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Carbon fluxes in soil food webs of increasing complexity revealed by ^{14}C labelling and ^{13}C natural abundance

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Abstract

Soil food webs are mainly based on three primary carbon (C) sources: root exudates, litter, and recalcitrant soil organic matter (SOM). These C sources vary in their availability and accessibility to soil organisms, which could lead to different pathways in soil food webs. The presence of three C isotopes (^{12}C, ^{13}C and ^{14}C) offers an unique opportunity to investigate all three C sources simultaneously. In a microcosm experiment we studied the effect of food web complexity on the utilization of the three carbon sources. We choose an incomplete three factorial design with (i) living plants, (ii) litter and (iii) food web complexity. The most complex food web consisted of autochthonous microorganisms, nematodes, collembola, predatory mites, endogeic and anecic earthworms. We traced C from all three sources in soil, in CO_2 efflux and in individual organism groups by using maize grown on soil developed under C_3 vegetation and application of ^{14}C labelled ryegrass shoots as a litter layer. The presence of living plants had a much greater effect on C pathways than food web complexity. Litter decomposition, measured as $^{14}CO_2$ efflux, was decreased in the presence of living plants from 71% to 33%. However, living plants increased the incorporation of litter C into microbial biomass and arrested carbon in the litter layer and in the upper soil layer. The only significant effect of food web complexity was on the litter C distribution in the soil layers. In treatments with fungivorous microarthropods (Collembola) the incorporation of litter carbon into mineral soil was reduced. Root exudates as C source were passed through rhizosphere microorganisms to the predator level (at least to the third trophic level). We conclude that living plants strongly affected C flows, directly by being a source of additional C, and indirectly by modifying the existing C flows within the food web including CO_2 efflux from the soil and litter decomposition.

Keywords: Carbon cycling; Isotopic markers; Microbial respiration; Microbial biomass; Soil fauna; Litter decomposition; Collembola; Mites; Earthworms; CO_2 partitioning; ^{14}C labelling; ^{13}C natural abundance; Food webs

1. Introduction

Primary production of plants is the main carbon (C) input to the soil (Wardle, 1999; Kuzyakov and Domanski, 2000). Two different forms of C input by plants into the soil can be distinguished. Living plants release root exudates that mainly consist of easily degradable organic compounds including carbohydrates (60–70%), N free carbonic acids (20–35%) and amino acids (2–25%) (Kraffczyk et al., 1984; Jones, 1998; Gransee and Wittenmayer, 2000). Dead plant tissues enter the soil food web by decomposition of leaf- and root-litter. The third C source is the soil organic matter (SOM) already stored in complex recalcitrant substances. Soil microorganisms use those three pools as growth and energy source at different rates. There is also direct feeding of soil organisms on living plant tissue of roots and shoots, but quantitatively this C source is generally thought to be of minor importance (Berg et al., 2001).

Based on their importance for utilisation by soil microorganisms, these three C sources can be ordered as follows: root exudates, litter, and SOM (Kuzyakov and Bol, 2006). Root exudates can be decomposed within a few hours (Jones et al., 2005). This rapid use, coupled with high local concentration of exudates around the roots leads to

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the rhizosphere effect (Hiltner, 1904). Various plant residues (litter) remaining on and in soil usually require weeks to months to be completely decomposed. Soil microorganisms utilize this C source much more slowly compared to root exudates. The decomposition rate of native SOM is still slower and, depending on the fraction considered, can take up to thousands of years. Therefore, utilization of SOM by microorganisms is extremely slow. However, the very large total stock of SOM compared to exudates and litter makes its contribution as a C source significant.

Soil microorganisms are the major primary decomposers and the nutritional basis for the entire soil food web (Hunt et al., 1987; De Ruiter et al., 1995; Berg et al., 2001). Although the experimental background on food webs especially in complex systems is still weak, various soil organisms use the three C sources to differing extents (Setälä et al., 1998; Mikola and Setälä, 1999; Mikola et al., 2001). As the C sources are distinct and this compartmentalization is maintained to higher trophic levels, Moore and Hunt (1988) introduced the concept of "energy channels". The basis of each energy channel in soil food webs is a distinct C source often accompanied by specific primary decomposers.

In model food web systems, the litter based C channel seems to be the most important (Berg et al., 2001; Uyl et al., 2002). Leaf litter is a C source that is only moderately available to soil microorganisms as a result of its spatial concentration on the soil surface and of its chemical composition with an often unfavourable C to N ratio. Therefore, litter decomposition is the result of a complex interaction between microorganisms and soil fauna (for example Anderson et al., 1983; Faber and Verhoef, 1991).

The importance of the root carbon channel is often underestimated, possibly because it is more difficult to study experimentally compared to the leaf litter channel. The root carbon channel may be more important for endogeic earthworms (Schmidt et al., 2004), microbi-detritivores like most collembola, and predatory mites (Garrett et al., 2001; Coleman et al., 2002).

The third C channel is the recalcitrant organic matter in soil. Due to its recalcitrance based on predominance of high molecular weight substances and their binding on clay minerals and sesquioxides it is hardly available for microorganisms. However, many studies have shown that soil microorganisms (bacteria or fungi) slowly decompose SOM with rates of 0.1 to 2% per year (review in Kuzyakov et al., 2000). Therefore, SOM derived C will be incorporated into microbial cells, and be easily available for the next trophic level such as nematodes or microbi-detritovores.

