorrhizal fungi as primary resource supports the majority of soil animals and represents the largest compartment. A shortcoming of the dual analysis of ^{15}N and ^{13}C is that ^{13}C values of ectomycorrhizal fungi

are similar to those of primary decomposers. Except for somewhat higher ^{15}N values, it is difficult to separate fungal feeders from predators feeding on primary decomposers. To further investigate trophic compartmentalization in soil animal food webs, additional stable isotopes, such as sulphur (^{34}S) that is not significantly fractionated per trophic level, and fatty acids allowing to separate fungal, bacterial and litter based diets need to be included.

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Chapter 3

Taking it to the next level: Trophic transfer of marker fatty acids from basal resource to predators

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Abstract

Fatty acid (FA) analysis is used increasingly to investigate the trophic structure of soil animal food webs as the technique allows separation of the role of detrital resources such as bacteria, fungi and plant material for consumer nutrition. The applicability of FAs as biomarkers for different diets has been verified for Collembola and Nematoda. However, for the analysis of whole food webs it is crucial to know whether marker FA are valid for different taxa and whether they are transferred along the food chain to higher trophic levels, i.e. predators. Top predators are integrators of lower level energy fluxes in food webs; therefore analysis of their FAs may allow to identify trophic pathways and to separate bacterial vs. fungal based energy channels. Chilopoda and Arachnida are among the main predators in soil food webs. Our aim was to test the applicability of marker FAs for these two predator taxa and to verify the trophic transfer of marker FAs of different basal resources via first order consumers into predators, i.e. over three trophic levels. Therefore, we investigated the transfer of FAs from different basal resources [fungi (*Chaetomium globosum*), plant leaf litter (*Tilia europaea*), Gram-positive (*Bacillus amyloliquefaciens*) and Gram-negative bacteria (*Stenotrophomonas maltophilia*)] via Collembola (*Heteromurus nitidus*) as first order consumers into predators [*Lithobius forficatus* (Chilopoda) and *Pardosa lugubris* (Arachnida)]. Fatty acid profiles of predators of food chains with different basal resources differed significantly. Marker FAs of basal resources were clearly detectable in predators, suggesting that FA analysis allows to separate trophic channels of soil food webs. By reflecting basal resources, FAs of predators allow tracking energy/resource fluxes through the food web and thereby clarifying the relative importance of bacterial vs. fungal vs. plant resources for soil animal food webs.

Keywords: fatty acid analysis, Collembola, Chilopoda, Lycosidae, food web, trophic transfer, biomarker, basal resources, bacteria, fungi, trophic channels

1. Introduction

Fatty acid (FA) analysis has been widely applied to study trophic relationships in aquatic systems (Howell et al., 2003; Richoux and Froneman, 2008; Lau et al., 2009; Sakdullah and Tsuchiya, 2009) and is increasingly used for analyzing terrestrial food webs (Ruess et al., 2005; Dungait et al., 2008; Thiemann et al., 2008; Haubert et al., 2009). In particular for understanding the structure of soil food webs FA analysis may allow significant progress, as the basal resource of these food webs is detritus which consists of a variety of components, including dead plant material, bacteria and fungi. Quantifying the relative importance of these different basal resources for soil animal food webs is notoriously difficult and has as yet hardly been accomplished.

The basis of FA analysis is the principle of dietary routing, i.e. the fact that it is energetically more efficient to incorporate fatty acids from the diet directly without modification into consumer tissue. Neutral lipid FAs of consumers, representing predominantly storage lipids, have been shown to mirror the FA composition of the diet (Ruess et al., 2002). Some FAs are specific for certain diets; e.g. branched chain (iso, anteiso) and cyclic FAs are specific markers for bacterial diets (Welch, 1991; Zelles, 1999; Haubert et al., 2006), whereas linoleic acid (18:2ω6,9) is a relative marker predominantly occurring in fungi (Frostegård and Bååth, 1996). Plants have a relatively high proportion of oleic acid (18:1ω9) and compared to fungi lower contents of linoleic acid (Ruess et al., 2005).

FA analysis has been used to analyse the role of bacteria, fungi and plant litter for Collembola nutrition in the field (Ruess et al., 2007). However, incorporation and synthesis of FAs may differ between taxa. Not all FAs in resources are routed to consumer tissue without modification; various FAs are synthesized de novo, preventing their use as dietary markers. Ruess et al. (2004) postulated the FA 20:1ω9 as a marker for nematode feeding in Collembola, but in the study of Chamberlain et al. (2005), this FA was also present when Collembola were fed with leaves, possibly due to desaturation of the abundant FA 20:0. Therefore, the use of marker FAs for different taxa and diets needs to be validated. Further, for evaluating the reliability of dietary routing incorporation of FAs of basal resources into higher trophic levels needs to be investigated at different environmental conditions. Laboratory experiments showed that FA composition in Collembola is only slightly influenced by developmental stage, temperature, food quality and starvation (Haubert et al., 2004, 2008) but this needs further testing for higher trophic levels.

To use FA analysis for investigating entire soil food webs, it is essential to know to which extent FAs are routed into higher trophic levels and if this varies among predator taxa. Ruess et al. (2004) observed the transfer of oleic acid over three trophic levels, from fungi to nematodes to Collembola. Ederington et al. (1995) found a similar trophic transfer

from bacteria to ciliates to copepods in aquatic systems. However, not all marker FAs are transferred equally to higher trophic levels. Hall et al. (2006) traced the transfer of two biomarker FAs into the first trophic level, while only one of them was transferred further to the second trophic level. Lack of enzymes or metabolic breakdown may prevent the incorporation of marker fatty acids into consumer tissue. Hence, marker FAs have to be tested separately for different taxa. Nutrient fluxes through the soil food web are integrated at higher trophic levels; FAs of top-predators therefore may reflect the relative importance of basal reources for soil food web functioning. Knowledge on the energy basis of top-predators is of particular importance as they may control the dynamics of prey populations (Post et al., 2000) and the flux of nutrients through food webs (de Ruiter et al., 1994).

Centipedes (Lithobiidae) and spiders, in particular hunting spiders, are large and abundant predators in soil food webs (Scheu and Falca, 2000). Both are likely bottom-up limited (Chen and Wise, 1999) and centipedes are able to efficiently control prey populations of Collembola and mites (Poser, 1988). While centipedes are sit-and-wait predators (Poser, 1988), lycosid spiders (Lycosidae) are free living spiders that actively hunt for prey, presumably resulting in different prey spectra in the field. Measurements of stable isotopes indicate that centipedes and lycosid spiders occupy high trophic positions in food webs (Halaj et al., 2005). However, whether these predators mainly prey on fungal, bacterial or plant feeders is not known. Scheu and Falca (2000) suggested that they predominantly prey on secondary decomposers, which are more nitrogen enriched, while Ponsard and Arditi (2000) assumed them to function as intraguild predators. Collembola contribute significantly to the nutrition of both centipedes and lycosid spiders (Poser, 1988; Lewis, 2007; Oelbermann et al., 2008). We used representatives of these two predator groups to evaluate the trophic transfer of marker fatty acids into higher trophic levels. Feeding experiments including three trophic levels were set up with bacteria (Gram-positive and Gram-negative) fungi and plant leaves as basal resources, Collembola (*Heteromurus nitidus*) as consumers of intermediate trophic level, and the centipede *Lithobius forficatus* and spider *Pardosa lugubris* as top predators.

We hypothesized that the different FA composition of Gram-positive and Gram-negative bacteria, fungi and plant leaves will be transferred to higher trophic levels i.e. will be detectable in Collembola and their predators, centipedes and lycosid spiders. The study aims at extending the use of FA analysis to higher trophic levels allowing to separate trophic channels and to evaluate the relative importance of different basal resources in soil food webs in the field.

2. Materials and methods

2.1. Food sources

The fungus *Chaetomium globosum*, two bacteria species, *Stenotrophomonas maltophilia* and *Bacillus amyloliquefaciens* FZB42, and lime leaves (*Tilia europaea*) were used as food source for Collembola in the experiments. *C. globosum* was cultivated at 15°C on potato dextrose agar (PDA, Merck, Darmstadt, Germany) with the agar being covered by a membrane filter (Millipore, 0.8 µm) to allow stripping off the fungal mycelium. Bacteria were cultivated in Standard I nutrient broth (Merck) at room temperature for three days. They were harvested from liquid cultures by centrifugation (3000 rpm, 3 min) and the pellet was washed twice with distilled water to clear it from the nutrient solution. Whole senescent lime leaves were harvested directly from trees before litter fall and washed with distilled water to remove adhering phyllosphere microorganisms.

2.2. Collembola

The Collembola *Heteromurus nitidus* (Templeton, 1835) was taken from laboratory cultures fed with bakers yeast. Specimens were put into plastic vessels (diameter 7 cm, height 4.5 cm) containing a layer of plaster mixed with activated charcoal (2:1) at the bottom. Vessels were stored at 15°C in darkness and kept moist with distilled water. Each vessel contained 20 individuals of Collembola. Eggs, pellets and exuvia were removed with a brush once a week. Collembola were fed the four different diets ad libitum for four weeks. Bacteria were offered as suspension (100 µl) placed on a piece of cellulose filter paper and put into the plastic vessels and renewed three times a week. The fungus *C. globosum* was offered as round cuts (diameter 10 mm) from actively growing mycelia of fungal colonies. Lime leaves were offered as pieces of 5-10 mm diameter. Both fungal cuts and leaf pieces were renewed once a week. Three replicates per treatment were taken for fatty acid analysis and the Collembola kept frozen at -20°C until analysis. The remaining Collembola were used as food for centipedes and spiders.

2.3 Centipedes

Juveniles (L3 and L4) of the centipede species *Lithobius forficatus* Linnaeus, 1758, were taken from laboratory cultures fed with *H. nitidus* from cultures fed with bakers yeast. Centipedes were placed separately into plastic vessels (Ø 4 cm) with a bottom layer of plaster mixed with activated charcoal (2:1). Vessels were stored at 15°C in darkness and kept moist with distilled water. Ten centipedes each were fed for four weeks with *H. nitidus* nourished on one of the four different diets described above. Collembola were added without remains of their previous diet and were resupplied three times a week. The initial and final weight of the centipedes was recorded. After four weeks centipedes were

frozen at -20°C until analysis. Prior to freezing, centipedes were starved for three days to ensure that there were no undigested prey remains in their gut.

2.4 Spiders

Juveniles and subadults of the spider *Pardosa lugubris* (Walckenaer, 1802) were captured by hand in a forest close to Darmstadt, Germany. They were put in the same plastic vessels as used for Collembola and stored in darkness at 15°C; some straw was added as structure. Sixteen spiders each were fed for four weeks with *H. nitidus* nourished on one of the four different diets described above. Initial and final weight was recorded and gender of the subadults was determined. After four weeks spiders were starved for three days and frozen at -20°C until analysis.

2.5 Analysis of fatty acids

Cellular lipids of Collembola, centipedes and spiders were extracted and divided into phospholipids and neutral lipids as described in Haubert et al. (2004). For the analysis of *C. globosum*, membrane filters were stripped off the agar; the adhering fungal mats and hyphae were harvested by scraping them off the filter with a sterile scalpel.

NLFA fractions of the Collembola, centipedes and spiders and total FA of *C. globosum*, bacteria and lime leaves were saponified and methylated following the procedures given for the Sherlock Microbial Identification System (MIDI Inc., Newark, USA). The lipid-containing phase was then transferred to test tubes and stored at -20˚C until analysis.

Fatty acid methyl esters (FAMEs) were analyzed by gas chromatography (GC) using a Perkin Elmer CLARUS 500 GC with a flame ionisation detector, equipped with a PE-5 capillary column (30 m x 0.32 mm i.d., film thickness 0.25 µm). The temperature program started with 60^{o}C (held for 1 min) and increased by 30^{o}C per minute to 160^{o}C followed by 3^{o}C per minute to 260^{o}C. The injection temperature was 250^{o}C and helium was used as carrier gas. FAMEs were identified by chromatographic retention time comparison with a standard mixture composed of 37 different FAMEs ranging from C11 to C24 (Sigma-Aldrich, St Louis, USA). To verify correct identification of the peaks some samples were analyzed using a Finnigan MAT Ion Trap 700 GC-MS system.

2.6 Statistical analysis

Differences in body weight of spiders and chilopods between start and end of the experiment were expressed as percent gain or loss of initial body weight. Percentage values were arcsin-radical-transformed and analysed using ANOVA. Means were compared using Tukey´s Honestly Significant Difference (HSD) test.

Differences in FA profiles of fungi, bacteria and leaves were arcsine-transformed and analysed by discrimant function analysis (DFA). Prior to analysis, dimensions were reduced to eight using multidimensional scaling (MDS). Differences in FA profiles of Collembola, centipedes and spiders were also arcsine-transformed and analyzed by DFA but without MDS. DFA and MDS were carried out using STATISTICA 7.0 for Windows (StatSoft, Tulsa, USA, 2001).

Differences between individual FAs were arcsine-transformed and analysed using protected ANOVAs. If significant effects were suggested, means were compared using Tukey´s Honestly Significant Difference (HSD) test.

ANOVAs and posthoc tests were performed using SAS 9.2 (SAS Institute; Cary, NC, USA).

3. Results

3.1. Fatty acid patterns of basal sources

The four food sources *T. europaea*, *C. globosum*, *S. maltophilia* and *B. amyloliquefaciens* and the potato-dextrose agar all differed significantly in their FA composition (DFA after multidimensional scaling; $F_{28,15}$=44.9, p<0.0001). Typical marker FAs were dominant in the respective food sources (Fig.1a). The fungus *C. globosum* contained a high amount of 18:2ω6,9 (56.7 ± 0.6 %) and, compared to *T. europaea,* a lower amount of 18:1ω9 (24.4 ± 0.9 %), while in leaves of *T. europaea* the relative marker for plants, 18:1ω9, was most abundant (62.5 ± 8.7 %). The ratio of 18:1ω9 to 18:2ω6,9 was 8.7 in *T. europaea*, but only 0.4 in the fungus *C. globosum*. The two species of bacteria, *S. maltophilia* and *B. amyloliquefaciens*, contained bacterial marker FAs such as the branched FAs i15:0 (28.3 ± 2.4 % and 14.1 ± 0.9 %, respectively) and a15:0 (20.1 ± 1.6 % and 50.3 ± 1.4 %, respectively).They also contained significantly higher amounts of 16:1ω7 than *T. europaea* and *C. globosum* (6.6 ± 0.8 % and 1.0 ± 0.3 %, respectively). *B. amyloliquefaciens* additionally contained 16.7 % of the FA a17:0, while *S. maltophilia* additionally contained the FA 17:0 (13.0 ± 1.0 %) and the Gram-negative marker cy17:0 (1.3 ± 0.3 %; not shown in graph). There were no C20 polyunsaturated FAs (PUFAs) in food sources.

3.2 Fatty acid patterns of Collembola

The FA patterns of food sources were transferred to the Collembola feeding on them (Fig. 1b). Collembola fed with *C. globosum* contained significantly more 18:2ω6,9 than those fed with *T. europaea* (21.9 ± 1.3 % and 12.5 ± 1.9 %, respectively), and Collembola fed with these diets had significantly higher amounts of this FA than Collembola fed with bacteria. Although all Collembola contained high amounts of the FA 18:1ω9, the ratio

between 18:1ω9 and 18:2ω6,9 was twice as high in *H. nitidus* fed with *T. europaea* than in *H. nitidus* fed with *C. globosum* (3.8 vs. 1.9). *H. nitidus* fed with *S. maltophilia* and *B. amyloliquefaciens* contained the bacterial marker FAs i15:0 (1.4 ± 0.4 % and 1.7 ± 0.7 %, respectively) and a15:0 (0.9 ± 0.3 % and 5.3 ± 2.4 %, respectively). *H. nitidus* fed with *B. amyloliquefaciens* additionally contained significantly more a17:0 (2.3 ± 1.5 %) than when fed with other diets. All Collembola contained the C20 PUFAs 20:4ω6,9,12,15 (1.7 ± 0.9 %) and 20:5ω3,6,9,12,15 (0.6 ± 0.3 %) in similar amounts.

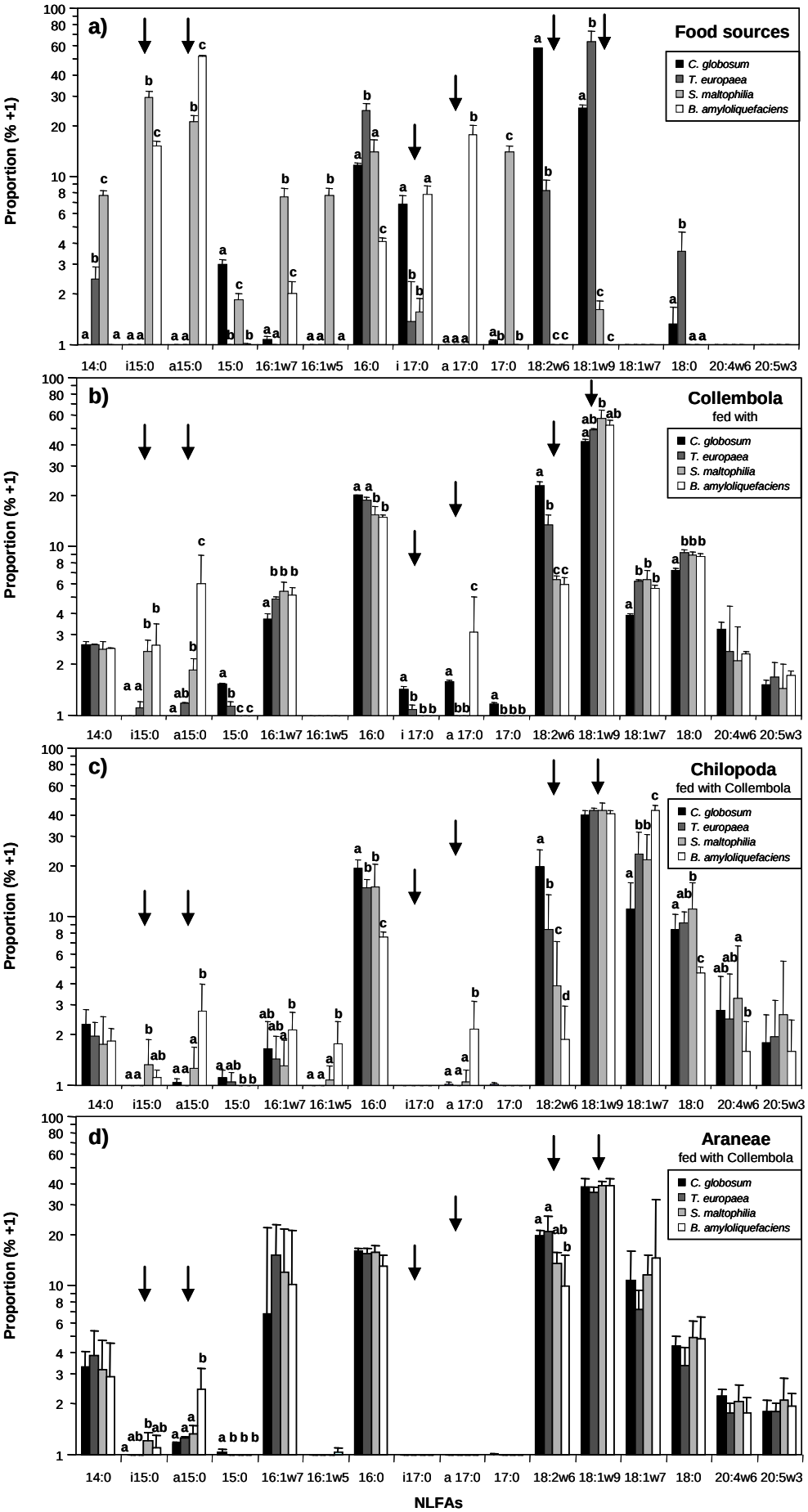

Fig. 1: Concentrations of fatty acids (percentages of total ± SD; note log scale) of (a) whole cellular lipids of food resources, the fungus *Chaetomium globosum*, leaves of *Tilia europaea*, the Gram-negative bacterium *Stenotrophomonas maltophilia* and the Gram-positive bacterium *Bacillus amyloliquefaciens*, and of NLFAs of (b) *Heteromurus nitidus* (Collembola) as first order consumer, and (c) *Lithobius forficatus* (Chilopoda) and (d) *Pardosa lugubris* (Araneae) as predators fed Collembola kept on the respective basal resources. Arrows indicate marker FAs. Different letters represent significant differences (Tukey´s Honestly Significant Difference (HSD) test; $p<0.05$).

3.3 Weight gain and fatty acid patterns of predators

Lithobius forficatus

Two of the 40 juveniles of *L. forficatus* (initial weight 2.3 ± 1.6 mg) escaped and two died during the experiment, all others survived. Most surviving centipedes gained weight (+19.4 ± 13.4 %). Whole FA composition of *L. forficatus* differed significantly between the treatments (DFA, Fig. 2, Table 1). Centipedes fed with differently nourished *H. nitidus* incorporated the marker FAs for the diets of the Collembola (Fig. 1c). *L. forficatus* fed with *H. nitidus* kept on *C. globosum* had a significantly higher amount of 18:2ω6,9 (19.1 ± 4.0 %) than when fed with *H. nitidus* kept on *T. europaea* (8.2 ± 3.8 %). When fed with Collembola kept on bacterial diets, the content of 18:2ω6,9 in *L. forficatus* was much lower (3.5 ± 2.2 % and 1.1 ± 1.2 %) for Collembola kept on *S. maltophilia* and *B. amyloliquefaciens*, respectively). The ratio of 18:1ω9 to 18:2ω6,9 in centipedes fed with *H. nitidus* kept on *C. globosum* was 2.1, whereas it was 5.1 in *L. forficatus* fed with *H. nitidus* kept on leaves of *T. europaea*. The bacterial marker FAs i15:0 and a15:0 were almost exclusively found in *L. forficatus* fed with *H. nitidus* kept on *S. maltophilia* (0.4 ± 0.5 % and 0.3 ± 0.4 %, respectively) and *B. amyloliquefaciens* (0.1 ± 0.1 % and 1.9 ± 0.7 %, respectively). However, they occurred in lower concentrations than in their Collembola prey. The FA a17:0, which mainly occurred in *B. amyloliquefaciens*, was also present in centipedes fed with Collembola kept on this diet (1.3 ± 0.7 %). Centipedes contained the C20 PUFAs 20:4ω6,9,12,15 (1.9 ± 1.9 %) and 20:5ω3,6,9,12,15 (1.3 ± 1.4 %).

