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REGULAR ARTICLE

Investigation of photosynthate-C allocation 27 days after ^{13}C -pulse labeling of *Zea mays* L. at different growth stages

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Abstract

Aims Pulse labeling of crops using ^{13}C is often employed to trace photosynthesized carbon (C) within crop-soil systems. However, few studies have compared the C distribution for different labeling periods. The overall aim of this study was to determine the length of the monitoring interval required after ^{13}C -pulse labeling to quantify photosynthate C allocation into plant, soil and rhizosphere respiration pools for the entire growing season of maize (*Zea mays* L.).

Methods Pot grown maize was pulse-labeled with $^{13}\text{CO}_2$ (98 at.%) at the beginning of emergence, elongation, heading and grainfilling growth stages. The routing of ^{13}C into shoot and root biomass, soil CO_2 flux and soil organic carbon (SOC) pools was monitored for 27-days after ^{13}C -pulse labeling at the beginning of each growth stage.

Results The majority of the ^{13}C was recovered after 27 d in the maize shoots, i.e., 57 %, 53 %, 70 % and 80 %, at

the emergence, elongation, heading, and grainfilling stages, respectively. More ^{13}C was recovered in the root biomass at elongation (27 %) compared to the least at the grainfilling stage (3 %). The amount recovered in the soil was the smallest pool of ^{13}C at emergence (2.3 %), elongation (3.8 %), heading and grainfilling (less than 2 %). The amount of ^{13}C recovered in rhizosphere respiration, i.e. $^{13}\text{CO}_2$, was greatest at emergence (26 %), and similar at the elongation, heading and grainfilling stages (~16 %). **Conclusions** At least 24 days is required to effectively monitor the recovery of ^{13}C after pulse labeling with $^{13}\text{CO}_2$ for maize in plant and soil pools. The recovery of ^{13}C differed between growth stages and corresponded to the changing metabolic requirements of the plant, which indicated labeling for the entire growth season would more accurately quantify the C budget in plant-soil system.

Keywords Soil organic carbon · Maize · ^{13}C pulse labeling · Rhizosphere respiration · Shoot · C balance

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Introduction

Knowledge of the processes that control the rates of soil organic carbon (SOC) stabilization or decomposition in cultivated soils is key to understanding whether agricultural land use is a sink or source of carbon (C). Calculating the C fluxes in soils associated with maize cropping is important because of its global importance as a food (grain and forage) and biofuel crop; the total land area

under maize production is estimated at 162×10^6 ha (FAO 2010). Plant roots are a major pathway for C input to soils (Jones et al. 2009) but the majority of photosynthesized C may be lost by rhizosphere respiration, i.e. the combined flux from autotrophic (root) respiration and heterotrophic (rhizomicrobial) respiration (Högberg and Read 2006). ^{13}C - and ^{14}C -isotope labeling studies have revealed the complex dynamics of photoassimilate routing during the growth cycles of cereal crops (see review by Kuzyakov and Domanski 2000). However, most studies used plants at early stages of maturity (less than 60 days after emergence) which may have caused under- or overestimation of C allocation to different soil pools because of changes in plant physiology during the growth cycle (Hütsch et al. 2002; Kuzyakov and Schneckenberger 2004; Amos and Walters 2006; Jones et al. 2009). For example, root respiration is decreased under mature plants (Kuzyakov 2006), when resources are diverted to reproduction and fruiting (Dungait et al. 2011), and the proportion of C translocated belowground and used for root growth, respiration and exudation decreases (Swinnen et al. 1994). In a survey of the literature, we found that above:below ground monitoring of pulse-labeled C ranged from 0.3 to 28 days (Table 1). Therefore, in order to properly understand the cycling of maize C, we hypothesised that longer term monitoring periods would provide a more reliable assessment of C dynamics in maize crops.

Partitioning rhizosphere respiration and SOC respiration is prerequisite to calculating gaseous losses derived from recently assimilated C or more stable SOC pools (Werth and Kuzyakov 2008). Estimates indicate that approximately 40 to 50 % (1.5 to 2.2 t C ha^{-1}) of net photosynthate-C is allocated belowground for the total vegetative period in graminaceous crops: 19 % in the root biomass, 12 % lost as rhizosphere respiration and 5 % in soil residues (Kuzyakov and Domanski

2000; Jones et al. 2009). Total CO_2 respiration rates of bare soil are often measured as a proxy for potential maximum heterotrophic respiration (e.g. Doetterl et al. 2012), but the processes contributing to CO_2 efflux from soil are greatly changed in the presence of growing roots (Kuzyakov 2006; Paterson et al. 2006). The contribution of root-derived CO_2 to total soil CO_2 efflux in experiments in grass species ranges from 36 to 70 % (Werth et al. 2006). The advent of experimental systems using ^{13}C pulse labeling has shown the potential for investigations of ^{13}C routing into the rhizosphere in intact, planted soils (e.g. Paterson et al. 2009; Hafner et al. 2012).

