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REGULAR ARTICLE

Determining the timepoint when ^{14}C tracer accurately reflect photosynthate use in the plant-soil system

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Abstract
Background and aims Only the carbon (C) isotope pulse labeling approach can provide time-resolved data concerning the input and turnover of plant-derived C in the soil, which are urgently needed to improve the performance of terrestrial C cycle models. However, there is currently very limited information about the point in time after pulse labeling at which the distribution of tracer C accurately represents the usage of photosynthates in different components of the plant-soil system. This should be the case as soon as the tracer has disappeared from the mobile C pool due to respiration, incorporation into the structural C pool of shoot and root tissue and exudation into the soil (rhizodeposition).

Methods Following $^{14}CO_2$ pulse labeling in laboratory and outdoor experiments with spring rye, the ^{14}C dilution rates of soluble fractions and different substances from the structural C pool of the shoot (molecular level), the release of labeled CO_2 by belowground respiration (component level), and the ^{14}C kinetics of shoot respiration and ^{14}C remaining in the plant-soil-soil gas continuum (system level) were analyzed during different stages of plant development.
Results At all three levels investigated, ^{14}C kinetics indicated that the C tracer levels changed very little between 15 and 21 days after labeling. Results also showed increasing tracer depletion in the mobile C pool. Consequently, only 0.42 % and 0.06 % of all ^{14}C was still available for shoot respiration 15 and 21 days after labeling, respectively.
Conclusions The similarities between ^{14}C tracer kinetics at the three investigated levels indicate that tracer disappearance from the mobile pool and distribution throughout the plant-soil system was nearly complete between 15 and 21 days after labeling. Therefore, this appears to be the point at which the pulse labeling approach provides sufficiently precise data concerning the use of C (assimilated during labeling) for root growth, rhizodeposition, root respiration and the microbial turnover of rhizodeposits.

Keywords Carbon allocation · ^{14}C pulse labeling · Partitioning coefficients · Carbon pool · Belowground respiration

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Introduction

It has become clear in recent years that precise time-resolved data concerning carbon (C) fluxes in the soil are urgently needed to improve the reliability of terrestrial C cycle models from the local to the global scale (Finzi et al. 2015; Kardol et al. 2013; Oikawa et al. 2014), as most processes regulating the C dynamics of the plant-soil system operate on shorter timescales than a growing season. For example, it was shown by Swinnen et al. (1994b) that the downward transfer of recently assimilated C from wheat is a highly dynamic process during which the C input into different components of the subsurface (e.g., the root, soil, and soil gas) can change substantially over the course of a few days. The only method that can be used to separate plant- and soil-derived C compounds with high temporal resolution is the C isotope pulse labeling approach, in which labeling is performed for a few hours at different plant developmental stages, such as emergence, tillering, elongation growth, flowering, and ripening (Meharg 1994; Kusyakov and Domanski 2000; Swinnen et al. 1994b; Werth and Kuzyakov 2008).

By contrast, the commonly used continuous labeling approach for estimating C input into the soil (Nguyen 2003) results in poor temporal resolution due to labeling over weeks or months. In addition, differentiating between labeled C originating from belowground C fluxes, such as root respiration or root exudation (loss of carbon from living roots), microbial turnover of rhizodeposits, and senescent or dead roots becomes increasingly difficult as labeling time increases to more than a few weeks (Meharg 1994). This drawback is accompanied by the considerably higher technical and financial resources necessary for continuous labeling compared with pulse labeling (Meharg 1994; Kuzyakov and Domanski 2000). Finally, ^{13}C discrimination experiments have insufficient temporal resolution and sensitivity, as the transfer of root-derived C to soil organic matter can only be investigated after more than 40 days of plant growth (Werth and Kuzyakov 2008). This is true to an even greater extent for the ^{14}C-bomb spike approach, which can only be used to investigate time ranges of one to several years (Richardson et al. 2015; Trumbore 2000).

To estimate the transfer of C into the soil, relative tracer partitioning coefficients are frequently used (Bolinder et al. 2007, 2012; Kuzyakov and Domanski 2000; Nguyen 2003; Warembourg and Estelrich 2001; Werth and Kuzyakov 2008). These partitioning coefficients represent the proportional distribution of tracer into shoots, roots, rhizodeposition, and belowground respiration following labeling, and they are calculated as the percentage of the applied tracer or total tracer found in the plant-soil-soil gas system at or until sampling (Nguyen 2003). To model the absolute transfer of freshly assimilated C into the subsurface, it is necessary to link the relative partitioning of the tracer to the absolute C flux of the plant, which can be the C increment of shoot growth per day (Swinnen et al. 1994b), developmental stage (Meng et al. 2013; Werth and Kuzyakov 2008), or the entire vegetation period (Kuzyakov and Domanski 2000).

The homogeneous labeling of total C tracer from pulse labeling fluxes and plant C pools is a mandatory prerequisite for accurately determining absolute C fluxes based on the distribution of a tracer within a plant. However, in contrast with continuous labeling, this is not the case if the pulse labeling method is applied from the start (Meharg 1994). This difference arises from the dynamics of C transfer and turnover in plants. As a result, there can be different ratios of labeled (new) and non-labeled (old) C used to build molecules, different turnover rates of labeled metabolites, or different transfer rates for the short and long distance transport of labeled photosynthates caused by the different C sink activities of tissues and organs (Ainsworth and Bush 2011; Bihmidine et al. 2013; Lemoine et al. 2013). Several pulse labeling experiments have repeatedly demonstrated that tracers can flow very quickly through a plant. For example, ^{14}C was detected in hydroponic solution around the roots only two hours after the labeling of wheat leaves (Dilkes et al. 2004), and Nguyen et al. (1999) found that the specific activity of ^{14}C in belowground respiration reached its maximum approximately 6 hours after the labeling of maize shoots. However, it is also known that photosynthates (substances formed by photosynthesis) are used to build temporary carbohydrate storage molecules such as starch and fructans (Bihmidine et al. 2013; Heldt and Piechulla 2008). Fructans are a group of water-soluble oligosaccharides and polysaccharides built of one or more fructose residues and a sucrose unit, and they primarily accumulate in the stems of cereals (Schnyder et al. 1993; Wardlaw and Willenbrink 2000). Lattanzi et al. (2005)

and Lehmeier et al. (2010) showed that temporary storage pools with mean residence times of 4 to 9 days are involved in the growth and respiration of grasses. Considering that not only sucrose but also glucose, fructose, raffinose, stachyose, and sugar alcohols can appear in phloem sap (van Bel and Hess 2008), it cannot be ruled out that the degradation of labeled temporary storage pools delays the distribution of tracers throughout the entire plant-soil-soil gas continuum. Therefore, the kinetics of the C tracer at the molecular level can also influence the distribution and turnover of tracer at both the component (e.g., shoot or soil gas) and whole plant-soil system levels.

Consequently, the relationship between the relative amount of tracer in the shoot and C shoot increment can only provide precise information about the use of assimilated C to produce shoot matter if the tracer in the mobile C pool is depleted. In other words, any tracer that is not used for shoot growth must have disappeared from the shoot via respiration or downward transfer. Moreover, any C tracer not used for the production of root matter must have disappeared from the root via respiration or exudation before the tracer distribution from pulse labeling experiments can be used to accurately calculate the use of recently assimilated C for the production of root matter and rhizodeposits, as well as for the root respiration and microbial turnover of fresh rhizodeposits.

However, there is limited information concerning the point in time after labeling at which the distribution of tracer most accurately represents the usage of photosynthates to produce plant matter in the various components of the investigated system. An analysis of different studies on C partitioning (differential distribution of photosynthates) into shoots, roots, rhizodeposition and belowground respiration showed that samples were collected at different times, ranging from 1 day (Gregory and Atwell 1991; Sey et al. 2010), 16 days (Pausch et al. 2013), 19 days (Swinnen et al. 1994b), 21 days (Stewart and Metherell 1999), and 27 days (Meng et al. 2013) after labeling. However, authors seldom justify their decisions concerning the sampling time after pulse labeling. Stewart and Metherell (1999) found more ^{13}C in roots 21 days after labeling compared with 1 hour after labeling. Swinnen et al. (1994b) analyzed the partitioning of ^{14}C in shoots and roots 5, 19, and 33 days after labeling, and they found that the distribution of ^{14}C between root and shoot reached an equilibrium 19 days after labeling, which was stable until 33 days after labeling. Furthermore, based on model assumptions, the authors calculated that more than 99 % of the assimilated, but not respired, ^{14}C was incorporated into structural plant compounds 19 days after labeling. Based on these results, Swinnen et al. (1994b) concluded that the distribution of the tracer must preliminarily be finished by 19 days after labeling. However, these authors did not ensure that their assumptions about the distribution of assimilated C tracer in the different C pools were correct.

An investigation of C tracer kinetics at three different system levels should provide sufficient information about the timing of tracer distribution in the plant-soil-soil gas system, as well as the point in time at which the tracer in the mobile C pool is depleted. At the molecular level, comparing the tracer dilution rates of water- and toluene-soluble fractions with those of substances from the structural C pool of shoot appears suitable for identifying the moment at which the incorporation of C tracer into structural substances of the shoot is complete. The underlying idea is that following the incorporation of the tracer into the structural substances of the shoot (e.g., α-cellulose, hemicellulose, and lignin), the specific activity of tracer in these substances should only decrease if dilution occurs following the incorporation of new ^{12}C. By contrast, the specific activity of tracer in the soluble fractions should decrease faster than in the structural C pool, because the soluble fractions likely include the majority of carbohydrates from the mobile C pool, and their tracer will be diluted due to respiratory turnover, transfer into the root, and incorporation into the structural C pool of the shoot, which require new ^{12}C to compensate C loss in the mobile C pool. Consequently, the tracer dilution rates of the soluble fractions should substantially change if the tracer disappears from the mobile C pool.