Previous studies have shown that food webs with increasing complexity may differently influence C fluxes (e.g. Couteaux et al., 1991, Mikola et al., 2001; Wardle et al., 2003). In our experiment, the basis of all food webs was the natural microorganisms community. The next trophic level was represented by bacteriovores (here: nematodes) and fungivores (here: collembola), both endogeic taxa. The third trophic level, the predators of collembola and nematodes, was a mesostigmatic mite that is known to feed on both prey. Endogeic earthworms and anecic earthworms contribute to fragmentation and comminution of plant residues (Couteaux et al., 1991; Bonkowski et al., 2000), and so modify the existing food webs by increasing the availability of C sources for primary decomposers. Thus, parallel to the main 3-level food web used in our study, we included an anecic earthworm species to study direct and indirect effect of an ecosystem engineer (Jones et al., 1997) on microorganisms, nematodes and microarthropods. The combination of these organisms' groups with the three possible C sources covers the most important interactions within the food web, competition and predation. We traced the fate of litter-derived C in the compartments SOM, microbial biomass, earthworms, and in CO_2 efflux. We disregarded an uptake of ^{14}C from decomposing litter by the plant (Cole et al., 2004).

Three different C isotopes: ^{12}C the most frequent (98.93%), the stable ^{13}C (1.07%), and the radioactive ^{14}C (10^{-8}%) are ideal for studying the three carbon channels in soil food webs (Scheu, 2002). We used ^{14}C labelled ryegrass shoots as litter and used the different ^{13}C signature of C_3 and C_4 plants (Tieszen and Boutton, 1989) to follow the SOM- and root exudate-derived carbon. In this way, all three carbon channels could be identified with a specific carbon isotope label.

Therefore, our objective in this study was to trace C from three sources having different availability in the food web. This is especially important, since the presence of additional C sources may strongly shift the utilization of C from other sources and their pathways through the various food web levels.

2. Material and Methods

2.1. Soil, plants and soil organisms

The soil was sampled from the Ap horizon of a loamy Haplic Luvisol (long-term field experimental station Karlshof of Hohenheim University). The soil was originated from loess, it contains no $CaCO_3$ and has the following characteristics: pH 6.0, C_{org} 1.2%, N_t 0.13%, clay 23%, silt 73%, and sand 4.4%. No C_4 plants were grown on this soil. The soil was air-dried and sieved on a 2-mm screen before the experiment.

Maize (*Zea mais*, cv. Tassilo) was germinated in Petri dishes in the dark on moist filter paper for 3 days. Seedlings with 1 cm root were planted in the microcosms on 17.09.2003, 2 weeks before the start of the experiment, so they could establish an undisturbed root system. On 30 September 2003 (input of ^{14}C labeled litter) the shoots were approx. 50 cm high on average. Microarthropods and nematodes were taken from synchronized cultures from the University of Bremen, Department of Ecology. Nematodes and collembola were fed with dried bakers' yeast,

predatory mites were fed with an astigmatic mite (*Caloglyphus* sp.), that was reared on dried bakers′ yeast. *Allolobophora chlorotica* were collected in the field by digging in long-term field plots of Bremen University and hand sorting. Only adult worms with a clitellum were used in the experiments. *Lumbricus terrestris* were bought as adult worms at a fisher bait shop, fresh weight was approximately 3 g/worm.

2.2. Microcosms

Microcosms were established in gas tight plastic vessels (Polycarbonate filtration device "CombiSart", volume 250 ml, Merck®-Laborkatalog, 2000). However, the real volume of the device including the volume under the lid is about 340 ml (7 cm height and 8 cm in diameter). The soil was separated from the outlet in the bottom of the CombiSart device by a perforated filter support, delivered together with the filtration device, overlain by two layers of perforated (holes = 0.5 mm) poly ethylene (exact description in Kuzyakov and Siniakina, 2001). 330 g dry soil was used in each microcosm. Water content was maintained at 60% of water holding capacity (determined by the standard method, Black, 1965) by daily weighting. After the addition of ^{14}C labelled litter (see below), the hole in the lid containing the maize stem was sealed with a 5 mm layer of non-phytotoxic silicon rubber paste (NG3170; Thauer and Co, Dresden, Germany). The sealing was tested for air leaks. Each microcosm was connected by tubings to washing flask containing 30 ml of 1 M NaOH for CO_2 trapping. After the trap, the CO_2 free air was pumped by a membrane pump back into the microcosm. Such closed air circulation avoided CO_2 losses. The microcosms were exposed to 27/22 °C day/night temperature, a 14-h photoperiod and 800 mM $m^{-2} s^{-1}$ light intensity.

The NaOH traps were exchanged every forth day. At the same time new air was supplied to microcosms to avoid anoxic conditions. The experiment lasted 30 days after addition of litter and soil organisms, and was terminated because roots occupied almost the whole microcosms.

2.3. Treatments

The effects of three factors were investigated: (1) presence of a growing plant; (2) presence of litter; and (3) food web complexity in an incomplete factorial design with three replicates for each treatment.

The first factor, presence of a growing plant, was investigated by comparison of unplanted soil with soil planted with maize (*Zea maize*, cv. Tassilo.). We used one plant per microcosm. The second factor, presence of litter, was evaluated by addition of shoot litter of *Lolium perenne* that was uniformly labeled with ^{14}C. This litter was a byproduct of a cutting experiment, in which the *Lolium* shoots were labeled 7 times during 2 months (Kuzyakov et al., 2002). 500 mg of dry *Lolium* litter cut in pieces of 2–5 mm containing 4.8 kBq g^{-1} ^{14}C activity were added to the soil surface of each microcosm. The litter was not mixed in the soil and formed a thin layer of 2–4 mm similar to an O horizon in the field.

