RESEARCH ARTICLE



What regulates the rhizodeposition of winter oilseed rape during growth?

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Received: 10 September 2021 / Accepted: 11 April 2022 / Published online: 22 June 2022 $\ensuremath{\mathbb{O}}$ The Author(s) 2022

Abstract

Purpose The goal of this work was to contribute to a better understanding of the process of rhizodeposition in crops and to find helpful approaches for creating a simple model of rhizodeposition. For this purpose, we tested three hypotheses about the relationships and changes in the relative C partitioning coefficients and their ratios. In particular, we analyzed the relationships between root growth, belowground respiration, rhizodeposition and other traits during plant growth. *Methods* The ranges of variation in ¹⁴C partitioning coefficients and various plant traits were determined

Responsible Editor: Doris Vetterlein.

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Supplementary Information The online version contains supplementary material available at https://doi. org/10.1007/s11104-022-05441-1.

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Research Area 2, Leibniz Centre for Agricultural Landscape Research, Eberswalder Str. 84, Müncheberg 15374, Germany e-mail: dluettschwager@zalf.de after ¹⁴C labeling of four winter oilseed rape genotypes in three developmental stages.

Result For all genotypes, we found very strong and significant correlations between the percentages of freshly assimilated C used for rhizodeposition and root growth. In addition, we showed that the ratios of the relative ¹⁴C fluxes in the root-soil-soil gas system changed significantly during plant development and that the relative and absolute C fluxes of rhizodeposition followed different trends. The root growth rate and the change in the ratio of the percentages of ¹⁴C in rhizodeposition and root tissue over time were the key factors that determined the absolute amount of rhizodeposited C. We also found that the C partitioning in a taproot system leading to root growth and rhizodeposition was similar to that of an adventitious root system.

Conclusion Based on our results, we conclude that using the identified key factors in combination with a root growth model, a simple model can be generated to describe rhizodeposition.

Keywords Rhizodeposition · Root growth · Belowground respiration · Brassica napus

Abbreviations

С	carbon
^{14}C	a radioactive isotope of carbon
$^{14}C_{\% \ belowgr. \ resp.}$	percentage of ¹⁴ C activity in below-
	ground respiration that was cap-
	tured during 21 days after labeling

$^{14}C_{\% \ rhizodep.}$	percentage of ¹⁴ C activity in
-	rhizodeposits 21 days after labeling
$^{14}C_{\% root}$	percentage of ¹⁴ C activity in root
	tissue 21 days after labeling
$^{14}C_{\% \ shoot}$	percentage of ¹⁴ C activity in shoot
	tissue 21 days after labeling
$\Delta C_{below. resp}$	C increment of belowground
1	respiration (mg per plant) resulting
	from C assimilation on the day of
	labeling
$\Delta C_{rhizodep.}$	C increment of rhizodeposits (mg
	per plant) resulting from C assimi-
	lation on the day of labeling
ΔC_{root}	C increment of root growth (mg per
	plant) resulting from C assimilation
	on the day of labeling
ΔC_{shoot}	C increment of shoot growth (mg
	per plant) on the day of labeling

Introduction

In 1904, Lorenz Hiltner defined the rhizosphere as root-influenced soil (Hartmann et al. 2008). Since then, a central question has remained: How does a plant influence its rhizosphere, and what is the significance of this influence for plant growth, soil formation, and subsequently, for the entire terrestrial ecosystem? In recent decades, significant features of the rhizosphere have been discovered. For example, roots release H⁺ or OH⁻ and thereby influence the pH of the rhizosphere (Hinsinger et al. 2003). In addition, living roots release organic compounds, which is called rhizodeposition (Shamoot et al. 1968); rhizodeposition is one of the most discussed phenomena in the rhizosphere. These organic components or rhizodeposits are a mixture of low-molecular-weight organic substances also known as exudates (e.g., sugars, amino acids, organic acids, and phenolics) and higher-molecular-weight components such as mucilage and enzymes. Rhizodeposition also includes the cells detached from the root (root hairs, root cap cells, cortical cells, and epidermal cells) and cell parts released into the soil after death and lysis. Thus, rhizodeposits include a wide range of root-derived organic matter with different structures and properties that is released into the soil through different pathways (Jones et al. 2009; Nguyen 2003).

Because of the complex composition of these rhizodeposits, they can influence many different processes in the rhizosphere. For example, root cap cells and mucilages facilitate root tip penetration into the soil and influence soil water retention (Jones et al. 2009; Oleghe et al. 2017; Naveed et al. 2019). Rhizodeposits play roles in the cycling and uptake of nutrients and in protecting plants from potentially toxic metals via complexation while also influencing the microbial and faunal communities in the rhizosphere (Henry et al. 2008; Jones et al. 2009; Mounier et al. 2004; Nguyen 2003; Toal et al. 2000; York et al. 2016). Furthermore, there is convincing evidence that rhizodeposits are involved in the soil aggregation that controls soil organic matter (SOM) stabilization (Poirier et al. 2018). Root-derived C also plays an important role in the accumulation of slow-cycling mineral-associated organic C as well as fast-cycling particulate organic C in soil (Rasse et al. 2005; Sokol et al. 2019).

In contrast, rhizodeposit-driven microbial activity also leads to increased decomposition or turnover of older SOM, a phenomenon termed the priming effect, for which several hypotheses have been proposed (Valadares et al. 2020). However, this decomposition may increase the availability of nutrients such as nitrogen and may be one of the most important rhizosphere effects in the context of plant nutrition (Finzi et al. 2015). This assumption is also supported by model simulations showing that rhizosphere processes could be an important supplement to soil derived N (Valadares et al. 2018).

Although rhizodeposition apparently has significant ecological importance, our quantitative understanding of the influence of rhizodeposition on rhizospheric processes is limited, and modeling the C flow in the rhizosphere is very difficult. Determining the root-derived C input to the soil is fundamental for better understanding terrestrial C cycling and for improving belowground process models (Stuart et al. 2009; Litton and Giardina 2008; Wiesmeier et al. 2014). Accurate quantification of C sources is therefore imperative (Toal et al. 2000). The significance of rhizodeposition is evident from the fact that predictions of biogeochemical models at global and ecosystem scales change dramatically when rhizodeposition is included (Roque-Malo et al. 2020). Unfortunately, rhizodeposits are often not measured due to various

operational and technical limitations (Hupe et al. 2016). Because the process of rhizodeposition occurs out of sight and in close proximity to microbes and SOM, all of which are continuously transforming, it is extremely difficult to distinguish and to quantify rhizodeposits from older C.

¹³C- and ¹⁴C-based experiments can help to overcome some of these difficulties. Such experiments have typically been applied to trace the fate of rootderived C compounds in a plant-soil-soil gas system over a period of days to months (Babst et al. 2005; Dilkes et al. 2004; Keith et al. 1986; Keutgen et al. 1995; Kuzyakov et al. 2001; Meng et al. 2013; Staddon et al. 2003; Swinnen et al. 1994a; Swinnen et al. 1994b; Wang et al. 1989; Warren et al. 2012). In ¹⁴C pulse labeling experiments, ¹⁴C partitioning coefficients are also often calculated to reflect the relative distribution of the total radioactivity of assimilated ¹⁴C in different parts of the whole plant-soil-soil gas system. For example, the ¹⁴C partitioning coefficient of the root gives the percentage of ¹⁴C found in the root to the ${}^{14}C$ of the whole system (100%). This calculation does not usually take into account the amount of C respired by the shoot (Nguyen 2003; Remus and Augustin 2016).

In addition to indicating the relative C fluxes into each part of the system, the ¹⁴C partitioning coefficients along with the shoot C increment (mg/plant) on the day of labeling can be used to calculate the absolute amounts of freshly assimilated C that have entered the roots or soil. Sometimes the release of plant-derived C needs to be estimated retrospectively. Therefore, the magnitude of rhizodeposition is often estimated using parameters such as aboveground biomass and the average or median of the empirically derived relative C partitioning coefficient of rhizodeposition from ¹³C or ¹⁴C isotope-based studies (Bolinder et al. 2007; Bolinder et al. 2012; Kuzyakov and Domanski 2000; Kuzyakov and Schneckenberger 2004; Nguyen 2003; Pausch et al. 2013; Pausch and Kuzyakov 2018; Wiesmeier et al. 2014).

However, an approach that uses average or median values of C partitioning coefficients to estimate the rhizodeposition-related C input into soil during the entire vegetation period can result in significant over- or underestimations, as shown in Remus and Augustin (2016). This uncertainty can be attributed to the fact that the C partitioning coefficients and the assimilation capacity of the plant vary during plant development. Therefore, the C partitioning coefficients and assimilation rates for each stage of plant development must be dynamically linked by a mathematical model to obtain precise information on the absolute amounts of C (mg C per plant or unit area) transferred to the soil at a given point in time or over a time interval (Remus and Augustin 2016). Even with this approach in its current form, root growth, belowground respiration and the release of detectable rhizodeposits can be determined only retrospectively after analyzing tracer distributions and plant growth.

An alternative approach is to develop a mathematical model that can predict the amount of root-derived C at any given time or in a time interval based on easily ascertainable parameters that can describe rhizodeposition. The realization of such a model depends on a thorough analysis of the process and the determination of the key parameters.

It is assumed that root exudates are the main component of rhizodeposits and that their exudation is a passive process that occurs by diffusion from roots to the surroundings (Jones et al. 2009; Nguyen 2003). Accordingly, Jones et al. (2009) developed a model to predict the rhizodeposition from maize plants by combining a thermal-time driven root architecture model with a diffusion model. Unfortunately, this model requires the temporal and spatial dynamics of the organic solutes in the root, particularly the actual concentration gradient across the cytoplasm and soil, and the membrane permeability coefficient, parameters whose determination and prediction are not straightforward (Jones et al. 2004; Jones et al. 2009). Moreover, this approach is not able to represent the entirety of rhizodeposits since it targets only the exudates.

To better quantify the rate of rhizodeposition and to identify the key parameters for modeling it, it is proposed to first conduct studies aimed at elucidating the plant physiological processes that regulate rhizodeposition (Nguyen 2003; Toal et al. 2000). If we assume that growing roots act as a sink for recently assimilated C and that most of rhizodeposition occurs passively by diffusion, rhizodeposition should follow the same trend as root growth, and there should be a strong positive correlation between the two.

However, the empirical evidence for a relationship between root growth and rhizodeposition is inconsistent. A positive correlation between the ¹⁴C in the root and soil ($r_{Pearson}=0.798^{***}$) was found for barley (Xu and Juma 1993), and strong positive correlations ($r_{Spearman} > 0.9^{***}$) were found between the ¹⁴C partitioning coefficients of the root, soil and belowground respiration for spring rye (Remus and Augustin 2016). Nguyen (2003), on the other hand, analyzed ¹⁴C partitioning coefficients from 43 labeling experiments and found only a weak positive correlation ($r_{Pearson} = 0.257^{**}$) between the ¹⁴C in the root and residues in pulse labeling experiments and a negative correlation ($r_{Pearson} = -0.043$) in continuous labeling experiments.

