Functional Ecology

British Ecological Society

Functional Ecology 2016, **30**, 479–489 doi: 10.1111/1365-2435.12512

Small but active – pool size does not matter for carbon incorporation in below-ground food webs

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Summary

1. The complexity of soil food webs and the cryptic habitat hamper the analyses of pools, fluxes and turnover rates of carbon (C) in organisms and the insight into their interactions. Stable isotope analysis has been increasingly used to disentangle soil food web structure, yet it has not been applied to quantitatively characterize C dynamics at the level of the entire soil food web.

2. The present study employed $^{13}CO_2$ pulse labelling to investigate the incorporation of maize root-derived C into major soil compartments and food web players in an arable field for 25 days. Bulk tissue and compound-specific (lipids) C isotope ratios were used to quantify pool sizes and ^{13}C incorporation in bacteria and fungi as primary decomposers, nematodes as key drivers of the microfood web and decomposers and predators among the meso- and macrofauna.

3. About 20% of the C assimilated by maize was transferred to below-ground pools. ^{13}C was predominantly incorporated into rhizosphere micro-organisms rather than in those of the bulk soil. ^{13}C in phospholipid fatty acid biomarkers revealed that root-derived C was incorporated into the soil food web mainly via saprotrophic fungi rather than via bacteria. Only small amounts of ^{13}C were transferred to higher trophic levels, predominantly into fungal-feeding nematodes and macrofauna decomposers.

4. Most importantly, C pool size and ^{13}C incorporation did not match closely. Although the fungal C stock was less than half that of bacteria, C transfers from fungi into higher trophic levels of the fungal energy pathway, that is fungal-feeding nematodes and meso- and macrofauna decomposers, by far exceed that of bacterial C. This challenges previous views on the dominance of bacteria in root C dynamics and suggests saprotrophic fungi to function as major agents channelling recent photoassimilates into the soil food web.

Key-words: bacteria, carbon pools and fluxes, fungi, macrofauna, mesofauna, nematodes, plant–soil (below-ground) interactions, pulse labelling, root-derived C, stable isotope probing of fatty acids

Introduction

Soils store *c*. 80% of global terrestrial organic carbon (C), and small changes of fluxes into and out of this pool may influence the atmospheric CO_2 concentrations and interact with ongoing climate change (IPCC 2007). Considerable information is available on the amounts, individual fractions and residence time of organic C in soil (Pendall & King 2007; Todorovic *et al*. 2010), but less is known on the C pools and fluxes of soil organisms entangled in multitrophic interactions that determine the critical balance between C mineralization and sequestration. Soil food

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webs have been analysed using a number of approaches such as descriptions of trophic links or energy and mass flow among food web compartments (De Ruiter *et al.* 1993; Moore, McCann & de Ruiter 2005). Basic assumptions of such classical models are aggregated trophic groups, equilibrium biomasses and constant metabolic rates. Quantitative food webs demonstrated that the distribution of interaction strengths (i.e. strong versus weak C flows) is related to characteristic biomass pyramids (De Ruiter, Neutel & Moore 1995). However, as soil food webs are highly complex, our knowledge on structure and function is still limited, as mirrored by Coleman (2008) questioning whether there are enough trophic niches for the high number of species in soil.

Over the last decade, stable C and nitrogen (N) isotope analyses have been used increasingly to disentangle soil food webs, with shifts in the natural abundance of ^{13}C and ^{15}N giving insight into C resources and trophic levels (Albers, Schaefer & Scheu 2006; Tiunov 2007; Crotty *et al.* 2014). Food web dynamics are predominantly driven by C inputs from plants, with two main sources: (i) litter, that is slowly decomposing plant material and (ii) rhizodeposits, that is readily available C substrates, resulting in three major soil C and energy pathways based on bacteria, fungi and roots (Moore, McCann & de Ruiter 2005; Scheu, Ruess & Bonkowski 2005; Ruf, Kuzyakov & Lopatovskaya 2006). The related C and N fluxes into decomposer systems were addressed by isotopic labelling of plants or litter (Leake *et al.* 2006; Ruf, Kuzyakov & Lopatovskaya 2006; Ostle *et al.* 2007; Elfstrand *et al.* 2008), suggesting that the majority of soil animals rely on root-derived sources rather than on litter (Albers, Schaefer & Scheu 2006; Pollierer *et al.* 2007). Recently, Crotty *et al.* (2014) showed that the divergence in energy channels within the soil food web is determined by ecosystem type. In particular, in arable systems, where plant biomass is harvested as crop, the internal terrestrial C cycle is strongly dependent on root-derived C rather than above-ground litter (Drigo *et al.* 2010). Surprisingly, only few studies using stable isotopes targeted at elucidating trophic interactions in agro-ecosystems, and no quantitative fluxes within food web compartments are at hand (Traugott *et al.* 2013).

In this study, we quantified the incorporation of root-derived C into the soil food web of an arable field and compiled a budget for C pools and shifts, including losses via CO_2 and dissolved organic carbon (DOC) and turnover rates in major food web compartments. This was addressed by a $^{13}CO_2$ pulse-labelling experiment of maize plants followed by a 25-day dynamic sampling of soil micro-organisms and animals for $\delta^{13}C$ analysis of lipids or bulk tissue. We hypothesize that root C is predominantly processed by rhizosphere bacteria, thereby fuelling the bacterial energy channel. Further, C pool size (i.e. organismic biomass) within the different food web compartments determines the ^{13}C incorporation into them. To the best of our knowledge, this is the first study presenting a comprehensive budget for plant C input into the food web of an arable soil.

Materials and methods

$^{13}CO_2$ PULSE LABELLING AND SAMPLING

The experiment was conducted in July 2010 on an arable field grown with maize (*Zea mays* L. cv. 'Fernandez') at the silking stage. This growth stage was chosen since maize root biomass is maximal just after anthesis (Amos & Walters 2006). The study site was located in the north-west of Göttingen, Germany. The soil, a loamy haplic Luvisol, had a pH ($CaCl_2$) of 6·0, a bulk density of 1·4 g cm^{-3}, an organic C content of 12·4 g kg^{-1}, a total N content of 1·3 g kg^{-1}, $\delta^{15}N$ and $\delta^{13}C$ values of 8·0 and −27·4‰, respectively. Soil properties as well as the soil management are described in detail in Kramer *et al.* (2012).

