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REGULAR ARTICLE

Assimilation dynamics of soil carbon and nitrogen by wheat roots and Collembola

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Abstract It has been demonstrated that plant roots can take up small amounts of low-molecular weight (LMW) compounds from the surrounding soil. Root uptake of LMW compounds have been investigated by applying isotopically labelled sugars or amino acids but not labelled organic matter. We tested whether wheat roots took up LMW compounds released from dual-labelled (^{13}C and ^{15}N) green manure by analysing for excess ^{13}C in roots. To estimate the fraction of green manure C that potentially was available for root uptake, excess ^{13}C and ^{15}N in the primary decomposers was estimated by analysing soil dwelling Collembola that primarily feed on fungi or microfauna. The experimental setup consisted of soil microcosm with wheat and dual-labelled green manure additions. Plant growth, plant N and recoveries of ^{13}C and ^{15}N in soil, roots, shoots and Collembola were measured at 27, 56 and 84 days. We found a small (<1%) but significant uptake of green manure derived ^{13}C in roots at the first but not the two last samplings. About 50% of green manure C was not recovered from the soil-plant system at 27 days and additional 8% was not recovered at 84 days. Up to 23% of C in collembolans derived from the green manure at 56 days (the 27 days sampling was lost). Using a linear mixing model we estimated that roots or root effluxes provided the main C source for collembolans (54−79%). We conclude that there is no solid support for claiming that roots assimilated green manure derived C due to very small or no recoveries of excess ^{13}C in wheat roots. During the incubation the pool of green manure derived C available for root uptake decreased due to decomposition. However, the isotopic composition in Collembola indicated that there was a considerable fraction of green manure derived C in the decomposer system at 56 days thus supporting the premise that LMW compounds containing C from the green manure was released throughout the incubation.

Keywords Dissolved organic carbon · Green manure · ^{15}N · ^{13}C · Linear mixing model · *Folsomia fimetaria* · *Proisotoma minuta* · *Protaphorura armata*

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Introduction

Plant roots have a large capacity to take up of low-molecular weight (LMW) compounds through the proton-coupled transporter system, whose primary function is to recapture compounds released from the roots (Farrar et al. 2003). In addition to recapturing root exudates, it has been documented that roots can take up LMW compounds such as amino acids (Nasholm et al. 2001; Lipson and Nasholm 2001) and B-vitamins (Mozafar 1994). Root uptake of LMW compounds has primarily been investigated using isotopically labelled glucose, amino acids or CO_2 (Boddy et al. 2007; Johnson et al. 1996; Kuzyakov and Jones 2006; Owen and Jones 2001). In these experiments very little (2–10%) of the labelled C was recovered in roots indicating that the capture of C from decomposing organic matter in soil does not have a significant impact on the plant's C budget (Kuzyakov and Jones 2006).

Most studies have investigated root uptake of LMW compounds with pulse injection (Bardgett et al. 2003; Nasholm et al. 2001; Weigelt et al. 2005) but to our knowledge only very few studies have used ^{13}C or ^{14}C labelled organic matter for the purpose of detecting root uptake of LMW compounds (Jones et al. 2005). Hodge et al. (1999, 2000) used dual-labelled organic matter in experiments with sedge grass but neither study found root uptake of C that derived from the added organic matter. However, their experimental setup was designed for different purposes than monitoring root uptake of C. Application of labelled organic matter might hold some advantages over pulse injection for monitoring root uptake of MSW compounds. (1) The compounds are released continuously and gradually over a longer period and (2) in local hot spots where the density of roots might be larger than in the surrounding, less nutrient rich soil. Compounds released in the rhizospehere are more likely to be taken up than those released outside the rhizosphere. In contrast, direct injection might favour microbial capture because the compounds are spread uniformly throughout the rhizosphere with the possibility that the significance of root C uptake is underestimated (Kuzyakov and Jones 2006).

The C cycle is closely linked to the N cycle but mineralization and immobilization processes lead to decoupling between C and N through respiration of CO_2, volatilisation of NH_3-N or trans- and deamination in amino acids (Magid et al. 2004). The decoupling might lead to over- or underestimation of the importance of root C uptake in long-term labelling studies if the sources of metabolically active C cannot be distinguished from each other. In studies using ^{13}C and ^{15}N labelled additions C and N decomposition processes can be estimated through isotopic analysis of soil fauna living on primary decomposers (Coleman et al. 2002; Garrett et al. 2001). Sampling and analysis of soil fauna is relatively simpler than that of micro-organisms and is especially suited for investigating mass fluxes in long-term studies (Albers et al. 2006).

