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Below-ground partitioning (^{14}C) and isotopic fractionation ($\delta^{13}C$) of carbon recently assimilated by maize

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Partitioning of carbon recently assimilated by maize between shoots, roots, exudates, and CO_2 from root respiration depending on three different levels of nutrient supply (full nutrient solution (NS), 10 times diluted NS, or deionised water) was estimated by ^{14}C pulse labelling. A ^{13}C fractionation in these compartments was investigated in relation to the nutrient supply.
With decreasing nutrient supply, ^{14}C allocation to the shoots and to the roots decreased from 76 % to 69 % and increased from 8 % to 13 % of ^{14}C recovery, respectively. Average percentage of ^{14}C in exudates and root-respired CO_2 was 0.5 % and 16 % of ^{14}C recovery, respectively. The concentration of the NS was not crucial for the amount of recently assimilated C recovered in exudates and CO_2, but for the amounts in shoots and roots.
For all three nutrient levels, roots were enriched in ^{13}C when compared with shoots and ^{13}C fractionation increased with decreasing nutrient supply up to 0.7 ‰. Further ^{13}C discrimination by exudation led to more ^{13}C in exudates when compared with the roots of full nutrient supply and less ^{13}C in exudates when compared with the roots grown in diluted NS and in deionised water. There were only small differences of <1.0 ‰ in $\delta^{13}C$ values between roots and CO_2 from root respiration. A ^{13}C fractionation of recently assimilated C occurred between roots and exudates but was negligible for the CO_2 respired by roots.

Keywords: Carbon-13; Carbon-14; Carbon assimilation; Maize

1. Introduction

Carbon dioxide (CO_2) efflux from soils is one of the most important fluxes of the global carbon (C) cycle. CO_2 efflux measurements from soil are commonly used to investigate short-term soil organic matter turnover. Carbon recently assimilated by plants and released into the soil by exudation is very important in this short-term turnover, because it is a readily available C source for microorganisms [1], which becomes decomposed to CO_2 within a few hours to days. Root respiration represents a further important contribution to the total CO_2 efflux from soil, which must be considered separately from soil organic matter turnover [2]. Different

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techniques based on C isotopes are used to separate these CO_2 flows and to quantify plant mediated C input into the soil as well as root-derived CO_2 fluxes.

Techniques like ^{13}C or ^{14}C labelling have been used to determine the carbon balance in a plant–soil system [1, 3–5]. The C translocation by plants into the soil and C partitioning for rhizodeposits and rhizosphere respiration were observed by these isotopic tracers and separated from native soil carbon. Total rhizosphere respiration has been quantified using continuous ^{14}C labelling [6–8] or ^{14}C pulse labelling [9–11]. Advantages and disadvantages of the two methods have been discussed [5, 12]. The ^{14}C fluxes from the plant to the soil or to any other growth medium (*e.g.* nutrient solution (NS)) and particularly the CO_2 efflux can be traced after every single pulse of a repeated ^{14}C pulse labelling. However, a series of labelling pulses produces more information about the C translocations in the soil–plant system when compared with continuous labelling [13].

Artificial ^{13}C or ^{14}C labelling is connected with many methodical difficulties and hence is mainly applied in laboratory studies. As an alternative, ^{13}C natural abundance has been frequently used in the last 15 years for estimation of below-ground C input and its partitioning [3, 14–19]. ^{13}C natural abundance has some advantages towards artificial labelling, *e.g.* ^{13}C distribution between different pools is more homogeneous when compared with pulse labelling and it is non-hazardous because no radioactive labels are used. Nevertheless, the results of any C balance obtained by ^{13}C natural abundance would be biased if any isotopic fractionation did occur by rhizodeposition and root respiration [20]. However, some studies have shown that there is no difference in $\delta^{13}C$ values of roots and of root respiration for winter wheat [17] and for bean and maize [21]. In incubation experiments, it was observed that CO_2 respired by microbial decomposition of roots was depleted by 1–10 ‰ [22–24]. Furthermore, C in exudation and in root respiration was mainly derived from recently assimilated C [25–27]. Hence, a fractionation of ^{13}C between roots and CO_2 or exudates must derive from a fractionation between recently assimilated C and CO_2 or exudates. Scartazza *et al.* [28] have found a linear relationship between the variation of $\delta^{13}C$ in plant respired CO_2 and the variation of $\delta^{13}C$ in recent photosynthetic products. In their study, ecosystem respired CO_2 was ~4 ‰ depleted in ^{13}C when compared with phloem sugars.