For $^{13}CO_2$ pulse labelling, four plots (1 × 1 m^2), containing nine maize plants each, were covered with labelling chambers (1 × 1 × 2 m^3) consisting of aluminium frames stringed with transparent LDPE foil with a total light transmission of ~90%. Prior to labelling, the CO_2 concentration, measured in one chamber with an infrared gas analyser (LI-820; LI-COR Biosciences, Lincoln, NE, USA), declined to zero. The $^{13}CO_2$ was released in each chamber by adding an excess of 1 M H_2SO_4 to 16 g of 99% ^{13}C-enriched Na_2CO_3 dissolved in water. Plants were labelled for 4 h (2 pm–6 pm) and then chambers were removed. A detailed description of the chamber system is given by Riederer *et al.* (2015).

Samples were taken shortly before labelling (^{13}C natural abundances) and 2, 5, 10 and 25 days after labelling. At each sampling, one plant per plot was harvested at 1 cm above the soil surface. Soil samples were taken at the position of the plant using a Riverside auger (ID 5 cm; Eijkelkamp, Giesbeek, the Netherlands) at 0–10 cm depth, where 50% of the maize roots were present (Pausch *et al.* 2013). For soil arthropods, two additional soil cores (ID 20 cm) were taken per chamber. To analyse the $\delta^{13}C$ value of the soil CO_2 efflux, two steel collars (ID 13 cm, height 15 cm) with beakers (ID 5 cm, height 5 cm) for NaOH (80 mL of 1 M NaOH) were inserted 5 cm deep into the soil at each labelling plot. In addition, eight collars were installed at the unlabelled plot. The NaOH solution was exchanged at each sampling date.

EXTRACTION PROCEDURES

Roots were separated from the soil by handpicking and the attached soil, removed by slight shaking, was taken as rhizosphere soil. The above-ground crown roots were separated, and exclusively the roots below the soil surface were used for further analysis. A subsample of the bulk and rhizosphere soil and shoot and root material was dried at 60 °C for 3 days, homogenized in a ball mill and analysed for $\delta^{13}C$ values.

The C concentration of the NaOH solution was measured by an N/C analyser (Multi N/C 2100; AnalytikJena, Jena, Germany). An aliquot of each sample was precipitated with $SrCl_2$ as $SrCO_3$ using the method by Harris, Porter & Paul (1997) and measured for the $\delta^{13}C$ value.

Total soil microbial biomass C (MBC) was determined in bulk and rhizosphere soil by chloroform fumigation–extraction method (Vance, Brookes & Jenkinson 1987) with the modification that fumigated and non-fumigated soil samples (5 g WW) were extracted (1 h at 200 rev min^{-1}) with 20 mL of 0·05 M K_2SO_4. After centrifugation of the extracts, the supernatant was filtered and an aliquot of 2 mL was used for measurement of total C. The remaining samples were dried (60 °C for 3 days) and analysed for $\delta^{13}C$. MBC (mg C g^{-1} soil) was calculated by dividing the

C-flush, that is the difference between the C content in fumigated and non-fumigated samples by a conversion factor of 0·45 to correct for C extraction efficiency (Wu *et al*. 1990). The C content of the non-fumigated soil extracts was used as a measure of the fraction of DOC. The isotopic composition of microbial biomass was determined based on a mass balance equation described by Werth & Kuzyakov (2009, eqn 3).

For phospholipid fatty acid (PLFA) analysis, lipids from 6 g of fresh soil (rhizosphere and bulk soil) were extracted with a Bligh & Dyer solution [chloroform, methanol, citrate buffer (pH = 4; 1 : 2 : 0·8; v/v/v)] according to Frostegård, Tunlid & Bååth (1991). Lipids were separated into glycolipids, neutral lipids and PLFAs with silica acid columns (0·5 g silicic acid, 3 mL; Varian Medical Systems, Palo Alto, CA, USA) and transformed into fatty acid methyl esters (FAMEs) with a mild alkaline methanolysis. FAMEs were measured by GC as described by Kramer *et al*. (2013). For quantification, internal FAME standard (Sigma-Aldrich, St. Louis, MO, USA) was added to the samples before methanolysis. For $\delta^{13}C$ determination, FAMEs were fractionated with Ag^{+}-SPE cartridges (6 mL; Supelco, Palo Alto, CA, USA) as described in Kramer *et al*. (2008). Stepwise elution results in four FAME fractions: saturated, monoenoic trans, monoenoic cis and dienoic. Only fraction one and four were used for $\delta^{13}C$ determination. Therefore, the fatty acids i15:0, a15:0, i16:0, i17:0 and cy17:0 were used as bacterial and 18:2ω6,9c as fungal biomarker (Ruess & Chamberlain 2010). For calculation of the C content, fungal PLFA concentration was multiplied with the conversion factor of 85 (Klamer & Bååth 2004). Please note that the usage of conversion factors and proportions to calculate fungal or bacterial biomass always is an approximation, though necessary to understand the relative dynamics of fungi and bacteria in soil. Since the microbial biomass C consists mainly of fungal and bacterial C, the bacterial C was calculated by subtracting fungal C values from mean MBC values of bulk or rhizosphere soil.

Nematodes were extracted from 50 to 80 g of fresh bulk soil using a modified Baermann's method (Ruess 1995) and fixed in cold 4% formaldehyde solution. For $\delta^{13}C$ analysis, specimens of each sample were sorted into trophic groups, placed in tin capsules (5 × 9 mm; HEKAtech GmbH, Wegberg, Germany) and dried for 2 days at 60 °C. The C content of the nematodes was assumed to be 50% C of dry mass (Schmidt *et al*. 2000).

Soil arthropods were extracted from the intact soil cores using a high-gradient heat extractor (Kempson, Lloyd & Ghelardi 1963), collected in saturated salt (NaCl) solution and stored at −10 °C until further analysis. Animals were determined to species level if possible, and body width was used to assign species to meso- (<2 mm) or macrofauna (>2 mm). Taxa with a collective dry weight of at least 0·1 mg were placed into tin capsules (3·2 × 4 mm; HEKAtech GmbH) for $\delta^{13}C$ and $\delta^{15}N$ analysis. Based on their natural $\delta^{15}N$ value, the species were ascribed to decomposers and predators with the threshold between predators and decomposers at 5·0‰ enrichment in ^{15}N. The threshold was set according to the mean ^{15}N signature of maize and wheat residues (3·9 ± 1·1‰) as baseline since both contributed to the nutrition of soil arthropods at the investigated field site, and assuming only low enrichment of ^{15}N in basal trophic levels (Scheu & Falca 2000; Potapov *et al*. 2013). Due to low body mass of mesofauna species, several individuals were pooled to allow stable isotope measurements; in macrofauna species, single individuals were analysed. However, low abundance of some taxa did not allow stable isotope analysis at all sampling dates.