In this study, we used a readily decomposable green manure enriched with ^{13}C and ^{15}N added to soil microcosms with Collembola and wheat (*Triticum aestivum*). Wheat was chosen due to its documented ability of taking up exogenously applied LMW compounds (Martens and Frankenberger 1994; Nasholm et al. 2001; Owen and Jones 2001). We tested whether organic C from the green manure was taken up by roots as it has been documented with injection of LMW compounds. Because ^{13}C labelled compounds are respired during incubation, the available pool of labelled LMW compounds are likely to decrease with time. To estimate the pool size of the metabolically active C derived from the green manure we analysed the isotopic values of three collembolan species: *Proisotoma minuta* (Tullberg), *Protaphorura armata* (Tullberg) and *Folsomia fimetaria* (L.) that were added at the initiation of the experiment. We used collembolans as ''sampling devices'' because they have very fast tissue turnover (Bakonyi 1998; Garrett et al. 2001) and are closely linked to the rhizosphere channel through their feeding on rhizosphere microbes and microfauna (Ostle et al. 2007). We expected that *P. armata* would have isotopic values closer to roots than the two other species because herbivory has been reported for *P. armata* and the closely related *Protaphorura fimata* but not for *F. fimetaria* or *P. minuta* (Brown 1985; Joosse and Koelman 1979; Ulber 1983). We further tested whether the presence of Collembola would affect plant growth and uptake of N since previous studies have yielded contradictory results on this matter (e.g. Cole et al. 2004; Filser 2002; Partsch et al. 2006).

Materials and methods

Soil, green manure and Collembola

A low-nutrient coarse sandy soil was collected from Jyndevad research station in early June 2004, Denmark. The soil was sieved through an 8 mm mesh-size sieve, freeze-thawed at −20 and +20 °C in three consecutive cycles over 2 weeks to remove the meso- and macrofauna and subsequently pre-incubated for 21 days at 20–25 °C before use. Soil water content was 14% (38% of gravimetric water holding capacity) and pH was 6.8. Additional properties of the soil are found under No Green Manure (NGM) soil in Table 1. The NGM soil is also referred to as the soil resource. The green manures consisted of ryegrass (*Lolium perenne* L.). The unlabelled ryegrass was grown at the National Environmental Research Institute in Silkeborg, Denmark and is henceforth referred to as NERI ryegrass. The ryegrass seedlings were grown outside between 1 June and 9 July 2004, in peat moss added clay granules and NPK (Stenrøgel Mosebrug A/S). An additional 50 mg urea l^{-1} peat moss was added 20 days after planting. The average temperature was 15.5 °C and the day length 17 h. The ^{13}C and ^{15}N enriched ryegrass was kindly provided by Plant Research International, Wageningen The Netherlands, and is henceforth referred to as Wageningen ryegrass. The ryegrass seedlings were grown for 35 days in a sandy loam soil fertilized with 79.6 mg NH_4NO_3 containing 7.6 mg $^{15}NH_4^+$ in 0.65-L pots in growth chambers, in which a continuously ^{13}C-labelled atmosphere was maintained (Gorissen et al. 1996). This setup ensured that all compounds in the labelled plant material were homogenously labelled. Growth conditions were: light 16 h, PAR 300 µmol m^{-2} s^{-1}, temperature shoot compartment 18/16 °C (day/night), root compartment 16/14 °C (day/night), relative humidity 70%/80% (day/night). After harvest both types of ryegrass were treated in a similar manner with air-drying in an oven with air circulation at 40 °C for 3 days. The ryegrass was ground (1 mm mesh size) to enable homogeneously mixing of the labelled and unlabelled material through the soil. The unlabelled green manure (UGM) consisted only of NERI ryegrass and was weighed into 3.30 g portions for each pot. The labelled green manure (LGM) was weighed into 3.425 g portions consisting of 0.275 g Wageningen

Table 1 Content of soil carbon and nitrogen (mean ± SE, w/GM; n = 12, NGM; n = 3)

Day	% C		% N		C : N (atom)		$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)		
	w/GM	NGM	w/GM	NGM	w/GM	NGM	LGM	UGM	NGM	LGM	UGM	NGM
0	1.84 ± 0.03	1.52 ± 0.02	0.155 ± 0.002	0.111 ± 0.001	13.9 ± 0.0	16.0 ± 0.1	16.0 ± 0.5	−22.6 ± 0.0	−22.7 ± 0.0	220 ± 2	5.0 ± 0.1	6.5 ± 0.5
27	1.71 ± 0.02	1.56 ± 0.00	0.143 ± 0.002	0.113 ± 0.000	13.9 ± 0.0	16.1 ± 0.0	−3.6 ± 0.1	−22.5 ± 0.1	−23.0 ± 0.0	163 ± 1	5.3 ± 0.1	5.4 ±0.0
56	1.70 ± 0.03	1.43 ± 0.02	0.134 ± 0.002	0.102 ± 0.001	14.8 ± 0.0	16.3 ± 0.0	−4.8 ± 0.4	−22.7 ± 0.1	−23.1 ± 0.1	126 ± 2	5.3 ± 0.1	5.9 ± 0.2
84	1.66 ± 0.02	1.54 ± 0.01	0.128 ± 0.002	0.112 ± 0.001	15.2 ± 0.1	16.1 ± 0.0	−6.8 ± 0.1	−22.8 ± 0.1	−22.6 ± 0.1	116 ± 1	5.1 ± 0.1	6.6 ± 0.2

w/GM with labelled and unlabelled green manure, *NGM* no green manure

Table 2 Carbon, nitrogen and staple isotopes in green manures (*GM*) (mean ± SE $n = 3$)

