



Beech trees fuel soil animal food webs via root-derived nitrogen

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Abstract

Root-derived resources are receiving increased attention as basal resources for soil animal food webs. They predominantly function as carbon and energy resources for microbial metabolism in the rhizosphere, however, root-derived nitrogen may also be important. We explored both the role of root-derived carbon (C) and nitrogen (N) for the nutrition of soil animal species. Using ¹³C and ¹⁵N pulse labeling we followed in situ the flux of shoot-derived C and N into the soil animal food web of young beech (*Fagus sylvatica*) and ash (*Fraxinus excelsior*) trees. For labeling with ¹³C, trees were exposed to increased atmospheric concentrations of ¹³CO₂ and for labeling with ¹⁵N leaves were immersed in a solution of Ca¹⁵NO₃. Twenty days after labeling root-derived N was detected in each of the studied soil animal species whereas incorporation of root-derived C was only detected in the ash rhizosphere. More root-derived N was incorporated into soil animals from the beech as compared to the ash rhizosphere, in spite of the higher ¹⁵N signatures in fine roots of ash as compared to beech. The results suggest that soil animal food webs not only rely on root C but also on root N with the contribution of root N to soil animal nutrition varying with tree species. This novel pathway of plant N highlights the importance of root-derived resources for soil animal food webs.

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Introduction

The close interrelationship between the decomposer system and plants is mediated by leaf litter input and rhizodeposition (Wardle 2002). As up to 90% of net primary plant

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production enters the soil as detritus (Cebrian 1999), litter has long been assumed to function as the main food source for soil animals, however, recent work has shown that other resources such as those derived from roots may be more important (Ruf, Kuzyakov, & Lopatovskaya 2006; Pollierer, Langel, Körner, Maraun, & Scheu 2007; Eissfeller, Beyer et al. 2013). A variety of substances are released actively or passively from roots into the soil as rhizodeposits (Curl & Truelove 1986; Jones, Nguyen, & Finlay 2009). Rhizodeposits are divided into exudates, leakages, secretions, mucilages, mucigel and lysates (Rovira, Foster, & Martin 1979; Curl & Truelove 1986), and include both carbon (C) and nitrogen (N) containing compounds with C compounds being most important. As nitrogen typically is transferred from soil to roots and incorporated into plants, the opposite pathway with nitrogen being transferred from plants into the soil is assumed to be only of significant importance in N fixing plants such as legumes (Ayres, Dromph, Cook, Ostle, & Bardgett 2007), but the role of this pathway in other plants has received little attention (Wichern, Eberhardt, Mayer, Joergensen, & Müller 2008). In addition to fueling microorganisms and fostering microbial biomass in the rhizosphere, rhizodeposits affect mutualistic and antagonistic interactions between soil microorganisms and plants (Bais, Weir, Perry, Gilroy, & Vivanco 2006).

Soil animal communities of deciduous forests are remarkably diverse (Anderson 1975; Schaefer 1991; Scheu 2005) and form complex food webs (Digel, Curtsdotter, Riede, Klamer, & Brose 2014; Ehnes et al. 2014). These food webs span a wide range of trophic levels including primary and secondary decomposers, and first, second and third order predators (Ponsard & Arditi 2000; Scheu & Falca 2000). Soil animals are affected by soil properties (Langenbruch, Helfrich, & Flessa 2012), plant species (Scheu 2005; Eissfeller, Langenbruch, Jacob, Maraun, & Scheu 2013) and soil microorganisms (Esperschütz et al. 2009; Koranda et al. 2011), with the latter two being mediated by rhizodeposits. Plant allocation of C to roots and into the rhizosphere received considerable attention in trees (Högberg et al. 2008; Subke et al. 2009; Kuzyakov & Gavrichkova 2010), but N allocation to roots and into the rhizosphere has been investigated for herbaceous plants in particular legumes (Ayres et al. 2007; Wichern et al. 2008), whereas information on trees is lacking.