Food web complexity was managed by addition of different organisms in order to establish model communities at four levels of complexity:

0: No addition of organisms: autochthonous microflora and microfauna only.
1: Addition of bacteriovorous nematodes (*Rhabditis* sp., 700–1000 specimens per replicate) and fungivorous collembola (*Folsomia candida*, 150 specimens per replicate). Here we assumed that there are no interactions between nematodes and collembola.
2: Treatment 1 plus addition of predatory mites (*Hypoaspis* (*Geolaelaps*) *aculeifer*, 35 adult females per replicate) and endogeic earthworms (*Allollobophora chlorotica*, 5 adult specimens per replicate). Here we assumed that there are no interactions between mites and earthworms.
3: Treatment 2 plus addition of an anecic earthworm (*Lumbricus terrestris*, 1 specimen per replicate).

In some microcosms, *L. terrestris* died during the first 2 weeks after their introduction. Those dead *L. terrestris* were replaced by new specimens. We had no information about the biomass of the fauna or about changes of biomass during the experiment.

2.4. Analyses

During the experiment, the CO_2 evolved from each microcosm was trapped in 30 ml 1 M NaOH, which was exchanged every fourth day. To compare the treatments we used mean CO_2 efflux rates per hour.

After the experiment was terminated, we carefully cut off the maize shoots at the soil surface level. Remaining *Lolium* litter was collected from the surface, dried and stored for analyses. After that each root-soil column was removed from the container. The roots were carefully separated from the soil and washed twice in 200 ml deionized water with the addition of Micropur containing Ag^+ to suppress the microbial decomposition of organic substances before analysis (Gransee and Wittenmayer, 2000). Roots were dried at 60 °C and then mixed and pulverized in a ball mill (Retsch) prior to ^{14}C and $\delta^{13}C$ analysis and determination of the total C and N content.

The remaining soil in the vessels was vertically divided into two parts: one was for extraction of soil fauna, the other for analysis of organic C, $\delta^{13}C$ and ^{14}C. The second half was cut into slices with different proximity to the soil surface containing labelled *Lolium* litter: two 5 mm thick slices from the soil surface, two 10 mm thick slices, and the rest (40 mm) was bulked to one sample. Earthworms were removed by hand from the soil and stored at 7 °C in the fridge overnight to allow them to empty their gut. After that they were dried, weighted, ground, and analysed for ^{14}C and $\delta^{13}C$.

The other soil fauna was extracted by two methods: Nematodes from a subsample of 40 g soil in a Baere apparatus, the rest of the soil sample was extracted with a modified Tullgren funnel apparatus to expel the microarthropods (for detailed description see Dunger and Fiedler, 1997). Nematodes and microarthropods were collected in water, air dried and pooled per treatment to get sufficient material for $\delta^{13}C$ analysis. Due to the minute dry weight of the organisms we could only measure one combined sample for all treatments with and one sample for all treatments without plants.

Microbial biomass was determined by the fumigation extraction method (Vance et al., 1987; Ross, 1990). 15 g of fumigated and non-fumigated soil samples (two replicates from each sample) were extracted with 15 ml of 0.5 M K_2SO_4 for 30 min and filtered through ashless filter paper '5893 Blue ribbon' (Schleicher & Schuell GmbH, Germany). An aliquot of K_2SO_4 solution was analysed for organic C with Dimatoc-100 and for ^{14}C activity by scintillation counting. The C amount and ^{14}C counts obtained from the fumigated soils represented the microbial-C and ^{14}C flush and converted to microbial biomass C using the relationship (Ross, 1990):

$$\text{Microbial C} = \text{C flush} * 2.34.$$

The ^{14}C activity in the K_2SO_4 extract without soil fumigation and ^{14}C activity found in water remaining after root washing were assumed to be ^{14}C in dissolved organic C (DOC). To consider the natural ^{40}K radioactivity in K_2SO_4 extracts, the 0.5 M K_2SO_4 extract from unlabelled soil was used as a background standard by scintillation counting.

The activity of ^{14}C–CO_2 trapped in the NaOH solution were determined on 2-ml aliquots added to 3 ml scintillation cocktail EcoPlus (Roth) on ß-spectrometer Rackbeta (Mod. 1419, Wallac). The C counting efficiency was about $89 \pm 1\%$ and the ^{14}C-activity measurement error did not exceed 2%. The absolute ^{14}C-activity was standardized by addition of NaOH solution as quencher to the scintillation cocktail and by using a two-channel ratio method of extended standard (tSIE). Radioactivity of soil samples and remaining litter of *Lolium perenne* was measured after combustion of 1 g of sample within an oxidizer unit (Canberra Packard Co. Ltd, Model 307) with the scintillation cocktail Permafluor E (Canberra Packard Co. Ltd). The total content of CO_2–C collected in NaOH solution was determined by titration with 0.2M HCl against phenolphthalein (pH change 8.2–9.8) after addition of 2.0M $BaCl_2$.

$^{13}C/^{12}C$ isotope ratios of soil, maize shoots and roots, and soil animals was measured on a mass spectrometer Finnigan MAT Delta E. Samples were burned in a Heräus Elementar-Analysator at 1000 °C, and purified in a Trapping Box before measuring. The standard was a mud flat sediment that was calibrated with the international standards IAEA-CH-6 (Sucrose) and IAEA-CH-7 (Polyethylene). Natural abundances of ^{13}C are expressed as $\delta^{13}C$ (‰) which represents the ratios of ^{13}C:^{12}C (R) relative to the PDB standard. The $\delta^{13}C$ values are defined as $[(R_{sample}–R_{standard})/R_{standard}] \times 1000$. The analytical precision of the $\delta^{13}C$ measurements was 0.11‰.

2.5. Statistics

The test design was three factorial (plant, litter, and food web complexity). For some dependent variables (CO_2 efflux and microbial biomass) we had repeated measurements (in time or in sampling depth). Therefore, the influence of effect parameters and their interactions were tested by a general linear model procedure (GLM), with the pairwise LSD post hoc test at 5% error probability. This test could only be applied for data with normal distribution and homogeneity of variances. Both requirements were tested with the Kolmogorov–Smirnov Test and the Levene-Test, respectively. If the requirements for an analysis of variance were not met, the Tamhane-Test for multiple comparisons was applied. Differences between treatments were quantified by comparing the mean values. All statistical calculations were done with the software package SPSS for Windows, 11.5.

