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RESEARCH ARTICLE

Assimilate partitioning affects ^{13}C fractionation of recently assimilated carbon in maize

M. Werth · Y. Kuzyakov

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Abstract Coupling ^{13}C natural abundance and ^{14}C pulse labelling enabled us to investigate the dependence of ^{13}C fractionation on assimilate partitioning between shoots, roots, exudates, and CO_2 respired by maize roots. The amount of recently assimilated C in these four pools was controlled by three levels of nutrient supply: full nutrient supply (NS), 10 times diluted nutrient supply (DNS), and deionised water (DW). After pulse labelling of maize shoots in a $^{14}CO_2$ atmosphere, ^{14}C was traced to determine the amounts of recently assimilated C in the four pools and the $\delta^{13}C$ values of the four pools were measured. Increasing amounts of recently assimilated C in the roots (from 8% to 10% of recovered ^{14}C in NS and DNS treatments) led to a 0.3‰ ^{13}C enrichment from NS to DNS treatments. A further increase of C allocation in the roots (from 10% to 13% of recovered ^{14}C in DNS and DW treatments) resulted in an additional enrichment of the roots from DNS to DW treatments by 0.3‰. These findings support the hypothesis that ^{13}C enrichment in a pool increases with an increasing amount of C transferred into that pool. $\delta^{13}C$ of CO_2 evolved by root respiration was similar to that of the roots in DNS and DW treatments. However, if the amount of recently assimilated C in root respiration was reduced (NS treatment), the respired CO_2 became 0.7‰ ^{13}C depleted compared to roots. Increasing amounts of recently assimilated C in the CO_2 from NS via DNS to DW treatments resulted in a 1.6‰ $\delta^{13}C$ increase of root respired CO_2 from NS to DW treatments. Thus, for both pools, i.e. roots and root respiration, increasing amounts of recently assimilated C in the pool led to a $\delta^{13}C$ increase. In DW and DNS plants there was no ^{13}C fractionation between roots and exudates. However, high nutrient supply decreased the amount of recently assimilated C in exudates compared to the other two treatments and led to a 5.3‰ ^{13}C enrichment in exudates compared to roots. We conclude that ^{13}C discrimination between plant pools and within processes such as exudation and root respiration is not constant but strongly depends on the amount of C in the respective pool and on partitioning of recently assimilated C between plant pools.

Keywords $\delta^{13}C$ · Exudation · ^{14}C Pulse labelling · Recently assimilated carbon · Root respiration · ^{13}C Isotopic fractionation

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M. Werth (✉) · Y. Kuzyakov
Institute of Soil Science and Land Evaluation, University of Hohenheim, Emil-Wolff-Str. 27, 70593 Stuttgart, Germany
e-mail: werth@uni-hohenheim.de

Y. Kuzyakov
Institute of Landscape Matter Dynamics, Leibniz-Centre for Agricultural Landscape Research (ZALF), Eberswalder Str. 84, 15374 Müncheberg, Germany

Abbreviations

NS Nutrient solution
DNS 10× diluted nutrient solution
DW Deionised water

Introduction

Labelling with ^{13}C or ^{14}C isotopes can be used to balance assimilated carbon (C) in a plant–soil system. These techniques are especially important because below-ground C fluxes and turnover of root-derived C in soil cannot be fully quantified without tracers. C translocation by plants into the soil and C partitioning of rhizodeposits and rhizosphere respiration can be observed by these tracers and separated from C of native soil organic matter. Total rhizosphere respiration has been quantified by either continuous ^{14}C labelling (Barber and Martin 1976; Liljeroth et al. 1994; Whipps and Lynch 1983) or ^{14}C pulse labelling (Cheng et al. 1993; Kuzyakov 2002; Kuzyakov et al. 1999). Advantages and disadvantages of the two methods have been discussed in detail by Kuzyakov and Domanski (2000) and Kuzyakov (2001). C fluxes from the plant to the soil or to any other growth medium (such as a nutrient solution) and the CO_2 efflux from the soil can be traced after every single pulse of a repeated ^{14}C pulse labelling. Compared to continuous labelling, a series of labelling pulses produces more information about C translocations in the plant–soil system (Warembourg and Estelrich 2000).

Artificial labelling entails many methodological difficulties and is mainly limited to laboratory studies. On-site ^{14}C labelling often requires obtaining special permissions, but has been performed in some pulse labelling studies (Swinnen et al. 1994a, b). ^{13}C labelling in the field has been done by pulse labelling (Stewart and Metherell 1999) or by continuous labelling, e.g. in free air carbon dioxide enrichment (FACE) studies (Søe et al. 2004). As a reasonable alternative to artificial labelling, natural ^{13}C labelling by planting C_4 plants on a soil developed under C_3 vegetation or vice versa has been frequently used in the last 15 years (Balesdent et al. 1987; Cheng 1996; Cheng et al. 2003; Gerzabek et al. 2001; John et al. 2003; Rochette et al. 1999; Rochette and Flanagan 1997). It has been used to estimate below-ground C input, its partitioning and separation of CO_2 sources from soil. However, the results of C balance and partitioning studies obtained by natural ^{13}C labelling and FACE studies can be biased by isotopic fractionation involving for example transport of assimilates, rhizodeposition, and root respiration. In studies with C_3 leaves, there is a strong evidence that dark respiratory CO_2 was significantly enriched in ^{13}C compared to the putative substrate (Duranceau et al. 1999; Ghashghaie et al. 2003; Ghashghaie et al. 2001; Tcherkez et al. 2003). Other studies, however, have shown no difference in $\delta^{13}C$ values of total root mass and of root respiration (Amundson et al. 1998; Cerling et al. 1991) or of total rhizosphere respiration (Cheng 1996; Fu and Cheng 2002). Incubation experiments reveal that CO_2 respired by microbial decomposition of root residues was depleted in ^{13}C by 1–10‰ (Kristiansen et al. 2004; Mary et al. 1992; Šantrůčková et al. 2000).

Carbon in exudation and root respiration derives mainly from C assimilated a few hours to days ago (Craine et al. 1999; Högberg et al. 2001; Kuzyakov and Cheng 2001). Moreover, the below-ground and rhizosphere processes respond very rapidly to changes in nutrient supply and environmental conditions (Ekblad and Högberg 2001; Kuzyakov and Cheng 2001). Therefore, knowledge of ^{13}C fractionation of this recently assimilated C is especially important in studies based on natural ^{13}C labelling, which focus on fast, short-term processes such as root respiration and microbial respiration of root exudates. The balance of recently assimilated C as well as its contribution to below-ground C fluxes can only be determined by pulse labelling of plant shoots in a $^{14}CO_2$ atmosphere or in an atmosphere which is enriched or depleted with $^{13}CO_2$ compared to ambient air. In order to determine the partitioning of recently assimilated C and the ^{13}C fractionation under natural conditions, i.e. without any influences from artificial ^{13}C labelling, we combined ^{14}C pulse labelling with ^{13}C natural abundance.