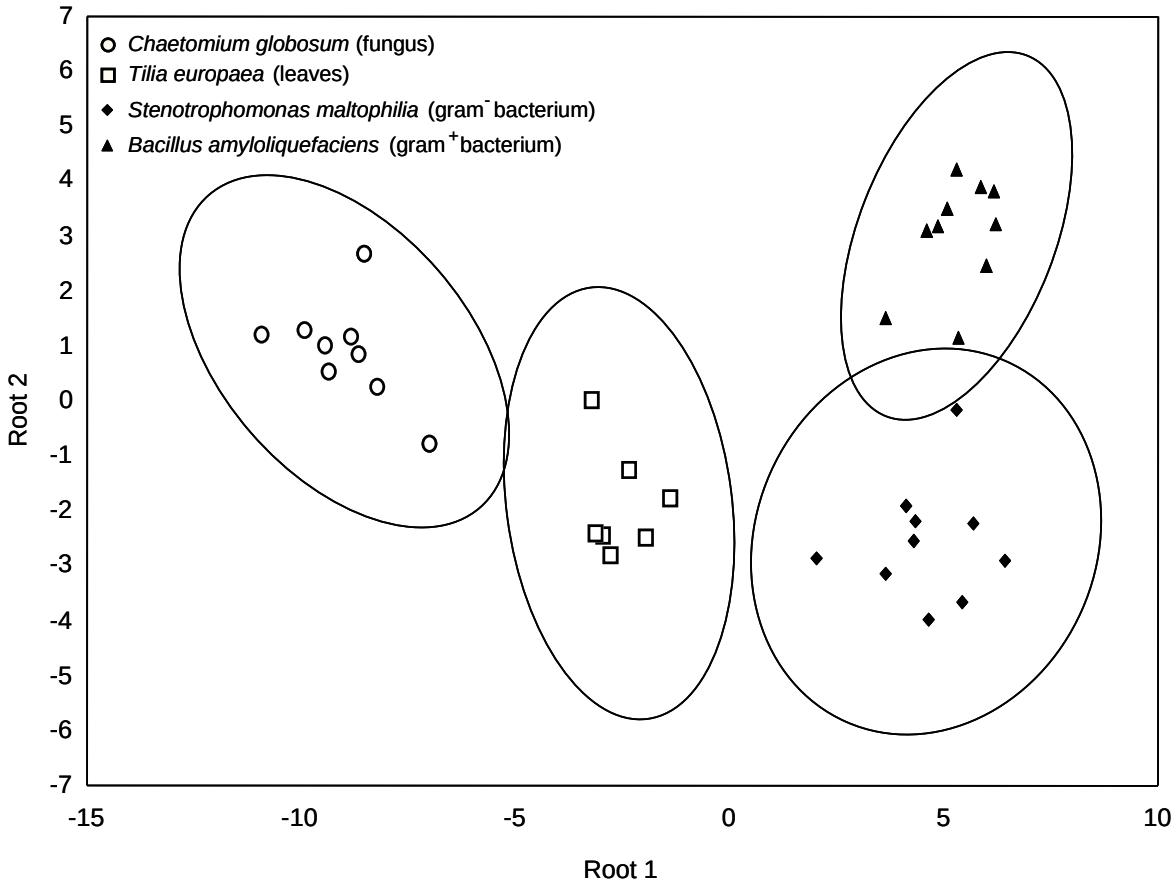

Fig. 2: Discriminant function analysis of NLFAs of *Lithobius forficatus* (Chilopoda) fed with *Heteromurus nitidus* (Collembola) kept on different diets (fungus, leaves, Gram-negative and Gram-positive bacteria). Ellipses represent confidence ranges α = 0.05.

Pardosa lugubris

The weight gain of *Pardosa lugubris* differed significantly between juvenile spiders, subadult males and subadult females ($F_{2,71}$ = 23.8, p<0.0001). Subadult male spiders (initial weight 12.5 ± 1.5 mg) lost weight during the 4 weeks of feeding (-2.0 ± 1.9 %) and subadult female spiders (initial weight 11.4 ± 3.5 mg) gained little weight (+1.5 ± 5.2 %). In contrast, juvenile spiders (initial weight 3.5 ± 1.6 mg) gained weight by 10.4 ± 6.6 %. This was also reflected in the FA patterns, with marker FAs being visible most clearly in juvenile spiders. However, in general the FA composition of *P. lugubris* differed significantly between treatments (DFA, Fig. 3, Table 1).

As the incorporation of marker FAs was most pronounced in juvenile spiders only these are presented here (Fig. 1d). *P. lugubris* fed with *H. nitidus* kept on *C. globosum* had higher amounts of the fungal marker 18:2ω6,9 (18.8 ± 1.7 %) than when fed with *H. nitidus* kept on bacterial diets. However, when *P. lugubris* was fed with *H. nitidus* kept on *T. europaea*, the amount of 18:2ω6,9 (20.3 ± 4.4 %) was similar to that of *P. lugubris* fed with *H. nitidus* kept on *C. globosum*. Also, the relative leaf and fungal marker FA 18:1ω9 did not differ between these treatments. The bacterial marker FA i15:0 was only found in

spiders fed with Collembola kept on *B. amyloliquefaciens* (0.1 ± 0.2 %) and *S. maltophilia* (0.2 ± 0.1 %). The bacterial marker FA a15:0 was found in small amounts in spiders of each of the treatments; only *P. lugubris* fed with *H. nitidus* kept on *B. amyloliquefaciens* contained significantly higher amounts of a15:0 (1.5 ± 0.7 %) than when fed with *H. nitidus* kept on other diets. The bacterial FAs i17:0 and a17:0 generally were not present in *P. lugubris*. All individuals of *P. lugubris* contained the C20 PUFAs 20:4ω6,9,12,15 (0.9 ± 0.3 %) and 20:5ω3,6,9,12,15 (1.2 ± 0.6 %) in similar amounts.

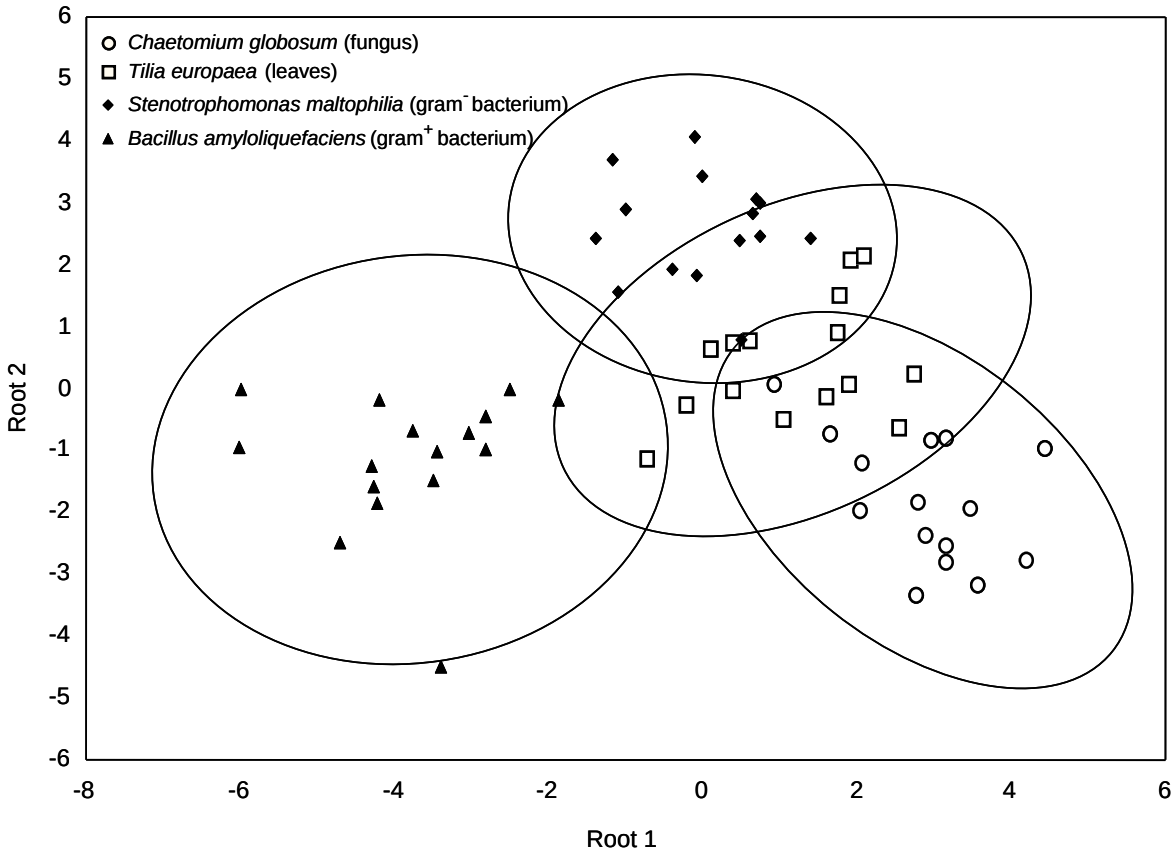

Fig. 3: Discriminant function analysis of NLFAs of *Pardosa lugubris* (Araneae) fed with *Heteromurus nitidus* (Collembola) on different diets (fungus, leaves, Gram-negative and Gram-positive bacteria). Ellipses represent confidence ranges α = 0.05.

4. Discussion

FA profiles were transferred over three trophic levels from basal resources (fungi, leaves and Gram-positive and Gram-negative bacteria) over Collembola to centipedes and spiders, suggesting that FA analysis allows tracing the role of basal resources for predator nutrition. The transfer of marker FAs along food chains therefore may allow separation of different energy channels in soil food webs, such as the bacterial vs. the fungal channel (Moore and Hunt, 1988).

Food resources differed significantly in their FA composition, which is an important precondition for further analyses of higher trophic levels. Food resource marker FAs were present, such as 18:2ω6,9 in fungi, 18:1ω9 in leaves and the branched FAs i15:0, a15:0, i17:0 and a17:0 and 16:1ω7 in bacteria. The fungus *C. globosum* and leaves of *T. europaea* could be distinguished by the relative proportions of 18:1ω9 and 18:2ω6,9. In *T. europaea*, the ratio was more than 20 times higher than in *C. globosum*. The Gram-negative bacterium *S. maltophilia* differed significantly from the Gram-positive *B. amyloliquefaciens* by the occurrence of 17:0 and cy17:0. The FA cy17:0 was postulated as a Gram-negative marker by Ruess et al. (2005). However, the percentage of cy17:0 (1.3 %) was low compared to the study of Haubert et al. (2006), who found a mean of 16.9 % and 31.4 % in the Gram-negative bacteria *Enterobacter aerogenes* and *Pseudomonas putida*. The Gram-positive *B. amyloliquefaciens* was characterized by high amounts of a17:0. Ruess et al. (2005) postulated branched chain FAs (iso, anteiso) as markers for Gram-positive bacteria. However, the Gram-negative *S. maltophilia* also contained significant amounts of the branched chain FAs i15:0 and a15:0, suggesting that these markers are specific for bacteria, but are not necessarily suitable to distinguish between Gram-positive and Gram-negative bacteria. Their abundance within total FAs likely is species-specific but may also depend on the culture medium.

Collembola feeding on the different diets retained diet specific marker FAs in their NLFAs The bacterial specific marker FAs i15:0 and a15:0 mainly occurred in *H. nitidus* fed with *S. maltophilia* and *B. amyloliquefaciens*, but also to a small extent in Collembola fed with leaves, possibly because the adhering phyllosphere had not been removed completely and was preferentially consumed by the Collembola. When fed with *B. amyloliquefaciens*, *H. nitidus* additionally contained a significant amount of a17:0. In contrast, 16:1ω7, which within food sources only occurred in bacteria, was almost equally abundant in *H. nitidus* of each of the treatments, suggesting that it is not suitable as biomarker in Collembola. In diet switching experiments Chamberlain et al. (2005) found a gradual decrease of 16:1ω7 in Collembola and suggested that they may not be able to synthesize this FA themselves, but that the high initial abundance was caused by the previous diet of bakers yeast. Since *H. nitidus* in this study was also fed with bakers yeast prior to the feeding experiments, it is possible that 16:1ω7 originated from the previous diet. The Gram-negative marker cy17:0 was not detected in Collembola fed with *S. maltophilia*, presumably because it only occurred in very low amounts in *S. maltophilia*. The relative fungal marker FA 18:2ω6,9, occurred in significantly higher amounts in Collembola fed with *C. globosum* than in those fed with *T. europaea*. Contents of 18:2ω6,9 in Collembola fed fungi or plant litter were generally higher than in those fed with bacteria. Contents of the relative plant marker, 18:1ω9, were similar in Collembola from each of the diet treatments, suggesting that they are able to synthesize this FA

themselves. However, the ratio of 18:1ω9 to 18:2ω6,9 was twice as high in Collembola fed with leaves than when fed with the fungus, suggesting that for Collembola, this ratio is a good indicator of plant vs. fungal diets. Ruess et al. (2007) also suggested that this ratio could be used to separate herbivory and fungivory in Collembola, with low to intermediate ratios corresponding to fungal feeding and high ratios to plant feeding. Collembola additionally contained C20 PUFAs, which they are able to synthesize themselves (Chamberlain et al., 2005) which contrasts to more derived insect lineages (Stanley-Samuelson et al., 1988).

FA profiles of *L. forficatus* fed with the differently nourished Collembola were well separated by DFA suggesting that for these predators the FA composition of the basal resources of their prey is imprinted in their FA profile, and that dietary FAs can be traced over three trophic levels. Although their percentage decreased with trophic level, bacterial marker FAs were present in significant amounts in centipedes suggesting that they can be used to trace bacteria based food chains and energy channels in the field to at least the third trophic level. Only the FA i17:0 was not transferred to centipedes, possibly due to low amounts in Collembola or metabolic breakdown. Further, the fraction of the fungal marker 18:2ω6,9 in the diet persisted in *L. forficatus* and the ratio of 18:1ω9 to 18:2ω6,9 was 2.4 times higher in *L. forficatus* fed with Collembola kept on a leaf diet, than when fed with Collembola on a fungal diet. This suggests that the 18:1ω9-to-18:2ω6,9 FA ratio can be used to differentiate between predators living on prey feeding on plant based vs. fungal based diets, which has been proposed previously for first order consumers (Ruess et al., 2007).

The second predator, the spider *P. lugubris*, did not gain weight uniformly as was the case in centipedes. Gain of weight depended on initial weight and gender. Only juvenile spiders with a mean initial weight of 3.5 mg gained substantially weight, although still only half the amount of *L. forficatus*. Subadult male spiders even lost weight, presumably because they invested time and energy into courtship and male functions. Similarly, the weight of subadult female spiders also changed little. *H. nitidus* is high quality prey for juvenile lycosid spiders (Oelbermann and Scheu, 2002), whereas larger subadult spiders may prefer different prey. Further, growth rates generally decline with age and the duration of the experiment (four weeks) may have been too short for subadult *P. lugubris* to gain substantial weight. The unequal gain of weight was also reflected in the FA profiles, with marker FAs being visible most clearly in juveniles. Therefore, only juveniles of *P. lugubris* were considered for the examination of marker FAs. The bacterial markers i15:0 and a15:0 were present in spiders fed with Collembola kept on bacterial diets. However, a15:0 was also present in small amounts in the other individuals. Presumably, these are remains of the earlier diet of these spiders in the field. The longer chained bacterial FAs i17:0 and a17:0 were not detectable in *P. lugubris*. Spiders may not be able

to incorporate these FAs due to a lack of proper enzymes or they may preferentially metabolize them. It was not possible to separate spiders fed with Collembola kept on fungi or leaves by the ratio of 18:2ω6,9 and 18:1ω9 since they contained equally high amounts of these FAs. For *P. lugubris*, the experimental period presumably was not long enough to substitute a substantial part of storage fat; therefore older FAs presumably masked effects of differing diets. Nevertheless, FA profiles of spiders differed significantly between each of the four prey treatments (Fig. 3), suggesting that FA analysis is suitable to distinguish between the different trophic chains spiders form part of in the field.

Both *L. forficatus* and *P. lugubris* contained C20 PUFAs in similar amounts as Collembola. These C20 PUFAs may either have originated from Collembola, or centipedes and spiders may have synthesized them in part themselves.

A drawback to FA analysis in the field is the lack of quantification of the dietary uptake of specific fatty acids. Future laboratory studies should aim at quantifying the contribution of dietary components to the storage fat of consumers. Considering the complexity of dietary components and the potential breakdown or conversion of marker FAs, additional methods, such as isotope labeling, may help to differentiate basal food sources in the field more precisely.

In conclusion, on the basis of two major macrofauna predators from two arthropod taxa of different phylogenetic affiliation (Chilopoda and Arachnida), this study showed that the transfer of marker FAs can be traced over at least three trophic levels. Marker FAs of bacteria, fungi and plant leaves as basal resources were incorporated via Collembola as first order consumers into predators as second order consumers. Hence, FA analysis presumably allows differentiating the trophic food chains which consumers of different trophic level form part of in the field. This is of particular importance for understanding soil food webs as it may allow differentiating between the major trophic chains in soil, i.e. bacterial and fungal based energy channels, and therefore evaluating the relative importance of basal resources for sustaining complex food webs with energy channeled to higher trophic levels.

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Chapter 4

Fatty acid patterns as biomarker for trophic interactions: Changes after dietary switch and starvation

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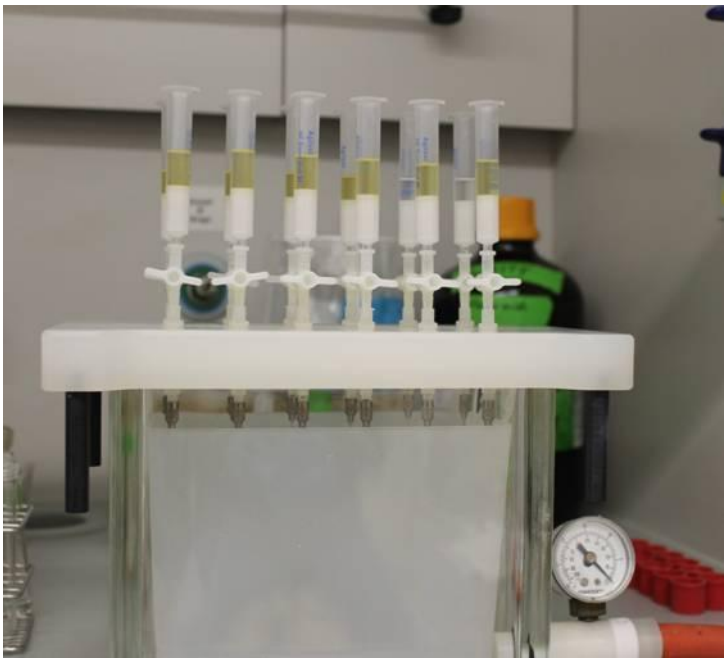

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Abstract

Fatty acid (FA) analysis is becoming increasingly important for investigating trophic interactions in soil food webs. FA profiles of neutral lipids are affected by diet, and the occurrence and amount of certain FAs can reflect feeding strategies. However, to draw conclusions on feeding strategies in the field, it is necessary to know physiological parameters of fatty acid metabolism such as the detection time and storage period of FAs. In this study we investigated the chronological change of FA biomarkers in the Collembola *Heteromurus nitidus*, when switched between different food sources: leaves (*Tilia europaea*), a fungus (*Chaetomium globosum*) and two bacteria (*Stenotrophomonas maltophilia*, *Bacillus amyloliquefaciens*). Additionally, we observed the change of bacterial FA biomarkers during starvation. After 14 days of food deprivation bacterial fatty acids were still detectable in a sufficient amount to use them as dietary markers. Switching diet experiments demonstrated that FAs typical for a specific diet are already present after one day and are still detectable after 14 days on a different food source, suggesting that FA analysis can integrate the food choice of Collembola over a longer period of time, in contrast to snapshot methods such as gut content analysis.

Keywords: fatty acid analysis, Collembola, food web, food source, diet, starvation, biomarker, dietary routing

1. Introduction

Lipid composition of bacteria and fungi is used as biomarker in environmental samples e.g., to quantify and classify microorganisms in soil (Tunlid and White, 1992; Frostegård and Bååth, 1996; Zelles, 1999). Only recently, fatty acids (FAs) were proposed as trophic markers for feeding strategies of soil animals (Ruess et al., 2004, 2005a; Chamberlain et al., 2005; Haubert et al., 2006). The method has been used more intensively in marine food web studies (Ederington et al., 1995; Meziane et al., 1997; Navarrete et al., 2000) and is now being applied to soil systems (Ruess et al., 2005b; Haubert et al., 2009). The method is based on the fact that it is energetically more efficient to incorporate dietary FAs directly into consumer tissue without degradation or modification ("dietary routing"), allowing to trace dietary signals in consumers by analyzing their fatty acid composition (Stanley-Samuelson et al., 1988). Therefore, FAs can be used as biomarkers for trophic links.

Collembola are widespread and abundant soil animals playing an important role in decomposition processes (Visser, 1985). In the laboratory Collembola can be reared on a wide range of different diets. However, recent measurements of stable isotopes in Collembola (Chahartaghi et al., 2005) suggest strong trophic niche differentiation in the field and very different trophic positions including detritivores, herbivores and carnivores. However, the composition of the diet of Collembola species and its variation in time remains to be explored.