ISOTOPE ANALYSES

Total C contents and $\delta^{13}C$ values of shoot, root and soil samples, as well as $\delta^{13}C$ values in soil CO_2 efflux, microbial biomass and meso- and macrofauna species, were measured using an elemental analyser (EA) NA 1500 (Carlo Erba Instruments, Milano, Italy) interfaced to a Delta plus isotope ratio mass spectrometer (IRMS; Thermo Fisher Scientific, Bremen, Germany). For meso- and macrofauna species, $\delta^{15}N$ values were also assessed.

$\delta^{13}C$ values of the separated fractions of FAMEs were measured using a GC (6890 series; Agilent Technologies, Santa Clara, CA, USA) equipped with a capillary column VS-23MS (Varian Medical Systems; 30 m × 250 μm, film thickness of 0·25 μm) coupled via a GC-combustion III Interface (Thermo Finnigan, Waltham, MA, USA) to a Delta Plus XP IRMS (Thermo Finnigan MAT, Bremen, Germany). Temperature of the oven and injector was 290 °C and 250 °C, respectively. The temperature program was set as follows: initial temperature was 80 °C and held for 2 min, increased by 10 °C min^{-1} to 140 °C and then increased by 4 °C min^{-1} to 240 °C and held for 5 min. The samples were measured in split less mode.

Nematode samples contained very low amounts of C (average of 1–24 μg per sample) and were measured on an Eurovector EA (Eurovector EA3000; Eurovector S.p.A. Milano, Italy) coupled to a Delta V Plus IRMS (Thermo Fisher Scientific). The EA was fitted with smaller oxidation and reduction reactor tubes (ID 7·8 mm, 450 mm length) to allow lower carrier gas flow and increased sensitivity (Langel & Dyckmans 2014). For all analysed materials, the standards (Australian National University sucrose and NBS 19) were calibrated with reference to the international standard (Vienna Pee Dee Belemnite).

CALCULATIONS

The excess (above background) of the ^{13}C tracer in a certain C pool (χ^{E} (^{13}C), atom%) was determined by subtracting the ^{13}C abundance in the respective pool before labelling ($\chi(^{13}C)_{Std}$, atom %) from the ^{13}C abundance in this pool (P) after labelling ($\chi(^{13}C)_{P}$, atom%):

$$\chi^{E}(^{13}C) = \chi(^{13}C)_{P} - \chi(^{13}C)_{Std} \qquad \text{eqn 1}$$

For $\chi(^{13}C)_{Std}$, the data from the stable isotope natural abundance measurements taken before the labelling were used. The natural abundance $\delta^{13}C$ value of the soil CO_2 efflux was determined by correcting the measured $\delta^{13}C$ values for the admixture of atmospheric CO_2, based on the Miller/Tans model and was −23·45 ± 2·03 (Miller & Tans 2003; Pausch & Kuzyakov 2012).

The ^{13}C excess mass of a particular C pool per square metre (n $(^{13}C_P)_F$, g ^{13}C m^{-2}) was calculated by multiplying the ^{13}C excess (χ^{E} (^{13}C), atom%) with the total C stock of the respective pool (n $(C_P)_F$, g C m^{-2}). The division by 100 converts atom% to absolute values:

$$n(^{13}C_P)_F = \frac{\chi^{E}(^{13}C)}{100} \cdot n(C_P)_F \qquad \text{eqn 2}$$

$$n(C_P)_F = z \cdot \rho \cdot n(C_P) \cdot 10 \qquad \text{eqn 3}$$

where F stands for the area at the arable field, z (cm) is the thickness of the respective soil layer, ρ (g cm^{-3}) is the soil bulk density (1·38 g cm^{-3} at Ap1 horizon), and n(C_P) is the C content (mg C g $soil^{-1}$) of the pool. The samples contained on average 27% rhizosphere and 73% bulk soil, which was considered for the calculations of the respective C pools. The absolute tracer amounts allowed investigating the dynamics of ^{13}C allocation within individual C pools independently from a dilution of ^{13}C by unlabelled recently assimilated C.

The total C budget for the plant–soil system was compiled based on the total C stocks of the investigated pools ($n(C_P)_F$, g C m^{-2}). The percentage of C of a certain pool ($r(C_P)_F$, %) was calculated by relating the C stock of the respective pool ($n(C_P)_F$, g C m^{-2}) to the sum of the C stocks of shoots, roots, bulk soil and rhizosphere soil ($n(C_T)_F$, g C m^{-2}).

$$r(C_p)_F = \frac{n(C_P)_F}{n(C_T)_F} \cdot 100 \qquad \text{eqn 4}$$

The ^{13}C excess measured in all pools is the result of the tracer influxes and outfluxes including ^{13}C respired to CO_2. Therefore, we cannot estimate the total amount of the tracer passed through each pool, but the net incorporation of tracer can be measured. The net incorporation of tracer into a certain C pool ($f(C_P)_F$, %) during the sampling period was calculated based on the ^{13}C excess mass of the respective pool ($n(^{13}C_P)_F$, g ^{13}C m^{-2}). For each pool, the maximum ^{13}C excess at one of the sampling dates ($n(^{13}C_P)_F$ (maximum), g ^{13}C m^{-2}) was related to the total ^{13}C excess recovered at sampling day 2 ($n(^{13}C_T)_F$ (day 2), g ^{13}C m^{-2}), that is to the sum of ^{13}C excess in shoots, roots, bulk soil, rhizosphere soil and soil CO_2 efflux:

$$f(C_P)_F = \frac{n(^{13}C_P)_F(\text{maximum})}{n(^{13}C_T)_F(\text{day 2})}. \qquad \text{eqn 5}$$

STATISTICAL ANALYSES

Values presented in figures and tables are given as means and standard errors of the means (±SEM). Significant differences ($P \leq 0{\cdot}05$) in ^{13}C excess between the sampling days were inspected using one-way analysis of variance (ANOVA) in combination with *post hoc* Tukey's HSD test. Differences in incorporation of ^{13}C in time between fungi and bacteria, trophic groups of nematodes and trophic groups of meso- and macrofauna were analysed by repeated measures ANOVAs followed by Wilk's lambda tests. In case of significant differences (Wilk's lambda), *post hoc* Tukey's HSD tests were used to further determine significant effects over time within one organism group. To test for significant differences in ^{13}C excess mass between bulk and rhizosphere soil, independent *t*-tests were used. Statistical analyses were performed with the statistical package STATISTICA for Windows (version 7.0; StatSoft Inc., Tulsa, OK, USA).

