Stem labeling results in different patterns of ^{14}C rhizorespiration and ^{15}N distribution in plants compared to natural assimilation pathways

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Abstract

To investigate C and N rhizodeposition, plants can be ^{13}C-^{15}N double-labeled with glucose and urea using a stem-feeding method (wick method). However, it is unclear how the ^{13}C applied as glucose is released into the soil as rhizorespiration in comparison with the ^{13}C applied as CO_2 using a natural uptake pathway. In the present study, we therefore compared the short-term fate of ^{14}C and ^{15}N in white lupine and pea plants applied either by the wick method or the natural pathways of C and N assimilation. Plants were pulse-labeled in $^{14}CO_2$-enriched atmosphere and ^{15}N urea was applied to the roots (atmosphere–soil) following the natural assimilation pathways, or plants were simultaneously labeled with ^{14}C and ^{15}N by applying a ^{14}C glucose–^{15}N urea solution into the stem using the wick method. Plant development, soil microbial biomass, total rhizorespiration, and distribution of N in plants were not affected by the labeling method used but by plant species. However, the ^{15}N : N ratio in plant parts was significantly ($p < 0.05$) affected by the labeling method, indicating more homogeneous ^{15}N enrichment of plants labeled *via* root uptake. After $^{14}CO_2$ atmosphere labeling of plants, the cumulated $^{14}CO_2$ release from roots and soil showed the common saturation dynamics. In contrast, after ^{14}C-glucose labeling by the wick method, the cumulated $^{14}CO_2$ release increased linearly. These results show that ^{14}C applied as glucose using the wick method is not rapidly transferred to the roots as compared to a short-term $^{14}CO_2$ pulse. This is partly due to a slower ^{14}C uptake and partly due to slow distribution within the plant. Consequently, ^{14}C-glucose application by the wick method is no pulse-labeling approach. However, the advantages of the wick method for ^{13}C-^{15}N double labeling for estimating rhizodeposition especially under field conditions requires further methodological research.

Key words: ^{14}C / belowground carbon / belowground nitrogen / double-labeling technique / ^{15}N / pulse labeling / stem-feeding method

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1 Introduction

Belowground C and N of legumes are significant quantitative pools of the plant-derived residue C and N contributing to C sequestration as well as to N nutrition in crop rotations (*Mayer* et al., 2003; *Jones* et al., 2009; *Yasmin* et al., 2010). However, our understanding of their contribution to the dynamics of microbially driven soil processes and nutrition of subsequent crops is limited (*Jones* et al., 2009; *Yasmin* et al., 2010). Estimation of belowground plant biomass (BGP) by physically detecting existing roots lacks accuracy as the rhizodeposition (release of ions, border cells, mucilage, exudates, secretes, root fragments, and fine roots from living plants during growth) is not taken into account (*Janzen*, 1990; *Uren*, 2001; *Paterson*, 2003; *Jones* et al., 2009).

Rhizodeposition accounts for half of the 30% to 40% net fixed C translocated belowground (*Kuzyakov* and *Domanski*, 2000; *Kuzyakov* and *Schneckenberger*, 2004; *Jones* et al., 2009). However, only a small proportion of the rhizodeposition remains in the soil because most of the C rhizodeposits are decomposed to CO_2 by microorganisms (*Nguyen*, 2003; *Kuzyakov* and *Schneckenberger*, 2004; *Jones* et al., 2009). Belowground N varies between 16% and 60% of total N in cereals (*Rroço* and *Mengel*, 2000; *Khan* et al., 2002) and between 14% and 74% in legumes (*e.g., Khan* et al., 2002; *Mayer* et al., 2003; *Wichern* et al., 2007a, b). In addition, N rhizodeposition varies widely between 4.3% and 56% of the net assimilated N in cereals and between 4% and 71% in legumes (*Wichern* et al., 2008; *Fustec* et al., 2010). Besides the fact that research on N rhizodeposition is still scarce (*Jones* et al., 2009), one possible reason for this high variability is the use of different methods for labeling plants with ^{15}N and varying experimental conditions.

Rhizodeposition in terms of C and N release can be quantitatively estimated by continuously labeling plants with ^{14}C or ^{13}C and ^{15}N and following their fate into the unlabeled soil.

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Prerequisite for this estimation is the assumption that the isotope signature of roots and rhizodeposits is the same and that this relationship remains throughout the investigation period (*Janzen* and *Bruinsma*, 1989). Plants are commonly labeled in $^{14}CO_2$-or $^{13}CO_2$-enriched atmosphere (for a review see *Kuzyakov*, 2002) using the physiological pathway of C assimilation. For ^{15}N labeling of plants, ^{15}N urea, $^{15}NO_3^-$, or $^{15}NH_4^+$ is applied *via* roots, leaf, or stem. Plants can also be labeled by exposing the shoots to $^{15}NH_3$ or by exposing their roots (only for legumes) to $^{15}N_2$-enriched air (*e.g., Janzen* and *Bruinsma*, 1989; *McNeill* et al., 1994). During the last years the "wick method", a shoot-labeling method, was increasingly used especially in grain legumes (*Mayer* et al., 2003; *Hertenberger* and *Wanek*, 2004; *Yasmin* et al., 2006; *Mahieu* et al., 2007; *Wichern* et al., 2007a, b, 2010). The wick method was developed for ^{15}N labeling of grain legumes (*Russell* and *Fillery*, 1996), later on compared with other ^{15}N-labeling methods and tested on various crop species (*Hertenberger* and *Wanek*, 2004; *Yasmin* et al., 2006; *Mahieu* et al., 2007; *Wichern* et al., 2010). Recently, plants were double-labeled by applying a highly enriched ^{13}C glucose–^{15}N urea solution using the wick method (*Wichern* et al., 2007a, b, 2010). These results as well as the results from the first study (*Russell* and *Fillery*, 1996) indicate that isotope distribution is not homogeneous among plant parts. Nevertheless, it is unclear if the ^{15}N distribution after pulse labeling becomes more homogeneous with time. Furthermore, it is unclear if pulse labeling plants with ^{13}C glucose using the wick method results in short term release of ^{13}C from roots as observed for $^{13}CO_2$ labeling. It is crucial to better understand the fate of the applied ^{13}C and ^{15}N tracers using the wick method.

