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Assimilation of plant-derived freshly fixed carbon by soil collembolans: Not only via roots?

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ABSTRACT

Large amounts of freshly fixed carbon in forest ecosystems are allocated belowground and fuel soil food webs. This supply is usually generalized as the 'root carbon', but particular pathways by which freshly fixed carbon is assimilated by soil animals remain poorly understood. We followed the appearance of the isotopic label in springtails inhabiting different layers of soil and litter (euedaphic, hemiedaphic and epigeic life forms) after *in situ* pulse-labelling of spruce trees with $^{13}C—CO_2$. Isotopic label was most frequently observed in euedaphic Collembola (26% of all samples), mainly in *Protaphorura armata*. The label was very rare in litter-dwelling (hemiedaphic) species (4%). Surprisingly, the label was often observed in epigeic Collembola (11%). During the period of 44 days after labelling, the proportion of labelled samples increased with time in euedaphic, but not in epigeic species, suggesting that freshly fixed carbon is assimilated by these collembolan groups via different pathways. Most likely, euedaphic species are trophically linked to plant roots, whereas epigeic species receive freshly fixed carbon from aboveground. The exact mechanisms and potential importance of the latter pathway should be evaluated in further research.

1. Introduction

A significant proportion of freshly fixed carbon (FFC) in forest ecosystems is allocated belowground and is further transferred to mycorrhizal fungi and other soil organisms closely associated with roots (Högberg and Read, 2006). This supply drives soil microbial activity (Högberg et al., 2001) and provides energy for soil food webs (Pollierer et al., 2007). Distribution of the root-derived FFC among soil animals was studied in several experiments using $^{13}CO_2$-canopy labelling (Pollierer et al., 2012; Eissfeller et al., 2013; Gilbert et al., 2014; Fujii et al., 2016), but particular pathways of the FFC entering the soil food webs remain poorly understood. Although direct consumption of living roots and/or extramatrical mycelium of mycorrhizal fungi can be suggested, studies using natural $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios do not support a widespread feeding of soil animals on mycorrhizal fungi (Potapov et al., 2013; Kudrin et al., 2015; Potapov and Tiunov, 2016). The FFC can be quickly assimilated by soil microorganisms of different functional groups, including saprotrophic fungi feeding on ectomycorrhizal mycelium necromass (Esperschütz et al., 2009; Drigo et al., 2012). In this situation, exact trophic links connecting living plant tissues and soil-dwelling animals can not be traced confidently. Indeed, detailed studies using compound-specific stable isotope analysis suggest that FFC can reach soil animals via several routes, including both, mycorrhizal and saprotrophic fungi, and also bacteria (Pollierer et al., 2012; Pausch et al., 2016; Scheunemann et al., 2016).

Mechanisms of the FFC acquisition by saprophagous and microbiphagous soil animals can be clarified by monitoring the appearance of the isotopic label in animals inhabiting different layers of soil and litter. In this respect, Collembola represent a suitable model group. In spite of having generally similar feeding mode (Ponge, 2000), collembolan species belonging to different life forms (sensu Gisin, 1943) inhabit different layers of soil and litter, and exploit different sources of carbon.

Here we present data from a short-term field experiment in which young spruce trees were pulse-labelled with $^{13}C–CO_2$. We followed the dynamics of FFC incorporation in euedaphic, hemiedaphic and epigeic Collembola. We expected that soil-dwelling (euedaphic) Collembola should be most strongly associated with

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root-derived carbon due to the spatial proximity to the roots, whereas epigeic species should not assimilate the root-derived FFC.

2. Material and methods

The experiment was conducted at the Chernogolovka biological station RAS, Moscow region (56°01′32″N, 38°25′47″E). In May 2012, root systems of five young spruce trees (height from 3.5 to 4.5 m) were surrounded by buried (40 cm in depth) steel fences at the distance of 1.7 m from the trunk. The distance between individual spruce trees was 10–20 m. Inside the fence, the ground vegetation was removed. In early September, these trees were labelled by injecting four litres of 99% $^{13}CO_2$ gas inside individual polypropylene chambers (3 × 3 m, 4 m tall) erected around the entire crown. The chambers were completely isolated from the ground by the polypropylene film and construction foam around the stem. The lower surfaces of the chambers were positioned 30–35 cm above ground. In each chamber five electric fans with a total capacity of 12 $m^3\,min^{-1}$ were installed. Sixty hours after labelling, chambers were removed. Sampling was performed immediately before ('control'), and every 3–5 days after labelling till day 44. During each sampling campaign, five soil cores (5 × 5 × 5 cm, including litter and upper mineral soil) were taken under each tree. Springtails were extracted in 70% ethanol using Tullgren funnels equipped with filament lamps. Extraction continued until the soil was dry (ca. 48 h). Animals were identified according to Fjellberg (1998, 2007) under the dissecting microscope. After the identification, collembolans were dried at 50° C for at least 48 h, weighted and wrapped into tin foil to conduct the stable isotope analysis. Collembolans extracted from each soil core were analysed separately if possible; otherwise animals from 2 to 5 cores were bulked. From 1 to 25 specimens were pooled in each sample (20–200 μg dry weight). Mean number of specimens per sample was lower in epigeic (2.6, SD 2.7) than in hemiedaphic (5.6, SD 5.0) and euedaphic (4.4, SD 4.7) species.

