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Short-term incorporation of freshly fixed plant carbon into the soil animal food web: field study in a spruce forest

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Abstract We analyzed the dynamics of the short-term incorporation of recently fixed carbon into the below-ground food web in a boreal forest. Five young spruce trees (*Picea abies*) were pulse-labeled with $^{13}CO_2$ and the isotopic label was traced in soil invertebrates during 5 weeks. The freshly fixed plant carbon quickly entered both litter-located and soil-located compartments of the detrital food web. Among invertebrates inhabiting the mineral soil layers, a trophic link to the root-derived C was most pronounced in species with higher $\delta^{15}N$ values, suggesting this energy source to be more important in deeper mineral soil horizons. The label appeared faster in saprophagous animals than in predators (the median time lag after labeling was 6 and 12 days, respectively), but the difference was not significant. The label was recovered in 15 of 38 species of saprophagous animals and in 20 of 63 species of predators. Among saprophages, the frequency and intensity of the label was relatively high in endogeic collembolans and in bibionid larvae, but earthworms and enchytraeids were not labeled. Several groups of predators, lithobiid centipedes in particular, quickly acquired the root-derived carbon, possibly indicating the feeding on live roots or mycorrhizal mycelium. In total, only 35 % of species or genera examined acquired the label. This suggests that majority of invertebrate taxa in the decomposer food web are unlikely to depend heavily on freshly fixed plant carbon provided by roots and root-associated microorganisms.

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Introduction

In forest ecosystems the main part of annual primary production is processed by the decomposer food web (Swift et al. 1979). Plant-fixed carbon reaches detrital food webs via several input channels: as above-ground litter, as below-ground litter and as a flux of root exudates that in forest ecosystems are largely directed into mycorrhizal fungi and other root-associated microorganisms (Smith and Read 1997; Bardgett et al. 2005; Bais et al. 2006). Up to half of the forest primary production is allocated below-ground as root litter and rhizodeposits (Litton et al. 2007). Mycorrhizal mycelium can contribute strongly to the formation of stabilized soil organic matter (Clemmensen et al. 2013; Ekblad et al. 2013), but the importance of 'root carbon' in the energy budget of detrital food webs remains poorly quantified (Pollierer et al. 2007; Metcalfe et al. 2011). Laboratory (Ruf et al. 2006; Eissfeller et al. 2013) and field studies (Högberg et al. 2010; Churchland et al. 2012) using whole-plant ^{13}C-labeling suggest that a broad array of soil invertebrates depend on recent photosynthates, although the importance of this trophic link differs widely between species. Rhizodeposits and exudates are consumed by root-associated fungi and bacteria that are in turn grazed upon by protozoans and microbivorous metazoan animals (Inderjit and Weston 2003; Bonkowski et al. 2009). Root carbon can therefore reach soil animals via both bacterial and fungal energy channels (Pollierer et al. 2012; Scheunemann et al. 2016). In addition, many saprophagous soil animals like earthworms can feed directly on live plant roots (Cortez and Bouché 1992; Gunn and Cherrett 1993).

A dependence on recently fixed carbon is likely to be most pronounced in certain groups of saprophagous invertebrates feeding on microorganisms in the rhizosphere. Soil saprophages inhabiting mineral soil are likely

to be limited by carbon availability (Maraun et al. 2001; Tiunov and Scheu 2004). A stronger importance of easily available root-derived carbon can therefore be suggested for endogeic (living in mineral soil) animals compared to litter dwellers. In contrast to this expectation, the label was often recovered in epigeic (litter-dwelling) collembolans, earthworms and millipedes in whole-tree ^{13}C–CO_2 labeling experiments (Pollierer et al. 2007, 2012; Gilbert et al. 2014). As far as we know, the distribution of root carbon in litter- and mineral soil-dwelling animals has never been explicitly tested. The importance of the root carbon should likely decrease towards higher trophic levels because predators unify trophic chains that are based on different sources, including above-ground and below-ground litter. Nevertheless, very strong trophic links of predatory animals to root-derived carbon were found in a laboratory experiment (Eissfeller et al. 2013). Exact mechanisms of the carbon transmission from roots to soil predators remain unclear.

The commonly reported phloem transport rate is about 1 m h^{-1} (Kuzyakov and Gavrichkova 2010), while the time lag between ^{13}C-labeling and $^{13}CO_2$ efflux from the soil under common forest tree species (pine, oak, beech) ranges within 0.5–1.5 days (Plain et al. 2009; Högberg et al. 2010; Epron et al. 2011). In forest ecosystems the label was detected in ectomycorrhizal fungi and soil mesofauna 2–4 days after labeling (Högberg et al. 2010, Churchland et al. 2012), whereas soil fauna in grasslands can start incorporating the label even faster, within less than 24 h (Ostle et al. 2007; Seeber et al. 2012). As the label is processed by a microbial community in the rhizosphere, it becomes available for the microbiphagous and saprophagous soil animals that belong to different compartments of below-ground food webs (Pollierer et al. 2009). Eventually, the label is incorporated in soil organic matter and can be acquired by the animals that seem to have no direct trophic links to plant roots (Pollierer et al. 2007; Gilbert et al. 2014). Short-term experiments are therefore needed for identifying invertebrates that acquire fresh photosynthates via relatively short trophic links.

The main purpose of this study was to analyze the dynamics of the short-term incorporation of recently fixed carbon into below-ground food webs. To this end, we pulse-labeled five young spruce trees (*Picea abies*) with $^{13}CO_2$ and traced the ^{13}C label in soil invertebrates during 5 weeks. We hypothesized that the isotopic label should be more intense and/or frequent, (1) in soil-dwelling animals compared to litter-dwellers, and (2) in saprophagous animals compared to predators.

Materials and methods

Study site and experimental setup

The field experiment was conducted at the Chernogolovka Biological Station of the Institute of Ecology and Evolution RAS, Moscow Region, Russia (56°01′32″N, 38°25′47″E). The dominating tree species at the study sites were Norway spruce (*Picea abies*), with an admixture of birch (*Betula pendula*) and ash (*Acer platanoides*). The herb layer was dominated by *Aegopodium podagraria*, *Carex pilosa* and *Bromopsis inermis*. Annual precipitation averages 669 mm, annual temperature is +5 °C, soils are acidic (pH 4.5–5.0), soil temperature (at a depth of 5 cm) ranged from +13 to +14 °C during tree labeling and the sampling of invertebrates.

