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Carbon flow into microbial and fungal biomass as a basis for the belowground food web of agroecosystems

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ABSTRACT

The origin and quantity of plant inputs to soil are primary factors controlling the size and structure of the soil microbial community. The present study aimed to elucidate and quantify the carbon (C) flow from both root and shoot litter residues into soil organic, extractable, microbial and fungal C pools. Using the shift in C stable isotope values associated with replacing C3 by C4 plants we followed root- *vs.* shoot litter-derived C resources into different soil C pools. We established the following treatments: Corn Maize (CM), Fodder Maize (FM), Wheat + maize Litter (WL) and Wheat (W) as reference. The Corn Maize treatment provided root- as well as shoot litter-derived C (without corn cobs) whereas Fodder Maize (FM) provided only root-derived C (aboveground shoot material was removed). Maize shoot litter was applied on the Wheat + maize Litter (WL) plots to trace the incorporation of C4 litter C into soil microorganisms. Soil samples were taken three times per year (summer, autumn, winter) over two growing seasons. Maize-derived C signal was detectable after three to six months in the following pools: soil organic C (C_{org}), extractable organic C (EOC), microbial biomass (C_{mic}) and fungal biomass (ergosterol). In spite of the lower amounts of root- than of shoot litter-derived C inputs, similar amounts were incorporated into each of the C pools in the FM and WL treatments, indicating greater importance of the root- than shoot litter-derived resources for the soil microorganisms as a basis for the belowground food web. In the CM plots twice as much maize-derived C was incorporated into the pools. After two years, maize-derived C in the CM treatment contributed 14.1, 24.7, 46.6 and 76.2% to C_{org}, EOC, C_{mic} and ergosterol pools, respectively. Fungi incorporated maize-derived C to a greater extent than did total soil microbial biomass.

Introduction

Detailed knowledge on the carbon (C) flow in terrestrial ecosystems is a prerequisite for understanding ecosystem services and for managing agricultural systems in a sustainable way. Considerable information is available on total amounts, individual fractions and residence time of C in soil (Amundson 2001; Janzen 2004; Marschner et al. 2008; Bol et al. 2009; Verchot et al. 2011). In contrast, the fate of belowground C, and especially the flux of C through the soil food web, is poorly understood. The transfer of C from plant roots into soil via rhizodeposition annually recycles around 10% of atmospheric CO_2, which is an order of magnitude greater than current rates of fossil fuel C combustion (Raich et al. 2002). A second pathway of C flow is via litter decomposition which is mediated by the microbial community, predominantly bacteria and fungi (Rosenbrock et al. 1995; Frankland 1998; Dilly et al. 2001). As primary decomposers of rhizodeposits and litter residues, soil microorganisms form the basis of the soil food web.

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Plant inputs are key determinants of microbial activity and community composition in soil. Rhizodeposits lead to a proliferation of microorganisms, altering community structure, and consequently changing the C transfer through the decomposer system (Kuzyakov 2002; Butler et al. 2003; Wasaki et al. 2005). As a result, the quality and quantity of plant C resources and therefore the composition of plant communities create feedbacks to soil microorganisms affecting microbial activity and C allocation in the rhizosphere (Eisenhauer et al. 2010; Ladygina and Hedlund 2010). Generally, labile and recalcitrant plant fractions are utilized by distinct microbial communities, affecting soil C transformation (Paterson et al. 2008; Kramer and Gleixner 2008). Further, root- and litter-derived substrates are processed by different soil organisms (Elfstrand et al. 2008) and root-derived C presumably is immobilized in soil to a greater extent than shoot litter-derived C (Puget and Drinkwater 2001; Rasse et al. 2005). Such effects of resource quality are important bottom-up drivers of soil food webs (Salamon et al. 2006).

Recently, the application of stable isotope tracer methods to determine fluxes within and between C pools in soil has been increased considerably (Bowling et al. 2008). Transformations of C have been followed by switching C3 to C4 plants or resources derived from C3 or C4 plants (Steinbeiss et al. 2008; Esperschütz et al. 2009; Nottingham et al. 2009), as well as by ^{13}C or ^{14}C pulse labeling or continuous labeling to introduce a distinct C signal into the soil system (Kuzyakov and Cheng 2001, 2004; Leake 2004; Leake et al. 2006; Williams et al. 2006; Werth and Kuzyakov 2008).

Only few studies have separated root- and shoot litter-derived C flow and followed C incorporation into different soil and microbial C pools. Soil microorganisms are of particular interest, as they form the basis of the soil food web. For this reason the present study aims to quantify the transfer of C from both root- and shoot litter-derived C as well as of each C source separately into soil C pools [soil organic C (C_{org}) and extractable organic carbon (EOC)], total microbial biomass (C_{mic}) and ergosterol as a proxy for fungal biomass (Djajakirana et al. 1996) using ^{13}C natural abundance techniques. Typically a mixture of sterols in fungi is present which one of these is dominant and contributed over 50% of the total sterol composition (Weete et al. 2010). Ergosterol is the predominant sterol in fungal cell membranes and only present in higher fungal phyla, *i.e.* Basidiomycota, Ascomycota and the majority of Zygomycota and does not occur in plants (Weete and Gandhi, 1997, 1999; Klamer and Bååth 2004). Conflicting data regarding the presence of ergosterol in membranes of arbuscular mycorrhizal fungi (AM fungi) have been reported in the literature. Hart and Reader (2002) used it to quantify the biomass of inoculated AM fungi in roots and soil, but in mycelium of AM fungi and colonised roots no ergosterol could be detected by Olsson et al. (2003). The major sterol in spores of AM fungi is 24-ethyl cholesterol and no ergosterol has been detected (Grandmougin-Ferjani et al. 1999).

A field experiment was established at an agricultural site with known long-term C3 cropping history (at least 25 years). In 2009 the following treatments were set up to investigate and quantify the flow of C into the belowground soil food web: Corn Maize (CM), where the new ^{13}C (or C4) signal entered the soil system via the shoot litter and root pathway, and Fodder Maize (FM), where the aboveground parts of plants were removed at harvest and C supplies were derived mainly from roots and rhizodeposits. To gain a C4 ^{13}C signal solely through the aboveground channel, maize litter was added to plots planted with wheat [Wheat + maize Litter (WL)]. Plots with Wheat (W) only served as C3 reference for the incorporation of the maize ^{13}C signal into the different C pools. In the present study soil microorganisms as the basis of the complex soil food webs were investigated and we hypothesized that the incorporation of C into soil pools (C_{org}, EOC, C_{mic} and fungal biomass) depends on the origin of C resources (root *vs.* shoot) entering the soil.