Isotopic discrimination of ^{13}C between the pools in a system strongly depends on the rate of processes and partitioning of C allocated in each pool. Thus, the different extents of ^{13}C discrimination observed in previous studies (von Fischer and Tieszen 1995; Scartazza et al. 2004) can be partly linked with the C partitioning between above- and below-ground pools

as well as between different below-ground pools. The root-to-shoot ratio is strongly affected by plants' requirements for nutrient and water acquisition (Andrews et al. 2001; Farrar and Jones 2000). Therefore, various nutrients and especially N are controlling C partitioning between above- and below-ground pools (Liljeroth et al. 1990; Merckx et al. 1987). Nitrogen fertilisation is known to decrease the amount of C translocated into roots (Brown et al. 1996). We therefore used different levels of nutrient supply to control the C partitioning between above- and below-ground pools and to affect below-ground processes. This allowed us to relate the ^{13}C fractionation to the changes in pools and to recent fluxes.

The specific objectives of this study were (1) to determine the amounts of recently assimilated C in maize (*Zea mays* L.) shoots, roots, exudates, and CO_2 derived from root respiration—in dependence of nutrient supply, (2) to estimate ^{13}C fractionation of recently assimilated C, especially by root respiration and exudation, and (3) to estimate the effect of different partitioning of recently assimilated C on the ^{13}C fractionation.

For these aims, maize shoots were pulse labelled three times in a $^{14}CO_2$ atmosphere using ^{14}C as a tracer for recently assimilated C in the four pools: shoots, roots, exudates and CO_2 from root respiration. In all four pools $\delta^{13}C$ values were measured to determine fractionations between these pools. As we hypothesized that ^{13}C fractionation depends on C partitioning, three different levels of nutrient supply were introduced to change C allocation pattern.

Materials and methods

Experimental set-up

Twenty-three maize plants (cv. Tassilo) were grown in standard nutrient solution under controlled laboratory conditions, one plant per container. The maize grains were germinated on wet filter paper first to a maximum leaf length of 5 cm. After 6 days the seedlings were transferred to 250 ml polycarbonate filtration devices (SM16510/11, Sartorius, Göttingen, Germany), used here as pots for plant growth. The pots were filled with the standard nutrient solution with full supply (K_2SO_4 153.4 mg l^{-1}, KCl 7.5 mg l^{-1}, $Ca(NO_3)_2 \cdot 4H_2O$ 472.3 mg l^{-1}, $MgSO_4 \cdot 7H_2O$ 246.5 mg l^{-1}, KH_2PO_4 34.0 mg L^{-1}, KNO_3 303.3 mg l^{-1}, H_3BO_3 0.0618 mg l^{-1}, $MnCl_2 \cdot 4H_2O$ 0.099 mg l^{-1}, $CuSO_4 \cdot 5H_2O$ 0.0499 mg l^{-1}, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 0.0247 mg l^{-1}, $ZnSO_4 \cdot 7H_2O$ 0.2875 mg l^{-1}, $C_{10}H_{12}FeN_2NaO_8$ aq. 36.7 mg l^{-1}). Air was pumped through these pots from bottom to top with one membrane pump (Type 113, Rietschle Thomas, Memmingen, Germany) connected by a tube to every single pot. Nutrient solution was exchanged for fresh solution on day 11 of maize growth. On day 14 after germination, the pots with the plants were sealed with non-phytotoxic silicone rubber (TACOSIL 145, Thauer & Co., Dresden, Germany) between shoots and roots and the seal was tested for air leaks. Another tube was connected from the top outlet of the filter devices to a CO_2-trapping tube filled with 20 ml 1 M NaOH solution. The output of the trapping tube was connected to the input of the membrane pump. Thus, air containing CO_2 evolved from root respiration circulated in a closed system: air was pumped through the nutrient solution, CO_2 from root respiration was trapped in NaOH solution, and the resulting CO_2-free air coming out of these trapping tubes was again pumped through the nutrient solution. A similar system is described in detail by Kuzyakov and Siniakina (2001). This circulation prevented contamination of the air inside the system by atmospheric CO_2 having a different $\delta^{13}C$.

Two hours after light on event on day 15, three treatments were started: full nutrient solution (NS), 10 times diluted NS (DNS), and deionised water (DW). So, the full NS provided for all plants before labelling was exchanged for one of the three treatments. The high-nutrient treatment consisted of 15 replications (to yield more biomass for further experiments) and the two low-nutrient treatments consisted of four replications.

Labelling and sampling

On day 15 after germination the maize was labelled for the first time. All sealed pots with plants were placed into a Plexiglas chamber ($0.5 \times 0.5 \times 0.6$ m^3) for the labelling procedure described in detail by Cheng et al. (1993). Briefly, the chamber was connected by tubing with a flask containing 2 ml 1 mM $Na_2^{14}CO_3$ solution to which 5 ml 9 M H_2SO_4 was added to produce $^{14}CO_2$. The $\delta^{13}C$ value of air in the chamber and in the laboratory was assumed to be −8.1‰ (calculated from a $\delta^{13}C$ value of −7.8‰ in

1991 (Boutton 1996; Ehleringer 1991) and a progressive ^{13}C reduction of 0.02‰ y^{-1} (Fung et al. 1997)). The plants were labelled during 1.5 h in the atmosphere containing 5 MBq ^{14}C and a concentration of 345 ppm atmospheric CO_2 plus the amount of labelled CO_2. Usually, about 30 min of labelling time are required for C_4 plants to reach the CO_2 compensation point (Kuzyakov and Cheng 2004). A longer time period was used in our experiment to increase the ^{14}C incorporation into plant biomass. Before opening the labelling chamber, the chamber air was pumped through 1 M NaOH solution to remove unassimilated $^{14}CO_2$. Activities of unassimilated $^{14}CO_2$ and of the ^{14}C residue in the $Na_2^{14}CO_3$ source were subtracted from the total ^{14}C, which was in the flask before the start of the labelling, in order to calculate the total ^{14}C input activity. The latter was divided by the number of plants in the labelling chamber resulting in an input activity of 214.5 kBq per plant. After the labelling, the chamber was opened and the trapping of CO_2 evolved by root respiration was started.