We used five spruce saplings (height from 3.5 to 4.5 m) for labeling. Four months before the labeling, a stainless steel fence was buried to a depth of 40 cm into the soil around each tree, at a distance of 1.7 m from the trunk. Inside the fence, all ground vegetation was removed. A rectangular wooden frame (3 × 3 m, 4 m tall) was erected around each tree. The frame was covered with a polypropylene film (180 μm thick). The bottom of the chambers was positioned 30–35 cm above the ground. In each chamber five electric fans with a total capacity of 12 m^3 min^{-1} were installed. The labeling was performed in early September 2012. At this season the flux of fresh photosynthates in mycorrhizal fungi and other root-associated microorganisms is most intense (Högberg et al. 2010; Epron et al. 2011). Four liters of 99 % ^{13}C–CO_2 were injected into each chamber on a cloudless morning (from 9 to 11 a.m.), immediately after the trees had completely been isolated by the chambers. The latter were removed after 60 h.

Sampling

One soil core (20 × 20 cm, 20 cm in depth) was taken inside each fenced area immediately before and 2 days after the labeling, and subsequently every 3–10 days till day 44. Each sample was divided into two horizons: litter layer (A_0) and soil layer (0–20 cm). Soil macroinvertebrates were obtained from each horizon by hand sorting the litter and soil. The animals collected were immediately frozen and stored at −10 °C until identification. Collembolans and mites were extracted from soil and litter using Tullgren funnels and stored in 70 % ethanol until identification. Aliquots of the soil and litter were collected, dried (48 h, 50 °C) and stored until analysis. From each soil sample, fine roots (< 2 mm) were extracted by wet sieving and a mixed sample of fungal mycelium was collected under a dissecting microscope from fermented litter. Along with soil samples, green needles were collected from labeled trees. For each tree, a few needles were taken at 0.5, 1.5 and 2.5 m heights, and subsequently bulked.

Stable isotope analysis

Needles, roots, litter and soil samples were dried and milled with a ball mill (MM 200, Retsch, Germany). Aliquots (1200–1700 μg) of a homogenized sample were sealed in 5 × 8 mm tin capsules. Samples of animals

ranged in weight from 50 to 600 μg. Large invertebrates like earthworms or carabid beetles were dissected under stereo microscope and samples of muscular tissues were taken for the isotope analysis. Smaller macroinvertebrates were analyzed individually as a whole. Several conspecific individuals of collembolans or mites were bulked into a single sample. Stable isotope ratios were measured using a Thermo Delta V Plus IRMS coupled to a Flash 1112 Elemental Analyzer at the A.N. Severtsov Institute of Ecology and Evolution RAS (Moscow, Russia). Stable isotope composition is reported as per mil (‰) using conventional delta notation: $\delta X = [(R_{sample}/R_{standard})-1]$, where X is ^{15}N or ^{13}C and R is the corresponding ratio of $^{15}N/^{14}N$ or $^{13}C/^{12}C$. Standard materials were atmospheric nitrogen and VPDB carbon. An internal laboratory standard (casein) was run every ten samples. The mass spectrometer was calibrated using three International Atomic Energy Agency reference materials (glutamic acid USGS 40 and USGS 41; cellulose IAEA-CH3). Measurement errors for USGS 40 [SD, n = 8] did not exceed ± 0.15 ‰ for $\delta^{13}C$ and $\delta^{15}N$. The $\delta^{15}N$ values of litter samples collected under individual labeled trees ranged from −2.2 to −2.7 ‰. For statistical analyses we therefore used litter-normalized $\delta^{15}N$ values of soil animals ($\Delta\delta^{15}N = \delta^{15}N_{animal}–\delta^{15}N_{litter}$).

Data analysis and statistics

The $\delta^{13}C$ values of soil, litter and animal samples collected before labeling never exceeded −23.0 ‰ (Fig. 1). This value was therefore adopted as the threshold unequivocally dividing 'labeled' and unlabeled samples. This approach produced a conservative estimate of label distribution.

In this study we tried to incorporate both, between-species and within-species variation in the ability to use freshly-fixed carbon. Average $\delta^{13}C$ values of individual taxa depend critically on the proportion of labeled individuals. Using average $\delta^{13}C$ values might therefore lead to overestimating the importance of the 'root carbon' in detrital food webs. Besides, average values (and associated parametric measures of variation) could not be used for $\delta^{13}C$ values in post-labeling samples due to strongly un-normal distribution. To describe the occurrence and intensity of the label, three main parameters were therefore used: (1) median $\delta^{13}C$ values of all samples collected after labeling, (2) median $\delta^{13}C$ values of labeled samples, hereafter denoted as $\delta^{13}C_L$, and (3) the proportion of labeled samples (LP) in the total number of samples collected after labeling, which reflects (although likely underestimates) the frequency of labeled animals of certain taxonomic groups. Based on the taxonomic identity, the collected animals were separated into sapro-microbiphages (such as bibionid larvae, earthworms, collembolans, oribatid mites) and predators (such as centipedes, spiders, cantharid larvae).