Results

CARBON STOCKS IN SOIL POOLS, PLANTS AND FOOD WEB COMPARTMENTS

The maize shoots contained 317 ± 48 g C m^{-2}, while the C stock of the roots was only 28 ± 4 g C m^{-2} (0–10 depth, Table 1). The soil of the upper 10 cm contained 1158 ± 15 and 453 ± 9 g C m^{-2} in bulk and rhizosphere soil, respectively. The dissolved organic C in rhizosphere soil exceeded that in bulk soil by a factor of ~2·5. Microbial biomass C was 29 ± 16 and 21 ± 8 g C m^{-2} in bulk and rhizosphere soil, respectively. Of this, about 66% in bulk and 69% in rhizosphere soil was bacterial C, reflecting the higher density of bacteria as compared to fungi in the arable soil.

The stock of C in nematode biomass ranged from 0·004 ± 0·002 to 0·068 ± 0·013 g C m^{-2} (Table 1). Predatory and omnivore nematodes as well as plant feeders accounted for <0·022 g C m^{-2}. Bacterial and fungal feeders contributed most to nematode biomass C with 0·051 ± 0·007 and 0·068 ± 0·013 g C m^{-2}, respectively. The biomass C of the mesofauna was about 10% of that of nematodes, with 0·0039 ± 0·0058 and 0·0071 ± 0·0012 g C m^{-2} in decomposers and predators, respectively (Table 1). In the macrofauna, mean biomass C was up to 37 times larger than in the mesofauna, with decomposers and predators accounting for 0·146 ± 0·296 and 0·140 ± 0·854 g C m^{-2}, respectively. The size of the C pool in the mesofauna reflected the abundance of species, with a maximum of 0·0034 ± 0·0006 g C m^{-2} in the dominant Collembola species *Protaphorura armata*. In contrast, the size of the C pool in the macrofauna varied with body size; among decomposers, it was highest in the diplopod species *Blaniulus guttulatus* and among predators in the centipede species *Necrophloeophagus longicornis*.

INCORPORATION OF ROOT-DERIVED C INTO THE SOIL FOOD WEB

The ^{13}C excess of microbial biomass did not differ significantly between rhizosphere and bulk soil (Table S2, Supporting information). As assigned by marker PLFAs, fungi incorporated 62 ± 28 and 26 ± 14 mg ^{13}C m^{-2} in rhizosphere and bulk soil, respectively, which was up to ten times more ^{13}C than that incorporated into bacteria. However, differences between these two groups were only significant in rhizosphere soil at days 5 and 10 after labelling (Fig. 1a). In the rhizosphere, the highest amount of ^{13}C was incorporated 2 days after labelling in both fungi and bacteria, followed by a decline until day 5 (not significant). At day 10 and 25 after labelling, the ^{13}C amount in rhizosphere bacteria was significantly lower compared to day 2. ^{13}C incorporation into bulk soil micro-organisms was more balanced and ^{13}C allocation did not differ significantly during the 25 days.

All functional groups of nematodes displayed a ^{13}C signal already at day 2 after labelling (Table S1, Supporting information, Fig. 1b). Fungal feeders incorporated the greatest amounts of ^{13}C, which significantly increased with time to 0·02 ± 0·01 mg ^{13}C m^{-2} at day 25. Higher trophic levels, that is predators and omnivores, showed a time lag in ^{13}C uptake compared to microbial consumers; however, this was not significant. The overall increase in ^{13}C excess over 25 days in all trophic groups reflects steady flux of plant C into the nematode community.

Also meso- and macrofauna species incorporated ^{13}C already 2 days after labelling (Fig. 1c). ^{13}C excess in macrofauna decomposers increased significantly at day 25 and reached 0·07 ± 0·03 mg ^{13}C m^{-2}, indicating gradual flux of root-derived C into higher trophic levels. While ^{13}C dynamics of macrofauna predators followed those of decomposers, mesofauna predators showed a humpback-shaped incorporation with highest ^{13}C excess mass at day 10.

Table 1. C stocks of maize plants and of major soil compartments (g C m^{-2} ± SEM), of the microbial community (g C m^{-2} ± SEM) in rhizosphere and bulk soil, of functional groups of nematodes (g C m^{-2} ± SEM) and of maso- and macrofauna taxa (mg C m^{-2} ± SEM) as mean values over time

Major C compartments	C stocks (g C m^{-2})	Meso-/Macrofauna	C stocks (mg C m^{-2})
Maize		Mesofauna decomposers	3·97 ± 0·58*
Shoot	317·4 ± 47·6	*Bourletiella hortensis*	0·38 ± 0·06
Roots	27·7 ± 3·9	*Protaphorura armata*	3·44 ± 0·56
		Tectocepheus velatus	0·15 ± 0·04
Soil			
Bulk	1157·5 ± 14·5	Mesofauna predators	7·13 ± 1·18*
DOC bulk	5·1 ± 0·3	*Alliphis siculus*	0·87 ± 0·37
Rhizosphere	453·3 ± 8·8	*Hypoaspis aculeifer*	0·30 ± 0·19
DOC rhizosphere	13·2 ± 4·5	*Isotoma viridis*	2·37 ± 0·54
		Lepidocyrtus cyaneus	1·18 ± 0·24
		Lysigamasus sp.	0·25 ± 0·02
Microbial community		*Nenteria unguiculata*	0·29 ± 0·19
Bulk soil		*Oppiella nova*	0·03 ± 0·03
Microbial biomass	28·9 ± 16·0	*Pergamasus* sp.	0·05 ± 0·03
Fungi	9·82 ± 0·65	*Pseudosinella alba*	1·35 ± 0·37
Bacteria	19·08 ± 0·65	*Willowsia buski*	0·69 ± 0·22
Rhizosphere soil		Macrofauna decomposers	145·89 ± 29·64*
Microbial biomass	20·9 ± 7·9	Aleocharinae	2·26 ± 0·84
Fungi	6·45 ± 0·52	*Blaniulus guttulatus*	103·62 ± 25·30
Bacteria	14·45 ± 0·52	*Haplothrips* sp.	2·38 ± 1·13
		Muscidae Larvae	0·24 ± 0·10
		Paederinae	4·06 ± 1·56
Nematodes		*Polydesmus superus*	30·64 ± 9·74
Bacterial feeders	0·051 ± 0·007	Staphylinidae Larvae	2·70 ± 0·66
Fungal feeders	0·068 ± 0·013		
Plant feeders	0·011 ± 0·002	Macrofauna predators	140·43 ± 85·39*
Omnivores	0·007 ± 0·002	Elateridae Larvae	17·07 ± 4·09
Predators	0·004 ± 0·002	*Lamyctes fulvicornis*	8·07 ± 1·40
		Meioneta sp.	11·24 ± 2·19
		Necrophloeophagus longicornis	87·64 ± 84·86
		Scopaeus sp.	2·39 ± 1·24
		Symphylella vulgaris	14·02 ± 3·45