Nguyen (2003) also observed no significant effect of plant age on the ¹⁴C partitioning between belowground components (root, rhizodeposition and belowground respiration). However, there are several reasons why the ratios of the three belowground C fluxes to each other should change during plant development. For example, an increasing temperature during the growing season should increase the turnover of freshly assimilated C in the root and rhizosphere (Boone et al. 1998; Lloyd and Taylor 1994; Turnbull et al. 2001), and as the root biomass increases, the maintenance respiration should also increase (Amthor 1984). Therefore, the ratio of released ¹⁴ C-labeled CO₂ in soil to ¹⁴C-labeled rhizodeposition or ¹⁴C-labeled root tissue should change during the growing season.

The ratio of ¹⁴C in rhizodeposits to the amount of ¹⁴C used to build root tissue should also change as the root system grows. The highest rates of rhizodeposit release have been observed at the growing root apex, with the majority of rhizodeposits passively leaving the root by diffusion (Jones et al. 2009). However, according to Personeni et al. (2007), root sections located behind the tip also release root-derived C, with the C efflux rates decreasing continuously with increasing distance from the root apex. For maize roots, the authors calculated C efflux rates at the apex of approximately 5 μ g C cm⁻² h⁻¹ and at a distance of 25 cm from the apex of greater than 1 μ g C cm⁻² h^{-1} . This suggests that as the root length increases and the root surface area increases, the fraction of C laterally entering the soil on its way to the growing root tip increases. Furthermore, we must consider that the death of root cortex cells is a programmed phenomenon that correlates with plant age (Gillespie and Deacon 1988; Liljeroth 1995). Increasing root turnover rates during development of spring wheat were also calculated by Swinnen et al. (1994b). After the death and lysis of root cells, additional C components are released into soil (Rovira 1969; Jones et al. 2009).

Furthermore, a ¹³C labeling experiment by Van de Broek et al. (2020) rejected the hypothesis that wheat cultivars with larger root biomass and deeper roots release more C in the form of rhizodeposits than cultivars with a smaller root system. This hypothesis should hold if we assume that most rhizodeposits passively leave the root by diffusion, which depends on the root surface. In addition, a larger root system should produce more rhizodeposits than a smaller root system because a larger root system should also have more root turnover than a smaller root system.

One aim of this study was to address the contradictions listed here and to improve our understanding of the process of rhizodeposition and C flux within the plant-soil-soil gas system. Therefore, we have studied the distribution and use of freshly assimilated C for root growth (incorporation into root tissue), rhizodeposition (release of C compounds from the root into soil), and belowground respiration (root respiration and degradation of root-derived C by microbes) and their relationship to each other during plant development. In more detail, the following hypotheses (H) are tested.

- H1. Increasing temperature and increasing root biomass during plant development should lead to an increase in plant tissue respiration and microbial turnover of fresh rhizodeposits. Therefore, we hypothesize that the ratio of ¹⁴C released as CO₂ in the rhizosphere to the amount of ¹⁴C used for the formation of root mass and rhizodeposits increases during the growing season.
- H2. Due to increases in root length and root surface area, as well as an increase in root turnover during plant development, the proportion of ¹⁴C released as rhizodeposits with respect to the amount of ¹⁴C in root tissue is expected to be higher in older plants than in younger plants. Therefore, we hypothesize that the ratio of ¹⁴C released by rhizodeposition to the amount of ¹⁴C used to build root tissue increases as the plant grows.
- H3. Considering that the most rhizodeposits passively leave the root by diffusion, we hypothesize that this behavior occurs regardless of the root system architecture or crop type. Consequently, dicot-

yledonous plants with a taproot system should exhibit strong relationships between the amounts of ¹⁴C used for root growth, rhizodeposition and belowground respiration, as has been observed previously in monocotyledonous plants with adventitious root systems (Remus and Augustin 2016; Xu and Juma 1993).

The investigation of the three hypotheses aims to better understand the relationships of the subsurface C fluxes with each other. However, the ¹⁴C partitioning coefficients that are the subject of the hypotheses provide information about only the relative C fluxes in the plant-soil-soil gas system. To advance the development of simple models to predict rhizodeposition, absolute C fluxes must also be considered, and the key factors that significantly influence them must be identified. Therefore, we also investigated the importance of absolute and relative carbon partitioning between the shoots, roots and soil of winter oilseed rape. To obtain a wide range of plant variation for the investigation, four genotypes were selected that were expected to differ at least in part in their shoot growth, root growth and shoot to root ratios.

Materials and methods

Plants and cultivation

We selected four genotypes of winter oilseed rape, namely, AVATAR, DH-1, DH-2 and VISBY, from Norddeutsche Pflanzenzucht, Hans-Georg Lembke KG (NPZ), Holtsee, Germany. AVATAR and VISBY are hybrid cultivars, while DH-1 and DH-2 are nonapproved doubled haploid lines. The plants were grown in plastic containers designed to allow airtight separation between the root and shoot spaces during and after ¹⁴C labeling (Remus et al. 2016). The containers (36 cm long with an inner diameter of 11.9 cm) were made from commercially available, hightemperature sewer pipes and accessories (HT DN 125 from Marley, Germany). The containers were filled with approximately 3.8 kg (dry mass) of albic luvisol topsoil obtained in Müncheberg (Germany). One seed was sown per container and allowed to germinate under controlled conditions at 21 °C in a growth chamber. After germination, the plants were grown under simulated autumn conditions. For this, the air temperature was gradually lowered from 20 °C during the day and 12 °C at night to 1 °C (day and night) over the course of 6 weeks. Likewise, the daylight duration was reduced from 16 h to 10 h with an intensity of 350 μ mol m⁻² s⁻¹ at the top of the container. For subsequent vernalization, the light intensity was halved, and the temperature was kept constant at 1 °C for 6 weeks. Afterwards, to acclimate the plants, the daytime temperature was raised to 13 °C and nighttime temperature to 6 °C over a period of 6 days. The plants were illuminated for 10 h at 350 μ mol m⁻² s⁻¹.

To initiate elongation growth, the day and night temperatures were subsequently increased to 18 °C and 9 °C, respectively, and the day length was set to 16 h. After flowering, the day and night temperatures were further increased to 23 °C and 12 °C, respectively. To avoid premature elongation growth, each plant was fertilized only slightly with 100 mg of kieserite (25% MgO and 50% SO₃) before vernalization. The first nitrogen fertilization took place during leaf development after vernalization, and each plant received 250 mg of ammonium nitrate (NH₄NO₃). The second nitrogen fertilization was performed during elongation growth, when each plant received 250 mg of NH₄NO₃ and 610 mg of Wopil, a commercially available mineral fertilizer (Siegfried W. Arnold e.K., Halle, Germany) containing 14 % nitrogen (N) (ammonium (NH₄) and nitrate (NO₃)), 2.6 % phosphorus (P), 19.9 % potassium (K), 3 % magnesium oxide (MgO) and low concentrations of boron (B), iron (Fe), copper (Cu), manganese (Mn), molybdenum (Mo), and zinc (Zn). The third nitrogen fertilization (250 mg of NH₄NO₃ per plant) was performed during flowering. The water content of the soil was monitored and adjusted to maintain 55 % of the soil water-holding capacity every 1 to 3 days with deionized water. The relative humidity of the air in the growth chamber was maintained at approximately 65 %.

¹⁴C pulse labeling - tracer application, preparation, and analysis

Tracer application

Plants were ¹⁴C labeled at three developmental stages: i. end of leaf development (shortly before beginning of elongation), ii. inflorescence emergence and iii. development of fruit (only a few flowers remained on the branches). Six plants per genotype were labeled at each developmental stage. The detailed procedure of pulse labeling is described in Remus et al. (2016) and Remus and Augustin (2016). Briefly, the plants to be labeled were moved to a different growth chamber equipped with the labeling system forty-eight hours prior to labeling. The labeling growth chamber was programmed to maintain climatic conditions identical to those in the chamber used for plant growth (see section 'Plants and cultivation'). Twenty-four hours before labeling, each plant container was closed using a perforated lid with two gas ports. The area around the inlet tubes was sealed using semisolid acid-free silicon rubber. The soil surface exposed in the inlet tubes was sealed with liquid silicon rubber (TACOSIL 170 with 3 % cross-linker no. 28 from Thauer & Co. KG, Germany). Just before labeling, the two gas ports in the caps were closed. This created an airtight headspace in the plant container separated from the atmosphere. The detailed procedure can be found in Remus et al. (2016).

Each plant was pulse labeled with ¹⁴C-labeled CO₂ individually. For this, each plant was covered with a specifically designed transparent plastic bag with a valve that allowed controlled injection of ¹⁴CO₂ into the bag. Each plant was labeled with 2 MBq of ¹⁴C. Six hours after the application of the ¹⁴C-labeled CO₂, the plastic bags were removed. The gas ports in the plant container were opened and connected to flexible tubes. The tubes were connected to a pump that allowed controlled flow of fresh air through the soil headspace. The CO₂ from the soil headspace was continuously trapped in a 1 M sodium hydroxide (NaOH) solution to determine the ¹⁴C activity associated with belowground respiration. The NaOH solution (3 x 12 ml 1 M NaOH) was renewed daily until harvest (21 days after labeling).

Plant and soil sample preparation

¹⁴C partitioning into structural biomass pools is assumed to be complete within 21 days (Swinnen et al. 1994b; Remus et al. 2016). This is an essential prerequisite for precisely measuring C partitioning among the biomass and soil carbon pools. Therefore, we harvested six plants of each genotype 21 days after labeling. At harvest, the shoot was cut close to the soil surface and then dried in an oven at 104 °C. The plant containers containing soil and intact roots were immediately frozen at -26 °C until further analysis. To measure root biomass, the plant containers were thawed overnight at 4 °C. The main taproot, lateral roots and visible root segments were extracted by hand. The soil adhering to these roots was defined as rhizosphere soil. To collect this rhizosphere soil, the roots were washed twice with 500 ml of deionized water by horizontal shaking in an Erlenmeyer flask for 1 h.