	% C	% N	C : N (atom)	$\delta^{13}C$	$\delta^{15}N$
Unlabelled GM	43.5 ± 0.1	6.78 ± 0.02	7.48 ± 0.0	−24.3 ± 0.0	0.07 ± 0.24
Labelled GM	42.8 ± 0.0	6.59 ± 0.04	7.58 ± 0.0	216 ± 2	827 ± 5

ryegrass and 3.15 g NERI ryegrass. The Wageningen ryegrass was used in small quantities due to the high costs of the material. Isotope, C and N-values of the labelled and UGMs are found in Table 2. The Wageningen ryegrass was analysed indirectly as the enrichments were too high for the analytical range of the mass spectrometer. Wageningen ryegrass characteristics were, on basis of the measurements of labelled and UGM, determined to: C : N-ratio 7.91 ± 0.05, 3,655 ± 2 $\delta^{13}C$, 15,389 ± 4 $\delta^{15}N$ (mean ± SE, $n = 3$). The collembolan species *P. minuta*, *P. armata* and *F. fimetaria* were obtained from laboratory cultures that had been raised on yeast for more than ten generations.

Experimental design

The microcosm setup consisted of 42 pots with three green manure treatments and two Collembola treatments: (1) Unlabelled Green Manure with Collembola (UGM with Collebola), (2) Unlabelled Green Manure without Collembola (UGM without Collembola), (3) Labelled Green Manure with Collembola (LGM with Collembola), (4) Labelled Green Manure without Collembola (LGM without Collembola) and (5) No Green Manure without Collembola (NGM). There were three replicates for each of the treatments. All pots were sown with wheat (*T. aestivum* L. cultivar Vinjett). Pots were sampled destructively three times at different plant ages: 27, 56 and 84 days.

The microcosms were contained in opaque PE cylinders with an inner diameter of 7.1 cm and height of 14.4 cm. The bottom and the top of each pot were closed with PVC lids. An acrylic cylinder with an inner diameter of 2.6 cm and height of 5.0 cm was inserted vertically through a hole in the top lid. All interfaces were sealed with gas tight silicone. An in- and outlet tube for air (PVC 4.0 × 0.8 mm) was inserted into the headspace of each cylinder and the outlet tube was connected to a vacuum pump continuously leading headspace air out of the growth chamber. The pots were placed in a 16 °C climate chamber with 14:10 h light/dark cycle PAR 400 µmol m^{-2} s^{-1}. Each portion of the green manure, 3.425 g LGM and 3.30 g UGM, was mixed into 547 g soil [dry weight (DW) equivalent] with a ladle. From this mixture, 50 g (DW equivalent) were collected for soil analysis. The 500 g soil (DW equivalent) and green manure remaining in each pot was equivalent to 126.3 kg DW m^{-2}. The amounts of C and N from the green manures were equivalent to 363 g C m^{-2} and 51.4 g N m^{-2} in the UGM treatment 370 g C m^{-2} and 51.8 g N m^{-2} in the LGM treatment. With an estimated N efficiency rate of ∼25% (Janzen and Schaalje 1992), the N application rate was similar to field application. After sampling, the soil was compacted to a height of 10 cm (1.25 g DW cm^{-3}). Then, 20 individuals of each of the three collembolan species were added to the soil surface (Collembola treatments only). Three wheat seeds were sown in each of the acrylic cylinders right after soil addition. After 5 days, the most viable plant was retained (equal to 253 plants m^{-2}) and the rest were removed. After 7 days, a 1 cm layer of a two-component silicone (Silastic 3,481, Dow Corning) was placed around each plant to seal the pots. Pots were watered with distilled water twice a week or more frequently when needed (max 40 ml water loss per pot) by adding water until the initial weight of the pot was reached. The initial weight was gradually adjusted for plant growth. Water addition was monitored throughout the 84 days that elapsed during the incubation in the five treatments in addition to the three pots without plants. Water loss was followed in all treatments to estimate water use efficiency for wheat.

Termination and analyses

Plants were collected at each sampling occasion around 11.00 AM, and divided into shoots and roots. Leaf fall during incubation was collected and pooled with the subsequent plant sampling. Roots were

thoroughly washed to ensure that all soil particles were removed. All plant materials were dried immediately at 60 °C until constant weight and then ball-milled (1 mm mesh size). The soil samples (20 g) were dried at 105 °C for 24 h and then ball-milled. About 4 mg plant material and 40 mg soil, respectively, were weighed into tin cups and analysed for the C, N, ^{13}C and ^{15}N with a Europa Scientific 20–20 IRMS at the Plant and Soil Science laboratory in Copenhagen. Mineral N (NO_3^- and NH_4^+) was measured on fresh soil samples in 1 M KCl, which were shaken and filtered, and subsequently analysed on a flow injection system by Steins Laboratorium A/S, Denmark. The replicates sampled for the mineral N analyses were pooled for each treatment to obtain sufficient material. Collembola were extracted from ~150 g soil (DW equivalent) with a Macfadyen high-gradient extractor. The animals were extracted into plastic containers with plaster of Paris and activated charcoal. The containers were kept at a constant 4 °C during the 5 days of extraction. After the extraction collembolans were separated into species and transferred to new substrates without activated charcoal and kept for 24 h at 20 °C to empty their guts. The animals were frozen (−18 °C) for 24 h and subsequently dried at 45 °C for 6 h. If any sample from a treatment contained <75 μg biomass the samples were pooled to obtain sufficient N for detection. The animals were analysed with a dual inlet IRMS Europa Hydra 20–20 at UC Davis Stable Isotope Facility, Department of Plant Sciences, California. The isotopic values of the Plant and Soil Science Laboratory were adjusted to those from UC Davis using standards of ethanenitrile.

