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Incorporation of plant carbon and microbial nitrogen into the rhizosphere food web of beech and ash

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ABSTRACT

We labeled tree saplings of beech and ash with ^{15}N and ^{13}C in a greenhouse. Carbon (C) was applied as $^{13}CO_2$ to plants and nitrogen (N) was added as $^{15}NH_4^{15}NO_3$ to the soil. We hypothesized that C will be transferred from plants to the rhizosphere, subsequently in beech to ectomycorrhiza (EM), in ash to arbuscular mycorrhiza (AM) and finally to soil animals. We expected the C signal to be more effectively transferred to soil animals in EM as compared to AM systems since EM forms more extensive extramatrical mycelia as compared to AM. For ^{15}N we hypothesized that it will be taken up by both saprotrophic microorganisms and mycorrhizal fungi and then channeled to soil animals. After five month, $\delta^{13}C$ and $\delta^{15}N$ signatures of soil animals, EM and fine roots of beech and ash were measured. Litter and soil were hardly enriched in ^{15}N whereas fine roots of beech and ash were highly enriched suggesting that nitrogen in $^{15}NH_4^{15}NO_3$ was predominantly taken up by plants and mycorrhizal fungi but little by saprotrophic microorganisms. Roots of beech and ash were highly enriched in ^{13}C with maximum values in EM proving that ^{13}C was translocated into roots and mycorrhizal fungi. Soil animals were a priori assigned to primary decomposers, secondary decomposers and predators. Generally, signatures of soil animals did not significantly vary between beech and ash and therefore were pooled. Primary decomposers had low $\delta^{13}C$ and $\delta^{15}N$ signatures similar to litter and soil confirming that rhizosphere C and microbial N are of limited importance for primary decomposer taxa. $\delta^{13}C$ and $\delta^{15}N$ signatures of secondary decomposers were higher than those of primary decomposers and spanned a large gradient indicating that certain secondary decomposers rely on root derived C and microbial N, however, none of the secondary decomposers had signatures pointing to exclusive feeding on EM. Unexpectedly, $\delta^{13}C$ and $\delta^{15}N$ signatures were highest in predators suggesting that they heavily preyed on secondary decomposer species such as the litter dwelling Collembola species *Lepidocyrtus cyaneus* and species not captured by the heat extraction procedure used for capturing prey taxa, presumably predominantly root associated nematodes. Overall, the results highlight that in particular higher trophic levels rely on carbon originating from other resources than litter with these resources channeled to dominant predators via litter dwelling Collembola species.

1. Introduction

In forest ecosystems most of the net primary production enters the decomposer community as detritus. This dead organic material usually is assumed to be the main source of nutrients for soil microbes (Swift, 1979; Berg and McClaugherty, 2008) and decomposer animals (Hättenschwiler and Gasser, 2005; Scheu, 2005). However, this view has been challenged recently by documenting that soil animals strongly rely on root-derived carbon (Ruf et al., 2006; Albers et al., 2006; Pollierer et al., 2007, 2012). In fact, a large fraction of plant fixed carbon enters the belowground system via roots and root exudates (Bardgett et al., 2005; Leake et al., 2006) and this carbon is more easily available for soil organisms than the recalcitrant carbon in plant litter since it comprises predominantly amino acids, sugars and peptides (Bais et al., 2006; Dennis et al., 2010).

Most plant roots are closely associated with mycorrhizal fungi channeling plant carbon to the outer rhizosphere (Smith and Read,

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1997; Wallander et al., 2009). In temperate forest ecosystems ectomycorrhizal fungi (EMF) dominate (e.g., in beech, oak, lime and hornbeam), but some tree species are associated with arbuscular mycorrhizal fungi (AMF; e.g., ash and acer; Lang and Polle, 2011; Lang et al., 2011). The transfer of carbon from the plant to the rhizosphere likely is more effective in the well-dispersed extramatrical mycelium of the EMF (Högberg et al., 2008; Cairney, 2012) than in AMF which do not form intensive extramatrical mycelia (Smith and Read, 1997).