When no more labeled mobile carbohydrates are available in the shoot, the tracer levels in shoot respiration should also substantially change, which should be detectable in tracer kinetics at the system level. In addition, the C tracer levels in the plant-soil-soil gas system, expressed as a percentage of gross ^{14}C assimilation, should indicate the point in time at which the C tracer amount in the entire plant-soil-soil gas system no longer changes.

It is known that the magnitude and velocity of C transfer within the plant can be influenced by plant age

and environmental factors (Brüggemann et al. 2011; Lambers et al. 2008; Yasumura 2009), so therefore, an investigation of C tracer kinetics at the component level is required. Considering that the soil gas component is located furthest from the shoot, any potential influences of plant age or environmental conditions on the timing of C tracer distribution within the plant should be apparent in this component.

The aim of this investigation was to help fill our knowledge gaps concerning the dynamics of C tracer distribution and its timing in the plant-soil system using the pulse labeling approach. We investigated ^{14}C tracer kinetics after pulse labeling in spring rye as a model plant at the molecular, component, and system levels. ^{14}C was used as a tracer because even low-level ^{14}C pulse labeling is more sensitive than ^{13}C labeling (Carbone et al. 2007). The simultaneous analysis of tracer kinetics at these three levels should allow us to determine the point in time at which the distribution of a tracer within the plant is preliminarily complete. Consequently, at this point in time, the partitioning of tracer within the plant-soil-soil gas system represents the proportional use of C for shoot and root growth, as well as for the production of rhizodeposits, given our assumptions are correct.

Materials and methods

Cultivation of plants

To investigate ^{14}C tracer kinetics at different levels and to determine ^{14}C partitioning within the plant-soil-soil gas and plant-soil-soil gas-atmosphere systems, several pulse labeling experiments were performed under controlled and outdoor conditions using spring rye (*Secale cereale*), cultivar Sorom, as a model plant. Under controlled conditions, 4 plants were grown per pot. Each pot had a depth and diameter of 20 cm and was filled with 6 kg albic luvisol topsoil obtained in Müncheberg, Germany. Temperatures in the growth chamber were maintained at 24 °C for 14 h during the day (light intensity: 350 $\mu E\ m^{-2}\ s^{-1}$) and at 18 °C for 10 h during the night. Pots were fertilized with 350 mL nutrient solution (3 mM K_2HPO_4, 10.3 mM NH_4NO_3, 1.2 mM K_2SO_4, 2.4 mM $MgSO_4$, 0.6 mM $CaCl_2$ and 0.24 mM Fe-Ethylene diamine tetraacetic acid (Fe-EDTA, $C_{10}H_{12}N_2O_8FeNa$, H_2O)) before sowing and after emergence each fourth week. To maintain a gravimetric water content of approximately 0.17 g / g dry soil, pots were watered with deionized water.

Additionally, a field experiment was conducted on loamy sand (albic luvisol) at the Leibniz Center for Agricultural Landscape Research (ZALF), Müncheberg, Germany (N 52°31', E 14° 07'). Approximately two weeks before rye seeds were sown in the field, several plastic pots (Fig. 1, length: 110 cm, outer diameter: 16 cm, inner diameter: 15 cm) made of commercially available KG sewer pipes and accessories (KG DN 160 from Marley, Germany) were placed in the field using a specially manufactured driller (hole depth of 110 cm). The soil obtained during drilling was placed stepwise in portions representing approximately 10 cm soil depth on a plastic tarp. Afterward, the pots were embedded in the field and filled with the obtained soil portions to create a 100 cm long soil column with a similar soil profile as in the field. The dry mass of the soil (including stones) in the pots was approximately 28 kg. The field (including the open embedded pots) was fertilized with 94 kg Ca ha^{-1}, 114 kg K ha^{-1}, 12 kg Mg ha^{-1}, 20 kg P ha^{-1}, and 16 kg S ha^{-1} using a commercial mineral fertilizer (Thomaskali, K+S KALI GmbH, Germany) on the day before sowing. Subsequently, the field was fertilized with N by applying liquid fertilizer (60 kg N ha^{-1} per application; NTS urea-ammonium nitrate solution, Nutri-Tech Solutions, Australia) 4 and 40 days after sowing. Consequently, each pot received 189 mg Ca, 201 mg K, 24 mg Mg, 35 mg P and 28 mg S through fertilization with Thomaskali, as well as 106 mg N on each of the two N application dates.

Sowing of rye in the field was performed after pot installation, ensuring that the plants in the pots grew as part of the normal rye stock. A single plant was grown in a reduction tube inside the pots (Fig. 1). Between sowing and harvesting, the average daytime temperature was 11 °C. Total precipitation over the whole year was 549 L m^{-2} and did not differ from the long-term average precipitation at this site. At the time of labeling, three to four pots were excavated and transferred to an open space that was equipped for labeling and collection of CO_2 from the head space of the pots (see the "^{14}C pulse labeling"). During the investigation periods (21 days after labeling), pots were irrigated with deionized water with an amount equivalent to the local precipitation.

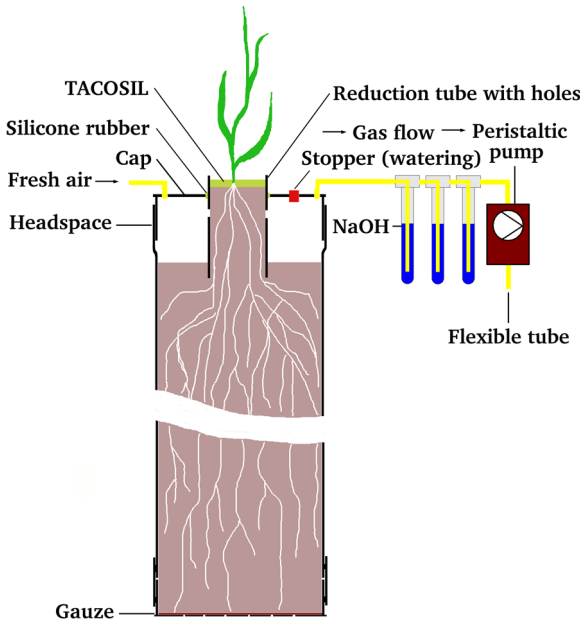

Fig. 1 Design of the pots for ^{14}C pulse labeling

Tracer application, preparation, and analysis

^{14}C pulse labeling

In the outdoor experiment, each plant was labeled separately with $^{14}CO_2$ at six different developmental stages between emergence and late milk-ripening stage (see the "Investigation of ^{14}C at the component level"). The top of each pot was closed with a perforated cap with two tubing connections (inlet and outlet, Fig. 1) 24 h before labeling. Afterward, holes in the cap around the reduction tubes as well as holes in the bottom of the pot were sealed with semi-solid acid-free silicon rubber. The soil surface within the reduction tube was sealed with liquid silicon rubber (TACOSIL 170 with 3 % cross-linker no. 28 from Thauer & Co. KG, Germany) to separate the soil gas in the pots from the atmosphere. As shown in Fig. 1 the use of the reduction tube allowed for a natural vertical gas exchange between soil and atmosphere due to the creation of a headspace. Moreover, the use of a reduction tube leads to a reduction in the area that has to be sealed with silicone rubber to separate the shoot from the subsurface. In addition, the upper layer of soil in the reduction tube can easily be removed to place silicon rubber exactly at the shoot basis. Gas exchange between the soil of the reduction tube and the headspace was promoted by lateral holes (see Fig. 1). Before labeling, tubing connections in the caps were closed and plants were covered with transparent plastic bags. In the next step, each plant was labeled with up to 10 MBq ^{14}C using the locking opening of the bag. After removing the plastic bags, tubing connections were opened and connected to flexible tubes for fresh air supply and for sucking gas out of the soil headspace. The CO_2 of the soil gas was continuously trapped in a NaOH solution to determine the ^{14}C activity of belowground respiration. The NaOH solution (3 x 12 mL 1 M NaOH) was renewed daily until harvesting (21 days after labeling).

^{14}C labeling was conducted under controlled conditions in a two-piece box (width: 56 cm, length: 118 cm, height: 96 or 196 cm, the latter including an extension for tall plants) with a transparent Plexiglas top inside a growth chamber. Two rubber straps (window seals) between the top and bottom rim of the box were used to seal the box. The temperature in the box was maintained at 24 °C for 14 h during the day and at 18 °C for 10 h during the night. For each investigated developmental stage (see the "Investigation of ^{14}C at the molecular level" and "Investigation of ^{14}C at the system level"), 3 to 5 pots with 4 plants each were labeled with up to 25 MBq $^{14}CO_2$. A 60 mM $NaH^{14}CO_3$ solution with 8 MBq ml^{-1} was placed into a beaker containing 10 mL 16 M H_3PO_4 to produce $^{14}CO_2$ inside the Plexiglas chamber. After 10 h, the air around the shoots inside the Plexiglas box (shoot space) was pumped through NaOH granules to absorb free leftover $^{14}CO_2$ from the labeling process. After removing free $^{14}CO_2$ from the shoot space, the continuous flow of fresh air through the Plexiglas box was established using a vacuum pump. A bypass between the outlet of the box and the pump was established to obtain a portion of the labeled CO_2 released by shoot respiration. CO_2 from the air flow was trapped continuously in 3 test tubes containing 12 mL 1 M NaOH each to determine the ^{14}C activity of the shoot space. Both the main flow of fresh air through the Plexiglas box and the flow through the bypass were controlled by flow meters.