Information on the extend of isotopic fractionation of recently assimilated C is especially important for studies on balancing carbon in the plant–soil system using ^{13}C natural abundance. The balance of recently assimilated C as well as its contribution to the below-ground C fluxes can be most easily obtained by pulse labelling of plant shoots in a $^{13}CO_2$ or $^{14}CO_2$ atmosphere or by natural labelling, *i.e.* planting C_4-plants on a soil developed under C_3-vegetation or *vice versa.* However, any studies into investigation of ^{13}C fractionation at ^{13}C natural abundance levels cannot be combined with artificial ^{13}C labelling. In the present study, we therefore determined the fractionation and partitioning of recently assimilated C by combining ^{14}C pulse labelling with ^{13}C natural abundance measurements.

The specific objectives of this study were (1) to quantify the amount of recently assimilated C in maize shoots and roots, exudates, and CO_2 derived from root respiration, (2) to examine the extend of ^{13}C fractionation of recently assimilated C, especially by root respiration and exudation, and (3) to assess the effect of different nutrient supplies on C partitioning and the ^{13}C fractionation.

Therefore, maize shoots were pulse labelled three times in a $^{14}CO_2$ atmosphere and ^{14}C was traced in the four compartments: shoots, roots, exudates, and CO_2 from root respiration. The $\delta^{13}C$ natural abundance of shoots, roots, exudates, and CO_2 deriving from root respiration was also determined to quantify the ^{13}C fractionation between maize roots, exudates, and CO_2 deriving from root respiration. Three maize treatments were established with full NS, 10 times diluted NS, and deionised water to correlate the ^{13}C isotope fractionation to the plant nutrient status.

2. Materials and methods

2.1 *Experimental setup*

Twelve maize plants (cv. Tassilo) were grown in NS (table 1) under controlled laboratory conditions, one plant per container. The maize seedlings were germinated on wet filter paper first to a maximum height of 5 cm. After 6 days, they were transferred to 250 ml polycarbonate filtration devices (SM16510/11, Sartorius, Germany) filled with a full supply of air. Air was pumped through these pots from bottom to top with one membrane pump (Type 113, Rietschle Thomas, Germany) connected by a tube to every single pot. Fourteen days after germination, the pots with the plants were sealed with silicone rubber (TACOSIL 145, Thauer & Co., Germany) between roots and shoots and the seal was tested for air leaks. Another tube was connected to the top outlet of the filter devices and 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. Therefore, the air containing CO_2 evolved from root respiration was circulated in a closed system, *i.e.* the air was pumped through the NS with any CO_2 from root respiration being trapped in NaOH solution and the resulting CO_2-free air coming from the trapping tube again being pumped through the NS (see [29] for further details).

The full NS provided for all plants before labelling was exchanged for one of three treatments, each treatment consisting of four plants. Two hours after light on event on day 15, these three treatments were initiated: full NS (table 1), 10 times diluted NS (0.1 × NS), and deionised water (DI-H_2O).

2.2 *Labelling and sampling*

Fifteen days after germination, the maize was labelled for the first time. All sealed pots with plants were placed into a Plexiglas chamber for the labelling procedure according to ref. [9]. Briefly, the chamber was connected by tubing with a flask containing 2 ml $Na_2{}^{14}CO_3$ solution to which 5 M H_2SO_4 was added to produce $^{14}CO_2$. The plants were labelled during 1.5 h in the atmosphere containing 5 MBq ^{14}C, resulting in an input activity of 214.5 kBq per plant. Before opening the labelling chamber, the chamber air was pumped through 1 M NaOH solution to remove unassimilated $^{14}CO_2$. This unassimilated $^{14}CO_2$ was considered to calculate the total assimilated $^{14}CO_2$. Then the chamber was opened and the trapping of CO_2 evolved by root respiration was started.