Nitrogen is of crucial importance for soil microorganisms and plants. During decomposition of litter material and for microbial growth in general microorganisms immobilize mineral nutrients in soil thereby competing with plants for these resources (Chapman et al., 2006; Geissler et al., 2010). Tree roots take up nitrogen from soil, but in temperate forests most nitrogen is channeled to plants via EMF (Hobbie and Hobbie, 2006; Van der Heijden et al., 2008). In soil food webs carbon is channeled along two main energy pathways, the fungal and bacterial energy channel (Moore and Hunt, 1988; Moore et al., 2005; Crotty et al., 2011). In temperate forests litter quality typically is low and litter is mainly processed by saprotrophic fungi (Wardle et al., 2004). Together with EMF saprotrophic fungi form the main source of N for the fungal energy channel of soil food webs (Moore-Kucera and Dick, 2008). In contrast, bacteria predominantly consume root exudates and serve as source for N (and other elements) for the bacterial energy channel (Crotty et al., 2011).

From a trophic level point of view the soil food web might be separated into primary decomposers, secondary decomposers and predators (Scheu and Falca, 2000; Scheu, 2002). Primary decomposers, such as Diplopoda, and certain species of Oribatida and Lumbricidae, are assumed to feed mainly on litter material (Pollierer et al., 2009). Secondary decomposers, such as most Oribatida, Collembola and certain species of Isopoda and Lumbricidae, are assumed to feed predominantly on fungi and microbial residues (Maraun et al., 1998; Scheu and Falca, 2000). Predators, such as Lithobiidae or Araneida, have been assumed to rely predominantly on secondary decomposers as food (Scheu, 2002; Pollierer et al., 2012; Ferlian et al., 2012).

Natural variations in stable isotope ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) have been shown to be a powerful tool for investigating nutrient fluxes and trophic interactions in soil food webs (Scheu and Falca, 2000; Illig et al., 2005; Tiunov, 2007; Pollierer et al., 2009). However, labeling experiments with enriched ^{13}C and ^{15}N compounds are indispensable for tracing carbon and nitrogen fluxes through decomposer systems (Ruf et al., 2006; Pollierer et al., 2007; Sticht et al., 2008; Högberg et al., 2010).

We conducted a $^{13}CO_2$ labeling experiment in the greenhouse to follow the flux of carbon from plant shoots to the rhizosphere and into the soil animal food web. In parallel, we used ^{15}N labeled mineral nitrogen (NH_4NO_3) to follow the flux of nitrogen via saprotrophic microorganisms and mycorrhiza into the soil animal food web. Saplings of European beech and European ash were excavated in the field, potted into mesocosms including rhizosphere soil and the associated soil animal community. After five months of labeling i.e., after one vegetation period, $\delta^{13}C$ and $\delta^{15}N$ signatures of beech and ash roots, ectomycorrhiza and soil animals were measured.

We investigated the following hypotheses: (1) Plant carbon is translocated via roots and mycorrhiza into fungal feeding soil invertebrates. (2) Carbon as well as nitrogen is transferred mainly to lower trophic levels and is diluted toward higher trophic levels due to predators incorporating prey relying in part on root and in part on litter carbon. (3) Carbon and nitrogen transfer into the soil animal food web is more pronounced in beech than in ash due to the more extensive extramatrical mycelium in EMF than in AMF. (4) Mineral nitrogen is translocated to higher trophic levels via both saprotrophic microorganisms and mycorrhizal fungi and subsequently into soil animals.