We therefore conducted a comparative study in which white lupine (*Lupinus albus* L.) and pea (*Pisum sativum* L.) plants were pulse-labeled either (1) with $^{14}CO_2$ *via* assimilation by shoots and ^{15}N urea applied to the soil for root uptake, or (2) double-labeled by applying a ^{14}C glucose–^{15}N urea mixture into the stem using the wick method. Our objective was to compare the dynamics and distribution of ^{14}C and ^{15}N in plant and soil compartments after pulse labeling plants using the wick method in comparison with the natural C and N uptake. Additionally, we investigated if the labeling methods had any effect on plant C and N allocation and soil microbial biomass. The hypotheses were: (1) Plant growth is not influenced by the labeling methods; (2) rhizosphere processes are stronger affected by plant species than by the labeling methods; (3) ^{14}C applied as glucose using the wick method shows the same pattern of ^{14}C rhizorespiration as ^{14}C applied using a short pulse of $^{14}CO_2$; (4) ^{15}N in plant parts after application using the wick method is less homogeneously distributed in comparison with ^{15}N applied using the natural pathway *via* soil but becomes more homogeneously distributed with time.

2 Materials and methods

2.1 Experimental set-up

Twenty white lupine (*Lupinus albus* L., cv. Amiga) and twenty pea (*Pisum sativum* L., cv. Santana) plants were grown under controlled laboratory conditions (average temperature of 25°C, 12 h/12 h day/night interval, with a light intensity of 500 μmol m^{-2} s^{-1}) on a silt-loam soil (Haplic Luvisol) taken from the top 10 cm (A_P horizon) of a long-term field experiment at Karlshof, University of Hohenheim, SW Germany. The soil contained 4% sand, 73% silt, and 23% clay. The pH ($CaCl_2$) was 6.0; the contents of organic C and total N were 1.2% and 0.13%, respectively. The soil (100 g dry weight) was placed into 100 mL containers, which were closed with a lid containing a hole for the plant and two holes to connect a pump for air circulation. Soil moisture was gravimetrically maintained at 40% to 60% of its maximal water-holding capacity throughout the experiment. Seeds of white lupine and peas were germinated in plastic Petri dishes. The seedlings were then transplanted into the soil containers (one plant per pot).

The CO_2 in the soil air was measured according to the procedure described by *Werth* et al. (2006). Trapping of CO_2 from soil air started at 7–8 leaves unfolded for lupine and at 9–10 leaves unfolded for peas in a closed-air-circulation system for each plant. One day prior to the start of CO_2 trapping, the holes in the lid were sealed around the plant shoots using silicone (TACOSIL 145, Thauer & Co., Germany). Air was pumped through every single pot using a membrane pump (Type 113, Rietschle Thomas, Germany; pumping rate 100 mL min^{-1}) connected to the pot by a plastic tube. The outlet of the container was connected to a CO_2-trapping glass tube containing 3 mL of 1 M NaOH solution. The output of the trapping tube was connected to the input of the membrane pump. Consequently, the air that evolved from soil respiration circulated in a closed system, with the containing CO_2 being trapped in NaOH and the CO_2-free air being transferred to the container and reused continuously.

2.2 Labeling plants with ^{14}C and ^{15}N

Lupine plants were labeled at 7 to 8 leaves unfolded and pea plants at 9 to 10 leaves unfolded. Two chase periods were investigated with plants being harvested 6 and 11 d after beginning of labeling. Plants were either pulse-labeled with $^{14}CO_2$ applied to the atmosphere and ^{15}N urea applied to the soil ("atmosphere–soil") or by applying a ^{14}C glucose–^{15}N urea solution to the plant using the wick method. In the labeling approach atmosphere–soil, the plants were pulse-labeled with ^{14}C (8.9×10^6 DPM per plant) by exposition to $^{14}CO_2$-enriched atmosphere for 4 h in an air-tight chamber. Plants were labeled with ^{15}N by applying 0.5 mL of an aqueous solution (0.08%, w/v) of ^{15}N-enriched urea (99 atom%) to the soil. In the labeling approach wick method, the plants were labeled with 0.5 mL of a aqueous solution, with 7% (w/v) glucose enriched with ^{14}C (1.6×10^6 DPM in lupine and 2.5×10^6 DPM in peas) and 0.8% (w/v) ^{15}N-enriched (99 atom%) urea using a wick method for solution transfer into the plant as described by *Wichern* et al. (2007a, b). Briefly, a cotton wick was passed through a hole in the stem, which was drilled with a 0.5 mm drill ≈ 2 cm above the soil surface. The ends of the wick were passed through a silicone tube and inserted into a 2 mL vial with a lid containing the solution. To prevent transpiration losses, the connections at the plant stem and at the lid were sealed with plasticine (Teroson, Henkel, Düsseldorf, Germany). All vials and materials used in the system were steam-sterilized for 20 min at 121°C. All solu-

tions were produced using sterile deionized water and filtrated (< 0.2 μm) before application. Where the solution was taken up from the vials, 0.2 mL deionized water was applied to the vials allowing remaining substances to be taken up. After harvest of the plants, the wicks, tubes, and vials were extracted with 50 mL 0.05 M K_2SO_4 by 30 min horizontal shaking at 200 rev min^{-1}. To prevent loss of ^{14}C from glucose by microorganisms, two drops of chloroform were added to the vials prior to extraction. The ^{14}C, which remained in the vial and wick, was subtracted from the applied ^{14}C to determine the actual ^{14}C uptake by individual plants. Solution uptake ranged from ≈ 28% to 100% of the applied solution in lupine (on average 69%) and from 65% to 100% in pea plants (on average 94%). On average, lupine plants took up 50% of the solution within 5 d, whereas pea plants needed only 3 d. Complete solution uptake was achieved for 2 of the 10 lupine plants after 7 and 8 d. In contrast, complete solution uptake was achieved for 7 of 10 pea plants after 1 to 11 d.

2.3 Plant and soil sampling

At harvest, where present, vials and wicks were carefully removed and plants were cut off directly above the soil surface. Plants were separated into leaves and stem. The soil was separated into rhizosphere and bulk soil. Bulk soil was recovered by carefully taking out the plants including roots and adhering soil. The soil adhering to the roots was carefully collected manually and defined as rhizosphere soil. All visible roots and root fragments of rhizosphere and bulk soil were collected manually and washed with 20 mL 0.001 M $CaCl_2$ and dried at 60°C for 72 h. Soil samples were dried at 60°C, plant and soil material ground to fine powder using a ball mill, and analyzed for total C, ^{14}C activity, total N, and the isotope ratio ^{15}N : ^{14}N (see below).