The bottoms and tops of each pot were sealed with semi-solid acid-free silicon rubber and a liquid silicon rubber (TACOSIL 170 with 3 % cross-linker no. 28 from Thauer & Co. KG, Germany) 24 h before labeling, similar to the outdoor experiment. The inlet and outlet tubing connections of the pot caps were

connected with the box wall via flexible tubes to supply the pots with fresh outside air (inlet) as well as to suck soil gas out of the soil headspace (outlet). Outside of the box, CO_2 within the soil gas was trapped identically as under outdoor conditions.

During and after labeling, until the harvesting of the pots, the CO_2 concentration inside the box was kept at a constant level of approximately 390 ppm, which was monitored using an infrared CO_2 measurement device (Guardian plus, Pewatron AG, Zürich, Swiss) connected to the shoot space of the box in a closed loop (flexible tube). The influx of fresh CO_2 was determined using an electronic valve, which was placed between the box and a CO_2 gas bottle and controlled by the CO_2 measurement device. A cooler inside the box connected to a cryostat was used to control the air temperature during irradiation. In addition, a continuously running fan located near the cooler was used to mix the air inside the box.

Therefore, labeling under controlled and outdoor conditions differed principally with respect to the regulation of CO_2 concentration, temperature, and soil humidity (see also the "Cultivation of plants") during and after labeling, the number of plants at labeling (labeling of single plants under outdoor conditions and simultaneously labeling of several plants in one Plexiglas box under controlled conditions), and the geometry of the used pots.

Sample preparation

Pots were harvested at different points in time, ranging from 2 to 28 days after labeling, depending on the objective of the experiment. Rye shoots were cut at their base at harvest and dried in an oven at 104 °C. Afterward, pots from the outdoor experiment were dissected into five layers, each 20 up to 30 cm thick. The soil (including roots) of each layer was immediately frozen at -26 °C and later defrosted separately at 4 °C overnight to separate the roots from the soil. The soil from the small pots used in the experiments under controlled conditions was not divided, but was also frozen at -26 °C immediately after harvesting the shoots. After hand-picking the roots from soil portions of approximately 5 to 6 kg, the roots were washed with 250 mL deionized water in an Erlenmeyer flask on a horizontal shaker for 1 h to remove adhesive soil. Next, the roots were dried at 104 °C. The soil that was separated from the roots by washing was operationally defined as rhizosphere soil. Washing water and rhizosphere soil were transferred to a sieve with 300-μm mesh size. Using a wet-sieving procedure, all root fragments ($\geq$ 300 μm) which released from the roots during the washing procedure were obtained. Sieving was considered to be complete when only white sand was visible on the top of the sieve. Then, sand and root residues remaining in the sieve were dried at 104 °C. The soil fractions that passed through the sieve were dried at 104 °C after the complete evaporation of water (approximately 1 to 2 L) at 80 °C to obtain all ^{14}C labeled material from the rhizosphere soil fraction $<$ 300 μm.

To determine the amount of root fragments $\geq$ 300 μm, which could not be selected by hand-picking, aliquots of bulk soil were analyzed. After hand-picking of the roots, each soil portion ($\approx$ 5-6 kg) was homogenized by hand, and two bulk soil aliquots (50 g each) were taken from each soil portion, totaling two and ten aliquots for the small and large pots, respectively. Next, each bulk soil aliquot was dissolved in 250 mL deionized water and shaken in an Erlenmeyer flask on a horizontal shaker for 1 h. Afterward, a wet-sieving procedure was used to separate root fragments $\geq$ 300 μm and material $<$ 300 μm. Sieving was considered to be complete when only white sand remained on a sieve with 300-μm mesh size. Sand (including root fragments) from the top of the sieve, washing water ($\approx$ 1-2 L) and the soil fractions $<$ 300 μm of bulk soil aliquots were treated like the fractions of rhizosphere soil to calculate all ^{14}C in root fragments $\geq$ 300 μm and labeled material of the bulk soil fraction $<$ 300 μm. Apart from organic material, the soil fraction $\geq$ 300 μm comprised mostly sand while the fraction $<$ 300 μm comprised mostly silt and clay. In addition, the dry soil mass of each bulk soil portion was determined using two soil samples with a fresh weight of 50 g.

Extraction of mobile and structural C pool fractions

Toluene-, water-, and hot-water-soluble fractions, as well as α-cellulose, hemicellulose, and lignin, were separated from the rye shoot material using a modified version of the extraction procedure described by Allen et al. (1974). Toluene-, water-, and hot-water-soluble fractions should include the majority of sugars, sugar derivatives and amino acids, which can flow through the plant, whereas α-cellulose, hemicellulose, and

lignin represent substances from the structural plant C pool.

Approximately 12 to 17 g of shoot powder from each sampling time were used for extraction. First, samples were boiled three times (for 3 h each) in a round-bottom flask with 165 mL toluene under a continuous flow of N_2. After each boiling treatment, the solvent was vacuumed through a fritted glass filter and concentrated using a rotary evaporator. The concentrated toluene-soluble fraction was dissolved with toluene and water at a ratio of 1:1 and incubated for 18 h at 4 °C. Next, this mixture was centrifuged (RCF = 200 g, 5 min, room temperature) to separate the toluene phase (with the toluene-soluble fraction) from the water-soluble fraction.

The residue in the round-bottom flask was boiled three times in 650 mL water (for 3 h each). The hot water was vacuum-filtered through a fritted glass filter and concentrated via rotary evaporator and dried to obtain the hot-water-soluble fraction. The residue in the round-bottom flask was washed with hot water on a fritted glass filter, dried and divided further to extract the α-cellulose, hemicellulose, and lignin.

For the lignin extraction, 5 to 9 g residue was cooled down to 15 °C and slowly mixed with 26 mL 72% H_2SO_4 in a round-bottom flask before incubation for 2 h at 20 °C. After incubation, 800 mL H_2O was added, and the mixture was boiled for 9 h under a continuous flow of N_2. The solvent was vacuumed through a fritted glass filter and discarded. This procedure was repeated three times, and the material was washed with hot water to maintain a neutral pH. The remaining material was dried on a fritted glass filter to obtain lignin.

For the extraction of α-cellulose and hemicellulose, 5 to 9 g residue from the hot water extraction was mixed with 480 mL H_2O, 16 mL of 10 % acetic acid, and 4.8 g sodium chlorite. This mixture was shaken at 75 °C under a continuous flow of N_2 for 1 h. The procedure was repeated three times. Next, the mixture was cooled down to 0 °C, and the solvent was vacuumed through a fritted glass filter and discarded. The residue was washed ten times with cold water and three times with acetone on a fritted glass filter before drying to obtain holocellulose. The dried holocellulose was mixed with 100 mL 24 % KOH and incubated on a shaker for 4 h at 20 °C. After incubation, the solvent was vacuumed through a fritted glass filter and adjusted to pH 4 with acetic acid. The dissolved material was precipitated by adding 350 mL ethanol (96 %) and incubating overnight. Subsequently, the precipitate was washed with ethanol on a fritted glass filter and dried to obtain hemicellulose.

The residue was washed with H_2O to maintain neutral pH and then swirled in a crucible with 25 mL 5 % acetic acid. Next, the residue was washed again with water to achieve a neutral pH. Subsequently, the material was washed three times with acetone before drying to obtain α-cellulose. A flow chart of the extraction procedure is shown in Fig. 2.

C analysis

C content and ^{14}C activity from the soluble fractions and substances of the structural C pools of the shoot (see the "Extraction of mobile and structural C pool fractions") were determined by combustion at 1200 °C under continuous O_2 flow. The CO_2 from the elemental analyzer (CS 500, ELTRA GmbH, Germany) was

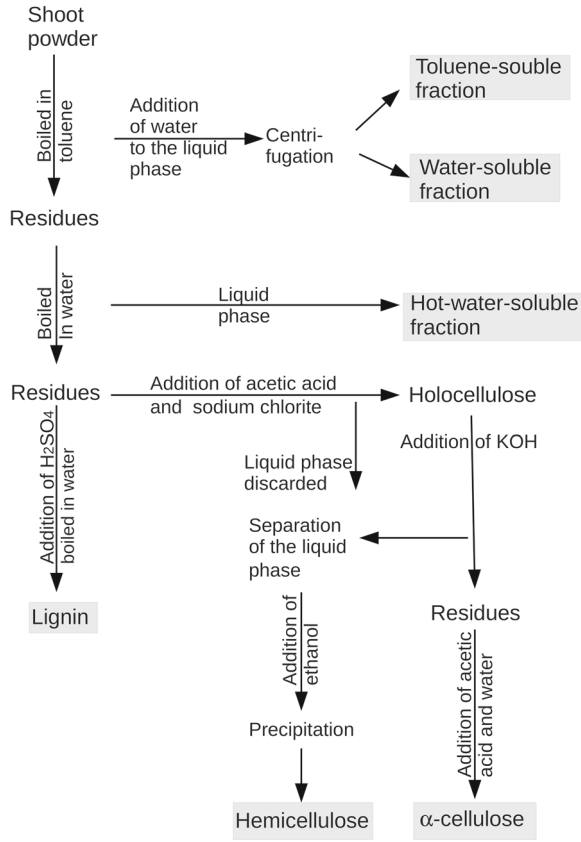

Fig. 2 Flow chart of the extraction procedure to obtain the fractions and substances of the mobile and structural C pools

absorbed in a CO_2 trap (Qureshi et al. 1985), which was filled with 7 mL Carbo-Sorb E (PerkinElmer, Rodgau, Germany). Next, 3 mL aliquots of CarboSorb E were mixed with 12 mL Permafluor-Scintillator (PerkinElmer) to measure ^{14}C activity using a liquid scintillation counter (LS 6000SC, Beckman, Germany).