2. Material and methods

2.1. Study site and experimental setup

Tree saplings were collected at two locations (Thiemsburg and Lindig) in the south east of the Hainich National Park, Thuringia, Germany (51°05′28″N, 10°31′24″E). The Hainich is the largest cohesive deciduous forest in Germany and was declared National Park in 1997. In the sampling area, forest cover was present since the mid 18th century. In the last four decades, the area was used for military training and has been managed little (Schmidt et al., 2009). The dominating tree species at the study sites is beech (*Fagus sylvatica* L.), but ash (*Fraxinus excelsior* L.), maple (*Acer pseudplatanus* L.) and lime (*Tilia platyphyllos* Scop. and *Tilia cordata* P. Mill.) are interspersed. The herb layer of the Hainich is dominated by *Allium ursinum* (L.), *Anemone nemorosa* (L.) and *Galium odoratum* (L.) (Vockenhuber et al., 2011). The mean annual temperature ranges from 7.5 to 8.0 °C and the mean annual precipitation is 600 mm (Leuschner et al., 2009). The area represents a slightly sloping plateau of the Triassic Upper Limestone formation covered by Pleistocene loess (Leuschner et al., 2009).

At the study sites 15 saplings of *F. sylvatica* and 14 saplings of *F. excelsior* (height ca. 60 cm) were excavated together with the surrounding intact soil (depth 25 cm and 2–3 cm litter layer) and placed into containers (diameter 25 cm, height 45 cm) equipped with drainage at the bottom. For ^{13}C labeling tree saplings were exposed to $^{13}CO_2$ enriched atmosphere (maximum CO_2 concentration 1200 ppm) in a greenhouse for five months at 23 °C and 70% humidity. For ^{15}N labeling the mesocosms were irrigated daily with a Hoagland-based nutrient solution containing 0.1 mM $^{15}NO_3^{15}NH_4$ and 0.6 mM $CaCl_2$, 0.4 mM $MgSO_4$, 0.01 mM $FeCl_3$, 0.4 mM K_3PO_4, 1.8 μM $MnSO_4$, 0.064 μM CuCl, 0.15 μM $ZnCl_2$, 0.1 μM MoO_3, 0.01 mM H_3BO_3 and 5 mM NO_3NH_4 (Euriso-top, Saint-Aubin, Essonne, France). The soil was moistened at regular intervals by adding tap water.

2.2. Sampling of soil, litter, plants and ectomycorrhiza

At the end of the experiment the soil was divided into two horizons, 0–10 cm (A1 horizon including litter) and 10–25 cm (A2 horizon). Aliquots of soil material for stable isotope analyses were collected from the A1 horizon, dried and stored in plastic bags until analysis. From the litter layer and A1 horizon large soil animals were picked by hand. From the A1 and A2 layer roots were washed, divided in coarse (>2 mm) and fine roots (<2 mm), dried (48 h, 70 °C) and weighed. Aliquots of the litter were taken, dried and stored in plastic bags until stable isotope analysis. Root caps of beech with EMF were collected and twenty samples were analyzed for stable isotope ratios.

2.3. Sampling of soil animals

Animals of the litter and A1 layer were extracted by heat using a high-gradient canister method effectively extracting mobile soil animals such as arthropods and (non-dormant) earthworms (Kempson et al., 1963). Soil animals were transferred into 70% ethanol and sorted to groups. Individuals were counted and determined to family, genus or species level (see Appendix). Based on natural variations in stable isotope ratios ($^{15}N/^{14}N$), feeding experiments, analyses of fatty acids and gut content analyses soil animal species were classified into primary decomposers, secondary decomposers and predators (see Appendix). Primary

decomposers included twelve species i.e., *Octolasion tyrtaeum* (Lumbricidae), two species of Diplopoda and nine taxa of Oribatida. Secondary decomposers comprised 15 taxa, i.e., four taxa of Lumbricidae, four taxa of Isopoda, *Craspedosoma* sp. (Diplopoda), four taxa of Oribatida, and *Sinella*/*Pseudosinella* spp. and *Lepidocyrtus cyaneus* (Collembola). Fifteen soil arthropod taxa were classified as predators including *Neobisium carcinoides* (Pseudoscorpionida), six taxa of Chilopoda, three taxa of Araneida, three taxa of Opilionida and *Acrogalumna longipluma* and *Hypochthonius luteus* (Oribatida).