The cleaned roots were then dried at 104 °C. The wash water containing the rhizosphere soil was subjected to a wet sieving procedure using a $300-\mu m$ mesh sieve until only light mineral particles remained on top of the sieve. This yielded two fractions: soil particles + small root fragments (> 300 μ m) and fine soil + rhizodeposits (< 300 μ m). The water in the two fractions was evaporated overnight at 80 °C before the material could be dried at 104 °C. After separation of visible roots, the bulk soil was homogenized by hand, and four subsamples of 50 g each were randomly withdrawn. Two of these subsamples were used to estimate the dry mass of the bulk soil. The other two were subjected to the wet sieving procedure described for the rhizosphere soil to further obtain two fractions: soil particles + root fragments > 300 μ m that could not be obtained by hand extraction and soil particles $< 300 \ \mu m + root$ -derived C or rhizodeposits. The shoot, taproot, lateral roots, and fractions of rhizosphere and bulk soil > and < 300µm were separately dried at 104 °C and pulverized for measurement of C contents and ¹⁴C activities.

Carbon analysis and calculations for all components and fractions

The C content and ¹⁴C activity in the biomass (shoot, taproot and lateral roots) and soil fractions were measured using an elemental analyzer (multi EA 4000, Analytik Jena, Germany). The samples were combusted under a nonlimiting O_2 supply at 1200 °C. The CO₂ thus produced was absorbed in a CO₂ trap (Qureshi et al. 1985) filled with Carbo-Sorb E (PerkinElmer, Rodgau, Germany). The resultant Carbo-Sorb E sample was mixed with Permafluor (PerkinElmer) at a ratio of 1:4 (v/v) and then analyzed for

¹⁴C activity using a liquid scintillation counter (Tri-Carb 2900TR, PerkinElmer, Rodgau, Germany).

To measure the ¹⁴C activity associated with belowground respiration, 3 ml of each NaOH solution from the traps was mixed with 12 ml of UltimaGold (PerkinElmer) and then analyzed on a liquid scintillation counter. The liquid scintillation counter was calibrated using ¹⁴C, ³H and background standards from PerkinElmer.

The specific ¹⁴C activities in the respective samples were calculated as Bq per ml of NaOH in the case of belowground respiration and Bq per mg for roots, shoots, and rhizosphere and bulk soil. To obtain the total ¹⁴C in the parts of the system, the respective specific ¹⁴C activities were multiplied by the total size of the system components. For instance, the total ¹⁴C of the soil fraction $< 300 \ \mu m$ was estimated by multiplying the specific ¹⁴C activity (Bq per mg) of that fraction by the total amount in the entire container. Similarly, the total ¹⁴C activity in the shoots and handextracted roots was calculated by multiplying the specific ¹⁴C activity, i.e., Bq per mg of biomass, by the total biomass of the shoot and root, respectively. The ¹⁴C activity of the entire root system was obtained by adding the ¹⁴C activities of hand-extracted roots (taproots and lateral roots) and the ¹⁴C activity of the soil sample, which included root fragments > 300 μ m. Total ¹⁴C activity associated with respiration over the 21-day period was calculated by adding the activities estimated for belowground respiration each day.

Calculation of relative ${}^{14}C$ partitioning and absolute C fluxes

The relative ¹⁴C partitioning within the plant-soil-soil gas system was calculated for each plant container individually (see section 'Carbon analysis and calculations for all components and fractions'). The total ¹⁴C activities in the analyzed pools (shoots, roots, rhizodeposition and belowground respiration over the 21-day period from labeling to harvest) were set equal to 100 % for each plant container, and the percentage ¹⁴C activity in each of the system components was calculated.

The absolute fluxes of C (mg of C per plant) assimilated during pulse labeling and subsequently transferred to the subsurface components (roots, soil, and soil gas) were estimated following Swinnen

et al. (1994b). This approach assumes that the ratio of ¹⁴C incorporated into the structural C pool of the shoot biomass and the shoot growth rate is equal to the ratio of ¹⁴C incorporated into the structural C pool of the root and the root growth rate on the day of labeling (Eq. 1). The denominator on the left side of the equation is the C increment of shoot growth (mg of C per day) on the day of labeling (ΔC_{shoot}). The denominator on the right represents the C increment (mg of C per day) of root growth (ΔC_{root}).

$$\frac{{}^{14}C_{\%\ shoot}}{\Delta C_{shoot}} = \frac{{}^{14}C_{\%\ root}}{\Delta C_{root}}$$
(1)

where ${}^{14}C_{\% \ shoot}$ and ${}^{14}C_{\% \ root}$ are the ${}^{14}C$ partitioning coefficients of the shoot and root, respectively.

Rearranging Eq. 1, the root growth rate $(\Delta C_{root}, Eq. 2)$ can be expressed in terms of the daily C increment (mg of C per root per day). This enables calculating the total amount of C fixed during labeling and subsequently used for root growth.

$$\Delta C_{root} = \frac{{}^{14}C_{\% \ root}}{{}^{14}C_{\% \ shoot}} * \Delta C_{shoot}$$
(2)

The absolute C flux rates for detectable rhizodeposition and belowground respiration were estimated by substituting the 14 C incorporated into the roots with 14 C partitioning coefficients for the respective C pools (see Eq. 3 and Eq. 4).

$$\Delta C_{rhizodep.} = \frac{{}^{14}C_{\% \ rhizodep.}}{{}^{14}C_{\% \ shoot}} * \Delta C_{shoot}$$
(3)

$$\Delta C_{belowgr.\ resp.} = \frac{{}^{14}C_{\%\ belowgr.\ resp.}}{{}^{14}C_{\%\ shoot}} * \Delta C_{shoot} \tag{4}$$

A proven approach for determining the shoot growth rate on the day of ¹⁴C labeling is to differentiate the modeled function of cumulative shoot growth (Remus and Augustin 2016; Swinnen et al. 1994b). This method, however, is very costly because four growth curves of plants grown in growth chambers are required and the plants need to be vernalized. Therefore, we instead used an empirical approach to obtain a good approximation of the shoot growth rates. Just before each of the three ¹⁴C pulse labeling procedures, six plants per genotype of approximately the same size as those used for labeling were harvested, and the C amounts in their shoots were determined. For this purpose, the shoots were dried at 104 °C immediately after harvest and then pulverized. The C analysis was performed in the same way as that for the labeled shoots (see section 'Carbon analysis and calculations for all components and fractions'). The difference between the average C amounts of the plants harvested before and 21 days after labeling was used to calculate daily shoot growth rates for each genotype, as summarized in Table 1.

The values in Table 1 are only estimates of the exact shoot C increments on the days of labeling, but they should be good approximations with which to calculate the rhizodeposition and root growth rates using Eq. 2 and Eq. 3.

Calculation of the influence of genotypic variation in different traits on rhizodeposition

To investigate whether the variation among the selected genotypes is the major driver of the magnitude of rhizodeposition, a sensitivity analysis was performed. For this, the smallest and the largest mean values of a trait (¹⁴C partitioning coefficients of shoots, roots, and rhizodeposition as well as shoot and root growth rates) for one of the four genotypes were gradually introduced into Eq. 3 or 5 while using the mean values across all four genotypes for the remaining traits. Subsequently, for each of the three labeling points, the maximum range of the amount of rhizodeposited C for the greatest observed genotypic variation of each trait (¹⁴C_{% shoot}, ¹⁴C_{% root}, ¹⁴C_{% root}, ¹⁴C_{% root}, ^{AC}_{shoot}, ΔC_{root}) was calculated.

Table 1 Estimated shoot growth rates of the four genotypes (AVATAR, DH-1, DH-2 and VISBY) at ${}^{14}C$ labeling at the leaf development, inflorescence emergence and fruit development stages

Shoot growth rates [mg of C plant ⁻¹ day ⁻¹] at ${}^{1+}C$ labeling at							
Genotype	leaf development	inflores- cence emergence	development of fruit				
AVATAR	20.5	162.5	229.5				
DH-1	16.6	146.7	221.6				
DH-2	31.1	149.8	208.1				
VISBY	23.9	146.0	245.8				

$$\Delta C_{rhizodep.} = \frac{{}^{14}C_{\% \ rhizodep.}}{{}^{14}C_{\% \ root}} * \Delta C_{root}$$
(5)

To compare the strengths of the influence of trait variation between the three labeling experiments, the relative deviation from the respective average rhizodeposition of all four cultivars was calculated.

Visualization of the ¹⁴C distribution in the root system

To visualize the distribution of freshly assimilated C in an old root system of oilseed rape, autoradiograms were produced. For this purpose, plant shoots were labeled with ¹⁴CO₂ in the final stage of fruit development and harvested 21 days later. The cultivation and labeling were analogous to those of the other plants in this study. The washed and dried roots were positioned in a light-tight box (Hypercassette, Amersham Biosciences) directly on Kodak BioMax MR film for an exposure of 5 days. To develop and fix the films, Kodak GBX Developer and Kodak GBX Fixer were used. An Epson Expression 12000XL scanner was used to digitize the autoradiograms and to produce white light scans of the roots. In addition, the software Mathematica (version 12, Wolfram Research) and Gimp (version 2.10) were used for background subtraction and colorizing, respectively.

Statistics

The statistical analyses were performed using Mathematica (version 12, Wolfram Research). Homogeneity of variances was tested by the Brown-Forsythe test (a modification of the Levene test) with $\alpha = 5\%$. In cases of homogeneous variances, analysis of variance (ANOVA) and the Student-Newman-Keuls test with α = 5% were used to detect differences between means. Otherwise, the Nemenyi test was performed to find groups of data differing at the same level of probability. Because Mathematica version 12 does not offer the Nemenyi test, an algorithm was created to generate n*k ranks. Afterwards, for each treatment or genotype, the rank sums and absolute values of their differences were calculated and compared with the corresponding table value (Sachs 1992). To identify the strength of a correlation between two variables, the Pearson correlation coefficient (for linear relationships) or the Spearman rank correlation coefficient ($r_{Spearman}$) (for nonlinear relationships) was calculated. Additionally, regression analyses were performed to find suitable models to describe the relationships under investigation. For linear regression analyses, the adjusted R-squared value was used as the coefficient of determination. For the selection of suitable nonlinear models, the coefficient of determination (R_{nl}^2) was calculated according to the formula $R_{nl}^2 = 1 - \sum_{i=1}^{n} (y_i - \hat{y}_i)^2 / \sum_{i=1}^{n} (y_i - \bar{y})^2$, where \hat{y}_i and \bar{y} are the predicted value of y and the mean of all measured values of y represented, respectively. We used this coefficient of determination because it is the most appropriate of the known coefficients of determination for nonlinear models (Kvålseth 1985).