The experiment consisted of three cycles started on days 14, 19, and 24. Each cycle included: (1) supply of the plants with full NS for recovery from DNS or DW for 1 day before labelling (CO_2 and exudates were collected during this period before second and third labelling), (2) ^{14}C labelling for 1.5 h, and (3) trapping of CO_2 in NaOH and of exudates released into NS, DNS, or DW for 4 days. Due to nutrient solution uptake, the plants were provided with 300 ml instead of 250 ml solution before the second and the third labelling. Plants were harvested on day 29 (after three cycles), divided into shoots and roots, dried at 40°C, and ground in a ball-mill. Immediately after sampling of root exudates accumulated in the nutrient solutions, Micropur (Katadyn, Wallisellen, Switzerland) containing Ag^+ ions was added to the flask to suppress microbial decomposition of exudates (Gransee and Wittenmayer 2000; Kuzyakov and Siniakina 2001) and the samples were stored at 4°C before analysis.

Sample analyses

Total dissolved C released as exudates into NS was measured by a Dimatoc-100 TOC/TIC analyser (Dimatec, Essen, Germany). C in shoots and roots was measured by a Euro EA C/N analyser (EuroVector, Milan, Italy). CO_2 trapped in NaOH solution during the sampling was precipitated with 0.5 M $BaCl_2$ solution and then the NaOH was titrated with 0.2 M HCl against phenolphthalein indicator (Zibilske 1994).

The ^{14}C activity of $^{14}CO_2$ trapped in NaOH solution or of exudates in nutrient solution was measured in 2 ml aliquots added to 4 ml scintillation cocktail Rotiszint Eco Plus (Carl Roth, Karlsruhe, Germany) after decay of chemiluminescence (for NaOH). ^{14}C activity was measured using a Wallac 1411 Liquid Scintillation Counter (Wallac Oy, Turku, Finland). The ^{14}C counting efficiency was about 87% and the ^{14}C activity measurement error did not exceed 2%. The absolute ^{14}C activity was standardised by addition of NaOH solution as quencher to the scintillation cocktail and using the spectrum of an external standard (SQP(E) method). ^{14}C in solid samples (dried shoots and roots) was measured after combustion of 200 mg of sample within an oxidiser unit (Model 307, Canberra Packard Ltd., Meriden, USA), absorption of the ^{14}C in Carbo-Sorb E (Perkin Elmer, Inc., Boston, USA), and addition of the scintillation cocktail Permafluor E^+ (Perkin Elmer, Inc.).

For $\delta^{13}C$, 1 mg of ground maize shoots or roots was weighed out into tin capsules and analysed on a Thermo Finnigan MAT DELTA^{plus} Advantage isotope ratio mass spectrometer (IRMS from Thermo Electron Corporation, Waltham, USA) coupled to the Euro EA C/N analyser. For $\delta^{13}C$ analysis of CO_2 trapped in NaOH, an excess of 0.5 M $BaCl_2$ solution was added to the NaOH trapping solution to form a precipitate of $BaCO_3$. The $BaCO_3$ precipitate was carefully washed ten times with deionised water until pH of 7 was achieved. Washed $BaCO_3$ was dried at 60°C and about 0.8 mg of dried $BaCO_3$ were weighed out into tin capsules for $\delta^{13}C$ analysis on IRMS. To prepare exudates for IRMS analyses, 60 ml of nutrient solution containing exudates was dried at 60°C in a Petri dish for each sample. The dry residue was scratched out of the dish with a steel spatula and weighed out into tin capsules resulting in 25 μg exudates C per sample for IRMS analyses.

Statistical analyses

The experiment was conducted with four replicates for the low-nutrient treatments and 15 replicates for the high-nutrient treatment. All replicates were

analysed on ^{14}C activity, C- and N-contents in shoots, roots, exudates, and CO_2. Only a choice of samples from each pool was analysed on $\delta^{13}C$ values (six, three, and four samples for NS, DNS, and DW treatments, respectively). ^{14}C data are presented as percentage of ^{14}C recovered in shoots, roots, exudates, and CO_2 after 29 days at the end of the experiment. So, the ^{14}C percentages are related to the ^{14}C activity recovered after three labelling pulses. Standard deviation (SD) was calculated as a variability parameter.

Significance of differences between treatments was analysed for each sampling by one-way ANOVA. We have calculated the least significant difference (LSD 0.05) in a post hoc Newman–Keuls test to identify differing treatments. Linear regression were calculated between plant parts' $\delta^{13}C$ values and biomass and between the $\delta^{13}C$ values of various plant parts. To relate $\delta^{13}C$ values to recently assimilated C, linear regressions between ^{14}C (representing recently assimilated C) and $\delta^{13}C$ values were calculated. In the first step, the dependence between $\delta^{13}C$ and ^{14}C within each treatment for each parameter was calculated. Since no significant regressions were found within the treatments, we accepted that the variations of $\delta^{13}C$ and ^{14}C within each treatment were randomly distributed and the means and standard deviations were calculated for each treatment. In the second step, we calculated regressions between means of $\delta^{13}C$ values (dependent variable) and means of ^{14}C (independent variable).

Results

Total carbon in different plant pools

The shoot dry matter of DNS and DW treatments was the same (Table 1). The value in the full supply NS treatment was up to 1.8 g significantly higher than in the other two treatments ($P<0.001$). There were also significant differences in the root mass between NS and DNS or DW treatments ($P<0.01$), whereas root mass in the NS treatment was up to 0.3 g higher. The root dry matter of DNS and DW treatments was the same. No significant differences between the three treatments were found for the C content in shoots (391 mg g^{-1} on average). The C content in roots of DNS was 405 mg g^{-1} and was, at about 46 mg g^{-1}, significantly higher ($P<0.05$) than that of full NS (Table 1). No significant difference was found between shoots and roots of any treatment, this means that the shoots and roots had the same C contents within each treatment.

Contrasting to the total C content in shoots, there was a significantly lower total nitrogen (N) content of 21 mg g^{-1} in DNS compared to 29 mg g^{-1} in full NS treatment ($P<0.01$). Consequently, the C/N ratio increased with decreasing nutrient supply from 13 for NS to 19 for DNS ($P<0.01$). The same effect of decreasing N content and increasing C/N with decreasing nutrient supply was recorded for the roots ($P<0.05$ for N; $P<0.001$ for C/N). The N and C/N of shoots and of roots also differed significantly between NS and DW treatments, but not between DNS and DW treatments. In all three treatments, the N content in the shoots was up to 9 mg g^{-1} higher than that of the roots. Considering the equal C contents in shoots and roots, the C/N ratios of shoots and roots were larger in DW than in NS. Thus, changing the nutrient supply affected the allocation of total N, but not of total C, to shoots and roots.