2.4 Analyses

The CO_2 trapped in NaOH was sampled three times daily during the first 36 h, twice during the following 5 d, and once from day 6 to day 11. To estimate total CO_2 efflux, the CO_2 trapped in the NaOH solution was precipitated with a 0.5 M $BaCl_2$ solution and titrated with 0.1 M HCl against phenolphthalein indicator (*Zibilske*, 1994). Total C and total N and the isotope ratio ^{15}N : ^{14}N in plant and soil samples were determined using Carlo Erba NA 1500 gas chromatograph (Carlo Erba Instruments, Milan, Italy) coupled on isotope-ratio mass spectrometer (Delta plus IRMS 251, Finnigan Mat, Bremen, Germany). The ^{14}C activity of $^{14}CO_2$ trapped in NaOH solution was measured in 1 mL aliquots added to 2 mL scintillation cocktail Rotiszint Eco Plus (Carl Roth, Karlsruhe, Germany) after decay of chemoluminescence. ^{14}C activity was measured using a MicroBeta Liquid Scintillation Counter (Perkin Elmer). The ^{14}C counting efficiency was ≈ 80%, and the ^{14}C-activity measurement error did not exceed 2%.

Microbial biomass C and N in the soil were estimated by chloroform-fumigation extraction (*Brookes* et al., 1985; *Vance* et al., 1987), including a pre-extraction step to remove living roots and root fragments (*Mueller* et al., 1992). Briefly, one portion of soil (2 to 50 g) was extracted with 0.05 M K_2SO_4 at an extraction ratio of 1:5 by 30 min horizontal shaking at 200 rev min^{-1}. The soil suspension was completely transferred into a beaker, and all visible root fragments were taken out manually. After that, the soil suspension was filtered. Then, one portion of the extracted soil was immediately fumigated for 24 h at 25°C with ethanol-free $CHCl_3$. Following fumigant removal, the sample was extracted with 0.05 M K_2SO_4 at an extraction ratio of 1:4 by 30 min horizontal shaking at 200 rev min^{-1} and filtered to collect the supernatant for determination of organic C, total N, and the ^{14}C activity. The nonfumigated subsample of the soil was extracted similarly at the time when fumigation commenced. Organic C in all extracts was measured as CO_2 by infrared absorption after combustion at 850°C using a Dimatoc 100 automatic analyzer (Dimatec, Essen, Germany). Microbial biomass C was calculated as E_C / k_{EC}, where E_C = (organic C extracted from fumigated soils) – (organic C extracted from nonfumigated soils) and k_{EC} = 0.45 (*Wu* et al., 1990). Total N in the extracts was measured by chemoluminescence detection after combustion using a Dima-N analyzer. Microbial biomass N was calculated as E_N / k_{EC}, where E_N = (total N extracted from fumigated soils) – (total N extracted from nonfumigated soils) and k_{EN} = 0.54 (*Brookes* et al., 1985; *Joergensen* and *Mueller*, 1996).

2.5 Calculations and statistical analysis

Results in the tables are presented on an oven-dry basis (about 24 h at 105°C for soil samples and ≈ 72 h at 60°C for plant parts). The effect of the labeling approach, plant species, and harvest time on plant development, total CO_2, $^{14}CO_2$ release, and ^{15}N distribution among plant parts were analyzed by analysis of variance (ANOVA). Data were log-transformed prior to ANOVA if they did not follow a normal distribution. In addition, the effect of method, plant species, harvest time, and location (bulk *vs.* rhizosphere soil) on microbial biomass C and N was analyzed by ANOVA. All statistical analyses were performed using JMP 6 (SAS Institute Inc., Cary, USA).

3 Results

3.1 Effect of labeling methods on plant and soil properties

Neither atmospheric $^{14}CO_2$ labeling of plants and ^{15}N-urea application in the root system, nor stem feeding plants with a ^{14}C glucose–^{15}N urea solution using the wick method had any effect on total plant and root dry matter (Tab. 1). Both labeling methods had also no effect on soil microbial biomass C and N. The microbial biomass C content was higher in the rhizosphere soil of white lupine in comparison with the bulk soil, and it was higher in comparison with the microbial biomass C content in rhizosphere and bulk soil of pea plants. Microbial biomass C in the rhizosphere of pea plants (Tab. 2) was similar or slightly lower in comparison with the respective bulk soil. Microbial biomass N, on the other hand, was neither influenced by plant species nor by harvest time, but followed a similar plant-specific distribution pattern between rhizosphere and bulk soil. The release of total CO_2 from soil and

Table 1: Total plant dry matter, root dry matter, cumulated CO_2 and $^{14}CO_2$ release after harvest at 6 and 11 d after labeling. The table shows *F* values from the analysis of variance; degrees of freedom: 1; $n = 5$. Different letters show significant differences between the various treatments ($p < 0.05$, Tukey-Kramer test).

Crop and method	Harvest after day	Total plant	Root	Cumulated CO_2	Cumulated $^{14}CO_2$
		dry matter		/ mg C pot^{-1}	/ % of applied ^{14}C
		/ g plant^{-1}			
White lupine, wick method	6	0.75 b	0.12 b	27.4 b	2.1 ab
	11	1.15 a	0.37 a	54.8 a	1.1 b
White lupine, atmosphere–soil	6	0.79 ab	0.16 b	31.4 b	8.2 a
	11	0.91 ab	0.20 b	47.0 ab	5.0 ab
Pea, wick method	6	0.72 b	0.15 b	36.5 ab	5.8 ab
	11	0.84 ab	0.18 b	42.7 ab	5.7 ab
Pea, atmosphere–soil	6	0.79 ab	0.17 b	28.0 b	6.2 ab
	11	0.80 ab	0.18 b	46.9 ab	5.5 ab
Analysis of variance					
Plant species		3.17	1.59	0.25	1.94
Labeling **M**ethod		0.33	0.13	0.39	8.43 **
Harvest time		8.46 **	14.20 ***	27.86 ***	0.44
P × **M**		0.43	0.82	0.00	10.92 **
P × **H**		1.75	5.98 *	0.00	3.62
M × **H**		2.59	5.72 *	1.91	0.04

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

roots followed a similar pattern for both labeling methods and both plant species. Also the cumulated total CO_2 release was similar for both labeling methods and plant species (Tab. 1). However, the dynamics of $^{14}CO_2$ release varied between the two labeling methods (Fig. 1). When using atmospheric labeling of plants, $^{14}CO_2$ release from roots and soil was faster in comparison with ^{14}C labeling using the wick method. After stem feeding, $^{14}CO_2$ release increased nearly linear, whereas $^{14}CO_2$ release following atmospheric labeling increased with exponential saturation to the maximum (Fig. 1). This difference between the methods was more pronounced in white lupine than in pea plants. In addition, cumulated $^{14}CO_2$ (% of applied ^{14}C) release from roots and soil was significantly different between the two labeling methods in lupine plants but showed no significant difference in pea plants (Tab. 1).