2.4. Stable isotope analyses

Dry plant tissues of leaves, stems, coarse roots and fine roots, aliquots of litter and non-rhizosphere soil of the A1 horizon were dried and milled with a ball mill (Type MM 2, Retsch, Haan, Germany), dried again at 70 °C for 24 h and kept in a desiccator until analysis. Aliquots of the samples and of EM root tips (ca. 1 mg) were weighed into tin capsules for stable isotope analysis ($^{13}C/^{12}C$ and $^{15}N/^{14}N$). For stable isotope analyses of soil animals, individual or bulked specimens corresponding to a minimum of 5 μg N were used. Large species were dried, fragmented mechanically and a subsample was analyzed. The capsules were dried at 60 °C for 24 h and stored in a desiccator prior to the analysis.

Stable isotope ratios were analyzed with a coupled system consisting of an elemental analyzer (NA 1500, Carlo Erba, Mailand) and a mass spectrometer (MAT 251, Finnigan, Bremen, Germany). Abundances of ^{13}C and ^{15}N are expressed using the δ notation with δ_{sample} [‰] = $[(R_{sample} - R_{standard})/R_{standard}] \times 1000$; 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 (PBD) and for ^{15}N atmospheric nitrogen served as the primary standard. Acetanilide (C_8H_9NO, Merck, Darmstadt) was used for internal calibration.

2.5. Statistical analyses

Differences in $\delta^{13}C$ and $\delta^{15}N$ signatures of the three groups of soil animal taxa, i.e., primary decomposers, secondary decomposers and predators, were analyzed with single factor analysis of variance (ANOVA) with the general linear model (GLM) procedure using SAS 9.13 (SAS Institute, Cary, NC, USA). Homogeneity of variances was inspected using Levene test. For post-hoc comparison of means, Scheffé test was used. Differences in $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of soil animals between beech and ash trees were tested with single factor ANOVA. As data from beech and ash generally did not differ significantly animal taxa of the two tree species were pooled. Data given in text and figures represent means and standard errors.

3. Results

3.1. Soil, plants and ectomycorrhiza

$\delta^{13}C$ values in litter and soil (−20.1 ± 2.2 and −23.1 ± 0.5‰, respectively) were slightly increased compared to natural variations in the field (respective values of −26.8 ± 0.1 and −27.8 ± 0.2‰). In contrast, $\delta^{15}N$ values of litter and soil (744.1 ± 164.8 and 230.3 ± 38.6‰, respectively) were markedly increased compared to natural variations (respective values of −1.8 ± 1.7 and −0.2 ± 0.3‰).

In saplings both $\delta^{13}C$ and $\delta^{15}N$ signatures were markedly increased with the signatures increasing from stems (41.3 ± 3.6‰ and 5434 ± 327.8‰, respectively) to leaves (80.4 ± 8.2‰ and 3506 ± 322‰) to coarse roots (48.0 ± 6.6‰ and 6152 ± 676.8‰) to fine roots (113.3 ± 7.7‰ and 9328 ± 1066‰).

On average 96.0 ± 3.5‰ of vital root tips of beech were colonized by EMF. $\delta^{13}C$ and $\delta^{15}N$ values averaged 114.6 ± 8.2‰ and 13,484 ± 1929‰, respectively (Fig. 1).

3.2. Soil animals

In total 42 taxa of soil animals were analyzed (see Appendix). The overall mean $\delta^{13}C$ and $\delta^{15}N$ signatures of soil arthropods were −0.2 ± 6.6‰ and 2282 ± 507.5‰, respectively, markedly exceeding those of the soil and litter layer, but being lower than those of plant roots and in particular EMF (Fig. 1). Notably, this was true for each of the three trophic groups including predators with the highest stable isotope signatures. $\delta^{13}C$ and $\delta^{15}N$ signatures spanned from for *Eupelops plicatus* (−28.0 ± 0.7‰ and 82.9 ± 57.8‰, respectively) to *Clubiona compta* (76.0 ± 11.7‰ and 13,673 ± 1522‰).