Results

To test our hypotheses, we determined the ¹⁴C partitioning coefficients in three stages of plant development. The ¹⁴C partitioning coefficients found in the whole plant-soil-soil gas system for the four genotypes 21 days after labeling at the end of leaf development, inflorescence emergence and the beginning of fruit development are summarized in Table 2. The table shows that the percentage of ¹⁴C retained in the shoots increased significantly during plant development. On average, 92.1% of the C assimilated into the plants at the beginning of fruit development was used to build shoot biomass. The percentage of ¹⁴C entering the roots, rhizodepositis and belowground respiration decreased during plant development. However, this decrease was not equally strong for all the three belowground components. When the average percent ¹⁴C value (16.6) across the roots of all four genotypes from the end of the leaf development stage (1st labeling experiment) is divided by the corresponding value (3.6) of the 2nd labeling experiment (inflorescence emergence), we obtain a significant decrease in ¹⁴C incorporation into the root, by a factor of 4.6. For the average ¹⁴C values of belowground respiration and rhizodeposition, however, the calculation results in reductions only by factors of 2 and 3, respectively, in the same period. The calculation with the average ¹⁴C percentages of the 2nd and 3rd experiments shows that the proportion of ¹⁴C in the belowground respiration and the rhizodeposition between the inflorescence emergence and the development of fruits decreased only by a factor of 2, while the incorporation of ¹⁴C into the root decreased by a factor of 3.

The ratio of the ¹⁴C from belowground respiration to the sum of the ¹⁴C in the roots and rhizodeposits during plant development

As formulated in H1, increases in root system size and temperature should have significant effects on the use of ¹⁴C for growth and maintenance respiration and on its turnover rate in root tissue and soil. To test this, the ratio of ¹⁴C in belowground respiration (${}^{14}C_{\% \ belowgr. \ resp.}$) to that remaining in the root and soil (${}^{14}C_{\% \ root} + {}^{14}C_{\% \ rhizodep.}$) seemed to be the most appropriate index. The sum of the ¹⁴C in the root and soil was used because the ¹⁴C of belowground respiration can come from both sources. In Fig. 1, the position of the medians and middle 50%of the data (closed square) show an increasing trend for each successive labeling experiment. This trend was confirmed by the significant differences between the average values of the ratios from the first and second labeling experiments for all individual genotypes and their pooled data. In contrast, when the second and third labeling experiments were compared, a significant increase in the ratio was detected only for VISBY, although all average values of the third experiment were larger than those of the second experiment.

However, the average ratios for each genotype and the pooled data for the third labeling differed significantly from those of the first labeling.

To determine the strength of the correlation between the ratio of the released ¹⁴C-labeled CO_2 from the root-soil-system per unit of ¹⁴C in rhizodeposits and root tissue (${}^{14}C_{\% \ belowgr. \ resp}$ /(${}^{14}C_{\% \ root} + {}^{14}C_{\% \ rhizodep.}$)) and the gradually increasing air temperature as well as the C mass of the root system 21 days after labeling, two correlation analyses (with n=72) were performed. The analyses showed i) a weak correlation ($r_{Spearman}$: 0.552***) between the ratio (${}^{14}C_{\% \ belowgr. \ resp}$ /(${}^{14}C_{\% \ root} + {}^{14}C_{\% \ rhizodep.}$)) and the C mass of the root system and ii) a strong correlation ($r_{Spearman}$: 0.8455***) between this ratio and the average air temperature.

The ratio of rhizodeposition to root growth during plant development

To test our second hypothesis, that the proportion of recently assimilated C in rhizodeposition with respect to **Table 2** Relative partitioning of ¹⁴C [%] among shoots, taproots, lateral roots, whole roots, belowground respiration, and detectable rhizodeposition (root-derived material < 300 μ m) in four oilseed rape genotypes (AVATAR, DH-1, DH2 and VISBY) 21 days after labeling at the end of leaf development (shortly before the start of elongation growth), inflorescence emergence and the development of fruit. Values are represented as means ± standard deviations. Means followed by the same Latin or Greek letter are not significantly different according to a Student-Newman-Keuls or Nemenyi test (p-level: 0.05), respectively. Different small letters indicate significant differences within a developmental stage, while different capital letters indicate significant differences between developmental stages. Errors are calculated as standard deviations. In addition, the coefficients of variation (CVs) are also presented. All: means, standard deviations and CVs of all data for the same component and the same developmental stage of the four genotypes. The values for the taproots and lateral roots have been grayed out to indicate that they are included in the values for the whole root

		¹⁴ C labeling at												
		end of leaf			inflorescence			d	development					
		dev	elo	pme	nt		emergence			C	of fruit			
Component	Genotype	Mean	,	s	CV		Mean		s	CV	Mea	n	s	CV
	AVATAR	57.6 ^b	\pm	2.5	4.3		84.3 ^b	\pm	2.1	2.5	93.0	^b ±	0.7	0.8
	DH-1	52.6 ^a	\pm	3.3	6.3		81.5 ^a	\pm	2.2	2.7	90.0	a ±	2.1	2.3
Shoot	DH-2	54.7 ^{ab}	\pm	2.1	3.8		80.0 ^a	\pm	3.0	3.8	91.4	$ab \pm$	1.3	1.4
	VISBY	55.7 ^{ab}	\pm	3.0	5.4		86.4^{b}	\pm	1.9	2.2	93.9	^{bc} ±	1.5	1.6
	All	55.2 ^A	±	3.2	5.8		83.0 ^B	±	3.4	4.0	92.1	^C ±	2.0	2.2
	AVATAR	21.3 ^a	\pm	2.0	9.5		11.2 ^{ab}	\pm	1.9	17.2	5.1 ^a	' ±	0.6	11.5
Belowground	DH-1	23.9 ^a	\pm	2.0	8.3		12.2^{b}	\pm	1.9	15.5	7.2^{c}	±	2.1	28.9
ragnization	DH-2	23.1^{a}	\pm	1.6	6.9		14.1^{b}	\pm	2.9	20.5	6.7 ^b	; ±	1.4	21.6
respiration	VISBY	23.5 ^a	\pm	3.5	14.7		9.0^{a}	\pm	1.8	20.2	4.5 ^a	+	1.1	25.2
	All	22.9 ^A	±	2.4	10.6		11.6 ^B	±	2.8	23.8	5.8 ^C	±	: 1.7	29.8
	AVATAR	16.2 ^{<i>a</i>}	±	1.1	7.1		2.8 ^a	\pm	0.4	14.3	1.1 ^a	β_{\pm}	0.1	12.9
	DH-1	18.3 ^{<i>a</i>}	\pm	1.9	10.5		4.5^{b}	\pm	0.4	9.6	1.7^{β}	+	0.4	21.7
Whole root	DH-2	15.7 ^a	\pm	3.1	19.9		4.1^{b}	\pm	0.6	15.1	1.1^{α}	β_{\pm}	0.3	26.1
	VISBY	16.1 ^a	\pm	2.1	13.3		2.9^{a}	\pm	0.5	17.8	0.9^{α}	+	0.2	24.8
	All	16.6 ^A	±	2.3	13.8		3.6 ^B	±	0.9	25.1	1.2^{Γ}	±	0.4	34.2
	AVATAR	5.3 ^{ab}	\pm	1.5	28.3		0.7 ^a	\pm	0.2	24.3	0.10	±	0.0	24.3
	DH-1	6.1^{b}	\pm	0.8	13.3		1.4^{b}	\pm	0.3	18.6	0.2^{β}	+	0.1	33.8
Taproot	DH-2	4.3 ^a	\pm	0.6	14.1		0.9 ^a	\pm	0.3	31.4	0.1 ^a	$\beta \pm$	0.0	30.8
	VISBY	5.4 ^{ab}	\pm	0.6	11.4		0.7 ^a	\pm	0.1	15.1	0.1 ^a	+	0.0	32.0
	All	5.3 ^A	\pm	1.1	20.8		0.9 ^B	\pm	0.3	36.5	0.1^{Γ}	+	0.1	56.6
	AVATAR	10.9 ^a	\pm	1.6	14.5		2.1 ^a	\pm	0.3	13.5	1.1 ⁰	±	0.1	13.5
	DH-1	12.1^{a}	\pm	1.7	13.9		3.2^{b}	\pm	0.3	9.8	1.5^{β}	+	0.3	20.5
Lateral roots	DH-2	11.3 ^a	\pm	3.0	26.9		3.2^{b}	\pm	0.4	13.2	1.00	+	0.3	26.0
	VISBY	10.7^{a}	\pm	2.1	19.3		2.2^{a}	\pm	0.4	19.7	0.8 ^a	+	0.2	24.5
	All	11.3^{A}	\pm	2.1	18.6		2.7 ^{<i>B</i>}	\pm	0.6	24.2	1.1^{Γ}	+	0.4	32.9
Detectable	AVATAR	4.9 ^a	±	0.6	12.9		1.7 ^a	±	0.2	14.7	0.8^{a}	±	0.2	22.0
rhizodeposits in	DH-1	5.3 ^a	\pm	0.3	6.2		1.8 ^a	\pm	0.3	15.0	1.1^{b}	±	0.2	17.7
the rhizosphere	DH-2	6.6^{b}	\pm	1.0	14.9		1.9 ^a	\pm	0.2	12.3	0.8^{a}	±	0.2	25.4
and bulk soil	VISBY	4.7 ^a	\pm	0.8	16.8		1.7 ^a	\pm	0.3	18.7	0.8^{a}	±	0.2	22.7
$(< 300 \mu{\rm m})$	All	5.3 ^A	±	1.0	19.1		1.8 ^B	±	0.3	14.8	0.9 ^Г	±	0.2	25.1

the C used for root growth increases over the course of plant development, the ¹⁴C ratios (${}^{14}C_{\% \ rhizodep}!$)¹⁴C_{$\% \ root}) of all three ¹⁴C pulse labeling experiments were analyzed and are plotted in Fig. 2). In this boxplot, the position of the medians and the middle 50% of the data (closed squares) increase with subsequent labelings across plant development. This trend is confirmed by the significant</sub>$

differences between the averages of the pooled data (n=4x6=24) of the individual labeling experiments. Analyses of individual genotypes showed a significant increase in the ratios across the three developmental stages in the cases of DH-1 and VISBY.

Moreover, Fig. 3 clearly shows that the ratio of ¹⁴C released by rhizodeposition to the ¹⁴C used to build the root can be described by a nonlinear function ($y=\alpha/x^{\beta}$).



Fig. 1 Each boxplot represents 24 ratios of the percentages of ¹⁴C associated with belowground respiration (${}^{14}C_{\% \ belowgr, resp.}$) and the sum of the ¹⁴C in the roots and rhizodeposits (${}^{14}C_{\% \ root}$ + ${}^{14}C_{\% \ rhizodep.}$) of all four genotypes at the time of harvest, 21 days after labeling at leaf development, inflorescence emergence and development of fruit. Below the boxplots are the corresponding means and standard deviations of the different genotypes (based on n=6 at each harvest) and all genotypes together (n=24 at each harvest). Different letters indicate significant differences between the developmental stages according to a Student-Newman-Keuls or Nemenyi test (p-level: 0.05)

The curve in Fig. 3 shows that the amount of freshly assimilated C that flows into rhizodeposits is approximately the same as that used to build root tissue when less than 1% of the assimilated C is used for root development. In contrast, in young plants, where a much higher percentage of assimilated C is used to build root tissue, the ratio of the ¹⁴C of rhizodeposits to the ¹⁴C of root tissue is much smaller.