3.2 Distribution of ^{14}C within plants

The phosphor imaging screens clearly showed the more branched and dense rooting system of pea in comparison with lupine plants. Furthermore, the screens show an inhomogeneous distribution of the applied ^{14}C for both plant species and both labeling techniques (for examples see Figs. 2 and 3). Distribution was more homogeneous between plant organs and between leaves in pea than in lupine plants. Using the wick method, the position of the stem where the wick was applied was most strongly enriched. This effect was more pronounced in white lupine than in pea plants. Younger leaves, stem, stipples, and roots were stronger enriched than older leaves in pea plants after 6 d for both techniques (data not shown). The contrast in the ^{14}C distribution between younger and older leaves of pea plants was much stronger pronounced by atmospheric labeling compared to the wick method labeling approach (data not shown). In general, the shoot was stronger enriched than leaves using the wick method, whereas leaves were stronger enriched than the stem when using atmospheric labeling. For atmospheric labeling in lupine, the young leaves present at the time of labeling and those evolving shortly thereafter were most strongly enriched. Enrichment of roots and older leaves was similar and lower than enrichment of young leaves and stem for both labeling techniques.

3.3 Distribution of N

At harvest, a significantly higher portion of plant N (recovered in roots, stem, and leaves) was present in the leaves than in stem and roots (Tab. 3). There was no significant difference between plant species, methods used, or harvest time. The same was observed for the distribution of ^{15}N recovered in roots, stem, and leaves. Roots of the atmosphere–soil labeling tend to recover more ^{15}N in comparison with the wick method. When using the wick method, the stem was stronger enriched in ^{15}N in comparison with the atmosphere–soil treatment. The ratio of ^{15}N distribution (^{15}N in a certain plant part as a percentage of total ^{15}N recovered in the plant) and N distribution (N in a certain plant part as a percentage of total N recovered in the plant) (^{15}N : N) was significantly different between plant parts for the labeling methods. Using the wick method, the stem had a significantly higher ^{15}N : N than

Table 2: Microbial biomass C (MBC) and N (MBN) in the soil after 6 and 11 d of incubation. The lower part of the table shows *F* values from the analysis of variance; $n = 5$. Different letters within one treatment (crop and method) show significant differences ($p < 0.05$, Tukey-Kramer test) for location or harvest time.

Crop and method	Harvest after day	Location	MBC / μg (g soil)$^{-1}$	MBN / μg (g soil)$^{-1}$
White lupine, wick method	6	rhizosphere soil	319 a	26 a
	11	rhizosphere soil	330 a	32 a
	6	bulk soil	215 b	18 b
	11	bulk soil	134 b	11 b
White lupine, atmosphere–soil	6	rhizosphere soil	387 a	32 a
	11	rhizosphere soil	242 b	23 ab
	6	bulk soil	233 b	21 b
	11	bulk soil	167 b	15 b
Pea, wick method	6	rhizosphere soil	183 b	21 a
	11	rhizosphere soil	203 b	23 a
	6	bulk soil	192 b	22 a
	11	bulk soil	213 a	22 a
Pea, atmosphere–soil	6	rhizosphere soil	121 b	21 a
	11	rhizosphere soil	139 ab	22 a
	6	bulk soil	225 a	25 a
	11	bulk soil	236 ab	25 a
Analysis of variance				
Plant species			21.42 ***	0.05
Method			0.10	1.09
Harvest time			3.58	1.75
Location (bulk and rhizosphere soil)			7.67 **	16.94 ***
P × **M**			0.83	0.01
P × **H**			10.12 **	3.14
P × **L**			45.47 ***	27.87 ***
M × **H**			1.88	1.54
M × **L**			5.08 *	2.03
CV			41.1	33.4

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

leaves and roots and varied between 1.8 and 3.0. The ^{15}N : N of roots and leaves ranged from 0.4 to 1.2. The atmosphere–soil treatment showed no significant difference in the ^{15}N : N of roots, stem, and leaves, even though the ratio varied between 0.7 and 1.6. The ^{15}N : N ratio did not change significantly between the two harvests within the investigation period.

4 Discussion

4.1 Effect of plant species on microbial biomass distribution

Even though total plant and root dry matter of lupine and pea plants was not significantly different, soil microbial biomass C content and its distribution in rhizosphere and bulk soil was significantly different between the two crops, indicating plant-species-specific influence on rhizosphere processes. This reflects the different rooting patterns of the two plant species also highlighted by the phosphor imaging screens. The higher content of microbial biomass C, especially in the rhizosphere soil, and a similar content of microbial biomass N indicate a higher availability of easily available substrate for microorganisms in lupine in comparison with pea plants. However, this was not reflected by a higher total CO_2 release because the rhizosphere soil reflects only a small part of the total soil volume. Overall, plant-specific effects on the rhizosphere are more pronounced than the effects of the labeling methods. In a previous study (*Wichern* et al., 2010), it was also shown that the wick method had no effect on plant growth or C and N partitioning, which might be expected when harming the plant. In the present study, we were able to confirm this observation and furthermore to show that the same holds