Primary decomposers included 12 taxa with mean $\delta^{13}C$ and $\delta^{15}N$ values of −21.6 ± 4.8‰ and 666.8 ± 584.9‰ (Appendix, Fig. 2). $\delta^{13}C$ and $\delta^{15}N$ values ranged from *E. plicatus* (−28.0 ± 0.7‰ and 82.9 ± 57.8‰, respectively) to *Glomeris undulata* (−11.2 ± 3.7‰ and 1928 ± 465.1‰) (Fig. 2).

Secondary decomposers included 15 taxa with mean $\delta^{13}C$ and $\delta^{15}N$ values of 2.2 ± 8.0‰ and 2105 ± 504.8‰, respectively. $\delta^{13}C$ and $\delta^{15}N$ values were lowest in *Nothrus palustris* with −23.8 ± 1.5‰ and 951.8 ± 285.4‰, respectively, and highest in *Craspedosoma* sp.

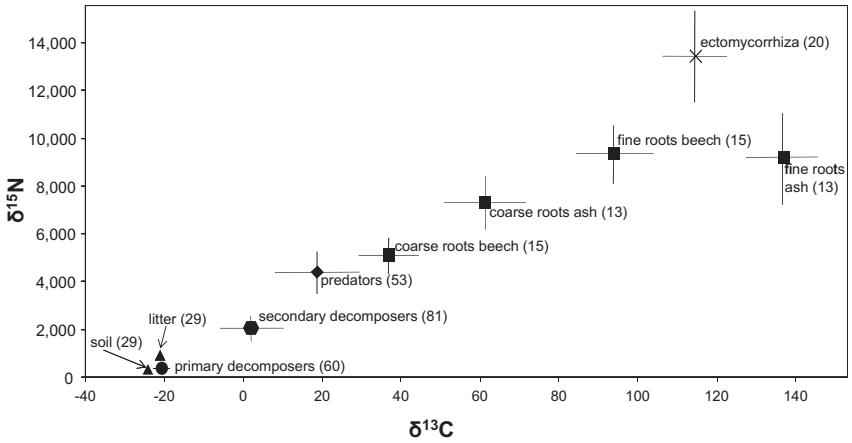

Fig. 1. Mean (±standard error) $\delta^{13}C$ and $\delta^{15}N$ value of primary decomposers (circle), secondary decomposers (hexagon) and predators (diamond). Means (±standard error) of $\delta^{13}C$ and $\delta^{15}N$ signatures of the soil and leaf litter (triangles) and coarse roots and fine roots of *Fagus sylvatica* and *Fraxinus excelsior* (squares) and of ectomycorrhiza (cross). Numbers of replicates are given in brackets.