Materials and methods

Study site

The experimental agricultural field is located on a terrace plain of the river Leine north–north-west of the city of Göttingen (Niedersachsen, Germany). The local climate, with a mean annual temperature of 8.7 °C and mean annual precipitation of 645 mm, represents a temperate climate zone, affected by the transgression from the maritime Atlantic climate to the west to the continental climate to the east. The elevation of the plane is 155–160 m, a.s.l., striking towards north-west with a mean base slope of approximately 2%.

Geologically the area belongs to the Leinegraben, a rift formation embedded within the Harz Mountains to the east and the Weserbergland Mountains to the west. Up to 15 m of quaternary materials deposited mainly during the Weichsel glacial period, form the stratum on top of Mesozoic rocks, predominantly limestones and mudstones of the Mittlerer Keuper. The quaternary deposits are composed of clayey and fine sandy materials interbedded with silty loess materials, which are now decalcified in the upper part of the profile. These deposits are parent material for the actual soil formation.

According to IUSS (2007), the dominant soil types are Cambisols (Braunerden, KA5 2005), Luvisols (Parabraunerden, KA5 2005) and stagnic Luvisols (Pseudogley, KA5 2005). However, long agricultural use has severely affected the build up of the soil profiles. The albic horizon typically found for these soils can no longer be detected in the field due to centuries of intensive tillage. In general, two plough layers (0.2 m and 0.3 m below surface) can be detected, with strong compaction below the second plough layer in particular. This is especially evident in the relatively high bulk density (1.6 g cm^{-3}) in and below the second plough layer (Table 1).

Establishment of the experiment

Before the start of the experiment the isotopic signature of C and N in soil were screened in 10 cm layers to 90 cm depth in April 2009. The $\delta^{13}C$ values of the Ap horizon were typical for C3 vegetation, with a mean value of -27.3 ± 0.04‰ (Fig. S1 top). The $\delta^{13}C$ values increased with depth (data not shown). The mean C and N contents of the Ap horizon were 11.6 ± 0.2 (Fig. S1 bottom) and 1.2 ± 0.02 mg g^{-1} dry weight, respectively, and the average C/N ratio was 9.7 ± 0.1. Background screening revealed comparable isotopic compositions and total soil C and N contents across the experimental field site.

The experimental plots were arranged in a factorial design in April 2009 (Fig. S1). The plots were aligned in two west-east striking rows to facilitate tillage during the experiment, 10 experimental plots (24 m × 24 m) with maize (*Zea mays* L.) in one row and 10 plots with wheat (*Triticum aestivum* L.) in the other. Wheat seedlings on the maize plots were removed using a non-selective herbicide ("Round-up", Monsanto Agrar, Düsseldorf, Germany). Maize plots were then tilled with a chisel plough to a depth of 12 cm and hybrid maize ("Ronaldinio", KWS Saat Ag, Einbeck, Germany) was sown in April 2009 at a density of 11.5 grains m^{-2} (34 kg ha^{-1}). N fertilizer (ammonium nitrate urea solution: 122.4 kg N ha^{-1}) and NP fertilizer (diammonium phosphate: 32.4 kg N ha^{-1}, 82.8 kg P ha^{-1}) were added shortly before and after seeding to improve growth of maize plants. Winter wheat ("Julius", KWS Saat AG, Einbeck, Germany) was sown in October 2008 at a density of 380 grains m^{-2} (224.0 kg ha^{-1}). Fertilizers to wheat were applied as follows: NS fertilizer (granular SSA: 21.0 kg N ha^{-1}, 24.0 kg S ha^{-1}) in March 2009 and ammonium nitrate urea solution two times in April (50.4 kg N ha^{-1} each) and once in May and once in June 2009 (39.5 kg N ha^{-1} each). During the growing season different herbicide combinations were applied twice on the maize plots

Table 1
Selected soil properties of the study site.

Horizon[a]	Depth [m]	Texture[b] clay/silt/sand, % (w/w)	pH (H_2O)	pH ($CaCl_2$)	K_s [cm d^{-1}]	bd [g cm^{-3}]	Porosity [cm^3 cm^{-3}]
Ap1	0–0.25	7.0/87.2/5.8	6.6	6.0	3.0	1.38	0.44
A(l)p2	0.25–0.37	7.1/87.8/5.0	6.9	6.2	1.8	1.61	0.38
Bv1	0.37–0.65	7.1/87.7/5.1	7.3	6.6	4.0	1.55	0.40
Bv2	>0.65	6.8/88.4/4.8	7.7	7.0	n.d.	1.68	0.38

K_s: Saturated hydraulic conductivity; bd: bulk density.
[a] Classification according to KA5.
[b] Texture according to the German classification system.

(Mesurol liquid 0.2 l ha^{-1}, TMTD 98% Satec 0.1 kg ha^{-1}), whereas the wheat plots received different herbicide combinations six times (22.10.2008: Arena C 0.41 l ha^{-1}; 03.04.2009: ARTUS 25.0 g ha^{-1}, Attribut 70.0 g ha^{-1}, CCC 720 1.0 l ha^{-1}, PRIMUS 50.0 ml ha^{-1}; 25.04.2009: BRAVO 500 SC 0.5 l ha^{-1}, CCC 720 0.5 l ha^{-1}, Input 0.8 l ha^{-1}, Moddus 0.1 l ha^{-1}; 19.05.2009: Matador 300 0.6 l ha^{-1}, U 46 M-Fluid 1.3 l ha^{-1}; 05.06.2009: Bulldock 0.3 l ha^{-1}, Matador 300 0.5 l ha^{-1}, Taspa 0.3 l ha^{-1}, Primor Granulate 0.2 kg ha^{-1}).

In August 2009 wheat plants were harvested and the straw was removed from the wheat plots. After harvest of maize plants which were separated from corncobs and then chopped, four treatments were established in early November differing in the source of C4 C input: Corn Maize (CM), Fodder Maize (FM), Wheat + maize Litter (WL), and Wheat as a reference (W). In the CM plots the C4-derived ^{13}C signal was introduced belowground through rhizodeposition during the growing period and the decomposition of dead roots after harvest, and aboveground through maize shoot litter addition after harvest. On the FM plots no maize shoot litter was applied, therefore, the C4 signal derived from roots and rhizodeposits. In the WL plots the C4 signal derived only from added maize shoot litter. The W plots did not receive any C4 plant input and served as reference plots. The cultivation of wheat on the W and WL plots was necessary to maintain habitat functions of the soil without changing its isotopic C signature. To establish the CM and WL treatment the maize shoot litter (0.8 kg dry weight m^{-2}, equivalent to 0.35 kg C m^{-2}) was applied to half of both the maize and wheat plots. Each treatment was replicated five times. Because one plot of every treatment was saved for ^{13}C and ^{15}N labeling experiments, 16 plots (4 replicates of each treatment) were sampled for this study.