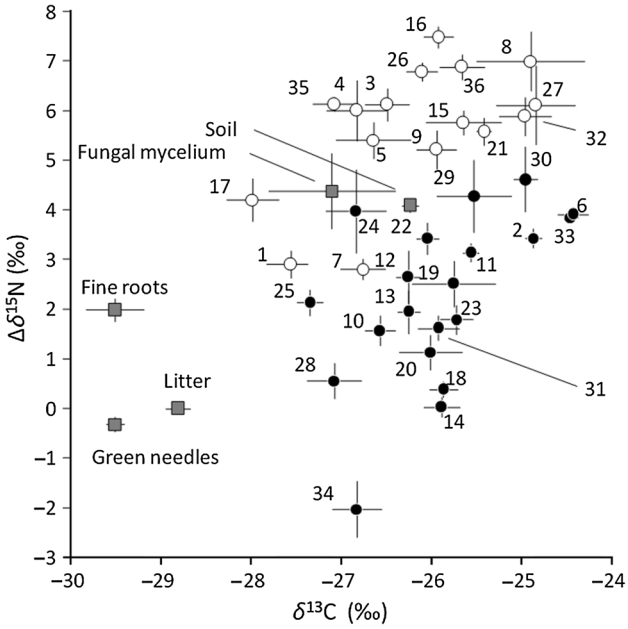

Fig. 1 Stable isotope composition (mean $\delta^{13}C$ and $\Delta\delta^{15}N$ values and 1 SE) of soil animals before labeling. *Open dots* represent predators, *closed dots* are saprophages, *grey squares* are plants, fungi and soil. *1—Amara brunnea* (Carabidae), *2*—Lumbricidae, *3*—Asilidae, *4—Athous niger* (Elateridae), *5—Athous subfuscus* (Elateridae), *6—Bibio* sp. (Bibionidae), *7—Cantharis pellucida* (Cantharidae), *8—Centromerus brevivulvatus* (Linyphiidae), *9—Dalopius marginatus* (Elateridae), *10—Diapterobates humeralis* (Ceratozetidae), *11*—Enchytraeidae, *12—Epidamaeus* sp. (Damaeidae), *13—Euzetes globulus* (Euzetidae), *14—Folsomia quadrioculata* (Isotomidae), *15—Hahnia ononidum* (Hahniidae), *16—Haplodrassus* sp. (Gnaphosidae), *17—Harpalus latus* (Carabidae), *18—Parisotoma notabilis* (Isotomidae), *19—Isotomiella minor* (Isotomidae), *20—Lepidocyrtus* sp. (Entomobryidae), *21—Lithobius curtipes* (Lithobiidae), *22—Nothrus sylvestris* (Nothridae), *23*—other Oribatida, *24—Otiorhynchus* sp. (Curculionidae), *25—Pachybrachius luridus* (Lygaeidae), *26—Pachimerium ferrugineum* (Geophilidae), *27—Pergamasus* sp. (Parasitidae), *28—Pogonognathellus* sp. (Tomoceridae), *29—Protaphorura armata* (Onychiuridae), *30—Pseudachorutes* sp. (Neanuridae), *31—Pseudosinella alba* (Entomobryidae), *32—Rhagio* sp. (Rhagionidae), *33*—Sciaridae, *34*—Sminthuridae, *35*—Staphylinidae, *36—Urodiaspis tecta* (Uropodidae)

We used R software to perform a generalized linear mixed-effects analysis (GLMM) as realized in *lme4* (Bates et al. 2015). Two variables were fitted using different models: $\delta^{13}C$ values (linear regression model) and the proportion of labeled samples (logistic regression model). Factors '$\Delta\delta^{15}N$' (continuous), 'Feeding type' (saprophages or predators), 'Time' (days after labeling) and 'Soil horizon' (litter or soil), as well as pairwise interactions between these factors were fitted as fixed effects. $\Delta\delta^{15}N$ values were included as a predictor because they reflect both the trophic level and habitat depth of soil animals (Tiunov 2007). To account for the

deviations from the predicted values that were not due to fixed effects, factors 'Species' and 'Block' (individual spruce tree) were fitted as random effects. The adjusted $\delta^{13}C$ and LP values were produced by the linear regression model and by the logistic regression model, respectively, by leveling off the interspecific and interplot variation.

Visual inspections of residual plots did not reveal deviations from either homoscedasticity or normality. *P*-values were obtained by likelihood ratio tests of the full model with the effect in question against the model without the effect in question.

The $\delta^{13}C$ signatures of different groups of soil animals were compared using Kruskal–Wallis test (KW). Two-sample test for proportions (P test) were used to compare the proportions of labeled samples (both tests performed in the 'Stats' package version 3.2.4). Data on the abundance of soil animals and natural $\delta^{13}C$ and $\Delta\delta^{15}N$ values are given as means ± 1 SE. Data on the $\delta^{13}C$ values after labeling are given as median and 1st and 3rd quartiles (25th and 75th percentiles) in brackets.

Results

Soil animal community

Especially abundant macroinvertebrates were larvae of March flies (Bibionidae, *Bibio* sp., 122 ± 22 ind. m^{-2}), centipedes (Lithobiidae, *Lithobius curtipes*, 82 ± 13 ind. m^{-2}), larvae of soldier beetles (Cantharidae: 22 ± 7 ind. m^{-2}) and small soil spiders (Linyphiidae: 22 ± 5 ind. m^{-2}, Hahniidae: 5 ± 2 ind. m^{-2}, Theridiidae: 3 ± 1 ind. m^{-2}). All larger spiders (Lycosidae and Gnaphosidae) collected were juveniles, with the abundances of 4 ± 1 and 6 ± 2 ind. m^{-2} respectively. Carabids were collected both as larvae (*Amara brunnea*, 8 ± 2 ind. m^{-2}) and as adults (*Pterostichus oblongopunctatus*, 6 ± 2 ind. m^{-2}).

The mean $\delta^{13}C$ and $\delta^{15}N$ values of litter collected before labeling were −28.8 ± 0.1 and −2.4 ± 0.1 ‰ respectively. The trophic structure of the soil community as reflected in natural (before labeling) $\delta^{13}C$ and $\Delta\delta^{15}N$ values was typical of temperate forests (Korobushkin et al. 2014). With a few exceptions, predators were enriched in ^{15}N relative to saprophagous or microbiphagous animals. The total range of mean $\delta^{13}C$ values was relatively narrow, from −28 to −24 ‰ (Fig. 1; Table S1 in Supplementary Material). Although we separated animals into two trophic groups only (saprophages and predators), several species, including the larvae of *Amara brunnea* (Carabidae) and the heteropterans *Pachybrachius luridus*, had relatively low $\delta^{13}C$ values, indicating phytophagous feeding. Saprophagous dipteran larvae (Bibionidae and Sciaridae) showed the highest $\delta^{13}C$ values. The $\Delta\delta^{15}N$ values spanned about 10 ‰. Epigeic collembolans (Sminthuridae, Tomoceridae, Entomobryidae) demonstrated the lowest $\Delta\delta^{15}N$ values, ranging from −2 to 2 ‰, whereas wolf spiders (Lycosidae) were most enriched in ^{15}N ($\Delta\delta^{15}N$ up to 8.2 ‰) (Table S1).