The cumulative C exudation and the cumulative CO_2 efflux were calculated from the first labelling to the end of the experiment. The C exudation in full NS was significantly higher ($P<0.001$) for all sampling dates compared to the other two treatments (Fig. 1a). The cumulative amount of exuded C in full NS reached 111 ± 15 mg C per plant in 14 days, which was about four times higher than in the nutrient-deficient treatments. No significant differences were found in the amount of exuded C between DNS and DW treatments (Fig. 1a).

The cumulative CO_2 efflux from NS treatment was 205 ± 45 mg C after 14 days and was always higher compared to the other two treatments, which was significant on sampling days 24, 25, and 29 ($P<0.05$). No significant differences were found in the cumulative CO_2 efflux between treatments on sampling days 19 and 20 (Fig. 1b).

Recently assimilated carbon (^{14}C)

We labelled plants in a $^{14}CO_2$ atmosphere to estimate the contribution of recently assimilated C to root respiration and exudation, and we changed the nutrient supply to vary the partitioning of recently assimilated C. The total ^{14}C recovery from shoots,

Table 1 Dry matter, total C content, total N content, and C/N-ratio of 29-day-old maize (means ± SD)

	Nutrient solution	0.1 × nutrient solution	Deionised H_2O	*P*	LSD (α=0.05)
Dry matter (g)					
Shoots	3.3 ± 0.4	1.8 ± 0.3	1.5 ± 0.3	***	0.4
Roots	0.9 ± 0.2	0.7 ± 0.1	0.6 ± 0.1	**	0.2
Total C (mg g^{-1})					
Shoots	364.6 ± 28.9	406.1 ± 17.3	402.7 ± 26.5	ns	41.3
Roots	358.5 ± 24.0	405.3 ± 11.4	390.1 ± 23.0	*	34.2
Total N (mg g^{-1})					
Shoots	29.4 ± 4.7	21.3 ± 1.9	17.9 ± 4.9	**	6.8
Roots	20.3 ± 2.2	16.1 ± 0.5	16.9 ± 1.9	*	3.0
C/N					
Shoots	12.7 ± 2.4	19.1 ± 0.9	23.6 ± 5.8	**	5.7
Roots	17.8 ± 1.8	25.1 ± 1.4	23.2 ± 2.4	***	3.0

*P<0.05, **P<0.01, ***P<0.001, ns: not significant

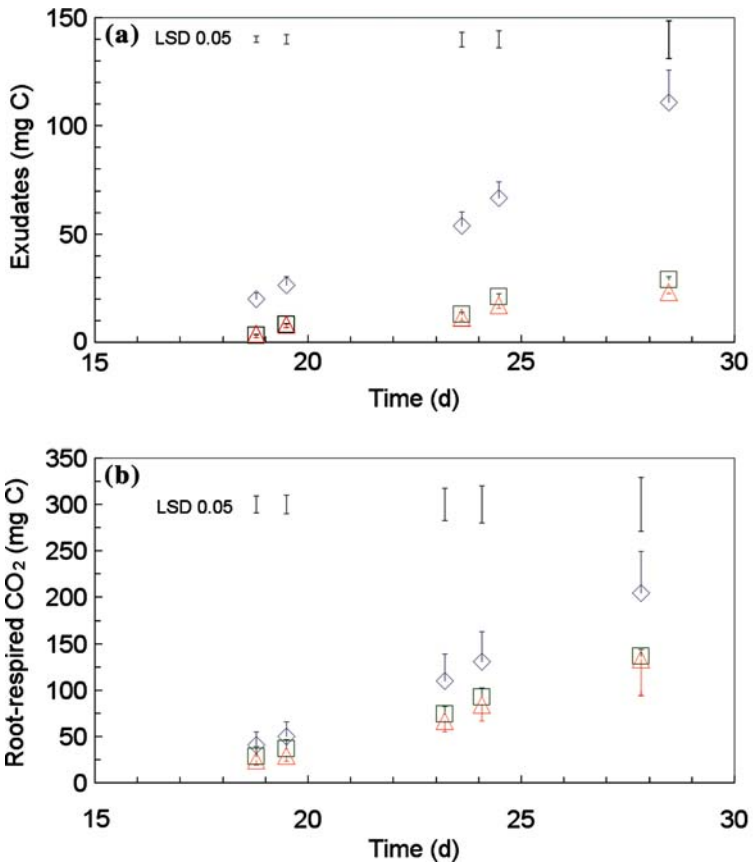

Fig. 1 Cumulative exudation **a** and cumulative root respiration **b** from maize between days 15 and 29 depending on growth media: ◇ full nutrient supply (NS), □ 10 × diluted nutrient supply (DNS), and Δ deionised water (DW). Standard deviation shown to one side of the symbol only, least significant difference (LSD, α=0.05) shown for each sampling date in bars on top. Note different range of *y*-scale

roots, exudates, and CO_2 from root respiration in relation to an input of 214.5 kBq per plant was not significantly different between the three treatments. We found 44–53% ^{14}C of the input per plant in all pools, i.e. the maize plants respired 47–56% of the ^{14}C input via the shoots during 14 days after the first labelling. The distribution of recently assimilated C of three-times labelled 29-day-old maize was: 76%, 73%, and 69% of recovered ^{14}C in the shoots and 8%, 10%, and 13% of recovered ^{14}C in the roots for NS, DNS, and DW treatments, respectively (Table 2). The amount of recovered ^{14}C

Table 2 Distribution of ^{14}C recovered in maize shoots, roots, exudates, and CO_2 from root respiration after three pulses of ^{14}C labelling in relation to total ^{14}C recovery per pot and sum of ^{14}C activity in all four pools in relation to ^{14}C input per pot (means ± SD)

	Nutrient solution	0.1 × nutrient solution	Deionised H_2O	*P*	LSD (α=0.05)
^{14}C (% of ^{14}C recovery)					
Shoots	76.1 ± 5.8	73.0 ± 6.0	69.4 ± 3.9	ns	9.0
Roots	8.3 ± 2.5	10.1 ± 1.3	12.5 ± 1.9	*	3.8
Exudates	0.4 ± 0.1	0.5 ± 0.1	0.8 ± 0.2	**	0.2
Root-derived CO_2	15.1 ± 4.6	16.4 ± 4.6	16.9 ± 3.1	ns	7.2
^{14}C (% of ^{14}C input)					
Sum of pools	52.8 ± 12.2	43.7 ± 0.2	45.8 ± 6.7	ns	17.4

*$P<0.05$, **$P<0.01$, ns: not significant

allocated in maize shoots was not significantly different in the three treatments. The allocation of ^{14}C in the roots was similar for DNS and DW and for DNS and NS treatments, whereas the incorporation of ^{14}C into roots of the NS treatment was significantly lower than in the DW treatment ($P<0.05$). Only 0.4%, 0.5%, and 0.8% of recovered ^{14}C were allocated to exudates, whereas 15%, 16%, and 17% of recovered ^{14}C were used for root respiration and were trapped as CO_2 for NS, DNS, and DW treatments, respectively. Recovery of ^{14}C in exudates was the same for NS and DNS treatments. There was no significant difference of ^{14}C in CO_2 of the three treatments.