3. Results

Microbial biomass in the microcosms and CO_2 efflux were mainly determined by the presence of living plants (Table 1). Food web complexity affected CO_2 efflux only at the highest level when anecic earthworms were present. Neither nematodes, nor microarthropods, nor endogeic earthworms had significant effects on CO_2 efflux or on microbial biomass.

3.1. Litter-Carbon pathways in the system

3.1.1. The role of maize plants

In treatments with litter, we traced ^{14}C in all compartments of the experimental system: respired as $^{14}CO_2$, remaining in the litter, incorporated into microbial biomass, and transferred to soil organic matter. Over all treatments, the largest part of the added litter C was detected in CO_2. However, there was a clear difference between treatments with plants and treatments without plants (Fig. 1, Table 2). When calculated over the whole experimental period, living plants decreased the loss of litter C with CO_2 efflux by a factor greater than 2. A considerable amount of the added ^{14}C remained in the litter layer. Again there was a significant difference between treatments with plants and without (Fig. 2, Table 3). In treatments with plants almost 30% of the added ^{14}C could be detected in the litter layer, whereas only 20% were recorded in treatments without plants. Living plants arrested litter C in the litter layer. Only small ^{14}C amounts (approx. 5% of added litter ^{14}C) were incorporated into microbial biomass in the absence of living plants. Plants strongly enhanced incorporation of litter-derived C into

Table 1
GLM results of the effect of the two factors: (1) presence of maize plants; and (2) food web complexity as well as their interactions, on the two parameters: CO_2 efflux and microbial biomass (MB) measured by fumigation extraction method (FEM).

Source	Plant		Complexity		Plant × complexity	
Variable	CO_2	MB	CO_2	MB	CO_2	MB
df	1	1	3	3	3	3
F-value	230.62	24.96	16.85	1.09	1.88	.14
Significance	.000	.000	.000	.372	.156	.937

Complexity: food web complexity; plant: maize plant present or absent. CO_2: CO_2 efflux from soil, MB: microbial biomass. For MB variances were equal between treatments (Levene test: $F = 6.909$), for CO_2 not (Levene test: $F = 1.695$).

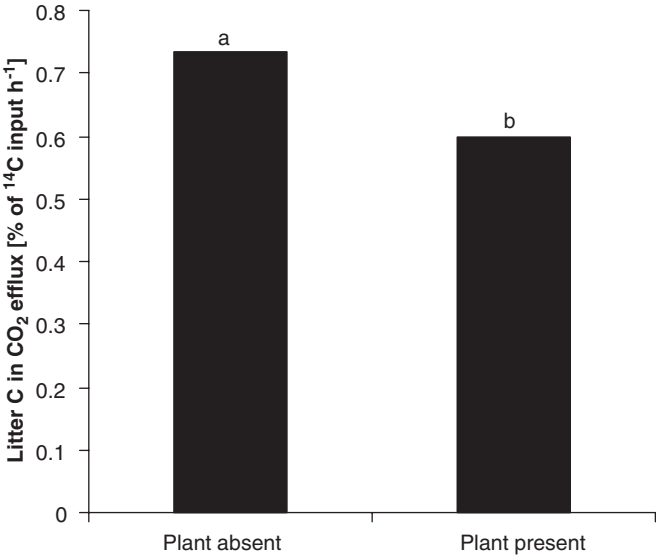

Fig. 1. Litter-derived C in CO_2 efflux from microcosms in % of ^{14}C input per hour. Values are means for all replicates of treatments with and without plants. Statistical tests were post-hoc tests after a GLM Analysis with $P<0.05$ ($N = 12$, $F = 5.43$, $P = 0.034$).

Table 2
Results of a GLM analysis of the effect of the two factors: (1) presence of maize plants; and (2) food web complexity on the ^{14}C content in microbial biomass (MB) and in CO_2 efflux at the end of the experiment in percent of the ^{14}C input.

Source	Plant		Complexity	
Variable	^{14}C in MB	^{14}C in CO_2	^{14}C in MB	^{14}C in CO_2
Df	1	1	3	3
F-value	10.859	5.425	1.560	1.699
Significance	.005	.034	.238	.210

Complexity: food web complexity; plant: maize plant present or absent; MB: Microbial biomass. Variances were equal between treatments (Levene test: $F = 3.549$ for MB; $F = 2.982$ for CO_2).

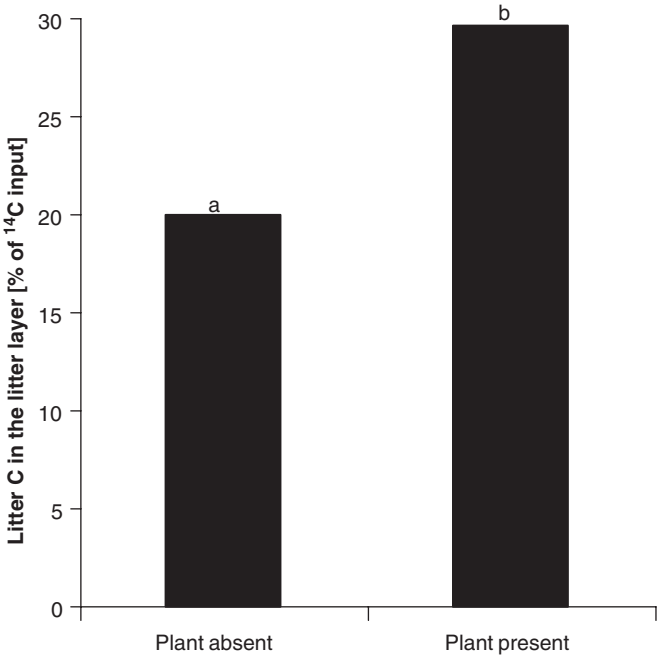

Fig. 2. Litter-derived C that remained in the litter layer at the end of the experiment in % of ^{14}C input. Values are means for all replicates of treatments with and without plants ($N = 12$). For results of statistical tests see Table 3.