Appearance of the ^{13}C-label in roots and fungal mycelium

The carbon isotope composition ($\delta^{13}C$ values) of spruce needles, roots and fungal mycelium collected before the application of ^{13}C–CO_2 averaged −29.5 ± 0.1, −29.5 ± 0.3 and −27.1 ± 0.7 ‰ respectively. The maximum ^{13}C enrichment ($\delta^{13}C$ = 145.8 ‰) of spruce needles was observed 24 h after the injection of the label. At days 3 and 4 after labeling an increase in $\delta^{13}C$ values was detected in fine roots (median $\delta^{13}C$ = −26.2 ‰ (1st quartile = −27.5; 3rd quartile = −18.9), maximum $\delta^{13}C$ = 94.6 ‰) and fungal mycelium (median $\delta^{13}C$ = 21.4 (−26.1; −18.0), maximum $\delta^{13}C$ = 87.2 ‰). Thereafter the $\delta^{13}C$ values of plant and fungal tissues decreased gradually. During the whole sampling period after label application, the median $\delta^{13}C$ values of aboveground spruce needles, fine roots and fungal mycelium amounted to −10.3 ‰ (−19.6; 3.1), −22.7 ‰ (−27.3; −9.7), and −25.8 ‰ (−27.7; −22.7), respectively. The proportion of labeled samples (LP) reached 97 % in green needles, 50 % in roots and 26 % in fungal mycelium (Fig. 2). Isotopic label was detected neither in the litter nor soil samples.

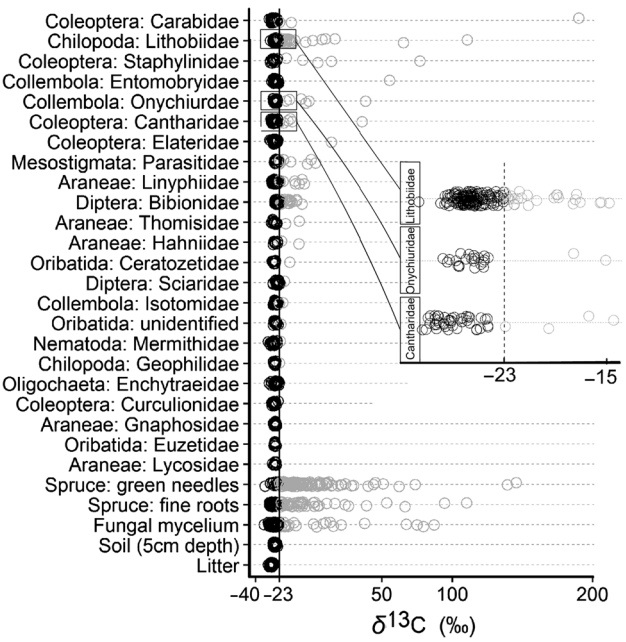

Fig. 2 Distribution of the $\delta^{13}C$ values of abundant groups of soil animals (n > 10), plants, soil and fungal mycelium after ^{13}C–CO_2 whole-tree labeling. Data from all sampling events (days 1–44) are bulked. *Points* show individual samples. A $\delta^{13}C$ value of −23.0 ‰ was taken as a threshold unequivocally indicating the labeled samples (see "Methods")

The dynamics of the ^{13}C-label in soil animals

Both, the δ^{13}C values and the proportion of labeled samples in soil saprophages varied relatively little during the sampling period, and reached maximum about 30 days after labeling (Fig. 3). In contrast, δ^{13}C and LP values in predators steadily increased during the sampling period. Nevertheless, the Time × Feeding type interaction in GLMM was not significant, reflecting huge variation in δ^{13}C and LP values within both trophic groups (Table 1). On the other hand, there was a significant $\Delta\delta^{15}$N × Time interaction indicating that the label appeared faster in species or individuals feeding at lower trophic level (thus depleted in ^{15}N) compared to species or individuals feeding at higher trophic level (enriched in ^{15}N). On average, saprophages started being labeled on day 6 (median with species/groups as replicates), while predators on day 12. However, this difference was not significant (KW: $H_{1, 30} = 2.1$, $P = 0.147$).

The label appeared in collembolans on day 6 after labeling. During 1 month the median δ^{13}C values of collembolans increased significantly from −26.9 ‰ (−26.6; −27.1) to −25.4 ‰ (−25.9; −24.1) (KW: $H_{6, 82} = 23$, $P < 0.001$) (Fig. 4a). The label occurred rarely (LP < 10 %) in litter-dwelling Entomobryidae, Isotomidae and Tomoceridae collembolans, while a significantly larger proportion (29 %) of soil-dwelling Onychiuridae were labeled (P test: $\chi^2 = 9.9$, $P = 0.002$). Overall, 9 % of collembolan samples were labeled (i.e., had δ^{13}C > −23.0 ‰). In oribatid mites the label started appearing on day 15 after labeling. The label was detected in Ceratozetidae (LP = 6 %, n = 16) and Peloppiidae (LP = 50 %, n = 4), but not in Damaeidae, Euzetidae and Nothridae (Fig. 2). In

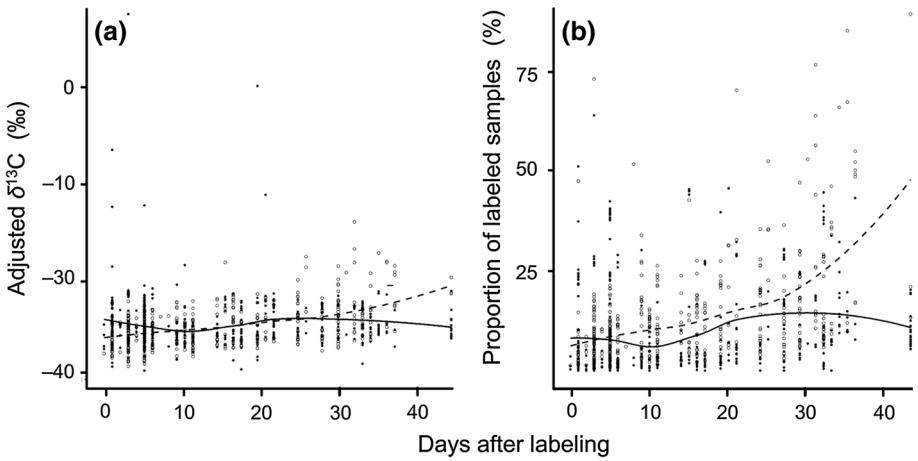

Fig. 3 Temporal dynamics of the adjusted δ^{13}C values (**a**) and the proportion of labeled samples (adjusted LP values) (**b**) of saprophagous and predatory soil animals. *Open dots* represent predators, *closed dots* are saprophages. *Lines* represent polynomial approximation for temporal dynamics of predators (*dashed line*) and saprophages (*soild line*) values. The adjusted δ^{13}C and LP values were produced by generalized linear mixed-effects analysis that leveled off interspecific and interplot variation (see text for further details)