The same procedure was used to determine the C content and ^{14}C activity of the shoot and root samples, the soil fractions ≥ 300 μm and < 300 μm of the rhizosphere, and bulk soil. The ^{14}C activity of the shoot and belowground respiration was determined using 3 mL aliquots of the NaOH solution from the CO_2 traps of the labeling experiments after they were mixed with 12 mL UltimaGold-Scintillator (PerkinElmer). The liquid scintillation counter was calibrated using ^{14}C, ^{3}H, and background standards from Beckman, according to the device manual.

^{14}C calculation for all components

The ^{14}C amounts resulting from belowground or shoot respiration were calculated using the specific ^{14}C activity of the NaOH solution in the CO_2 traps (expressed as Bq per mL NaOH) multiplied by the total volume of NaOH in the trap. Finally, the respired ^{14}C activity values of each day were summed to obtain the total ^{14}C activity of belowground and shoot respiration.

The total amount of ^{14}C in shoots and hand-picked roots was calculated by multiplying the specific ^{14}C activity (Bq per mg shoot or root material) by the total weight of the shoots and roots, respectively. The total ^{14}C amount of the root fragments that could not be recovered by hand was calculated using the specific ^{14}C activity (Bq per mg) of the samples ≥ 300 μm of rhizosphere and bulk soil and the weights of the fractions of rhizosphere and bulk soil from the entire pot. Next, the amount of ^{14}C in the root fragments and roots was summed to obtain the total ^{14}C amount in the entire root system. The amount of ^{14}C in the fractions < 300 μm, which was obtained by wet-sieving rhizosphere soil and bulk soil aliquots, was determined by multiplying their specific ^{14}C activities by the total weights of these fractions in the whole root system and pot. The resulting ^{14}C activity values were summed to obtain the entire ^{14}C activity of the total soil fraction < 300 μm in the pot. This total ^{14}C activity in soil fractions < 300 μm was operationally defined as ^{14}C from detectable rhizodeposition, as a portion of the ^{14}C from labeled rhizodeposits must have been released by microbial respiration between labeling and sampling.

Investigation of ^{14}C at the molecular level

To determine the point in time at which ^{14}C disappeared from the mobile C pool, the specific activity of ^{14}C in the soluble fractions and substances of the structural C pool were determined on days 11, 15, 19, 25 and 28 after labeling. For this determination, the toluene-, water-, and hot-water-soluble fractions, as well as the α-cellulose, hemicellulose and lignin fractions (structural C pool), were separated from spring rye shoots after drying and grinding of the plant material (see the "Sample preparation"). Separating these different substances and fractions is very labor-intensive, as described in the "Extraction of mobile and structural C pool fractions". Therefore, only one set of plants (5 pots with 4 plants per pot) was labeled under controlled conditions (see the "Cultivation of plants" and "^{14}C pulse labeling") at tillering for this investigation, and all four shoots from one pot were pooled for each of the five sampling times. After determining the specific ^{14}C activity of each sample, the ^{14}C dilution in each substance or fraction was modeled using the best-fit two-parameter model found via non-linear regression. The first derivatives of the modeled ^{14}C concentration from each substance or fraction were used to investigate changes in the ^{14}C dilution rates over time. In addition, the half-life of the specific ^{14}C activity was calculated for the investigated timespan using Eq. 2 when possible (i.e., when time (t) was found in the exponent of the function model, as in Eq. 1). Particularly when considering the structural C pool, the half-life provides the time at which the specific ^{14}C activity will have decreased by one-half due to the incorporation of ^{12}C into the structural C pool.

$$^{14}C\ concentration = a * e^{-b*t} \quad (1)$$

$$t_{1/2} = \frac{-ln(2)}{-b} \quad (2)$$

Investigation of ^{14}C at the component level

At the component level, the influence of plant age under outdoor conditions on the release of $^{14}CO_2$ by belowground respiration was analyzed for 21 days after pulse labeling at emergence, stem elongation, and ear emergence. For this investigation, three plants per plant set (total of 9 plants) were labeled and harvested 21 days after labeling. The ^{14}C that was released each day via belowground respiration was calculated as the percentage of total ^{14}C found in the shoot, root, and detectable rhizodeposition on the day of harvest and the total ^{14}C amount released by belowground respiration between labeling and harvest. The experimental design used here could not distinguish between root and microbial respiration. Therefore, the ^{14}C-labeled belowground respiration includes ^{14}C-labeled CO_2 released by root respiration and microbial turnover of ^{14}C-labeled rhizodeposits.

In addition to analyzing the daily release of ^{14}C-labeled CO_2 and its cumulative curves, the normalized ^{14}C-labeled belowground respiration of each plant set was investigated. For this investigation, the accumulated ^{14}C amount of belowground respiration for each plant set until harvest (expressed as the percentage of total ^{14}C found in the plant-soil-soil gas system) was normalized to 100 %.

Investigation of ^{14}C at the system level

At the system level, the ^{14}C dynamics in the plant-soil-soil gas system, expressed as a percentage of gross ^{14}C assimilation, were analyzed to identify the time at which the ^{14}C amount of the entire plant-soil-soil gas system no longer changed. For this investigation, three plant sets were grown to elongation, ear emergence and late milk-ripening stages under controlled conditions (see the "Cultivation of plants"). Subsequently, plants were pulse labeled (see the "^{14}C pulse labeling"), and plants from each set were harvested at different times (days 2, 7, 10, 11, 14, 15, 19 and 21 after labeling). During and after labeling, the plants were grown in a Plexiglas box, which made it possible to capture shoot respiration from labeling until harvest. The ^{14}C activity trapped in the shoot space (Plexiglas box) was divided by the number of pots within the box to calculate the average ^{14}C shoot respiration per pot. In addition, CO_2 from belowground respiration for each pot was also collected between labeling and harvest to determine its ^{14}C amount. After harvest, the ^{14}C amounts of the shoots, roots, and detectable rhizodeposition were determined as described above (see the "^{14}C calculation for all components"). The total ^{14}C found in shoots, roots, detectable rhizodeposition, belowground respiration, and shoot respiration was defined as gross ^{14}C assimilation. Gross ^{14}C assimilation and the total ^{14}C amount in the plant-soil-soil gas system were equalized at the day of pulse labeling by adjusting to 100 %. A curve representing the progression of ^{14}C amounts in the plant-soil-soil gas system (expressed as a percentage of gross ^{14}C assimilation) was modeled using non-linear regression analysis. Additionally, linear regression analyses were used to determine the slope of the measured ^{14}C values between days 7 and 15, as well as days 14 and 21 after labeling.

Statistical analyses

Non-linear regression was used to analyze the turnover of ^{14}C in each soluble fraction or substance of the structural C pool, to investigate the dynamics of the ^{14}C-labeled belowground respiration, as well as to describe the dynamics of the total ^{14}C amount in the plant-soil-soil gas system after pulse labeling. The coefficient of determination (R^2) for all non-linear regression analyses was calculated using 1-(SS error / SS corrected total), according to Kvålseth (1985). All statistical calculations were carried out using the Mathematica 9 software package (Wolfram Research).

Results

Dynamics of ^{14}C dilution in the mobile and structural C pool

^{14}C tracer kinetics at the molecular level show that the ^{14}C activity in all investigated fractions and substances of the rye shoots decreased after pulse labeling (Fig. 3). As shown in Fig. 3, the coefficients of determination (R^2) of the utilized models ranged from 0.951 to 0.999. The p-values of the two parameters, a and b, ranged from < 0.001 to < 0.02 and from < 0.001 to < 0.01, respectively. Both, the coefficients of determination and the statistically significant

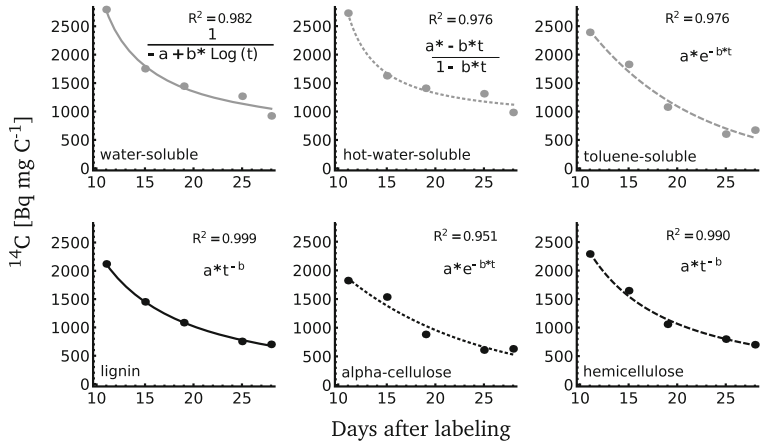

Fig. 3 Specific ^{14}C activity (expressed as Bq per mg C) of different substances and fractions of spring rye shoots after ^{14}C pulse labeling. Each time point represents a pooled sample from the pooled material of four shoots

parameters, a and b, indicate a good model fit for each investigated ^{14}C dilution.

The water-soluble fractions showed a different curve progression of the ^{14}C dilution compared with the toluene-soluble fraction and the substances of the structural C pool (α-cellulose, hemicellulose, and lignin). Moreover, the curve progression of the hot-water-soluble fraction gives the impression that the ^{14}C turnover decreased slower during the final days of the investigation period compared with all other soluble fractions and substances of the structural C pool, although ^{14}C was still detectable in the hot-water-soluble fraction until the end of the investigation, 28 days after labeling.

The differences between the highest and lowest values of the specific ^{14}C activities plotted in Fig. 3 were calculated, and they showed that the fastest ^{14}C dilution occurred in the toluene-soluble fraction. The specific activity of this fraction decreased by 1886 Bq/mg C between days 11 and 28 after labeling. The slowest ^{14}C dilution was observed for α-cellulose, where the specific activity decreased by 1327 Bq/mg C between days 11 and 28 after labeling, yielding a biological half-life of 9.4 days. Thus, the α-cellulose fraction doubled over 9.4 days with the addition of recently produced non-radioactive α-cellulose.