Trophic transfer of FAs from fungal food sources to nematode or Collembola grazers has been reported by Ruess et al. (2002, 2004, 2005a) and Chamberlain et al. (2004). FA biomarkers for different trophic guilds have been assigned (Chamberlain et al., 2005; Ruess et al., 2005b; Haubert et al., 2006), i.e., i14:0, i15:0, a15:0, i17:0, cy17:0 and 16:1ω5 for bacterial feeders, high proportion of 18:2ω6,9 for fungal feeders and high proportion of 18:1ω9 and 18:2ω6,9 for species feeding on leaf litter. Additionally, the ratio of 18:1ω9 to 18:2ω6,9 has been proposed to reflect the relative contribution of fungi and leaf litter to the diet of Collembola being higher when feeding on leaves (Pollierer et al., 2010). The composition of neutral lipid fatty acids (NLFAs) in the fat body of consumers results from different processes including storage of dietary lipids, de novo synthesis, degradation and subsequent release for mobilisation to sites where they are metabolized (Beenakkers et al., 1985). However, specific FA markers for food sources in Collembola showed some robustness against variations in temperature, food quality, food deprivation and developmental stage (Haubert et al., 2004, 2008). Further, Haubert et al. (2006) showed that NLFA composition depends more on the food source than on Collembola species.

The results of these laboratory experiments have been used to determine trophic interactions in the field. Ruess et al. (2005b) determined food preferences of Collembola in three different forests and Haubert et al. (2009) investigated trophic links between belowground prey and aboveground predators in differently managed agricultural fields. Both studies showed that FA analysis allows novel insights into trophic interactions in soil food webs. However, to allow rigorous interpretation of field data more information is needed on how fast marker FAs occur in the fat body of consumers after consumption of respective diets and for how long prey FAs can be traced after switching to another diet. This study therefore introduces time as variable in FA analysis by investigating (1) the time necessary for being able to trace FA markers in NLFAs of consumers (Collembola) and (2) the duration FA markers can be traced after switching diet or starvation.

2. Materials and methods

2.1. Food sources

The fungus *Chaetomium globosum*, two bacteria *Stenotrophomonas maltophilia* and *Bacillus amyloliquefaciens* FZB42, and lime leaves (*Tilia europaea*) were used as food source for Collembola in the experiments. *C. globosum* was cultivated on potato dextrose agar (PDA, Merck, Darmstadt, Germany). For the analysis of fatty acids *C. globosum* was grown on PDA covered by a membrane filter (Millipore, 0.8 µm). Fungi were kept at 15°C. Bacteria were cultivated in Standard I nutrient broth (Merck) at room temperature for three days. They were harvested from liquid cultures by centrifugation (3000 rpm, 3 min) and the pellet washed twice with sterile distilled water to clear from the nutrient solution. Senescent lime leaves were collected in the field and washed with sterile water to remove adhering phyllosphere microorganisms.

2.2. Collembola

The Collembola *Heteromurus nitidus* (Templeton, 1835) was taken from laboratory cultures fed with bakers yeast. Specimens were put into plastic vessels (diameter 7 cm, height 4.5 cm) containing a layer of plaster mixed with activated charcoal (2:1) at the bottom. Vessels were stored at 15°C in darkness and kept moist with distilled water. Each vessel contained 20 individuals of Collembola. Eggs, pellets and exuvia were removed with a brush once a week. Collembola were fed the four different diets ad libitum for eight weeks. Bacteria were offered as suspension (100 µl) placed on a piece of cellulose filter paper and put into the plastic vessels and renewed three times a week. The fungus *C. globosum* was offered as round cuts (diameter 10 mm) from actively growing mycelia of fungal colonies. Lime leaves were offered as pieces of 5-10 mm diameter. Both fungal

cuts and leaf pieces were renewed once a week. After eight weeks the diet was switched and specimens were sampled at day 0 and after 1, 7 and 14 days. Three replicates per treatment were taken and the Collembola kept frozen at -20°C until analysis.

2.3. Analysis of fatty acids

Cellular lipids of Collembola were extracted and divided into phospholipids and neutral lipids as described in Haubert et al. (2004). For the analysis of *C. globosum*, membrane filters were stripped of the agar; the adhering fungal mats and hyphae were harvested by scraping them off the filter with a sterile scalpel.

NLFA fractions of the Collembola and total FA of *C. globosum*, bacteria and lime leaves were saponified and methylated following the procedures given for the Sherlock Microbial Identification System (MIDI Inc., Newark, USA). The lipid-containing phase was then transferred to test tubes and stored at -20˚C until analysis.

Fatty acid methyl esters (FAMEs) were analyzed by gas chromatography (GC) using a Perkin Elmer CLARUS 500 GC with a flame ionisation detector, equipped with a PE-5 capillary column (30 m x 0.32 mm i.d., film thickness 0.25 µm). The temperature program started with 60^{o}C (held for 1 min) and increased by 30^{o}C per minute to 160^{o}C followed by 3^{o}C per minute to 260^{o}C. The injection temperature was 250^{o}C and helium was used as carrier gas. FAMEs were identified by chromatographic retention time comparison with a standard mixture composed of 37 different FAMEs ranging from C11 to C24 (Sigma-Aldrich, St Louis, USA). To verify correct identification of the peaks some samples were analyzed using a Finnigan MAT Ion Trap 700 GC-MS system.

2.4. Statistical analysis

Differences in FA profiles of fungi, bacteria, leaves and Collembola were arcsin-radical-transformed and analyzed using ANOVA. If significant effects were suggested, means were compared using Tukey´s honestly significant difference test. Statistical analyses were performed using STATISTICA 7.1 (StatSoft Inc., Tulsa, USA).

3. Results

3.1. Fatty acid patterns

FA patterns of food sources were dominated by the typical FAs already proposed as marker FAs (Table 1). Leaves of *T. europaea* contained a high amount of 18:1ω9 (62.5%) and *C. globosum* of 18:2ω6,9 (56.7%). In bacteria branched FAs were most abundant, i15:0 and a15:0 in *B. amyloliquefaciens* and *S. maltophilia* (14.1% and 50.3%, and 28.3% and 20.1%, respectively); *B. amyloliquefaciens* additionally contained 16.7% of the FA a17:0. In Collembola 15 different FAs with a chain length of 14 – 20 carbon atoms were detected. The FA pattern significantly varied with food sources (Table 1). The FAs i15:0 and a15:0 only occurred in animals fed bacteria, with a15:0 (18.8% compared to 1.6%) and also a17:0 (7.1% compared to 0.6%) being much more abundant in Collembola fed *B. amyloliquefaciens* than in those fed S. maltophilia. Further, FA 18:1ω7 was abundant in Collembola fed S.maltophilia (19.3%). FAs 15:0 (0.6%) and 17:0 (0.1%) only occurred in Collembola fed *C. globosum*, whereas FA 18:2ω6,9 was abundant in Collembola fed *C. globosum* (22.9%) and *T. europaea* (14.8%). Further, Collembola fed *T. europaea* contained 50.7% of the FA 18:1ω9.

Table 1
Fatty acid pattern (means in % of total FAs ± s.d.) of *Tilia europaea* leaves, the fungus *Chaetomium globosum* and the bacteria *Stenotrophomonas maltophilia* and *Bacillus amyloliquefaciens* used as diet for Collembola, and the neutral lipid fatty acid pattern of the Collembola *Heteromurus nitidus* fed with these diets.

Diet					*T. europaea*	*C. globosum*	*B. amyloliquefaciens*	*S. maltophilia*
Organism	*T. europaea*	*C. globosum*	*B. amyloliquefaciens*	*S. maltophilia*	*H. nitidus*			
14:0	1.5 ±0.4	-	-	6.7 ±0.4	1.2 ±0.2	1.5 ±0.2	0.8 ±0.7	1.1 ±0.2
i15:0	**-**	**-**	**14.1 ±0.9**	**28.3 ±2.4**	**- a**	**- a**	**3.0 ±1.8 b**	**2.8 ±0.7 b**
a15:0	**-**	**-**	**50.3 ±1.4**	**20.1 ±1.6**	**- a**	**0.1 a**	**18.8 ±4.3 c**	**1.6 ±0.4 b**
15:0	**-**	**2.0 ±0.2**	**-**	**0.9 ±0.2**	**- a**	**0.6 ±0.1 b**	**- a**	**- a**
i16:0	-	-	2.2 ±0.1	0.5 ±0.3	-	-	-	-
16:1ω7	-	0.1 ±0.1	1.0 ±0.3	6.6 ±0.8	2.9 ±0.4	2.5 ±0.1	1.0 ±0.9	2.2 ±0.3
16:1ω5	-	-	-	6.7 ±0.8	-	-	-	-
16:0	23.4 ±2.5	10.6 ±0.4	3.1 ±0.2	13.2 ±2.3	17.7 ±0.5	18.5 ±0.5	17.9 ±3.7	17.0 ±1.1
cyc 17:0	-	-	-	1.3 ±0.3	-	-	-	-
i 17:0	0.5 ±0.9	5.8 ±0.9	6.9 ±0.9	0.6 ±0.3	-	0.6 ±0.2	1.2 ±1.1	-
a 17:0	**-**	**-**	**16.7 ±2.2**	**-**	**- a**	**0.6 ±0.2 b**	**7.1 ±1.7 c**	**- a**
17:0	-	-	-	13.0 ±1.0	- a	0.2 ±0.1 b	- a	- a
18:2ω6,9	**7.2 ±1.2**	**56.7 ±0.6**	**-**	**-**	**14.8 ±2.0 a**	**22.9 ±3.6 a**	**2.8 ±4.0 b**	**1.4 ±0.4 b**
18:1ω9	**62.5 ±8.7**	**24.4 ±0.9**	**-**	**0.6 ±0.2**	**50.7 ±2.0 c**	**39.6 ±4.2 b**	**30.9 ±4.5 a**	**41.5 ±0.9 b**
18:1ω7	**-**	**-**	**-**	**-**	**3.6 ±0.7 a**	**3.2 ±1.3 a**	**4.4 ±3.2 a**	**19.3 ±3.5 b**
18:0	2.7 ±1.0	0.4 ±0.3	-	-	8.0 ±0.9 ab	6.0 ±0.3 a	9.8 ±2.4 b	9.8 ±0.3 b
20:4ω6,9,12,15	-	-	-	-	0.9 ±0.2	2.4 ±0.7	1.3 ±1.6	1.9 ±0.3
20:5ω3,6,9,12,15	-	-	-	-	0.1 ±0.1	0.7 ±0.2	0.8 ±1.0	1.4 ±0.1

Marker FAs are in bold. Means of *H. nitidus* within a row sharing the same letter or not marked by letters are not significantly different from each other (Tukey´s HSD test, P<0.05) -: not detected or trace amount.

3.2. Diet switching

When the diet was switched from *T. europaea* to *S. maltophilia* (Table 2) the typical leaf FAs 18:2ω6,9 and 18:1ω9 decreased from 14.8% to 9.6% ($F_{3,11}$=2.38, P=0.146) and from 50.7% to 45.7% ($F_{3,11}$=4.67, P=0.036), respectively, within two weeks. The typical *S. maltophilia* FAs i15:0 and a15:0 were already detectable after one day (1.3% and 1.0%, respectively) and increased significantly to 4.0% ($F_{3,11}$=22.96, P<0.001) and 2.5% ($F_{3,11}$=44.80, P<0.001), respectively, within two weeks. A dietary switch from *C. globosum* to *S. maltophilia* led to a significant increase in the FAs i15:0 and a15:0 from 0% to 0.5% ($F_{3,11}$=4.93, P=0.032) and from 0.1% to 0.4% ($F_{3,11}$=3.74, P=0.060), respectively; with these FAs being already detectable after one day. The decrease in the fungal FA 18:2ω6,9 was not significant after two weeks ($F_{3,11}$=0.94, P=0.466; Table 3). A switch in the opposite direction (Table 4) led to a non-significant decrease in i15:0 and a15:0 from 2.8% to 0.7% ($F_{3,11}$=2.04, P=0.187) and 1.6% to 0.5% ($F_{3,11}$=1.75, P=0.234), respectively. FA 18:1ω7 significantly decreased from 19.3% to 3.0% ($F_{3,11}$=16.90, P<0.001). The typical fungal FA 18:2ω6,9 increased significantly from 1.4% to 18.6% ($F_{3,11}$=89.49, P<0.001) within two weeks; the increase already occurred after one day (5.8%). FA 15:0 increased significantly from 0% to 0.7% ($F_{3,11}$=87.92, P<0.001). A similar increase occurred when the diet was switched from *B. amyloliquefaciens* to *C. globosum* (Table 5). FA 18:2ω6,9 increased significantly from 2.8% to 22.1% ($F_{3,11}$=11.12, P=0.003) and 15:0 from 0 to 0.9% ($F_{3,11}$=33.25, P<0.001). The typical *B. amyloliquefaciens* FAs i15:0, a15:0 and a17:0 decreased ($F_{3,11}$=3.32, P=0.078, $F_{3,11}$=7.81, P=0.009, and $F_{3,11}$=19.12, P<0.001, respectively), but were still well detectable after two weeks with 0.9%, 3.7% and 1.9%, respectively.

In Collembola formerly fed with *B. amyloliquefaciens*, starvation for two weeks led to a decrease in the marker FAs i15:0, a15:0 and a17:0, but the decrease was only significant for a15:0 ($F_{1,5}$=10.03, P=0.034; Table 6). All three marker FAs were still detectable after two weeks. Starvation of Collembola fed *S. maltophilia* showed no significant changes in i15:0 and a15:0, only 18:1ω7 decreased significantly ($F_{1,5}$=60.92, P=0.001). Only Collembola switched from *B. amyloliquefaciens* to *C. globosum* gained weight during the two weeks, all others lost weight (data not presented).

Table 2
Neutral lipid fatty acid pattern (means in % of total FAs ± s.d.) of the Collembola *Heteromurus nitidus* 0, 1, 7 and 14 days after switching diet from leaves (*Tilia europaea*) to bacteria (*Stenotrophomonas maltophilia*).

days after switching diet	0	1	7	14
14:0	1.2±0.2	1.4±0.1	1.6±0.4	2.1±0.7
i15:0	**- a**	**1.3±1.1 b**	**1.2±0.4 b**	**4.0±1.1 c**
a15:0	**- a**	**1.0±0.5 b**	**1.2±0.2 b**	**2.5±0.4 c**
15:0	-	0.1±0.1	0.1±0.1	-
16:1ω7	2.9±0.4 a	3.9±0.6 ab	5.0±0.8 b	4.0±0.1 ab
16:0	17.7±0.5	17.6±0.8	17.7±0.6	18.7±2.0
18:2ω6,9	**14.8±2.0**	**13.1±3.0**	**11.3±1.8**	**9.6±3.0**
18:1ω9	**50.7±2.0 a**	**46.7±2.1 ab**	**45.1±0.8 b**	**45.7±2.7 ab**
18:1ω7	3.6±0.7	4.4±1.0	5.5±0.6	3.5±1.5
18:0	8.0±0.9	7.9±1.1	8.0±0.6	8.2±1.2
20:4ω6,9,12,15	0.9±0.2	1.7±0.3	2.0±0.6	0.9±0.7
20:5ω3,6,9,12,15	0.1±0.1 a	0.7±0.2 ab	1.0±0.3 b	0.4±0.3 ab

Marker FAs are in bold. Means within a row sharing the same letter or not marked by letters are not significantly different from each other (Tukey´s HSD test, P<0.05) -: not detected or trace amount.

Table 3
Neutral lipid fatty acid pattern (means in % of total FAs ± s.d.) of the Collembola *Heteromurus nitidus* 0, 1, 7 and 14 days after switching diet from fungi (*Chaetomium globosum*) to bacteria (*Stenotrophomonas maltophilia*).

days after switching diet	0	1	7	14
14:0	1.5±0.2	1.2±0.1	1.3±0.1	1.3±0.1
i15:0	**- a**	**0.1±0.2 ab**	**0.1±0.1 ab**	**0.5±0.2 b**
a15:0	**0.1±0**	**0.2±0.1**	**0.1±0.1**	**0.4±0.2**
15:0	0.6±0.1	0.4±0.2	0.5±0	0.4±0.1
16:1ω7	2.5±0.1	2.1±0.6	2.6±0.4	2.6±0.1
16:0	18.5±0.5	17.0±1.6	18.6±0.5	17.8±0.5
i 17:0	0.6±0.2	0.3±0.3	0.4±0	0.3±0.1
a 17:0	0.6±0.2	0.5±0.3	0.6±0.1	0.5±0.2
17:0	0.2±0.1	0.2±0.1	0.2±0.1	0.2±0.1
18:2ω6,9	**22.9±3.6**	**19.1±4.4**	**21.4±1.0**	**19.8±1.9**
18:1ω9	39.6±4.2	45.0±6.3	40.2±2.1	41.4±3.7
18:1ω7	3.2±1.3	4.6±0.7	3.9±0.6	4.7±0.3
18:0	6.0±0.3	6.9±1.1	6.7±0.5	6.8±0.3
20:4ω6,9,12,15	2.4±0.7	1.8±0.6	2.4±0.1	2.3±0.3
20:5ω3,6,9,12,15	0.7±0.2	0.5±0.3	0.8±0.1	0.8±0.2

Marker FAs are in bold. Means within a row sharing the same letter or not marked by letters are not significantly different from each other (Tukey´s HSD test, P<0.05) -: not detected or trace amount.

Table 4
Neutral lipid fatty acid pattern (means in % of total FAs ± s.d.) of the Collembola *Heteromurus nitidus* 0, 1, 7 and 14 days after dietary switch from bacteria (*Stenotrophomonas maltophilia*) to fungi *(Chaetomium globosum*).

days after switching diet	0	1	7	14
14:0	1.1±0.2	0.9±0.7	1.6±0.4	1.6±0
i15:0	**2.8±0.7**	**0.8±1.3**	**2.4±2.3**	**0.7±0.4**
a15:0	**1.6±0.4**	**0.6±1.0**	**1.4±1.3**	**0.5±0.3**
15:0	**- a**	**- a**	**0.2±0.1 b**	**0.7±0.2 c**
i16:0	-	-	-	-
16:1ω7	2.2±0.3 a	0.6±0.8 b	1.9±0.5 ab	1.5±0.2 ab
16:0	17.0±1.1	18.0±2.5	19.3±0.9	19.3±1.1
i 17:0	-	-	-	0.1±0.1
a 17:0	- a	- a	0.1±0.1 a	0.3±0.2 b
17:0	-	-	0.6±1.0	0.2±0.1
18:2ω69	**1.4±0.4 a**	**5.8±1.6 b**	**12.8±2.4 c**	**18.6±0.4 d**
18:1ω9	41.5±0.9	43.5±4.0	39.9±1.3	41.7±2.1
18:1ω7	**19.3±3.5 a**	**19.3±6.8 a**	**8.4±3.3 b**	**3.0±0.6 b**
18:0	9.8±0.3 a	9.1±1.2 a	8.2±0.9 b	7.9±0.2 b
20:4ω6,9,12,15	1.9±0.3 ab	0.9±0.5 a	2.4±0.3 b	3.0±0.7 b
20:5ω3,6,9,12,15	1.4±0.1	0.5±0.4	0.7±0.2	0.8±0.4

Marker FAs are in bold. Means within a row sharing the same letter or not marked by letters are not significantly different from each other (Tukey´s HSD test, P<0.05) -: not detected or trace amount.

Table 5
Neutral lipid fatty acid pattern (means in % of total FAs ± s.d.) of the Collembola *Heteromurus nitidus* 0, 1, 7 and 14 days after dietary switch from bacteria (*Bacillus amyloliquefaciens*) to fungi (*Chaetomium globosum*).

days after switching diet	0	1	7	14
14:0	0.8±0.7	1.3±0.3	1.8±0.1	2.2±0.3
i15:0	**3.0±1.8 a**	**3.3±1.0 ab**	**1.9±0.7 b**	**0.9±0.4 b**
a15:0	**18.8±4.3 a**	**12.6±2.7 ab**	**4.1±3.7 b**	**3.7±1.8 b**
15:0	**- a**	**0.1±0.1 a**	**0.5±0.1 b**	**0.9±0.1 b**
i16:0	0.1±0.1 a	0.1±0.2 a	- b	- b
16:1ω7	1.0±0.9	2.0±1.1	1.7±0.2	1.9±0.4
16:0	17.9±3.7	16.0±0.4	17.8±0.9	13.2±9.5
i 17:0	1.2±1.1	0.9±0.6	0.7±0.3	0.3±0.1
a 17:0	**7.1±1.7 a**	**6.3±1.0 a**	**3.2±0.9 b**	**1.9±0.5 b**
17:0	-	-	-	0.2±0.3
18:2ω6,9	**2.8±4.0 a**	**7.7±4.2 ab**	**16.8±1.8 b**	**22.1±3.4 b**
18:1ω9	30.9±4.5	30.7±3.0	36.7±0.8	39.3±6.3
18:1ω7	4.4±3.2	5.8±0.2	4.3±0.4	3.6±0.5
18:0	9.8±2.4	8.2±1.8	6.1±0.6	5.7±1.1
20:4ω6,9,12,15	1.3±1.6	2.9±0.6	3.2±0.1	3.1±0.1
20:5ω3,6,9,12,15	0.8±1.0	1.9±0.5	1.2±0.1	1.0±0.2

Marker FAs are in bold. Means within a row sharing the same letter or not marked with letters are not significantly different from each other (Tukey´s HSD test, $P<0.05$) -: not detected or trace amount.