Table 1 χ^2 and *P*-values of fixed effects ($\Delta\delta^{15}$N values, Time, Feeding type and Soil horizon) on the δ^{13}C values of soil animals collected after labeling and on the proportion of labeled samples (LP values) as estimated by GLMM

	δ^{13}C		LP	
	χ^2	*P* value	χ^2	*P* value
Δ^{15}N	1.6	0.205	0.4	0.531
Time	8.4	**0.004**	14.9	**< 0.001**
Feeding type	1.9	0.169	0.6	0.424
Soil horizon	2.3	0.130	0.9	0.345
Δ^{15}N × Time	11.6	**< 0.001**	4.3	**0.037**
Δ^{15}N × Feeding type	7.8	**0.005**	< 0.1	0.989
Δ^{15}N × Soil horizon	21.6	**< 0.001**	16.7	**< 0.001**
Time × Feeding type	0.0	0.854	0.4	0.544
Time × Soil horizon	0.1	0.793	0.0	0.873
Feeding type × Soil horizon	0.4	0.513	0.4	0.545

Variances of intercepts of random effects 'Species' and 'Block' were 63.4, and 0.1 ‰ (for δ^{13}C values), 1.1 and 0.1 % (for LP values), respectively. Multiple R^2 values were 0.25 and 0.42 for the adjusted δ^{13}C and LP values, respectively. Significant effects are given in bold

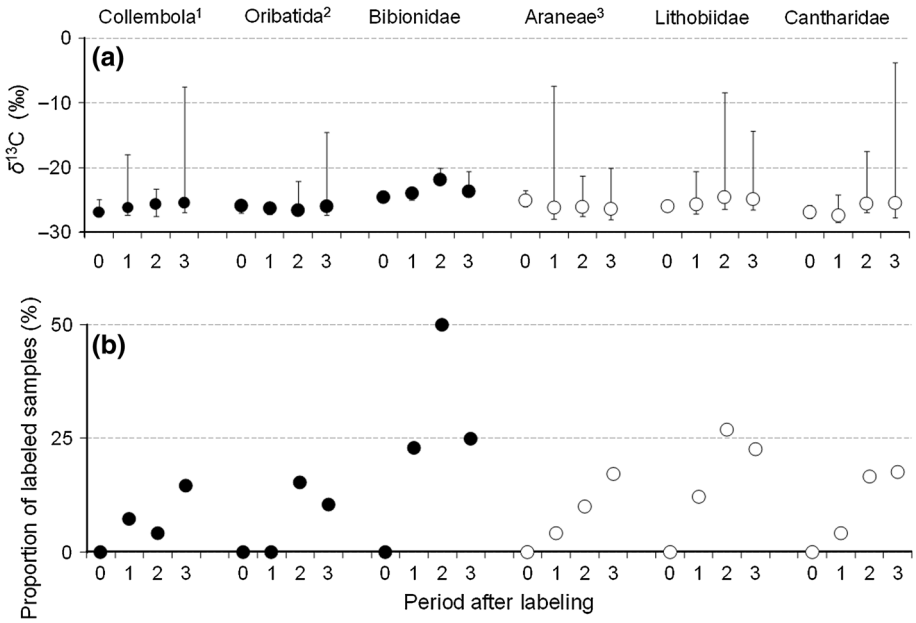

Fig. 4 The dynamics of the ^{13}C label in selected groups of soil animals. **a** Median $\delta^{13}C$-values; *whiskers* show 5 and 95 % percentiles. **b** Proportion of labeled samples or individuals (%). *Closed dots* represent saprophages, *open dots* are predators. Time periods: (0) before labeling, (1) days 1–10, (2) days 11–20, (3) days 21–44 after labeling. [1]Collembola = Entomobryidae + Isotomidae + Onychiuridae + Tomoceridae. [2]Oribatida = Ceratozetidae + Damaeidae + Euzetidae + Peloppiidae. [3]Araneae = Hahniidae + Linyphiidae + Theridiidae

total, 12.5 % of oribatid samples contained the label. All labeled predatory mites belonged to Parasitidae (mainly *Pergamasus* sp.). The first labeled samples of gamasids were collected on day 2 after labeling. A significant proportion of gamasids was labeled (LP = 41 %, n = 22), the median $\delta^{13}C_L$ values being −19.7 ‰ (−22.2; −2.8).

Among macrofauna, saprophagous bibionid larvae *Bibio* sp. became labeled on day 4. The highest frequency of the label (50 %) and the highest median $\delta^{13}C$ values were detected on days 11–20 (Fig. 4). In total, 23 % of collected bibionid larvae were labeled. In the less abundant groups, high proportions of the label were found in *Ectobius sylvestris* cockroaches (two of the three specimens were labeled). Adults and larvae from the spider families Hahniidae, Linyphiidae and Theridiidae became labeled on days 4–6. Both the frequency of the label (15, 10 and 13 %, respectively) and the $\delta^{13}C_L$ values (−15.2 ± 6.1, −18.6 ± 2.2 and −19.2 ‰ respectively) varied little between these families. None of the larval Gnaphosidae (n = 19) or Lycosidae (n = 11) spiders were labeled. The first labeled specimens of predatory centipedes, *Lithobius curtipes*, were sampled on day 4. Ten days after labeling the proportion of labeled individuals reached 31 % (Fig. 4). Nevertheless, the median $\delta^{13}C$ value of centipedes collected after labeling was −25.2 ‰ (−25.9; −22.5) and exceeded the natural values of −25.9 ‰ (−26.2; −25.7) only by 0.7 ‰ (KW: $H_{1,195} = 4.6$, $P = 0.033$). The first predatory Cantharidae larvae became labeled on day 10. The frequency and intensity of the ^{13}C label in Cantharidae increased slowly during the sampling period. In total, the fraction of labeled individuals amounted to 12.5 % (Figs. 2, 4).

Distribution of the label in different groups of soil animals

The distribution of the isotopic label was highly uneven both between and within species (Fig. 2). The proportion of labeled samples or individuals in different families of saprophagous and predatory invertebrates varied from 3 to 50 % and from 7 to 41 %, respectively, averaging 9 % across all species or higher taxonomic groups analyzed (species with n = 1 were excluded from calculations). During the entire sampling period of 44 days, the label was recovered in 15 of 38 species of saprophagous animals (including those with n = 1), and in 20 of 63 species of predators (39 and 32 %, respectively).