In April 2010 all experimental plots were tilled with a chisel plough to a depth of 12 cm, and hybrid maize ("Fernandez", KWS Saat Ag, Einbeck, Germany) was sown at a density of 12.1 grains m^{-2} (25.6 kg ha^{-1}) with additional N (ammonium nitrate urea solution: 79.2 kg N ha^{-1}) and NP fertilizer (diammonium phosphate: 32.4 kg N ha^{-1}, 82.8 kg P ha^{-1}). To improve comparability of maize and wheat plots during the growing season of 2010, summer wheat ("Melon", Saaten-Union GmbH, Isernhagen, Germany) instead of winter wheat (2009) was sown at a density of 440 grains m^{-2} (224 kg ha^{-1}), and N fertilizer (ammonium nitrate urea solution) was added in April and June (61.3 kg N ha^{-1} and 39.5 kg N ha^{-1}, respectively). During the 2010 growing season two herbicide combinations were applied on the maize plots (14.04.2010: Mesurol liquid 0.2 l ha^{-1}, TMTD 98% Satec 0.1 kg ha^{-1}; 05.06.2010: Click 1.0 l ha^{-1}, Milagro 0.5 l ha^{-1}, Peak 14.0 g ha^{-1}), and one herbicide combination on the wheat plots (29.04.2010: Biathlon 70.0 g ha^{-1}, MCPA Berghoff 1.3 l ha^{-1}). In early November 2010 maize (without corncobs) and wheat plants were harvested after which chaffed maize straw was applied to the respective plots (0.8 kg dry weight m^{-2}, equivalent to 0.35 kg C m^{-2}).

Determination of root biomass and rhizodeposition

Root biomass of maize and wheat was determined three times in 2009. At the maize plots samples were taken directly at the position of the maize plants, 12.5 and 25 cm away from the plants within rows, 20 and 40 cm away from the plants in inter-row locations, and 23.5 and 47 cm away from the plant at the diagonal between row and inter-row. At the wheat plots frames (25 cm × 25 cm) were placed randomly within four of the wheat plots and soil samples were taken at 5 positions within each frame. A soil corer (Riverside auger, inner diameter 5 cm; Eijkelkamp, Giesbeek, The Netherlands) was used and samples were taken to a depth of 50 cm in 10 cm layers. All roots were washed free of soil, dried and weighed.

In addition to root biomass, the input of C through rhizodeposition was estimated for maize based on the rhizodeposition-to-root ratio determined under controlled conditions (J. Pausch, unpubl. data).

Plant carbon and $\delta^{13}C$

Wheat shoot material (summer 2010), maize leaves (senescent; litter) and maize roots (from respective soil sampling dates) were ground and about 3 mg was analyzed by an elemental analyser (Euro EA 3000, EuroVector, Milan, Italy) coupled with an isotope ratio mass spectrometer (IRMS, Delta Plus XP, Thermo Finnigan MAT, Bremen, Germany) for estimation of C and N content and $\delta^{13}C$. Glutamic acid USGS-40 (IAEA, Vienna; $\delta^{13}C$ -26.39 ± 0.04‰) was used as reference material for calibration of CO_2 reference gas. Acetanilide (C_8H_9NO, Merck, Darmstadt) was used as a secondary laboratory reference material for internal calibration and determination of C and N content. $\delta^{13}C$ values are expressed relative to Vienna Pee Dee belemnite (V-PDB).

Soil sampling

Soil was sampled in summer shortly before plant flowering (highest exudation), autumn (shortly before maize harvest) and winter (highest translocation of mobile organic particles). Summer sampling was conducted in July 2009 and 2010, autumn sampling in September 2009 and 2010, and winter sampling in December 2009 and in January 2011 because of heavy snow in December 2010 (Fig. S2).

In each plot 10 soil samples were taken to 70 cm depth with a soil corer in 10 cm layers randomly between two plants within a row. The samples from each layer were thoroughly mixed and homogenized by hand. Samples were transported in a cooling box to the laboratory and stored at 4 °C until sieving (no longer than one week). After sieving (<2 mm) and careful removal of plant particles, the soil was stored at −28 °C until analysis. Water content of samples was determined gravimetrically after drying at 105 °C for 24 h. Here we focus on the top soil layer at 0–10 cm. All data presented are related to dry weight of soil.

Soil organic carbon and $\delta^{13}C$

For estimation of C_{org} and $\delta^{13}C$ about 3 g of soil was dried at 105 °C for 24 h and subsequently ground. Soil subsamples of 15–30 mg were measured with a coupled system consisting of an

elemental analyser (NA 1500, Carlo Erba, Milan, Italy) and an isotope ratio mass spectrometer (MAT 251, Thermo Finnigan, Bremen, Germany).

Microbial biomass

C_{mic} was estimated by chloroform-fumigation-extraction (Vance et al. 1987). In brief, 10 g soil (fresh weight) of a homogeneous subsample of each plot was fumigated under vacuum with ethanol-free chloroform in a desiccator for 24 h. After removing the chloroform, samples were extracted by adding 40 ml of a 0.025 M K_2SO_4-solution (1:4, w/v, soil/extractant ratio), shaken for 30 min at 250 rev min^{-1} on a horizontal shaker and centrifuged for 30 min at 4422 × *g*. A second subsample of 10 g was treated similarly but without fumigation for the estimation of 0.025 M extractable organic C (EOC). Organic C in the supernatants was measured with a DOC/TN-analyser (Dimatoc 100, Dimatec, Essen, Germany). EOC content of the fumigated samples was subtracted from C content of the non-fumigated samples and resulted in extractable C_{mic}. For estimation of total C_{mic} a k_{ec} factor of 0.45 was used (Joergensen 1996).

$\delta^{13}C$ of microbial biomass

For analysis of $\delta^{13}C$ values of EOC and C_{mic}, 10 ml aliquots of the supernatants of both non-fumigated and fumigated samples were dried in a vacuum rotary evaporator (RVC 2-25, Martin Christ, Osterode am Harz, Germany) at 60 °C. The remnant was ground, weighed into tin capsules within a range of 7–30 mg (minimum of 10 μg C) per capsule (Marhan et al. 2010), and analyzed as described for the plant material.