Table 6
Neutral lipid fatty acid pattern (means in % of total FAs ± s.d.) of the Collembola *Heteromurus nitidus* fed with the bacteria *Bacillus amyloliquefaciens* or *Stenotrophomonas maltophilia* before and after starvation for 14 days.

diet	*B. amyloliquefaciens*		*S. maltophila*	
days of starvation	0	14	0	14
14:0	0.8±0.7	0.9±0.1	1.1±0.2	1.4±0.7
i15:0	**3.0±1.8**	**1.8±1.1**	**2.8±0.7**	**3.2±4.2**
a15:0	**18.8±4.3 a**	**8.5±3.7 b**	**1.6±0.4**	**2.4±2.3**
15:0	-	-	-	-
i16:0	0.1±0.1	-	-	-
16:1ω7	1.0±0.9	1.1±0.1	2.2±0.3	2.2±1.5
16:0	17.9±3.7	15.6±1.0	17.0±1.1	18.5±0.6
i 17:0	1.2±1.1	0.5±0.6	-	0.3±0.5
a 17:0	**7.1±1.7**	**4.2±2.2**	-	**0.1±0.1**
17:0	-	-	-	-
18:2ω6,9	2.8±4.0	2.2±0.3	1.4±0.4	1.9±0.8
18:1ω9	**30.9±4.5 a**	**47.7±4.3 b**	**41.5±0.9 a**	**52.6±10.3 b**
18:1ω7	4.4±3.2	1.9±0.1	19.3±3.5	2.8±1.6
18:0	9.8±2.4	12.2±1.5	9.8±0.3	12.1±2.8
20:4ω6,9,12,15	1.3±1.6	1.3±0.3	1.9±0.3	0.4±0.8
20:5ω3,6,9,12,15	0.8±1.0	1.1±0.3	1.4±0.1	0.2±0.4

Marker FAs are in bold. Means within a row sharing the same letter or not marked with letters are not significantly different from each other (Tukey´s HSD test, $P<0.05$) -: not detected or trace amount.

4. Discussion

FA patterns of animals were proposed as marker of food sources in the field (Ruess et al., 2004, 2005a; Chamberlain et al., 2005; Haubert et al., 2006). However, for successfully applying the method to field populations information on the incorporation and turnover of FAs in consumers is needed. Chamberlain et al. (2004) investigated the time span needed to replace the carbon in FAs of Collembola. They observed a half-life for carbon turnover in FAs between 1.5 and 5.8 days. We determined how long specific marker FAs can be detected after diet switch or starvation and investigated the time span until they can be detected and used as indicator for a specific food source. In Collembola two kinds of FA markers have been defined: (1) absolute markers that the consumer cannot synthesise and that only occur in the lipid pattern when feeding on a certain diet, and (2) relative markers which can be derived from the lipid metabolism of the consumer, but are incorporated to a large extent from the diet (Chamberlain et al., 2005; Haubert et al., 2006; Ruess et al., 2004, 2005a). In our experiment we assigned 18:2ω6,9 as a relative fungal marker (Ruess et al., 2002; Haubert et al., 2004). The FA 15:0 also only occurred in fungal feeding Collembola in our experiment, but has been detected in Collembola feeding on different food sources in previous experiments (Haubert et al., 2006; Ruess et al., 2002, 2004). Relative marker FAs for *T. europaea* were 18:1ω9 and 18:2ω6,9 (Ruess et al., 2005b). Bacteria contain typical FAs which cannot be synthesised by animals. Therefore, absolute markers for bacterial diets could be determined (Haubert et al., 2006). The FAs i15:0 and a15:0 have been proposed as markers for a bacterial diet and only occurred in Collembola fed with *S. maltophilia* or B. amyloliquefaciens, with a much higher abundance in the latter (18.8%). Additionally, in our experiment a17:0 was a marker for *B. amyloliquefaciens* and 18:1ω7 for *S. maltophilia* even though it was not detected in *S. maltophilia* itself.

Switching diet generally led to a decrease in marker FAs of the previous food source and an increase in marker FAs of the present food source as indicated previously (Chamberlain et al., 2005). A switch from leaves of *T. europaea* to the bacterium *S. maltophilia* led to a decrease in typical leaf FAs 18:2ω6,9 (from 14.8% to 9.6%) and 18:1ω9 (from 50.7% to 45.7%), and to the occurrence of typical bacterial FAs including i15:0 (4.0%) and a15:0 (2.5%). These FAs were already detectable after one day. Despite the decrease in the leaf marker FAs 18:2ω6,9 and 18:1ω9, they were still more abundant than in Collembola fed only *S. maltophilia* for 8 weeks. This indicates that these markers can be used to trace consumption of leaf litter for longer than the two week period of the study. When switching diet from *C. globosum* to *S. maltophilia*, the fungal marker 18:2ω6,9 decreased, although this decreasewas not significant, and the bacterial marker FAs i15:0 and a15:0 increased slightly but significantly. Changes in FAs were less

pronounced than in the dietary switch from *T. europaea* to *S. maltophilia*, presumably because Collembola lost weight during the experiment. Although the signal was incorporated only to a small extent, FAs of *S. maltophilia* were detectable. Switching diet in the opposite direction, from *S. maltophilia* to *C. globosum*, led to a decrease in the FAs i15:0 and a15:0, however, both were still detectable after two weeks. The abundance of 18:2ω6,9 increased already after one day from 1.4% to 5.8% and reached 18.6% after two weeks. Similar observations were made by switching from *B. amyloliquefaciens* to *C. globosum*; 18:2ω6,9 increased from 2.8% to 22.1%, and i15:0, a15:0 and a17:0, typical for *B. amyloliquefaciens*, decreased, but were still detectable after two weeks. The results suggest that in general FAs typical for a specific diet are already present after one day and remain detectable for at least two weeks after switching to a different food source, documenting that FA analysis integrates the food consumed by Collembola over a longer period of time.

Although not all FAs from the food sources were incorporated unmodified into the body tissue of the consumer, in our experiment the marker FAs were sufficient to determine the specific food sources. Starvation of Collembola previously fed with the bacteria *B. amyloliquefaciens* or *S. maltophilia* for 14 days led to slight differences in FA composition. However, concentrations of characteristic bacterial FAs such as a15:0 and i15:0 were still high enough for detection and use as dietary markers.

Laboratory experiments necessarily are artificial in some respect and the fact that some Collembola lost weight during the study demonstrates that the offered diets may not be the preferred diet in the field. Nevertheless, the results present strong evidence that FAs provide reliable information on the diet of Collembola, even if they lose weight.

5. Conclusions

Fatty acids as biomarker for trophic relationships in soil food webs have become increasingly important over the last years. This study showed that they allow both the detection of the actual diet of consumers but also tracing the diet of the past. The method therefore provides an integrative measure of the diet of consumers and complements other measures such as stable isotope, microscopic and molecular gut content analysis. Compared to stable isotope analysis it allows more detailed insight into the role of major components of the diet of decomposer taxa such as bacteria and fungi; compared to molecular gut content analysis it provides more time integrated information on dietary components which have been assimilated rather than just consumed. FA analysis therefore is a promising tool for shedding light on trophic interactions in belowground food webs and their interrelationship with the aboveground system.

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Chapter 5

Carbon flux through fungi and bacteria into the forest soil animal food web as indicated by compound specific ^{13}C fatty acid analysis

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Summary

1. Soil food webs are compartmentalized and comprise of major energy channels, such as the plant litter, fungal and bacterial channel. The relative contributions of basal resources of these channels for nutrition of higher trophic levels are largely unknown.
2. The study took advantage of the Swiss Canopy Crane Project, where a mature temperate forest was labelled with ^{13}C-depleted CO_2. By exchanging leaf litter between the $^{13}CO_2$-enriched area and an unlabelled control area, we were able to separate carbon fluxes originating from leaf litter and roots, respectively.
3. Fatty acid composition and $\delta^{13}C$ signatures of individual fatty acids of basal resources and soil animals, including decomposers and predators, were analysed. Marker fatty acids allowed differentiating the contribution of plant litter, fungi and bacteria to the nutrition of higher trophic levels.
4. Our findings suggest that root derived carbon is of major importance for the soil animal food web and that it enters the soil animal food web mainly via feeding on ectomycorrhizal fungi.
5. In contrast to previous studies that emphasize the dominance of the fungal energy channel, significant amounts of bacterial fatty acids in each of the predator species studied indicate that the bacterial energy channel is considerably more important for the nutrition of higher trophic levels than previously assumed. The distribution of energy between the two channels presumably contributes to food web stability.
6. Compound specific ^{13}C fatty acid analysis of basal resources and soil animals allowed to separate energy fluxes originating from ectomycorrhizal vs. saprotrophic fungi, from bacteria feeding on leaf litter vs. bacteria feeding on root exudates, and from leaf litter itself, providing information on the partitioning of these different energy channels in unprecedented detail.

Key-words: bacterial energy channel, basal resources, carbon fluxes, CO_2 labelling, decomposers, food web stability, fungal energy channel, predators, resource partitioning

1. Introduction

Soil animal food webs in forests are based on different resources such as leaf litter, roots, fungi and bacteria. It is difficult to disentangle the use of these resources and their relative importance, since the opaqueness of the habitat prevents direct observation of feeding. Methods used to infer trophic relationships circumventing these difficulties include stable isotope analysis, molecular gut content analysis and fatty acid (FA) analysis, each having specific advantages and limitations. Stable isotope analysis provides a time-integrated measure of trophic niches, but interpretation of the data is hampered by different degrees of fractionation, missing signatures of specific basal resources such as ectomycorrhizal fungi or bacteria (Pollierer et al., 2009), or similar isotopic signatures of different resources. Molecular gut content analysis has the potential to uncover the actual diet of an animal, but represents a snapshot technique that is not quantitative. Without specific primers, prey cannot be detected; you only find what you're looking for (Harper et al., 2006). Other problems include scavenging and secondary predation (King et al., 2008). Fatty acid analysis has the advantage of providing specific and relative markers for bacteria and fungi (Ruess & Chamberlain, 2010), allowing to separate fundamentally different basal resources and the trophic chains they support i.e., the bacterial and fungal energy channel in soils (Moore et al., 2005). Additionally, the ratio of plant and fungal markers has been used to separate primary decomposers feeding on plant litter from secondary decomposers predominantly living on a fungal diet (Ruess et al., 2007; Ngosong et al., 2009). Recently, it has been shown that marker FAs are transferred to higher trophic levels (Ruess et al., 2004; Pollierer et al., 2010), rendering possible the analysis of whole food webs including predatory taxa using FA analysis.

Stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analyses suggest that soil animal food webs are compartmentalized (Pollierer et al., 2009). For predators litter feeding species, such as millipedes, likely are unsuitable prey due to large size and/or strong sclerotization, whereas fungal feeding species, such as springtails, substantially contribute to predator nutrition. Generally, there is increasing evidence that root derived carbon is of major importance for soil animal food webs (Albers et al., 2006; Ruf et al., 2006; Pollierer et al. 2007). However, the specific way by which carbon enters the soil food web still remains largely unknown. It may either be taken up by root feeding, by feeding on bacteria living on root exudates, or by feeding on ectomycorrhizal fungi that acquire carbon from tree roots. Compound specific FA analysis, where $\delta^{13}C$ values of individual fatty acids are measured, has the potential of distinguishing between these pathways since the isotopic signal of labelled leaf litter or labelled root derived carbon can be detected in individual fungal or bacterial marker FAs. Their relative contribution to the diet of animals consuming these microorganisms then can be traced by comparing the relative abundance of fungal

vs. bacterial marker FAs in their tissue. The bacterial and fungal energy channels process organic matter in independent ways. Since bacteria and fungi and their respective consumers have different turnover rates, the bacterial energy channel is considered fast cycle whereas the fungal energy channel is considerd slow cycle (Coleman et al., 1983). The bacterial energy channel processes labile organic matter and has been found to dominate in fertile and productive ecosystems such as grasslands. The more resistant fractions of organic matter are processed predominantly by the fungal energy channel which is favoured in infertile and unproductive ecosystems with litter containing low amounts of nitrogen and high amounts of lignin and other structural compounds (Wardle et al., 2004). Therefore, in forest soil food webs the fungal energy channel is likely to dominate. It has been assumed that compared to the fungal energy channel the bacterial food chain is short (Scheu and Setälä, 2002), supporting mainly bacteriovorous nematodes and mites preying upon them (Hunt et al., 1987). The majority of secondary decomposers such as oribatid mites and collembolans are thought to mainly feed on fungi (Scheu and Falca, 2000). Since they constitute the major prey for larger predators such as centipedes and spiders the fungal energy channel appears to be most important for forest soil animal food webs.

In the Swiss Canopy Crane CO_2 enrichment project (Körner et al., 2005) a mature temperate forest was labelled with CO_2 depleted in ^{13}C, allowing to trace the flux of carbon from tree crowns into the soil animal food web via leaves or roots. As in the study of Pollierer et al. (2007), we mutually exchanged leaf litter from the labelled site and an adjacent unlabelled site, allowing to separate the flux of leaf and root derived carbon. However, rather than analysing bulk isotopic signatures of soil animals as done previously (Pollierer et al., 2007), we analysed $\delta^{13}C$ values of individual fatty acids in soil animals and basal resources (leaf litter, roots and soil), allowing to separate energy channels based on ectomycorrhizal (root derived carbon) or saprotrophic (litter derived carbon) fungi, and bacteria acquiring their carbon resources from root exudates or litter. We hypothesized that root derived carbon forms the main carbon source for the soil animal food web and that it enters the food web mainly via feeding on ectomycorrhizal fungi.

2. Materials and methods

2.1 Study site and CO_2 enrichment

The study site forms part of the Swiss Canopy Crane (SCC) CO_2 enrichment project and is located in a mature temperate forest near Basel, Switzerland (47°28´ N, 7°30´ E, 550 m a.s.l.). The plant community is dominated by beech (*Fagus sylvatica* L.), oak (*Quercus petraea* Mattuschka) and hornbeam (*Carpinus betulus* L.), but also includes lime (*Tilia platyphylla* Scop.), wild cherry (*Prunus avium* L.) and maple (*Acer campestre* L.). Trees are 80 to 120 years old and 30-35 m high and have a basal area of about 46 m^2 ha^{-1}. Details on the experiment and the experimental site can be found in Pepin & Körner (2002) and Steinmann et al. (2004). The climate is temperate with an annual precipitation of 990 mm and the soil is a mesophilic Rendzina (pH 5.8).

The web-FACE (Free Air CO_2 Enrichment) technique ensures that FACE is restricted to the tree crowns (Pepin and Körner, 2002; Fig. 1), therefore allowing isotopic tracing from the tree crowns via roots to the soil animal food web without interfering effects of understorey vegetation and air above soil (Keel et al., 2006). The CO_2 used in this experiment was of fossil origin and therefore strongly depleted in ^{13}C ($\delta^{13}C$ of -29.7 ± 0.3 ‰ *vs*. -8 ‰ in ambient air; Keel et al., 2006). Pulses of pure CO_2 were released in the tree crowns through punctured irrigation tubes, which were installed by a 40 m tall canopy crane. An elevated concentration of 550 ppm (compared to 370 ppm in ambient air) was constantly maintained during daylight hours throughout the growing season.

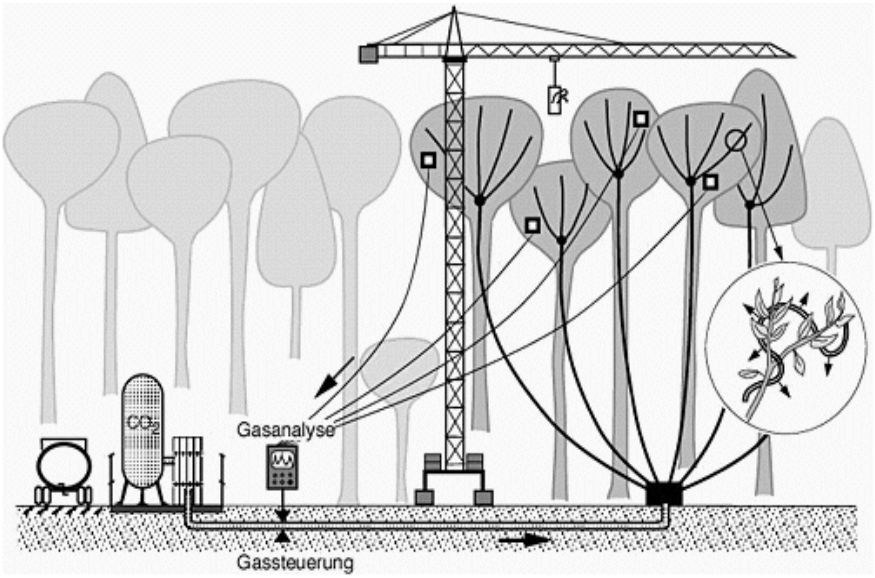

Fig. 1 Schematic setup of the canopy crane and web-FACE (source: http://pages.unibas.ch/botschoen/scc/2.gif)

2.2 Experimental design

Labelled leaf litter was harvested in November 2006 directly from labelled trees by hand-picking from the canopy crane. Unlabelled leaf litter was harvested from outside the canopy crane area. In April 2007 six circular plots with a diameter of 50 cm (Fig. 2) were set up under beech trees in the SCC CO_2 enrichment area. Six control plots were set up c. 50 m away from the enriched area. The plots were surrounded by plastic fences dug 5 cm into the soil and covered by netting to prevent litter from falling in. Three plots per area were filled with 150 g dry wt of labelled beech leaf litter and three plots with unlabelled beech leaf litter, in a way that four different treatments with three replicates each were obtained: (i) both leaf litter and roots labelled, (ii) only leaf litter labelled, (iii) only roots labelled and (iv) unlabelled control.

In October 2007 leaf litter and the top 5 cm of soil were taken from each of the plots. Animals were extracted alive into water using a high gradient extractor for two weeks (Macfadyen, 1961; Kempson et al., 1963). They were collected daily, determined with a dissecting microscope and frozen until analysis. Separate samples of leaf litter and soil were frozen at -20ºC for extraction of whole cellular lipids and PLFAs, respectively. Fine roots were picked by hand and also frozen until extraction of whole cellular lipids. Earthworms were extracted from each plot using the electrical octet method (Thielemann, 1986; Eisenhauer et al., 2008b).

Fig. 2 One of the experimental plots surrounded by plastic fencing and covered by netting.

2.3 Analysis of fatty acids

Phospholipid fatty acids (PLFAs) were extracted from soil and fractionated as described in Frostegård et al. (1993). From animals fatty acids were extracted as described in Zelles (1999) and Haubert et al. (2004). Neutral lipid fatty acid (NLFA) fractions of all investigated animals, whole cellular FAs of leaves and roots and PLFAs from soil were saponified and methylated following the procedures given for the Sherlock Microbial Identification System (MIDI Inc., Newark, USA; see Ruess et al., 2002) The lipid containing phase was then transferred to test tubes and stored at -20˚ C until analysis.

2.4 Analysis of $^{13}C/^{12}C$ ratios of fatty acids

The isotopic composition of individual FAs in basal resources and animals was determined using a Thermo Finnigan Trace GC coupled via a GP interface to a Delta Plus mass spectrometer (Finnigan, Bremen, Germany). The GC was equipped with a fused silica capillary column (Phenomenex Zebron ZB-5, 30 m, 0.32 mm i.d., film thickness 0.25 µm, Torrance, CA). The temperature program started with 60˚C (held for 1 min) and increased by 6˚C per minute to 310˚C and held for 15 min at 310˚C. The injection temperature was 250˚C and helium was used as carrier gas. The flow rate of helium was 2.2 ml/min. FAMEs were identified by chromatographic retention time comparison with standard mixtures composed of 37 different FAMEs (Fatty Acid Methyl Esters) ranging from C11 to C24 and 26 BAMEs (Bacterial Fatty Acid Methyl Esters; Sigma Aldrich, St Louis, USA). To verify correct identification of the peaks some samples were analyzed using GC–MS (Varian CP-3800 chromatograph coupled to a 1200L mass spectrometer). The GC-MS was equipped with a fused silica column (Phenomenex Zebron ZB-5MS, 30 m, 0.25 µm film thickness, i.d. 0.32 mm). The carbon isotope composition is reported in δ notation (‰) relative to Vienna Pee Dee Belemnite standard (V-PDB) as $\delta^{13}C$ [‰] = $(({}^{13}C/{}^{12}C)_{sample}/({}^{13}C/{}^{12}C)_{standard} - 1) * 1000$. To obtain isotope ratios of the FAs, measured isotope ratios of FAMEs were corrected for the isotope ratio of the methyl moiety originating from methanol using the formula $\delta^{13}C_{FA} = [(C_n + 1) * \delta^{13}C_{FAME} - \delta^{13}C_{MeOH}]/C_n$, with $\delta^{13}C_{FA}$ the $\delta^{13}C$ of the fatty acid, C_n the number of carbons in the fatty acid, $\delta^{13}C_{FAME}$ the $\delta^{13}C$ of the fatty acid methyl ester, and $\delta^{13}C_{MeOH}$ the $\delta^{13}C$ of the methanol (-38,8‰) used for methylation (Abraham et al., 1998).

2.5 Statistical analysis

FA profiles of soil animals were arcsine transformed and analyzed by discriminant function analysis (DFA). FA $\delta^{13}C$ signatures of leaf litter and fine roots were also analyzed using DFA. Where necessary, dimensions were reduced by non-metric multidimensional scaling (NMDS). DFA and NMDS were carried out using STATISTICA 7.0 for Windows (StatSoft, Tulsa, USA, 2001).

Differences in $\delta^{13}C$ signatures of individual FAs in basal resources and soil animals were analysed using Type IV General Linear Model (GLM). GLMs were performed using SAS 9.2 (SAS Institute, Cary, NC, USA).