The highly sophisticated instrumentation required for continuous isotopic labeling and sampling are rare (e.g. Paterson et al. 2009), especially for periods equivalent to the life cycle of a large crop plant such as maize. Alternatively, ^{13}C isotope pulse labeling at different growth stages of a plant provides discrete information about C dynamics associated with particular growth stages (Swinnen et al. 1994). Therefore, to test the hypothesis that longer monitoring times are required to quantify C dynamics in maize, we applied ^{13}C -pulse labeling to pot grown maize plants at the beginning of the four major growth stages in the whole plant lifecycle, and followed plant:soil:atmosphere ^{13}C dynamics by trapping $^{13}\text{CO}_2$ from soil respiration and destructive analysis of plant biomass and soils over time.

Materials and methods

Soil preparation

Soil samples were collected from the plough layer (0–30 cm) of an arable plot at the China Agricultural

Table 1 Recovered pulse-C partitioning at elongation growth stage in maize. Incubation period is days after $^{13}\text{C}/^{14}\text{C}$ labeling to final sampling. Lit. ref. = literature reference. Literature references: *This

Literature reference	*	a	b	c	d	e	f	g	h	i
Incubation period (days)	27	3.8	28	10	7	0.3	3	0.3	14	16
	% total recovered C									
Total aboveground (shoots)	53.5	72.9	71.0	68.5	68.0	65.5	56.0–86.0	80.0	76.1	50.9
Total belowground	46.5	27.1	29.0	31.5	32.0	34.5	24.0–44.0	20.0	23.9	49.1
Roots	26.7	12.6	11.3	17.5	9.0	22.8	2.5–4.9	12.7	8.3	28
Rhizosphere respiration	16.0	11.0	13.4	11.2	21.0	3.5	7.0–12.0	2.3	15.2	16.2
Soil	3.8	3.5	4.3	2.8	2.0	8.2	1.3–4.4	5.0	0.4	4.9

study; ^aNguyen et al. 1999; ^bWhipps 1985; ^cLiljeroth et al. 1994; ^dWerth and Kuzyakov 2008; ^eHolland et al. 1996; ^fFan et al. 2008; ^gFischer et al. 2010; ^hWerth and Kuzyakov 2005; ⁱPausch et al. 2012

University Quzhou Experimental Station in Hebei province, Northern China (36°52'N; 115°01'E). The plot had been under winter wheat-summer maize double cropping management since the 1990s. The station is located in a warm, semi-humid and continental temperate monsoon zone, with a mean temperature of 13.1 °C a⁻¹ and a mean precipitation of 556 mm a⁻¹. The soil is classified as a Cambisol and has a silty loam texture (sand 62 %, silt 29 % and clay 10 %), with a pH of 7.7 (soil:water ratio of 1:25), total organic carbon content of 7.6 g kg⁻¹, total inorganic carbon content 7.8 g kg⁻¹, and total nitrogen of 0.66 g kg⁻¹. The $\delta^{13}\text{C}$ values of the SOC and soil inorganic carbon (SIC) were $-22.4\pm 0.2\text{‰}$ and $-3.4\pm 0.2\text{‰}$, respectively. After collection the soil was bulked and air-dried then sieved (5 mm).

Maize growth conditions

Two maize seeds (*Zea mays* L., Cultivar Jiyuan 1) were sown directly into pots (35×20 cm i.d.) containing 9.5 kg air-dried soil pre-mixed with fertilizer at the rate of 0.55 g N, 0.19 g P, 0.31 g K kg⁻¹ soil (equivalent to field application rates to the plough layer). An equal number of pots were prepared without seeds for the determination of soil respiration without plants. The maize was cultivated between May and September in a net house to provide similar light and temperature to field conditions. The soil water content of each container was controlled gravimetrically to simulate local growing conditions at each of the growth stages, and was adjusted daily to 60 % (emergence), 70–75 % (elongation), 75–80 % (heading) and 70–75 % (grainfilling) field capacity. Emergence occurred after 6 days. After emergence of the third leaf, the weaker of the seedlings was removed. The maize was grown for 100 days, and four different growth stages were recognized as days after emergence (DAE): (i) emergence (0–24 DAE), (ii) elongation (25–53 DAE), (iii) heading (44–66 DAE), and (iv) grainfilling (67–99 DAE).