To investigate the flux of C and N from plants into the belowground system stable isotopes are increasingly used (Hertenberger & Wanek 2004; Högberg et al. 2008). Adopting this approach we conducted a pulse labeling experiment in the field. By exposing trees to increased atmospheric ^{13}C concentrations and by immersing leaves in a $\text{Ca}^{15}\text{NO}_3$ solution we followed the flux of C and N into the soil animal food web. ^{13}C labeling by exposing trees to increased atmospheric ^{13}C concentrations is widely used (Högberg et al. 2008; Eissfeller, Beyer et al. 2013; Goncharov, Tsurikov, Potapov, & Tiunov 2016). For aboveground ^{15}N labeling, leaf feeding is commonly used (Wichern et al. 2008). Besides urea (Høgh-Jensen & Schjoerring 2000), nitrate has been used in

leaf feeding (Brumme, Leimcke, & Matzner 1992; Sierra, Daudin, Domenach, Nygren, & Desfontaines 2007; Jalonen, Nygren, & Sierra 2009). Using European beech (*Fagus sylvatica*) and common ash (*Fraxinus excelsior*) we examined if the effect of trees on the soil animal food web via rhizodeposits varies between tree species. Beech and ash were chosen as they differ in nutrient allocation patterns and mycorrhizal types. Beech roots are associated with ectomycorrhizal (EM) and ash roots with arbuscular mycorrhizal (AM) fungi. The following hypotheses were investigated: (1) incorporation of root-derived carbon into the soil animal food web varies between tree species and is more pronounced in EM beech than AM ash trees, and (2) root-derived nitrogen is of minor importance for soil animal nutrition and therefore, incorporation into the soil animal food web varies little with tree species.

Materials and methods

Study site

The experiment was conducted in a temperate deciduous beech forest in the Hainich National Park (Thüringen, Germany) near Weberstedt (51°05'N, 10°28'E) at 300 m asl. Mean annual precipitation is 670 mm and mean annual air temperature 7.5 °C. With 16,000 ha the Hainich National Park is the largest continuous deciduous forest in Germany and has been declared World Heritage Nature Site in June 2011. The forest predominantly consists of beech (*F. sylvatica*) stocking on Luvisol developed on loess underlain by Triassic Shell Limestone. The forest floor is classified as mull-like moder and the mean thickness of the litter layer is 2.8 ± 0.1 cm (Jacob, Viedenz, Polle, & Thomas 2010; Langenbruch, Helfrich, Joergensen, Gordon, & Flessa 2014). The topsoil (0–10 cm) is rather acidic with a pH_{KCl} of 3.3 (Mölder, Bernhardt-Römermann, & Schmidt 2006; Guckland, Jacob, Flessa, Thomas, & Leuschner 2009).

Labeling

In August 2011 eight young trees, four beech and four ash, were selected for labeling. Two beech trees and two ash trees served as controls. Trees were 2.5–4.0 m in height and 5 m or more apart from each other. The young trees grew in the understory of a closed-canopy beech forest. Around each tree used for labeling an area of 1×1 m was trenched by inserting polyethylene (PE) panels (thickness: 3 mm). The panels extended 10 cm into the soil and 10 cm above the soil surface to avoid immigration of animals.

For ^{15}N labeling 36 g $\text{Ca}^{15}\text{NO}_3$ (99.23 atom% ^{15}N , Campro Scientific GmbH, Berlin, Germany) was dissolved in 1200 ml sterile water resulting in a 0.18 M solution. Leaves were fed with this solution by inserting three leaves of beech or three leaflets of the compound leaves of ash into a vial con-

taining 9.7 ml Ca¹⁵NO₃ solution. Three vials per tree were installed and leaves were incubated for 72 h. To increase the uptake of N, leaves were scratched prior to placement into the vials. To avoid leakage vials were covered with plastic film and enclosed into plastic bags.

For ¹³C labeling, trees were enclosed in plastic foil (thickness: 0.08 mm) fixed to wooden poles erected around the trees. Enclosed trees were labeled with ¹³CO₂ by adding 60 ml 5 M H₂SO₄ to a solution containing 6.85 g Na₂¹³CO₃ (99.0 atom% ¹³C; Cambridge Isotope Laboratories, Tewksbury, USA) dissolved in 100 ml distilled water. Air inside the enclosures was mixed using a ventilator. The labeling started at about 11 a.m. and lasted about 5 h. Thereafter, the plastic foil was removed.