3. Results

3.1 FA composition of basal resources

In leaf litter, the most abundant FAs were 22:0 (16.4 ± 11.9%), 16:0 (16.0 ± 11.5%), 18:1ω9 (11.9 ± 8.2%), 18:3ω3 (10.3 ± 12.8%) and 24:0 (8.6 ± 8.4%). Bacterial FAs only had low abundances (4.4 ± 5.1%). The ratio between the relative plant (18:1ω9) and fungal marker FA (18:2ω6) was 1.8 ± 1.6. In soil, the most abundant PLFAs were 18:1ω7 (16.4 ± 1.4%), 18:2ω6 (11.7 ± 2.3%), 16:0 (10.2 ± 0.5%) and 18:1ω9 (9.2 ± 1.1%). Bacterial FAs, including the specific bacterial markers i15:0, a15:0, i16:0, i17:0, a17:0, cy17:0 and cy19:0, the vaccenic type FAs 16:1ω7 and 18:1ω7 and the hydroxy substituted FA 2-OH 12:0, made up half of all PLFAs in soil (49.6 ± 2.4%). Fine roots only contained seven different FAs, with the most abundant being 18:2ω6 (36.4 ± 3.6%), 16:0 (25.8 ± 3.5%), 18:1ω9 (12.3 ± 3.4%) and 18:3ω3 (12.1 ± 4.8%). For details on FA composition of basal resources see Figure S1 in Supporting Information.

3.2 Labelling of basal resources

$\delta^{13}C$ signatures of leaf litter FAs differed significantly between the four treatments (i) leaf litter and roots labelled, (ii) only leaf litter labelled, (iii) only roots labelled and (iv) unlabelled control (DFA after multidimensional scaling; $F_{15,11} = 3.6$, $P < 0.019$). Individual FAs were depleted in ^{13}C by up to 12‰ in treatments where leaf litter was labelled. Mean depletion was 6.8‰ (SD = 2.7) when both leaf litter and soil were labelled and 5.5‰ (SD = 3.1) when only leaf litter was labelled, whereas FA $\delta^{13}C$ signatures in treatments where only soil was labelled were similar to the unlabelled control (Fig. 3a). For FA $\delta^{13}C$ signatures of soil, no statistical analysis could be performed due to insufficient number of replicates for the treatment with only leaf litter labelled. However, $\delta^{13}C$ signatures of FAs were depleted by 2.2‰ (SD = 1.1) and 2.1‰ (SD = 0.7), respectively, in treatments where soil and leaf litter and where only soil was labelled. When only leaf litter was labelled, soil FAs were not depleted compared to the unlabelled treatment (mean difference 0.2‰ ± 0.5; Fig. 3b).

$\delta^{13}C$ signatures of FAs of fine roots differed significantly between the four treatments (DFA; $F_{15,11} = 3.7$, $P < 0.017$). The FAs 16:0, 18:2ω6,9c and 18:3ω3 were significantly depleted in $\delta^{13}C$ in the leaf litter and roots labelled, and in the roots labelled treatment (between 2.6 and 3.9‰), whereas $\delta^{13}C$ signatures in the treatment with only leaf litter labelled were similar to the unlabelled control (Fig. 3c).

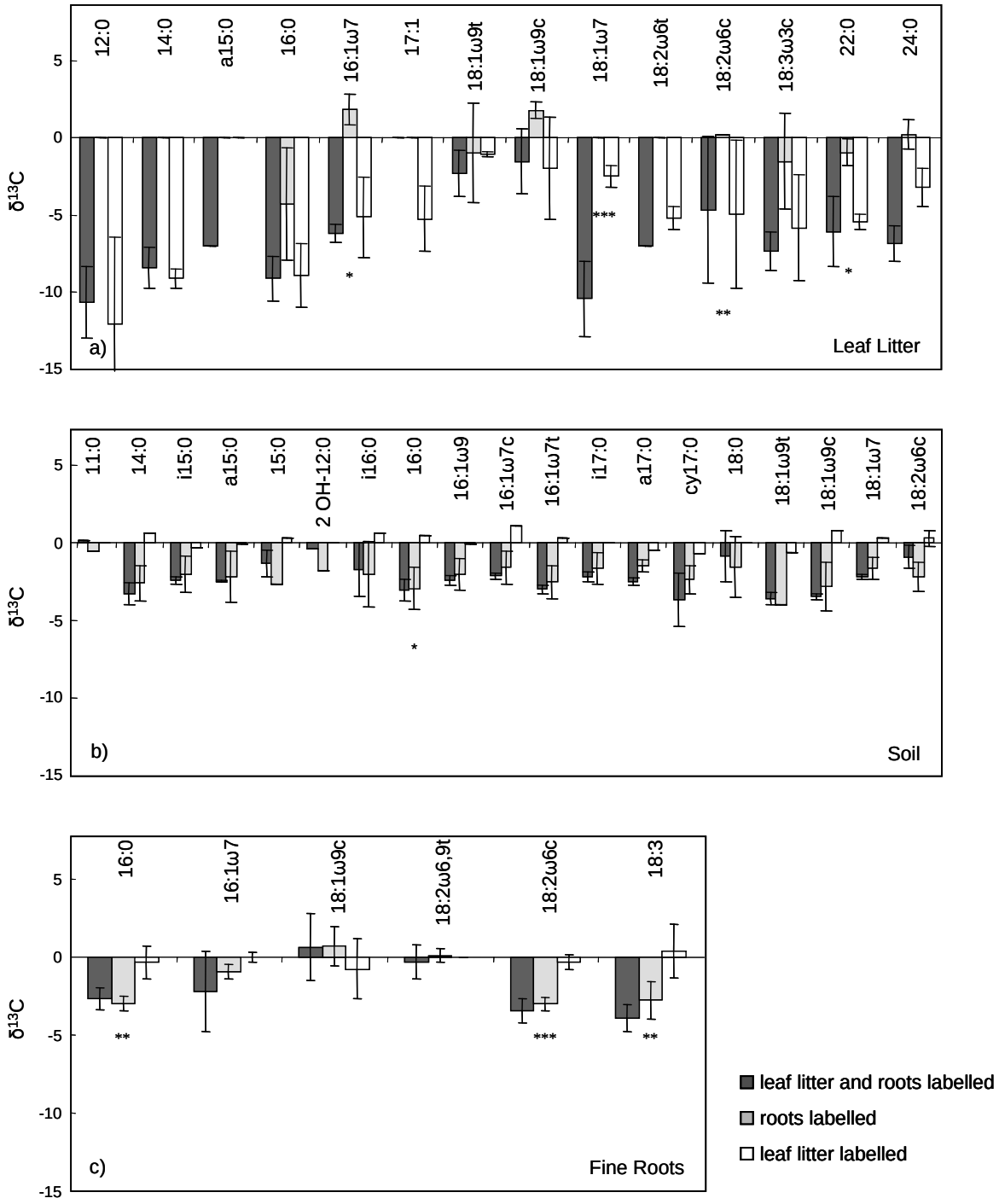

Fig. 3 Differences in $\delta^{13}C$ signatures of individual fatty acids between the control and treatments with leaf litter and roots labelled, roots labelled and leaf litter labelled for the basal resources (a) leaf litter, (b) soil and (c) fine roots. Data are presented as mean ± SD (bars without SD are single measurements). *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$

3.3 FA composition of soil animals

Soil animals differed significantly in their FA composition (DFA; $F_{210,775}$ = 18.05, P < 0.0001; Fig. 4, Table 1). The investigated oribatid mites, except *Hypochthonius rufulus* C.L. Koch, had a similar FA composition, with the highest amounts of plant and fungal specific FAs (18:1ω9, 18:2ω6, 18:3ω3) of all investigated animals (up to ~80 % of total FAs; Table 2). In *Achipteria coleoptrata* (Linnaeus), *Steganacarus magnus* (Nicolet) and *Platynothrus peltifer* (C.L. Koch) the ratio between the relative plant (18:1ω9) and fungal marker FA (18:2ω6) was high, whereas this ratio was low in *Euzetes globulus* (Nicolet; Table 2). Bacterial FAs, including specific bacterial markers and vaccenic type FAs (16:1ω7 and 18:1ω7), made up 5.3 to 10.5% of total FAs in oribatid mites (excluding *H. rufulus*; Table 2).

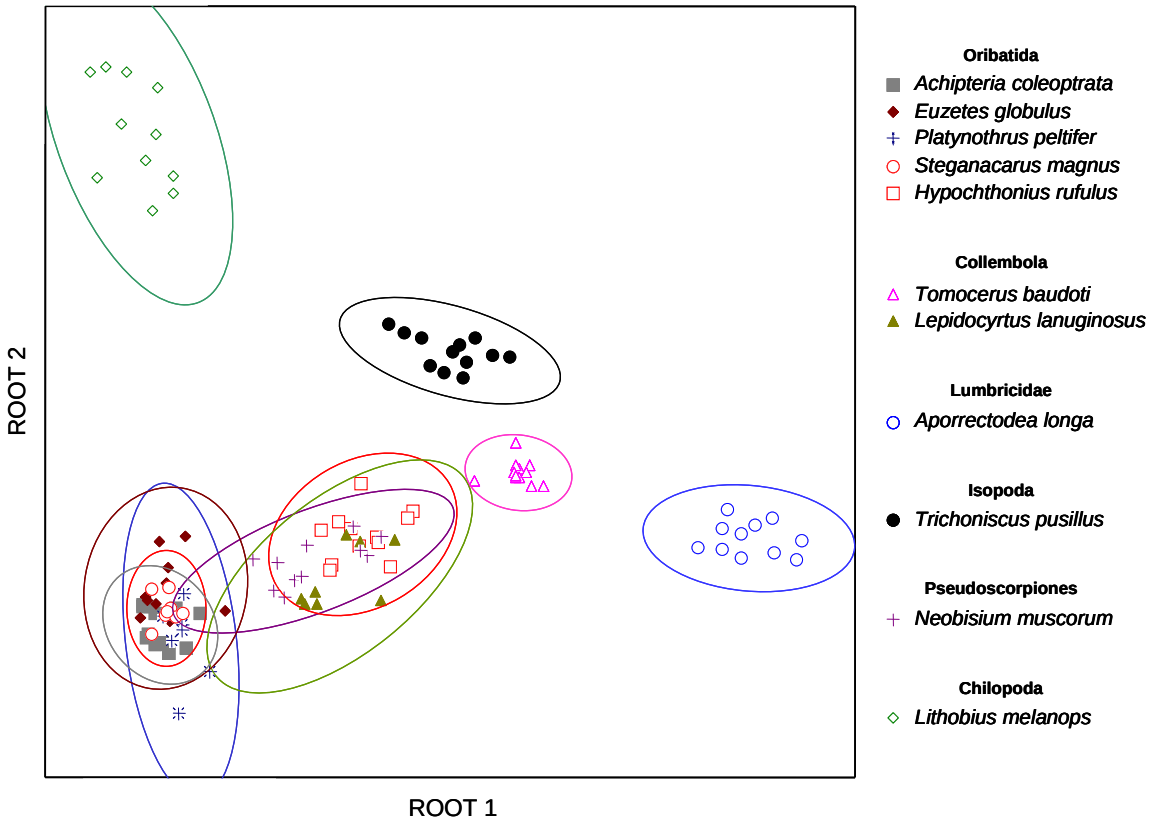

Fig. 4 Discriminant function analysis of the NLFA composition of soil animals (Oribatida, Collembola, Lumbricidae, Isopoda, Pseudoscorpiones and Chilopoda). Ellipses represent confidence intervals at P = 0.05

Table 1
Squared Mahalanobis distances between group centroids and reliability of discrimination for NLFA composition of investigated soil animals.

	H. rufulus	*L. melanops*	*T. baudoti*	*T. pusillus*	*A. coleoptrata*	*E. globulus*	*L. lanuginosus*	*N. muscorum*	*P. peltifer*	*S. magnus*
A. longa	262.4***	683.8***	178.2***	236.9***	451.6***	436.0***	307.5***	296.3***	433.5***	454.2***
H. rufulus	-	329.2***	109.3***	117.0***	116.7***	91.9***	127.9***	95.5***	111.4***	91.7***
L. melanops		-	376.0***	246.2***	333.7***	287.2***	358.5***	308.5***	359.2***	315.9***
T. baudoti			-	93.4***	239.5***	221.6***	91.1***	89.5***	225.3***	213.5***
T. pusillus				-	232.1***	209.8***	187.6***	132.7***	230.4***	217.5***
A. coleoptrata					-	18.8***	119.6***	69.0***	8.1	23.1***
E. globulus						-	110.8***	68.4***	26.8***	10.1*
L. lanuginosus							-	32.3***	108.8***	98.2***
N. muscorum								-	66.7***	57.4***
P. peltifer									-	27.4***

*** p<0.0001, * p<0.05.

Table 2
Concentrations (means ± SD) of bacterial marker FAs (iso/anteiso methyl-branched FAs and FAs with a cyclopropyl ring), vaccenic type FAs (16:1ω7 and 18:1ω7), total bacterial FAs and plant/fungal specific FAs (18:1ω9, 18:2ω6 and 18:3ω3) and the ratio between the relative plant and fungal markers 18:1ω9 and 18:2ω6. Predators are underlined.

Species	Bacterial markers (%)	Species	Vaccenic type FAs (%)	Species	Bact. FAs total (%)	Species	18:1+18:2 +18:3 (%)	Species	18:1/18:2
Trichoniscus pusillus	0.44 ± 0.55	***A. coleoptrata***	0.15 ± 0.51	***S. magnus***	5.33 ± 3.27	***A. longa***	9.64 ± 1.30	***A. longa***	0.51 ± 0.41
Tomocerus baudoti	0.46 ± 0.61	***S. magnus***	0.6 ± 0.71	***N. muscorum***	5.88 ± 5.91	***L. lanuginosus***	39.31 ± 9.93	***L. melanops***	0.80 ± 0.29
Lithobius melanops	0.7 ± 1.17	***E. globulus***	1.16 ± 2.02	***E. globulus***	7.86 ± 7.97	***T. baudoti***	40.36 ± 7.17	***T. pusillus***	1.12 ± 0.38
Neobisium muscorum	3.06 ± 4.12	***L. lanuginosus***	1.54 ± 2.96	***L. lanuginosus***	8.75 ± 7.65	***H. rufulus***	51.16 ± 6.56	***E. globulus***	1.32 ± 0.53
Steganacarus magnus	4.73 ± 3.33	***P. peltifer***	2.01 ± 2.54	***L. melanops***	8.81 ± 4.40	***N. muscorum***	51.20 ± 10.08	***N. muscorum***	1.49 ± 0.35
Euzetes globulus	6.70 ± 6.25	***N. muscorum***	2.83 ± 2.93	***A. coleoptrata***	8.86 ± 6.12	***L. melanops***	53.79 ± 14.20	***L. lanuginosus***	1.49 ± 0.23
Lepidocyrtus lanuginosus	7.21 ± 7.39	***L. melanops***	8.11 ± 4.32	***P. peltifer***	10.52 ± 7.49	***T. pusillus***	55.71 ± 8.82	***A. coleoptrata***	1.74 ± 0.55
Hypochthonius rufulus	8.07 ± 4.41	***H. rufulus***	11.22 ± 2.61	***T. baudoti***	12.29 ± 1.56	***P. peltifer***	64.10 ± 12.42	***P. peltifer***	1.93 ± 0.59
Platynothrus peltifer	8.51 ± 8.45	***T. baudoti***	11.83 ± 1.60	***T. pusillus***	13.37 ± 3.04	***A. coleoptrata***	68.17 ± 9.71	***S. magnus***	1.95 ± 0.19
Achipteria coleoptrata	8.71 ± 6.14	***T. pusillus***	12.93 ± 2.84	***H. rufulus***	19.28 ± 6.18	***E. globulus***	77.38 ± 15.06	***H. rufulus***	2.30 ± 0.89
Aporrectodea longa	10.23 ± 2.59	***A. longa***	15.33 ± 1.83	***A. longa***	25.56 ± 2.30	***S. magnus***	79.3 ± 4.97	***T. baudoti***	2.66 ± 0.41

The Collembola species *Lepidocyrtus lanuginosus* (Gmelin) had similar amounts of bacterial FAs as oribatid mites and a similar ratio between the relative plant (18:1ω9) and fungal marker FA (18:2ω6). However, *L. lanuginosus* had only about half as much plant and fungal specific FAs as oribatid mites (Table 2). *Tomocerus baudoti* Denis contained low amounts of specific bacterial markers, but high amounts of vaccenic type (ω7) FAs. The amounts of plant and fungal specific FAs were similar to *L. lanuginosus*. The ratio between the relative plant (18:1ω9) and fungal marker FA (18:2ω6) was considerably higher in *T. baudoti* than in *L. lanuginosus* (Table 2).

The isopod species *Trichoniscus pusillus* Brandt contained low amounts of absolute bacterial marker FAs, but high amounts of vaccenic type FAs (Table 2). Amounts of plant and fungal specific FAs were intermediate. The ratio between the relative plant (18:1ω9) and fungal marker FA (18:2ω6) was low compared to oribatid mites and collembolans (Table 2).

The lumbricid earthworm species *Aporrectodea longa* (Ude) differed greatly in FA composition from the other investigated animals (Fig. 4, Table 1). While it contained the greatest amounts of total bacterial FAs, it had the lowest amounts of plant and fungal specific FAs. Further, *A. longa* had the lowest ratio between the relative plant (18:1ω9) and fungal marker FA (18:2ω6) (Table 2).

The three investigated predators *Lithobius melanops* Newport (Chilopoda), *Neobisium muscorum* (Leach) (Pseudoscorpionida) and *H. rufulus* (Oribatida) contained similar amounts of plant and fungal specific FAs each ca. 50% of total FAs. However, the ratio between the relative plant (18:1ω9) and fungal marker FA (18:2ω6) differed between the three species; it was low in *L. melanops* (0.80 ± 0.29), intermediate in *N. muscorum* (1.49 ± 0.35) and high in *H. rufulus* (2.30 ± 0.89). Further, *H. rufulus* contained high amounts of bacterial FAs while *N. muscorum* and *L. melanops* contained low amounts (Table 2). For details on FA composition of soil animals see Figure S1.

3.4 Labelling of soil animals

FA $\delta^{13}C$ signatures of the oribatid mite species *S. magnus*, *A. coleoptrata* and *P. peltifer* were generally more depleted in treatments where leaf litter was labelled (Fig. 5a,b,c). However, some FAs were also depleted in treatments where only roots were labelled e.g., 18:0 in *A. coleoptrata* (Fig. 5b, $F_{3,8}$ = 5.77, P = 0.04; see Table S2 for details). In *A. coleoptrata* the bacterial marker FA i16:0 was only significantly depleted when roots were labelled ($F_{3,6}$ = 13.04, P = 0.01). In *E. globulus*, the relative plant and fungal marker FAs 18:1ω9 and 18:2ω6 were only significantly depleted in roots labelled treatments ($F_{3,5}$ = 8.13, P = 0.04 and $F_{3,7}$ = 7.84, P = 0.03; Fig. 5d).

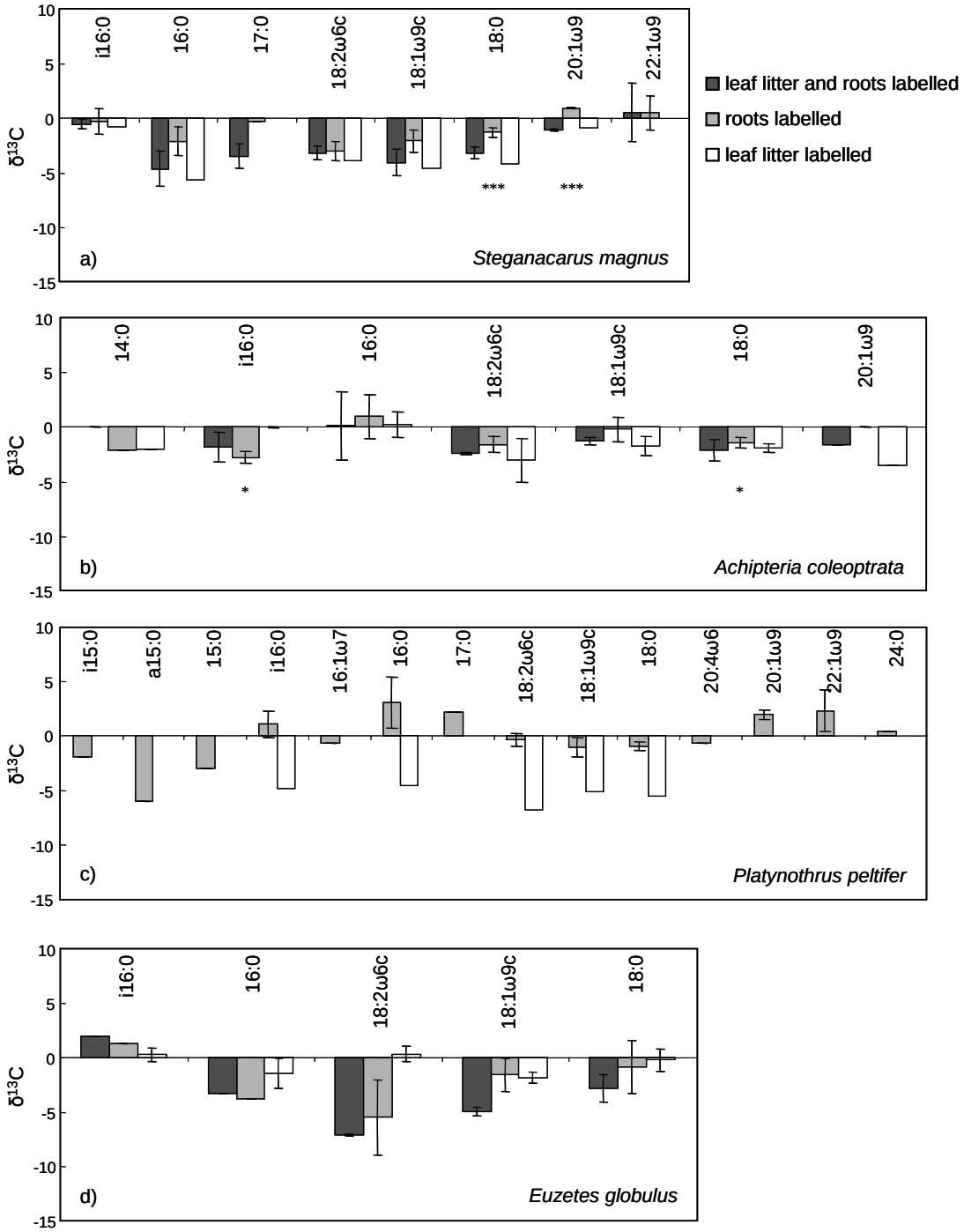

Fig. 5 Differences in $\delta^{13}C$ signatures of individual fatty acids between the control and treatments with leaf litter and roots labelled, roots labelled and leaf litter labelled for the oribatid mite species (a) *Steganacarus magnus*, (b) *Achipteria coleoptrata*, (c) *Platynothrus peltifer* and (d) *Euzetes globulus*. Data are presented as mean ± SD (bars without SD are single measurements). *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$

In the Collembola species *L. lanuginosus*, FAs were not labelled significantly (Fig. 6a). FAs of *T. baudoti* were most depleted in treatments where leaf litter was labelled (Fig. 6b). However, the fungal marker FA 18:2ω6, the vaccenic type FA 18:1ω7 and the FA 18:3ω3, occurring in fungi and plants, and the PUFAs 20:4 and 20:5 were also significantly depleted in the roots labelled treatment (see Table S2). Most FAs of the isopod species *T. pusillus* were significantly depleted when roots were labelled, whereas labelled leaf litter did not affect FA $\delta^{13}C$ signatures (Fig. 6c). Similarly, FAs of the lumbricid earthworm species *A. longa* were also only significantly depleted in treatments with labelled roots (Fig. 6d; see Table S2).