^{13}C discrimination depending on dry mass in shoots and roots and on $\delta^{13}C$ in the source compartment

Analysis of variance showed that the $\delta^{13}C$ values of shoots were the same for DNS and DW treatments (−15.2‰; Table 3). These ^{13}C enriched values (by 0.2‰ compared to NS) were also observed for the whole plant. Shoots and roots $\delta^{13}C$ values of the full NS treatment were significantly lower ($P<0.05$; $P<0.01$; respectively) than those of the other two treatments. In all three treatments the $\delta^{13}C$ value of the roots was higher than that of the shoots ($P<0.01$ in the NS treatment). The difference of $\delta^{13}C$ values between shoots and roots increased from 0.3‰ to 0.6‰ with decreasing nutrient concentration. Nutrient limitation led to smaller shoot and root dry mass, yielding higher $\delta^{13}C$ values in shoots ($P<0.05$) and roots ($P<0.05$), i.e. less ^{13}C discrimination (Fig. 2, Table 4). These increasing $\delta^{13}C$ values in the roots were significantly related to increasing $\delta^{13}C$ values in the shoots (Fig. 3a, Table 4).

The $\delta^{13}C$ value of exudates in the NS treatment collected from days 15 to 19 was at −7.9‰, significantly higher than the values of DNS and DW treatments ($P<0.001$). Higher $\delta^{13}C$ values of the NS versus the other two treatments were also found for sampling days 24 ($P<0.05$) and 29 ($P<0.01$). Data for sampling days 20 and 25 are not shown, because all treatments provided full nutrient supply one day before that sampling. Differences for these two sampling dates were not significant.

Comparing the mean $\delta^{13}C$ values of exudates from sampling days 19, 24, and 29 with the $\delta^{13}C$ values of maize roots, significant differences were found only in the NS treatment ($P<0.001$). The $\delta^{13}C$ value of exudates was 5.3‰ higher compared to maize roots (−15.1‰). For the DW and DNS treatments, the $\delta^{13}C$ values of exudates were 2.1–1.9‰ lower compared to −14.5 and −14.8‰ of maize roots, but this was not significant. The relationship between $\delta^{13}C$ in exudates and roots had a significant R^2 of 0.51 (Fig. 3b, Table 4).

Significant differences were found between $\delta^{13}C$ of CO_2 evolved in NS and in DNS or DW treatments ($P<0.05$ to $P<0.001$) on all sampling days. The $\delta^{13}C$ value of CO_2 respired by roots grown in NS was 0.7‰ lower ($P<0.01$) than the root value (−15.1‰). This significant difference was recorded only for full nutrient supply. Increasing $\delta^{13}C$ values in CO_2 from root respiration were very highly significantly related to increasing $\delta^{13}C$ values in the roots (Fig. 3c, Table 4).

^{13}C discrimination of recently assimilated C

Despite a very high R^2, the linear regression between recently assimilated C (^{14}C) allocated to shoots (Table 2) and their $\delta^{13}C$ values (Table 3) was not significant (Table 4). For the roots a significant

Table 3 $\delta^{13}C$ values of shoots, roots, exudates, and CO_2 from root respiration from maize grown for 14 days in three different types of nutrient solution (means ± SD)

	$\delta^{13}C$ (‰)			*P*	LSD (α=0.05)
	Nutrient solution	0.1 × nutrient solution	Deionised H_2O		
Maize (29 days old)					
Plants	−15.3 ± 0.1	−15.1 ± 0.0	−15.0 ± 0.1	***	0.1
Shoots	−15.4 ± 0.1	−15.2 ± 0.1	−15.2 ± 0.0	*	0.2
Roots	−15.1 ± 0.1	−14.8 ± 0.1	−14.5 ± 0.3	**	0.3
Exudates on days					
19	−7.9 ± 1.1	−17.0 ± 0.4	−16.3 ± 0.7	***	1.6
24	-9.9 ± 2.6	−15.8 ± 0.3	−16.2 ± 0.2	*	3.7
29	−11.6 ± 1.4	−17.3 ± 0.8	−17.5 ± 1.0	**	2.8
Mean	−9.8 ± 1.9	−16.7 ± 0.8	−16.7 ± 0.8	***	2.5
CO_2 on days					
19	−15.9 ± 0.6	−14.8 ± 0.9	−13.8 ± 0.3	***	1.0
24	−16.1 ± 0.5	−14.4 ± 0.1	−14.2 ± 0.3	***	0.6
29	−15.4 ± 0.2	−14.6 ± 0.6	−14.7 ± 0.5	*	0.8
Mean	−15.8 ± 0.3	−14.6 ± 0.2	−14.2 ± 0.5	**	0.7
Differences for					
Roots − shoots	0.3 ± 0.1**	0.3 ± 0.1^{ns}	0.6 ± 0.2^{ns}		
Roots − exudates					
19	−7.2 ± 0.9***	2.2 ± 0.3^{ns}	1.7 ± 0.6^{ns}		
24	−5.2 ± 2.1**	1.0 ± 0.3^{ns}	1.7 ± 0.3^{ns}		
29	−3.5 ± 1.1***	2.4 ± 0.7^{ns}	3.0 ± 0.9^{ns}		
Mean	−5.3 ± 1.6***	1.9 ± 0.7^{ns}	2.1 ± 0.7^{ns}		
Roots − CO_2					
19	0.8 ± 0.5**	0.0 ± 0.7^{ns}	-0.7 ± 0.3^{ns}		
24	1.0 ± 0.4***	-0.5 ± 0.1^{ns}	-0.3 ± 0.3^{ns}		
29	0.3 ± 0.2**	-0.2 ± 0.5^{ns}	0.2 ± 0.5^{ns}		
Mean	0.7 ± 0.3**	-0.2 ± 0.2^{ns}	-0.3 ± 0.5^{ns}		

Values for exudates and CO_2 are shown for three of five sampling times; values for shoots and roots are shown for 29-day-old maize
*P<0.05, **P<0.01, ***P<0.001, ns: not significant

relationship was comprised of decreasing nutrient supply, increasing amounts of recently assimilated C, and increasing $\delta^{13}C$ values (Tables 2, 3, 4). Regression parameters of exudates $\delta^{13}C$ on recently assimilated C were not significant (Table 4); thus, the former (Table 3) was not linearly related with the latter (Table 2). Similarly to the roots, a significant relationship was found for CO_2 from root respiration (Table 4), i.e. with decreasing nutrient supply, the $\delta^{13}C$ value increased with increasing amounts of recently assimilated C (^{14}C) (Tables 2, 3).