The experiment consisted of three cycles. Each cycle included: (1) supply of the plants with full NS for recovery at least 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

Table 1. Composition of the NS used for hydro-culture of maize plants.

c (mg/l)			
Macronutrients		Micronutrients	
K_2SO_4	153.4	H_3BO_3	0.0618
KCl	7.5	$MnCl_2 \cdot 4\,H_2O$	0.0845
$Ca(NO_3)_2 \cdot 4\,H_2O$	472.3	$CuSO_4$	0.0499
$MgSO_4$	246.5	$(NH_4)_6Mo_7O_{24}$	0.0247
KH_2PO_4	34.0	$ZnSO_4$	0.2875
KNO_3	303.0	Fe(III)EDTA	36.7100

CO_2 in NaOH and exudates in NS, 0.1 × NS, or DI-H_2O for 4 days. Owing to NS uptake, the plants were provided with 300 ml instead of 250 ml solution (maximum capacity of the filter devices) before the second and the third labelling. Plants were harvested on day 29, divided into shoots and roots, and dried at 40 °C. Immediately after sampling of the NSs, Micropur (Katadyn, Switzerland) containing Ag^+ ions was added to the flask containing exuded organic substances to suppress their microbial decomposition before analysis [29, 30].

2.3 *Sample analysis and calculations*

Dissolved C released as exudates into NS was measured by a Dimatoc-100 TOC/TIC analyser (Dimatec, Germany). The C content in shoots and roots was measured by a Euro EA C/N analyser (EuroVector, Italy). The 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 [31].

The ^{14}C activity collected as $^{14}CO_2$ in NaOH solution or as exudates in NS was measured in 2 ml aliquots added to 4 ml scintillation cocktail Rotiszint Eco Plus (Carl Roth, Germany) after decay of chemiluminescence (for NaOH). The ^{14}C measurements were done by a Wallac 1411 Liquid Scintillation Counter (Wallac Oy, Finland). The ^{14}C counting efficiency was ∼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 external standard (SQP(E) method). The ^{14}C in solid samples (dried shoots and roots) was measured after combustion of 200 mg of sample within an oxidizer unit (Model 307, Canberra Packard Ltd., USA), absorption of the ^{14}C in Carbo-Sorb E (Perkin Elmer Inc., USA), and addition of the scintillation cocktail Permafluor E^+ (Perkin Elmer Inc.).

For $\delta^{13}C$, only solid samples could be analysed by a Thermo Finnigan MAT Delta plus advantage isotope ratio mass spectrometer (IRMS) coupled to the Euro EA C/N analyser. Maize roots and shoots could hence be analysed directly, but the CO_2 and exudates samples had to be specifically prepared. Any CO_2 trapped in NaOH as Na_2CO_3 was precipitated with an excess of 0.5 M $BaCl_2$ aqueous solution. To prevent fractionation in this step, CO_3^{2-} was completely precipitated to a maximum of 1.4×10^{-5} % of the total C remaining in the solution. The precipitated $BaCO_3$ was then washed at least 10 times with deionised water to remove NaOH and to reach a pH of 7. Keeping the tubes open for washing as short as possible prevented contamination by atmospheric CO_2 during sample preparation. After washing, the remaining water was removed from the vials and the $BaCO_3$ was dried at 105 °C. The $BaCO_3$ could then be analysed to get $\delta^{13}C$ values on the IRMS. For the exudates, the NS containing the exudates was dried at 60 °C and then be analysed by the IRMS. We preferred drying of exudate samples towards freeze-drying, to avoid loss of easily volatile compounds and thus a possible source of ^{13}C fractionation. An aliquot of ∼25, 50, and 295 μg C of dried exudates, $BaCO_3$, and maize roots or shoots, respectively, was analysed by IRMS. Samples from days 20 and 25 were excluded from mass spectrometry analyses because of the high salt contents in the NS.

The experiment was conducted with four replicates for every treatment. The total ^{14}C activity recovered in the shoots and roots at the end of the experiment was divided by three to get the total mean ^{14}C activity after each of the three labelling pulses. On days 20, 25, and 29, the total ^{14}C activity of plants, exudates, and CO_2 was summed up to get the total ^{14}C recovery after each labelling. The ^{14}C-CO_2 and -exudates data are presented here as percentages of the total ^{14}C recovery after each labelling. Standard deviation (SD) was calculated as a variability parameter.

Significance of differences between treatments was analysed for each sampling by one-way ANOVA. Significance of differences between two sampling dates within one treatment was analysed in the same way.