FAs of the three investigated predators, *N. muscorum*, *L. melanops* and *H. rufulus,* were most depleted in the leaf litter and roots labelled treatments. In treatments where only roots were labelled, FAs were more depleted than in treatments where only leaf litter was labelled (Fig. 7a,b,c). This was most pronounced in *L. melanops* where only three of ten FAs that were significantly affected by labelled roots were also significantly affected by labelled leaf litter; the FAs not affected by leaf litter included the relative plant and fungal markers 18:1ω9 and 18:2ω6. In *N. muscorum* one of the four significantly affected FAs was also influenced by labelled leaf litter. In *H. rufulus* a larger fraction of FAs (four out of six) which were significantly affected responded to both labelled leaf litter and labelled roots, including the relative plant and fungal markers 18:1ω9 and 18:2ω6 (see Table S2).

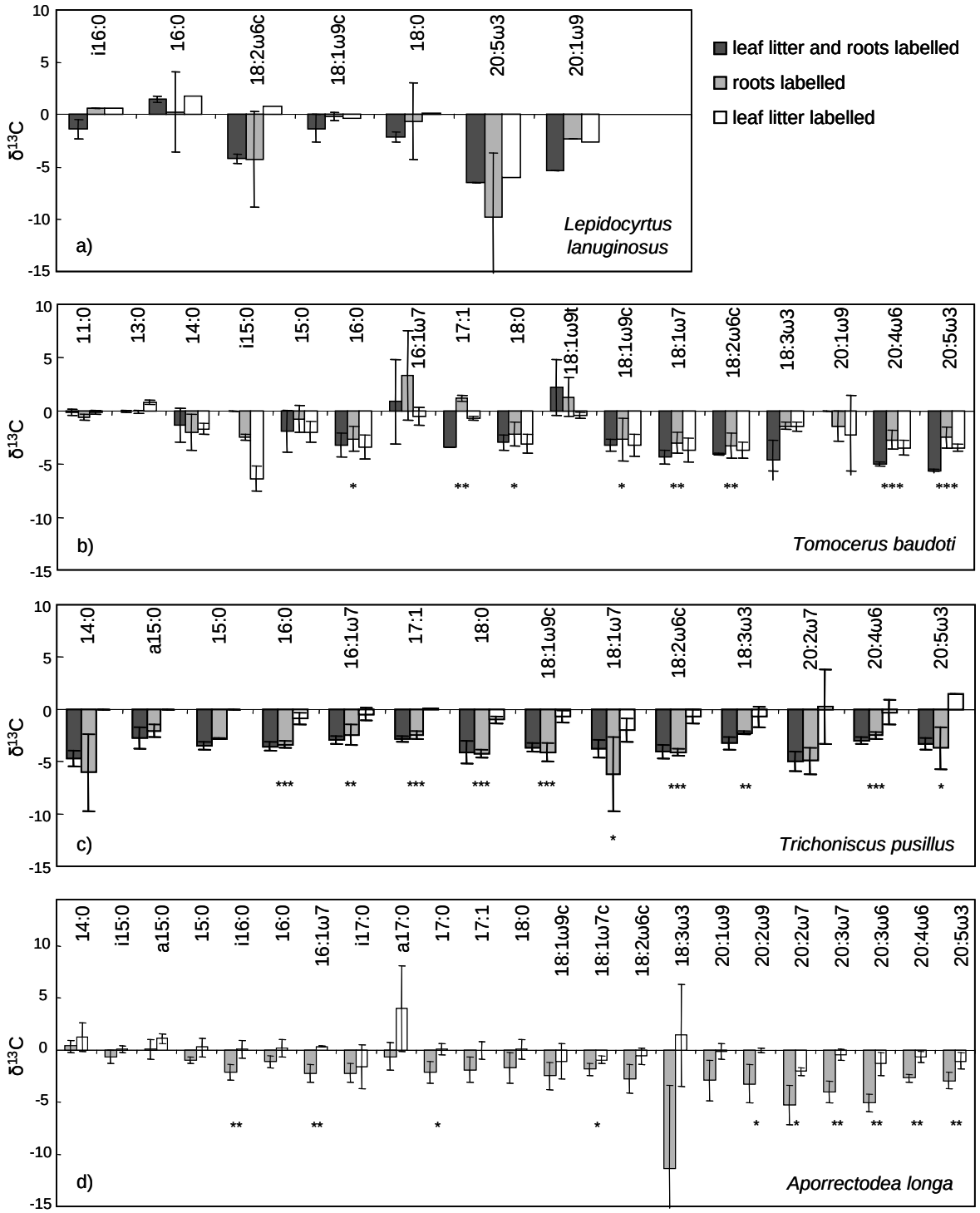

Fig. 6 Differences in $\delta^{13}C$ signatures of individual fatty acids between the control and treatments with leaf litter and roots labelled, roots labelled and leaf litter labelled for the Collembola species (a) *Lepidocyrtus lanuginosus* and (b) *Tomocerus baudoti*, (c) the isopod species *Trichoniscus pusillus*, and (d) the lumbricid earthworm species *Aporrectodea longa*. Data are presented as mean ± SD (bars without SD are single measurements). *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$

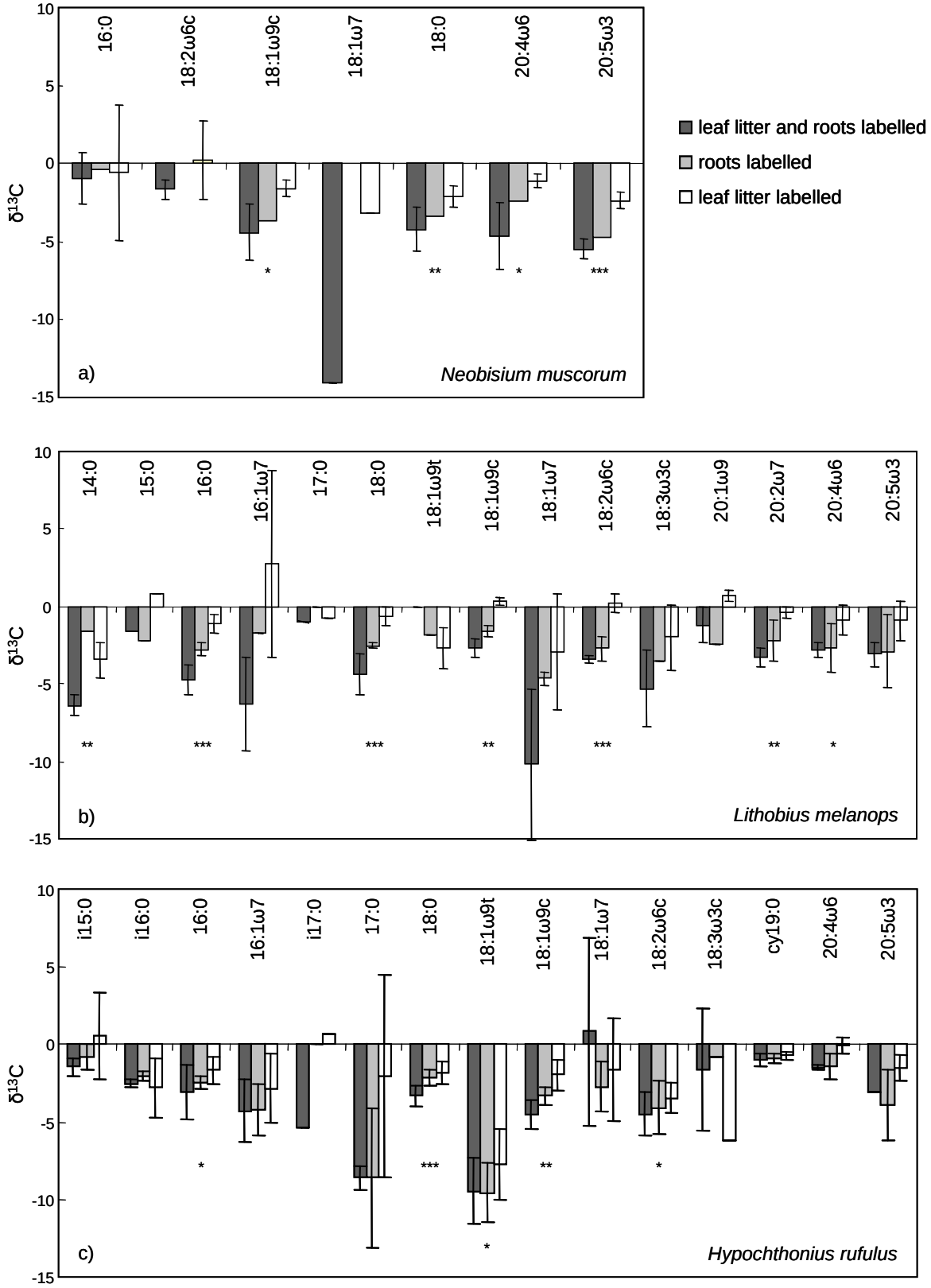

Fig. 7 Differences in $\delta^{13}C$ signatures of individual fatty acids between the control and treatments with leaf litter and roots labelled, roots labelled and leaf litter labelled for the predators (a) *Neobisium muscorum* (Pseudoscorpionida), (b) *Lithobius melanops* (Chilopoda) and (c) *Hypochthonius rufulus* (Oribatida). Data are presented as mean ± SD (bars without SD are single measurements). *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$

4. Discussion

4.1 FA composition and isotopic labelling of basal resources

Leaf litter mainly contained plant (22:0, 18:3ω3) and fungal (18:2ω6, 18:1ω9) derived FAs (Ruess et al., 2007), whereas abundances of bacterial FAs were low, suggesting that leaf litter was primarily colonized by saprotrophic fungi. In contrast, in soil bacterial and fungal marker PLFAs contributed ca. 50% and 20% of total of total PLFAs, respectively, suggesting that microorganisms in soil are dominated by bacteria. PLFAs in fine roots were dominated by the relative fungal marker 18:2ω6 indicating that they were heavily colonized by mycorrhizal fungi. Conform to our experimental setup FAs of leaf litter were strongly depleted in $\delta^{13}C$ in treatments where leaf litter was labelled. Conversely, FAs in soil were depleted in treatments where roots were labelled, however, the depletion was not as strong as in the treatment with labelled leaf litter (~2‰ as compared to ~6‰). PLFAs in fine roots were also significantly depleted in treatments where roots were labelled with the depletion being similar to that of soil PLFAs. The differential depletion in the labelled leaf litter and labelled roots treatment needs to be considered when interpreting $\delta^{13}C$ signatures of soil animals. As in beech forests leaf litter only plays a minor role for the nutrition of soil animals (Pollierer et al., 2007) the higher depletion may help to identify taxa that obtain at least some carbon from leaf litter.

4.2 FA composition and isotopic labelling of soil animals

The oribatid mites *P. peltifer*, *A. coleoptrata*, *S. magnus* and *E. globulus* had similar FA composition with high amounts of plant and fungal specific FAs and high ratios between the relative plant and fungal markers 18:1ω9 and 18:2ω6, suggesting a similar diet mainly based on leaf litter and fungi. This is in line with the $\delta^{15}N$ measurements of Schneider et al. (2004), who assigned these species to primary decomposers feeding predominantly on litter. Interestingly, ratios between 18:1ω9 and 18:2ω6 were low compared to those of litter feeders having a ratio of ~4 (Pollierer et al., 2010). However, it has to be taken into account that the ratio between 18:1ω9 and 18:2ω6 in leaf litter in our study was lower than in the study of Pollierer et al. (2010), consequently leading to a lower ratio in primary decomposers. *P. peltifer* differed significantly in its FA composition from *S. magnus* and *E. globulus*; and *A. coleoptrata* differed significantly from *S. magnus* and *E. globulus*, indicating trophic niche differentiation between these species. Indeed, *S. magnus* and *E. globulus* contained lower amounts of bacterial FAs than the other two species. The ratio between 18:1ω9 and 18:2ω6 was lower in *E. globulus* than in the other species, suggesting a higher proportion of fungi in its diet. $\delta^{13}C$ measurements of FAs also suggest that leaf litter was the dominating food resource of *P. peltifer*, *A. coleoptrata* and *S. magnus*, since the majority of FAs was more depleted in treatments with labelled leaf

litter. However, some FAs were also significantly affected by labelled roots e.g., stearic acid (18:0) in *A. coleoptrata*. The bacterial marker FA i16:0 in *A. coleoptrata* was even exclusively affected by labelled roots, suggesting that the diet of this species includes soil bacteria or consumers of bacteria. In *E. globulus*, the relative plant and fungal markers 18:1ω9 and 18:2ω6 were only significantly depleted in $\delta^{13}C$ in treatments where roots were labelled, suggesting that *E. globulus* mainly feeds on ectomycorrhizal fungi obtaining carbon via tree roots. This is in line with the findings of Pollierer et al. (2007), showing that the $\delta^{13}C$ signature of *E. globulus* was mainly affected by labelled roots.

The Collembola species *T. baudoti* contained lower amounts of plant and fungal specific FAs and higher amounts of bacterial FAs, especially vaccenic type FAs, than the studied oribatid mites, suggesting that bacteria are an integral part of its diet. Collembola usually are considered fungal feeders (Chen et al., 1995, 1996), however, it has been shown recently that Entomobryomorpha consume bacteria in considerable amounts (Crotty et al., 2011). The high ratio of the relative plant and fungal markers 18:1ω9 to 18:2ω6, and the low fraction of the relative fungal marker 18:2ω6 indicate that the species does not heavily feed on fungi. Ngosong et al. (2009) also observed that the fungal energy channel was of minor importance for Collembola nutrition in an arable field. FAs of *T. baudoti* were most depleted in treatments with labelled leaf litter. However, a large proportion of FAs, including the fungal marker 18:2ω6 and the vaccenic type FA 18:1ω7, was significantly affected by both labelled leaf litter and labelled roots, suggesting that *T. baudoti* obtains carbon from both above- and belowground plant inputs.

In *L. lanuginosus* the ratio between the relative plant and fungal markers 18:1ω9 and 18:2ω6 was lower and the fraction of the relative fungal marker 18:2ω6 was about twice as high as in *T. baudoti*, suggesting that it consumes fungi to a greater extent. This is in line with the findings of Chahartaghi et al. (2005), who designated species of *Lepidocyrtus* as secondary decomposers. *L. lanuginosus* contained higher amounts of absolute bacterial markers, but much lower amounts of vaccenic type FAs than *T. baudoti*, suggesting that the bacterial diet of these two species is different. Variable $\delta^{13}C$ signatures of FAs in *L. lanuginosus* suggest that the species may opportunistically feed on both resources fuelled by plant carbon inputs from above- and below the ground.

Similar to *T. baudoti*, the isopod species *T. pusillus* contained very low amounts of absolute bacterial markers, but high amounts of vaccenic type FAs. However, the high fraction of relative plant and fungal markers and the ratio between plant and fungal FAs close to one indicate that *T. pusillus* predominantly feeds on fungi. $\delta^{13}C$ signatures of FAs of *T. pusillus* were almost exclusively affected by labelled roots, suggesting that *T. pusillus* primarily consumes ectomycorrhizal fungi obtaining carbon from tree roots.

The earthworm species *A. longa* differed greatly in FA composition from all other investigated animals, suggesting a markedly different diet. The very low fraction of plant

and fungal FAs and the high fraction of bacterial FAs indicate a mainly bacteria-based diet. The extremely low ratio between plant and fungal FAs suggests that leaf litter is irrelevant for the nutrition of *A. longa*. This is in line with the $\delta^{13}C$ signatures of FAs, which were only significantly depleted when roots were labelled. These findings are surprising since *A. longa* commonly is grouped as anecic earthworm species (Curry and Schmidt, 2007) which typically feed on litter. However, examining the feeding behaviour of several anecic earthworm species Eisenhauer et al. (2008a) suggested that in contrast to *Lumbricus* species, *A. longa* does not consume litter but rather almost exclusively feeds on mineral soil, thereby resembling endogeic species; our results support this conclusion.

While the three investigated predators, *L. melanops*, *N. muscorum* and *H. rufulus*, contained similar fractions of plant and fungal specific FAs of about 50% each, the ratio between the relative plant and fungal markers 18:1ω9 and 18:2ω6 differed substantially, suggesting that their integration into soil energy channels differs. In the centipede *L. melanops* the ratio was very low, suggesting that this species mainly consumes fungal feeding prey. Since $\delta^{13}C$ signatures of most FAs of *L. melanops*, including plant and fungal markers, were only significantly affected by labelled roots, this predator presumably relies predominantly on prey species feeding on ectomycorrhizal fungi. However, *L. melanops* also contained bacterial FAs, especially vaccenic type FAs, indicating that its prey species to some extent also consume bacteria. The pseudoscorpion *N. muscorum* had an intermediate to low ratio between plant and fungal FAs, with the fraction of bacterial FAs being low, suggesting that this species mainly consumes fungal feeders. Its $\delta^{13}C$ signatures of FAs were more depleted in treatments with labelled roots than in treatments with labelled leaf litter, indicating that the fungal-derived carbon mainly originates from ectomycorrhiza. The FA profile of *N. muscorum* closely resembled that of *L. lanuginosus*, suggesting that *L. lanuginosus* may form part of its diet.

In the predatory oribatid mite *H. rufulus* the ratio between plant and fungal marker FAs was very high, with the fraction of bacterial FAs being also high. Presumably, therefore, *H. rufulus* predominantly feeds on bacterial feeding prey, such as bacterial feeding nematodes. Supporting this conclusion Ruf et al. (2006) also found predatory mites to be closely linked to the bacterial rather than the fungal energy channel in soil. In *H. rufulus* the majority of FAs, including the fraction of plant and fungal markers, was significantly influenced by both labelled roots and leaf litter, suggesting that its prey relies on both plant carbon from above-and belowground inputs.

Generally, FAs of each of the investigated predator species were most depleted in $\delta^{13}C$ in treatments with labelled roots. As predators integrate energy fluxes of lower trophic levels, this confirms that soil animal food webs of temperate beech forests predominantly rely on root derived rather than leave litter derived carbon (Pollierer et al., 2007). However, labelled leaf litter also significantly affected $\delta^{13}C$ signatures of some FAs,

indicating that leaf litter derived carbon also contributes to nourishment of species of higher trophic levels.

The bacterial food chain has been proposed to be short (Scheu and Setälä, 2002). In contrast to this view, all investigated animals contained bacterial FAs. Both the oribatid mite and Collembola species contained bacterial marker FAs in amounts similar to that of Collembola kept on bacterial diets for several weeks (Pollierer et al., 2010), suggesting that bacteria form an important part of the diet of these taxa. In predators, the fraction of bacterial FAs ranged from ~6 to almost 20%, indicating considerable flux of energy through the bacterial channel to higher trophic levels. This presumably contributes to food web stability, since theory suggests that systems possessing multiple pathways of energy flow can recover more easily from disturbances and have a greater chance to persist than systems with fewer pathways (MacArthur, 1955; Teng and McCann, 2004). Moore et al. (2005) showed that the relative strength of the bacterial vs. fungal energy channels is crucial for food web stability, with stability being increased when energy is more evenly distributed between the two channels.

As yet fluxes through different energy channels in soil cannot be quantified using FA analysis. FA profiles of animal species can only be interpreted in respect to similarities of profiles with basal resources and other animal taxa, allowing to delineate dietary relationships in a qualitative rather than quantitative way. More laboratory studies are needed investigating variations in the fractions of different marker FAs in consumers feeding on mixed diets of e.g., bacteria, fungi and plant litter.

In conclusion, by taking advantage of a $\delta^{13}C$ labelling of a mature temperate forest using compound specific FA analysis in combination with FA profiles of soil animals, we were able to trace the flux of carbon through the soil animal food web in unprecedented detail. The results underline the importance of root derived carbon for food web functioning, but also show that leaf litter derived carbon is utilized to some extend, especially by oribatid mites, collembolans and predators. Fungal feeders predominantly rely on belowground carbon suggesting that they consume in particular ectomycorrhizal fungi. Further, the results suggest that bacterial derived carbon, originating from both above- and belowground plant inputs, is more important for sustaining soil animal food webs than previously assumed and is channelled to higher trophic levels including predators.