Twenty days after labeling the trees, soil samples (diameter: 21 cm, depth: 10 cm) were taken from the trenched quadrates. Two samples spaced at least 10 cm were taken from each unlabeled and labeled tree. Soil animals were extracted by heat using a high-gradient canister method (Kempson, Lloyd, & Ghelardi 1963) and stored in concentrated salt water at -7 °C. Fresh fine roots (diameter <2 mm) were sampled after excavating trees immediately after taking soil cores for extracting soil animals. Soil particles were carefully removed from the roots before samples were freeze-dried, ground in a ball mill (Retsch Schwingmuehle MM2, Haan, Germany) and stored in a desiccator until further analysis. For stable isotope analysis 1.5 mg of fine root dry weight were filled into tin capsules.

Stable isotope analysis

Seven species of moss mites (Oribatida: *Chamobates* sp. Hull, 1916, *Damaeus gracilipes* Kulczynski, 1902, *Damaeus riparius* Nicolet, 1855, *Damaeus onustus* C.L. Koch, 1844, *Phthiracarus* sp, Perty, 1841, *Steganacarus magnus* Nicolet, 1855, *Xenillus tegeocranus* Hermann, 1804), one woodlouse species (Isopoda: *Porcellium conspersum* C.L. Koch, 1841) and two centipede species (Chilopoda: *Lithobius mutabilis* L. Koch, 1862, *Strigamia acuminata* Leach 1814) were prepared for stable isotope analysis with one to eight replicates per individual tree. The species represent major taxa of mesofauna detritivores (Oribatida), macrofauna detritivores (Isopoda) and macrofauna predators (Chilopoda). For *D. gracilipes*, *S. magnus* and *X. tegeocranus* four replicates for each tree species were used, while for *D. riparius* and *P. conspersum* only three replicates were used as they did not occur in soil of each of the labeled trees. For dual C and N stable isotope ratio analysis 100–300 µg of animal tissue were transferred into tin capsules and dried at 40 °C for 24 h. Single individuals of large Oribatida were used, but for most Oribatida species several individuals had to be pooled. In the Isopoda only the head was used to prevent including food material in the gut. Stable isotope ratios were analyzed using a system consisting of an elemental analyzer (NA 1500, Fisons-Instruments, Rodano, Milan, Italy) and a mass spectrometer

(Delta plus, Finnigan MAT, Bremen, Germany) coupled by a ConFlo III interface (Thermo Electron Corporation, Bremen, Germany). The computer controlled system allows on-line measurement of stable isotopes (¹³C and ¹⁵N). Their abundance (δ_x) is expressed using the δ notation as

$$\delta_x[\text{‰}] = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000$$

with R_{sample} and R_{standard} representing ¹³C/¹²C and ¹⁵N/¹⁴N ratios of samples and standard, respectively. For ¹³C PD belemnite (PDB) and for ¹⁵N atmospheric nitrogen served as the primary standard. Acetanilide (C₈H₉NO, Merck, Darmstadt, Germany) was used for internal calibration. The precision of the measurement is <0.2 ‰ (Reineking, Langel, & Schikowski 1993).

Calculation of Δ¹³C and Δ¹⁵N values

For analyzing the enrichment in ¹⁵N and ¹³C of soil animals and fine roots we calculated the difference in delta values between animals and fine roots from labeled and unlabeled trees as Δ_{element} = δ_{label} - δ_{control} with Δ_{element} the Δ¹³C and Δ¹⁵N values, and δ_{control} the mean values of animal species from unlabeled beech and ash trees. Values with mean Δ¹³C and Δ¹⁵N in the range of two standard deviations of δ¹³C and δ¹⁵N of samples from unlabeled trees were assumed not to be enriched and set to zero.

Statistical analysis

Statistical analyses were performed using R v.3.2.4 (R Core Team 2016) and the 'nlme' package (Pinheiro, Bates, DebRoy, & Sarkar 2017). Stable isotope signatures of fine roots were used as covariate, but as variations in the signatures of the animals were not significantly related to isotope signatures of roots (C: F_{1,6} = 0.64, P = 0.455; N: F_{1,6} = 0.34, P = 0.579) of the respective tree, the covariate was removed from the final model. Δ¹³C and Δ¹⁵N values of fine roots were analyzed separately using one-factorial ANOVA to test for the effect of tree species on ¹³C and ¹⁵N enrichment in fine roots.