¹³CO₂ pulse labeling and sampling

The analysis of soil respiration in calcareous soils presents particular challenges for the source apportionment of ¹³CO₂ (Setia et al. 2010; Tamir et al. 2011). The sources of CO₂ respiration from soils with an alkali pH may be: (i) autotrophic respiration (i.e. maize in this experiment, $\delta^{13}\text{C}=-14\text{‰}$), (ii) heterotrophic respiration, (iii) CO₂ from either autotrophic or heterotrophic

respiration that has been retained in the soil solution or (iv) exchanged with soil carbonate, or (v) released directly from carbonate (i.e. in this experiment $\delta^{13}\text{C}=-3.4\text{‰}$). Therefore, we applied the method described by Fan et al. (2008) for tracking ¹³C-pulse label in an Aridisol. This approach accommodates the potential release of soil carbonate-derived CO₂, by using a control treatment (unlabeled maize) and exploiting the $\Delta^{13}\text{C}$ value (i.e. the difference between the $\delta^{13}\text{C}$ values of the unlabeled versus ¹³C-labeled treatments) to determine the percentage contribution of the ¹³C-pulse label to rhizosphere respiration (which includes CO₂ from root respiration and the decomposition of rhizodeposits).

The maize plants were ¹³C pulse labeled with ¹³CO₂ at the beginning of each growth stage. On each occasion, three maize plants were randomly selected for ¹³C labeling, and an additional three maize plants were selected as unlabeled controls (the unlabeled controls were kept separately from the labeled plants). A chamber (1.05 m long×2.15 m high) adapted from (Swinnen et al. 1994) was used for ¹³C labeling. The surface of the soil was covered with a PVC board and sealed with silicon, including around the maize stems. A leak check was carried out to confirm air tightness (Kuzyakov et al. 2002). A beaker containing Ba¹³CO₃ (98 at.% ¹³C; 4.5, 8.5, 8.0 and 8.0 g each for the 4 labeling occasions, respectively) was placed into the chamber and an aliquot of 1 M HCl was injected into the beaker every 1.5 h for 7 h. CO₂ concentration within the chamber was semi-quantitatively detected by GXH305 infrared detector (Beijing Analytical Equipment Co.) because of the differing wavelengths for maximum absorption of ¹³CO₂ and ¹²CO₂. If the rate of CO₂ concentration decline slowed considerably, air (CO₂ $\delta^{13}\text{C}=-6.6\text{‰}$) was pumped into the chamber until ¹³CO₂+¹²CO₂ concentrations increased to approximately 360 $\mu\text{L L}^{-1}$. The ¹³C abundance of CO₂ within the chamber was calculated within 55 at.%–65 at.% for the 4 pulse labeling stages. An electric fan was used to homogenize the gases. The plants were removed from the chamber after labeling to prevent reassimilation of shoot-respired ¹³CO₂. Sodium hydroxide (NaOH; 3.5 mol L⁻¹) was placed in a small glass beaker under the PVC board to trap headspace CO₂ (rhizosphere respiration) and changed every 3 days after the start of labeling, until the end of tracing period (27 days). Excess BaCl₂ was added to the NaOH solutions containing trapped CO₂ to precipitate BaCO₃ which was washed with deionized water three times after back-titration with HCl (0.3 mol L⁻¹) for determination of the total soil CO₂ flux. The BaCO₃

precipitates were combined into 4 homogenised samples for periods: 3 to 9, 12 to 18, 21 to 24, and 25 to 27 days after the start of each growth stage. The precipitates were dried at 60 °C and ground to <500 µm, before suspension in deionized water and acidification (1 mol L⁻¹ H₂SO₄) to release CO₂ into an air-tight syringe. The evolved CO₂ was injected into an evacuated sample vial for ¹³C isotope analysis.

Shoot, root and soil sampling

Control and ¹³C labeled maize plants and soils were destructively sampled 27 days after labeling at each growth stage. Visible roots >2 mm were separated from the soil by careful hand-picking followed by repeated sieving (2 mm) to remove finer roots. All root material was soaked in 2.5 L deionized water, gently shaken for 20 min and then sieved (1 mm mesh). All the washing water was saved and added to the remaining pot soil to attempt to completely recover dissolved organic carbon and other rhizodeposits. The soil was spread on plastic film overnight to reduce water content. Then the soil was thoroughly mixed and repeatedly half sampled by gridding to achieve a fully representative 50 g sub-sample. Twenty grams of the 50 g sub-sample was placed on a white plate and any remaining visible fine roots were separated from soil by careful hand-picking. The 20 g soil sample was then soaked in 100 mL deionized water and shaken for 30 min and left to settle under gravity. The supernatant was collected in beaker and HCl (3 mol L⁻¹) added until pH was less than 3 to removed inorganic carbon. For the soil slurry, 50 ml of HCl (3 mol L⁻¹) was added and completely mixed. After 2 day, the slurry was washed to neutralise the pH, and mixed with the supernatant, and then dried at 105 °C. Shoots (stem and leaves) and roots were also oven-dried at 60 °C. All samples were ground (<500 µm) prior to analysis.