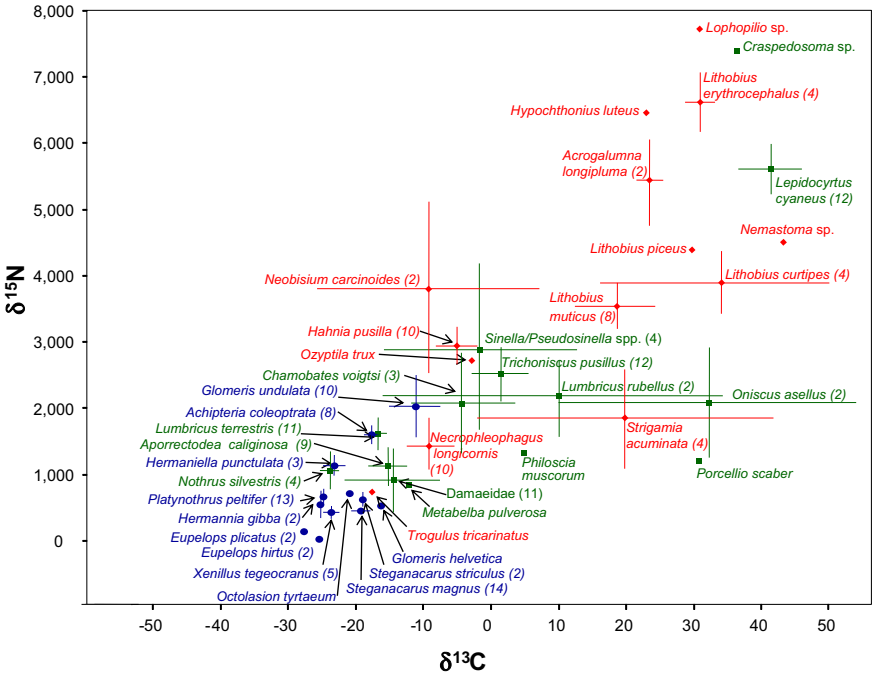

Fig. 2. Mean (±standard error) $\delta^{13}C$ and $\delta^{15}N$ signatures of primary decomposers (blue circles), secondary decomposers (green squares) and predators (red diamonds) (for details see Appendix). $\delta^{13}C$ and $\delta^{15}N$ values of *Clubiona compta* were 76.0 ± 11.7‰ and 13,673 ± 1522‰, respectively (not shown). Numbers of replicates are given in brackets. Dots without standard error represent single measurements.

with respective values of 38.8‰ and 7383‰ (both single measurements; Fig. 2).

Predators included 15 taxa with mean $\delta^{13}C$ and $\delta^{15}N$ values of 18.8 ± 10.8‰ and 4443 ± 876.8‰, respectively, differing significantly from respective values of primary and secondary decomposers ($F_{2,38} = 35.47$, $p < 0.0001$ and $F_{2,38} = 17.36$, $p < 0.0001$, respectively). $\delta^{13}C$ and $\delta^{15}N$ values were lowest in *Necrophloeophagus longicornis* with −9.2 ± 3.6‰ and 1331 ± 391.3‰, respectively, and highest in *C. compta* with respective values of 76.0 ± 11.7‰ and 13,673 ± 1522‰.

4. Discussion

The main objective of this study was to follow the flux of plant carbon and soil mineral nitrogen into the soil animal food web of temperate forests. Therefore, we labeled ash and beech tree saplings with $^{13}CO_2$ and added $^{15}NO_3^{15}NH_4$ to their rhizosphere. Ash and beech saplings were used for investigating the food web in the rhizosphere of plants colonized by EMF (beech) as compared to AMF (ash). The plants assimilated the $^{13}CO_2$, translocated the label to roots and in beech transferred it to EMF but little ^{13}C was transferred into soil and litter. Mineral nitrogen ($^{15}NO_3^{15}NH_4$) added to soil was transported via mycorrhizal fungi to plant roots as indicated by the signature of EMF exceeding that of beech fine roots. Similar to plant carbon, mineral nitrogen was only little incorporated into the soil but to some extent into litter probably by unspecific soaking during irrigation but $\delta^{15}N$ values in fine roots exceeded those in litter by more than a factor of twelve indicating that $^{15}NO_3^{15}NH_4$ was primarily assimilated by mycorrhizal fungi and transported to plant roots rather than immobilized by saprotrophic microorganisms and incorporated into litter (Lummer et al., 2012). Incorporation of label into higher trophic levels therefore likely was mainly via animals feeding on roots and/or AMF or EMF. However, in part $^{15}NO_3^{15}NH_4$ may also have been assimilated by algae potentially contributing to increased litter $\delta^{15}N$ signatures.

In contrast to our expectations, $\delta^{13}C$ and $\delta^{15}N$ signatures of soil animal species did not differ significantly between beech and ash treatments. The similar stable isotope signatures of soil animal species suggest that morphological and structural differences between the EMF rhizosphere of beech and the AMF rhizosphere of ash little affected the incorporation of label into higher order consumers. Potentially, stronger incorporation of label into soil animals via EMF in beech treatments was compensated by stronger transfer of label into soil animals via rhizosphere bacteria in ash treatments (Cesarz et al., 2013).