3. Results

3.1 *Total carbon fluxes by exudation and respiration*

The cumulative C exudation and the cumulative CO_2 efflux were calculated from the first labelling to the end of the experiment. There was no significant difference in the cumulative C exudation between 0.1 × NS and DI-H_2O treatments for the first three sampling dates (table 2), but there was a difference for the last two ones ($P < 0.01$). The C exudation in full NS was significantly higher ($P < 0.001$) for all sampling dates when compared with the other two treatments. It amounted to an accumulated 44 mg C per plant in 14 days, which was more than double the amount when compared with the nutrient deficient treatments.

No significant differences were found in the cumulative CO_2 efflux from 0.1 × NS and DI-H_2O treatments and from 0.1 × NS and NS treatments except for the last sampling. The cumulative CO_2 efflux from NS treatment amounted 205 mg C after 14 days and was always higher ($P < 0.05$) than that from DI-H_2O treatment (133 mg C).

No significant treatment differences were found in the shoot C content (39 % C) (table 3). The C content in roots of the 0.1 × NS treatment was at 39 % C ~3 % significantly higher ($P < 0.05$) than the C content of the full NS treatment. No significant difference was found between shoots and roots of any treatment. Contrasting to the total shoot C content, the total N content was significantly lower at 2.1 % N in 0.1 × NS treatment when compared with 2.9 % N in full NS treatment ($P < 0.05$). Consequently, the C/N ratio was increasing with decreasing nutrient supply from 13 for NS to 19 for 0.1 × NS ($P < 0.01$). The same effect of decreasing N content with decreasing nutrient supply occurred for the roots ($P < 0.05$ for N; $P < 0.001$ for C/N). Significant differences in N content and C/N ratio were also found between NS and DI-H_2O treatments in shoots ($P < 0.01$) and roots ($P < 0.05$ for N; $P < 0.01$ for C/N). In all three treatments, up to 0.9 % more N was allocated to shoots when compared with roots.

Table 2. Cumulative exudation of organic substances and cumulative root respiration of maize between day 15 and day 29 depending on growth media: full NS, 0.1 × NS, and DI-H_2O (mean ± SD) treatments.

	Cumulative [mg C]		
Maize growth [d]	NS	0.1 × NS	Deionised H_2O
Exudation			
19	12.1 ± 1.2	1.9 ± 0.2	2.7 ± 1.3
20	18.1 ± 2.0	6.5 ± 0.3	6.6 ± 1.0
24	28.8 ± 1.7	9.0 ± 0.5	8.7 ± 1.0
25	39.3 ± 2.1	16.3 ± 0.9	14.0 ± 0.8
29	44.2 ± 2.2	19.2 ± 0.4	17.2 ± 0.4
CO_2 efflux			
19	40.3 ± 14.6	27.9 ± 11.2	24.3 ± 4.9
20	49.5 ± 15.8	36.6 ± 10.3	29.4 ± 6.5
24	110.0 ± 28.8	73.7 ± 8.7	66.8 ± 11.5
25	130.2 ± 32.9	92.1 ± 10.1	84.2 ± 18.0
29	204.6 ± 44.8	136.4 ± 7.3	132.7 ± 38.5

Table 3. Total C, total N, C/N-ratio, and ^{14}C activity after three times of labelling of maize shoots and roots grown for 14 days in three different types of NS and total ^{14}C activity of exudates and CO_2 from root respiration (mean ± SD).

	NS	0.1 × NS	Deionised H_2O
Total C [%]			
Shoots	36.5 ± 2.9	40.6 ± 1.7	40.3 ± 2.6
Roots	35.9 ± 2.4	40.5 ± 1.1	39.0 ± 2.3
Total N [%]			
Shoots	2.9 ± 0.5	2.1 ± 0.2	1.8 ± 0.5
Roots	2.0 ± 0.2	1.6 ± 0.0	1.7 ± 0.2
C/N			
Shoots	12.7 ± 2.4	19.1 ± 0.9	23.6 ± 5.8
Roots	17.8 ± 1.8	25.1 ± 1.4	23.2 ± 2.4
^{14}C [% of ^{14}C recovery]			
Shoots	76.1 ± 5.8	73.0 ± 6.0	69.4 ± 3.9
Roots	8.3 ± 2.5	10.1 ± 1.3	12.5 ± 1.9
Exudates	0.4 ± 0.1	0.5 ± 0.1	0.8 ± 0.2
Root-respired CO_2	15.1 ± 4.6	16.4 ± 4.6	16.9 ± 3.1
^{14}C [% of ^{14}C input]			
Sum of all compartments	52.8 ± 12.2	43.7 ± 0.2	45.8 ± 6.7

Considering equal C contents between shoots and roots, this led to broader C/N ratios of roots by dilution of NS.