Discussion

^{13}C fractionation of recently assimilated C in below-ground fluxes

Recently assimilated C by maize was traced by ^{14}C labelling to quantify its balance in dependence on differently concentrated nutrient solutions. With decreasing nutrient supply, ^{14}C decreased in the shoots and increased in the roots (Table 2). This confirms the frequently reported increase of C allocation into the roots at decreasing nutrient availability (Kuzyakov et al. 2002; Liljeroth et al. 1990; Merckx et al. 1987; Paponov and Engels 2005) and supports the functional equilibrium hypothesis of C allocation to the roots (Farrar and Jones 2000). A lower translocation of recently assimilated C into the roots at increased N fertilisation (Table 2) was overcompensated by the higher total plant matter production of fertilised plants in our experiment (Table 1, compare Kuzyakov et al. (2002)). Differences between the treatments in ^{14}C of exudates and of CO_2 were significant only for the exudates, but ^{14}C in root respiration tended to increase with decreasing nutrient supply (Table 2).

An allocation shift of recently assimilated C related to nutrient supply allowed us to investigate

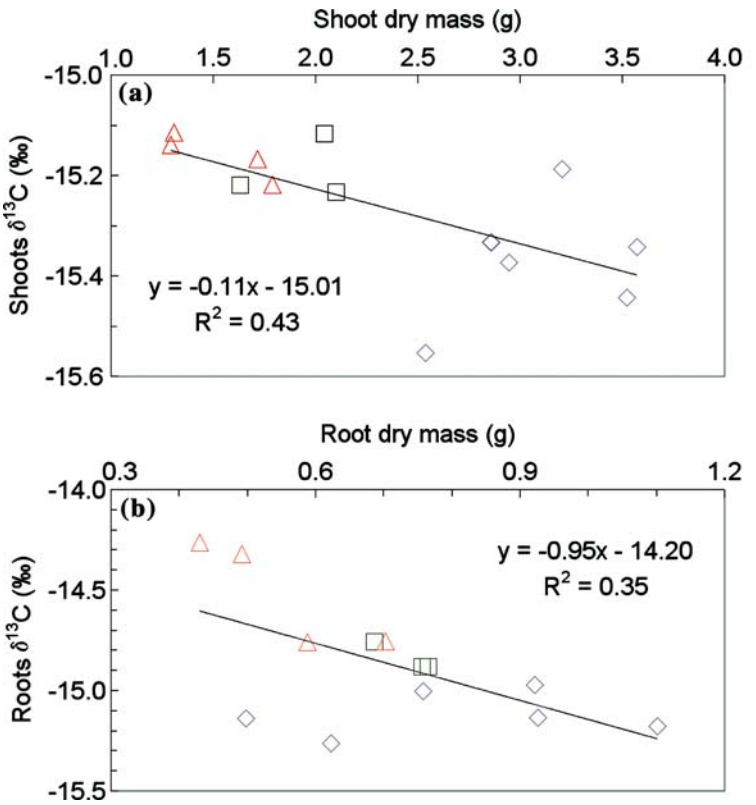

Fig. 2 $\delta^{13}C$ values of **a** shoots, and **b** roots versus dry mass of total maize plant parts (n=13). Nutrient concentrations are: ◇ full nutrient supply (NS), □ 10× diluted nutrient supply (DNS), and Δ deionised water (DW). Note different range of x- and y-scales

the dependence of ^{13}C isotopic fractionation on amounts of such C allocated to various plant parts or C fluxes. The increase in shoot $\delta^{13}C$ values from nutrient-rich to nutrient-poor conditions followed the slight decrease in recently assimilated C in the shoots (Tables 2, 3). This increased translocation of photosynthate from shoots to roots under N stress may have altered $\delta^{13}C$ in shoots, independent of discrimination during carbon fixation (Brown et al. 1996). Although the regression of $\delta^{13}C$ versus ^{14}C in the shoots had a high R^2 of 0.82, none of the regression parameters was significant (Table 4). This absence of significant differences is connected with the fact that about 50% of the shoot mass was already present before the treatments with the three levels of nutrient supply were started. Another explanation lies in the use of only three points for the regression calculation (see Tables 2, 3), because the number of point pairs is crucial for the significance level. Finally, a non-linearity of the relationship is possible.

We found a strong relationship between ^{14}C and $\delta^{13}C$ of maize roots: the higher the amount of recently assimilated C translocated to the roots, the higher the root $\delta^{13}C$ value (Tables 2, 3). Since $\delta^{13}C$ values of roots and of CO_2 from root respiration were significantly different only for the NS treatment (Table 3), fractionation due to respiration cannot be considered to be the main process. The coefficient of determination allows us to conclude that the variance of root $\delta^{13}C$ can be entirely explained by the variance of recently assimilated ^{14}C.

For the exudates, the regression between $\delta^{13}C$ and ^{14}C was not significant, i.e. we found no linear relationship between the amount of recently assimilated C and ^{13}C discrimination in exudates (Tables 2, 3, 4). More data points are needed to identify a significant relationship, which might be a non-linear one. The significant relationship for CO_2 from root respiration can be described as follows: the more recently assimilated C was respired by the roots with decreasing nutrient supply, the more enriched in ^{13}C was the CO_2 (Tables 2, 3, 4). This supports the following hypothesis: the more C is translocated to a certain pool, the more enriched is that pool in ^{13}C.