To determine whether the size or age of the root system is responsible for the observed relationship, several correlation analyses were performed. We assumed that in oilseed rape, the carbon mass and surface area of the roots are positively related. Therefore, the C mass of the root system observed at 21 days after ¹⁴C labeling was used as a proxy for its size. The ratio of ¹⁴C in rhizodeposits to ¹⁴C in the roots was significantly but weakly correlated with the C mass of the root system ($r_{Spearman}=0.462^{***}$). In contrast, there was a stronger correlation ($r_{Spearman}=0.809^{***}$) between this ratio (${}^{14}C_{\% rhizodep}$. / ${}^{14}C_{\% root}$) and plant age on the day of labeling.



Fig. 2 Each boxplot represents 24 ratios of the percentages of ¹⁴C of rhizodeposits (¹⁴C_{% rhizodep.}) and ¹⁴C incorporated into the root (¹⁴C_{% root.}) for all four genotypes 21 days after labeling at leaf development, inflorescence emergence and development of fruit. Below the boxplots are the corresponding means and standard deviations of the different genotypes (based on n=6 at each harvest) and all genotypes together (n=24 at each harvest). Different Latin or Greek letters indicate significant differences between developmental stages determined by a Student-Newman-Keuls or Nemenyi test (p-level: 0.05), respectively

¹⁴C distribution in the root system

Autoradiograms were produced to find out which parts of the root system were supplied with freshly assimilated C up to the end of fruit development.



Fig. 3 Ratio of ¹⁴C in rhizodeposits and root tissue (¹⁴C_{% rhizodep}, / ¹⁴C_{% root}) in relation to the ¹⁴C found in root tissue of all four genotypes (AVATAR, DH-1, DH-2 and VISBY). Model: y= 0.803837/x^{0.33883}. Both parameters had p-values < 0.001, n=72

Figure 4 shows that the recently assimilated ¹⁴C was increasingly used for the production of root branches and root tips. However, clearly, even old parts of the roots, including the upper part of the taproot, were supplied with ¹⁴C in the final phase of fruit development.

Relationships between the relative C fluxes of root growth, rhizodeposition and belowground respiration

To detect relationships between root growth, rhizodeposition and belowground respiration (the third hypothesis), the ¹⁴C percentages of these three system components were paired with each other, and correlation analyses were performed. For this purpose, the





pooled data sets for all four genotypes (with n=72) as well as the corresponding subsets (n=18) of the individual genotypes were paired separately. The results of all correlation analyses are summarized in Table 3.

As shown in Table 3, the correlation coefficients of the 15 analyses range from 0.843 to 0.979. To determine the type of the relationships, linear and nonlinear regression analyses were performed. Nonlinear models were found to be more suitable than linear models in describing the relationships between the subsurface C fluxes. Furthermore, the model y= $\alpha (1 - e^{(-b + x)})$ was found to be suitable for describing the relationships between the fluxes of C of the three system components for all genotypes together as well as individually. In graph a of Fig. 5, the slope of the ¹⁴C released in the form of rhizodeposits was steeper in plants that used a relatively low percentage of assimilated ¹⁴C for root growth (predominantly old plants) than in plants that incorporated a higher percentage of freshly assimilated C into roots (predominantly young plants). The same dynamics were observed for the release of ¹⁴C-labeled CO₂, regardless of whether the percentage of ¹⁴C-labeled belowground respiration was plotted against ¹⁴C in root tissue (b) or rhizodeposits (c).

In addition to the three regression analyses of the pooled data from all four genotypes (Fig. 5), 12 further regression analyses were performed for the individual genotypes. In 14 of 15 regression analyses, the p-values of the parameters α and β were less than 0.001.

Table 3 Spearman rank correlation coefficients for the ¹⁴C percentages of roots (${}^{14}C_{\% root}$), rhizodeposition (${}^{14}C_{\% rhizodep}$) and belowground respiration (${}^{14}C_{\% helowgr.resp.}$) for individual genotypes (n=3*6=18) and pooled data from all four genotypes (n=4*3*6=72). *** indicates that the p-value of the correlation coefficient is < 0.001.

	Comparison of							
	¹⁴ C _{% root}	¹⁴ C _{% root}	¹⁴ C _{% rhizodep.}					
	vs.	vs.	vs.					
Genotype	¹⁴ C _{% rhizodep.}	¹⁴ C _{% belowgr. resp.}	$^{14}C_{\% \ belowgr. \ resp}$					
AVATAR	0.878***	0.928***	0.920***					
DH-1	0.940***	0.872***	0.903***					
DH-2	0.911***	0.843***	0.894***					
VISBY	0.897***	0.901***	0.979***					
All	0.917***	0.906***	0.923***					

The coefficient of determination for the regression analysis of individual genotypes (n=18) was higher than that of the pooled data sets for all four genotypes (n=72, Fig. 5). The statistics of the relationship between the percentages of 14 C incorporated into root tissue and from detectable



Fig. 5 Relationships between the percentages of ¹⁴C incorporated into root tissue (${}^{14}C_{\% root}$) and ${}^{14}C$ of detectable rhizodeposits (${}^{14}C_{\% rhizodep.}$) (a), between the percentages of ${}^{14}C$ incorporated into root tissue and ${}^{14}C$ released as CO₂ (${}^{14}C_{\% belowgr.resp.}$) from the soil (b) and between the percentages of ${}^{14}C$ of detectable rhizodeposits and ${}^{14}C$ of belowground respiration (c) for all four oilseed rape genotypes (n=72)

rhizodeposition for each genotype are summarized in Table 4.

Comparison of spring rye and winter oilseed rape regarding C partitioning for root growth and rhizodeposition.

To compare the relationships between the percentages of ¹⁴C incorporated into root tissue (¹⁴ $C_{\% root}$) and the ¹⁴C of the detectable rhizodeposits (¹⁴ $C_{\% \ rhizodep.}$) in winter oilseed rape (a dicotyledonous plant with a taproot system) and spring rye (a monocotyledonous plant with an adventitious root system), the corresponding data points and models were plotted, as shown in Fig. 6. The spring rye model and related data were taken from a previously conducted study Remus and Augustin (2016). It should be noted that the spring rye data set covers a larger developmental period. For the spring rye, the first labeling was performed on 16-day-old plants in the emergence stage, whereas the first labeling of oilseed rape was performed after vernalization at the end of leaf development when the plants were already 88 days old.

Figure 6 shows that the release of ${}^{14}C$ via rhizodeposition in spring rye and oilseed rape followed a similar pattern. In fact, as the plant age increased, the curves continued to converge. However, the figure also shows that a lower percentage of ${}^{14}C$ was found in rhizodeposits of oilseed rape than in spring rye when a high percentage (15-20%) of ${}^{14}C$ was used to build root matter.

Table 4 Coefficients of determination (R_{nl}^2) and parameters of the model (α and β) used for the nonlinear regression analyses of the relationship between root growth $({}^{14}C_{\% \ root})$ and rhizodeposition $({}^{14}C_{\% \ rhizodep})$ of the individual genotypes. *** indicates that the p-value of the parameter is < 0.001

Genotype	R_{nl}^2	α	β
AVATAR	0.947	5.501***	0.131***
DH-1	0.973	7.419***	0.068***
DH-2	0.883	9.101***	0.076***
VISBY	0.903	4.916***	0.170***



Fig. 6 Data points and modeled relationships between the percentages of ¹⁴C used for root growth (${}^{14}C_{\% root}$) and ${}^{14}C$ of detectable rhizodeposits (${}^{14}C_{\% rhizodep.}$) in spring rye (gray, n=19, Remus and Augustin (2016)) and winter oilseed rape (black, n=72). Since the data are from different stages of plant development, arrows have been added to help classify the data over time

What determines the absolute amounts of rhizodeposited C?

To investigate the influence of various factors on the absolute amount of rhizodeposited C, it is imperative to investigate the variations in absolute rhizodeposition along with potential influential factors. Therefore, we first addressed the changes in shoot and root growth during plant development and the genotypic differences.

Changes in shoot and root growth during plant development

To describe the changes in shoot and root growth of the investigated genotypes, the amounts of C accumulated in the whole plant and individual plant parts at each harvest, i.e., at elongation growth, flowering and the end of fruit development, were analyzed. As shown in Fig. 7, a significant increase in the amount of C was observed in the whole plant (shoots and roots (> 300 μ m)) across the sampling points, with an average doubling of the C amount between elongation growth and flowering as well as between flowering and the end of fruit development. At the end of fruit development, the largest difference in the amount of C accumulated in the whole plant was found between AVATAR and DH-2. When the C amount in DH-2 was set to 100%, AVATAR accumulated 18% more



Fig. 7 The C amounts in the whole plants (shoots and roots (> $300 \ \mu m$)) of four winter oilseed rape genotypes at elongation growth, flowering and the end of fruit development. C amounts represented by bars and bar portions with the same lowercase Latin letters are not significantly different according to the Student-Newman-Keuls test (p-level: 0.05, n=6) for the individual sampling point. Different uppercase Latin letters indicate significant differences between plant developmental stages regarding the means of C accumulated in the entire plants of all four genotypes according to the Nemenyi test (p-level: 0.05, n=24)

C in the whole plant than DH-2. On average, the C amount in the shoot accounted for 84% of the total C mass of the plant.

Similar to the results observed for the amount of C accumulated in the whole plant, a significant change in the shoot to root ratio was observed between the developmental stages. A comparison of the individual genotypes showed that DH-1 accumulated a higher proportion of assimilated C in the root in two of three development stages, which was reflected in its low shoot to root ratio (Table 5) and revealed significant differences between the shoot to root ratios of DH-1 and other genotypes at flowering and the end of fruit development.