3.2 *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 as well as its dependence on nutrient supply. After each labelling, maize assimilated almost the total amount of $^{14}CO_2$ applied to the shoots during 1.5 h. Less than 0.02 % of the $^{14}CO_2$ input remained in the $^{14}CO_2$ source and in the NaOH trap of the labelling chamber. 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 of NS, 0.1 × NS, and DI-H_2O treatments, respectively (table 3). 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 allocated to CO_2 deriving from root respiration of NS, 0.1 × NS, and DI-H_2O treatments, respectively.

The amount of recovered ^{14}C allocated in maize shoots was similar for all three treatments. The allocation of ^{14}C in the roots of 0.1 × NS and DI-H_2O treatments was similar, whereas the incorporation in roots of NS treatment was significantly lower when compared with DI-H_2O treatment ($P < 0.05$). At the end of the experiment, after three ^{14}C labelling pulses, up to eight times more ^{14}C ($P < 0.001$) was found in the shoots of all treatments when compared with the roots. Recovery of ^{14}C in exudates and CO_2 was only significantly different for exudates of NS and DI-H_2O treatments ($P < 0.01$).

No significant differences between the three treatments were found in the cumulative ^{14}C exudation after the first and second labelling (figure 1). Exudation was stable at ∼0.5 % of ^{14}C recovery between days 19 and 20, but then increased between days 24 and 25 from 0.5% to 0.8 % of ^{14}C recovery. In the last sampling, ^{14}C in DI-H_2O exudates (0.6 % of ^{14}C recovery) was significantly higher when compared with NS (0.2 % of ^{14}C recovery, $P < 0.01$) and 0.1 × NS (0.4 % of ^{14}C recovery, $P < 0.05$). This shows that about 14 days of different nutrient supply

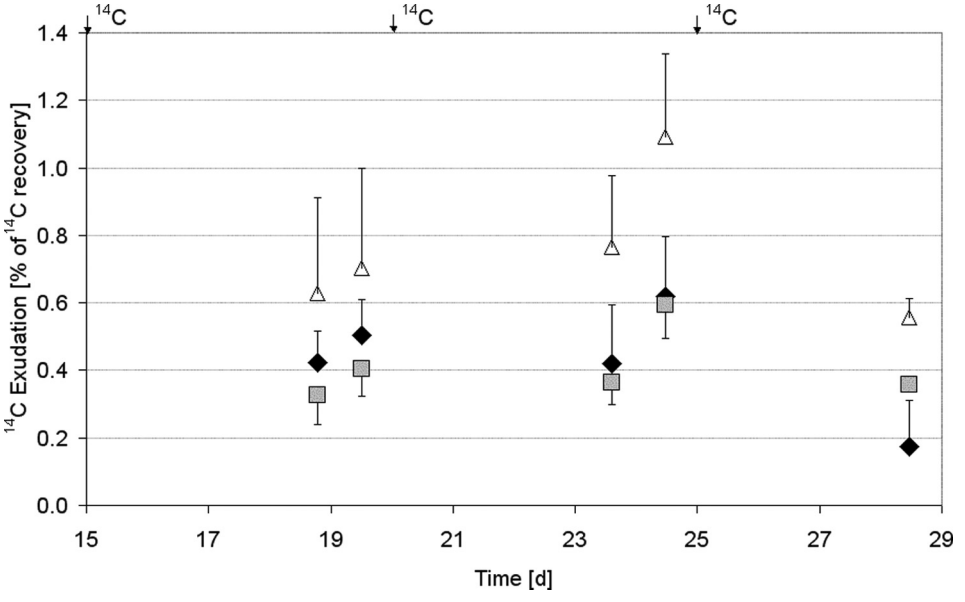

Figure 1. ^{14}C in root exudates of maize grown in three different types of NS after three times of labelling (♦ full nutrient supply, □ 0.1 × nutrient supply, △ deionised water; SD only shown to one side of the symbol, ↓ ^{14}C labelling pulses). Time scale is shown in days of maize growth.

was necessary to obtain significant differences in the amount of exuded substances between the treatments.