To calculate the $\delta^{13}C$ of C_{mic} following equation was used:

$$\delta^{13}C_{mic} = \frac{c_{nf} \times \delta_{nf} - c_f \times \delta_f}{c_{nf} - c_f},$$

where c_{nf} and c_f are the corresponding extracted organic C contents (μg C g^{-1} soil) of the non-fumigated and fumigated sample and δ_{nf} and δ_f are the corresponding $\delta^{13}C$ values.

Ergosterol

Ergosterol was extracted using a modified method of Djajakirana et al. (1996). To 2 g soil (fresh weight) 25 ml ethanol was added and shaken at 250 rev min^{-1} on a horizontal shaker for 30 min. Extracts were then centrifuged for 30 min at 4422 × *g* and 10 ml of supernatant per sample was dried at 50 °C in a vacuum rotary evaporator (Martin Christ, RVC 2-25, Osterode am Harz, Germany). To dissolve the extracts 1 ml methanol was added and samples were transferred into 2 ml brown glass HPLC vials with cellulose-acetate filters (0.45 μm; Sartorius Stedim Biotech GmbH, Göttingen, Germany). Ergosterol in samples was quantitatively determined by HPLC analysis (Beckmann Coulter, System Gold 125, Fullerton, USA) using a 250 mm × 4.6 mm Spherisorb ODS II 5 μm column with a mobile phase of pure methanol, a flow rate of 1 ml min^{-1} and a detection wavelength of 282 nm (Beckmann Coulter, System Gold 166 UV-detector, Fullerton, USA). For calibration pure ergosterol (Sigma–Aldrich, St. Louis, USA) was dissolved in methanol and diluted to give final concentrations of 0.1, 0.2, 0.5, 1 and 2 μg ergosterol ml^{-1}.

^{13}C ergosterol

For extraction of ergosterol for $\delta^{13}C$ determination, 11–15 g soil (fresh weight) was mixed with 170 ml ethanol and 5 g NaOH, homogenized in an ultrasonic bath and saponified (30 min at 80 °C). After cooling, samples were filtered through folded filters (Ø15 cm; Macherey-Nagel, Oensingen, Switzerland). Filtered extracts were mixed with 100 ml of deionised H_2O and 80 ml of petroleum ether and shaken for 1 min, separating into two phases. The upper phase was saved and the lower phase was mixed again with 50 ml petroleum ether and shaken for another 1 min. Both resulting upper phases were pooled and 20 μl of ethylene glycol was added. Samples were evaporated to near dryness in a rotary evaporator at 300–500 mbar at 40 °C. Residues were re-dissolved in 2 ml of methanol/water (95/5, v/v), transferred to Eppendorf vials and centrifuged for 2 min at 12,000 × *g*. Supernatants were transferred into brown glass HPLC-vials.

Concentration and cleaning of the extracts was performed with a Varian preparative HPLC with a Varian Pro-Star 210 pump and 701 Fraction Collector equipped with a Grohm Nucleosil 120 C4 (250 mm × 16 mm) column. The eluent was methanol/water (95/5, v/v) with a flow rate of 6 ml min^{-1}. One ml of each sample was injected and the ergosterol fraction was collected. Retention time was monitored using the Galaxy Chromatography Data System (Version 1.7.4.5) software. The fraction was than evaporated under nitrogen at 60 °C, dissolved in 70 μl isooctane and transferred to a brown glass vial. A GC-C-IRMS system was used to determine the isotopic C composition of ergosterol. The system consists of a gas chromatograph (6890 series, Agilent Technologies, USA) coupled via a gas chromatography-combustion III Interface (Thermo Finnigan, Waltham, USA) to a Delta Plus XP mass spectrometer (Thermo Finnigan MAT, Bremen, Germany). An Rtx-5 (30 m × 0.25 mm, film thickness of 0.25 μm) column with helium as the carrier gas (flow rate of 1.5 ml min^{-1}) was used. The combustion reactor had a temperature of 940 °C and the reduction reactor 640 °C. The GC program was set as follows: initial temperature was 160 °C and held for 1 min, temperature was increased to 270 °C at a starting rate of 5 °C min^{-1} followed by a rate of 2 °C min^{-1} to 300 °C then held for 10 min. The injector temperature was 280 °C and the samples were measured in a splitless mode.

Each sample was measured at least two times. For internal calibration and to check fractionation during separation of ergosterol with preparative HPLC, $\delta^{13}C$ of ergosterol standards (50 μg ml^{-1}) (ACROS Organics, Geel, Belgium, purity: 98%) was also determined with every series of measurement. No ^{13}C isotopic fractionation could be detected as a result of cutting the ergosterol with the preparative HPLC (−10.11 ± 0.08‰ *vs.* −10.05 ± 0.14‰, ergosterol standards unprocessed and processed with preparative HPLC, respectively).

Calculation of maize-derived C

For calculation of the relative amounts of maize C in C_{org}, EOC, C_{mic}, and ergosterol the following mixing model was used:

$$\%\text{C-maize} = \frac{\delta_{sample} - \delta_{reference}}{\delta_{maize} - \delta_{wheat}},$$

where δ_{sample} is the $\delta^{13}C$ value of the respective sample, and $\delta_{reference}$ is the $\delta^{13}C$ mean value of a sample from the reference plots (with wheat crop alone). δ_{maize} is the $\delta^{13}C$ value of the maize residues. An average $\delta^{13}C$ value of maize material (−13.01‰) was used for calculation of maize C because there were only small differences in $\delta^{13}C$ values between maize litter (2009: −13.04 ± 0.04‰; 2010: −13.34 ± 0.13‰) and maize roots (−12.82 ± 0.09‰), as well as over time. To assess the relative amount of maize C in EOC, C_{mic} and ergosterol, the average $\delta^{13}C_{org}$ value (δ_{wheat}) over two years of the reference plots was used (−26.99 ± 0.03‰). For calculation of relative amounts of maize C in the C_{org} for δ_{wheat} the $\delta^{13}C$ value of the wheat plants was used (−28.31 ± 0.16‰). $\delta^{13}C$ mean values and standard errors of all samples in the different pools are given in Table S1.

Absolute amounts of maize-derived C were calculated by multiplying relative amounts of maize-derived C by C content of the respective samples.

Statistical analyses

Treatment and date effects on C_{org}, EOC, C_{mic} and ergosterol as well as on the amounts of maize-derived C in these pools were analyzed by ANOVA with sampling dates as repeated factors. In addition separate ANOVAs with post hoc tests (Fischer LSD) for comparison of means were performed for each sampling date. Best fitted transformation (log or reciprocal transformation) was used to improve homogeneity of variance (tested by Levene's test). For statistical analyses the software STATISTICA 6.0 (Tulsa, OK, USA) was used. All errors are reported as standard error.