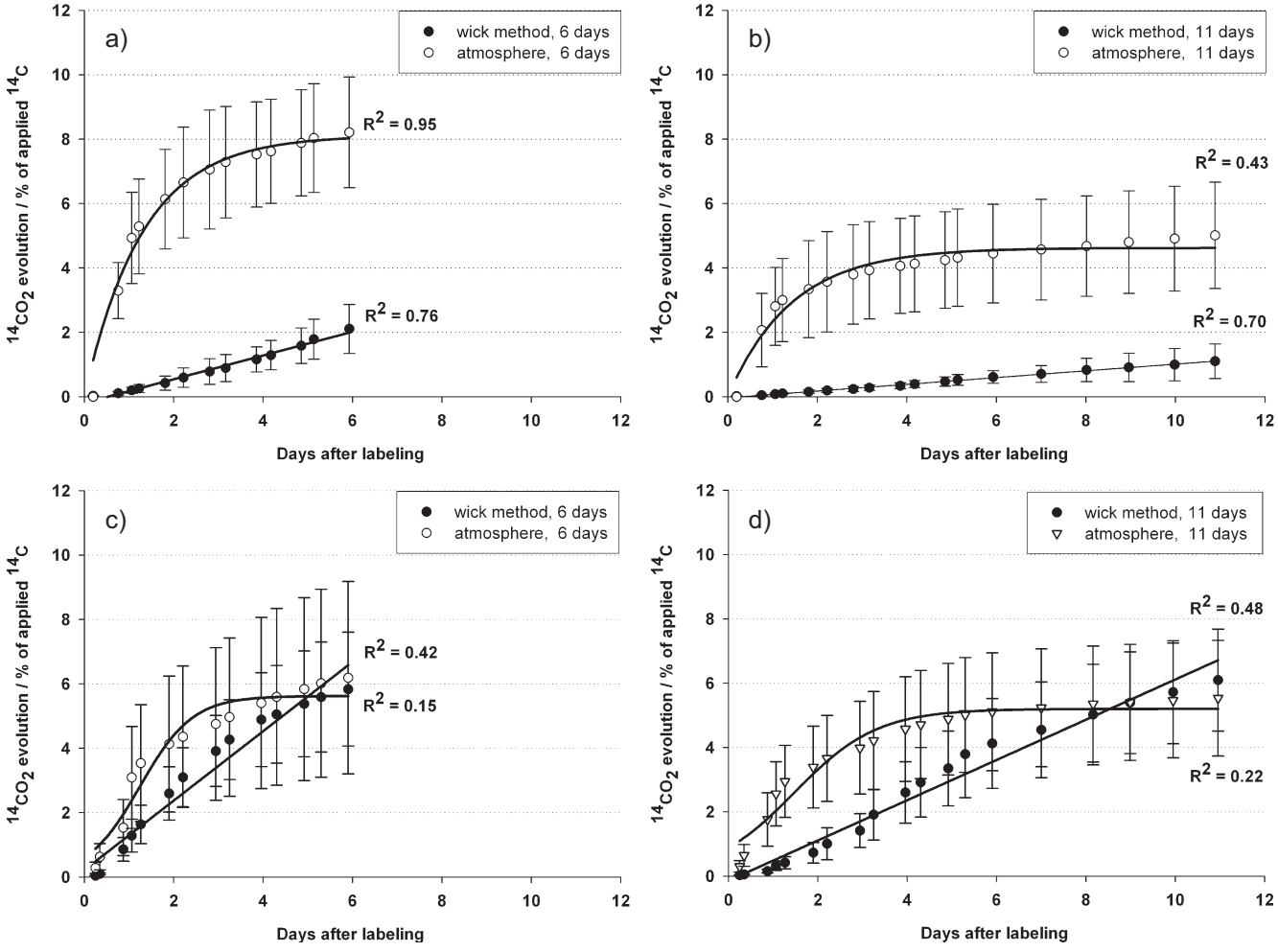

Figure 1: Cumulative $^{14}CO_2$ efflux (% of applied ^{14}C) from rhizosphere of white lupine (a, b) and peas (c, d) 6 and 11 d after labeling plants with ^{14}C either by using the wick method (^{14}C glucose) or by atmospheric labeling ($^{14}CO_2$). Values show means ± standard error of the mean ($n = 5$).

true for the atmosphere–soil ^{14}C-^{15}N double-labeling approach. Moreover, also the microbial biomass, which is a sensitive indicator of short-term increases in the rhizosphere (*De Neergaard* and *Magid,* 2001; *Schenck zu Schweinsberg-Mickan* et al., 2010), was not influenced by the labeling methods but by plant species. This demonstrates that labeling methods had no substantial influence on rhizosphere processes supporting our hypothesis.

4.2 Label distribution varies between methods

The ^{14}C phosphor images and the ^{15}N distribution showed that pulse-labeling plant shoots with ^{14}C or ^{15}N resulted in inhomogeneous label distribution, with aboveground plant parts being stronger enriched than roots as expected. This has been shown for ^{14}C and ^{15}N separately (*Hill* et al., 2007; *Russell* and *Fillery*, 1996; *Mahieu* et al., 2007; *Mayer* et al., 2003) and for ^{13}C and ^{15}N simultaneously before (*Wichern* et al., 2007a). However, we were able to show that a high portion of the applied ^{14}C stayed at the area of labeling and was not relocated any further. For the atmosphere–soil treatment, this area was the young growing leaves present at labeling and for the wick method, it was the stem. Additionally, a high proportion of the ^{15}N applied *via* wick was present in the stem where the wick was attached. In the atmosphere–soil treatment, however, ^{15}N was applied *via* roots and therefore distributed relatively homogeneously among plant organs, indicated by the ^{15}N : N being not significantly different between plant parts, even though slight differences were observed. Furthermore, no change in ^{15}N or N distribution between plant organs could be observed between the harvest times when using the wick method. Consequently, no significant changes of the ^{15}N : N ratio were observed, indicating that no ^{15}N and N redistribution occurred during the short time period investigated. Overall, no short-term changes in ^{15}N distribution have been observed. For this reason, ^{15}N distribution remained nonhomogeneous. This supports our hypothesis that ^{15}N distribution using the wick method results in a less homogeneous distribution of ^{15}N in comparison with the application of ^{15}N *via* roots. However, the second part of our hypothesis namely that the label distribution becomes more homogeneous, could not be verified.