Table 3
Results of a GLM analysis of the effect of the two factors: (1) presence of maize plants; and (2) food web complexity on the ^{14}C content in litter and soil at the end of the experiment in percent of the ^{14}C input.

Source	Plant		Complexity	
Variable	^{14}C in litter	^{14}C in 0-5 mm	^{14}C in litter	^{14}C in 0-5 mm
Df	1	1	3	3
F-value	5.137	29.526	.329	5.760
Significance	.039	.000	.804	.008

Complexity: food web complexity; plant: maize plant present or absent. For litter, variances were different between treatments (Levene test: $F = 1.821$), but they were equal for 0–5 mm (Levene test: $F = 3.415$).

microbial biomass; ^{14}C amounts reached 30% of added litter ^{14}C (Fig. 3). The difference of ^{14}C in the microbial biomass between treatments with and without plants was highly significant (Table 2), although variation within treatments was very high. We can conclude that one month after litter input, living plants facilitated incorporation of litter C into microbial biomass and simultaneously reduced its complete mineralization to CO_2.

After removing the residues of ^{14}C labelled *Lolium* litter from the soil surface at the end of the experiment, most

remaining ^{14}C was found in the upper 5 mm of the soil. Again, the greatest difference occurred between planted and unplanted soil. In the absence of plants, there was significantly less ^{14}C in the upper 0-5 cm soil layer (Fig. 4, Table 3). Plants arrested litter C near the soil surface.

A considerable amount of ^{14}C was measured in anecic earthworm tissue, up to 4% (mean 3%) of the initially added ^{14}C was found in *Lumbricus terrestris* (Table 4). The endogeic species did not show any ^{14}C signal unless *L. terrestris* was present. In the presence of anecic species (*L. terrestris*), the endogeic species incorporated approximately the same ^{14}C amount as *L. terrestris*. The feeding and borrowing activity of *L. terrestris* made the litter carbon available for the endogeic earthworms, clearly showing indirect interactions between different earthworm species.

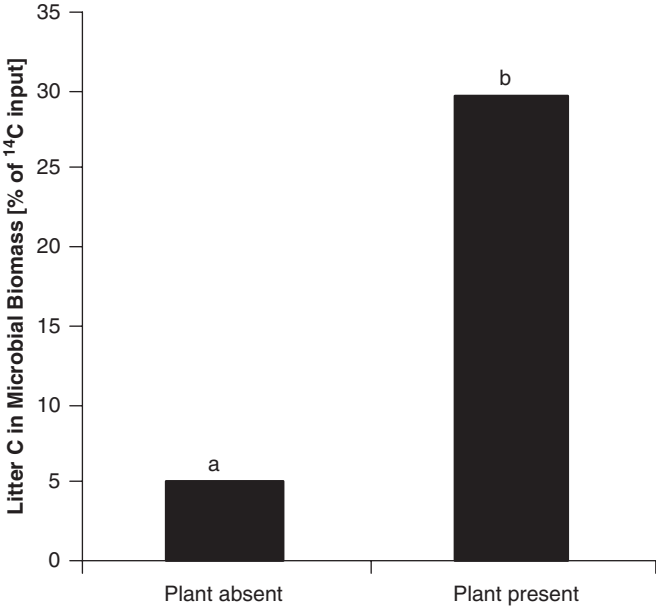

Fig. 3. Litter-derived C that has been incorporated in the microbial biomass the end of the experiment in % of ^{14}C input. Values are means for all replicates of treatments with and without plants. Statistical tests were post-hoc tests after a GLM Analysis with $P<0.05$ ($N = 12$, $F = 10.8643$, $P = 0.005$).

In summary, the pathways of litter C as estimated by ^{14}C were mainly determined by living plants (Fig. 5). In the presence of plants, litter-derived C was divided almost equally between three sinks: (1) it was respired as CO_2, (2) incorporated in microbial biomass, and (3) remained unchanged in the litter. Other sinks, such as incorporation to the soil organic matter or to earthworm tissue were statistically significant but of minor ecological importance. In contrast, without plants, the litter-derived C was distributed differently. The major part (more than 70%) of litter C was mineralized to CO_2, only 20% remained in the litter and less than 10% was incorporated into microbial biomass.

3.1.2. The role of food web complexity

The general effect of food web complexity on litter C in CO_2 efflux was not significant (Table 2), although treatments with anecic earthworms had the lowest ^{14}C values (data not shown). Food web complexity had no significant effect on the remaining litter nor on the percentage of ^{14}C in microbial biomass (Table 2), although highest ^{14}C values were measured in the treatment without fauna (data not shown). In treatments with soil fauna there was significantly less ^{14}C in the upper soil layer (Fig. 4, Table 3) reflecting a mixing of organic residues or infiltration of dissolved carbon compounds into deeper soil layers. Interestingly, the most pronounced effect of food web complexity was in complexity level 2 where all