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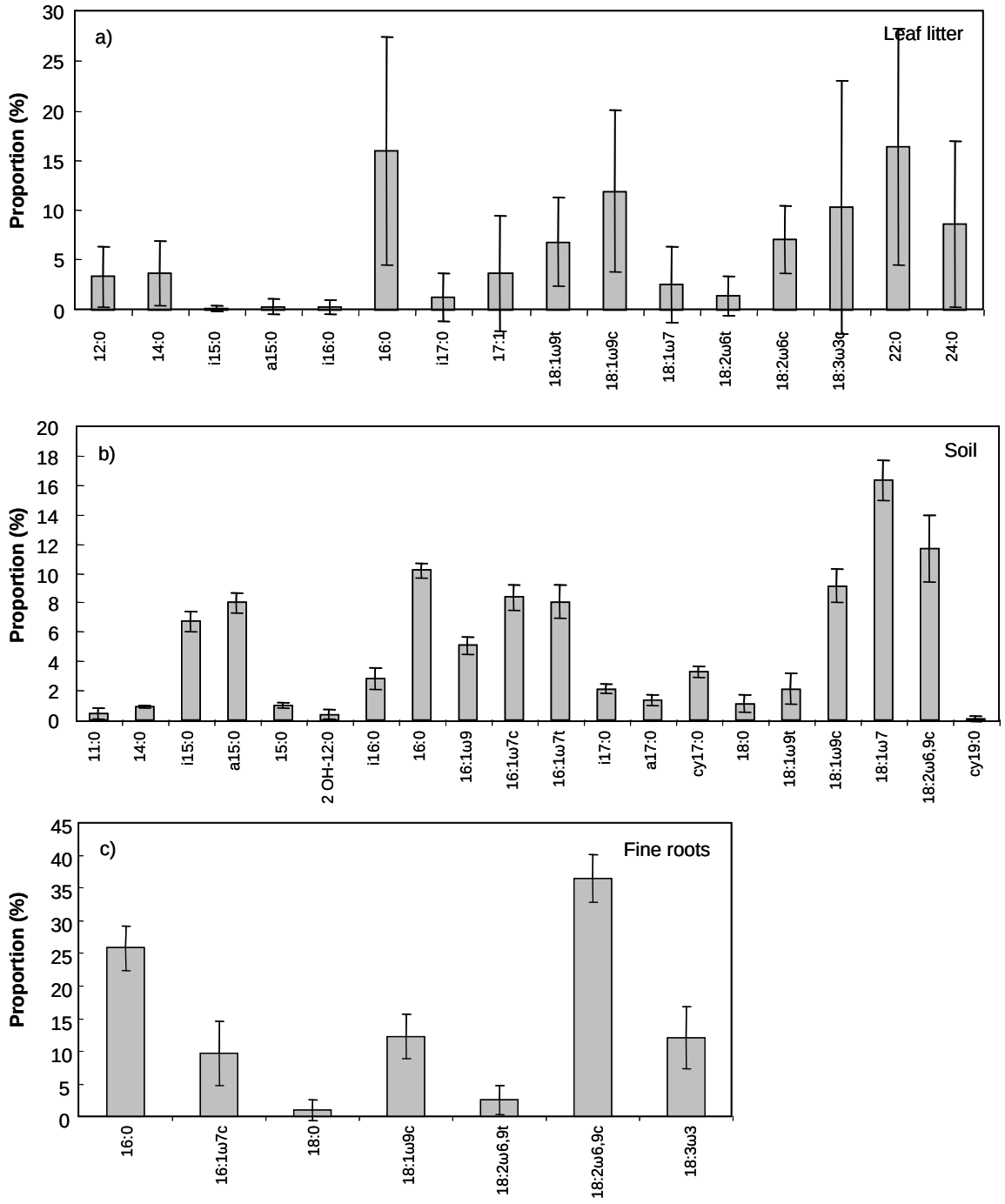
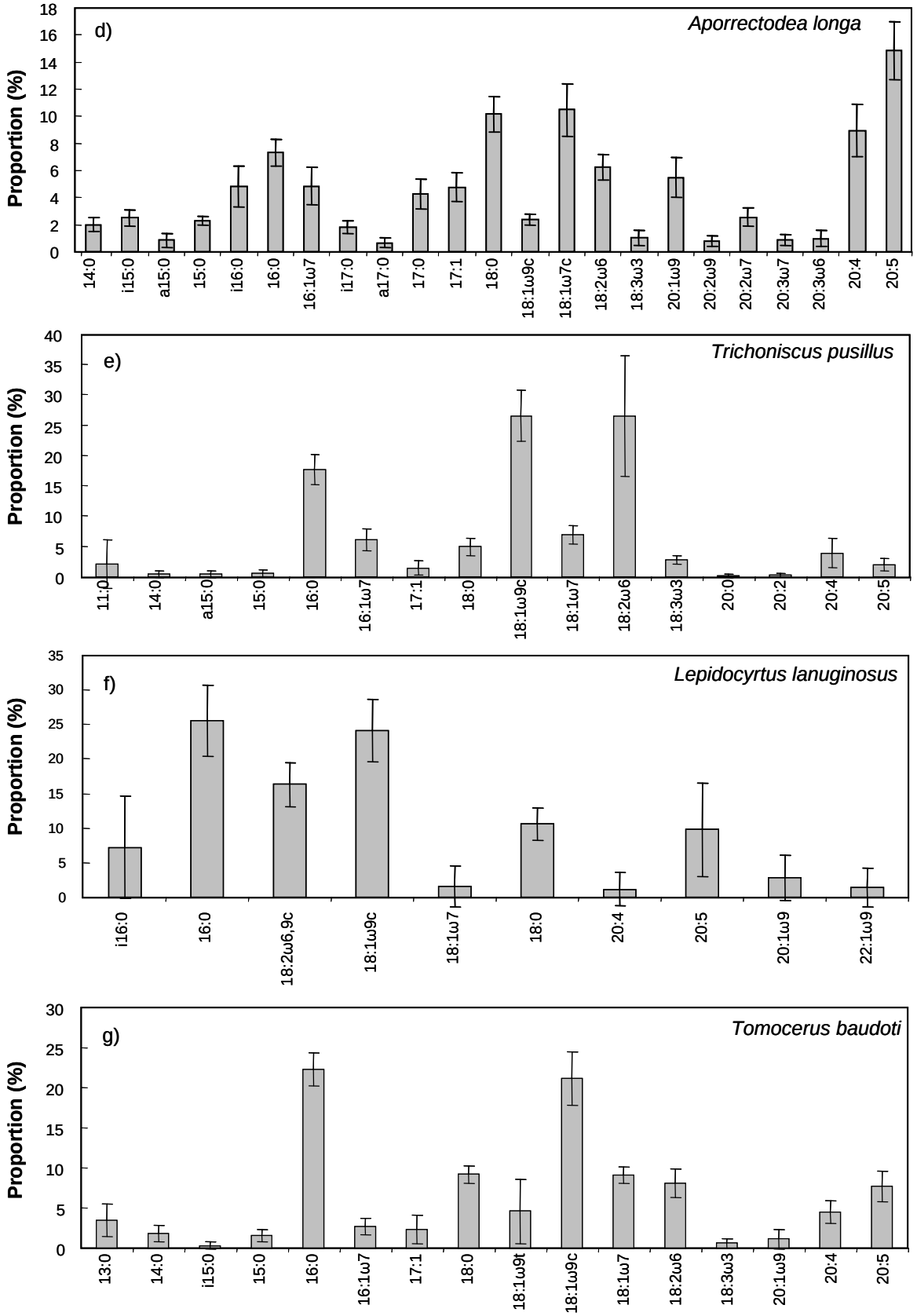
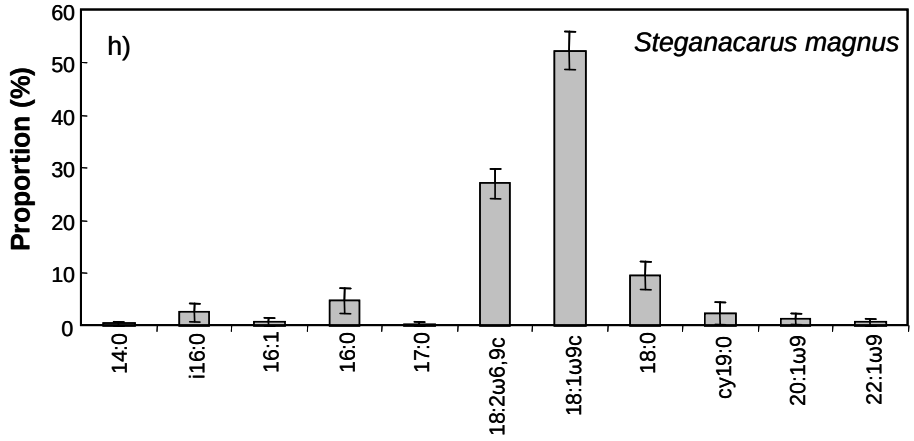
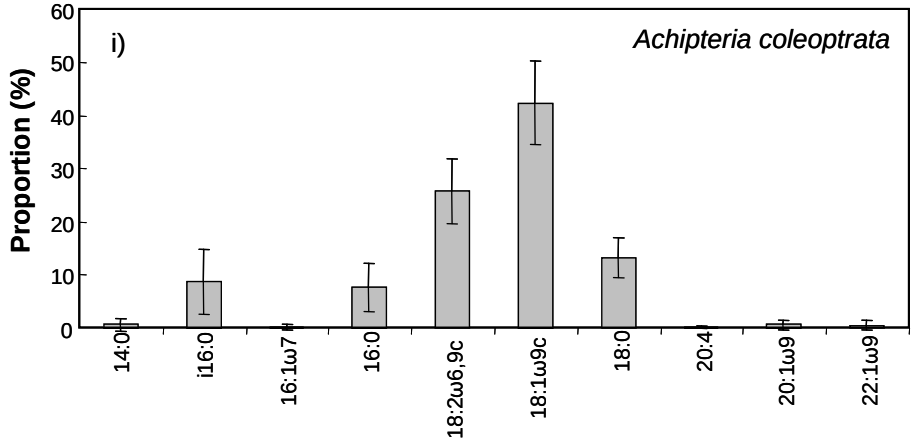
SUPPORTING INFORMATION

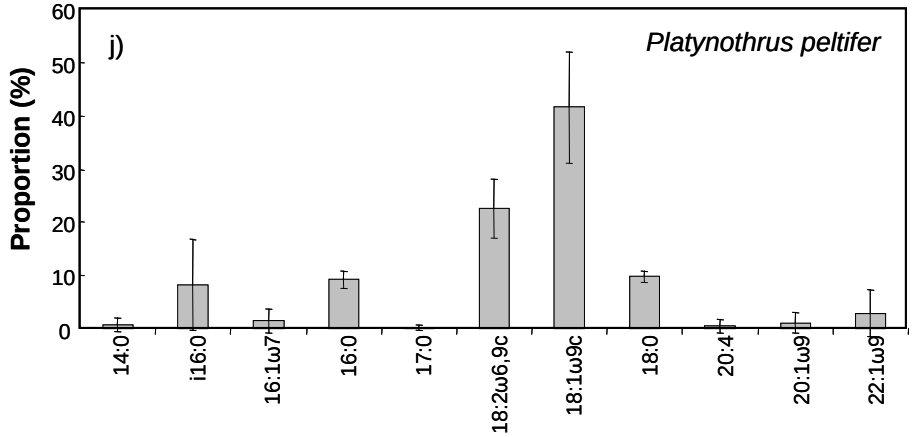
Figure S1 Concentrations of fatty acids (percentages of total ± SD) of a) whole cellular lipids in leaf litter, b) PLFAs in soil, c) whole cellular lipids in fine roots, and d) to n) NLFAs in soil animals.




d)
Aporrectodea longa
Proportion (%)
14:0
i15:0
a15:0
15:0
i16:0
16:0
16:1ω7
i17:0
a17:0
17:0
17:1
18:0
18:1ω9c
18:1ω7c
18:2ω6
18:3ω3
20:1ω9
20:2ω9
20:2ω7
20:3ω7
20:3ω6
20:4
20:5
e)
Trichoniscus pusillus
Proportion (%)
11:0
14:0
a15:0
15:0
16:0
16:1ω7
17:1
18:0
18:1ω9c
18:1ω7
18:2ω6
18:3ω3
20:0
20:2
20:4
20:5
f)
Lepidocyrtus lanuginosus
Proportion (%)
i16:0
16:0
18:2ω6,9c
18:1ω9c
18:1ω7
18:0
20:4
20:5
20:1ω9
22:1ω9
g)
Tomocerus baudoti
Proportion (%)
13:0
14:0
i15:0
15:0
16:0
16:1ω7
17:1
18:0
18:1ω9t
18:1ω9c
18:1ω7
18:2ω6
18:3ω3
20:1ω9
20:4
20:5


h)
Steganacarus magnus
Proportion (%)
0
10
20
30
40
50
60
14:0
i16:0
16:1
16:0
17:0
18:2ω6,9c
18:1ω9c
18:0
cy19:0
20:1ω9
22:1ω9


i)
Achipteria coleoptrata
Proportion (%)
0
10
20
30
40
50
60
14:0
i16:0
16:1ω7
16:0
18:2ω6,9c
18:1ω9c
18:0
20:4
20:1ω9
22:1ω9


j)
Platynothrus peltifer
Proportion (%)
0
10
20
30
40
50
60
14:0
i16:0
16:1ω7
16:0
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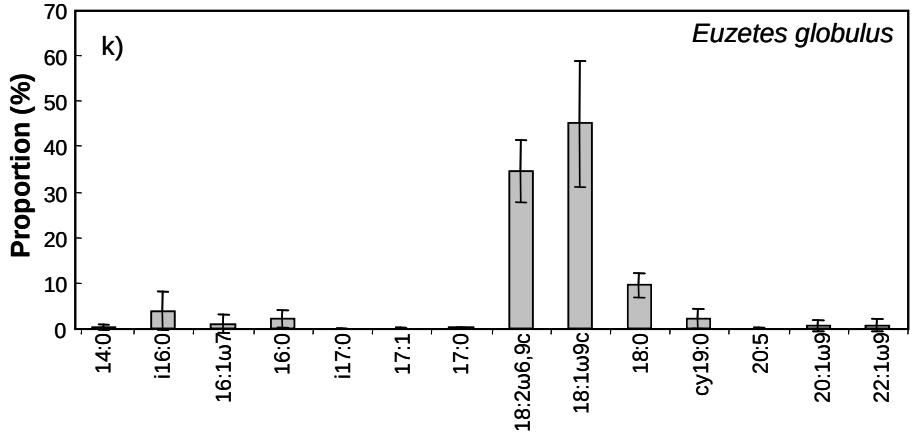
18:1ω9c
18:0
20:4
20:1ω9
22:1ω9


k)
Euzetes globulus
Proportion (%)
0
10
20
30
40
50
60
70
14:0
i16:0
16:1ω7
16:0
i17:0
17:1
17:0
18:2ω6,9c
18:1ω9c
18:0
cy19:0
20:5
20:1ω9
22:1ω9

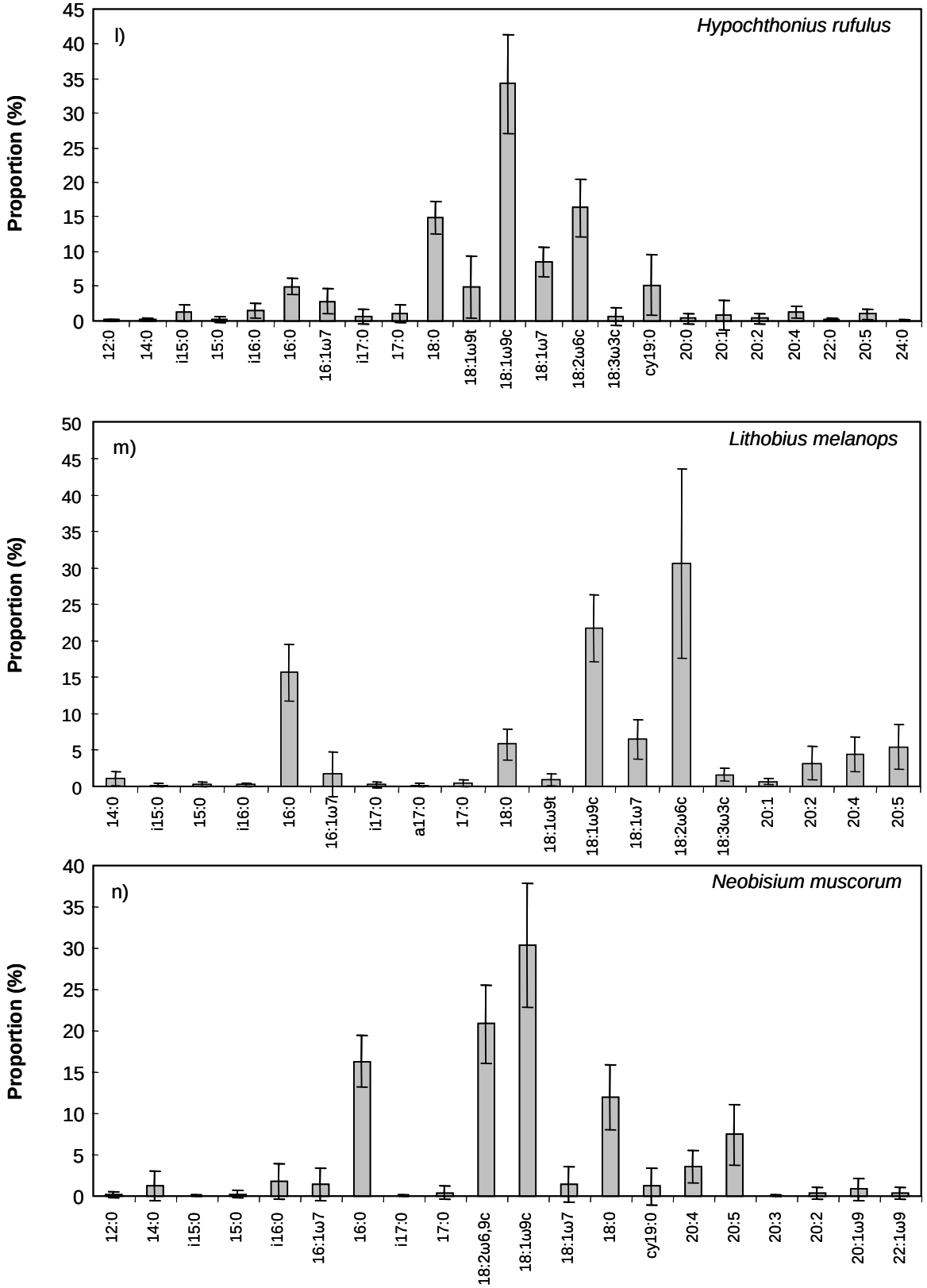

l)
Hypochthonius rufulus
Proportion (%)
12:0
14:0
i15:0
15:0
i16:0
16:0
16:1ω7
i17:0
17:0
18:0
18:1ω9t
18:1ω9c
18:1ω7
18:2ω6c
18:3ω3c
cy19:0
20:0
20:1
20:2
20:4
22:0
20:5
24:0
m)
Lithobius melanops
Proportion (%)
14:0
i15:0
15:0
i16:0
16:0
16:1ω7
i17:0
a17:0
17:0
18:0
18:1ω9t
18:1ω9c
18:1ω7
18:2ω6c
18:3ω3c
20:1
20:2
20:4
20:5
n)
Neobisium muscorum
Proportion (%)
12:0
14:0
i15:0
15:0
i16:0
16:1ω7
16:0
i17:0
17:0
18:2ω6,9c
18:1ω9c
18:1ω7
18:0
cy19:0
20:4
20:5
20:3
20:2
20:1ω9
22:1ω9

Table S2: GLM table of F-values for the effect of $\delta^{13}C$ labelled root and leaf litter material on the FA $\delta^{13}C$ signatures of soil animal taxa. Significant effects are in bold.