DOC, dissolved organic carbon.
*Total pool size of the trophic group calculated as sum of all pool sizes of the single species.

C BUDGET AND NET C INCORPORATION INTO THE SOIL FOOD WEB

The largest C pool was the soil comprising 82% of the total C per m^{-2}, with 60% hold in the bulk soil (Fig. 2). The maize crop contained 16·2% and 1·4% C m^{-2} in shoots and roots, respectively. Soil micro-organisms accounted for 2·5% C m^{-2}, with 1·5% in bulk and 1·1% in rhizosphere soil. The C pool in higher trophic levels was much lower, with only 0·0072% C m^{-2} in nematodes and 0·0152% in the meso- and macrofauna.

The C pool size did not determine the amount of incorporated tracer (Fig. 2). Despite the large C stock of the bulk soil, the net tracer incorporation into this pool was only 2·4%. In contrast, the much smaller rhizosphere C pool received more than 13% of the assimilated C, which also provoked a higher tracer incorporation into DOC and micro-organisms in this compartment. Rhizosphere micro-organisms received about 5% of the ^{13}C tracer, much more than bulk soil micro-organisms with only 1% of total ^{13}C recovered at day 2 after labelling. Saprotrophic fungi were the strongest sink for root-derived ^{13}C, even though the bacterial C pool was greater than the fungal pool in both rhizosphere and bulk soil. Moreover, due to high turnover of plant-derived C in the rhizosphere, the incorporation of ^{13}C into rhizosphere fungi exceeded that into bulk soil fungi by more than a factor of two.

Changes in the amount of ^{13}C in micro-organisms were reflected by respective shifts in the soil microfauna. Although bacterial- and fungal-feeding nematodes accounted for comparable amounts of C, ^{13}C incorporation was highest in fungal feeders (Table 1, Fig. 2) indicating high C turnover in the fungal energy channel. In contrast, the incorporation into plant feeders, directly consuming living roots, was low with 0·0001% of total ^{13}C tracer recovered at day 2 after labelling. Comparable amounts of plant C were incorporated into higher trophic levels, that is omnivores and predators. In sum, the incorporation of root-derived ^{13}C into the nematode microfood web was about 0·0022%.

Meso- and macrofauna incorporated about 0·0069% of root-derived ^{13}C (Fig. 2). C pools and net ^{13}C incorporation into the mesofauna were small, whereas macrofauna decomposers incorporated highest amounts of C. Compared to the latter, ^{13}C incorporation into macrofauna predators, although accounting for a similar C pool, was much lower with about 0·0009% of total ^{13}C tracer reflecting slow C turnover.

Assuming steady-state conditions, that is fixed values for pool size and flux rates during the experiment, relative turnover rates for single food web pools were calculated according to the values given in Fig. 2 by dividing the net tracer incorporation by the pool size (Table 2). However, the calculated rates do not represent absolute turnover rates as they depend on the amount of ^{13}C tracer added during labelling. The relative turnover rates of micro-organisms were much higher in rhizosphere compared to bulk soil and in fungi compared to bacteria. The mean turnover rate of nematodes (0·31) and meso- and macrofauna (0·45) was a magnitude lower than that of fungi but not of bacteria (Table 2). Within the nematode microfood web, C flux was dominated by omnivores and fungal feeders and within larger animals by macrofauna decomposers.