The measured $\delta^{13}C$ values in the labeled samples ranged widely, but the median $\delta^{13}C_L$ values varied relatively little in different families of predators (from −21.3 ‰ in Linyphiidae to −4.4 ‰ in Staphylinidae). In saprophages, the range of $\delta^{13}C_L$ values was considerably wider (from −21.8 ‰ in Isotomidae to 11.5 ‰ in Onychiuridae). Nevertheless, saprophages and predators failed to differ both in the intensity of label acquisition expressed as $\delta^{13}C_L$ values (KW test: $H_{1,\ 36} = 3.4$, $P = 0.180$) and in the proportion of labeled samples (P test: $\chi^2 = 0.002$, $P = 0.965$).

In some predatory insects (Carabidae, Staphylinidae), both larvae and adults were collected. In these

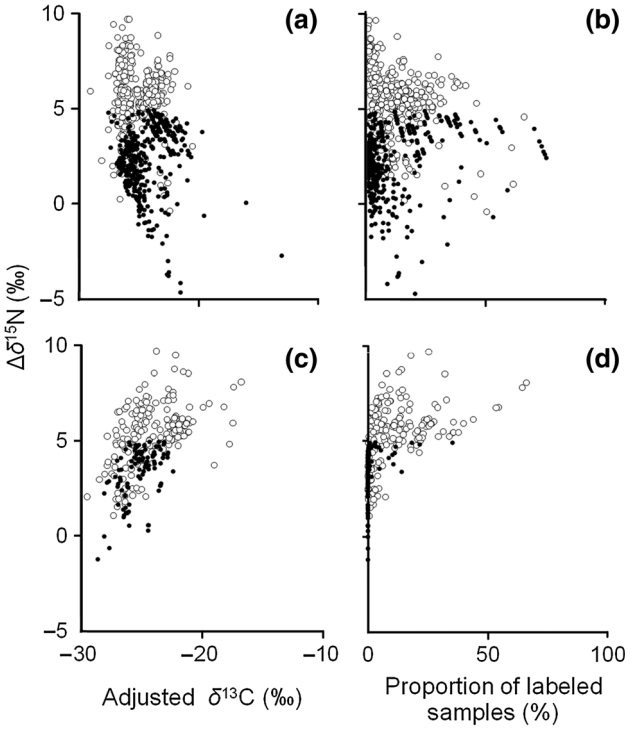

Fig. 5 The adjusted $\delta^{13}C$ values (**a**, **c**) and the proportion of labeled samples (adjusted LP values) (**b**, **d**) of the litter-dwelling (**a**, **b**) and in the soil-dwelling (**c**, **d**) animals as dependent on $\Delta\delta^{15}N$ values in animal tissues. *Open dots* represent predators, *closed dots* are saprophages. The adjusted $\delta^{13}C$ and LP values were produced by generalized linear mixed-effects analysis that leveled off interspecific and interplot variation

cases, only larvae were ^{13}C-labeled, the difference in label frequency being significant (P test: $\chi^2 = 5.5$, $P = 0.019$). All collected larvae (five specimens) of rove beetles were labeled with a median $\delta^{13}C_L$ of 4.5 ‰ (−6.3; 14.3). Among the 34 larvae of ground beetles taken, three (9 %) were labeled with a $\delta^{13}C_L$ of −14.3 (−18.5; 87.9). Similarly, larvae of *Lithobius curtipes* centipedes were labeled more frequently than adults (24 and 17 %, respectively); albeit the difference was not significant (P test: $\chi^2 = 1.0$, $P = 0.325$).

The main factors that affected the distribution of isotopic labels among soil animals were further assessed using the adjusted $\delta^{13}C$ and LP values produced by generalized linear mixed-effects analysis by leveling off the interspecific and interplot variation. Against our expectations, the adjusted $\delta^{13}C$ values of soil animals and the proportion of labeled samples (adjusted LP values) failed to depend on the primary factors 'Feeding type' or 'Soil horizon'. The median $\delta^{13}C$ values of soil animals collected in the litter [−25.6 ‰ (−25.9; −25.5)] and in the soil [−25.4 ‰ (−26.1, −25.3)] did not differ, although the proportion of labeled samples was slightly higher in litter as compared to soil: 13 versus 11 % (P test: $\chi^2 = 1.3$, $P = 0.258$). Similarly, there was no significant difference (KW: $H_{1, 102} = 2.3$, $P = 0.127$) in the median $\delta^{13}C$ values of saprophages [−25.3 ‰ (−26.0; −24.1)] and predators [−25.9 ‰ (−26.8, −24.7)].

Nevertheless, several interactions were significant (Table 1). Both the $\delta^{13}C$ and LP values depended strongly on the '$\Delta\delta^{15}N$' × 'Soil horizon' interaction ($P < 0.001$, Table 1). Indeed, the intensity and frequency of the label generally increased with a rise in $\Delta\delta^{15}N$ values in soil-dwelling animals, but not in litter-dwellers (Fig. 5). In the litter samples, the adjusted $\delta^{13}C$ values increased with a decrease in $\Delta\delta^{15}N$ values in saprophages, but not in predators. This led to a significant '$\Delta\delta^{15}N$' × 'Feeding type' interaction (Table 1).

Discussion

Intensity and frequency of the isotopic label

Our results are in line with several published experiments that used whole-tree isotopic labeling either in the field (Pollierer et al. 2007, 2012; Högberg et al. 2010; Churchland et al. 2012; Gilbert et al. 2014) or in the laboratory (Eissfeller et al. 2013). Those studies used a wide range of isotopic label intensity, from a few to hundreds delta units; the time lag between the beginning of tree labeling and the collecting of soil animals varied from a few days to several years. Overall, our results confirm that recently fixed root-derived carbon contributes to the energy budget of soil animals. On the other hand, the frequency of the isotopic label in soil animals was relatively low and increased very slowly during the sampling period (Figs. 2, 3). Only 186 of 1141 samples collected showed the $\delta^{13}C$ values above −23.0 ‰, i.e. were unequivocally labeled. The capability for assimilating freshly fixed carbon varied strongly between species and taxonomic groups of soil animals. During the whole experiment, the label was either never or only rarely recovered in saprophagous enchytraeids and earthworms, predatory dipteran larvae (Asilidae and Rhagionidae) and many other groups of soil animals (Fig. 2, Table S1). Overall, the label was detected in about 35 % of the species analyzed.