Prior to statistical analysis of soil animals mean Δ¹⁵N and Δ¹³C per species and individual tree were calculated to avoid pseudo-replication. Only five species (*D. gracilipes*, *D. riparius*, *P. conspersum*, *S. magnus*, *X. tegeocranus*) with three to four replicates per tree species (beech and ash) were included in the statistical analyses. Δ¹⁵N and Δ¹³C values of soil animals were analyzed separately using linear mixed effects models including a random effect of tree identity to allow testing for the effect of tree species and C and N incorporation into soil animal species avoiding pseudo-replication of soil animal species of the same tree. Fixed factors were Tree species (beech, ash) and Animal species (*D. gracilipes*, *D. riparius*, *P. conspersum*, *S. magnus*, *X. tegeocranus*). The interaction term was removed from the final models as it was

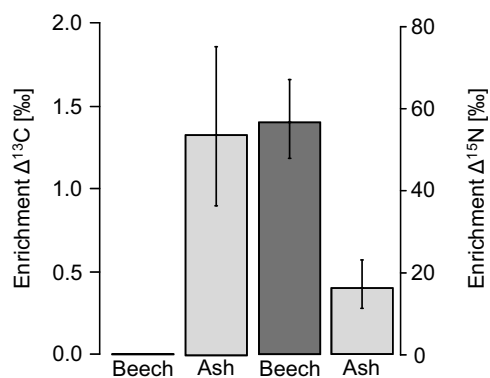


Fig. 1. Enrichment in $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$ in soil animals (average of all species analyzed) under beech (dark grey) and ash (light grey) 20 days after labeling with $^{13}\text{CO}_2$ and $\text{Ca}^{15}\text{NO}_3$. Means and standard errors (SE) are back-transformed values of log-transformed data.

not significant. $\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$ values were log-transformed to improve homogeneity of variance. Data given in text and figures represent means and standard errors.

Results

For five detritivore species (*D. gracilipes*, *D. riparius*, *P. conspersum*, *S. magnus*, *X. tegeocranus*) enough individuals were found to include into the statistical analyses. Additionally, two species of predatory macrofauna and two species of detritivore mesofauna were analyzed.

Natural ^{15}N signatures in the five analyzed soil animal species increased in the order *P. conspersum* (-2.40 ± 0.2 ‰) < *S. magnus* (-2.19 ± 0.25 ‰) < *X. tegeocranus* (-2.18 ± 0.48 ‰) < *D. gracilipes* (-0.69 ± 0.07 ‰) < *D. riparius* (-0.57 ± 0.39 ‰). Respective values for $\delta^{13}\text{C}$ were -25.39 ± 0.38 , -20.8 ± 0.47 , -24.26 ± 0.44 , -25.26 ± 0.25 , -25.61 ± 0.61 ‰. Enrichment in ^{13}C and ^{15}N did not differ significantly between primary and secondary decomposers ($F_{1,7} = 2.77$, $P = 0.14$ and $F_{1,7} = 0.44$, $P = 0.53$, respectively).

Overall, detritivore species (*D. gracilipes*, *D. riparius*, *P. conspersum*, *S. magnus*, *X. tegeocranus*) did not differ significantly in ^{13}C enrichment ($F_{4,26} = 1.21$, $P = 0.33$). $\Delta^{13}\text{C}$ values of detritivore species differed between tree species ($F_{1,6} = 20.56$, $P = 0.004$); detritivore species under beech generally were not enriched in ^{13}C , whereas under ash they were slightly enriched but the enrichment varied strongly (0.56 ± 0.14 ‰; Fig. 1) between individuals. $\Delta^{13}\text{C}$ values of fine roots of beech and ash did not differ significantly, although they were higher in ash (36.57 ± 17.78 ‰) than in beech (19.36 ± 12.72 ‰; $F_{1,6} = 1.32$, $P = 0.295$, Fig. 2) matching the enrichment observed in detritivore species.