In contrast with the α-cellulose and toluene-soluble fractions, which showed a constant dilution of ^{14}C between days 11 and 28 after labeling, the ^{14}C dilution of the water-soluble and hot-water-soluble fractions changed significantly between days 11 and 15 after pulse labeling. During this period, the specific activity of the hot-water-soluble fraction decreased by 1051 Bq/mg C, which was more than twice the dilution of α-cellulose during that period. This large change is clear in Fig. 4, showing that the ^{14}C activities of the water-soluble and hot-water-soluble fractions decreased (negative sign) by approximately 450 and 600 Bq per mg C, respectively, on day 11. The ^{14}C dilution rate (Bq per mg C per day) of these fractions on day 15 after pulse labeling was nearly the same as that for the structural C pool substances.

Moreover, Fig. 4 shows that the ^{14}C dilution rates of all soluble fractions and substances from the structural C pool converged following day 15 after labeling until the end of the experiment. Considering that the soluble fractions include the majority of substances from the mobile C pool, these findings imply that the biggest change in ^{14}C activity for this C pool occurs before day 15 after pulse labeling, and then gradually decreases.

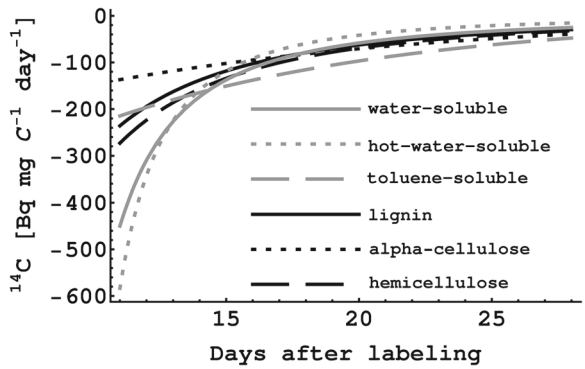

Fig. 4 Dilution rates of the specific ^{14}C activity of different substances and fractions of spring rye shoots after pulse labeling expressed as Bq per mg C per day. Plotted curves are first derivatives of the functions shown in Fig. 3

^{14}C dynamics of the plant-soil-soil gas system

The progression of the ^{14}C amount in the plant-soil-soil gas system of three plant sets labeled at the elongation, end of ear emergence, and late milk-ripening stages was used to investigate ^{14}C tracer kinetics at the system level (see Fig. 5). This progression of the ^{14}C amount is expressed as a percentage of gross ^{14}C assimilation. Therefore, the area above the modeled curve shown in Fig. 5 represents the amount of ^{14}C that was released by shoot respiration, whereas the area below the modeled curve represents the amount of ^{14}C found in the plant-soil-soil gas system. Fig. 5 shows a rapid decrease in the ^{14}C amount in the plant-soil-soil gas system a few days after labeling, which reached approximately the same level between days 14 and 21 after labeling in all three plant sets, regardless of plant age.

Regression analysis showed that an asymptotic model was suitable for describing the changes in ^{14}C amount in the plant-soil-soil gas system after pulse labeling because the coefficient of determination (R^2) was 0.993 and all three parameters, a, b and c, were statistically significant ($p < 0.001$), indicating a good model fit. After the rapid decrease in ^{14}C, the fitted curve shows an asymptotic curve progression. Measured ^{14}C amounts averaged 50.7 % of gross ^{14}C assimilation between days 15 and 21 after labeling, and the modeled ^{14}C amount of the plant-soil-soil gas system tended towards 50 % of gross ^{14}C assimilation 21 days after labeling. Therefore, both measured and modeled values between 15 and 21 days after labeling were close to the asymptote, which was 49.9 % at infinity. A comparison of the modeled curve and its asymptote indicated that at 15 and 21 days after labeling, only 0.42 % and 0.06 % of ^{14}C, respectively, in the whole system was available for shoot respiration.

In addition to the non-linear regression, two linear regression analyses were conducted to investigate the slope of the ^{14}C amount in the plant-soil-soil gas system between days 7 and 15, as well as between days 14 and 21, after labeling (Fig. 5). These analyses indicated a slope of -0.44 between days 7 and 15 after labeling, which was significantly different from 0 ($p = 0.007$). The slope of 0.06 observed between days 14 and 21 after labeling was not significantly different from 0 ($p = 0.872$). Taken together, these results indicate that the turnover of ^{14}C by shoot respiration

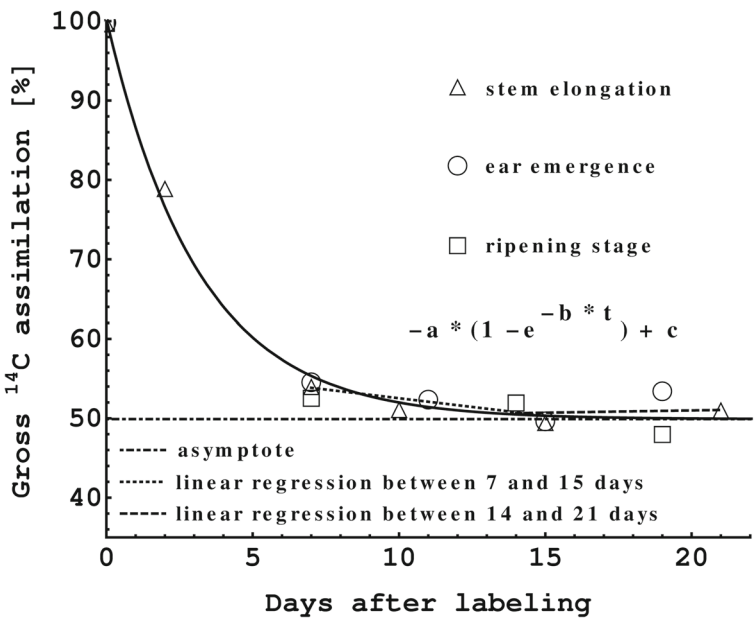

Fig. 5 Changes in the ^{14}C amount of the plant-soil-soil gas system expressed as the percentage of gross ^{14}C assimilation in spring rye after labeling at the elongation growth, ear emergence, and late milk-ripening stages. The sum of ^{14}C recovered in the shoots, roots, and detectable rhizodeposits on the day of harvest and ^{14}C from belowground respiration collected after labeling until harvest was defined as the ^{14}C amount of the plant-soil-soil gas system. The gross ^{14}C assimilation is defined as the sum of the ^{14}C amount of the plant-soil-soil gas system and the amount of ^{14}C respired by shoots

is nearly complete between days 15 and 21 after labeling and that the ^{14}C amount in the plant-soil-soil gas system does not substantially change during this time range. A later re-translocation of ^{14}C from root to shoot due to remobilization was not considered in this analysis, but is discussed in the "^{14}C kinetics at the system level".

Dynamics of ^{14}C labeled belowground respiration

Shortly after labeling at emergence, stem elongation, and ear emergence, the rate of ^{14}C-labeled belowground respiration increased rapidly during the first 24 h after pulse labeling and gradually declined over the following days until harvest. The cumulative ^{14}C activity of the belowground respiration of plants labeled at emergence, stem elongation, and ear emergence averaged 27.4 %, 7.8 %, and 5.0 %, respectively, of the total ^{14}C amount recovered in the plant-soil-soil gas system 21 days after labeling (Fig. 6). These values clearly show that the amount of ^{14}C released by belowground respiration was substantially different between plants of different ages.

However, Fig. 6 also shows that one asymptotic model was suitable for describing the cumulative belowground respiration of ^{14}C-labeled metabolites at all three developmental stages. The coefficient of determination (R^2) ranged from 0.998 to 0.999, and the parameters a and b were both statistically significant (p-value < 0.001). Thus, the curve progression of the cumulative belowground respiration was similar at all three plant developmental stages.

The asymptotic model approaches an upper limit at infinity, which corresponds to 33.2 %, 9.7 %, and 5.9 % of the ^{14}C amount in the plant-soil-soil gas system after labeling at emergence, stem elongation, and ear emergence, respectively. The convergence of the cumulative belowground respiration values with the corresponding asymptote was different for plants of different ages.

However, if the upper limit at infinity of each curve is adjusted to 100 % (33.2 %, 9.7 % or 5.9 % $\triangleq$ 100 %), the cumulative ^{14}C-labeled belowground respiration on day 21 for all three developmental stages averaged 82.5 % of the respective upper limit. Thus, independent of the developmental stage, collection of the belowground respiration until 21 days after labeling provides nearly the same fraction (82.5 ± 2.1 %) of the maximum expected ^{14}C-labeled CO_2 released by root respiration and microbial turnover of labeled rhizodeposits at infinity, assuming that this release of labeled CO_2 follows the model.

According to an additional analysis, the cumulative ^{14}C-labeled belowground respiration of the three plant sets on day 21 after labeling were set to 100 % (27.4 %, 7.8 % or 5.0 % $\triangleq$ 100 %) to normalize the ^{14}C-labeled belowground respiration of the different developmental stages. Afterward, the normalized daily releases of $^{14}CO_2$ of the three plant sets were calculated and plotted in Fig. 7.