4.1. Primary decomposers

As expected, plant C and microbial N were little incorporated into primary decomposers supporting the assumption that they almost exclusively rely on litter and soil organic matter resources rather than root derived C and microbial N. This is consistent with findings of Pollierer et al. (2007) who also suggested that *Steganacarus magnus* and *Glomeris* sp. function as primary decomposers. However, primary decomposers were not trophically uniform. Rather, they formed a gradient of taxa that incorporated virtually no plant C and microbial N [*E. hirtus*, *E. plicatus*, *S. magnus*, *Steganacarus striculus*, *Platynothrus peltifer*, *Hermannia gibba*, *Xenillus tegeocranus* (all oribatid mites), *Glomeris helvetica* (Diplopoda) and *O. tyrtaeum* (Lumbricidae)] to those also incorporating plant C and microbial N [*Hermaniella punctulata*, *Achipteria coleoptrata* (both oribatid mites) and *Glomeris undulata* (Diplopoda)]. Presumably, in addition to dead organic matter the latter species to some extent also digested microorganisms that colonized these resources.

4.2. Secondary decomposers

Secondary decomposers incorporated more ^{13}C and ^{15}N than primary decomposers supporting the hypothesis that secondary

decomposers essentially rely on plant C and microbial N. However, ^{15}N and ^{13}C signatures of some secondary decomposer species overlapped with those of primary decomposers reflecting that in fact decomposer soil invertebrates form a gradient from species exclusively incorporating litter C to those exclusively feeding on microorganisms (Scheu and Falca, 2000). In fact, species rich taxa previously assumed to predominantly feed on fungi, such as Collembola and Oribatida, have been shown to partition resources ranging from plant litter to microorganisms to even higher order animal consumers (Schneider et al., 2004; Chahartaghi et al., 2005). In the present study, secondary decomposers of the lower end of this gradient included Damaeidae, *Metabelba pulverosa*, *N. palustris* (Oribatida), *Aporrectodea caliginosa*, *Lumbricus terrestris* (Lumbricidae), *Philoscia muscorum* and *Porcellio scaber* (Isopoda) whereas those at the higher end included *Chamobates voigtsi* (Oribatida), *Sinella/Pseudosinella* spp. (Collembola), *Lumbricus rubellus* (Lumbricidae), *Trichoniscus pusillus* and *Oniscus asellus* (Isopoda). $\delta^{15}N$ signatures of two secondary decomposers, i.e., *L. cyaneus* (Collembola) and *Craspedosoma* sp. (Diplopoda) were exceptionally high pointing to specific food resources. *L. cyaneus* is known to feed on algae (Scheunemann et al., 2010) and this may explain its high signature as algae on litter presumably directly incorporated ^{13}C and ^{15}N from the labeled atmospheric CO_2 and NH_4NO_3 in irrigation water. Unfortunately, measuring stable isotope signatures of algae growing on leaf litter is virtually impossible. For high stable isotope signatures of Craspedosomatidae the same as for *L. cyaneus* may apply. Notably, ^{13}C and ^{15}N signatures of secondary decomposers were considerably lower than those of EMF or roots indicating that none of them exclusively fed on mycorrhizal fungi; rather, the data suggest that they fed on a combined diet of mycorrhizal and saprotrophic fungi.