In contrast to ^{13}C , $\Delta^{15}\text{N}$ values significantly differed between detritivore species ($F_{4,26} = 3.32$, $P = 0.025$) and declined in the order *P. conspersum* > *X. tegeocranus* > *D. gracilipes* > *S. magnus* > *D. riparius* (Fig. 3). Again, in con-

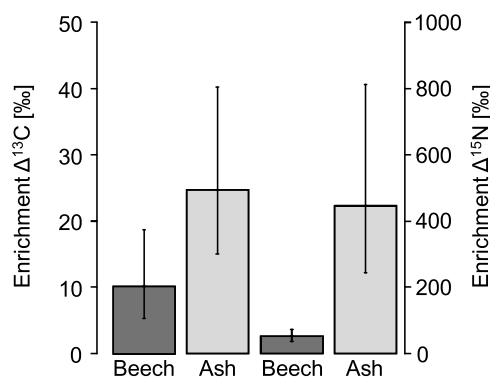


Fig. 2. Enrichment in $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$ in fine roots in beech (dark grey) and ash (light grey) 20 days after labeling with $^{13}\text{CO}_2$ and $\text{Ca}^{15}\text{NO}_3$. Means and standard errors (SE) are back-transformed values of log-transformed data.

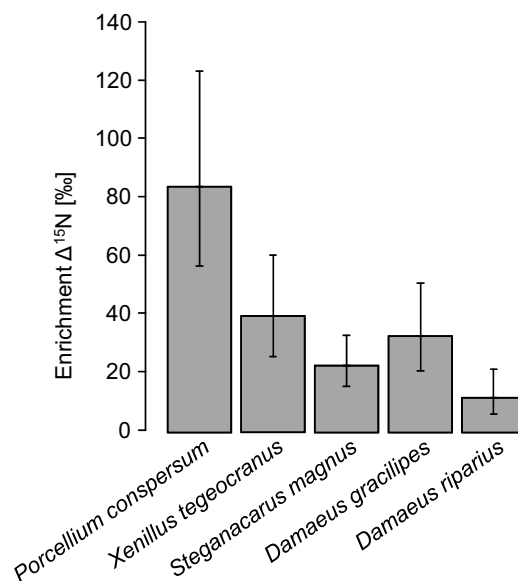


Fig. 3. Enrichment in $\Delta^{15}\text{N}$ in soil animal species 20 days after labeling with $^{13}\text{CO}_2$ and $\text{Ca}^{15}\text{NO}_3$. Means and standard errors (SE) are back-transformed values of log-transformed data.

trast to ^{13}C , $\Delta^{15}\text{N}$ values of detritivore species varied markedly between tree species ($F_{1,6} = 13.85$, $P = 0.010$); on average, detritivore species under beech (74.65 ± 13.61 ‰) were more enriched than those under ash (43.77 ± 15.61 ‰). $\Delta^{15}\text{N}$ values of fine roots of beech and ash also significantly differed, but contrary to animal signatures; $\Delta^{15}\text{N}$ values in ash fine roots (727.56 ± 364 ‰) markedly exceeded those in beech fine roots (62.19 ± 20.19 ‰; $F_{1,6} = 9.98$, $P = 0.020$, Fig. 2). The predatory macrofauna (*L. mutabilis*, *S. acuminata*) and detritivore mesofauna (*Chamobates* sp., *D. onustus*, *Phthiracarus* sp.), which were not replicated over the full design also were not enriched in ^{13}C but highly enriched in ^{15}N (see Appendix A).

Discussion

Incorporation of root carbon

Contrary to our expectations, detritivore species incorporated little root-derived C. As it is well established that soil animals heavily rely on root-derived carbon (Ruf et al. 2006; Pollierer et al. 2007; Eissfeller, Beyer et al. 2013), the experimental set-up and the amount of ^{13}C for labeling may have been responsible for this result. Unlike to most previous studies we used an in situ approach by labeling understory trees in the field. Under these conditions, a number of factors may have contributed to low uptake of $^{13}\text{CO}_2$ by the trees, most importantly shading by mature trees resulting in low photosynthetic activity. Further, the amount of label and the duration of the labeling period may not have been sufficient to allow tracing the signal in soil animals as typically only 2–4% of the C fixed by plants is transferred into the soil (Jones, Hodge, & Kuzyakov 2004). Also, heterogeneously distributed roots, typical for field growing trees, and a mismatch between the localities where rhizodeposits were released and where soil cores for extracting soil animals were taken may have contributed to the low incorporation of root C into the soil animals analyzed.