Statistics and calculations

The isotopic ratios are reported with units of per mil (‰) difference according to:

$$\delta_{sample} = (R_{sample}/R_{std} - 1) \times 1000, \qquad (1)$$

where δ is ^{13}C or ^{15}N, R_{sample} is the ratio of the rare to abundant isotope, R_{std} refers to the isotope standards, R_C = 0.011180 (VPDB) for $^{13}C/^{12}C$ and air, R_N = 0.0036765 (air) for $^{15}N/^{14}N$ (Fry 2006). Assuming that two phases, the sink and its source, is in equilibrium, the isotopic fractionation α is described as:

$$\alpha = R_{sink}/R_{source} = (1000 + \delta_{sink})/(1000 + \delta_{source}) \qquad (2)$$

and the isotopic enrichment is

$$\varepsilon = (\alpha - 1) \times 1000. \qquad (3)$$

The fraction f of C or N derived from the labelled element in a sample is:

$$f_{sample} = \left(\delta_{sample} - \delta_{background}\right)/\left(\delta_{labelled} - \delta_{background}\right), \qquad (4)$$

where $\delta_{background}$ is the isotopic value of the system without the labelled material and $\delta_{labelled}$ is the isotopic value of the labelled material. The mass of labelled derived C or N in the system, l_{sample}:

$$l_{sample} = f_{sample} \times m_{sample}, \qquad (5)$$

where m_{sample} is the mass of total C or N in the system. l_{sample} is used to calculate the total recovery r of labelled C and N in the system according to:

$$r^{t=1}_{sample} = l^{t=1}_{sample}/l^{t=0}_{sample}, \qquad (6)$$

where t = 0 is time at initiation and t = 1 is the time of sampling.

A linear mixing model according to Phillips and Gregg (2003) was applied to the isotope values of Collembola in the LGM treatment to distinguish between the contribution of C and N from three sources: roots, soil resource and LGM. This approach was used instead of directly applying mixing models since it provides all the source combinations that may explain a given sink isotopic composition within given tolerance values. The source increment was set to 0.01 and the tolerance of variability in $\delta^{13}C$ and $\delta^{15}N$-values to 4‰. The fractionation values used in the model were taken from laboratory reared Collembola on yeast diet (Table 3, day 0). The isotope values of the yeast were −16.6‰ $\delta^{13}C$ and −0.1‰ $\delta^{15}N$. C : N ratios of the three sources were not taken into account because Collembola were assumed not to feed directly on any of these sources but on the primary decomposers.

All statistical analyses were performed with S-Plus Version 6.2. Student's t-test and ANOVA (untransformed data, tested for normal distribution) were used

Table 3 Collembola carbon, nitrogen, isotopic composition, individual weight, total biomass (dry weight) (mean ± SE, w/GM n = 6, LGM and UGM n = 3, lab[a]; n = 3)

Day	Species	% C	% N	C : N (atom)	$\delta^{13}C$ (‰)[b]		$\delta^{15}N$ (‰)		μg individual^{-1}	Mg dw m^{-2}	Cumulated release[c]
		w/GM	w/GM	w/GM	LGM	lab/UGM	LGM	lab/UGM			mg NH_4^+–N m^{-2}
0	*P. minuta*	48.4 ± 0.1	11.2 ± 0.1	5.02 ± 0.04		−16.6 ± 0.1		3.6 ± 0.3	4.14 ± 0.22	20.9 ± 1.1	
	P. armata	53.2 ± 0.3	9.81 ± 0.12	6.32 ± 0.06		−17.4 ± 0.1		2.9 ± 0.7	10.0 ± 0.9	50.5 ± 4.4	
	F. fimetaria	49.3 ± 0.1	9.91 ± 0.11	5.80 ± 0.06		−16.1 ± 0.1		3.0 ± 0.0	5.02 ± 0.58	25.4 ± 2.9	
56	*P. minuta*	48.4 ± 0.2	9.58 ± 0.09	5.90 ± 0.03	34.7	−21.2	574	−1.0	1.34 ± 0.12	41.2 ± 8.0	5.9
	P. armata	49.0 ± 0.8	10.5 ± 0.3	5.44 ± 0.16	−4.6 ± 0.6	−20.7 ± 0.1	599 ± 4	0.0 ± 1.2	4.23 ± 0.50	162 ± 21	6.6
	F. fimetaria	49.0 ± 0.7	8.74 ± 0.31	6.54 ± 0.24	20.3	−21.2	562	0.1	1.77 ± 0.18	55.7 ± 16.3	7.8
84	*P. minuta*	48.4 ± 0.7	9.61 ± 0.10	5.88 ± 0.09	12.2	−21.0 ± 0.1	565	−2.3 ± 2.1	1.37 ± 0.12	82.1 ± 12.0	21.6
	P. armata	48.0 ± 0.4	11.3 ± 0.2	4.97 ± 0.06	−4.8 ± 1.2	−21.4 ± 0.2	588 ± 15	3.2 ± 0.3	6.26 ± 0.33	524 ± 46	47.3
	F. fimetaria	49.2 ± 0.1	9.04 ± 0.53	6.34 ± 0.28	12.4	−21.6 ± 0.1	521	−0.9 ± 2.5	1.73 ± 0.14	85.5 ± 27.2	20.2

w/GM soils with labelled and unlabelled green manure, *LGM* labelled green manure soils, *UGM* unlabelled green manure soils