Results

Root biomass and C_{org}

In July 2009 wheat root biomass was 0.47 ± 0.17 mg C g^{-1} soil, while maize root biomass (without crown roots) was only 0.15 ± 0.04 mg C g^{-1} soil. Before this sampling calculated rhizodeposit-derived C had been 0.37 ± 0.10 mg C g^{-1} soil.

During the sampling period C_{org} content of soils ranged from 10.59 to 13.74 mg g^{-1} soil (Fig. 1a). C_{org} was significantly affected by treatment only at the last sampling date in winter 2010/2011 (date × treatment effect: $F_{15,60} = 2.34$, $P = 0.011$). For this date C_{org} was significantly different between treatments ($F_{3,12} = 9.82$, $P = 0.001$), with 23% lower values in the FM in comparison to the WL treatment. Maize-derived C was detected in the C_{org} of the FM and CM plots starting with the first sampling in summer 2009 and the maize signal in samples of the WL plots were detected one month after litter amendment in winter 2009/2010 (Fig. 1a and b). During the vegetation period 2010, the highest amount of maize-derived C was present in the CM plots, unlike both the FM and the WL plots, which showed similar amounts of maize-derived C. Maize-derived C_{org} increased continuously in each of the treatments during the two years (Fig. 1a and b). After two years the amount of maize-derived C in C_{org} was 7.28, 7.50 and 14.12% in the FM, WL and CM plots, respectively (Fig. 1b).

Extractable organic carbon

During the sampling period EOC content varied between 10.99 and 34.52 μg C g^{-1} soil (Fig. 2a). Effects of treatment on EOC differed over the sampling period (date × treatment effect: $F_{15,60} = 4.21$; $P < 0.001$). Although similar amounts of EOC were detected in all of the treatments in summer and autumn 2009, EOC content in soils was significantly higher in the WL than in the FM plots during the following soil sampling dates. In 2010, EOC content tended to be higher in the wheat plots (WL and W), but the difference was only significant in autumn 2010 ($F_{3,12} = 15.41$; $P < 0.001$). In winter 2010/2011 EOC content in the FM plots was significantly different ($F_{3,12} = 6.86$; $P = 0.006$) between treatments, with about 50% lower contents in the FM than in the other treatments.

Maize-derived C in EOC was first detected in the FM and CM plots in autumn 2009 (Fig. 2a and b). In winter 2009/2010, one month after addition of maize litter, maize-derived C was also detected in the WL plots. After summer 2010 the relative amount of

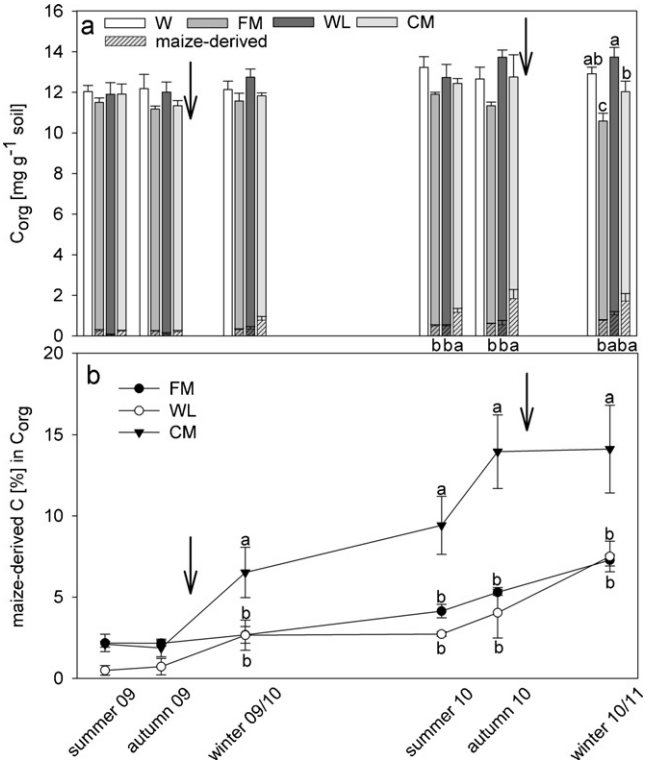

Fig. 1. Means and standard errors of C_{org} and absolute amounts of maize-derived C (a) and relative amounts of maize-derived C (b) in the different treatments Wheat (W), Fodder Maize (FM), Wheat + maize Litter (WL) and Corn Maize (CM) at respective soil sampling dates. Different letters indicate statistically significant differences between treatments at this date (Fisher LSD: $P < 0.05$). In (a) letters above bars specify significance for total C_{org} and letters below bars for absolute amounts of maize-derived C in this pool. Arrows indicate litter application.

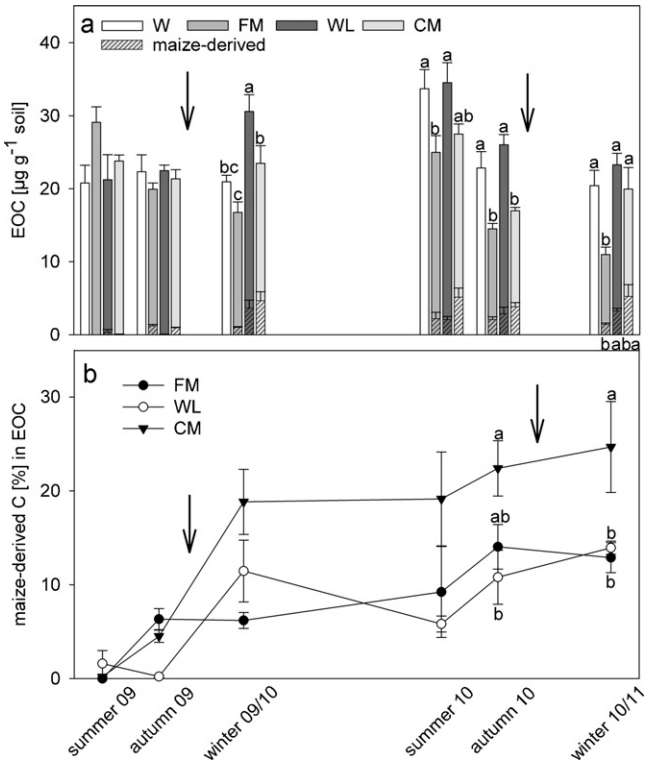

Fig. 2. Means and standard errors of total extractable organic C (EOC) and absolute amounts of maize-derived C (a) and relative amounts of maize-derived C (b) in the different treatments at respective soil sampling dates. Different letters indicate statistically significant differences between treatments at this date (Fisher LSD: $P < 0.05$). In (a) letters above bars specify significance for total EOC and letters below bars for absolute amounts of maize-derived C in this pool. Arrows indicate litter application; for legend see Fig. 1.