Although incorporation of root C was generally low, in the rhizosphere of ash it exceeded that in beech where no incorporation was detectable suggesting that our first hypothesis has to be rejected. The low incorporation under beech trees is surprising as extramatrical hyphae of EM fungi associated with these trees enhance carbon translocation into soil (Högberg et al. 2008; Esperschütz et al. 2009; Cairney 2012) and the soil food web (Cesarz et al. 2013). Indeed, beech trees released more ^{13}C into the soil than ash trees (Sommer, Dippold, Flessa, & Kuzyakov 2016). Potentially, the complex rhizosphere community resulted in increased incorporation of ^{13}C into soil animals in the ash rhizosphere, however, understanding the more pronounced incorporation into the ash rhizosphere needs further attention.

Incorporation of root nitrogen

In contrast to our second hypothesis soil animals were markedly enriched in ^{15}N demonstrating that root-derived N forms part of the resources fueling soil animal food webs. Foliar nitrate uptake is well known (Brumme et al. 1992; Rennenberg & Gessler 1999) followed by translocation of the assimilated nitrogen within the plant and toward roots (Uscola, Villar-Salvador, Olet, & Warren 2014). Presumably, N compounds entered the rhizosphere and the surrounding soil via rhizodeposition (Curl & Truelove 1986; Merbach et al. 1999; Bais et al. 2006). Except for legumes this has been shown mainly for grasses (Hertenberger & Wanek 2004; Wichern et al. 2008) but not for trees. Root-derived N compounds are likely to be taken up quickly by microorganisms, and via microbivorous soil animals they are incorporated into

the soil animal food web. However, feeding on living or dead roots may also contribute to the flux of root N into soil animal food webs as some detritivores occasionally also feed on roots (Endlweber, Ruess, & Scheu 2009). In the present experiment nitrogen assimilated by leaves was transferred to the roots (Sommer et al. 2016), which is in agreement with results of an earlier study on beech (Brumme et al. 1992).

Notably, incorporation of root-derived N into soil animals varied between species and decreased in the order *P. conspersum* > *X. tegeocranus* > *D. gracilipes* > *S. magnus* > *D. riparius*. According to natural variations in stable isotope signatures in the present experiment *P. conspersum*, *X. tegeocranus* and *S. magnus* functioned as primary decomposers while *D. gracilipes* and *D. riparius* functioned as secondary decomposers which conforms to previous studies (Scheu & Falca 2000; Schneider et al. 2004; Maraun et al. 2011). Incorporation of root-derived N into these species was unlikely due to feeding on roots as ^{15}N signatures of fine roots were not correlated with those of animals (see Material and methods). Rather, root-derived N likely was taken up by microorganisms and thereby also transferred into leaf litter (Lummer, Scheu, & Butenschoen 2012) and, via feeding on litter, it was incorporated into primary decomposer animals such as *P. conspersum*, *X. tegeocranus* and *S. magnus* (Rihani, Cancela da Fonseca, & Kiffer 1995; David & Gillon 2002). In secondary decomposers root-derived N likely was incorporated by feeding on microorganisms in particular fungal hyphae. Further, in certain soil mite species such as *S. magnus* root-derived N may have been incorporated via feeding on microbivorous or root-feeding nematodes (Heidemann, Scheu, Ruess, & Maraun 2011). This suggests that root-derived N propagates quickly into soil decomposers with nematodes likely contributing to this process (Heidemann, Ruess, Scheu, & Maraun 2014).

Each of the studied soil animal species incorporated more ^{15}N under beech than under ash and this contrasted the concentrations of ^{15}N in fine roots of beech and ash. Conform to the incorporation into soil animals, beech rhizosphere soil was more enriched in ^{15}N as compared to ash (Sommer et al. 2016). This suggests that rhizodeposition in beech including N compounds exceeds that in ash which supports recent findings (Holzwarth, Daenner, & Flessa 2011; Cesarz et al. 2013).

Conclusion

Incorporation of root-derived C into soil animals was low in this experiment, but this likely was due to low uptake of $^{13}\text{CO}_2$ by the studied understory trees and low transfer of ^{13}C into the rhizosphere. Low ^{13}C addition may have contributed to these findings. Surprisingly, root-derived N was incorporated into soil animals and this was more pronounced under beech associated with EM fungi as compared to ash associated with AM fungi. The results therefore support recent findings that the effect of EM beech on rhizosphere

microorganisms exceeds that of AM ash (Cesarz et al. 2013). Notably, all animal species studied incorporated root-derived N with the incorporation varying with animal species but not with trophic group, reflecting that soil animals within trophic groups incorporate root-derived resources to different extents.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.baec.2017.06.006>.

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