			Oribatida			**Collembola**			**Lumbricidae**	**Isopoda**	**Pseudoscorp.**	**Chilopoda**
			S. magnus	*A. coleoptrata*	*E. globulus*	*H. rufulus*	*L. lanuginosus*	*T. baudoti*	*A. longa*	*T. pusillus*	*N. muscorum*	*L. melanops*
14:0	roots labelled	df							2,6			3,4
		F							0.14			**10.75**
		p							0.720			**0.031**
	leaf litter labelled	*F*							1.32			**35.12**
		p							0.295			**0.004**
	roots labelled × leaf litter labelled	*F*							-			0.88
		p							-			0.401
i15:0	roots labelled	df				3,4			2,6			
		F				1.77			1.24			
		p				0.254			0.309			
	leaf litter labelled	*F*				0.00			0.03			
		p				0.954			0.876			
	roots labelled × leaf litter labelled	*F*				0.32			-			
		p				0.603			-			
a15:0	roots labelled	df							2,4			
		F							0.00			
		p							0.949			
	leaf litter labelled	*F*							1.50			
		p							0.287			
	roots labelled × leaf litter labelled	*F*							-			
		p							-			
15:0	roots labelled	df						3,6	2,6			
		F						0.13	2.11			
		p						0.729	0.197			
	leaf litter labelled	*F*						2.85	0.13			
		p						0.143	0.729			
	roots labelled × leaf litter labelled	*F*						0.20	-			
		p						0.667	-			
i16:0	roots labelled	df	3,3	3,6	3,4	3,5			2,7			
		F	0.00	**13.04**	4.75	1.12			**10.70**			
		p	0.98	**0.011**	0.095	0.338			**0.014**			
	leaf litter labelled	*F*	0.56	0.54	0.49	3.73			0.02			
		p	0.51	0.490	0.524	0.111			0.890			
	roots labelled × leaf litter labelled	*F*	0.15	0.56	0.08	1.91			-			
		p	0.73	0.482	0.793	0.226			-			
16:0	roots labelled	df	3,3	3,6	4,3	3,8	3.4	3,8	2,7	3,8	3,6	3,7
		F	0.16	0.25	2.32	**10.69**	0.00	3.46	3.29	**182.19**	0.00	**65.87**
		p	0.715	0.633	0.202	**0.011**	0.996	0.100	0.113	**<.0001**	0.984	**<.0001**
	leaf litter labelled	*F*	**10.25**	0.18	0.38	3.68	0.55	**9.71**	0.11	5.16	0.00	**14.80**
		p	**0.049**	0.686	0.572	0.091	0.499	**0.014**	0.746	0.053	0.949	**0.006**
	roots labelled × leaf litter labelled	*F*	1.45	0.02	1.03	0.73	0.02	4.60	-	2.08	0.03	1.20
		p	0.315	0.885	0.368	0.417	0.90	0.064	-	0.187	0.879	0.310
16:1w7	roots labelled	df				3,7		3,8	2,7	3,8		3,2
		F				3.99		1.83	**16.87**	**45.49**		1.71
		p				0.086		0.214	**0.005**	**0.000**		0.3212
	leaf litter labelled	*F*				1.04		0.70	0.30	1.69		0.04
		p				0.342		0.426	0.599	0.230		0.8527
	roots labelled × leaf litter labelled	*F*				0.91		0.32	-	0.00		0.78
		p				0.372		0.585	-	0.991		0.4694

			Oribatida				**Collembola**		**Lumbricidae**	**Isopoda**	**Pseudoscorp.**	**Chilopoda**
			S. magnus	*A. coleoptrata*	*E. globulus*	*H. rufulus*	*L. lanuginosus*	*T. baudoti*	*A. longa*	*T. pusillus*	*N. muscorum*	*L. melanops*
i17:0	roots labelled	df							2,7			
		F							3.54			
		p							0.102			
	leaf litter labelled	*F*							1.57			
		p							0.250			
	roots labelled × leaf litter labelled	*F*							-			
		p							-			
a17:0	roots labelled	df							2,6			
		F							0.05			
		p							0.836			
	leaf litter labelled	*F*							1.73			
		p							0.236			
	roots labelled × leaf litter labelled	*F*							-			
		p							-			
17:0	roots labelled	df	2,1			3,3			2,7			3,1
		F	0.02			4.34			**7.55**			0.07
		p	0.900			0.129			**0.029**			0.8331
	leaf litter labelled	*F*	5.39			0.08			0.01			1.65
		p	0.259			0.796			0.945			0.4208
	roots labelled × leaf litter labelled	*F*	-			0.08			-			0.03
		p	-			0.800			-			0.8944
17:1	roots labelled	df						3,6	2,7	3,6		
		F						1.98	4.18	**125.20**		
		p						0.209	0.080	**<.0001**		
	leaf litter labelled	*F*						**25.69**	0.00	0.32		
		p						**0.002**	0.991	0.590		
	roots labelled × leaf litter labelled	*F*						**14.09**	-	0.77		
		p						**0.010**	-	0.415		
18:0	roots labelled	df	3,4	3,8	3,6	3,8	3,4	3,8	2,7	3,8	3,6	3,7
		F	0.08	**5.77**	3.36	**31.48**	0.53	3.89	2.55	**109.17**	**16.36**	**49.8**
		p	0.798	**0.043**	0.116	**0.001**	0.506	0.084	0.155	**<.0001**	**0.007**	**0.0002**
	leaf litter labelled	*F*	**54.36**	**13.33**	1.12	**20.43**	0.11	**13.94**	0.01	1.41	4.37	**8.04**
		p	**0.002**	**0.007**	0.330	**0.002**	0.755	**0.006**	0.939	0.270	0.081	**0.0252**
	roots labelled × leaf litter labelled	*F*	**7.99**	2.99	0.80	0.91	0.18	4.93	-	2.65	0.60	1.85
		p	**0.048**	0.122	0.406	0.367	0.69	0.057	-	0.142	0.467	0.2164
18:1w9t	roots labelled	df				3,6		3,8				
		F				**15.65**		4.20				
		p				**0.008**		0.075				
	leaf litter labelled	*F*				**7.24**		0.06				
		p				**0.036**		0.809				
	roots labelled × leaf litter labelled	*F*				**7.62**		0.45				
		p				**0.033**		0.522				
18:1w9c	roots labelled	df	3,4	3,8	3,5	3,8	3,4	3,8	2,7	3,8	3,6	3,7
		F	0.72	0.07	**8.13**	**34.69**	1.00	3.34	2.76	**111.63**	**16.42**	**34.89**
		p	0.445	0.793	**0.036**	**0.000**	0.374	0.105	0.141	**<.0001**	**0.007**	**0.0006**
	leaf litter labelled	*F*	**13.03**	**7.69**	4.84	**9.81**	1.81	**6.42**	0.43	0.09	2.74	1.09
		p	**0.023**	**0.024**	0.079	**0.014**	0.250	**0.035**	0.534	0.772	0.149	0.3303
	roots labelled × leaf litter labelled	*F*	1.99	0.40	0.11	0.59	0.53	3.31	-	2.97	0.61	3.39
		p	0.231	0.544	0.759	0.463	0.51	0.107	-	0.123	0.463	0.1081
18:1w7	roots labelled	df				3,8		3,8	2,7	3,8		3,7
		F				0.00		**9.98**	**9.90**	**13.23**		**7.42**
		p				0.952		**0.013**	**0.016**	**0.007**		**0.0296**
	leaf litter labelled	*F*				0.17		**19.55**	2.14	0.05		3.71
		p				0.687		**0.002**	0.187	0.833		0.0955
	roots labelled × leaf litter labelled	*F*				1.23		4.15	-	3.97		0.36
		p				0.300		0.076	-	0.082		0.5671
18:2w6c	roots labelled	df	3,4	3,8	3,7	3,8	3,4	3,8	2,7	3,8	3,6	3,7
		F	3.31	0.33	**7.84**	**10.10**	3.25	**10.50**	3.99	**164.21**	1.14	**72.5**
		p	0.143	0.582	**0.027**	**0.013**	0.146	**0.012**	0.086	**<.0001**	0.326	**<.0001**
	leaf litter labelled	*F*	**9.50**	**5.75**	0.01	**5.90**	0.03	**15.80**	0.15	1.04	0.94	0.46
		p	**0.037**	**0.043**	0.919	**0.041**	0.877	**0.004**	0.709	0.337	0.369	0.5205
	roots labelled × leaf litter labelled	*F*	**8.42**	1.94	0.01	3.54	0.02	**6.66**	-	1.90	0.12	1.42
		p	**0.044**	0.202	0.926	0.097	0.89	**0.033**	-	0.206	0.737	0.2725

			Oribatida			Collembola			Lumbricidae	Isopoda	Pseudoscorp.	Chilopoda
			S. magnus	*A. coleoptrata*	*E. globulus*	*H. rufulus*	*L. lanuginosus*	*T. baudoti*	*A. longa*	*T. pusillus*	*N. muscorum*	*L. melanops*
18:3w3	roots labelled	df				3,1		3,4	2,6	3,8		3,6
		F				0.26		**8.98**	2.14	**39.07**		5.19
		p				0.701		**0.040**	0.194	**0.000**		0.063
	leaf litter labelled	*F*				0.90		**9.42**	0.03	5.11		1.57
		p				0.516		**0.037**	0.871	0.054		0.2569
	roots labelled	*F*				0.53		1.32	-	0.17		0.01
	× leaf litter labelled	*p*				0.599		0.315	-	0.691		0.9398
cy19:0	roots labelled	df	2,3			3,7						
		F	2.73			4.79						
		p	0.197			0.065						
	leaf litter labelled	*F*	1.11			2.23						
		p	0.369			0.179						
	roots labelled	*F*	-			1.13						
	× leaf litter labelled	*p*	-			0.322						
20:1w9	roots labelled	df	2,3						2,7			3,4
		F	31.02						3.80			**12.02**
		p	0.031						0.092			**0.0257**
	leaf litter labelled	*F*	459.84						0.00			2.33
		p	0.002						0.948			0.2013
	roots labelled	*F*	70.70						-			0.21
	× leaf litter labelled	*p*	0.014						-			0.6692
20:2	roots labelled	df							2,7	3,5		3,6
		F							**8.46**	**13.82**		**33.46**
		p							**0.0227**	**0.0137**		**0.0012**
	leaf litter labelled	*F*							1.07	0.01		2.92
		p							0.3353	0.9311		0.1386
	roots labelled	*F*							-	0.02		0.59
	× leaf litter labelled	*p*							-	0.9055		0.4699
20:3w7	roots labelled	df							2,6			
		F							**15.82**			
		p							**0.0073**			
	leaf litter labelled	*F*							0.19			
		p							0.6814			
	roots labelled	*F*							-			
	× leaf litter labelled	*p*							-			
20:3w6	roots labelled	df							2,6			
		F							**25.24**			
		p							**0.0024**			
	leaf litter labelled	*F*							1.58			
		p							0.256			
	roots labelled	*F*							-			
	× leaf litter labelled	*p*							-			
20:4	roots labelled	df				3,5		3,8	2,7	3,8	3,6	3,7
		F				**7.86**		**23.73**	**25.13**	**48.6**	**7.87**	**13.38**
		p				**0.0378**		**0.0012**	**0.0015**	**0.0001**	**0.0309**	**0.0081**
	leaf litter labelled	*F*				0.02		**43.6**	1.19	1.14	2.46	0.58
		p				0.8871		**0.0002**	0.3122	0.3167	0.168	0.4702
	roots labelled	*F*				0		1.95	-	0.1	0.27	0.45
	× leaf litter labelled	*p*				0.9746		0.1999	-	0.7632	0.6213	0.5257
20:5	roots labelled	df				3,4	3,3	3,8	2,7	3,6	3,6	3,7
		F				4.63	0.37	**57.35**	**18.47**	**23.83**	**47.98**	**6.77**
		p				0.0978	0.5846	**<.0001**	**0.0036**	**0.0028**	**0.0004**	**0.0353**
	leaf litter labelled	*F*				0.08	0.02	**114.91**	2.04	1.21	**7.73**	0.34
		p				0.7915	0.8989	**<.0001**	0.1965	0.3128	**0.032**	0.5774
	roots labelled	*F*				0.91	1.72	0.22	-	0.42	1.97	0.13
	× leaf litter labelled	*p*				0.394	0.2805	0.6507	-	0.5425	0.2105	0.7288
22:1w9	roots labelled	df	2,2									
		F	0.04									
		p	0.8612									
	leaf litter labelled	*F*	0									
		p	0.9904									
	roots labelled	*F*	-									
	× leaf litter labelled	*p*	-									

Chapter 6

General Discussion

General discussion

Results of this thesis represent major advances in our understanding of forest soil animal food webs by identifying trophic compartments and by tracing energy fluxes via different energy channels, thereby obtaining information on food web structure and functioning in unprecedented detail.

The first study of this thesis, which took advantage of the natural variation of $\delta^{15}N$ and $\delta^{13}C$ in soil animals and food resources, gave evidence on the compartmentalization of the soil animal food web (Chapter 2). Together with the findings of Pollierer et al. (2007) we could show that the trophic compartment of primary decomposers feeding directly on leaf litter is comparatively small and of minor importance for the nutrition of higher trophic levels, presumably because primary decomposers such as diplopods and oribatid mites are often large and/or strongly sclerotized. A small compartment comprised of predators with high $\delta^{15}N$ and high $\delta^{13}C$ signatures, including mesostigmate mites and the oribatid mite *Hypochthonius rufulus*, which presumably fed on enchytraeids or nematodes. Earthworms, especially endogeic species, had the highest $\delta^{13}C$ signatures of all investigated species and presumably assimilate old carbon that is enclosed in soil aggregates. The largest compartment consisted of secondary decomposers such as damaeid oribatid mites, entomobryomorph collembolans and diplurans, which were assumed to mainly feed on fungi, and of their predators, e.g. centipedes and staphylinid beetles. The dual analysis of $\delta^{15}N$ and $\delta^{13}C$ was not capable of separating predators from ectomycorrhizal fungal feeders within this compartment by their isotopic signatures due to similar $\delta^{13}C$ signatures of primary decomposers and ectomycorrhizal fungi. Therefore, we could not obtain a finer resolution of feeding preferences within the largest compartment of the soil animal food web and the potential of stable isotope analysis had reached its limits at this point. Consequently, we had to employ a new method to further resolve energy fluxes into different compartments of the soil animal food web.

Fatty acid analysis has the potential to separate the fungal, plant and bacterial energy channel by using specific or relative marker fatty acids for these basal resources. However, in soil animal food webs fatty acid analyses so far had only been applied to analyze soil or leaf litter microbial community composition (Frostegard et al., 1993, 2011; Moore-Kucera and Dick, 2008; Baath, 2003) and some decomposer animals (mainly collembolans; Ruess et al., 2004, 2005a, 2007; Chamberlain et al., 2005, 2006). There was only one study by Ruess et al. (2005b) showing that marker fatty acids were transferred to higher trophic levels; in this case from fungi via fungal feeding nematodes to Collembola. We therefore had to investigate whether marker fatty acids are transferred to higher trophic levels including top predators and whether they reliably can be used to differentiate feeding strategies and energy fluxes within a whole food web. Consequently,

we set up a laboratory experiment (Chapter 3) in which we fed two top predators of soil animal food webs, the centipede *Lithobius forficatus* and the spider *Pardosa lugubris*, with collembolans (*Heteromurus nitidus*) kept on different diets, including $Gram^+$ and $Gram^-$ bacteria, fungi and tree leaves. By analyzing fatty acid profiles of predators, prey, and basal resources, we showed that marker fatty acids of the different resources were transferred over three trophic levels, i.e. were detectable in predators. Predators could be reliably separated by discriminant function analysis according to their diet, suggesting that fatty acid analysis is suitable to trace energy fluxes from different basal resources to higher trophic levels, allowing the analysis of whole food webs.

In addition to the verification of marker fatty acids and their transfer to higher trophic levels, physiological parameters of fatty acid metabolism need to be known to investigate feeding strategies in the field. While it had been shown that marker fatty acids show some robustness against variations in temperature, food quality, food deprivation and developmental stage (Haubert et al., 2004, 2008), the detection time and storage period of marker fatty acids in soil animals after switching to a new food source was not known yet. In another laboratory experiment (Chapter 4) we therefore investigated the chronological change of fatty acid biomarkers in collembolans (*Heteromurus nitidus*) switched from one diet to another and we investigated the change of bacterial fatty acid biomarkers during starvation. Fatty acids that were specific to a certain food source were already detectable in the neutral lipids of consumers one day after consumption. After switching to a different diet, marker fatty acids of the previous diet were detectable even after 14 days of feeding on the new food source, suggesting that fatty acid analysis provides an integrative measure of food consumption that allows to follow both fast changes in diet and medium-term dietary intake. During starvation, the composition of fatty acids changed only little and marker fatty acids were still detectable in sufficient amounts after 14 days of food deprivation, suggesting that fatty acids provide reliable information on the diet of soil animals even after periods of starvation.

With these preconditions fulfilled, we used fatty acid analysis to investigate dietary choices and energy fluxes from basal resources to higher trophic levels in the field (Chapter 5). For our field study we took advantage of the Swiss Canopy Crane Project, where the canopy of a mature temperate forest was labeled with CO_2 depleted in ^{13}C (for details see Pepin and Körner, 2002 and Steinmann et al., 2004). By exchanging leaf litter from the labeled canopy crane area with an unlabeled control site, we were able to separate carbon fluxes originating from aboveground, via consumption of leaf litter, and from belowground, via roots and root exudates. In a previous study using stable isotope analyses and a similar design (Pollierer et al., 2007), we had shown that the majority of soil animals obtain carbon via roots and that leaf litter only plays a minor role for soil animal nutrition. However, the exact way by which this root-derived carbon enters the soil

animal food web could not be determined. We hypothesized that consumption of ectomycorrhizal fungi played the most important role for soil animal nutrition, but stable isotope analyses did not allow to prove this hypothesis. By employing compound specific fatty acid analyses in our field study, where the $\delta^{13}C$ of individual fatty acids is measured, we were able to trace the origin of carbon in individual fatty acids. In combination with the use of marker fatty acids for specific diets, we gained insight into soil animal nutrition in unprecedented detail. Generally, fatty acids of all investigated predators were more depleted, i.e. labeled, in treatments where roots were labeled. Since predators integrate lower level energy fluxes, this confirms the major importance of root derived carbon for soil animal food webs. However, fatty acids of predators were also labeled to some extend in treatments with labeled leaf litter, indicating that leaf litter derived carbon is also channeled to higher trophic levels. This is in line with the findings of a new study by Eisenhauer and Reich (2012) which also point to the dual importance of above- and belowground plant inputs for soil food webs. The fungal marker linoleic acid (18:2ω6,9) in fungal feeders contained mainly belowground derived carbon, supporting the hypothesis that ectomycorrhizal fungi form the main food resource for fungal feeders. Generally, it is assumed that the fungal energy channel is most important for forest soil animal food webs (Wardle et al., 2004; Klironomos and Kendrick, 1995; Scheu and Falca, 2000) whereas the bacterial food chain is supposedly short (Scheu and Setälä, 2002). However, results of our study showed that virtually all soil animals contain considerable amounts of bacterial marker fatty acids, suggesting that the predominance of the fungal energy channel may have been overrated. The partitioning between the fungal and bacterial energy channel presumably contributes to food web stability, since systems relying on multiple pathways of energy fluxes have been found to be less susceptible and to recover more easily from disturbances than those relying on only few pathways (MacArthur, 1955; Teng and McCann, 2004). Models of trophic pathways in soils (Moore et al., 2005) also pointed to an enhanced stability when energy is distributed more evenly between the fungal and bacterial channel.

Since all investigated animals, including fungal feeders, contained some bacterial fatty acids, results of our field study (Chapter 5) put the findings of our first study (Chapter 2) that soil animal food webs are strongly compartmentalized, into a somewhat different perspective. The observed compartmentalization of the soil animal food web may not be as strongly linked to different energy channels than previously assumed (Moore et al., 2005). Most animals obtain carbon from roots as well as in smaller portions also from leaf litter, and they utilize fungi as well as bacteria, suggesting that the compartmentalization may either originate from the different partitioning of resource utilization in the respective species, or from the exploitation of carbon sources of different age.

As yet, energy fluxes through the fungal, bacterial and plant energy channel can only be analyzed in a qualitative rather than a quantitative manner by comparing fatty acid composition of basal resources and consumers. There is a need for more laboratory experiments relating the relative uptake of specific food materials, such as fungi, bacteria or plant leaves, to the relative composition of marker fatty acids within different species of soil animals. This could be accomplished by feeding defined mixtures of food materials to different species of soil animals with varying body sizes, allowing for calibration of the resulting neutral lipid fatty acid pattern and possibly for extrapolation to other species.

Results of this thesis represent major advances in the understanding of soil animal food web structure and functioning. We identified different feeding strategies on the species level. In doing so, we could not only show that root derived carbon is of major importance for soil animal nutrition, but we also traced the pathways by which this carbon enters different compartments within the soil animal food web, depicting different energy channels, such as the bacterial and fungal energy channel. We showed that the bacterial energy channel is more important than previously assumed and proposed that this contributes to food web stability.

The potential of fatty acid analyses for the investigation of soil animal food webs has not been fully exploited yet. Especially compound specific ^{13}C fatty acid analyses in combination with labeling experiments are a promising tool for the further investigation of energy fluxes within food webs. By introducing a more quantitative perspective to fatty acid analyses, future studies should aim at constructing quantitative models of energy fluxes through soil animal food webs. Potentially, the additional use of other metabolically relevant molecules such as amino acids may help to further refine these models by providing a second element (nitrogen) of which compound specific stable isotopes can be evaluated, thereby allowing the analysis of dual labeling experiments with labeled carbon as well as labeled nitrogen. A novel method introduced by Larsen et al. (2009) takes advantage of $\delta^{13}C$ signatures of essential amino acids and is termed stable isotope fingerprinting. This method benefits from the differing pathways in amino acid synthesis in plants, fungi and bacteria which result in unique patterns of carbon stable isotope signatures. By ascribing consumers to their respective diet using the $\delta^{13}C$ value of their essential amino acids, stable isotope fingerprinting may complement compound specific fatty acid analyses in further clarifying the relative contribution of different basal resources to soil animal nutrition.

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List of Publications

(Please note that for publications I continue to use my maiden name Pollierer)

Pollierer, M.M., Dyckmans, J., Scheu, S., Haubert, D., 2012. Carbon flux through fungi and bacteria into the forest soil animal food web as indicated by compound-specific ^{13}C fatty acid analysis. Functional Ecology 26, 978-990.

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Pollierer, M.M., Langel, R., Koerner, C., Maraun, M., Scheu, S., 2007. The underestimated importance of belowground carbon input for soil animal food webs. Ecology Letters 10, 729-736.

Thesis declarations

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

Chapters 2 to 4 comprise manuscripts that have been published in peer-reviewed journals; Chapter 5 comprises a mansucript that is currently submitted to a peer reviewed journal. In all manuscripts except that presented in Chapter 4 I am the first author and I have collected and analyzed the data, written the manuscripts, developed the main ideas, created tables, figures and appendices and contributed significantly to the study design. To the studies presented in Chapters 3 and 4 Dominique Haubert and I have equally contributed to the design, data collection and data analysis. The study presented in Chapter 4 was mainly written by D. Haubert. All co-authors contributed to finalising the manuscripts.

Plagiarism declaration

I declare that I have written this doctoral thesis independently. All persons contributing to the manuscripts have been named so. 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.

Melanie Mira Maraun
Göttingen, January 2012

Coloured cover images by Göttingen Centre for Biodiversity and Ecology
(legend top to bottom)

1 Mixed deciduous forest in the Hainich region (Central Germany)
2 Different insect taxa on the flowers of a thistle (*Cirsium* sp.)
3 *Glomeris* sp., a member of the decomposing soil fauna in forest ecosystems
4 *Pleodorina californica* (Chlorophyceae), colony-forming freshwater phytoplankton species
5 Grasshopper *Tettigonia cantans*, distributed from the Pyrenees to Northeastern China
6 *Microcebus berthae* (Cheirogaleidae), the smallest extant Primate species (Madagascar)
7 Tropical rain forest (Greater Daintree, Australia)
8 *Lethocolea glossophylla* (Acrobolbaceae), a liverwort of alpine mountain ranges in South America
9 Part of a coral reef in the Red Sea

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