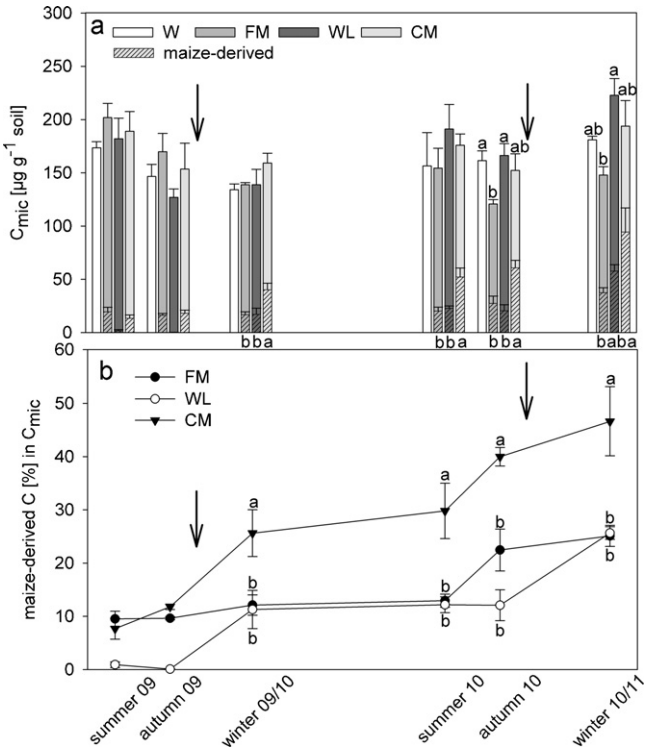

Fig. 3. Means and standard errors of total microbial biomass (C_{mic}) and absolute amounts of maize-derived C (a) and relative amounts of maize-derived C (b) in the different treatments at respective soil sampling dates. Different letters indicate statistically significant differences between treatments at this date (Fisher LSD: $P < 0.05$). In (a) letters above bars specify significance for total C_{mic} and letters below bars for absolute amounts of maize-derived C in this pool. Arrows indicate litter application; for legend see Fig. 1.

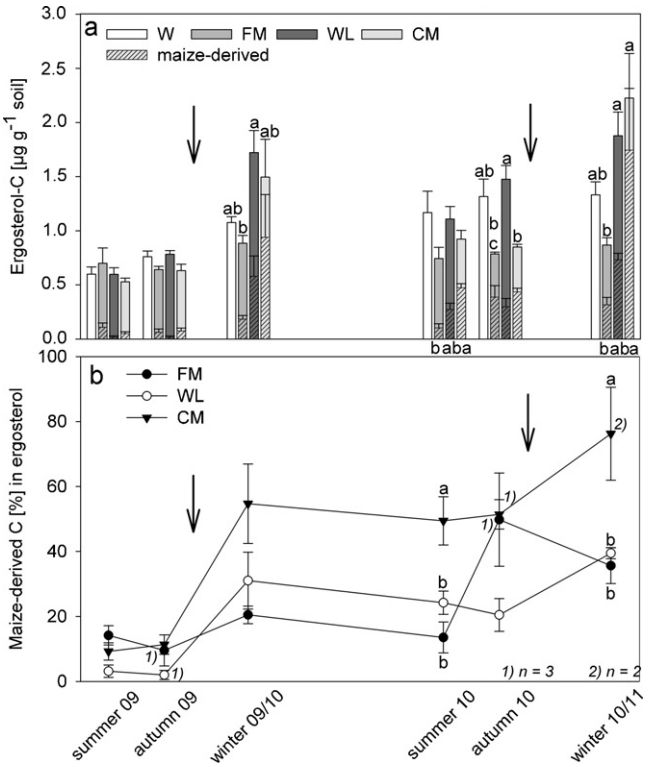

Fig. 4. Means and with standard errors of total ergosterol C and absolute amounts of maize-derived C (a) and relative amounts of maize-derived C (b) in the different treatments at respective soil sampling dates. Different letters indicate statistically significant differences between treatments at this date (Fisher LSD: $P < 0.05$). In (a) letters above bars specify significance for total ergosterol C and letters below bars for absolute amounts of maize-derived C in this pool. Arrows indicate litter application. Note numbers on some data points in (b) which also apply to (a); for legend see Fig. 1.

maize-derived C was higher in the CM plots but no significant differences could be detected between the FM and WL plots. This was reflected at the end of this study by 12.88, 13.94 and 24.67% maize C incorporation into EOC in the FM, WL and CM plots, respectively.

Microbial biomass C

Microbial biomass C ranged from 120.7 to 222.8 µg g^{-1} soil (Fig. 3a). The date of sampling significantly affected C_{mic} ($F_{5,60} = 7.41$, $P < 0.001$) but the variations did not show a clear seasonal trend. In autumn 2010 C_{mic} in the FM plots was significantly lower than in WL and W plots ($F_{3,12} = 3.59$, $P = 0.046$). In winter 2010/2011 C_{mic} significantly differed between treatments ($F_{3,12} = 4.30$, $P = 0.028$) and was 34% lower in the FM than in the WL plots (Fig. 3a).

Maize C derived from rhizodeposition was detected in C_{mic} in the FM and CM plots at the first sampling in summer 2009 (Fig. 3a and b). In winter 2009/2010, maize litter-derived C was also detected in C_{mic} of the WL plots. Subsequently, the amounts of maize-derived C in FM and WL plots were similar, while in the CM plots they were about twice as high (Fig. 3a and b). The maize-derived signal in the C_{mic} increased continuously during the two years resulting in 25.1, 25.6 and 46.6% maize C in C_{mic} in the FM, WL and CM plots at the end of the two-year period, respectively.

Ergosterol C

Amounts of ergosterol C ranged from 0.53 to 2.23 µg C g^{-1} soil. In both winter 2009/2010 and 2010/2011 amounts of ergosterol C tended to be higher in the WL and CM plots than in the W and FM plots (Fig. 4a). During the vegetation period 2010, ergosterol C tended to be higher in the wheat (WL and W) plots compared to the maize plots but significant differences between treatments were only found in autumn 2010 ($F_{3,10} = 7.87$, $P = 0.005$).