[a] Values from day 0 are from laboratory animals

[b] Samples without ±SE were pooled from three samples to obtain sufficient biomass for analysis

[c] Estimated

to detect differences unless otherwise noted. Wilcoxon rank sum tests were applied when the errors were not normally distributed. Precision of the sample means are shown with standard error of means. In the isotope mixing models, errors were propagated according to Fry (2006).

Results

Wheat

The presence of Collembola did not affect shoot biomass (Collembola × sampling day interaction, $F_{2,12}$ = 0.04, P = 0.96), N content ($F_{2,12}$ = 0.31, P = 0.74) or $\delta^{15}N$ ($F_{2,12}$ = 1.32, P = 0.30). Therefore, GM treatments with and without Collembola were pooled. Likewise, shoot biomass (GM treatment × sampling day interaction, $F_{2,30}$ = 1.33, P = 0.28) and N content ($F_{2,30}$ = 2.84, P = 0.07) were similar between both LGM and UGM treatments and these parameters were therefore pooled (Table 4). Shoots in the NGM treatment had a much lower growth reaching about 6% of the DW of green manure treatments at 84 days.

$\delta^{13}C$-values of LGM roots were significantly higher than UGM roots at 27 days but only by 0.9‰ (Fig. 1b; Wilcox, P < 0.05). If it is assumed that this was due to uptake of LGM-C, the percentage derived from LGM C (%Cdf LGM) would be 0.38 ± 1.16% (Table 5). This was the only occasion with significant difference between the labelled and unlabelled treatments for roots as well as shoots (Fig. 1a, b). In contrast, $\delta^{13}C$-values of NGM shoots and roots were 2.7–7.1‰ lower than those of UGM. This difference was correlated with the water use efficiency that was around three times lower per unit dry matter in NGM compared to the GM treatments at 27 days and 4.5 times lower at 56 and 84 days.

Plant $\delta^{15}N$-values in the LGM treatment were between 670 and 750‰ indicating that most of the N derived from the added GM. In the shoots, between 87 and 90% of N derived from LGM (%Ndf LGM) and in the roots between 82 and 85% (Table 5). Shoots contained 5.7 ± 0.3% less N from soil resources than roots. The amount of LGM-N taken up by plants was 42% at 84 days. This amount is underestimated because of root loss during washing. The only source of N for plants in the NGM soil was

Table 4 Content of wheat carbon, nitrogen, biomass (dry weight) yield and isotopic difference, $\varepsilon^{15}N$ (‰) relative to sources (mean ± SE, w/GM $n = 12$, UGM $n = 6$, NGM $n = 3$)

	Day	% C		% N		C : N (atom)		g dw m^{-2}		$\varepsilon^{15}N$ (‰)	
		w/GM	NGM	w/GM	NGM	w/GM	NGM	w/GM	NGM	UGM[a]	NGM
Shoots	27	40.8 ± 0.1	37.8 ± 1.1	5.54 ± 0.07	4.10 ± 0.22	8.59 ± 0.08	10.8 ± 0.48	176 ± 5	26.1 ± 5.1	0.4 ± 0.5	0.1 ± 0.3
	56	44.2 ± 0.1	37.8 ± 1.7	1.96 ± 0.04	2.39 ± 0.24	26.3 ± 0.5	18.5 ± 0.9	983 ± 21	66.5 ± 6.6	2.8 ± 0.5	0.2 ± 0.8
	84	44.2 ± 0.0	41.0 ± 0.8	1.37 ± 0.04	1.77 ± 0.30	37.7 ± 0.9	27.0 ± 3.4	1,792 ± 47	101 ± 13	2.2 ± 0.5	−1.1 ± 0.3
Roots	27	41.9 ± 0.5	42.7 ± 0.3	2.57 ± 0.14	2.23 ± 0.27	19.0 ± 0.9	22.4 ± 2.7	47 ± 5	20.2 ± 7.7	−1.5 ± 0.8	−4.5 ± 0.3
	56	44.0 ± 0.2	42.6 ± 0.2	1.49 ± 0.09	1.31 ± 0.06	34.5 ± 2.0	38.0 ± 1.0	149 ± 5	17.7 ± 3.9	−0.6 ± 0.6	−4.2 ± 0.3
	84	44.4 ± 0.2	43.7 ± 0.2	1.15 ± 0.05	1.29 ± 0.07	44.9 ± 1.7	39.4 ± 1.4	118 ± 6	20.2 ± 5.3	−1.5 ± 0.3	-4.7 ± 0.2

w/GM with labelled and unlabelled green manure, *UGM* unlabelled green manure, *NGM* no green manure