Stable isotope analysis was conducted using a Thermo Delta V Plus continuous-flow IRMS located at the Institute of Ecology and Evolution, Moscow. The isotopic composition of C was expressed in the conventional δ-notation relative to VPDB (Vienna PeeDee belemnite). Samples were analysed with reference gas calibrated against IAEA (International Atomic Energy Agency, Vienna, Austria) reference materials (USGS 40, USGS 41 and IAEA-CH3). Drift correction was performed using the internal laboratory standards (casein and acetanilide). The standard deviation of $\delta^{13}C$ values of reference materials (n = 6–8) was < 0.15‰. Special precautions were taken when small samples (< 50 μg) were analyzed. In this case, correspondingly small amounts of reference material (from 10 to 50 μg) were measured after every second sample. These data were used to compensate for a mass-related drift using a set of linear or log-linear regression equations (not shown). After this correction, the accuracy of our measurements was better than ± 0.2‰.

We chose not to analyse the measured $\delta^{13}C$ values directly due to very uneven distribution of these values, which is typical of short-term labelling studies (Ostle et al., 2007). Instead, we used $\delta^{13}C$ values of springtails collected before the injection of the label (mean $\delta^{13}C = -26.3$‰, with 95% confidence intervals of −26.9 and −25.8, n = 21) as a reference to determine whether subsequent samples should be considered labelled or not. More specifically, a sample was considered as labelled if its $\delta^{13}C$ value exceeded the maximum value measured before labelling ($\delta^{13}C = -23.6$‰, vertical dotted line, Fig. 1). The occurrence of labelled samples was subsequently analysed using generalized linear models for a binomial variable. As a first step, we modelled the labelled/non labelled variable as a function of time (number of days since the injection of $^{13}CO_2$) and of an interaction between time and species identity (n = 14) using the *glm* function for the binomial variable. To

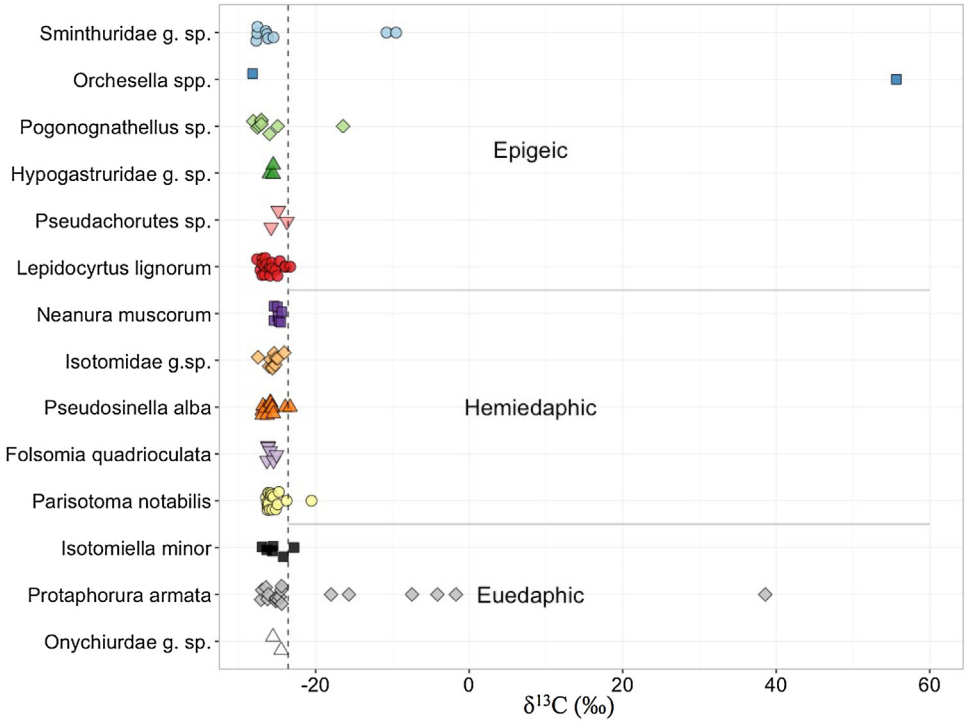

Fig. 1. Isotope composition ($\delta^{13}C$ values) of different collembolan species collected during 44 days after labelling. The vertical dotted line represents the maximum $\delta^{13}C$ value observed before labelling (−23.6‰). This value was used as a threshold to determine whether the samples were labelled or not.