In summer 2009, maize C in ergosterol was detected in both FM and CM plots, and in winter 2009, one month after litter addition, it also appeared in the WL plots (Fig. 4a and b). During the growing seasons in both WL and CM plots, the amount of maize C tended to be highest at the winter samplings. Over the whole sampling period no significant differences in the relative amounts of maize-derived C could be detected between the FM and WL plots. After two years the amount of maize C in ergosterol accounted for 35.6, 39.5 and 76.2% in the FM, WL and CM plots, respectively.

Discussion

The origin and quantity of plant inputs to soil are primary factors controlling the size and structure of the soil microbial community. The present study aimed to elucidate and quantify the C flow from both root and shoot litter residues into soil organic, extractable, microbial and fungal C pools. Our experimental approach allowed separating the flux of root-derived from shoot litter-derived C by switching from C3 (wheat) to C4 (maize) crops and by the addition of C4 shoot litter to plots with C3 plants.

Plant effects

Wheat was grown on the W and WL plots to maintain habitat functions of the soil without changing its isotopic C signature. However, the type of crop influenced the total amounts of C_{org}, EOC, C_{mic} and ergosterol, with typically greater amounts in treatments

with wheat plants (W *vs.* FM and WL *vs.* CM treatment). The effect of wheat was generally more pronounced in 2010 than in 2009. In July 2009 wheat root biomass was 0.47 mg C g^{-1} soil, while maize root biomass (excluding crown roots) was only 0.15 mg C g^{-1} soil. Therefore, we suggest that higher substrate (rhizodeposit) availability in treatments with wheat plants was responsible for greater amounts of C in the organic C, microbial, and fungal pools. Furthermore, we cannot exclude the possibility that microbial diversity differences in the wheat and maize plots (WL and CM) result in assimilation of substrates by varying species. However, nearly additive amounts of maize-derived C in FM and WL to CM plots indicate that assimilated C quantities were independent of maize or wheat growing plots and the respective microorganisms present.

The wheat-derived C input, with its depleted $\delta^{13}C$ signature compared to maize, probably led to an underestimation of maize C incorporation into the different pools of the WL treatment. The increase in the EOC pool in the WL (and W) treatment during summer 2010, when the plots were planted with wheat, indicates that a certain amount of EOC was derived from the rhizodeposition of wheat. In contrast, the amount of maize C in the EOC pool of the WL treatment decreased. The wheat-derived C presumably was mineralized quickly as the total amount of EOC decreased but the maize-derived C in the EOC pool increased until the following soil sampling in autumn 2010. Similar increases in maize-derived C in almost all pools in the WL and CM treatments after litter addition between the autumn and winter samplings further indicate that the contribution of wheat root material to the $\delta^{13}C$ signal of the different pools was negligible in the WL treatment.

Total C input

In the first year of the experiment (2009), total root-derived C input of maize was 0.52 mg C g^{-1} soil (0.37 mg C g^{-1} of total rhizodeposits until July 2009 plus 0.15 mg C g^{-1} root biomass in July 2009). In spring 2010 the maize crown roots (aboveground root material) were incorporated into the soil by chisel plough tillage, contributing to an additional input of 8.0 mg C g^{-1} into soil. Taking the incorporation of the crown root material into account, over the study period the total C input by roots was almost two times higher (9.04 mg C g^{-1} soil) than the C input derived from litter (5.08 mg C g^{-1} soil). A decomposition experiment with litter-bags in the field showed that after one year only 42% of maize root material (without crown roots), but up to 88% of maize shoot litter material was decomposed (N. Scheunemann, unpubl. data) indicating faster decomposition of shoot than root litter. Presumably, after incorporation into soil, the decomposition of the crown root material was further slowed down in comparison to the belowground root biomass due to its more compact and solid structure. We assume therefore that access and utilization of crown root material by decomposers was strongly limited and that the contribution of this material to the investigated C pools as a source for C was negligible. With the assumption that the root biomass and rhizodeposition were equal in both years (although maize varieties changed) root C input in the maize plots (without the crown root material) was only 1.04 mg C g^{-1} soil during the study period, which is only one fifth of the maize shoot litter input and similar to the root biomass C (without rhizodeposition) input in the wheat plots (see above). Indeed, total quantities of C in all pools of the FM treatment were slightly lower as compared to the other treatments in the second year of the study.

Absolute incorporation of maize C into different soil C pools

The pool of C_{org} is assumed to be stable with long turnover times (Flessa et al. 2008; von Lützow et al. 2008). However, maize-derived C in C_{org} was detected almost immediately, beginning with the first growing period. Balesdent and Balabane (1992) also found the $\delta^{13}C$ values of different soil particle size fractions to differ significantly in the first year after replacing C3 by C4 plants.

The total amount of C_{org} varied little over time; it decreased only slightly in the FM treatment and increased slightly in the WL treatment with significantly lower amounts in the FM treatment only in winter 2010/2011. However, the concentration of maize-derived C increased with time in each of the treatments indicating that the decrease in C_{org} in the FM treatment was due to the mineralization of C3-derived C. Clapp et al. (2000) investigated the SOC of maize cultivated fields over 13 years and showed that the SOC amount declined in treatments where aboveground plant parts were removed from the soil, which is comparable to the FM treatment in the present study.

The EOC pool represents a more mobile fraction of soil organic C than C_{org} and is assumed to be an important C source for the soil microbial community (Marschner and Bredow 2002). In the present study no maize-derived C from rhizodeposition was detected in the EOC pool at the first sampling date in summer 2009, three months after seeding of maize, but it was detected in C_{mic} and C_{org}. The minor contribution of maize exudates to the EOC pool in soil presumably was due to the fact that bulk soil was sampled 25 cm away from the maize plants and not directly in the rhizosphere. Marx et al. (2007) found only low amounts of C derived from rhizodeposits in the rhizosphere of maize and wheat plants, while in bulk soil rhizodeposit C was present in C_{mic} and CO_2. Relative amounts of water soluble C were shown to decrease with increasing distance to wheat roots (Merbach et al. 1999). Hütsch et al. (2002) concluded that extractable organic compounds derived from rhizodeposits were assimilated immediately by microorganisms and/or stabilized in C_{org}.