[a] Estimated values

that of the soil resource. Consequently, the isotopic difference, $\varepsilon^{15}N$, between plant parts and soil resource could be readily determined (Table 4). On average, NGM roots were depleted by 4.2 ± 0.4‰ compared to NGM shoots. In the UGM treatment $\varepsilon^{15}N$-values were estimated on the basis on the fraction of plant N that derived from soil resource N and LGM-N in the LGM treatment. On average, LGM roots were depleted by 2.9 ± 0.6‰ compared to LGM shoots (Table 4). Addition of GM also increased the utilization of the soil resource N compared to NGM except for the first plant sampling at day 27. At 84 days NGM plants had taken up 2.05 g N from soil resources versus 3.50 g N in the LGM treatment.

Collembola

Collembola samples from day 27 were lost due to a breakdown of the extractor. At 56 and 84 days the $\delta^{15}N$-values in the LGM treatment were between 520 and 600‰ and the $\delta^{13}C$-values between -5 and 35‰ (Table 3). Most of Collembola body N, 64–73%, derived from LGM. In contrast, C mainly derived from soil resources or root exudates with 7 and 24% from LGM-C (Table 5). On Fig. 2, the isotopic values of Collembola are placed relative to the three sources of C and N: (1) roots, (2) soil resource and (3) LGM. Collembola are placed relatively closer to roots than the two other sources. The linear mixing model by Phillips and Gregg (2003) predicted that from 77 to 79% of the *P. armata* body mass derived from roots opposed to 54–67% for *P. minuta* (Table 5). The fraction of C and N derived from roots increased between 56 and 84 days. *P. armata* obtained less of their mass from LGM compared to *P. minuta* and *F. fimetaria*. The isotope values of Collembola in the NGM treatment were very close and could not be used to infer sources (Table 3).

The numbers of collembolans were 101,600 ± 18,100 m^{-2} at day 56 and 202,900 ± 13,800 m^{-2} at day 84. The only significant difference between species was at 84 days where *P. armata* was more abundant than *F. fimetaria* ($P < 0.05$). Excretion of NH_4^+-N was estimated from collembolan biomass (Table 3) and the ammonium excretion values by Larsen et al. (2007). The estimated accumulated excretion of NH_4^+-N at 84 days was 89 mg N m^{-2}. This amount corresponded to

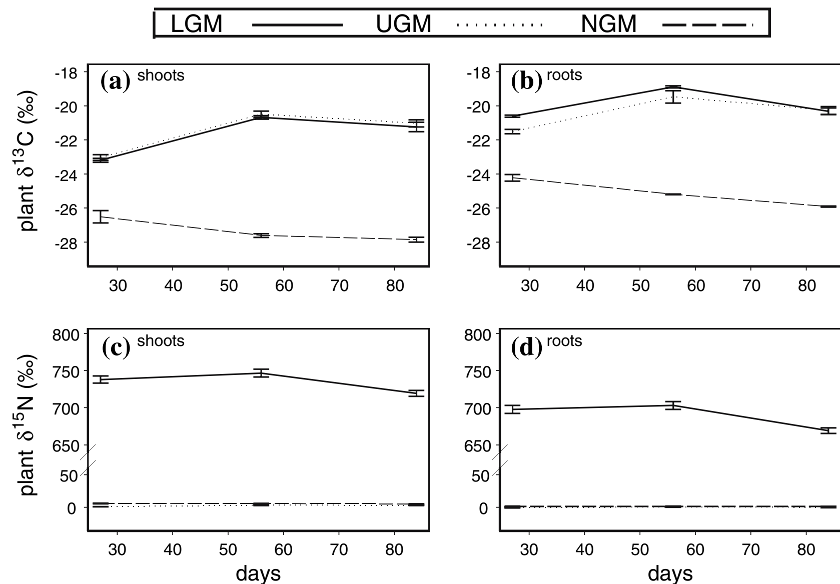

Fig. 1 Isotope values, δ^{13}C (‰) and δ^{15}N (‰), in shoots (**a** and **c**) and roots (**b** and **d**)

about 0.3% compared to total plant N uptake at 84 days (Table 4).

Recoveries of LGM-C and -N

Recovery of LGM-C from soil was 49% at 27 days. At 84 days, recovery of LGM-C had had decreased to 41% ($P < 0.01$, Fig. 3). Total recovery of LGM-N included soil and plant N and was similar at 27, 56 and 84 days with 90% ($P < 0.05$, Fig. 3). The following values of mineral N were found at day 0: For NO_3^--N, 3.16, 2.78 and 1.52 g m^{-2} for the LGM, UGM and NGM treated soils, respectively; for NH_4^+-N, 6.69, 6.44 and 0.417 g m^{-2} the LGM, UGM and NGM treated soils, respectively. Thus, an amount of ~10 g N m^{-2} was readily available in mineral form and predominantly NH_4^+-N right after addition to soil, which was about one-fifth of total GM added.