Both the intensity and frequency of the label in our study could be underestimated, because we used a relatively high 'labeling threshold', i.e. −23.0 ‰. Nevertheless, in most taxa that were classified as 'unlabeled', the mean $\delta^{13}C$ values before labeling and the median $\delta^{13}C$ values after labeling were nearly identical (Table S1). There were only three exceptions from this pattern. In the collembolan *Pseudosinella alba* and in earthworms the mean $\delta^{13}C$ values before labeling (–26.8 and −25.4 ‰, respectively) were lower than the median $\delta^{13}C$ values after labeling (−25.8 and −24.7 ‰, respectively), but still within the corresponding inter-quartile intervals. The $\delta^{13}C$ values in enchytraeids before labeling (−27.1 ‰) were 1.6 ‰ lower than after labeling (−25.5 ‰), but in this case only two samples were taken before labeling. Considering a large number of post-la-

beling replicates (n = 22, 67, and 104 for *P. alba*, earthworms, and enchytraeids, respectively) the total lack of samples with $\delta^{13}C$ values above −23.0 ‰ clearly indicates that these animals did not assimilate a significant amount of freshly-fixed carbon.

In this respect our results differ strongly from those reported in the long-term studies (Pollierer et al. 2007, 2012; Gilbert et al. 2014). This confirms the observation of Högberg et al. (2010) that 'the longer the labeling period and the time elapsed after labeling, the greater the numbers of individuals and animal groups that are likely to be labeled'. In contrast, short-term studies partly support our results. Ostle et al. (2007) found a pronounced consumption of the freshly fixed C by endogeic earthworms *Allolobophora chlorotica* in a grassland ecosystem, but a very small $\delta^{13}C$ increase in enchytraeids. Similarly, Högberg et al. 2010 did not detect the use of the 'root carbon' by enchytraeids in a pine forest.

It can be suggested that invertebrates having smaller body size (e.g., microarthropods) can be labeled faster and more intensively than larger animals that often have sclerotized cuticle and other inert tissues (e.g., insects or millipedes). To account for the 'body size' factor, we divided all invertebrates into three size classes, including mesofauna (Collembola, Acari, Enchytraeidae), 'small macrofauna' (most of macroarthropods) and 'large macrofauna' (Lumbricidae, Carabidae, Diplopoda, Scarbaeidae), and performed a separate GLMM analysis. Both, $\delta^{13}C$ values and the proportion of labeled samples depended on the time since labeling ($P = 0.004$ and $P < 0.001$, respectively), but did not depend significantly on the size class ($P = 0.529$ and $P = 0.711$, respectively) or on the 'Time × Size class' interaction ($P = 0.597$ and $P = 0.784$, respectively). In fact, animals of large size were labeled somewhat more frequently than smaller animals (Supplementary Fig. S1).

Saprophages

As noted above, our results contradict several previous studies where earthworms and/or enchytraeids actively assimilated recent plant photosynthate (Ostle et al. 2007; Churchland et al. 2012). Nevertheless, our data are consistent with earlier studies showing that enchytraeids and endogeic earthworms utilize old soil carbon (5–10 years old) (Briones et al. 2005; Hyodo et al. 2008). Collembolans were not labeled in the short-term experiment of Churchland et al. (2012), while in our study the Onychiuridae showed the highest mean $\delta^{13}C_L$ values among all saprophages, and a relatively high frequency of the label (Fig. 2). Collembolans are largely mycophagous (Hopkin 1997), with hyphae of mycorrhizal fungi to be assumed as the most probable link connecting recent photosynthate and endogeic Onychiuridae. High $\delta^{13}C$ values of several samples of epigeic collembolans (*Orchesella* sp., Sminthuridae) can indicate their feeding on some plant or animal-produced material falling down from the tree crown, e.g., honeydew (Seeger and Filser 2008; Potapov et al. 2016). Trophic links of epigeic collembolans with recently fixed carbon are likely to be opportunistic in nature. For instance, in our study, *Lepidocyrtus* sp. was not labeled; similarly, *Lepidocyrtus lanuginosus* did not contain labeled fatty acids in the field experiment of Pollierer et al. (2012). In contrast, *Lepidocyrtus cyaneus* was among the most labeled saprophagous species in the experiment of Eissfeller et al. (2013). Among other sapro/microbiphagous species, the label was most abundant in bibionid larvae. This group was not listed among soil animals dependent on root-derived carbon in previous tree-labeling experiments, although bibionid larvae are known to damage plant roots in agroecosystems (D'Arcy-Burt and Blackshaw 1991). The first ^{13}C-enriched dipteran larvae (Bibionidae and Sciaridae) were collected the next day after the label had appeared in fine roots (Fig. 4). Such a rapid transport of the label may be caused by feeding on live roots or mycorrhizal fungi. In oribatid mites, the frequency of the label was low except for Peloppiidae species. In particular, none of Damaeidae samples were labeled, although in some other studies Damaeidae clearly received root-derived carbon (Pollierer et al. 2007; Eissfeller et al. 2013). Overall, our data and comparisons with other studies indicate that recent plant photosynthate is assimilated by a limited subset of saprophagous species, implying a certain degree of isolation of this energy channel in soil food webs. On the other hand, the range of saprophagous species that actively use freshly fixed C differs in different ecosystems.

Predators

We hypothesized that in our short-term experiment the isotopic label would be most pronounced and appear first in sapro/microbiphagous animals compared to predators. This hypothesis was not directly supported. The intensity and frequency of the label did not differ significantly between saprophagous and predatory animals (Table 1), and the dynamics of label acquisition were similar in these groups. Only at the last sampling events the label became more frequent in predators than in saprophages (Fig. 3). The mechanism of recent photosynthate transmission from the phloem to soil predators remains unclear. It could certainly occur via feeding on soil saprophages, but a short time lag between the appearance of the isotopic label in fine roots and in soil predators suggests the presence of shorter trophic links. Omnivory is widespread in soil food webs (Wardle 2002), and some predators like centipedes (Lewis 1965), ground beetles (Hengeveld 1979), and rove beetles (Ashe 1984) were shown to feed on roots or fungal mycelium.