Considering the $^{14}CO_2$ efflux from root respiration of the maize plants, no significant difference between the three treatments was found at all for any sampling dates (figure 2). Comparing

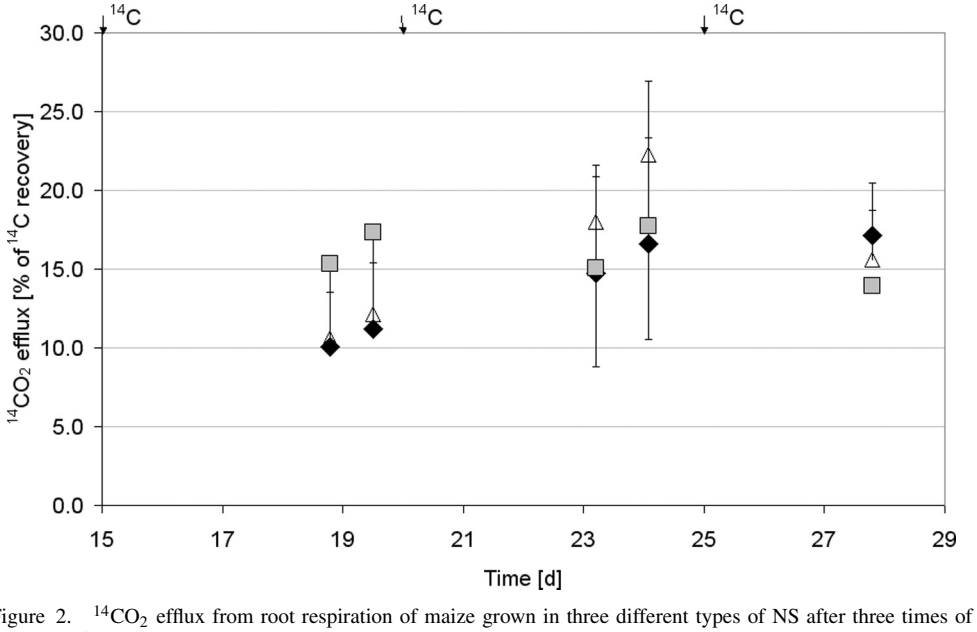

Figure 2. $^{14}CO_2$ efflux from root respiration of maize grown in three different types of NS after three times of labelling (♦ full nutrient supply, □ 0.1 × nutrient supply, △ deionised water; SD only shown to one side of the symbol, ↓ ^{14}C labelling pulses). Time scale is shown in days of maize growth.

the three 4-days sampling periods, the $^{14}CO_2$ efflux increased between sampling dates 1 and 3 from 12 % to 16 % of ^{14}C recovery and then stabilised at this value for the last sampling date. This showed that the root respiration was not affected by different concentrations of NS.

3.3 *Isotopic discrimination of ^{13}C in recently assimilated carbon*

The $\delta^{13}C$ values of shoots were the same in 0.1 × NS and DI-H_2O treatments (−15.2 ‰), but were significantly higher when compared with the NS treatment ($P < 0.05$) (table 4). In the same way, different nutrient supply did not affect the $\delta^{13}C$ values of roots of the two nutrient limited treatments. The $\delta^{13}C$ value of roots of the full NS treatment (−15.1 ‰) was significantly lower ($P < 0.01$) when compared with the other two treatments. The difference to NS was 0.2 ‰ and 0.2 ‰ in shoots and 0.3 ‰ and 0.6 ‰ in roots of 0.1 × NS and DI-H_2O treatments, respectively. In all three treatments, the $\delta^{13}C$ value of the roots was significantly higher ($P < 0.01$) when compared with the shoots. The difference of $\delta^{13}C$ values between shoots and roots increased from 0.3 ‰ to 0.7 ‰ with decreasing concentration of NS.

The $\delta^{13}C$ values of exudates in NS treatment collected in the first 4 days after the first labelling amounted to −7.9 ‰ and were significantly higher than the $\delta^{13}C$ values of 0.1 × NS and DI-H_2O treatments ($P < 0.001$). Higher $\delta^{13}C$ values of NS treatment were found also on day 24 ($P < 0.05$) and on day 29 ($P < 0.05$). In the 0.1 × NS treatment, 1.2 ‰ significantly higher $\delta^{13}C$ values of exudates were found on day 24 when compared with day 19 ($P < 0.05$).