4.3. Predators

Contrary to our expectations, predators incorporated the highest amount of ^{13}C and ^{15}N. We hypothesized that predators predominantly feed on secondary decomposers, such as Collembola and Isopoda, as suggested earlier (Scheu, 2002). In part this hypothesis is supported as $\delta^{15}N$ and $\delta^{13}C$ signatures of e.g., *N. carcinoides* (Pseudoscorpionida), *Hahnia pusilla* and *Ozyptila trux* (both Araneida) were similar to secondary decomposers, indicating that these predators predominantly feed on secondary decomposers such as *Sinella/Pseudosinella* spp. (Collembola), *T. pusillus* (Isopoda) and *C. voigtsi* (Oribatida). However, both ^{15}N and ^{13}C signatures of most predator taxa, including *Lithobius muticus*, *Lithobius curtipes*, *Lithobius piceus*, *Lithobius erythrocephalus* (all Chilopoda), *Lophopilio* sp., *Nemastoma* sp. (Opilionida) *H. luteus* and *A. longipluma* (Oribatida), considerably exceeded that of the great majority of secondary decomposers indicating that they fed on higher labeled prey species such as the two highly labeled secondary decomposers *L. cyaneus* (Collembola) and *Craspedosoma* sp. (Diplopoda) and potentially other species not measured in this study, such as small Collembola, Nematoda and Enchytraeidae. Lithobiidae predominantly hunt in the litter layer (Poser, 1990) which is colonized by epigeic Collembola such as *L. cyaneus*. High $\delta^{13}C$ and $\delta^{15}N$ signatures of *Lophopilio* sp. and *Nemastoma* sp. presumably are related to the wide feeding strategies of many Opilionida including intraguild predation and cannibalism (Martens, 1978). Further, Lithobiidae and Opilionda likely also fed on as the highly labeled secondary decomposers *L. cyaneus* and *Craspedosoma* sp. The high $\delta^{13}C$ and $\delta^{15}N$ signatures of *H. luteus* and *A. longipluma* (Oribatida) likely are due to feeding on prey closely connected to the rhizosphere and the high label of roots. Hypochthoniidae are known to rely on belowground carbon and presumably predominantly prey on nematodes (Pollierer et al., 2012) and this also applies to Galumnidae (Rockett and Woodring, 1966; Muraoka and Ishibashi, 1976). Therefore, high $\delta^{13}C$ and $\delta^{15}N$ signatures of *H. luteus* and *A. longipluma* likely resulted from feeding on nematodes which either directly fed on roots or on mycorrhizal fungi. High stable isotope signatures in predators therefore presumably resulted from incorporation of the label via two different pathways, the one based on algae and algal feeders, the other based on root derived resources and associated nematodes. Potentially, the first pathway was more pronounced since the canopies of the tree seedlings were rather open thereby allowing more light entering the soil surface resulting in more pronounced algal growth.

Two predator taxa, *N. longicornis* and *Strigamia acuminata* (both Geophilomorpha), had rather low $\delta^{15}N$ signatures indicating that they fed on prey with low $\delta^{15}N$ signature, potentially a mixture of Lumbricidae and Isopoda. Indeed, Geophilomorpha are known to hunt for Lumbricidae by following them in large soil pores (Poser, 1990; Wolters and Ekschmitt, 1997). Low $\delta^{15}N$ signatures of *S. acuminata* may also be related to feeding on earthworms; however, high $\delta^{13}C$ signatures exceeding those of Lumbricidae suggest that they included also other prey, potentially Isopoda such as *O. asellus* and *P. scaber*.

4.4. Conclusions

Results of this study showed that primary and secondary decomposers comprise a gradient of species relying to different degrees on root C and microbial N. High stable isotope incorporation into EMF and considerably lower signatures in soil animals suggest that the animal species studied do not exclusively feed on mycorrhizal fungi; but long-term studies exceeding the life span of the animals are needed to prove this assumption. Surprisingly, predators were most intensively labeled with plant C and root N. Presumably, this high label was due to both feeding on algal consumers, such as the Collembola species *L. cyaneus*, and on plant rhizosphere associated root or mycorrhiza feeding nematodes. The results indicate that predators in soil animal food webs rely on very different carbon resources including algae, roots and microorganisms which are channeled to higher trophic levels predominantly via Collembola, Nematoda and Lumbricidae. Notably, dominant predators of temperate forests such as Lithobiidae appear to predominantly prey on species of litter dwelling Collembola such as *L. cyaneus*.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2013.03.002.

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