The average amount of C in the entire root system across all four genotypes increased over time. However, this increase was statistically confirmed only between the stages of elongation growth and flowering, whereby the average amount of C increased from 1388 mg to 2611 mg. From flowering to the end of fruit development, the amount in the entire root system grew by only 226 mg C to a total of 2837 mg

Table 5 Shoot to root ratios of four oilseed rape genotypes based on the C amount [g] in the shoots and roots > 300μ m at three developmental stages. Errors are calculated as standard deviations. Means followed by the same lowercase Latin letter are not significantly different according to the Student-Newman-Keuls test (p-level: 0.05, n=6), and means followed by the same Greek letter are not significantly different according to the Nemenyi test (p-level: 0.05, n=6). All: means and stand-

to the Nemenyi test (p-level: 0.05, n=6). All: means and standard deviations of all data from the four genotypes at the same developmental stage. Different uppercase Latin letters indicate significant differences between the means of the shoot to root ratios of all four genotypes and the individual plant developmental stages according to the Student-Newman-Keuls test (p-level: 0.05, n=24)

	Harvest at		
Genotype	elongation growth	flowering	end of fruit development
AVATAR	$2.4^{b} \pm 0.4$	$2.6^{c} \pm 0.3$	$5.5^{\beta} \pm 0.6$
DH-1	$1.7^a \pm 0.1$	$1.9^{a} \pm 0.2$	$3.9^{\alpha} \pm 0.2$
DH-2	$2.4^b \pm 0.5$	$3.3^d \pm 0.3$	$6.1^{\beta\gamma} \pm 0.6$
VISBY	$1.9^a \pm 0.4$	$2.3^b \pm 0.3$	$6.1^{\gamma} \pm 0.4$
All	$2.1^{\rm A}\pm0.5$	$2.5^B \pm 0.6$	$5.4^{C}\pm1.0$

C. A comparison among the genotypes revealed that DH-1 had a significantly higher amount of C in the entire root system and taproot than the other three at flowering and at the end of fruit development (Fig. 8).

At the end of fruit development, the greatest difference between DH-1 and DH-2 was observed for the amount of C in the root system. When the amount of C in the entire root system of DH-2 was set equal to 100%, the root system of DH-1 had approximately 51% more C than that of DH-2.

Figure 8 also shows that between elongation growth and the end of fruit development, much more C was invested in the growth of the lateral roots than in taproots. During this period, the growth of the lateral roots was on average more than twice that of the taproot.

Changes in the absolute C amounts used for rhizodeposition and root growth

The amount of assimilated C on the day of labeling that was used to build root tissue and rhizodeposits was calculated by Eq. 2 and 3. As shown in Fig. 9, the absolute amount of C in the rhizodeposits changed significantly during plant development.



Fig. 8 C amount in the entire root system (taproot and lateral roots, including all root fragments > 300μ m) of four winter oilseed rape genotypes at elongation growth, flowering and the end of fruit development. C amounts represented by bars and bar portions with the same lowercase Latin letters are not significantly different according to the Student-Newman-Keuls test (p-level: 0.05, n=6) at the individual sampling point. Different uppercase Latin letters indicate significant differences between the means of C accumulated in the entire root system of all four genotypes and the plant developmental stages according to the Student-Newman-Keuls test (p-level: 0.05, n=24)

Rhizodeposition of the plants labeled at the time of inflorescence emergence was on average approximately 39% higher than that of the younger plants (labeled at the end of leaf development) and approximately 52% higher than that of the older plants (labeled at the start of fruit development). Additionally, there were significant differences in rhizodeposition between the genotypes in the first and third labeling experiments. The rankings in the first and third experiments were also different (Fig. 9). It is particularly noticeable that at 21 days after labeling at the time of leaf development, DH-2 showed a significantly higher rhizodeposition rate than DH-1 and AVATAR (Fig. 9). In addition, at the time of the first labeling experiment, DH-2 showed a significantly greater root growth rate than AVATAR and DH-1, as clearly shown by the corresponding mean values in Fig. 10. In contrast, significantly higher rates of rhizodeposition and root growth were found in DH-1 than in DH-2 21 days after labeling at the time of fruit development (Figs. 9 and 10). To investigate whether there was indeed a correlation between these



Fig. 9 Absolute C amounts in rhizodeposits built from C that was assimilated on the day of labeling and was detectable at harvest 21 days after labeling (at the end of leaf development, inflorescence emergence and the start of fruit development). Below the boxplots are the corresponding means and standard deviations for the different genotypes (based on n=6 at each harvest) and all genotypes together (n=24 per developmental stage). Different lowercase Greek and Latin letters indicate significant differences between means at the same developmental stage determined by the Nemenyi test and Student-Newman-Keuls (p-level: 0.05), respectively. Means followed by different capital letters are significantly different between developmental stages as determined by the Nemenyi test (p-level: 0.05)

two absolute C fluxes, correlation analyses were performed at each of the investigated time points. In the two labeling experiments (at leaf development and fruit development) where significant differences in detectable rhizodeposition among the four genotypes were found, significant positive correlations were also obtained. The Pearson correlation coefficients were 0.647*** and 0.791***, respectively. At the time of inflorescence emergence, when no significant differences in rhizodeposition among the genotypes were found, no significant correlation between the rates of rhizodeposition and root growth was obtained.

Sensitivity analysis for absolute amount of rhizodeposited C

The influence of genotypic variation in shoot (ΔC_{shoot}) and root (ΔC_{root}) growth rates as well as C partitioning within the plant (${}^{14}C_{\% shoot}$, ${}^{14}C_{\% root}$, ${}^{14}C_{\% rhizodep}$) on the absolute amount of C in the rhizodeposits was investigated through sensitivity analysis. The smallest and largest mean values of a trait found for one of the



Fig. 10 Absolute C amounts used for root growth originating from C that was assimilated on the day of labeling (at the end of leaf development, inflorescence emergence and the start of fruit development). Below the boxplots are the corresponding means and standard deviations for the different genotypes (based on n=6 at each harvest) and all genotypes together (n=24 per developmental stage). Different lowercase Greek and Latin letters indicate significant differences between the means of the same developmental stage as determined by the Nemenyi test and Student-Newman-Keuls test (p-level: 0.05), respectively. Means followed by different uppercase letters are significantly different between developmental stages as determined by the Student-Newman-Keuls test (p-level: 0.05)

four genotypes were gradually introduced into Eq. 3 or 5, while for the remaining traits, the mean values across all four genotypes were used (see section 'Calculation of the influence of genotypic variation in different traits on rhizodeposition' for more details). The results of these calculations are summarized in Table 6.

As shown in Table 6, the observed variation in ¹⁴C partitioning coefficients of the shoots (${}^{14}C_{\% \ shoot}$) had the weakest influence on rhizodeposition at each developmental stage because this trait showed the smallest difference in percentage deviation, which ranged from 9% at leaf development to 4% at fruit development. In contrast, the variations in the shoot growth rate (ΔC_{shoot}) and the ¹⁴C partitioning in the root had the greatest influence on rhizodeposition in the leaf development and fruit development stages because their percentage deviations from the mean (reference) were 63% and 70%, respectively. At inflorescence emergence, the percentage deviation of both the root growth rate (ΔC_{root}) and ¹⁴C partitioning in the root was 50%, showing that variation in these traits has a similar influence on rhizodeposition.

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Consequently, this also means that there is no single trait whose genotypic variation is the dominant driver of the amount of C in rhizodeposits at all three stages of development. Therefore, the ranges of absolute C amounts in rhizodeposits at all three developmental stages for each trait were added, and the sums of the traits were ranked by size. This resulted in the following ranking: $\Delta C_{root} >$ $^{14}C_{\% root} > \Delta C_{shoot} > {}^{14}C_{\% rhizodep.} > {}^{14}C_{\% shoot}$. This ranking shows that the genotypic variation in the root growth rate had the greatest influence on the absolute amount of C in rhizodeposits ($\Delta C_{rhizodep}$). This is also supported by the $R_{adjusted}^2 = 0.782$ of a multiple regression analysis in which the functional dependence of the average absolute rhizodeposition (C in mg per plant per day) on the genotypic means of the root growth rate and its ¹⁴C distribution $({}^{14}C_{\% root})$ was analyzed. The parameters of both variables and the intercept had p-values < 0.01. The multiple regression analysis using the last two variables (14C% rhizodep, 14C% shoot) of the ranked list yielded a coefficient of determination $(R_{adjusted}^2)$ of -0.018, which supports their weak influence on absolute rhizodeposition.

Influence of plant traits on the absolute amount of C in rhizodeposits

To better understand the dependence of the average absolute amount of rhizodeposited C on other factors, several multiple linear regressions were performed on ΔC_{root} combined with all other traits used in sensitivity analysis (see above). The highest coefficient of determination (= 0.782) was obtained for the combination of the genotypic averages of ΔC_{root} and ${}^{14}C_{\% root}$.

However, the investigations for testing hypothesis 2 showed that the ratio of freshly assimilated C used for rhizodeposition and root growth was not constant but changed significantly during plant development. Therefore, this relationship was included in the next analyses where the 12 genotypic mean values of the ¹⁴C_{% rhizodep.} / ¹⁴C_{% root} ratios were combined with the 12 mean root growth rates and set against the 12 mean absolute amounts of rhizodeposited C in a multiple linear regression analysis. This analysis yielded a coefficient of determination of 0.842, where the parameters of both variables and the intercept had p-values < 0.01. Afterwards, the same analysis was performed using the 72 individual values of each

Table 6 Sensitivity analysis for the amount of absolute C of detectable rhizodeposits from the C assimilated on the day of labeling 21 days before sampling. As references (100%), the average amounts of rhizodeposited C, which were calculated by the average rates of shoot growth (ΔC_{shoot}) and root growth (ΔC_{root}) from the day of labeling as well as the percentages

of¹⁴C incorporated into the shoot ($^{14}C_{\% shoot}$), root ($^{14}C_{\% shoot}$) and rhizodeposits ($^{14}C_{\% rhizodep.}$), of all four genotypes (AVA-TAR, DH-1, DH-2 or VISBY) were used. Percentages with positive and negative signs indicate the deviation from the reference (100%)