Analyses

Total organic carbon and nitrogen content of soils and plant materials were determined by elemental analyzer (Flash EA1112, ThermoFinnigan, Milan, Italy). The bulk δ¹³C values of soils, plant materials and gas samples were determined using isotope ratio mass spectrometry (DELTAplus XP, ThermoFinnigan, Bremen, Germany). The abundance of ¹³C was expressed as parts per mil (‰) relative to the international standard PDB (0‰) expressed as delta units (δ).

Calculations

The δ¹³C values of the biomass, CO₂ and SOC was converted into mg ¹³C (R) using the method described by Simard et al. (1997) and used to calculate the fractional abundance (Fi) of ¹³C relative to ¹³C+¹²C from R. The ¹³C assimilated (mg) in plant biomass, soil and respired CO₂ was calculated as the difference between the ¹³C contents of the labeled and unlabeled samples (Lu et al. 2002):

$$^{13}Ci = Ci \times (Fi - Ful) / 100 \times 1000 \quad (1)$$

Where *i* and *ul* indicate labeled and unlabeled samples, respectively, and *Ci* is the total C content of sample.

We defined the total amount of ¹³C allocated into the 4 measured pools (root biomass, shoot biomass, rhizosphere respiration and SOC) as ¹³C recovered. The ¹³C recovered did not include shoot respiration as the ¹³CO₂ respired by the shoots was returned to the atmosphere and would not affect SOC as described in previous ¹⁴C and ¹³C pulse labeling studies (Fan et al. 2008; Werth and Kuzyakov 2008). The total ¹³C recovered was the sum of the ¹³C content in the measured pools: shoots and root biomass, SOC, and respired CO₂ after labeling:

Net ¹³C recovered

$$= (^{13}C_{\text{root}} + ^{13}C_{\text{shoot}} + ^{13}C_{\text{respiration}} + ^{13}C_{\text{soil}}) \quad (2)$$

The percentage distribution of ¹³C (¹³Ci%) between pools was calculated as:

$$^{13}Ci\% = ^{13}Ci / \text{Net } ^{13}C \text{ recovered} \quad (3)$$

where, ¹³Ci is the ¹³C content in the measured pools: shoots and root biomass, SOC, and respired CO₂ after labeling.

The photosynthate-C distribution in the different pools of the maize-soil system at each growth stage was estimated using the method described by Gregory and Atwell (1991), Kuzyakov (2001), and Werth and Kuzyakov (2008):

$$SCi = \Delta C_{\text{shoot}} \times ^{13}Ci\% / ^{13}C_{\text{shoot}}\% \quad (4)$$

where, SCi is the biomass-C in each pool, and ΔC_{shoot} is the biomass-C change during each stage of the maize.

Statistical analysis

The experiment was implemented with three replications. Data were subjected to one-way analysis of variance (ANOVA) in which the growth stage was the variable factor. Fisher's LSD (least significance difference, $P < 0.05$) was used to test differences in variables among different growth stages of the maize. SAS 9.1 software (SAS Institute, Cary, NC, USA) was used for all statistical tests.

Results

Plant biomass

There was no significant difference between the mean weights of the aboveground (shoot) and belowground (root) biomass of the ^{13}C pulse labeled plants and the unlabeled control plants (data not shown) when they were harvested 27 days after labeling during each of the growth stages (Table 2). This indicated that ^{13}C pulse labeling did not affect plant growth. The shoot:root ratios were similar at emergence and elongation stages, and increased in the heading and grainfilling stages (Table 2).

The plant biomass $\delta^{13}\text{C}$ values were around -14‰ for both shoots and roots in the control plants (Table 3) which is within the range of $\delta^{13}\text{C}$ values for C_4 plants (Boutton 1991). In the ^{13}C labeled maize plants, the shoot $\delta^{13}\text{C}$ values were more ^{13}C enriched compared to the roots at emergence, heading, and grainfilling but not at elongation. This equated to more than half of the assimilated ^{13}C allocated to the shoots at each growth stage, with significantly increased proportions at heading (69.8 %) and

grainfilling stages (80.1 %) than at emergence (57.1 %) and elongation stages (53.5 %, Fig. 1). The allocation of ^{13}C to the roots (not including root respiration and rhizodeposition) was greater at elongation (26.6 %) than at emergence (14.9 %) and heading stage (12.1 %), and lowest at grainfilling (3.3 %).