In our case, predatory macroinvertebrates belonged to taxa showing different types of mouthparts: chewing (Lithobiidae centipedes, Carabidae, Staphylinidae), sucking with external digestion (spiders) or sucking with internal digestion (Cantharidae larvae). The median $\delta^{13}C$ values and LP values did not differ significantly

between these groups (data not shown), but the label was most frequent and the $\delta^{13}C_L$ values were at the maximum in species with chewing mouthparts. In particular, the label was frequent (20 %) in *Lithobius curtipes* centipedes. Comparable results were obtained in previous labeling studies: four species of the genus *Lithobius* (Eissfeller et al. 2013) and *Lithobius crassipes* (Pollierer et al. 2007) showed a strong linkage to root-derived carbon. A high frequency and a rapid appearance of the label in centipedes (Fig. 4) could be caused in part by consuming live roots and fungal hyphae (Lewis 1965). Similarly, the high $\delta^{13}C$ values of Staphylinidae larvae may be explained both by direct mycophagy and feeding on mycophages (Newton 1984). Omnivorous Carabidae regularly consume live plant tissues, including fine roots (Hengeveld 1979; Sasakawa et al. 2010). In our experiment, larvae but not adults of Carabidae and Staphylinidae beetles were linked to root-derived carbon, confirming that syntopic larvae and adults of predatory coleopterans can have considerably different trophic niches (Luff 1974).

Larvae of Cantharidae beetles and spiders have sucking mouthparts and can only feed on invertebrates. This coincided with the lack of extra-high $\delta^{13}C_L$ values among these predators (Fig. 2). Although most of the soil predators are generalist feeders (Scheu and Setälä 2002), the importance of root-derived carbon differs greatly between taxonomic groups. In our samples, Gnaphosidae and Lycosidae spiders were at the first larval stages, i.e. of the same size class as the smaller species of Theridiidae, Thomisidae and Linyphiidae. Nevertheless, Gnaphosidae and Lycosidae were not labeled, while the proportion of labeled specimens of Theridiidae, Thomisidae and Linyphiidae was about 15 %. Lycosid and gnaphosid spiders occupy higher trophic levels than the smaller spider species do ($\Delta\delta^{15}N$ values from 7.4 to 8.2 ‰ and from 4.4 to 7.1 ‰, respectively). Overall, the proportion of labeled individuals among litter-dwelling predators had a significant (Pearson correlation: $R = -0.18$, $P < 0.001$) tendency to decrease with an increase in $\Delta\delta^{15}N$ values (Fig. 5), likely indicating the dilution of the label while it moved from the lower to the higher trophic levels.

Soil-dwelling and litter-dwelling animals

Animals inhabiting mineral soil have direct access to the roots and root-associated microorganisms. We therefore expected that the isotopic label could be more intense and/or frequent in soil-dwelling animals compared to litter-dwellers. Although the soil horizon did not affect the distribution of the label as the main factor, there was a strong '$\Delta\delta^{15}N$' × 'Soil horizon' interaction (Table 1). The importance of root carbon as an energy source for soil animals should likely increase with soil depth, following a decrease both in the amount of total soil organic matter (SOM) and microbial biomass. As the $\delta^{15}N$ and $\delta^{13}C$ values of SOM and those of soil-dwelling microorganisms, both typically increase with depth (Högberg et al. 1996; Högberg 1997; Wallander et al. 2004; Wynn 2007), a positive correlation between the $\delta^{13}C$ and $\delta^{15}N$ values of soil-dwelling animals is to be expected. On the other hand, the fluxes of C and N in soil food webs are not necessarily tightly coupled (Pokarzhevskii et al. 2003). It can be therefore suggested that animals inhabiting deeper soil layers can depend on SOM-decomposing microorganisms as a source of nitrogen, but more on the root-derived carbon as a source of energy. This can account for an increase in the proportion of labeled animals with a rise of $\Delta\delta^{15}N$ values (Fig. 5c, d). In addition, this correlation could be rooted in feeding on mycorrhizal mycelium that is likely enriched in ^{15}N relative to SOM and saprotrophic microorganisms (Högberg et al. 1996; Hobbie and Ouimette 2009).

No positive correlation between $\Delta\delta^{15}N$ and $\delta^{13}C$ values was observed in litter-dwelling invertebrates (Fig. 5a, b). Instead, several samples of epigeic collembolan species with the lowest $\Delta\delta^{15}N$ values (Fig. 1) were strongly labeled. As noted above, they could have received the label via consuming ^{13}C-labeled material falling down from the tree crown. The frequency of the label (LP values) in litter-dwellers peaked at $\Delta\delta^{15}N$ values ranging from 3 to 4 ‰. These $\Delta\delta^{15}N$ values are typical of the 'secondary consumers' feeding on microorganisms (Scheu and Falca 2000; Chahartaghi et al. 2005), whereas the primary litter consumers with lower $\Delta\delta^{15}N$ were labeled less frequently. Similar results were obtained by Eissfeller et al. (2013) in a laboratory experiment.

Conclusions

The results of the present study show that freshly fixed plant carbon quickly enters both litter-located and soil-located compartments of the detrital food web. Nevertheless, this energy source is not used uniformly by the whole community of soil invertebrates, as indicated by a limited number of species consuming freshly fixed carbon (35 % of the species examined). Trophic links allowing the acquisition of fresh photosynthates by particular species or taxonomic groups of soil animals appear to be opportunistic and inconsistent across different studies. This generally conforms to the observations of a low importance of mycorrhizal fungi in the feeding of soil animals (recently reviewed in Potapov and Tiunov 2016). In our study, the label was quickly assimilated by endogeic collembolans, a few species of oribatid mites, and saprophagous dipteran larvae, but neither by earthworms nor enchytraeids. Several species of macrofauna predators with chewing mouthparts, lithobid centipedes in particular, acquired the freshly fixed plant carbon at high frequency and intensity rates. This can indicate an opportunistic feeding on live roots or mycorrhizal mycelium. Macrofauna predators with sucking mouthparts (cantharid larvae, spiders) also received the label, but this occurred later. Among the

animals that inhabited the mineral soil layers, a trophic link to root-derived C was the most pronounced in species with higher $\delta^{15}N$ values, suggesting this energy source being more important in deeper mineral soil horizons. In the litter-dwelling animals, the label was the most frequent in species showing medium $\delta^{15}N$ values, classified as microbiphagous secondary decomposers. The capability for using freshly fixed plant carbon provided by roots and root-associated microorganisms can be regarded as a relatively infrequent trait contributing to the diversification of trophic niches of soil animals.

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