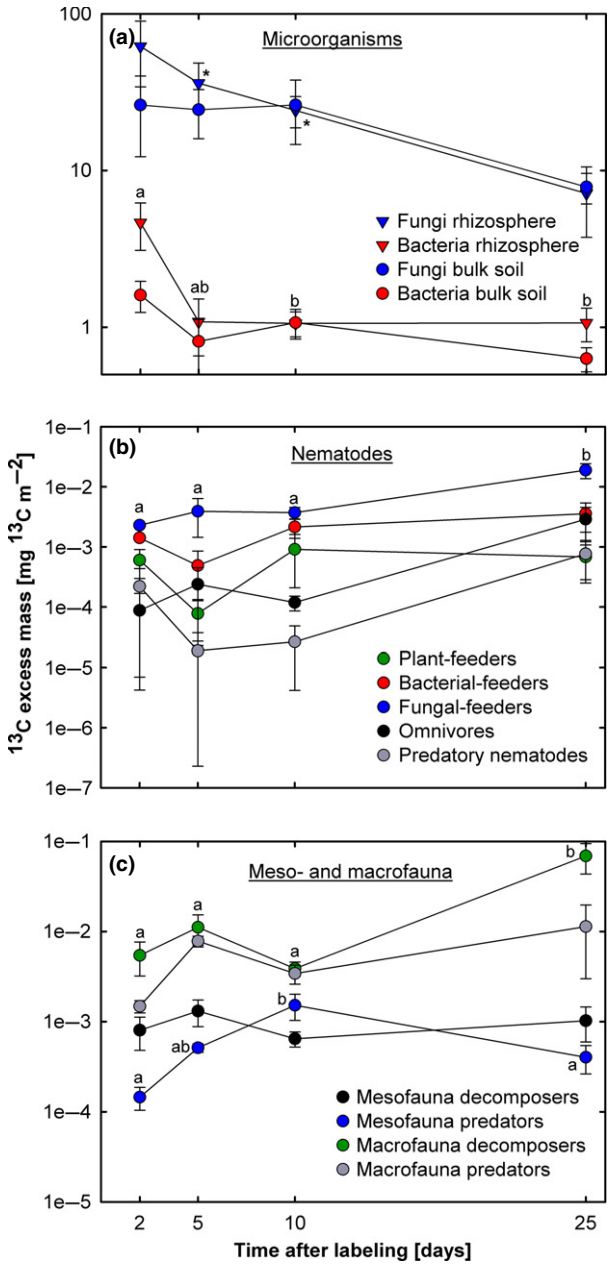

Fig. 1. ^{13}C excess mass (mg ^{13}C m^{-2} ± SEM) 2, 5, 10 and 25 days after $^{13}CO_2$ labelling of fungi and bacteria in the rhizosphere and bulk soil (0–10 cm) (a), of functional groups of nematodes (b) and of decomposers and predators of meso- and macrofauna (c). Values of different sampling dates with the same or no letters are not significantly different according to Tukey's HSD with $P \leq 0{\cdot}05$. The asterisk indicates significant differences ($P \leq 0{\cdot}05$) between fungi and bacteria according to Tukey's HSD. There were no significant differences between bulk and rhizosphere soil. Note the logarithmic scale of the *y*-axis.

Discussion

INCORPORATION OF ROOT C INTO THE SOIL FOOD WEB

Soil biota in agro-ecosystems are supplied with recent plant assimilates via root exudates, which are utilized in the rhizosphere within a few hours after release (Pausch *et al.* 2013). In our study, root-derived ^{13}C was quickly incorporated into bacteria and fungi, as indicated by distinct ^{13}C enrichment at day 2 after labelling. This confirms earlier studies reporting highest incorporation of ^{13}C into microbial PLFA markers within the first 3 days (De Deyn *et al.* 2011; Tavi *et al.* 2013). For both rhizosphere and bulk soil, a large proportion of the plant-fixed C was transferred to saprotrophic fungi, with only

Table 2. Relative turnover rates of the single organism C pools of an arable soil calculated by dividing the net tracer incorporation by the pool size (shown in Fig. 2)

Pool	Relative turnover rates
Microbial biomass C	2·35
Bulk soil	0·75
Fungi bulk soil	4·34
Bacteria bulk soil	0·14
Rhizosphere soil	4·56
Fungi rhizosphere soil	15·6
Bacteria rhizosphere soil	0·52
Nematodes	0·31
Bacterial feeders	0·11
Fungal feeders	0·45
Plant feeders	0·14
Omnivores	0·64
Predators	0·30
Meso- and macrofauna	0·45
Mesofauna decomposers	0·51
Mesofauna predators	0·35
Macrofauna decomposers	0·77
Macrofauna predators	0·13

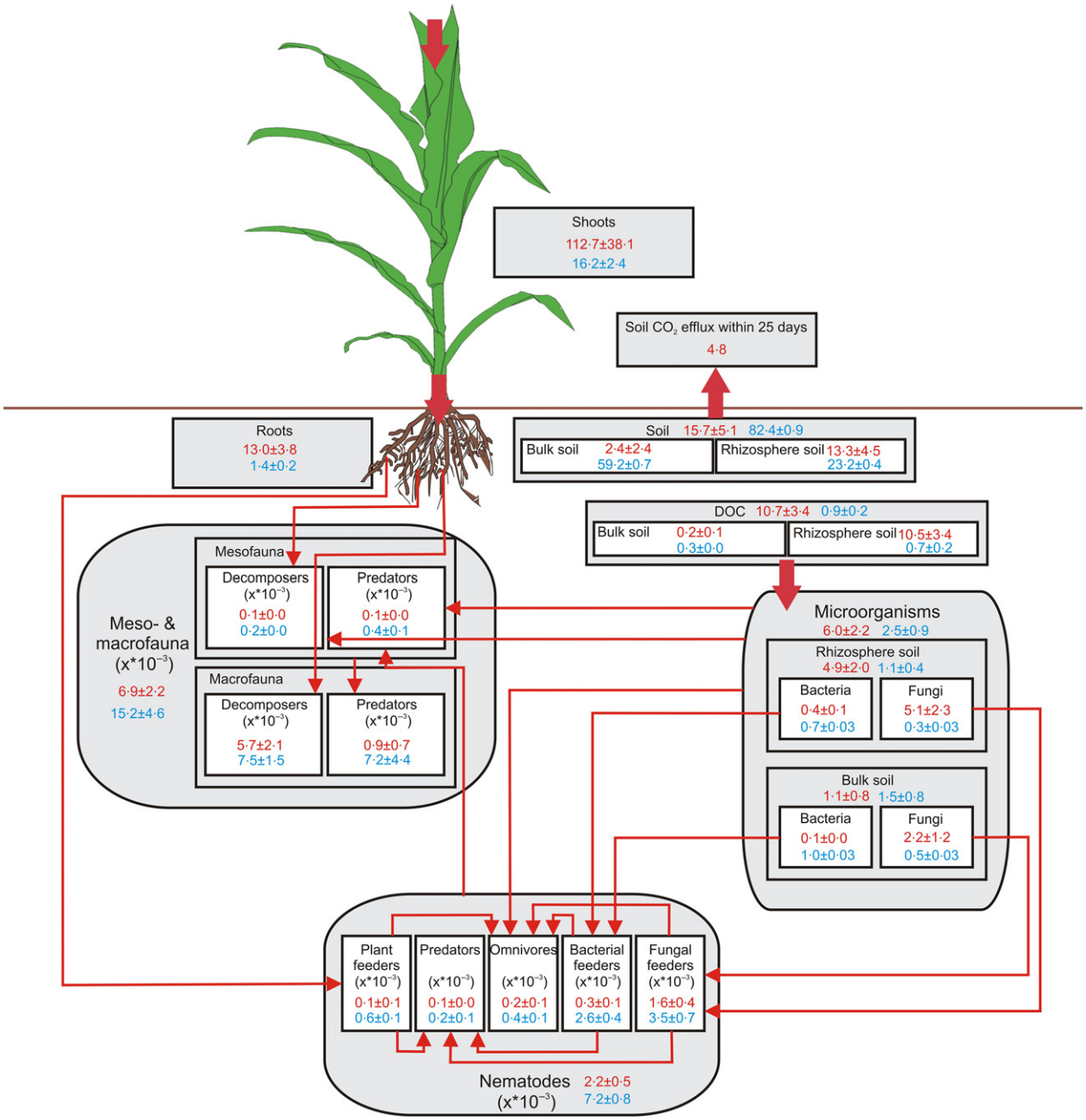

Fig. 2. Carbon budget within a soil food web of the upper 10 cm of an arable soil presented as % of total C per m^2 (blue values) and net tracer incorporation (red values) into each C pool of the food web determined within 25 days after $^{13}CO_2$ pulse labelling of maize. Tracer incorporation was calculated based on the maximal ^{13}C recovered in each pool and is given as % of total recovered ^{13}C on day 2 after labelling. The soil CO_2 efflux includes root respiration and CO_2 released from decomposition processes within the food web.