Bulk soil organic carbon (SOC)

The total organic carbon content of the original field soil was 0.8 %, and this did not change with incubation. The $\delta^{13}\text{C}$ values of the bulk SOC in the control soils at all growth stages were not significantly different from the value before planting (-22‰ ; Table 3). The $\delta^{13}\text{C}$ values of the bulk SOC in the ^{13}C labeled soils at all growth stages were significantly ^{13}C enriched by $\sim 2\text{‰}$ at emergence, heading and grainfilling, and 6‰ at elongation (Table 3). The ^{13}C remained in the soil was 2.3 %, 3.8 %, 1.5 % and 1.2 % for emergence, elongation, heading and grainfilling stage respectively (Fig. 1).

Rhizosphere respiration

In the control treatments, rhizosphere respiration was ^{13}C enriched ($\delta^{13}\text{C}$ value range = -13.5 to -15.3‰) compared to bulk soil values ($\delta^{13}\text{C} = -22.4\text{‰}$), and similar at all growth stages and sample times to the shoot and root values (-14.1‰), indicating the use of the natural abundance ^{13}C labeled maize-C for respiration by the root and microbial biomass (Table 3). In the ^{13}C pulse labeled treatments, rhizosphere respiration had the greatest $\delta^{13}\text{C}$ values up to 9 days after ^{13}C pulse labeling at all growth stages with the greatest values at emergence and elongation, compared with

Table 2 Biomass and recovered ^{13}C budget in the maize-soil system at each growth stage. The figure in brackets is the standard error. Different letters in the same row denote a significant difference (LSD, $P < 0.05$) between growth stages

Pools	Emergence	Elongation	Heading	Grainfilling
Biomass	g plant ⁻¹			
Shoots	48.7 (3.24) c	75.1 (4.60) b	125.2 (6.54) a	133.0 (5.04) a
Roots	22.0 (1.15) b	29.4 (1.45) a	28.4 (1.99) a	25.6 (1.28) ab
Shoot:root ratio	2.2 (0.12) c	2.6 (0.17) c	4.4 (0.09) b	5.2 (0.21) a
Recovered ^{13}C	mg plant ⁻¹			
Shoots	33.0 (3.84) c	66.4 (4.17) b	81.5 (5.44) ab	86.5 (7.34) a
Roots	8.6 (1.39) bc	33.0 (3.13) a	14.1 (1.41) b	3.6 (0.39) c
Rhizosphere respiration	14.9 (1.47) b	19.8 (1.37) a	19.4 (1.44) a	16.7 (1.13) ab
Soil	1.3 (0.14) b	4.8 (0.26) a	1.8 (0.17) b	1.3 (0.11) b

Table 3 Mean $\delta^{13}\text{C}$ (‰) values ($n=3$) of rhizosphere respiration at 9, 18, 24 and 27 days, and shoot, root and bulk SOC at 27 days for each maize growth stage: emergence, elongation, heading and grainfilling in (a) ^{13}C pulse labeled treatments and (b) control (unlabeled). The figure in brackets is the standard error

Growth stage	Emergence				Elongation				Heading				Grainfilling			
	9	18	24	27	9	18	24	27	9	18	24	27	9	18	24	27
^{13}C pulse labeled maize																
Shoot biomass				135.4 (28.2)				177.9 (14.6)				127.0 (9.5)				125.3 (15.3)
Root biomass				78.8 (13.8)				247.5 (32.5)				99.9 (14.6)				16.3 (4.0)
Bulk SOC				-20.9 (0.1)				-16.5 (0.1)				-20.1 (0.3)				-20.6 (0.1)
Rhizosphere respiration	504.6 (40.5)	21.5 (6.9)	8.8 (2.1)	2.0 (0.4)	493.6 (22.8)	53.2 (3.9)	18.3 (2.1)	0.5 (0.1)	366.0 (32.5)	69.4 (4.6)	36.4 (2.6)	13.8 (1.0)	345.9 (21.6)	129.0 (2.0)	47.6 (1.3)	13.8 (0.5)
Control maize																
Shoot biomass				-14.1 (0.0)				-14.2 (0.1)				-14.1 (0.2)				-14.1 (0.2)
Root biomass				-14.2 (0.1)				-14.2 (0.1)				-14.1 (0.3)				-14.1 (0.2)
Bulk SOC				-22.5 (0.1)				-22.3 (0.1)				-22.3 (0.1)				-22.2 (0.0)
Rhizosphere respiration	-13.9 (0.3)	-14.0 (0.2)	-13.7 (0.3)	-14.1 (0.3)	-15.0 (0.2)	-14.5 (0.1)	-14.7 (0.4)	-14.6 (0.3)	-15.2 (0.2)	-14.1 (0.3)	-15.3 (0.3)	-14.4 (0.7)	-14.4 (0.3)	-14.2 (0.1)	-14.3 (0.3)	-14.3 (0.3)