In addition to the difference in ^{15}N-label homogeneity between both labeling approaches, also the $^{14}CO_2$ release from

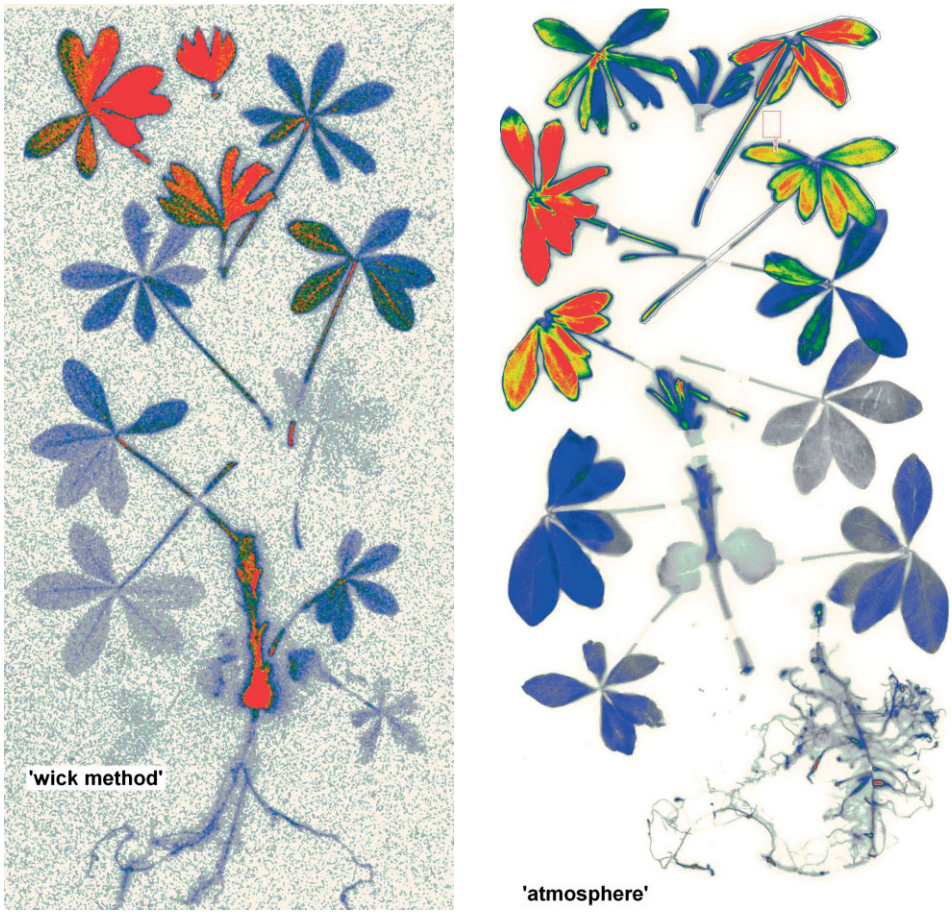

Figure 2: Phosphor imaging of white lupine plants after labeling plants by stem feeding ^{14}C glucose using the wick method (a) or by atmospheric labeling (atmosphere) with $^{14}CO_2$ (b).

soil and roots showed different patterns. In the atmosphere–soil labeling treatment, cumulated $^{14}CO_2$ release rose with exponential saturation to the maximum, whereas after ^{14}C labeling using the wick method, the cumulated $^{14}CO_2$ release increased nearly linear. This effect was more pronounced in white lupine than in pea plants. It was expected that the ^{14}C glucose would be transferred rapidly to the location of active plant growth and consequently released from roots as rhizodeposition, especially as root exudates or respired from roots to a similar extend as recently assimilated C. This, however, was not the case. One reason is the slower uptake of the solution containing ^{14}C glucose in the wick method treatments, taking between 1 and 10 d. In contrast, labeling *via* $^{14}CO_2$ assimilation was finished within 2 h, where recently assimilated ^{14}C has been transferred to actively growing plant parts (*Jones* et al., 2009). In comparison with the ^{14}C pulse labeling of plants by atmospheric $^{14}CO_2$ application, the wick method cannot be named as pulse-labeling approach.

5 Conclusions

The two methods applied for labeling plants with ^{14}C and ^{15}N showed no effect on plant growth and soil microbial biomass. However, the latter was clearly influenced by plant species. Independent of the labeling method, ^{14}C distribution among plant parts varied between plant species, with a more homogeneous label distribution in peas. This indicates an interactive effect of plant species and labeling method, which has to be taken into account when investigating C rhizodeposition. On the other hand, ^{15}N applied by the wick method was less homogeneously distributed among plant parts than the ^{15}N taken up by roots. A reasonable compromise for double labeling plants therefore is to apply multiple pulses of ^{14}C glucose in combination with ^{15}N urea using the wick method as suggested before (*Russell* and *Fillery*, 1996; *Mayer* et al., 2003), providing more a continuous labeling than a pulse-labeling approach. However, the reliability of this approach needs further verification. Future methodological research on the quantification of rhizodeposition should focus on the question to what extent roots and rhizodeposition can be homogeneously labeled using other pathways than the natural ones. It would be also helpful to gain more information on the degree of the bias introduced by inhomogeneous labeling.