As shown in Fig. 7, normalizing ^{14}C-labeled belowground respiration revealed similar dynamics of ^{14}C-labeled CO_2 production from roots and microbial turnover of fresh rhizodeposits after pulse labeling for

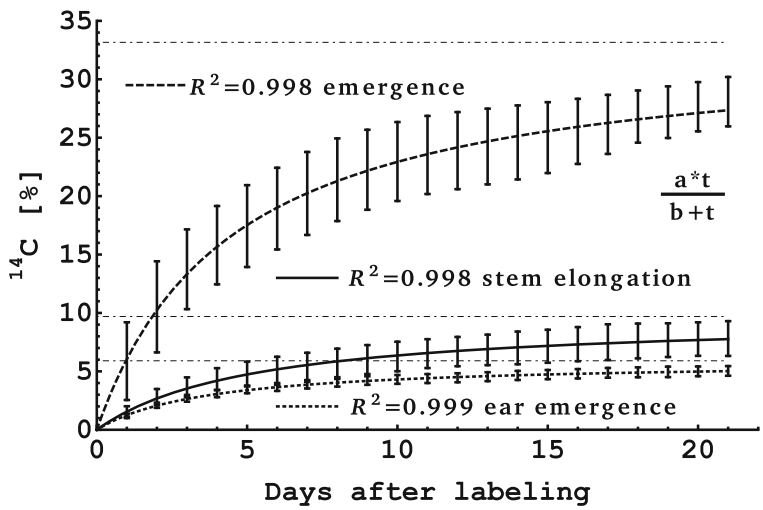

Fig. 6 Cumulative curves of ^{14}C-labeled belowground respiration expressed as percentages of the total ^{14}C amount recovered in the plant-soil-soil gas system 21 days after labeling at emergence, stem elongation, and ear emergence. The error bars represent the standard deviation (n=3). The dashed/dotted lines represent the upper limits of the modeled curves

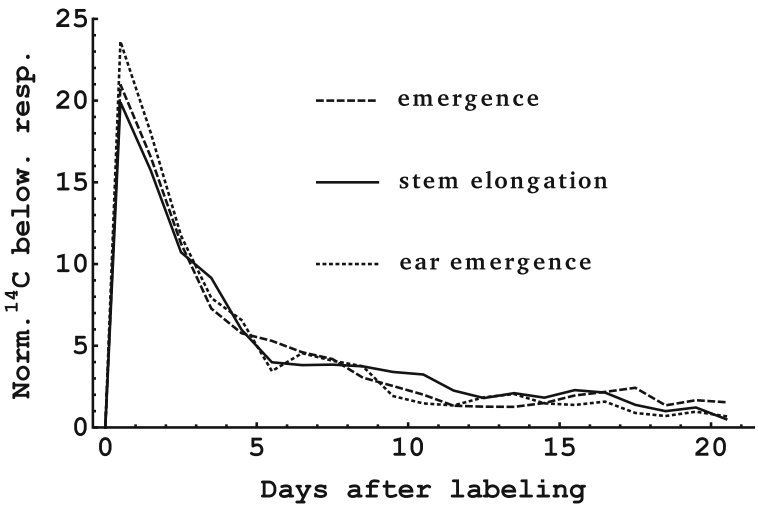

Fig. 7 Normalized ^{14}C-labeled belowground respiration of three plant sets after pulse labeling at emergence, stem elongation and ear emergence. Each line represents the average daily release of ^{14}C-labeled CO_2 (n=3 plants), which was normalized by adjusting the cumulative ^{14}C activity of each plant set to 100 %

plants of different ages under outdoor conditions. An analysis of the normalized ^{14}C-labeled belowground respiration showed that approximately 80 % of the ^{14}C-labeled CO_2 that was captured until day 21 after labeling was released before day 8 or 9, regardless of plant developmental stage.

Discussion

The ^{14}C kinetics at three different levels (molecular, component, and system) were investigated to determine the point in time after pulse labeling at which the distribution of a C tracer within a plant-soil-soil gas system most accurately represents the use of assimilated C to produce plant matter. As already mentioned, this point in time after pulse labeling should be reached if the tracer is disappeared from the mobile C pool. The investigation at the molecular level shows that the ^{14}C amount of the water-soluble fractions was diluted by new ^{12}C, similar to the substances (α-cellulose, hemicellulose, and lignin) from the structural C pool of rye shoots 15 days after labeling. At the same time, the ^{14}C kinetics at the system level indicated that only 0.42 % of total ^{14}C was still available for shoot respiration. ^{14}C kinetics also showed that the ^{14}C activities of all three investigated levels changed only minimal between 15 and 21 days after labeling. Moreover, the release of ^{14}C-labeled CO_2 through belowground respiration (component level) indicated that the flow of ^{14}C through the plant and it release by root respiration and microbial turnover in the soil was not affected by different plant developmental stages under favorable conditions.

^{14}C kinetics at the molecular level

As assumed, our investigation at the molecular level clearly showed that the ^{14}C dilution in soluble fractions of the rye shoot progressed faster than the ^{14}C dilution in the substances of the structural C pool. Considering that the investigated soluble fractions likely included the majority of carbohydrates from the mobile C pool of the shoot, the faster ^{14}C dilution in these fractions can be explained by respiratory turnover of ^{14}C in the shoot, incorporation into structural C pool of the shoot, and transfer of labeled carbohydrates from the shoot to the root, which would induce rapid dilution with new ^{12}C. In contrast with this, it can be assumed that ^{14}C in lignin and α-cellulose in the shoot will only be diluted by the incorporation of new ^{12}C, as these substances cannot be recycled and used as carbon reserves; this should also apply to hemicellulose because the plants were not stressed (Hoch 2007).

Moreover, our comparison of the ^{14}C dilution rates of the different fractions showed substantial changes in the ^{14}C dilution rates in the two water-soluble fractions of the rye shoots. The ^{14}C in the two water-soluble fractions was diluted rapidly between days 11 and 15, and the ^{14}C concentration in these two fractions was diluted at nearly the same rate as the ^{14}C in the structural C pool 15 days after labeling. This finding indicates that the remaining ^{14}C in the

water-soluble fractions will be diluted by new ^{12}C 15 days after labeling, similar to the substances of the structural C pool. Consequently, ^{14}C in the mobile C pool of the shoot must be nearly depleted and the incorporation of ^{14}C into the structural C pool, the transfer of labeled carbohydrates from shoot into root, and the respiratory turnover of ^{14}C in the shoot must be nearly finished by 15 days after labeling. The latter result is supported by our investigation of ^{14}C tracer kinetics at the system level, because in the time range between days 15 and 21 after labeling, only 0.06% to 0.42% of the assimilated ^{14}C were available for shoot respiration. This finding, as well as the low ^{14}C dilution rates of the water-soluble fractions between days 15 and 28 after labeling which were similar to the ^{14}C dilution rates of substances from the structural C pool at this time lead us to assume that the ^{14}C remaining in these fractions after 15 days is primarily found in substances of the structural C pool, which became soluble during the extraction procedure. Admittedly, only an investigation of the most important sugars, sugar derivatives, amino acids, and temporal C storages and their ^{14}C content between days 15 and 28 after labeling can provide more certainty on this issue.

^{14}C kinetics at the system level

At the system level, the ^{14}C kinetics of rye plants after labeling at the elongation growth, ear emergence, and late milk-ripening stages under controlled conditions were analyzed. Similar to Swinnen et al. (1994b), we observed a flat curve progression after a rapid decrease of total ^{14}C within the plant-soil-soil gas system. This curve progression implies that the turnover of ^{14}C by shoot respiration is nearly complete between days 14 and 21 after labeling for all three investigated developmental stages, as the measured total ^{14}C amount only deviated by 0.1% from the asymptote of the regression curve 21 days after pulse labeling. Moreover, the model analysis showed that only 0.06 % to 0.42 % of the assimilated ^{14}C was available for shoot respiration. This is in accordance with the model calculation of Swinnen et al. (1994b), who showed that more than 99 % of the assimilated ^{14}C that was not respired after pulse labeling but was rather incorporated into the structural compounds of wheat plant 19 days after labeling.

Moreover, the investigation indicated that use of the tracer by shoot respiration was not substantially influenced by plant age because the measured ^{14}C amounts of the plant-soil-soil gas system (expressed as percentages of gross ^{14}C assimilation) between days 7 and 21 after labeling at stem elongation, ear emergence, and late milk-ripening were quite similar. This aspect was particularly obvious in the linear regression analysis, which indicated that the slope of the measured total ^{14}C amount of the three plant sets was close to zero during the final days of the investigation.

We note that our investigation showed that the ^{14}C amount of the plant-soil-soil gas system reached an average value of approximately 51 % of gross ^{14}C assimilation between days 14 and 21 after pulse labeling (Fig. 5). By contrast, Swinnen et al. (1994b) observed a total ^{14}C amount of approximately 65% of gross ^{14}C assimilation in a spring wheat field experiment in a temperate humid climate. Our investigation at the system level was performed under controlled conditions at 24 °C for 14 h during the day and at 18 °C for 10 h during the night. This temperature was substantially higher than the temperature at which cereals grow normally in temperate humid climates. For example, in our outdoor experiment, the average daytime temperature between sowing and harvesting was 11 °C. The higher temperatures in our experiment are one potential reason for the lower ^{14}C amount in the plant-soil-soil gas system, because both photorespiration and dark respiration of plants increase with increasing temperature (Schopfer and Brennecke 2006; Turnbull et al. 2001). In addition to temperature, the different crops (spring rye and spring wheat) could also explain the difference in ^{14}C turnover by shoot respiration, as both factors can substantially influence the respiratory turnover of fixed C (Millenaar and Lambers 2003).

However, similar to the study of Swinnen et al. (1994b), where the authors concluded that the turnover of ^{14}C by shoot respiration is nearly complete by 19 days after labeling of spring wheat, we found that this turnover is nearly complete between days 15 and 21 after labeling of spring rye, independent of plant age. Considering this fact, the transfer of labeled photosynthates into the root should also be completed at this time, providing that only ^{14}C from the mobile C pool will be respired and transferred from the shoot to the root.