Table 4 Parameters of linear regressions of $\delta^{13}C$ on plant dry mass (see Fig. 2); $\delta^{13}C$ in roots on shoots, exudates, and CO_2 from root respiration (see Fig. 3); and $\delta^{13}C$ on recently assimilated C (measured as ^{14}C) of maize shoots, roots, exudates and CO_2 from root respiration for means of NS, DNS, and DW treatments (see Tables 2, 3)

Regression variables	Intercept	Slope	R^2
$\delta^{13}C$–dry mass			
Shoots	−15.01***	−0.11*	0.43*
Roots	−14.20***	−0.95*	0.35*
$\delta^{13}C$–$\delta^{13}C$			
Roots–shoots	8.14 ^{ns}	1.51*	0.44*
Exudates–roots	−138.26**	−8.37*	0.51*
CO_2–roots	21.52**	2.46***	0.81***
$\delta^{13}C$–^{14}C			
Shoots	−12.98 ^{ns}	−0.03 ^{ns}	0.82 ^{ns}
Roots	−16.28**	0.14*	0.99*
Exudates	−7.55 ^{ns}	−12.61 ^{ns}	0.35 ^{ns}
CO_2	−29.21*	0.89*	0.99*

*$P<0.05$, **$P<0.01$, ***$P<0.001$, ^{ns}: not significant

Comparisons of $\delta^{13}C$ values of plant pools and recently assimilated C (represented by ^{14}C) were only partly correct. The ^{14}C labelled CO_2 was assimilated by the shoots and was allocated to different plant pools. Thus, ^{14}C in fact represented recently assimilated carbon. However, the $\delta^{13}C$ values of different plant pools (especially of the shoots and roots) were build not only by recently assimilated C, but also by carbon assimilated before the ^{14}C labelling. Consequently, the $\delta^{13}C$ value of recently assimilated C contributes to the pools' $\delta^{13}C$ values, but does not represent them completely. Comparisons of allocation of recently assimilated C (measured as ^{14}C) into the main photosynthetic products like sucrose or starch and their $\delta^{13}C$ values could provide more specific and precise information to ^{13}C fractionation. Such studies presume ^{13}C compound specific analyses (Hobbie and Werner 2004; Nogués et al. 2004), which were not available in our laboratory.

Dependence of ^{13}C fractionation on biomass and C pools

A relationship between shoot $\delta^{13}C$ values and shoot biomass has been frequently reported for wheat (Araus et al. 1998; Condon et al. 1987; Fischer et al. 1998; Monneveux et al. 2005). In our experiment with maize, we have found the same significant relationship (Fig. 2a, Table 4). The maize of our DNS and DW treatments produced less phytomass and showed higher shoot $\delta^{13}C$ values compared to the full-supply NS treatment. Low nutrient concentrations, especially N, generally result in a reduced water use efficiency (Caviglia and Sadras 2001; Raven et al., 2004; Stout and Schnabel 1997), which is linked to an increased stomatal conductance or a decreased net carbon assimilation. However, nitrogen deficiency reduces stomatal conductance to some extent (Jacob et al. 1990). Thus, a decrease of net carbon assimilation must be the reason for a low water use efficiency. The carbon assimilation between the three treatments was not significantly different, but a trend of decreased assimilation by low nutrient supply could be seen. This decrease of assimilation in the nutrient-poor treatments would have yielded a lower water use efficiency, a higher ratio of intercellular space to atmospheric partial pressure of CO_2 (p_i/p_a-ratio) and a higher ^{13}C isotope discrimination by photosynthesis compared to nutrient-rich treatments. This increased discrimination, i.e. more negative $\delta^{13}C$ values, could not be found in our nutrient-poor treatments (Table 3). The p_i/p_a-ratio was not measured in our experiment to check the internal CO_2 concentration. Nor could we calculate the p_i/p_a-ratio from shoot $\delta^{13}C$ values since there is another unknown in C_4 plant ^{13}C discrimination: ϕ, i.e. the proportion of carbon fixed by PEP carboxylase that subsequently leaks out of the bundle sheath (Farquhar 1983; Farquhar et al. 1989). Farquhar (1983) presented increased $\delta^{13}C$ values in C_4 plant shoots for a $\phi<0.4$. Thus, a small value ϕ representing the leakage could compensate an increased p_i/p_a-ratio caused by nutrient deficiency and consequently lead to more positive $\delta^{13}C$ values in the

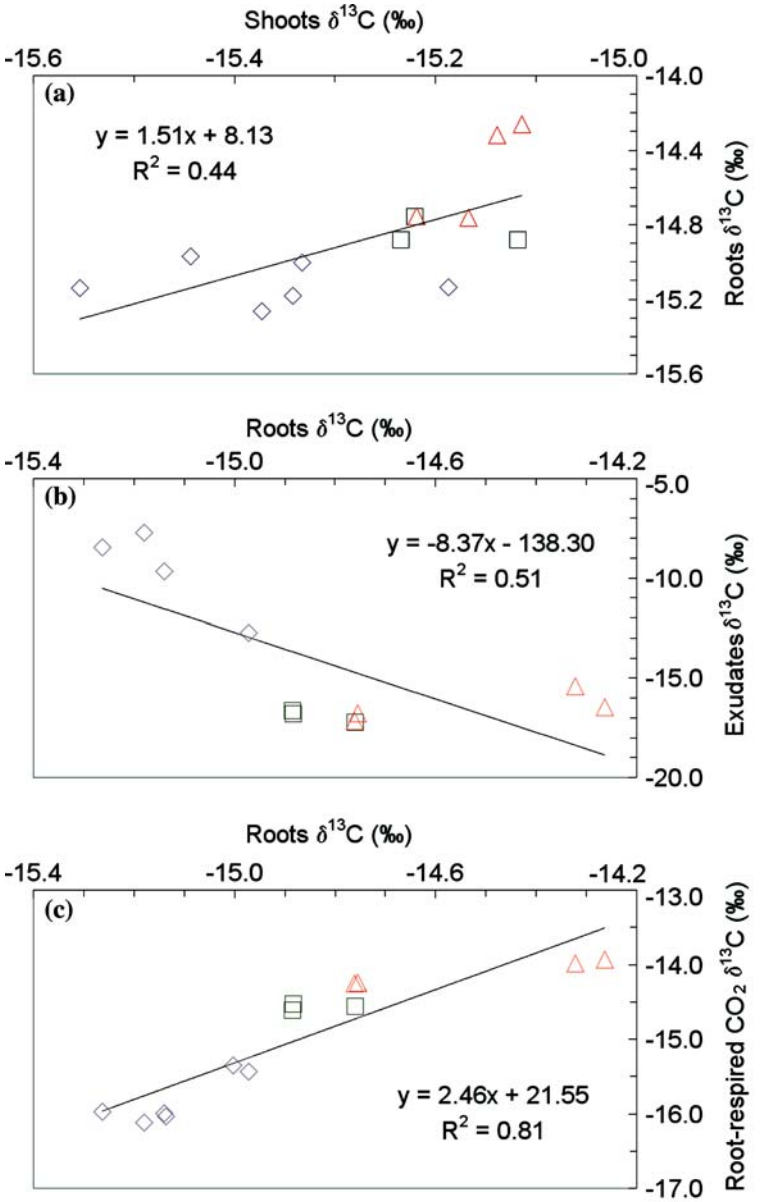

Fig. 3 $\delta^{13}C$ values of **a** roots versus shoots (n=13), **b** exudates versus roots (n=11), and **c** root-respired CO_2 versus roots of maize (n=13). Nutrient concentrations are: ◇ full nutrient supply (NS), □ 10× diluted nutrient supply (DNS), and Δ deionised water (DW). Note different range of x- and y-scales