a minor fraction transferred to bacteria. Generally, the $\delta^{13}C$ value of the fungal biomarker linoleic acid could be influenced by an additional ^{13}C signal derived from linoleic acid from root hairs. However, plant roots are much larger than fungal hyphae, with a lower surface area-to-volume ratio, and therefore, the contribution of root tissue to the soil PLFA pool is relatively small. Thus, the pool size of fungal-derived linoleic acid is sufficiently high to use it as a biomarker (Ruess & Chamberlain 2010). Moreover, according to Ngosong, Gabriel & Ruess (2014), linoleic acid accounts only for about 14% of total lipids in maize roots, whereas it ranged from 33·6% to 87·6% of total PLFAs across 15 different fungal species (Ruess *et al.* 2002). Together with the findings of Kaiser *et al.* (2010) who estimated a contribution of roots to the biomarker linoleic acid in sieved soil samples of 0·61% only, the influence of root-derived linoleic acid to the ^{13}C signal is negligible. This is further supported by a relatively low $\delta^{13}C$ value of maize roots (~510‰ at day 2 after labelling) compared to that of the fungal biomarker in the rhizosphere (~960‰ at day 2 after labelling; Table S1, Supporting information).

The large proportion of ^{13}C in saprotrophic fungi contrast our hypotheses that rhizosphere bacteria are the major functional group allocating and processing plant root C. On the other hand, results from pulse-labelling studies using PLFA markers in grassland (Butler *et al.* 2003; Denef *et al.* 2009) and organic arable fields (Tavi *et al.* 2013) comparably indicated a considerable importance of the fungal energy pathway. Thus, despite their low C pool, saprotrophic fungi showed a much higher ^{13}C incorporation and turnover rate than bacteria. This highlights their role as major conduit in the transfer of C between plants and soil in arable systems. The high turnover of fungi is likely due to translocation within fungal hyphae (Frey, Six & Elliott 2003), thereby transporting root-derived C into compartments further away from roots such as bulk soil.

Arbuscular mycorrhiza (AM) fungi were shown to release plant-fixed ^{13}C from their mycelium to bacterial and fungal populations in the (myco-) rhizosphere (Drigo *et al.* 2010). Since we did not analyse the neutral lipid fatty acid or ^{13}C in the PLFA biomarker for AM fungi, we are not able to separate the direct C flux from the roots and the indirect path via AM fungi into bacteria and saprotrophic fungi. However, our results are in line with natural stable isotope studies at the field site, revealing predominant root C translocation into ergosterol, that is saprotrophic rather than AM fungi (Kramer *et al.* 2012). Moreover, as hyphae of saprotrophic fungi can be closely associated with roots (Butler *et al.* 2003), they likely compete effectively with AM fungi on root-derived carbon resources.

Bacteria mainly associated with organo-mineral surfaces are more dependent on the transport of substrates than fungi exploring their substrates with hyphal growth (Poll *et al.* 2008). At our site, about 65% of total precipitation is lost via transpiration, and only 13% of the seepage water is available in the plough zone (Dibbern *et al.* 2014). Therefore, bacteria likely have less access to substrates under water limited conditions typically occurring in summer periods at temperate arable fields. High incorporation of root-derived C into Gram-negative PLFA markers 16:1ω7 and 18:1ω7 has been reported earlier (Treonis *et al.* 2004; Denef *et al.* 2009; Tavi *et al.* 2013). Due to methodological restrictions, we only analysed the PLFA cy17:0 as marker for Gram-negative bacteria, which might have resulted in an underestimation of the ^{13}C in the bacterial pool. Nevertheless, the high ^{13}C flux into fungal biomass and the fast C turnover (~30 times higher than that of bacteria) suggests fungi to represent the dominant micro-organisms processing root-derived C. This also is reflected by the high transfer of C into fungal-feeding nematodes heavily relying on saprotrophic fungi as prey (Ruess, Garzía Zapata & Dighton 2000).

Among nematodes, plant feeders directly consume root tissue and thus are expected to show the fastest and strongest incorporation of ^{13}C (Yeates & Bongers 1999). In contrast, in our study, ^{13}C uptake was consistently higher in bacterial and fungal feeders compared to plant feeders. Besides higher densities of these microbial grazers, this may also be due to their preferential feeding in the rhizosphere. The high ^{13}C excess mass in fungal-feeding nematodes corresponds to the fast turnover of the fungal community, indicating a major flux of root-derived C through the fungal energy channel. This is in line with C incorporation 4·3 and turnover rates 4·9 times lower in bacterial feeders compared to fungal feeders.

No diminishing of the ^{13}C signal, for example via respiration, was observed within the nematode community over the experimental period, suggesting a continuous flux of root C into higher trophic levels. In contrast to our hypotheses, omnivores and predators displayed low C pool sizes but high C fluxes and turnover rates. This contradicts the general opinion that disturbed soil food webs tend to be bottom heavy (Ferris 2010) and indicates a notable top-down control even in perturbed arable soil. While omnivores likely incorporated plant C from lower trophic levels, thereby bypassing food web linkages, C flux into predators suggests tritrophic cascades to be of major importance. Presumably, omnivory and indirect trophic interactions regulate soil food webs to a higher extent as currently considered in energy flow models.

At present, no specific fatty acid biomarker for protozoa is available as the frequently used 20:4ω6,9,12,15 does occur across all soil fauna (Ruess & Chamberlain 2010). Consistent with the low biomass C in soil animals, the amount of this biomarker was low in our study (rhizosphere soil: $0{\cdot}0014 \pm 0{\cdot}0003$ g C m^{-2}, bulk soil: $0{\cdot}0038 \pm 0{\cdot}0004$ g C m^{-2}). Thus, unfortunately, we cannot infer its size relative to that of nematodes the second major consumers of bacteria in soil (Bonkowski 2004). Recently, it has been stressed that protozoa may channel significant amounts of C to meso- and macrofauna consumers via feeding on living and dead protozoan cells (Crotty *et al.* 2012).