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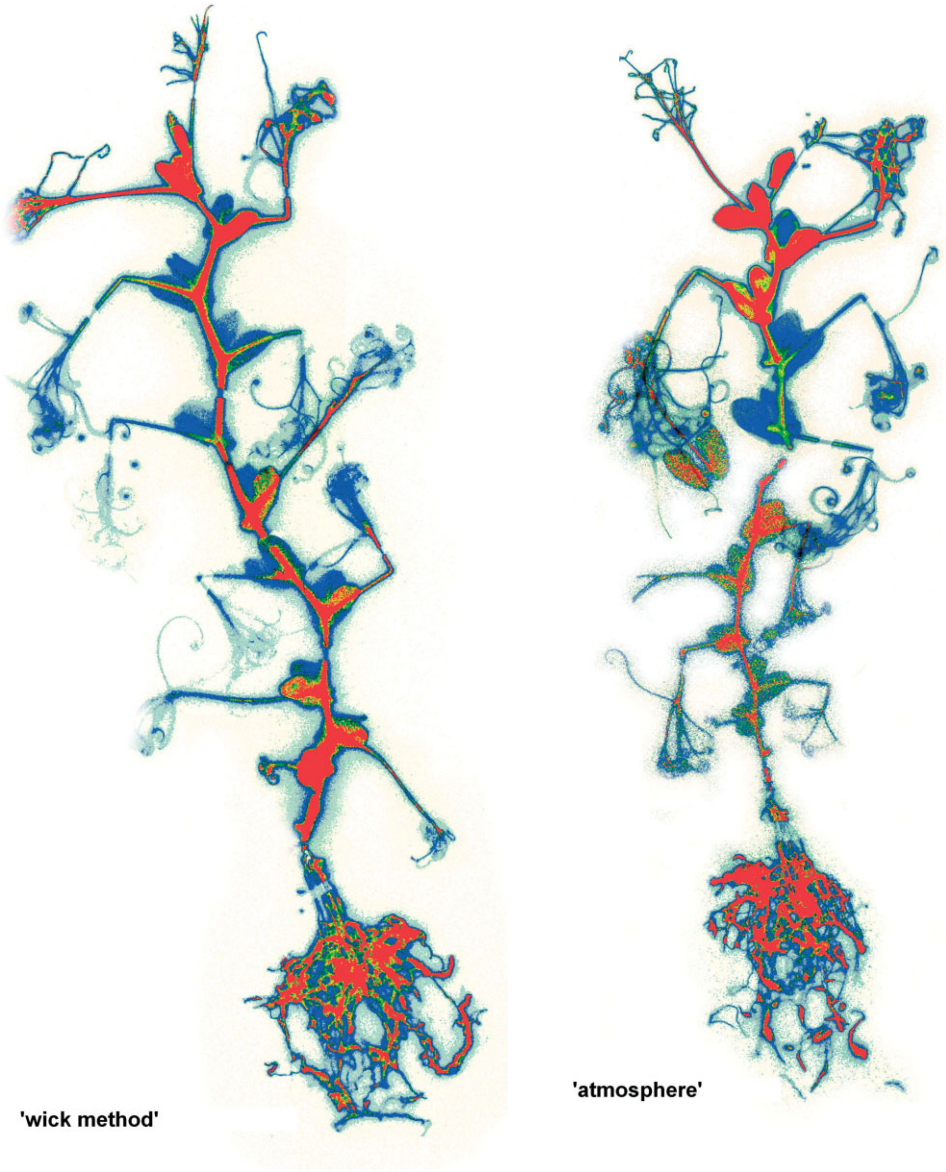

Figure 3: Phosphor imaging of pea plants after labeling plants by stem feeding ^{14}C glucose using the wick method (a) or by atmospheric labeling (atmosphere) with $^{14}CO_2$ (b).

Table 3: Nitrogen (N) and ^{15}N distribution (%) and the ratio of the percentage distribution of ^{15}N and N in different plant parts of white lupine and peas after ^{15}N labeling either by the wick method or *via* roots. The lower part of the table shows *F* values from the analysis of variance; $n = 5$. (‡, $n = 4$). Different letters within harvest time show significant differences ($p < 0.05$, Tukey-Kramer test) between plant parts.

Crop, labeling method	**Harvest after day**	**Plant part**	**Distribution /** %		
			^{15}N	N	^{15}N:N
White lupine, wick method	6	roots	3.9 b	8.3 b	0.5 b
		leaves	57.1 a	78.9 a	0.7 b
		stem	39.0 a	12.8 b	3.0 a
	11	roots	‡ 7.3 b	14.6 b	‡ 0.4 b
		leaves	‡ 52.6 a	70.7 a	‡ 0.7 b
		stem	‡ 40.1 a	14.7 b	‡ 2.7 a
White lupine, atmosphere–soil	6	roots	17.1 b	10.8 b	1.6 a
		leaves	66.2 a	72.8 a	0.9 a
		stem	16.7 b	16.4 b	1.1 a
	11	roots	‡ 16.5 b	11.4 b	‡ 1.4 a
		leaves	‡ 69.6 a	76.1 a	‡ 0.9 a
		stem	‡ 14.0 b	12.4 b	‡ 1.1 a
Pea, wick method	6	roots	10.7 b	16.8 b	0.6 b
		leaves	63.5 a	68.5 a	0.9 ab
		stem	25.7 b	14.6 b	1.8 a
	11	roots	21.3 b	18.6 b	1.2 b
		leaves	48.1 a	67.1 a	0.7 b
		stem	30.6 ab	14.3 b	2.2 a
Pea, atmosphere–soil	6	roots	26.0 b	19.4 b	1.4 a
		leaves	58.8 a	69.1 a	0.9 a
		stem	15.2 b	11.5 b	1.3 a
	11	roots	13.5 b	20.9 b	0.7 a
		leaves	68.8 a	64.7 a	1.1 a
		stem	17.7 b	14.3 b	1.2 a
Analysis of variance					
Plant species			< 0.01	0.01	1.69
Method			< 0.01	0.29	5.08 *
Harvest time			< 0.01	< 0.01	0.19
Plant **p**art			265.16 ***	2184.60 ***	63.97 ***
P × Pp			4.19 *	27.62 ***	2.81
M × Pp			27.76 ***	0.26	55.14 ***
Pp × H			0.26	3.97 *	0.26
P × M × Pp			3.37 *	1.94	13.84 ***
M × H × Pp			6.72 **	1.22	4.16 *
P × M × H × Pp			2.40	3.76 *	2.29
CV			68.6	81.7	61.0

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

References

Brookes, P. C., Landman, A., Pruden, G., Jenkinson, D. S. (1985): Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method for measuring microbial biomass nitrogen in soil. *Soil Biol. Biochem.* 17, 837–842.

De Neergaard, A., Magid, J. (2001): Influence of the rhizosphere on microbial biomass and recently formed organic matter. *Eur. J. Soil Sci.* 52, 377–384.

Fustec, J., Lesuffleur, F., Mahieu, S., Cliquet, J.-B. (2010): Nitrogen rhizodeposition of legumes: A review. *Agron. Sust. Dev.* 30, 57–66.

Hertenberger, G., Wanek, W. (2004): Evaluation of methods to measure differential N-15 labelling of soil and root N pools for studies of root exudation. *Rap. Com. Mass Spec.* 18, 2415–2425.

Hill, P. W., Marshall, C., Williams, G. G., Blum, H., Harmens, H., Jones, D. L., Farrar, J. F. (2007): The fate of photosynthetically-fixed carbon in *Lolium perenne* grassland as modified by elevated CO_2 and sward management. *New Phytol.* 173, 766–777.