Root-derived C also was already detected in fungal biomass (ergosterol) at the first sampling in summer 2009. Since only limited amounts of ergosterol were found in Glomeromycota (see review of Weete et al. 2010), we assume minor contribution of AM fungi-derived C in the ergosterol fraction in our experiment. Only low AM colonisation of maize roots in the same field experiment during two sampling times in 2009 were detected (J. Moll, pers. communication). Therefore, we conclude that mainly saprotrophic fungi might have incorporated maize-derived C from rhizodeposits (and shoot litter) into the ergosterol fraction.

Generally, fungi can play an important role in C cycling in bulk soil because their hyphae can grow in the direction of the substrate source or from substrate into the bulk soil (Frey et al. 2003; Butenschoen et al. 2007). Esperschütz et al. (2009) found that fungi are involved in transporting ^{13}C compounds from labeled rhizodeposits into bulk soil. Soon after labeling the fungal PLFA 18:2ω6,9 was highly enriched in the rhizosphere, later it was also enriched in bulk soil. The translocation of the assimilated substrates within the hyphal network is an advantage fungi have over bacteria which depend more on external transport processes, such as the flux of water through soil (Poll et al. 2006).

In contrast to root-derived C, litter-derived C was detected in the EOC pool at the first sampling one month after litter addition (winter 2009/2010). Even distribution of litter on the plots presumably fostered direct leaching of water soluble C from the litter into the underlying bulk soil. Diffusion of soluble C and advective transport from labeled rye litter into the soil were also found by Poll et al. (2008). A similar quick uptake of maize litter C was also detected in C_{mic} and ergosterol in winter 2009/2010. While total C_{mic} did not generally respond to litter addition, fungal biomass increased in winter in the litter addition treatments. Increased fungal biomass by the addition of maize litter has been shown previously (Helfrich et al. 2008; Potthoff et al. 2008; Rottmann et al. 2010), suggesting that fungi in bulk soil benefit from aboveground litter resources in arable systems.

Relative contribution of root- and litter-derived C to the different C pools

After two vegetation periods the proportion of maize-derived C increased in the order C_{org} < EOC < C_{mic} < ergosterol. With the exception of ergosterol, which has not been investigated before, this is in accordance with previous studies (Gregorich et al. 2000; Liang et al. 2002). For example, Gregorich et al. (2000) simulated changes in $\delta^{13}C$ values in different soil pools which had been under maize monoculture from 4 to 37 years. They calculated exponential enrichment in the first two years with higher enrichments in C_{mic} than in water soluble C, and very slow enrichment in humus C. Liang et al. (2002) found in a 110 day greenhouse study with maize 11.5, 23.3 and 48.0% maize-derived C in SOC, water soluble organic C (WSOC) and C_{mic}, respectively. Due to higher proportions of maize-derived C in WSOC and C_{mic} they concluded that recent plant C was more bioavailable than the older soil-derived soluble C pool.

After two vegetation periods, the relative incorporation of maize-derived C was at a maximum in fungal biomass (ergosterol) with up to 76.2% maize C in ergosterol in the CM treatment. Similarly, the fungal biomarker PLFA, 18:2ω6,9, was highly enriched in comparison to bacterial PLFAs due to the addition of litter or by rhizodeposition (Butler et al. 2003; Paterson et al. 2008; Rubino et al. 2009; Jin and Evans 2010). Flessa et al. (2008) calculated from the data of Kramer and Gleixner (2006) that after 23 years of maize cultivation 93.8% of the C in the fungal biomarker PLFA 18:2ω6,9 derived from the C4 plant. In current concepts of food webs, the fungal energy channel, which is favoured by recalcitrant organic materials and high C/N ratio in soils, is considered a slow cycle (Scheu et al. 2005; Joergensen and Wichern 2008). Fungi are thought to use organic substrates more efficiently than bacteria (Sakamoto and Oba 1994) and to be more resistant to mortality factors as compared to bacteria (Guggenberger et al. 1999). In contrast, readily decomposable substrates are favoured by the bacterial energy channel which is characterized by rapid growth, turnover of C, and fast cycling of nutrients (Holtkamp et al. 2008; Ingwersen et al. 2008).

In spite of different C input quantities of the resources (root *vs.* litter) similar relative amounts of maize-derived C had been incorporated in each of the four soil C pools by the end of both vegetation periods (winter) with the effects of root and litter input being additive. The similar relative incorporation or assimilation of maize C in the treatments with only maize root or shoot litter is noteworthy because the total size of the different pools varied between treatments (with mostly lower total amounts in the FM plots; see above).

Generally, a large fraction of root-derived C is assumed to be stable in soil and hence has longer turnover time in comparison to shoot-derived C (Rasse et al. 2005). Balesdent and Balabane (1996) found more root-derived than shoot-derived maize C to be stable in different soil fractions four years after changing from C3 to C4 plants. They assumed high production but slow decomposition of belowground C compounds. Higher stability of root-derived C was also found by Puget and Drinkwater (2001). After one growing season of labeled hairy vetch (*Vicia villosa* Roth subsp. *villosa*) 48.8% of root-derived C and 13.2% of litter-derived C in C_{org} were retrieved, while almost equal relative amounts of root C (4.1%) and shoot C (6.5%) were recovered in C_{mic}. They concluded that even though the amount of shoot C input into soil was threefold higher than that of root C, relatively more root-derived C was assimilated by C_{mic}. Williams et al. (2006) detected similar proportions of ryegrass root- and straw-derived C in the fungal biomarker PLFA 18:2ω6,9 two months after mixing ryegrass and crimson clover litter into the soil, although input of straw litter was 4.5-fold greater than the root biomass.

In our experiment root-derived input of C was lower than the shoot-derived input but similar relative amounts of root- and shoot-derived C were recovered in the C_{org}, EOC, C_{mic} and fungal (ergosterol) pools. Data from the present study underline the results of the above mentioned studies in which typically more root- than shoot-litter derived C is stabilized in the soil organic C pools and assimilated in the total soil microbial and the fungal biomass.

Our results indicate that not only for soil food webs in forests (Pollierer et al. 2007) but also for the basis of food webs in agricultural ecosystems belowground C input via roots is of great importance.

Conclusions

We presented data on the incorporation of root and shoot litter C into organic and microbial C pools under field conditions over a period of two years, providing a basis for future modelling of C transfer through the belowground food web. Notably, similar amounts of C derived from the two resources differing in substrate quality and amount were incorporated into the C_{org}, EOC, C_{mic} and ergosterol pools over time, indicating the importance of root-derived C for the soil food web. High incorporation of maize C (up to 76.2%) into ergosterol suggests fast and high assimilation of maize C into fungal biomass, with major implications for the flux of C through the bacterial and fungal energy channels of arable systems.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.pedobi.2011.12.001.

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