The C pool size of mesofauna decomposers was only half that of mesofauna predators, due to the low number of decomposer species. Despite that, decomposers and predators incorporated similar amounts of ^{13}C, indicating that mesofauna decomposers essentially rely on root-derived C. For instance, high ^{13}C enrichment in *P. armata*, the most abundant Collembola species at our study site (N. Scheunemann, pers. comm.) confirms earlier results reporting Collembola to incorporate more ^{13}C than macrofauna (Ostle *et al.* 2007; Murray *et al.* 2009), with a signal corresponding to fungal-feeding nematodes. Supporting these findings, the latter were a major component for ^{13}C flux and turnover in our study. The high ^{13}C label in many Collembola assigned as predators, identifies them as another key group of the fungal energy channel, presumably due to directly feeding on saprotrophic fungi and/or on fungal-feeding nematodes (Heidemann *et al.* 2014), indicating top-down effects of microarthropods on microfauna. Also, mesofauna decomposers may have incorporated ^{13}C via feeding on maize roots as recently shown for

Protaphorura fimata (Endlweber, Ruess & Scheu 2009), a closely related species of *P. armata*. Notably, mesofauna decomposers continuously incorporated ^{13}C during the experiment, while the ^{13}C flux into mesofauna predators peaked at day 10, suggesting fewer trophic links to prey with access to root-derived C with time, that is a diet switch. In sum, the diverse resources derived from different trophic levels highlight the role of mesofauna species for soil food web and soil C dynamics.

Based on the much higher C pool size, macrofauna species contributed more to the flux of C through the investigated food web than mesofauna species. While the C pool in macrofauna predators and decomposers was similar, the latter incorporated up to six times more ^{13}C, most evident at day 25. Moreover, the turnover rate was highest for macrofauna decomposers, indicating that they essentially rely on root-derived C as suggested earlier (Albers, Schaefer & Scheu 2006; Pollierer *et al.* 2007). Notably, diplopods had the highest abundance and biomass and incorporated high amounts of ^{13}C. This either may be due to their function as primary decomposers feeding on dead plant material (Semenyuk & Tiunov 2011), thereby participating in high ^{13}C flux into saprotrophic fungi, or result from directly feeding on roots as observed by Gunn & Cherrett (1993) in a grassland soil. Thus, despite the small C pool, there was a considerable C flux into diplopods, presumably due to their function as both primary decomposers and herbivores.

C BUDGET AND NET C INCORPORATION INTO THE SOIL FOOD WEB

The detailed analysis of C stocks of soil food web compartments and root-derived C incorporation into these compartments suggests that these two parameters are not closely linked. The discrepancy between pool sizes and incorporation intensity results from different turnover rates of individual pools. While C stocks in bulk soil and rhizosphere micro-organisms were similar, processing of ^{13}C was about 4·5 times faster in the rhizosphere, likely due to the quick utilization of easily available root-derived C compounds. Further, predation by the rhizosphere fauna likely contributed to the fast turnover of C in rhizosphere micro-organisms (Griffiths 1994; Bonkowski 2004). Most interestingly, C flux was highest through the saprotrophic fungal energy channel and root-derived C was incorporated heavily into higher trophic levels, with turnover rates of fungal-feeding exceeding those of bacterial-feeding nematodes and of meso- and macrofauna decomposers (i.e. saprophages) exceeding those of predators. This contrasts with the widely accepted notion that the fungal energy channel is favoured in systems with complex organic resources of lower quality (i.e. forests), whereas systems driven by labile root exudates (i.e. arable land) are dominated by the bacterial energy pathway (Ruess & Ferris 2004; Wardle *et al.* 2004). Our results clearly showed that the fungal community and their faunal grazers dominate and control the flux of easily available root C through the food web of arable soils.

In summary, the present findings shed new light on widely held ideas and theory underlying quantitative food web models. In contrast to our initial hypotheses that C pool size of food web compartments determines C incorporation into them, our results indicate: (i) despite smaller biomass, the C turnover rate of bacteria and fungi is higher in rhizosphere than bulk soil, (ii) the C turnover of micro-organisms as major primary decomposers exceeds that of higher trophic levels, (iii) bottom-up effects are not necessarily driven by C stocks as small pools (the fungal energy channel in this study) with high turnover rates can dominate the flux of C through food webs and (iv) contrasting traditional views, the flux of easily available root-derived C in arable soils is predominantly channelled through the fungal and not the bacterial energy pathway.

Acknowledgements

We would like to thank Dr. Jens Dyckmans (Centre of Stable Isotope Research and Analysis, University of Göttingen) for his support with stable isotope measurements. Thanks also to Martin Rimmler for his help during this experiment. This study was supported by the German Research Foundation (DFG) within the Research Unit 'Carbon flow in below-ground food webs assessed by isotope tracers' (FOR 918).

Data accessibility

$\delta^{13}C$ values (‰ ± SEM) of maize plants and of major soil compartments, of soil CO_2 efflux, of the microbial community in rhizosphere and bulk soil, of functional groups of nematodes and of maso- and macrofauna taxa over time and ^{13}C excess mass (mg ^{13}C m^{-2} ± SEM) 2, 5, 10 and 25 days after $^{13}CO_2$ labelling of maize shoots and roots, soil CO_2 efflux (mg ^{13}C day^{-1} m^{-2} ± SEM), and of the following rhizosphere and bulk soil compartments at 0–10 cm depth: soil, DOC and total microbial biomass C (MBC) are uploaded as Supporting Information.

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Received 13 September 2014; accepted 2 July 2015
Handling Editor: Kathleen Treseder

Supporting Information

Additional Supporting information may be found in the online version of this article:

Table S1. $\delta^{13}C$ values (mean ‰, SEM ‰) of maize plants and of major soil compartments, of soil CO_2 efflux, of the microbial community in rhizosphere and bulk soil, of functional groups of nematodes and of maso- and macrofauna taxa over time.

Table S2. ^{13}C excess mass (mean (mg ^{13}C m^{-2}); SEM (mg ^{13}C m^{-2})) 2, 5, 10, and 25 days after $^{13}CO_2$ labelling of maize shoots and roots, soil CO_2 efflux (mg ^{13}C day^{-1} m^{-2} ± SEM), and of the following rhizosphere and bulk soil compartments at 0–10 cm depth: soil, dissolved organic C (DOC), and total microbial biomass C (MBC).