The mean $\delta^{13}C$ values of exudates from all three samplings and the $\delta^{13}C$ values of maize roots were significantly different for all three treatments. The $\delta^{13}C$ value of exudates in the

Table 4. $\delta^{13}C$ values of maize plants, exudates and root-respired CO_2 from maize grown in three different types of NS for 14 days (mean ± SD, $n = 4$).

	$\delta^{13}C$ [‰]		
	NS	0.1 × NS	Deionised H_2O
Maize (29-day-old)			
Shoots	−15.4 ± 0.1	−15.2 ± 0.1	−15.2 ± 0.0
Roots	−15.1 ± 0.1	−14.8 ± 0.1	−14.5 ± 0.3
Exudates on days			
19	−7.9 ± 1.1	−17.0 ± 0.4	−16.3 ± 0.7
24	−9.9 ± 2.6	−15.8 ± 0.3	−16.2 ± 0.2
29	−11.6 ± 1.4	−17.3 ± 0.8	−17.5 ± 1.0
Mean	−9.8 ± 1.9	−16.7 ± 0.8	−16.7 ± 0.8
CO_2 on days			
19	−15.9 ± 0.6	−14.8 ± 0.9	−13.8 ± 0.3
24	−16.1 ± 0.5	−14.4 ± 0.1	−14.2 ± 0.3
29	−15.4 ± 0.2	−14.6 ± 0.6	−14.7 ± 0.5
Mean	−15.8 ± 0.3	−14.6 ± 0.2	−14.2 ± 0.5
Differences			
Roots–shoots	0.3 ± 0.1	0.3 ± 0.1	0.6 ± 0.2
Roots–exudates			
19	−7.2 ± 0.9	2.2 ± 0.3	1.7 ± 0.6
24	−5.2 ± 2.1	1.0 ± 0.3	1.7 ± 0.3
29	−3.5 ± 1.1	2.4 ± 0.7	3.0 ± 0.9
Mean	−5.3 ± 1.6	1.9 ± 0.7	2.1 ± 0.7
Roots–CO_2			
19	0.8 ± 0.5	0.0 ± 0.7	−0.7 ± 0.3
24	1.0 ± 0.4	−0.5 ± 0.1	−0.3 ± 0.3
29	0.3 ± 0.2	−0.2 ± 0.5	0.2 ± 0.5
Mean	0.7 ± 0.3	−0.2 ± 0.2	−0.3 ± 0.5

NS treatment was 5.3 ‰ higher ($P < 0.001$) when compared with −15.1 ‰ of maize roots. The $\delta^{13}C$ values of exudates in the DI-H_2O and 0.1 × NS treatments were 2.1–1.9 ‰ lower when compared with −14.5 ‰ and −14.8 ‰ of maize roots, respectively.

Significant differences of 0.7–2.1 ‰ were found between $\delta^{13}C$ of CO_2 evolved in NS and DI-H_2O treatments ($P < 0.05$). In NS and 0.1 × NS treatments, significant differences of 1.7 ‰ and 0.7 ‰ were observed for the third and fifth sampling, respectively ($P < 0.05$). Within treatments, only one significant difference of 0.7 ‰ was obtained between days 24 and 29 of the NS treatment ($P < 0.01$).

The $\delta^{13}C$ value of CO_2 respired by roots grown in NS was 0.7 ‰ lower ($P < 0.01$) than the $\delta^{13}C$ value of roots (−15.1 ‰). This difference of $\delta^{13}C$ values of CO_2 and roots was pronounced only for full nutrient supply.

4. Discussion

The ^{14}C labelling in this experiment was performed to quantify the balance of recently assimilated carbon by maize grown in different concentrated NSs. With decreasing nutrient supply, ^{14}C decreased in the shoots and increased in the roots. This confirms the frequently reported increase of C allocation into the roots by decreasing nutrient supply [32, 33]. The differences between the treatments in ^{14}C in exudates and in CO_2 were not significant in most of the cases, but some trends of ^{14}C increase in exudates and in CO_2 with decreasing nutrient supply were observed.