Table 5 Percentages of C and N derived from labelled green manure (*df* LGM) and estimated food sources from linear mixing modelling (mean ± SE, plant; n = 6, Collembola; n = 3)

	Day	Elements		Linear mixing model		
		%C *df* LGM	%N *df* LGM	Roots	Soil resource	LGM
Shoots	27	0	89.7 ± 0.0			
	56	0	90.4 ± 0.0			
	84	0	87.2 ± 0.0			
Roots	27	0.38 ± 1.16	85.0 ± 0.0			
	56	0	85.5 ± 0.0			
	84	0	81.5 ± 0.0			
P. minuta	56	23.4 ± 0.2	69.9 ± 0.3	54 ± 1	23 ± 0	23 ± 1
	84	13.9 ± 0.1	69.0 ± 0.3	67 ± 2	19 ± 0	14 ± 1
P. armata	56	6.7 ± 0.5	72.9 ± 0.0	77 ± 2	16 ± 0	7 ± 1
	84	6.9 ± 0.9	71.2 ± 0.2	79 ± 2	14 ± 1	7 ± 1
F. fimetaria	56	17.4 ± 0.1	68.4 ± 0.3	59 ± 2	24 ± 1	17 ± 1
	84	14.2 ± 0.1	63.5 ± 0.3	60 ± 1	26 ± 0	14 ± 1

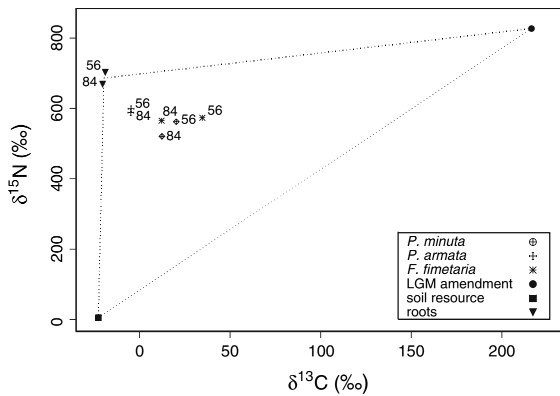

Fig. 2 Source mixing triangle for Collembola. The numbers next to symbols signify sampling day

Discussion

The study was designed to test whether wheat roots would take up ^{13}C derived from the dual-labelled green manure, LGM-C. LGM roots were enriched more than UGM roots at 27 days but the isotopic difference was very small ($P < 0.05$, ~1‰) and we estimated the amount of C derived from LGM to be 0.38 ± 1.16% (Table 5). We cannot account for the fraction of LGM-C taken up by roots that was subsequently respired but Kuzyakov and Jones (2006) found this to be 45% for maize roots that had taken up exogenously applied glucose. Thus, the amount of LGM-C taken up by roots would still represent <1% of total plant C. There is a possibility that the small root uptake of exogenously C was an artefact because of contamination of the root surface with labelled C. However, the ^{13}C signatures in roots at the two last samplings were similar and it supports the premise that the washing procedure neatly cleaned off labelled particles from the root surface. The identical ^{13}C signatures of the LGM and UGM shoots show that the difference in roots cannot be attributed to different water use efficiencies as for the NGM plants (Farquhar and Richards 1984). Because of the very small or total lack of recovery of excess ^{13}C in wheat roots, we do not find that there is solid support for claiming that roots assimilated green manure derived carbon. This supports findings from injection studies that uptake of exogenously applied LMW compounds is small or insignificant indicating that roots are poor competitors for LMW compounds (Boddy et al. 2007; Despland and Noseworthy 2006; Kuzyakov and Jones 2006; Owen and Jones 2001).

Most of plant N derived from the green manure but apparently there was a smaller fraction of LGM-N in roots than in shoots (Table 5). This variation can partly be explained by differences in isotope values between the two plant compartments observed at natural abundance. In the UGM treatment, roots were estimated to be depleted by about 3‰ compared to shoots, which also agrees with previous natural abundance studies (Evans et al. 1996; Lopes and Araus 2006; Lopes et al. 2004). Assuming a similar

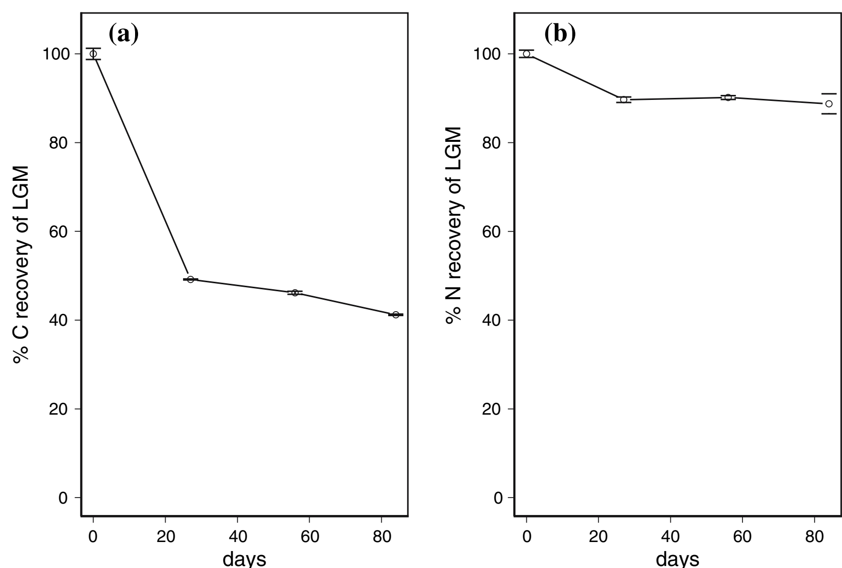

Fig. 3 Total recovery of labelled green manure (*LGM*) C (**a**) and N (**b**) from plant plus soil (mean ± SE, $n = 6$)