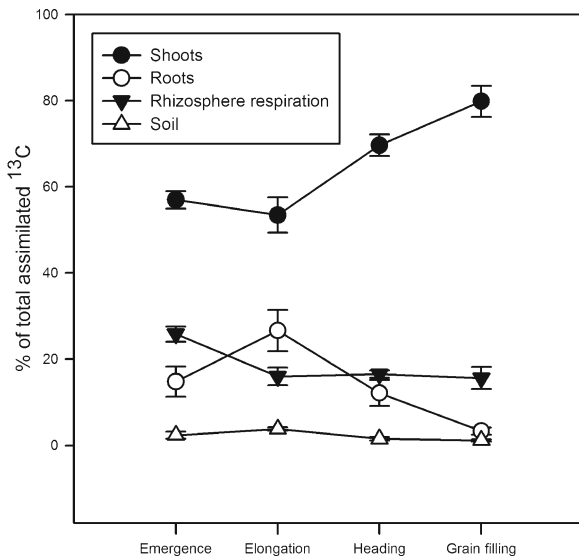


Fig. 1 Percentage ¹³C (n=3) shoot biomass, root biomass, rhizosphere respiration and bulk SOC 27 days after ¹³C labeling at emergence, elongation, heading and grainfilling growth stages in maize

heading and grainfilling stages (Table 3). The δ¹³C values of the respired CO₂ at all growth stages reduced rapidly at 18 days, and more slowly thereafter.

The ¹³C recovered as ¹³CO₂ (rhizosphere respiration) declined with time in all growth stages after pulse-labeling, and there were significant differences between

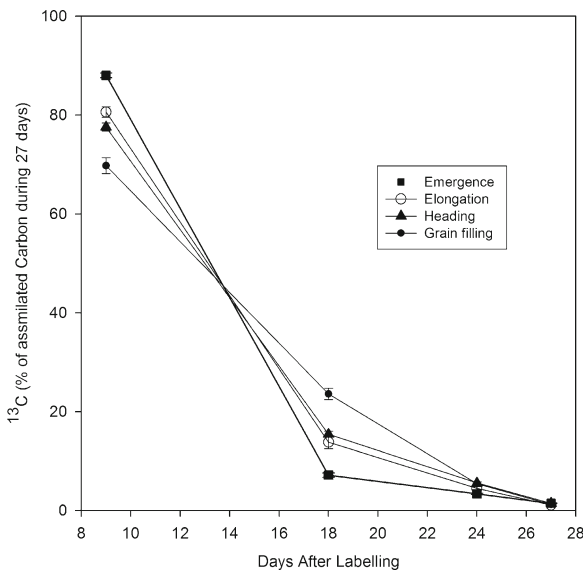


Fig. 2 Recovery of ¹³C label in rhizosphere respiration at 9, 18, 24 and 27 days after the start of labeling for 4 maize growth stages. Error bars indicate standard error (n=3)

¹³C recovered between growth stages (Fig. 2). The contribution of ¹³C to rhizosphere respiration at 9 days was significantly increased during emergence, and significantly decreased during grainfilling compared with elongation and heading. The opposite was observed at 18 days when rhizosphere respiration of ¹³C was significantly decreased during emergence, and significantly increased during grainfilling, compared with elongation and heading. After 27 days, the amount of ¹³C lost as rhizosphere respiration (Fig. 1) was only significantly different at the emergence stage, accounting for 25.8 % of the total recovered ¹³C, or 14.9 mg ¹³C plant⁻¹ (Table 2), compared with that at other three stages (elongation: 16.0 %, heading: 16.6 %, and grainfilling: 15.5 %).

Above: below ground allocation of photosynthate after 27 days

The amount of photosynthate allocated below and above-ground after 27 days changed with each growth stage (Fig. 1). The percentage ¹³C of the total recovered ¹³C in the shoot biomass was similar at emergence and elongation and increased during heading and grainfilling stages. This equated to 33.0, 66.4, 81.5 and 86.5 mg ¹³C plant⁻¹ at emergence, elongation, heading and grainfilling, respectively. Allocation belowground was greatest at elongation (57.6 mg ¹³C plant⁻¹; Table 2), when 26.6 % of the total recovered ¹³C was determined in the root biomass, 16.1 % in rhizosphere respiration and 3.8 % in soil, compared with the smallest quantity of 21.6 mg plant⁻¹ to belowground during grainfilling.