shoots of nutrient-poor treatments. In order to determine the effect of p_i/p_a or ϕ on ^{13}C discrimination in dependence of nutrient supply, p_i/p_a should be measured in future observations. Furthermore, the photosynthetic carbon isotope discrimination in leaves towards air is needed to use the equation to calculate p_i/p_a or ϕ. Our results represent only the $\delta^{13}C$ values of shoots referred to the PDB standard. Since we did not measure $\delta^{13}C$ of source air, we cannot calculate the discrimination in shoots referred to air CO_2. The $\delta^{13}C$ of source air should be determined in future observations to determine the photosynthetic carbon isotope discrimination.

Besides water use efficiency and leakage, Rubisco activity can also be influenced by altering nutrient supply. Ehleringer and Osmond (1989) reported a decrease in Rubisco activity at low leaf N concentrations. This decrease in Rubisco activity should lead to an increased CO_2 concentration in the bundle sheath cells that might induce increased CO_2 losses

from this compartment by leaking. According to the equation for C_4 plants by Farquhar (1983), increased leakage in nutrient-poor treatments of our experiment causes amplified ^{13}C discrimination in the shoots. However, we observed a minor ^{13}C discrimination in the shoots of nutrient-poor treatments than in the full NS treatment (Table 3). Hence, low Rubisco activity and increased leakage cannot be the cause of the ^{13}C enriched shoots of nutrient-poor treatments compared to the full NS treatment. Furthermore, a reduced Rubisco activity in the nutrient-poor treatments is very unlikely since our plants were grown in the same nutrient solution up to day 15 of the experiment and received full nutrient supply one day before ^{14}C labelling. Thus, a reduced Rubisco activity and an increased CO_2 concentration in the bundle sheath cells cannot be assumed for our nutrient-poor treatments compared to the NS treatment.

Dependent on the C allocation pattern, isotopic fractionation of ^{13}C occurred between shoots and roots of maize; this was more pronounced for higher C allocation to roots versus shoots (Table 3). Roots were significantly enriched in ^{13}C compared to shoots, as reported in several other studies (Andreux et al. 1990; Badeck et al. 2005; Boutton 1996; von Fischer and Tieszen 1995; Hobbie et al. 2002; Tieszen and Boutton 1989). Such ^{13}C enrichments in the roots could arise by two plausible post-photosynthetic fractionation mechanisms (Badeck et al. 2005; Hobbie et al. 2004): (1) the formation of ^{13}C enriched carbohydrates, the primary transfer compounds in plants, or (2) discrimination against ^{13}C during root respiration. Hobbie and Werner (2004) explained the former mechanism by the synthesis of ^{13}C depleted compounds in the leaves like lipids and lignin leading to ^{13}C enriched unreacted mobile sugars. These ^{13}C enriched sugars are then transported to the roots, where again lipids and lignin are synthesized from these sugars. In this second fractionation lipids and lignin are now more enriched then in the leaves due to the transport of the ^{13}C enriched substrate to the roots in contrast to the original substrate in the leaves.

In the NS treatment, exudates were between 4‰ and 7‰ enriched in ^{13}C compared to roots (Table 3). This enrichment could be due to a fractionation of recently assimilated C between roots and exudates. Hobbie et al. (2004) found a 2‰ isotopic enrichment of ^{13}C in newly incorporated soil carbon relative to Douglas-fir (*Pseudotsuga mensiezii* (Mirbel) Franco) root carbon. Cernusak et al. (2005) reported an enrichment range of −0.3 to +4.6‰ in phloem sap sugars relative to Tasmanian bluegum (*Eucalyptus globulus* Labill.) leaf dry matter. In a similar study on *Triticum aestivum* L. Yoneyama et al. (1997) found an enrichment range of 1.4–1.6‰ in phloem relative to leaf blades. Since exudates and phloem sap sugars both consist of recently assimilated C, the latter two studies can also be compared to our one. Those three studies and our study show that ^{13}C fractionation between roots and exudates occurs in a wide range for C_3 and C_4 plants and has to be further investigated for different species and nutrient supply. The extend of ^{13}C fractionation between roots and exudates could depend on the type of source compound used to produce exudates. Fractionation could depend for example on the type of sucrose as one of the main exudation components. Tcherkez et al. (2004) gave some evidence that sucrose produced in the night from starch breakdown is isotopically heavier than sucrose produced in the light from triose-phosphate.

Considering the $\delta^{13}C$ of CO_2 in the NS treatment, we found a significant depletion of about 0.7‰ compared to the roots (Table 3). Badeck et al. (2005) found a preferred release of the lighter isotope relative to roots of kidney bean (*Phaseolus vulgaris* L.), leading to a 1.3‰ depletion of CO_2 in root respiration. Similar results were found in a study on common sunflower (*Helianthus annuus* L.), perennial ryegrass (*Lolium perenne* L.), and alfalfa (*Medicago sativa* L.), where the respiratory CO_2 of whole roots was depleted by 0.5–5.4‰ relative to root tissues (Klumpp et al. 2005). While the NS treatment showed a significant fractionation, the two nutrient-poor treatments showed no significant fractionation between roots and CO_2 from root respiration (Table 3). This means that earlier results pointing to the absence of ^{13}C fractionation by root respiration (Amundson et al. 1998; Cerling et al. 1991; Cheng 1996; Fu and Cheng 2002) must be interpreted with caution and related to the C allocation pattern. ^{13}C fractionation during root respiration could also be a cause of roots' ^{13}C enrichment in contrast to the shoots as suggested by Badeck et al. (2005).

Conclusions

A separate evaluation of the effects of C pools and of recent C fluxes on the fractionation of ^{13}C in shoots,

roots, exudates and root respiration was possible by a unique coupling of ^{14}C tracing after pulse labelling with measurements of $\delta^{13}C$ values. Roots and root-respired CO_2 were less depleted in ^{13}C when increasing amounts of recently assimilated C were translocated to these pools. This relationship shows that the allocation of recently assimilated C, which depends on nutrient supply and changing environmental conditions, may be an important factor controlling $\delta^{13}C$ fractionation within the plant–soil system. Therefore, the allocation pattern of assimilated C within the plant should be considered for corrections of ^{13}C fractionation in studies based on ^{13}C natural abundance.

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