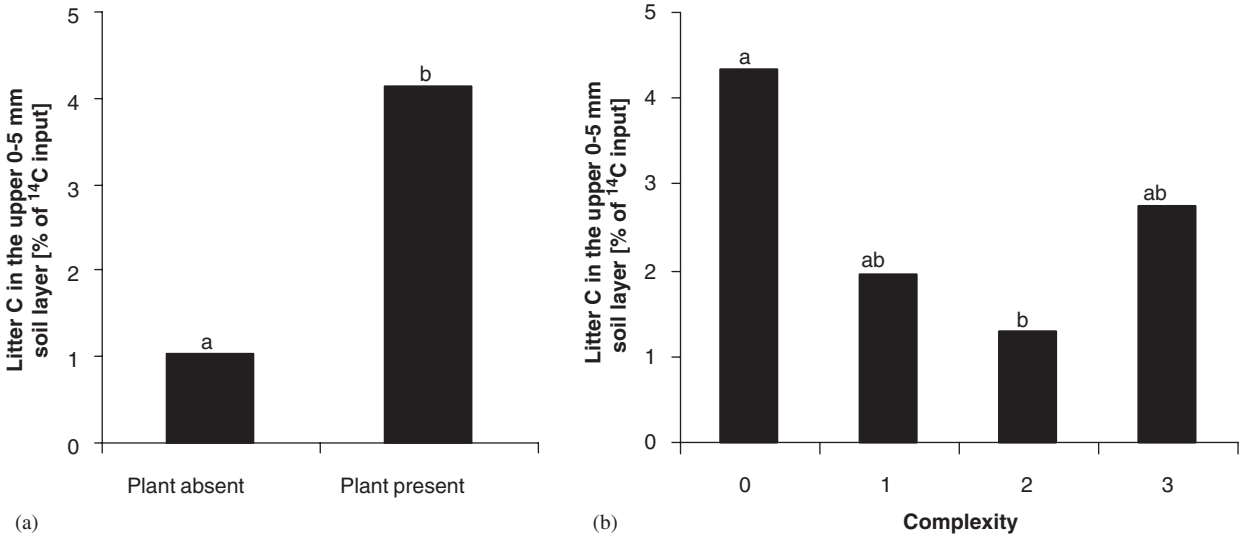

Fig. 4. Litter-derived C that was transferred to the upper 0–5 mm soil layer at the end of the experiment in % of ^{14}C input. Values are means for all replicates with the respective treatment. For results of statistical tests see Table 3: (a) Treatments without plants compared with treatments with plants ($N = 12$); (b) Treatments with different levels of food web complexity, for details see text ($N = 6$).

Table 4
Content of litter-derived C (measured as ^{14}C) in tissue of two earthworm species, *Alollobophora chlorotica* and *Lumbricus terrestris* in each replicate ($N = 3$).

	Presence of *L. terrestris*	DPM mg^{-1}		% of Input	
		Mean	SE	Mean	SE
A. chlorotica	No	3.9	0.1	0.3	0.011
A. chlorotica	No	1.5	0.1	0.1	0.006
A. chlorotica	No	3.8	0.1	0.3	0.009
A. chlorotica	Yes	16	0.3	2.4	0.042
L. terrestris	Yes	2.9	0.1	1.5	0.054
L. terrestris	Yes	8.3	0.3	4.4	0.134

Measurements were based on mixed samples of dried and ground specimens per replicate. SE: standard error, DPM mg^{-1} means DPM ^{14}C per mg earthworm dry weight, % of input means the percentage of added ^{14}C with *Lolium* litter, that was detected in earthworm tissue.

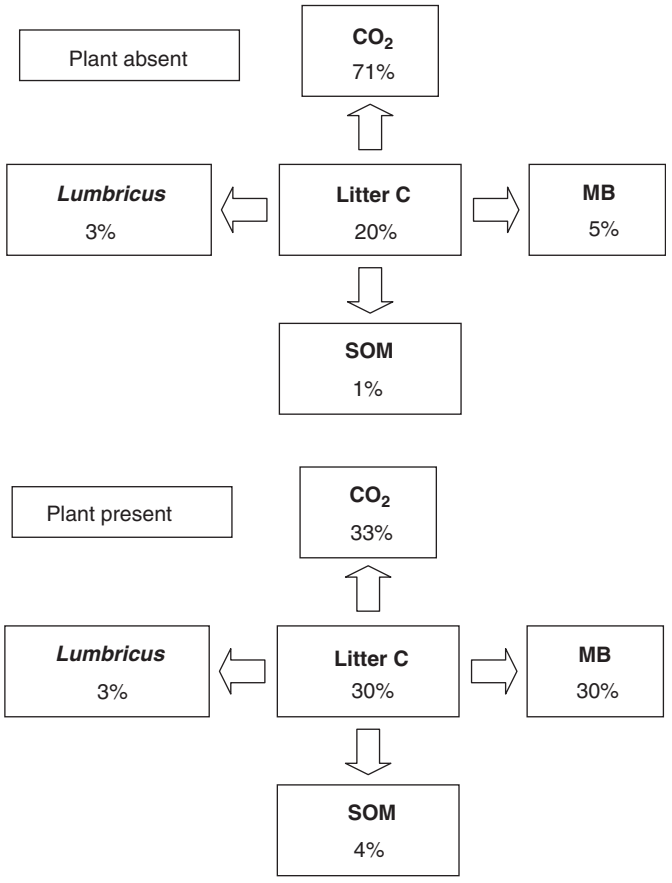

Fig. 5. Fate of litter-derived C after 30 days depending on the presence of living plants. The percentage of litter C in CO_2 was calculated as difference of the sum of the others components to 100%, because measurements were done in intervals and not throughout the whole experiment.