However, the present approach assumes that the distribution of a tracer within the plant is preliminarily finished and stable several days after labeling. Hence, a later re-translocation of C from the root to the shoot due to remobilization will not be considered for the estimation of the C transfer into soil. Preliminary investigations (Remus et al., unpublished) indicate that in case of spring rye, a significant later re-translocation of ^{14}C form root to shoot does not occur, although a re-translocation within the shoot during grain filling was observed. This phenomenon requires further research, but in the case of spring rye, later re-translocations from root to shoot appear to be minimal.

^{14}C kinetics at the component level

At the component level, the ^{14}C tracer kinetics of the belowground respiration were analyzed after labeling at emergence, stem elongation, and ear emergence. The dynamics of the relative and absolute downward transfer of C at these developmental stages is higher than in later developmental stages. As shown in Remus and Augustin (2016) the highest relative amount of assimilated ^{14}C is transferred downward at emergence, although the absolute amount of transferred C at this stage is very low. By contrast, the absolute downward transfer of C during stem elongation and ear emergence is higher than in all other developmental stages, although the relative amount of ^{14}C transferred downward is substantially lower than at emergence. Therefore, these developmental stages were chosen to analyze the potential effects of plant age under outdoor conditions on the timing of the C transfer within the plant.

The cumulative curves of the ^{14}C-labeled belowground respiration (Fig. 6) showed that relatively more assimilated ^{14}C (expressed as the percentage of the ^{14}C amount recovered in the plant-soil-soil gas system) was respired by younger plants than by older plants. This finding indicates that plant developmental stage influences the magnitude of the relative amount of ^{14}C released through belowground respiration, thus confirming studies of Carbone and Trumbore (2007), Keith et al. (1986), Sauerbeck et al. (1976), Sey et al. (2010), and Xu and Juma (1993).

However, regardless of plant age, the same fraction (82.5± 2.1 %) of the maximum expected ^{14}C-labeled CO_2 released through root respiration and the microbial turnover of rhizodeposits was captured until day 21 after labeling. This implies that the timing of the release of ^{14}C-labeled CO_2 into the soil gas was similar during all three investigated plant developmental stages (emergence, stem elongation and ear emergence). This was particularly obvious when comparing the graphs of the normalized ^{14}C-labeled belowground respiration (see Fig. 7). Therefore, it can be assumed that the flow of freshly assimilated C through the plant and its partial release as CO_2 is not substantially influenced by different plant developmental stages and favorable environmental conditions.

Consistent with several studies of belowground respiration (Nguyen et al. 1999; Swinnen et al. 1994a; Kuzyakov et al. 2001; Warembourg and Estelrich 2000), a large peak of $^{14}CO_2$ in the soil gas was observed within the first 24 h after pulse labeling (see Fig. 7). We also observed that 80 % of the ^{14}C-labeled CO_2 captured until day 21 after labeling at emergence, stem elongation, and ear emergence was already released before days 8 or 9. In contrast with this result, Warembourg and Estelrich (2000) calculated that over 80 % of the CO_2 efflux from the soil occurred prior to day 4 after pulse labeling in perennial bromegrass. The reason for this difference could have been due to the different species examined or the length of the investigated period, as Warembourg and Estelrich (2000) finished their investigation 92 h after pulse labeling, when ^{14}C was still detectable in the released CO_2. This example highlights the difficulty in comparing the release of ^{14}C through belowground respiration using different experimental designs, because in our experiment, ^{14}C was still detectable in the released CO_2 21 days after labeling. It is possible that modeling of the maximal expected release of ^{14}C-labeled CO_2, as shown in this study, can enhance the comparability of different experiments.

Analysis of ^{14}C kinetics at all levels

The main aim of this study was to determine the time after pulse labeling at which the distribution and turnover of labeled carbohydrates is finished, and consequently, the distribution of the applied tracer represents the use of assimilated C to produce plant matter. Our results indicate that the respiratory turnover and the incorporation of ^{14}C into the structural C pool

of the shoot are nearly complete between days 15 and 21 after labeling, as less than 1 % of ^{14}C in the plant-soil-soil gas system was available for modeled or expected shoot respiration. Moreover, similar ^{14}C dilution rates of soluble fractions and substances from the structural C pool at this time also indicate that ^{14}C in the mobile C pool must be nearly depleted in this timespan. Considering that shoot and root respiration use the same C pool (Lehmeier et al. 2008) and that ^{14}C flows with a velocity of 0.87 meter per hour through wheat stems (MacRobbie 1971), root respiration should also be complete between days 15 and 21. Any further increase in ^{14}C-labeled belowground respiration by extending the period between labeling and harvesting beyond 21 days will likely be derived from the turnover of ^{14}C-labeled rhizodeposits or senescent roots.

A comparison of our results with those of Swinnen et al. (1994b) indicates that the distribution and turnover of ^{14}C after pulse labeling of spring rye shows similar dynamics to that of spring wheat. Both studies show that the distribution and turnover of ^{14}C in the plant-soil-soil gas system after pulse labeling is nearly complete by 15 or 19 days after pulse labeling. In contrast with this, Gordon et al. (1980) found that the amount of C remaining in the leaf 24 h after assimilation was only 6 % of the total assimilated C during the photo-period, implying rapid removal from the leaf. Moreover, Smith and Stitt (2007) reported that the starch found in leaves is mostly remobilized at the end of the night when plants are grown with adequate nutrient levels and at favorable temperatures. However, in case of cereals, fructans will accumulate in stems (Bancal and Triboi 1993; Borrell et al. 1989; Schnyder et al. 1993; Thome and Kuhbauch 1985) and can serve as temporary C storage (Wardlaw and Willenbrink 2000). Moreover, Lattanzi et al. (2005), and Lehmeier et al. (2010) showed that temporary storage pools are used for growth and respiration in grasses. Therefore, it cannot be ruled out that the turnover of ^{14}C in the plant-soil-soil gas system after pulse labeling by the degradation of labeled temporary C storage will also be delayed in spring rye and wheat, assuming that between 15 and 19 days are necessary to respire, distribute and incorporate nearly all assimilated ^{14}C. Considering that less than 1 % of assimilated ^{14}C is available for shoot respiration 15 days after labeling, collecting CO_2 released by belowground respiration until this time and harvesting the plants 15 days after labeling seems sufficient to obtain a good approximation of tracer distribution within the plant-soil-soil gas system independent of plant developmental stage. The resulting values should represent the amount of C assimilated during labeling for the production of plant matter.

However, it could be useful to expand sampling times until day 21 after labeling, especially under outdoor conditions, even if the avoidance of drought stress after labeling cannot be guaranteed. ^{14}C partitioning studies on oilseed rape (Remus et al., unpublished) showed a delay of ^{14}C turnover in soil and young leaves under severe drought stress after labeling, which was stimulated again after re-watering. Additionally, (Brüggemann et al. 2011) reported an increased mean residence time of recently assimilated C in leaf biomass under drought stress.

In addition, it is also necessary to consider that root growth and the decomposition of root matter might occur simultaneously (Milchunas 2009). Thus, an extension of the sampling period beyond 21 days would increase the risk of bias in C partitioning measurements due to the turnover of ^{14}C from senescent roots, as has been discussed for continuous labeling experiments (Meharg 1994).

Conclusions

Based on our analysis of ^{14}C kinetics at three different levels, we conclude that the proportional distribution of a C tracer into shoot, root, rhizodeposition, and belowground respiration between days 15 and 21 after pulse labeling should accurately represent the use of C (assimilated during labeling) for root growth and the production of detectable rhizodeposits, as well as for root respiration and the microbial turnover of fresh rhizodeposits, under favorable conditions. Moreover, it was possible to show that depletion of the tracer in the mobile C pool at three different plant ages required the same time, and that the release of labeled CO_2 in the soil gas at three different developmental stages featured the same dynamics. Consequently, the use of repeated single-pulse labeling experiments at different plant developmental stages during the vegetation period should provide precise relative C partitioning coefficients with high temporal resolution, which are necessary for good estimations and modeling of plant-derived C transfer to the subsurface.

The investigations in this study were performed using spring rye as model plant, which showed similar tracer kinetics as spring wheat. However, it cannot be excluded that other plant species or genotypes might show different timings of C partitioning and respiratory turnover. Therefore, studies of other plant species or genotypes with the same design should reveal the extent to which these variables influence tracer transfer into the subsurface.