	Rhizodeposition [mg C plant ^{-1} day ^{-1}] and [%] at							
Varied parameter Smallest genotype-specific mean of ΔC_{shoot} Largest genotype-specific mean of ΔC_{shoot} Smallest genotype-specific mean of ΔC_{root} Largest genotype-specific mean of ΔC_{root} Smallest genotype-specific mean of $^{14}C_{\% shoot}$ Largest genotype-specific mean of $^{14}C_{\% shoot}$	leaf development	[%]	infloresence emergence	[%]	develop- ment of fruit	[%]		
Smallest genotype-specific mean of ΔC_{shoot}	1.61	-28	3.09	-4	1.97	-8		
Largest genotype-specific mean of ΔC_{shoot}	3.02	+35	3.44	+7	2.33	+9		
Smallest genotype-specific mean of ΔC_{root}	1.87	-16	2.41	-25	1.77	-17		
Largest genotype-specific mean of ΔC_{root}	2.90	+30	4.02	+25	3.08	+44		
Smallest genotype-specific mean of ${}^{14}C_{\% \ shoot}$	2.34	+5	3.33	+4	2.19	+2		
Largest genotype-specific mean of ${}^{14}C_{\% \ shoot}$	2.14	-4	3.08	-4	2.10	-2		
Smallest genotype-specific mean of ${}^{14}C_{\% root}$	2.35	+5	4.13	+29	3.00	+40		
Largest genotype-specific mean of ${}^{14}C_{\% root}$	2.01	-10	2.54	-21	1.49	-30		
Smallest genotype-specific mean of ${}^{14}C_{\% \ rhizodep.}$	1.95	-13	3.05	-5	1.91	-11		
Largest genotype-specific mean of ${}^{14}C_{\% \ rhizodep.}$	2.76	+24	3.38	+5	2.68	+25		
Reference: means of all parameters over all genotypes	2.23	100	3.21	100	2.14	100		

variable. The graph of this multiple linear regression (mesh), data points, corresponding equation and statistics are presented in Fig. 11. Multiple linear regression with all 72 data points of the four genotypes resulted in a coefficient of determination ($R^2_{adjusted}$) of 0.766. This finding revealed that the change in the absolute amount of C used in rhizodeposition during plant development does not depend on a single

variable but on at least two variables. Using the averages of the root growth rates (ΔC_{root}) and ratios of ¹⁴C in the rhizodeposits and root matter (${}^{14}C_{\% \ rhizodep.}$ / ¹⁴ $C_{\% \ root}$) in the generated model ($\Delta C_{rhizodep.} = \alpha * \Delta C_{root} + \beta * {}^{14}C_{\% \ rhizodep.}$ / ¹⁴ $C_{\% \ root} -\gamma$) (Fig. 11), impacts of 55% and 45% from the first and second variables, respectively, on the calculated rate of rhizodeposition were observed.



Fig. 11 Relationships of the rate of rhizodeposition ($\Delta C_{rhizodep}$, mg of C per plant and day) with the rate of root growth (ΔC_{root} , mg of C per plant and day) and the ratio of recently assimilated C used for rhizodeposition per unit C incorporated into root tissue (${}^{14}C_{\% rhizodep}$, / ${}^{14}C_{\% root}$) over time. The coefficient

of determination ($R^2_{adjusted}$) for the model ($\Delta C_{rhizodep.} = 0.46 * \Delta C_{root} + 3.8 * {}^{14}C_{\%} {}_{rhizodep.} / {}^{14}C_{\%} {}_{root} -2$ (values are rounded) is 0.766, where the parameters of the two variables (ΔC_{root} and ${}^{14}C_{\%} {}_{rhizodep.} / {}^{14}C_{\%} {}_{root}$ ratio) as well as the intercept have p-values < 0.001, n=72

Additionally, note that the comparison of more than 200 different models with three parameters showed that neither the root growth rate nor the ratio of the two ¹⁴C partitioning coefficients alone was able to describe the changes in the absolute amount of rhizodeposited C since the largest coefficient of determination was only 0.291. Performing 25 correlation analyses revealed that no other trait (such as plant age; phenological stage of development on the BBCH scale; air temperature; C amount in shoots, root systems or lateral roots; shoot to root ratio; growth rate of the shoot or root; and ¹⁴C partitioning coefficients and their ratios) identified or calculated in this study was able to describe the changes in the absolute rhizodeposition values alone. These analyses showed only a weak relationship between the rates of root growth and the rates of rhizodeposition ($r_{Spearman} = 0.504^{***}$).

Finally, note that in Table S1 and S2 (Supplementary material), plant age, air temperature, C amounts of the shoot and root as well as ¹⁴C partitioning coefficients are listed individually for each pot.

Discussion

Statistical analyses showed that the chosen genotypes varied significantly with respect to shoot and root growth as well as shoot to root ratio, at least in part. This was also true for the ¹⁴C partitioning coefficients as well as the rates of absolute C fluxes of root growth and rhizodeposition. This indicates some degree of genotypic variation, which should increase the significance of the relationships found and described in this study. Moreover, a significant change in the relationship between rhizodeposition and root growth over time could be detected, which was not possible in the study of Nguyen (2003). We assume that the variance created by merging data from different studies masked any observable trends. Nevertheless, the significant differences that we found in the investigations of hypothesis 1 and 2 indicates the suitability of our experimental design and detection method.

First hypothesis

During plant development, a significant increase in the ¹⁴C-labeled CO₂ of belowground respiration per unit ¹⁴C used for root growth and in rhizodeposition could be

detected. Since significant growth of the root system was recorded, it can therefore be assumed that the increased release of 14C-labeled CO2 was caused by increased maintenance respiration of the enlarging root matter (Amthor 1984; Gifford 2003; Szaniawski and Kielkiewicz 1982; Thornley 1970). Indeed, the corresponding analysis showed a positive and significant yet weak correlation between the two variables (r_{Spearman}: 0.552***). One possible reason for the weak correlation may be the fact that, according to Lötscher et al. (2004), mainly older C compounds are used for maintenance respiration, whereas mainly fresh C is used for growth. However, note also that approximately 80% of the ¹⁴C-labeled CO₂ was released during the first five days after labeling, while the C mass of the root was determined at harvest 21 days after labeling. Therefore, the determined root C masses are only proxies of the real root C mass after labeling. They nevertheless should reflect the differences and trends of root C mass progression between the individual plants.

The rise in night and day air temperatures during the developmental stages, which was approximately 10 °C from leaf development up to the development of fruit, may also contribute to an increase in the turnover of rhizodeposits and/or root tissue respiration (Boone et al. 1998; Lloyd and Taylor 1994; Szaniawski and Kielkiewicz 1982; Turnbull et al. 2001). Because the corresponding correlation was strong ($r_{Spearman}$, 0.8455***), we assume that the temperature had a stronger effect on the ratio of ¹⁴C released as CO₂ in the rhizosphere to the sum of ¹⁴C in root tissue and rhizodeposits than the size of the root system.

Nevertheless, the findings suggest that older plants released more 14 C-labeled CO₂ per unit 14 C detected in root tissue and rhizodeposits than younger plants.

Second hypothesis

As expected in H2, the average ratio of the ¹⁴C in rhizodeposits to the ¹⁴C in root tissue across all four genotypes was not stable but increased significantly up to the development of fruit (Fig. 2). This result implies that older plants release significantly more freshly assimilated C through rhizodeposition than younger plants in relation to the C that they use to build root matter. Our investigation further revealed a nonlinear model (Fig. 3) that can be used to describe this dependency. As already mentioned, the increase in the ratio of ¹⁴C used for rhizodeposition to root growth

during plant development can be explained by a growing root system (root length and surface) over time if it is considered that root-derived C is released into the soil not only from the root tip but also from older root segments (Personeni et al. 2007) and that most of the rhizodeposits, the exudates, passively enter the soil by diffusion from the root (Jones et al. 2009).

However, there was strong root growth (1223 mg C) from the first to the second labeling experiment but only small root growth (226 mg C) between the 2nd and 3rd experiment, while the ratio of the ¹⁴C of rhizodeposition to the ¹⁴C in root tissue continuously increased from 0.3 to 0.5 and finally to 0.8. These results indicate a disproportionate relationship between root growth and the ¹⁴C ratio studied here. Furthermore, rank correlation analysis of the root C mass 21 days after ¹⁴C labeling and the ratio of the ¹⁴C of rhizodeposition to the ¹⁴C that was used for root growth of all 72 pairs of data showed a correlation coefficient (r_{Spearman}) of only 0.462***. The analysis of the data at each developmental stage (n=4x6=24) and of the pooled data from the first and second labeling experiments (n=48), where strong root growth was observed, did not reveal a correlation coefficient greater than 0.462 (analyses not shown). Hence, no closer relationship between the two traits could be identified.

The reason for this weak correlation is unlikely to be the use of root system C masses instead of root lengths because the increases in root lengths and their dry masses are relatively similar (Malagoli and Le Deunff 2014). Thus, at least one additional trait must be involved. This assumption is also supported by the fact that the increasing ratio of ¹⁴C used for rhizodeposition to ¹⁴C used for building root tissue (${}^{14}C_{\% \ rhizodep.}/{}^{14}C_{\% \ root}$) has a stronger correlation (r_{Spearman}=0.809***) with plant age. The progression of plant development has not only an effect on root size (root length, branching, and numbers of root tips and root cap cells) but also several other effects on the root system. The programmed cell death of root cells, with a predictable pattern, is probably an important effect. For several grass species, an increasing linear correlation between plant age and the death of root cortex cells was observed (Gillespie and Deacon 1988; Liljeroth 1995). Increasing root turnover rates during the growing season was also reported by Swinnen et al. (1994b), as already mentioned. An indication that in the presented study, increasing root turnover with plant development took place is given by the increasing percentages of radioactivity found in the soil fractions > 300 μ m. As described in sections: 'Plant and soil sample preparation' and 'Carbon analysis and calculations for all components and fractions', soil samples were sieved after root selection to determine the amount of root segments > 300 μ m that could not be hand-selected due to their small size. The analysis showed that in the sieved soil fractions (mostly white sand) > 300 μ m, 17%, 21%, and 35% of the total activity of lateral roots was found for the 1st, 2nd, and 3rd experiments, respectively, although the root mass increased only by approximately 8% between the 2nd and 3rd experiments. This finding clearly indicates an increase in detached small root fragments over time. The decomposition of these root segments is also likely to release C compounds, cell fragments, or whole cells into the soil that are detected as rhizodeposits. In addition, aging root cell walls are more susceptible to degrading microbial enzymes, which can reduce the integrity of the root surface and promote the release of root-derived C into the soil (Rovira 1969).

The fact that even in old winter rape plants, the entire root system, including the old root parts, is supplied with freshly assimilated C (Fig. 4) leads to the assumption that freshly assimilated C can diffuse into the soil over a large surface area and can also be released into the soil by the death and lysis of old root cells.