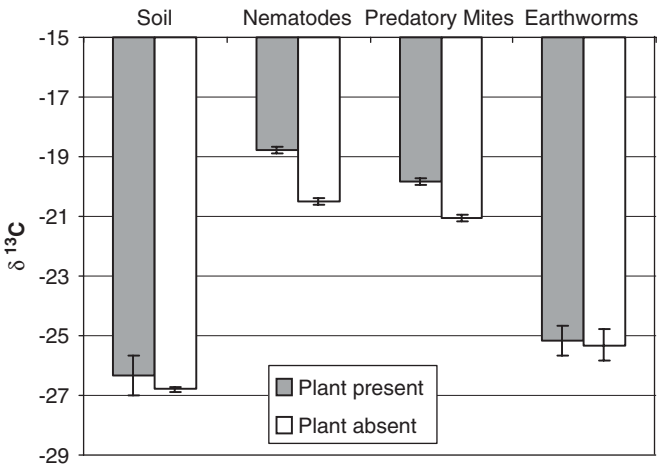

Fig. 6. $\delta^{13}C$ values of SOM and soil animals in treatments with and without living plants. For earthworms and SOM the mean and standard error are given for at least 12 subsamples; nematodes and predatory mites have been pooled to one sample, the error bar is the standard deviation of the reference samples for the mass-spectrometer.

functional groups of soil organisms except anecic earthworms were present.

In comparison to the strong effect of maize plants to litter C pathway, the effect of food web complexity was more subtle. Faunal activity distributed the litter-derived carbon from the surface to deeper soil layers, whereas the presence of plants arrested the litter-derived carbon in the upper soil.

3.2. Carbon originating from root exudates

The carbon from root exudates (with C_4 plant $\delta^{13}C$ signature) was followed through the food web to the third trophic level of secondary consumers (predatory mites). In the presence of maize, we found elevated $\delta^{13}C$ values not only in nematodes (+1.7‰), but also in predatory mites (+1.2‰)(Fig. 6). Higher $\delta^{13}C$ values of nematodes clearly showed that nematodes were closer to the root-derived C than predatory mites in the food web. However, this result can be biased by the faster turnover rates of nematodes compared to mites.

Carbon from root exudates appeared in bacteriovorous nematodes and, with an only slightly weaker signal, in the omnivorous predatory mite. Despite the low resolution of the ^{13}C natural abundance approach, root exudates were a carbon source for the entire food web detectable to at least three trophic levels. However, neither earthworms species did change their ^{13}C values during the short period of the

experiment. This is mainly connected with (i) slow turnover rates of C in earthworm tissue, (ii) high biomass of earthworms, and (iii) low resolution of the ^{13}C natural abundance approach. The same reasons lead to insignificant changes of $\delta^{13}C$ of SOM (Fig. 6).

4. Discussion

In our microcosm experiment, we demonstrated that the presence of a growing plant had major effects on carbon pathways in the system. The effect of food web complexity was of minor importance. The fact that plants increased the microbial activity and biomass is well known from laboratory and field studies (Hines et al., 1999; Fu et al., 2000). Microorganisms are thought to be resource controlled and any addition of C resources enhances their activity and biomass (Wardle et al., 2001; Coleman et al., 2002). An area of dispute is the influence of soil fauna on microbial activity and abundance (Hanlon and Anderson, 1979; Alphei et al., 1996; Filser, 2002; Bowman et al., 2004; Cole et al., 2004). In our experiment we found no effect of nematodes, microarthropods, or endogeic earthworms, but a significant increase of CO_2 efflux in treatments with anecic earthworms. The direct respiration of earthworms themselves is about 36 μg CO_2–C d^{-1} gFW^{-1} (Saetre, 1998) and therefore it is more than 500 times smaller than microbial respiration in the soil (*L. terrestris* ca. 3 g fresh weight (FW), soil ca. 390 g FW). So the direct contribution of earthworms to CO_2 efflux can be neglected in this calculation. The drilosphere and earthworm castings are known to have higher microbial activity. Considering higher CO_2 efflux and the unchanged microbial biomass in our experiment, we conclude that anecic earthworms increased the turnover rate of the microbial biomass. This effect is probably connected with increasing availability of organic substances after passing through earthworm guts (e.g. by enrichment with intestinal mucus and mutualistic microorganisms, Trigo and Lavelle, 1995) and incorporation of surface leaf litter into the soil. But it remains unclear why Alphei et al. (1996) and our experiment failed to show positive effects of endogeous earthworm species that were obvious in other studies (Bonkowski et al., 2001). Scheu et al. (2002) even found a reduced microbial biomass in the presence of endogeic earthworms and discussed that as an effect of competitive interaction between growing and non-growing microorganisms.

4.1. Living plants structured C pathways

Our experiment revealed an important effect of a growing maize plant on belowground C transformation. Plants enhanced CO_2 efflux, microbial biomass, and incorporation of litter-derived C in microbial biomass. However, plants decreased litter decomposition measured as the litter-derived $^{14}CO_2$. Moreover, plants contributed to higher incorporation of litter carbon, mainly into microbial biomass. This is connected with higher amount of microbial biomass in the presence of living plants and higher incorporation of litter C into growing microbial cells. That means that the plants favour the rhizosphere community that is thought to be dominated by bacteria (Bonkowski et al., 2000). On the other side plants suppressed litter decomposition. Fast decomposition rates are mostly due to bacterial based pathways (Mulder et al., 2005). So there seems to be competition between rhizosphere and litter decomposing bacteria and only the rhizosphere bacteria are favoured by the presence of plants. We could not examine whether this effect is direct, or is mediated by other organisms. The studied soil animals did not affect litter-derived carbon respiration significantly.

The results support the concept of basically separated C channels: the root exudate channel reacted within 4 weeks to the growing plant dramatically: both respiration and biomass was increased; whereas there was no positive effect on litter degradation. The litter channel, on the other hand, was not closely connected to the plant. Those microorganisms that benefited from the rhizosphere effect, mainly bacteria, were not involved in litter decomposition. Microorganisms (presumably fungi, the slow decomposers) incorporated litter-derived C but had a low turnover rate and did not mineralise it during the experiment.