Discussion

Determining ¹³C emission from soil with inorganic carbon

The δ¹³C value of the SOC calculated in the acid washed soil used in the experiment was -22 ‰. Published values for C3 soils are more ¹³C-depleted, e.g. ~ -27 ‰, which is similar to the δ¹³C value of C3 vegetation (Boutton 1991). Therefore, the comparative ¹³C-enrichment of the soil was assumed to reflect the contribution of maize cultivation (δ¹³C=-13‰ ~ -14‰) to the previous predominantly C3 SOC pool. The rhizosphere respiration in the control treatments had δ¹³C values in the range -13.5 to -15.3‰ compared to bulk soil values and was similar at all growth stages and sample times to the shoot and

root values (-14.1%). This suggested that maize-C was the principal source of the respired CO_2 from the soil because its $\delta^{13}\text{C}$ value was very similar to the value of the maize. However, the soil was slightly alkaline (pH 7.7), and consideration needs to be given to the influence of soil carbonate on the quantity and source of CO_2 in soils with a pH >6.7 (Bertrand et al. 2007; Tamir et al. 2011). Setia et al. (2010) found that in soils with low carbonate and inorganic N contents, CO_2 -C emission was not affected by CaCO_3 addition. Similar to Setia et al. (2010), the soil in this experiment had a low content of inorganic C and low inorganic N content ($\text{NH}_4\text{-N} <5 \text{ mg kg}^{-1}$, $\text{NO}_3\text{-N} <40 \text{ mg kg}^{-1}$, reported in Liu et al. 2003), indicating carbonate-derived CO_2 was unlikely to contribute substantially to total soil respiration. Moreover, the potential influence of carbonate to the respired $^{13}\text{CO}_2$ was resolved by subtracting the $\delta^{13}\text{C}$ value of the CO_2 from the unlabeled maize treatment in our study. However, the specific contribution of inorganic C to CO_2 was not determined and admittedly may decrease the reliability of the results herein. The application of ^{14}C -pulse labeling (e.g. Werth and Kuzyakov 2008) rather than ^{13}C would help to resolve this concern, but it is not possible to use this approach under field conditions.

Incorporation of $^{13}\text{CO}_2$ into maize biomass

The majority of photosynthate was incorporated into the aboveground (shoot) biomass at each growth stage of maize in this experiment, and this increased with maturity of the plant to a maximum of $>80\%$ in the grainfilling stage. Correspondingly, shoot biomass was generally more ^{13}C enriched than the roots in the ^{13}C pulse labeled treatments, except during elongation. This suggests that there was active routing of photosynthate belowground through the early establishment (i.e. elongation) of the maize during a period of active root growth, but there is lower allocation to the roots as plant becomes mature. This is similar to the plant:soil C dynamics observed previously during the cultivation of rice (Lu et al. 2002), wheat and barley (Keith et al. 1986; Gregory and Atwell 1991; Swinnen et al. 1994, 1995) and maize (Yang et al. 2006), but not for pasture grasses (Kuzyakov et al. 1999; Kuzyakov 2001; Warembourg and Estelrich 2001).

Incorporation of $^{13}\text{CO}_2$ into soil pools

A minor proportion of the total ^{13}C was recovered from the SOC pool at the four labeling stages, with the largest

($\sim 4\%$) at elongation. This was lower than the proportions reported in other studies, especially compared to those with shorter labeling periods (Table 1). This small but significant increase in $\delta^{13}\text{C}$ amount provided evidence of substantial $\text{C}_4\text{-C}$ (i.e. ^{13}C labeled) from photosynthate translocation belowground. Rhizosphere respiration (CO_2) in the planted control treatments was ^{13}C enriched compared with the bulk SOC at all stages, suggesting that the majority of the maize $\text{C}_4\text{-C}$ in the soil was labile and available for use as a respiratory substrate within the rhizosphere. Derrien et al. (2004) determined that 40% of root-derived C from wheat was soluble neutral sugars which are highly available for microbial respiration. However, a component will also have been derived from the decomposing maize roots and aerial crop residues within the SOC (Dungait et al. 2009). To determine the contribution of this pool requires differentiation of $\text{C}_4\text{-SOC}$ from novel maize rhizoexudates and root turnover, which is possible though the application of ^{14}C pulse labeling (e.g. Werth and Kuzyakov 2008). The proportion of maize photosynthate allocated to rhizosphere respiration changed with growth stage, but was greatest (25.8%) during emergence, and similar (15.5 to 16.6%) during elongation, heading and grainfilling growth stages. The latter is comparable to the value (12%) reported by Jones et al. (2009). However, the content of ^{13}C in rhizosphere respiration fluctuated less between growth stages, with a mean value of $18 \pm 2 \text{ mg } ^{13}\text{C plant}^{-1}$ at each growth stage (Table 2), suggesting a sustained supply of plant-C was directed to root exudation.