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References

Ainsworth EA, Bush DR (2011) Carbohydrate export from the leaf: A highly regulated process and target to enhance photosynthesis and productivity. Plant Physiol 155:64–69. doi:10.1104/pp.110.167684

Allen SE, Grimshaw HM, Parkinson JA, Quarmby C (1974) Chemical analysis of ecological materials. Black Well Scientific Publications, Oxford

Bancal P, Triboi E (1993) Temperature effect on fructan oligomer contents and fructan-related enzyme-activities in stems of wheat (Triticum-aestivum L.) during grain filling. New Phytol 123:247–253

van Bel AJE, Hess PH (2008) Hexoses as phloem transport sugars: the end of a dogma? J Exp Bot 59:261–272

Bihmidine S, Hunter CT, Johns CE, Koch KE, Braun DM (2013) Regulation of assimilate import into sink organs: update on molecular drivers of sink strength. Front Plant Sci 4:177. doi:10.3389/fpls.2013.00177

Bolinder M, Katterer T, Andren O, Parent L (2012) Estimating carbon inputs to soil in forage-based crop rotations and modeling the effects on soil carbon dynamics in a swedish long-term field experiment. Can J Soil Sci 92:821–833

Bolinder MA, Janzen HH, Gregorich EG, Angers DA, VandenBygaart AJ (2007) An approach for estimating net primary productivity and annual carbon inputs to soil for common agricultural crops in Canada. Agric Ecosyst Environ 118:29–42

Borrell AK, Incoll LD, Simpson RJ, Dalling MJ (1989) Partitioning of dry-matter and the deposition and use of stem reserves in a semi-dwarf wheat crop. Ann Bot 63:527–539

Brüggemann N, Gessler A, Kayler Z, Keel S, Badeck F, Barthel M, Boeckx P, Buchmann N, Brugnoli E, Esperschütz J, Gavrichkova O, Ghashghaie J, Gomez-Casanovas N, Keitel C, Knohl A, Kuptz D, Palacio S, Salmon Y, Uchida Y, Bahn M (2011) Carbon allocation and carbon isotope fluxes in the plant-soil-atmosphere continuum: a review. Biogeosciences 8:3457–3489

Carbone MS, Trumbore SE (2007) Contribution of new photosynthetic assimilates to respiration by perennial grasses and shrubs: residence times and allocation patterns. New Phytol 176:124–135

Carbone MS, Czimczik CI, McDuffee KE, Trumbore SE (2007) Allocation and residence time of photosynthetic products in a boreal forest using a low-level C-14 pulse-chase labeling technique. Glob Chang Biol 13:466–477. doi:10.1111/j.1365-2486.2006.01300.x

Dilkes NB, Jones DL, Farrar J (2004) Temporal dynamics of carbon partitioning and rhizodeposition in wheat. Plant Physiol 134:706–715

Finzi AC, Abramoff RZ, Spiller KS, Brzostek ER, Darby BA, Kramer MA, Phillips RP (2015) Rhizosphere processes are quantitatively important components of terrestrial carbon and nutrient cycles. Glob Chang Biol 21:2082–2094. doi:10.1111/gcb.12816

Gordon AJ, Ryle GJA, Powell CE, Mitchell D (1980) Export, mobilization, and respiration of assimilates in uniculm barley during light and darkness. J Exp Bot 31:461–473

Gregory PJ, Atwell BJ (1991) The fate of carbon in pulse-labeled crops of barley and wheat. Plant Soil 136:205–213

Heldt HW, Piechulla B (2008) Pflanzenbiochemie. Spectrum Akademischer Verlag, Heidelberg

Hoch G (2007) Cell wall hemicelluloses as mobile carbon stores in non-reproductive plant tissues. Funct Ecol 21:823–834

Kardol P, De Deyn GB, Laliberte E, Mariotte P, Hawkes CV (2013) Biotic plant-soil feedbacks across temporal scales. J Ecol 101:309–315. doi:10.1111/1365-2745.12046

Keith H, Oades JM, Martin JK (1986) Input of carbon to soil from wheat plants. Soil Biol Biochem 18:445–449

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

Kuzyakov Y, Ehrensberger H, Stahr K (2001) Carbon partitioning and below-ground translocation by lolium perenne. Soil Biol Biochem 33:61–74

Kvålseth TO (1985) Cautionary note about R^2. Am Stat 39:279–285

Lambers H, Chapin III FS, Pons TL (2008) Plant Physiological Ecology, 2nd edn. Springer

Lattanzi FA, Schnyder H, Thornton B (2005) The sources of carbon and nitrogen supplying leaf growth: Assessment of the role of stores with compartmental models. Plant Physiol 137:383–395

Lehmeier CA, Lattanzi FA, Schaeufele R, Wild M, Schnyder H (2008) Root and shoot respiration of perennial ryegrass are supplied by the same substrate pools: Assessment by dynamic C-13 labeling and compartmental analysis of tracer kinetics. Plant Physiol 148:1148–1158

Lehmeier CA, Lattanzi FA, Schaeufele R, Schnyder H (2010) Nitrogen deficiency increases the residence time of respiratory carbon in the respiratory substrate supply system of perennial ryegrass. Plant Cell Environ 33:76–87

Lemoine R, La Camera S, Atanassova R, Ddaldchamp F, Allario T, Pourtau N, Bonnemain JL, Laloi M, Coutos-Thvenot P, Maurousset L, Faucher M, Girousse C, Lemonnier P, Parrilla J, Durand M (2013) Source-to-sink

transport of sugar and regulation by environmental factors. Front Plant Sci 4:272. doi:10.3389/fpls.2013.00272
MacRobbie E (1971) Phloem translocation - facts and mechanisms - comparative survey. Biol Rev Camb Philos Soc 46:429–481
Meharg AA (1994) A critical-review of labeling techniques used to quantify rhizosphere carbon-flow. Plant Soil 166:55–62
Meng F, Dungait JAJ, Zhang X, He M, Guo Y, Wu W (2013) Investigation of photosynthate-C allocation 27 days after 13C-pulse labeling of Zea mays L. at different growth stages. Plant Soil 373:755–764
Milchunas D (2009) Estimating root production: Comparison of 11 methods in shortgrass steppe and review of biases. Ecosystems 12:1381–1402
Millenaar FF, Lambers H (2003) The alternative oxidase: in vivo regulation and function. Plant Biol 5:2–15
Nguyen C (2003) Rhizodeposition of organic C by plants: mechanisms and controls. Agronomie 23:375–396
Nguyen C, Todorovic C, Robin C, Christophe A, Guckert A (1999) Continuous monitoring of rhizosphere respiration after labelling of plant shoots with (CO2)-C-14. Plant Soil 212:191–201
Oikawa PY, Grantz DA, Chatterjee A, Eberwein JE, Allsman LA, Jenerette GD (2014) Unifying soil respiration pulses, inhibition, and temperature hysteresis through dynamics of labile soil carbon and O_2. J Geophys Res Biogeosci 119:521–536. doi:10.1002/2013JG002434
Pausch J, Tian J, Riederer M, Kuzyakov Y (2013) Estimation of rhizodeposition at field scale: upscaling of a C-14 labeling study. Plant Soil 364:273–285
Qureshi RM, Fritz P, Drimmie RJ (1985) The use of CO2 absorbers for the determination of specific 14C activities. Int J Appl Radiat Isot 36:165–170
Remus R, Augustin J (2016) Dynamic linking of 14C partitioning with shoot growth allows a precise determination of plant-derived C input to soil. Plant Soil. doi:10.1007/s11104-016-3006-y
Richardson AD, Carbone MS, Huggett BA, Furze ME, Czimczik CI, Walker JC, Xu X, Schaberg PG, Murakami P (2015) Distribution and mixing of old and new nonstructural carbon in two temperate trees. New Phytologist 206:590–597. doi:10.1111/nph.13273
Sauerbeck D, Johnen B, Six R (1976) Respiration, decomposition and excretion of wheat roots during their development. Landwirtschaftliche Forschung Sonderheft 32:49–58
Schnyder H, Gillenberg C, Hinz J (1993) Fructan contents and dry-matter deposition in different tissues of the wheat-grain during development. Plant Cell Environ 16:179–187
Schopfer P, Brennecke A (2006) Pflanzenphysiologie elsevier. Spektrum Akademischer Verlag, Heidelberg
Sey BK, Manceur AM, Whalen JK, Gregorich EG, Rochette P (2010) Root-derived respiration and nitrous oxide production as affected by crop phenology and nitrogen fertilization. Plant Soil 326:369–379
Smith AM, Stitt M (2007) Coordination of carbon supply and plant growth. Plant Cell Environ 30:1126–1149
Stewart DPC, Metherell AK (1999) Carbon C-13 uptake and allocation in pasture plants following field pulse-labelling. Plant Soil 210:61–73
Swinnen J, Van Veen JA, Merckx R (1994a) C-14 pulse-labeling of field-grown spring wheat - an evaluation of its use in rhizosphere carbon budget estimations. Soil Biol Biochem 26:161–170
Swinnen J, Van Veen JA, Merckx R (1994b) Rhizosphere carbon fluxes in field-grown spring wheat - model calculation based on C-14 partitioning after pulse-labeling. Soil Biol Biochem 26:171–182
Thome U, Kuhbauch W (1985) Change in the carbohydrate pattern in the cell content of wheat stems during grain-filling. J Agron Crop Sci 155:253–260
Trumbore S (2000) Age of soil organic matter and soil respiration: Radiocarbon constraints on belowground C dynamics. Ecol Appl 10:399–411. doi:10.2307/2641102
Turnbull MH, Whitehead D, Tissue DT, Schuster WSF, Brown KJ, Grifin KL (2001) Responses of leaf respiration to temperature and leaf characteristics in three deciduous tree species vary with site water availability. Tree Physiol 21:571–578
Wardlaw IF, Willenbrink J (2000) Mobilization of fructan reserves and changes in enzyme activities in wheat stems correlate with water stress during kernel filling. New Phytol 148:413–422
Warembourg FR, Estelrich HD (2000) Towards a better understanding of carbon flow in the rhizosphere: a time-dependent approach using carbon-14. Biol Fert Soils 30:528–534
Warembourg FR, Estelrich HD (2001) Plant phenology and soil fertility effects on below-ground carbon allocation for an annual (Bromus madritensis) and a perennial (Bromus erectus) grass species. Soil Biol Biochem 33:1291–1303
Werth M, Kuzyakov Y (2008) Root-derived carbon in soil respiration and microbial biomass determined by C-14 and C-13. Soil Biol Biochem 40:625–637
Xu JG, Juma NG (1993) Aboveground and belowground transformation of photosynthetically fixed carbon by 2 barley (Hordeum vulgare L.) cultivars in a typic cryoboroll. Soil Biol Biochem 25:1263–1272
Yasumura Y (2009) The effect of altered sink-source relations on photosynthetic traits and matter transport during the phase of reproductive growth in the annual herb chenopodium album. Photosynthetica 47:263–270