In contrast to this finding, long-term field studies showed a stimulating effect of rhizosphere activity on litter decomposition in forest soils (Subke et al., 2004) and in orchards (Wardle et al., 2001). The difference to our experiment is the time scale of the study. We expect that ^{14}C incorporated in the microbial biomass would be recovered in the CO_2-efflux, if the experiment would be prolonged for 2–4 months. Additionally, the tree dominated systems (Subke et al., 2004) may respond differently, because many trees grow fine roots in the litter layer, what the maize plants did not.

The amount of litter-derived carbon incorporated in soil organic matter was low, both in the presence and absence of plants, but was almost 4 times more in the former. This means that the elevated microbial biomass processed more litter-derived carbon to the long term storage in the soil organic matter.

4.2. Food web complexity

In contrast to the beneficial effects of anecic earthworms on CO_2 efflux, the respiration of litter-derived C was hindered in our *L. terrestris* treatments. Yet, there is ample evidence from field studies that earthworms (especially anecic earthworms) enhance decomposition rates in litter bags (Curry and Byrne, 1997; González et al., 2003). However, studies with litter bags do not allow tracing C to complete mineralization. Organic matter may just be translocated by leaching or incorporated into microbial biomass. Hendrix et al. (1986) described a shift from fungal to microbial decomposition of organic matter in the presence of anecic earthworms; the worms adversely affected fungal populations. This may be key for understanding the

reduced litter decomposition in our short term experiment. In treatments without earthworms we visually observed fungal hyphae weaving the *Lolium* litter. The fungal hyphae may be strongly disturbed by the earthworms, and the complete shift to bacterial decomposition had not yet been performed because of short duration of our experiment.

The food web complexity level that contained bacteriovorous nematodes, mainly fungivorous collembola, predatory mites, and endogeic earthworms had one significant effect on the vertical distribution of litter-derived C in the soil. Kisselle et al. (2001) suggested that litter-derived C is translocated by fungal hyphae to the underlying soil. So, the highest complexity level with all trophic groups, but without anecic earthworms, may just favour growth and dispersion of fungal hyphae due to moderate grazing. Tuffen et al. (2002) observed adverse effects of endogeic earthworm activity on fungal hyphae; so a damaged mycelium could be the reason for the inhibited translocation of carbon in soil.

In the food web complexity treatment we could again observe the separation of the litter channel. The litter channel is dominated by fungi and fungi interacted positively with the grazing microarthropods in the second complexity level. This positive effect was compensated by anecic earthworms that disrupt fungal hyphae nets. Moore (1994) and Hedlund et al. (2004) found, that organisms connected to the fungal channel were more sensitive to disturbance, so earthworms could be seen as a disturbance for the fungal channel organisms. On the other side, microorganisms that belong to the other C channel and are more resistant to disturbance, were favoured by anecic earthworms and not by any of the other faunal components.

4.3. Fate of root derived carbon

We observed higher $\delta^{13}C$ values in both nematodes and predatory mites in the presence of maize plants compared to unplanted soil. Thus root borne C was passed through the food web to the predatory mite level during the 4 weeks after organisms were introduced. The introduced nematode was a bacterial feeder and clearly fed on rhizosphere bacteria that used the root exudates as C source. Nematodes and other microfauna is known to have important functions in regulating rhizoshere microbial processes by grazing (Bonkowski et al., 2000). Although there was considerable grazing on the bacteria, there was no effect on respiration nor on microbial biomass. The predatory mite feeds on nematodes as well as on collembola. Considering the much slower turnover rate of the mite in comparison to the nematodes, the $\delta^{13}C$ value of the mites is surprising. Under the experimental conditions the mites were more closely connected to the rhizosphere/bacetrial channel than to the fungal channel. Garrett et al. (2001) and Coleman et al. (2002) could also trace root derived carbon in a group of microarthropods, consisting of collembola, Mesostigmata, Oribatida, and Prostigmata.

Fungal hyphae could also incorporate root derived C (Staddon et al., 1999) that shows the central role of root exudates in fuelling the soil food web. Schmidt et al. (2004) found that different earthworm species in the three ecological groups epigeic, endogeic, and anecic did not differ in their $\delta^{13}C$ value, but in their ^{15}N signature. This may be related with higher turnover rates of N within the earthworms compared to C. Also continuous recycling of mineralized N within the soil, lead to higher N incorporation into earthworms tissue compared to C. The $\delta^{13}C$ value in earthworm tissue was the same as that of the bulk soil. Therefore, we conclude that root derived carbon was not significantly incorporated in earthworm tissue in our short term experiment. This reflects the high body mass of earthworms, their slow turnover rates, and low sensitivity of ^{13}C natural abundance. It does not necessarily mean that earthworms are not connected to the rhizoshere channel, Bonkowski and Schaefer (1997) showed that rhizosphere protozoa are part of earthworm's diet.

5. Conclusions

In a 4 weeks experiment simulating agricultural system with three different C sources and 4 complexity levels of the belowground food web, we demonstrated that there were two factors mostly responsible for carbon pathways in the soil: plants and anecic earthworms. Both had beneficial effects on microorganisms and increased sequestration of litter-derived carbon in the soil system, mainly in microbial biomass. Food web complexity *per se* had only minor effects on the transformation and translocation of litter-derived carbon in the soil. Interactions between the three factors plants, litter, and food web complexity were rarely significant. Root exudates were an important C source for the entire food web and could be traced to the third trophic level. Living plants were the main modifiers of the soil food web and strongly affected C flows, directly: by being a source of additional C, and indirectly: by modification of existing C flows within the food web including CO_2 efflux from the soil and litter decomposition.

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