Longer term and entire growing season monitoring of $^{13}\text{CO}_2$ in plant: soil systems

The $\delta^{13}\text{C}$ values of respired CO_2 determined after ^{13}C pulse labeling were most elevated after the first sampling period of 9 days, and declined rapidly thereafter. However, even after 27 days, there was still evidence of the ^{13}C label in all C compartments, i.e. shoot biomass, root biomass, rhizosphere respiration and SOC at all growth stages. The majority of the ^{13}C allocated belowground at all growth stages was lost as CO_2 via rhizosphere respiration (autotrophic root respiration or heterotrophic mineralisation of rhizodeposits) rather than retained in SOC after 27 days. At least 98% of the total respired ^{13}C collected over the whole monitoring period (27 days) was recovered after 24 days and for last 3 days period (25nd to 27nd days after labeling), only about

1.1 %–1.4 % was recovered (Fig. 2). This monitoring period described specifically for the elongation growth stage in maize is the longest in duration reported in published studies (Table 1). Similar sustained sampling periods have been reported that rely on the hypothesis that distribution of assimilated C is assumed to be complete if losses of labeled CO₂ by respiration can no longer be detected (Kuzayakov 2001). Swinnen et al. (1994) reported that 3 weeks is appropriate to estimate C assimilate distribution at different development stages using ¹⁴C pulse labeling under field condition for spring wheat. Neergaard and Gorissen (2004) confirmed that 2 to 3 weeks is adequate to quantify ¹⁴C allocation for white clover and ryegrass in a pot experiment. Particularly, comparing the recovered ¹³C/¹⁴C allocation proportion within shoots, roots, rhizosphere respiration and SOC pools in our study and other literatures indicated the significance of longer labeling periods. The mean of the published values for recovered ¹³C/¹⁴C in the above-ground pool was 67.7±9.2 % (Table 1), significantly higher than this study. This was the opposite for root and rhizosphere respiration pools which were 13.9±7.8 % and 11.2±6.1 %, respectively, significantly lower than 26.7 % and 16.0 % in our study. The proportion for the SOC pool was similar (3.9±2.3 %) with our data (3.8 %). The metadata analysis reported by Amos and Walters (2006) found that net rhizodeposited C values for maize cultivated in growth chambers was 22.4 %, and significantly higher than that calculated NRC (ratio of net rhizodeposited C, i.e., soil microbial biomass and as soil residue, to total net root-derived belowground C, i.e., standing root biomass C+rhizodeposited C) for the emergence, elongation, heading and grainfilling stages in this study (i.e. 0.13, 0.13, 0.11 and 0.26, respectively). However, of the published values, only 4 were sampled for longer than 99 days. This concurs with our findings that sampling for the entire growth season of maize is required to accurately quantify the photosynthate distribution within crop-soil systems.

Conclusions

The longer timescale of the monitoring in this experiment provided a much more complete assessment of photoassimilate-C dynamics in the maize plant:soil system than previously described. We estimated the C budget for the entire life cycle of the maize plants in the experimental system based on the 27 day monitoring period of each

growth stage. The total photosynthate-C inputs were aboveground 57.5 g pot⁻¹ (63 %), root 16.2 g pot⁻¹ (17.8 %), rhizosphere respiration 2.4 g pot⁻¹ (2.7 %) and soil organic carbon 15.1 g pot⁻¹ (16.6 %). Using this calculation, we estimated the input of photosynthesized C to the maize-soil system under field conditions for the intensive winter wheat-summer maize farming systems of the North China Plain, where the typical aboveground net biomass of maize is 18 t ha⁻¹ (7.56 t C ha⁻¹). This translates to 4.6 t C ha⁻¹ translocated belowground annually, which is partitioned into 2.1 t C ha⁻¹ lost as CO₂ by rhizosphere respiration, 2.2 t C ha⁻¹ allocated to root biomass and 0.3 t C ha⁻¹ incorporated into SOC. However, the longevity of the latter maize-C pool in soil post-27 days remains to be determined, including the potential positive priming effect of maize rhizoexudation on extant SOC (Pausch et al. 2012).

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