Our results reported for the below-ground ^{14}C allocation were somewhat lower when compared with wheat and barley grown in loamy soil for 30 days [34]. The latter found 19–23 %, 1–3 %, and 12–13 % of total assimilated ^{14}C in root respiration, exudates, and roots, respectively. The maize in our experiment incorporated more ^{14}C in shoots (69–76 %) when compared with 58–64 % in the cereal shoots [34]. This difference was probably due to the larger above-ground biomass in our experiment. The amounts of exudates released into NS in our experiment must be underestimated when compared with soil conditions; *e.g.* due to non-sterile conditions in our plant pots, microorganisms would have partly decomposed the exudates to CO_2. In addition, the absence of exudates binding on soil particles as well as re-uptake of released compounds by roots from NS would decrease the estimated exudates amount. Some authors have found that carbon exudation from roots into soil can range from 5 % to 20 % of the carbon fixed photosynthetically by plants [7, 35], which is much more when compared with a maximum of 0.8 % in our experiment.

Kuzyakov *et al.* [36] found a cumulative $^{14}CO_2$ efflux for labelled maize grown on soil of 13–20 % of recovered ^{14}C in the first 3 days after pulse labelling. The results of this study confirm this range. Fourteen days after the first labelling, the amount of root-derived CO_2 was ∼56–63 % of the ^{14}C label, which was translocated below ground. The fraction related to the C amount translocated below ground increases with plant age [37]. According to Martens [38] ∼45 % of carbon translocated below ground by maize was respired by the roots in a few days. Veen [39] found in a study with NS that 49 % of the carbon translocated to roots was respired. The higher percentages of respired ^{14}C found in our experiment are due to the longer time of ^{14}C chase.

Isotopic fractionation of ^{13}C occurred between shoots and roots of maize dependent on the nutrient status and this was more pronounced for the less concentrated solutions. Roots were significantly enriched in ^{13}C when compared with shoots. This has been found by several other studies [40–43]. Roots contained more ^{13}C than exudates in the NS treatment, but the exudates of 0.1 × NS and DI-H_2O treatments were ^{13}C depleted when compared with the

roots. The $\delta^{13}C$ of CO_2 was significantly depleted by 0.7 ‰ compared with the roots in the NS treatment. This suggests that earlier obtained results of the absence of ^{13}C fractionation by root respiration [17, 21, 44] must be used with caution, because fractionation can be affected by nutrient status. The other two treatments showed no fractionation between roots and respired CO_2. The temporal $\delta^{13}C$ changes for the exudates and for the CO_2 did not follow a common pattern for the three treatments, *i.e.* a comparable increase or decrease of $\delta^{13}C$ for all three treatments. The $\delta^{13}C$ data of NS, 0.1 × NS, and DI-H_2O treatments were also differently affected by nutrient status. These two observations make it impossible to substitute $\delta^{13}C$ values of the NS treatment by data of the other two treatments. Hence, strongly diluted NS and water are no suitable substitutes of full NS to gain exudates for $\delta^{13}C$ mass spectrometry analyses.

Hence, other methods have to be found to make it possible to measure $\delta^{13}C$ in exudates from NS. Gransee and Wittenmayer [30] proposed a method of growing plants in quartz sand filled with NS and dipping the roots into distilled water to gain exudates. This method has been used with ^{14}C labelling, but it has not been tested with measuring of $\delta^{13}C$ values. Kuzyakov and Siniakina [29] used a similar setup like in our experiment, but they used soil instead of NS and eluted exudates from soil to analyse ^{14}C activity. This approach was not tested with ^{13}C measurements, but this could be further investigated.

5. Conclusions

The balance of recently assimilated C by maize plants grown in NS has been determined by repeated ^{14}C labelling pulses of the shoots. The concentration of the NS was not crucial for the amount of recently assimilated C recovered in exudates and CO_2. However, total below-ground recently assimilated C was higher at lower concentrations of NS. A ^{13}C fractionation of recently assimilated C occurred between roots and exudates, leading to more ^{13}C in exudates of NS treatment and less ^{13}C in exudates of nutrient-poor treatments. The $\delta^{13}C$ values of CO_2 respired by roots were close to the root values and were only ^{13}C depleted when compared with roots in the nutrient-rich treatment.

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