Janzen, H. H. (1990): Deposition of nitrogen into the rhizosphere by wheat roots. *Soil Biol. Biochem.* 22, 1155–1160.

Janzen, H. H., Bruinsma, Y. (1989): Methodology for the quantification of root and rhizosphere nitrogen dynamics by exposure of shoots to ^{15}N-labelled ammonia. *Soil Biol. Biochem.* 21, 189–196.

Joergensen, R. G., Mueller, T. (1996): The fumigation-extraction method to estimate soil microbial biomass: Calibration of the k_{EN} value. *Soil Biol. Biochem.* 28, 33–37.

Jones, D. L., Hodge, A., Kuzyakov, Y. (2004): Plant and mycorrhizal regulation of rhizodeposition. *New Phytol.* 163, 459–480.

Jones, D. L., Nguyen, C., Finlay, R. D. (2009): Carbon flow in the rhizosphere: carbon trading at the soil-root interface. *Plant Soil* 321, 5–33.

Khan, D. F., Peoples, M. B., Chalk, P. M., Herridge, D. F. (2002): Quantifying below-ground nitrogen of legumes. 2. A comparison of ^{15}N and non isotopic methods. *Plant Soil* 239, 277–289.

Kuzyakov, Y. (2002): Review: Factors affecting rhizosphere priming effects. *J. Plant Nutr. Soil Sci.* 165, 382–396.

Kuzyakov, Y., Domanski, G. (2000): Carbon input by plants into the soil. Review. *J. Plant Nutr. Soil Sci.* 163, 421–431.

Kuzyakov, Y., Schneckenberger, K. (2004): Review of estimation of plant rhizodeposition and their contribution to soil organic matter formation. *Arch. Agron. Soil Sci.* 50, 115–132.

Mahieu, S., Fustec, J., Faure, M.-L., Corre-Hellou, G., Crozat, Y. (2007): Comparison of two ^{15}N labelling methods for assessing nitrogen rhizodeposition. *Plant Soil* 295, 193–205.

Mayer, J., Buegger, F., Jensen, E. S., Schloter, M., Heß, J. (2003): Estimating N rhizodeposition of grain legumes using a ^{15}N in situ stem labelling method. *Soil Biol. Biochem.* 35, 21–28.

McNeill, A. M., Hood, R. C., Wood, M. (1994): Direct measurement of nitrogen fixation by *Trifolium repens* L. and *Alnus glutinosa* L. using $^{15}N_2$. *J. Exp. Bot.* 45, 749–755.

Mueller, T., Joergensen, R. G., Meyer, B. (1992): Estimation of soil microbial biomass C in the presence of living roots by fumigation-extraction. *Soil Biol. Biochem.* 24, 179–181.

Nguyen, C. (2003): Rhizodeposition of organic C by plants: mechanisms and controls. *Agron.* 23, 375–396.

Paterson, E. (2003): Importance of rhizodeposition in the coupling of plant and microbial productivity. *Eur. J. Soil Sci.* 54, 741–750.

Rroço, E., Mengel, K., (2000): Nitrogen losses from entire plants of spring wheat (*Triticum aestivum* L.) from tillering to maturation. *Eur. J. Agron.* 13, 101–110.

Russell, C. A., Fillery, I. R. P. (1996): In situ ^{15}N labelling of lupin below-ground biomass. *Austr. J. Agric. Res.* 47, 1035–1046.

Schenck zu Schweinsberg-Mickan, M., Joergensen, R. G., Müller, T. (2010): Fate of ^{13}C- and ^{15}N-labelled rhizodeposition of *Lolium perenne* as function of the distance to the root surface. *Soil Biol. Biochem.* 42, 910–918.

Uren, N. C. (2001): Types, amounts, and possible functions of compounds released into the rhizosphere by soil-grown plants, in Pinton, R., Varanini, Z., Nannipieri, P.: The Rhizosphere – Biogeochemistry and Organic Substances at the Soil-Plant interface. Marcel Dekker, New York.

Vance, E. D., Brookes, P. C., Jenkinson, D. S. (1987): An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* 19, 703–707.

Werth, M., Subbotina, I., Kuzyakov, Y. (2006): Three-source partitioning of CO_2 efflux from planted soil by ^{13}C natural abundance fails by inactive microbial biomass. *Soil Biol. Biochem.* 38, 2772–2781.

Wichern, F., Mayer, J., Joergensen, R. G., Müller, T. (2007a): Rhizodeposition of C and N in peas and oats after ^{13}C-^{15}N double labelling under field conditions. *Soil Biol. Biochem.* 39, 2527–2537.

Wichern, F., Mayer, J., Joergensen, R. G., Müller, T. (2007b): Release of C and N from roots of peas and oats and their availability to soil microorganisms. *Soil Biol. Biochem.* 39, 2829–2839.

Wichern, F., Eberhardt, E., Mayer, J., Joergensen, R. G., Müller, T. (2008): Nitrogen rhizodeposition in agricultural crops: Methods, estimates and future prospects. *Soil Biol. Biochem.* 40, 30–48.

Wichern, F., Mayer, J., Joergensen, R. G., Müller, T. (2010): Evaluation of the wick method for in situ ^{13}C and ^{15}N labelling of annual plants using sugar-urea mixtures. *Plant Soil* 329, 105–115.

Wu, J., Joergensen, R. G., Pommerening, B., Chaussod, R., Brookes, P. C. (1990): Measurement of soil microbial biomass-C by fumigation-extraction – an automated procedure. *Soil Biol. Biochem.* 22, 1167–1169.

Yasmin, K., Cadisch, K., Baggs, E. M. (2006): Comparing ^{15}N-labelling techniques for enriching above- and below-ground components of the plant-soil system. *Soil Biol. Biochem.* 38, 397–400.

Yasmin, K., Cadisch, K., Baggs, E. M. (2010): The significance of below-ground fractions when considering N and C partitioning within chickpea *(Cicer arietinum* L.). *Plant Soil* 327, 247–259.

Zibilske, L. M. (1994): Carbon Mineralization, in Weaver, R. W., Angle, S., Bottomley, P., Bezdicek, D., Smith, S., Tabatabai, A., Wollum, A.: Methods of Soil Analysis, Part 2, Microbiological and Biochemical Properties. Soil Science Society of America, Madison, WI, USA, pp. 835–864.