fractionation in the LGM treatment, only about one-eight of the depletion of ^{15}N in LGM roots can be explained by intra-plant fractionation. Another explanation for the isotopic differences between the two plant compartments could be that roots assimilated N at a later stage than shoots when the soil was more depleted in ^{15}N. However, the similar ^{15}N-values at 27–56 days (Fig. 1c, d) do not support the notion of differential assimilation events between the two plant compartments. So the intra-plant variation was probably caused by different patterns of assimilation. For example, significant intra-plant variation has been observed when NO_3^- is the primary nitrogen source, but little variation has been observed when NH_4^+ is the source (Evans et al. 1996; Yoneyama et al. 1991). NH_4^+ is assimilated immediately in the root why organic nitrogen in shoots and roots is a product of a single assimilation event. In contrast, NO_3^- assimilation that can occur in both roots and shoots (Evans 2001).

Collembola were primarily included as sampling devices of primary decomposers. The isotopic values of Collembola from 56 and 84 days depict that their tissue N mainly derived from LGM and that C mainly derived from roots or soil resources (Fig. 2). For *P. minuta* that do not eat plant roots (Ulber 1983), the fraction of LGM-C decreased from 23 to 14% between 56 and 84 days showing that C and N from LGM was increasingly but not completely decoupled in the decomposer system. The linear mixing model revealed that C from roots, the rhizosphere channel, was very important for Collembola in general and *P. armata* in particular (Table 5). This agrees with our hypothesis that *P. armata* would derive more of it resources from roots than the two other species because it can feed directly on roots. Our finding that the rhizosphere channel was the main supplier of energy in the soil food web is in line with previous findings (Garrett et al. 2001; Pelz et al. 2005; Ruf et al. 2006). However, the large loss of LGM-C at the beginning of the experiment indicates that LGM-C was very important for fuelling the microbial mediated release of mineral N and subsequent plant growth. Collembola generally incorporated more C derived from soil resources than from LGM. In contrast, Collembola showed a clear preference for newly incorporated C from litter over the soil resource in the study by Briones et al. (1999) without plants and with a slower decomposable green manure.

In spite of comparable or higher collembolan densities than at field conditions (Axelsen and Thorup-Kristensen 2000), neither plant biomass nor N content were not affected by the presence of Collembola. This is in line with Cole et al. (2004) who found no effect by Collembola on plant productivity in monocultures. Collembolan release of mineral N was estimated to 0.3% compared to plant N uptake and it is evident that the direct contribution by Collembola was insignificant. Collembolan effects are often ascribed to their grazing on fungal hyphae (Lussenhop 1996) or spreading of fungal propagules (Filser 2002) but fungi might have been less important for decomposition than bacteria in this study because the organic matter was readily decomposable through a rapid release and utilization of soluble carbon compounds (Van Ginkel and Gorissen 1998). Moreover, the fertilization level was high compared to normal agricultural settings because the 42% uptake the added LGM-N was higher than we expected. Thus, the ample supply of mineral N reduced the importance of collembolan mediated processes.

Magid et al. (2004) proposed that the decomposition of intracellular LMW compounds and proteins can be viewed as a process separate from the decomposition of macro-polymers in cell walls. In our study, these two processes seemed to be separated with an initial rapid loss of C that followed by a slower loss of C. N was also lost within the first 27 days. The loss was most likely due to a rapid breakdown of the proteins in the green manure or ammonium volatilisation due to senescence of micro-organisms (Hogberg 1997). It is unlikely that denitrification occurred since the soil was not water logged. The coincidence of C and N losses early in this study indicate that microbial decomposition decoupled substantial amounts of labelled compounds from the LGM giving the micro-organisms a pre-emptive competitive advantage in the acquisition of easily available LGM resources.

We stated in the introduction that application of labelled organic matter might hold an advantage over pulse injection of labelled compounds because LMW compounds are released gradually during the decomposition processes. It can be argued, however, that there was no gradual release of LMW compounds in this study as a lot of C was lost in the initial phase before the onset of plant growth. However, according

to the linear mixing model more than one-fifth of *P. minuta* body mass derived from LGM at 56 days indicating that not all labelled compounds from LGM were decoupled within the first 27 days. Also, an additional 8% of LGM-C was lost between 27 and 84 days showing that the remaining pool of C was recycled and released continuously. The sensitivity of detecting uptake of LGM-C in roots would have been higher if the enrichment of ^{13}C in LGM had been higher than in this study. But the conclusion that root uptake of C from exogenously applied organic matter was very small or insignificant would probably remain the same. However, the methodology of using ^{13}C and ^{15}N LGM in a plant-soil system in combination with isotopic analysis of soil fauna provided a lot of valuable information on C and N dynamics in soil and the significance of plant derived C for the soil food web.

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