Third hypothesis

The aim of the 3rd hypothesis was to determine whether the relative C fluxes of root growth, rhizodeposition and belowground respiration in winter oilseed rape (a dicotyledonous plant with a taproot system) are similarly correlated and show functional dependencies such as those described for spring rye (a monocotyledonous plant with an adventitious root system) (Remus and Augustin 2016). As can be seen from the analyses, very close relationships between root growth (incorporation of ¹⁴C into root tissue), rhizodeposition (release of ¹⁴C-labeled root-derived C into the soil), and belowground respiration (release of ¹⁴C-labeled CO₂ from the root-soil system) could be determined for winter oilseed rape. The high correlation coefficients found in all analyses of both the individual genotypes and the pooled data (Table 3) clearly indicates that root growth, rhizodeposition and belowground respiration, for which recently assimilated carbon is metabolized, were strongly positively correlated. The almost identical Spearman rank correlation coefficients found for the relationship of the percentage of ¹⁴C incorporated into the root tissue and the percentage of ¹⁴C detected in rhizodeposits in spring rye and winter oilseed rape, which had values of 0.912*** (Remus and Augustin 2016) and 0.917*** (Table 3), respectively, indicate that this relationship was almost equally correlated in both crops. In addition, the regression models of the pairwise analysis of the three subsurface C fluxes (Fig. 5) showed similar progressions to those obtained for spring rye (Remus and Augustin 2016). The direct comparison of the regression models for ¹⁴C incorporated into the root tissue and the percentage of ¹⁴C detected in rhizodeposits in winter oilseed rape and spring rye (Fig. 6) shows that in both curves, the slope of ${}^{14}C$ used for rhizodeposition flattened when a high percentage of ¹⁴C was incorporated into the root, which generally occurs in young plants. This indicates that in young plants that use a high proportion of assimilated ¹⁴C for root growth, the release of ¹⁴C in the form of rhizodeposits is limited. If it is correct that the majority of the rhizodeposits are C compounds that leave the root by diffusion (Jones et al. 2004; Jones et al. 2009) and that the exchange of C is determined by the root surface (Personeni et al. 2007), this flattening slope of rhizodeposition can be explained by a limitation of the diffusion due to a small root surface. In contrast, the curve slope is steeper in older plants, indicating that their rhizodeposition is not limited. However, the overall progression of both curves does not exclude the simultaneous release of root-derived C by diffusion and root turnover. Both curves in Fig. 6 show that a lower percentage of ¹⁴C was found in rhizodeposits in oilseed rape than in spring rye when a high percentage (15-20%) of ^{14}C was used to build the root matter. The different root architecture may be responsible for this. Considering that a taproot has a smaller surface area than lateral or adventitious roots with the same biomass, a growing taproot system should release fewer rhizodeposits than an adventitious root system when the same percentages of ¹⁴C are used to build the root matter. However, considering the strong correlation between the amount of ¹⁴C used for root growth and the formation of rhizodeposits and the similarity of their progression, it is reasonable to assume that the partitioning of recently assimilated C to rhizodeposition is influenced by the same mechanisms in both crops, despite their different root architectures (adventitious root versus taproot systems).

What determines the absolute amount of C associated with rhizodeposition?

One goal of this study was to identify the key factors controlling the process of rhizodeposition to improve the understanding of this process and to potentially find new approaches for its simplified modeling. Clearly, the absolute C amount of rhizodeposition depends on multiple factors. It is logical to assume that the absolute C amount of rhizodeposition depends on the relative distribution of C (C partitioning) within the plant-soil system and the absolute amount of assimilated C that can be distributed (Remus and Augustin 2016; Swinnen et al. 1994b). In addition, it is known that C assimilation is largely determined by the rate of Rubisco carboxylation, electron transport driving regeneration of ribulose-1,5-bisphosphate (RuBP) and triose-phosphate utilization (Long and Bernacchi 2003), while C partitioning depends on the sink capacities of individual plant organs (Bihmidine et al. 2013; Lemoine et al. 2013; Ludewig and Fluegge 2013; Yin et al. 2009). Although it is assumed that a large portion of the rhizodeposits, the exudates, enter the soil passively by diffusion, little is known about the quantitative influence of other processes, such as root turnover, on rhizodeposition (Jones et al. 2009), which was why we wanted to find the key factors that significantly regulate overall rhizodeposition. Therefore, we focused on C partitioning to obtain an overview of the relative C distribution within the plant-soil system and the biomass formation of individual plant organs, as they represent the assimilation efficiency and absolute C flux in the plant.

However, reliable statements can be made only on the basis of reliable data. Here, we discuss the most important findings to demonstrate that the observed C partitioning and formation of biomass were not unnatural and followed expected trends. A decrease in the percentage of fresh C transported to the root over the course of plant development (see Table 2) has been observed in other crops (Gregory and Atwell 1991; Keith et al. 1986; Remus and Augustin 2016; Sey et al. 2010; Swinnen et al. 1994b). Notably, the two nonapproved doubled haploid lines DH-1 and DH-2 showed less biomass development of the shoot up to the end of fruit development than the two hybrids AVATAR and VISBY. This is not surprising given that AVATAR and VISBY are high-yielding cultivars.

In contrast to that of the shoot, the C mass of the root systems of all four genotypes showed a significant increase only until flowering (see Fig. 8). This is also reflected in the calculated root growth rates (Fig. 10). A similar trend for root growth was described by Malagoli and Le Deunff (2014). The shoot to root ratio continuously increased over plant development in all four genotypes, which is true for most crops except root crops (Wilson 1988). This result is mainly attributable to the continuously decreasing percentage of assimilated C that is transported to the root during plant development (Table 2). This trend is also observed in some cereals (Remus and Augustin 2016; Swinnen et al. 1994b) and represents the varying axial sink during plant development (Lambers et al. 2008; Yasumura 2009).

At the start of elongation growth, i.e., shortly after the transition from the growth of the rosette body, all four genotypes showed a very low shoot to root ratio (based on their C amounts), ranging from 1.7 to 2.4. This range is narrower than that reported by Svečnjak and Rengel (2006), who examined 4 genotypes of oilseed rape during rosette formation and observed a shoot to root ratio (based on biomass) ranging from 2.4 to 3.6. This discrepancy may partly be because of different genotypes or because in the present study, in addition to the hand-picked roots, the mass of root fragments that could not be detected via manual selection were estimated.

Afterwards, the shoot to root ratio increased to 5.4 by the end of the fruit development stage (Table 5), which is smaller than that of cereals (Bolinder et al. 2012).

In contrast to the continuous decrease in the percentage of the ¹⁴C partitioning coefficient of rhizodeposition during plant development (Table 2), a significantly larger absolute C amount was found for rhizodeposition at the time of inflorescence emergence (2nd experiment) than for the stages of leaf development (1st experiment) and development of fruit (3rd experiment), as seen in Fig. 9. The same trend was also observed in spring rye and is based on the linkage between the relative C partitioning and absolute amount of assimilated C at the corresponding stage of development (Remus and Augustin 2016; Swinnen et al. 1994b). All these results showed that the C partitioning and formation of biomass were not unnatural and facilitated

the search for key factors determining the absolute amount of C associated with rhizodeposition.

Based on the sensitivity analysis, the significant positive correlation between root growth and rhizodeposition rate and the fact that oilseed rape genotypes with significantly higher root growth rates showed significantly higher rates of rhizodeposition, we concluded that the root growth rates significantly affected rhizodeposition rates. This result is also supported by the model for rhizodeposition rates shown in Fig. 11. Moreover, modeling revealed that in addition to the root growth rate, the ratio ${}^{14}C_{\% \ rhizodep.}$ / ${}^{14}C_{\% \ root}$, which is identical to the ratio of $\Delta C_{rhizodep}$ / ΔC_{root} (Eq. 5), determines the rate of rhizodeposition during plant growth. Moreover, a calculation showed that the two variables had nearly the same influence on the rate of rhizodeposition.

Nevertheless, we must consider that the present study investigated winter oilseed rape with a long vegetation period (more than 200 days). In the case of crops with shorter vegetation periods, the influence of age on rhizodeposition rates could be smaller, as suggested by the ${}^{14}C_{\% \ rhizodep.}$ / ${}^{14}C_{\% \ root}$ ratio of spring rye (Remus and Augustin 2016), which showed a maximum range of 0.32 to 0.46 between 16- and 84-day-old plants - clearly a smaller range than that observed in winter oilseed rape (Fig. 3).

Considering that four different genotypes were used in this study, the results of the multiple linear regression (Fig. 11) showed that the changes in rhizodeposition over time can be described reasonably accurately ($R_{adiusted}^2$ =0.766).

Moreover, Fig. 3 shows that the ratio of ${}^{14}C_{\% \ rhizodep.} / {}^{14}C_{\% \ root}$ changes monotonically with a continuous change in the proportion of ${}^{14}C$ used to build the root system. Since several studies have shown that the percentage of fresh C transported to the root of cereals during plant development continuously decreases (Gregory and Atwell 1991; Keith et al. 1986; Sey et al. 2010; Swinnen et al. 1994b), it can be assumed that this behavior is also true for oilseed rape, which is supported by the ${}^{14}C$ partitioning coefficients (Table 2). This suggests that the ratio of ${}^{14}C_{\% \ rhizodep.} / {}^{14}C_{\% \ root}$ should also be relatively easy to model for different plant ages or developmental stages. If this could be done,

based on the multiple linear model shown in Fig. 11 and root growth rates from root growth models, a simple function enabling predictions of the absolute amount of rhizodeposited C in oilseed rape could be generated.

However, our finding that the use of freshly assimilated C for root growth and rhizodeposition in winter oilseed rape shows a trend similar to that found in spring rye (Remus and Augustin 2016) despite their completely different root architectures allows us to assume that root growth and rhizodeposition rates should also be positively related in other crops but with potentially different extents of influence from plant age, which determines the ratio of ${}^{14}C_{\% \ roit}$ or $\Delta C_{rhizodep}$. / ΔC_{root} .

We also foresee that the determination of the relationship between rhizodeposition and root growth at a given plant age or phenological stage of development, e.g., a certain BBCH scale code, could help answer key questions such as whether plants can control the degradation of SOM in the rhizosphere by allocating photosynthetic C to soil via rhizodeposition (Henneron et al. 2020) or if rhizodeposition is determined purely mechanistically.

Detectable and gross rhizodeposition

In this study, sampling was performed 21 days after ¹⁴C labeling to allow sufficient time for the distribution of ¹⁴C in the plant-soil-soil gas system (Remus et al. 2016; Swinnen et al. 1994b). If soil samples are taken several days after ¹⁴C labeling, only the ¹⁴ C-labeled rhizodeposits that have not been microbiologically respired by then can be detected. Moreover, the ¹⁴C-labeled CO₂ released from the soil is composed of that from root respiration (growth and maintenance respiration) and the microbial turnover of rhizodeposits, which makes it extremely difficult to estimate gross rhizodeposition. We have also seen that root mass, root growth and rhizodeposition rates vary during plant development and that temperature has a significant influence on the turnover of freshly assimilated C in the rhizosphere. This should also explain why the rate of C released in the form of CO₂ relative to the C input into the root-soil-soil gas system increased from 51% in the first experiment (labeling at leaf development) to 74% in the third experiment (labeling at fruit development), as shown in Table 7. However, these values are in the upper part of the range of 20-80% for the rhizosphere respiration fraction of C transferred to the belowground system of annual plants reported by Cheng et al. (1993).

Nevertheless, estimation of gross rhizodeposition would be useful for several reasons. Different approaches for estimating gross rhizodeposition were analyzed and recommended by Kuzyakov (2002). The isotope dilution and ${}^{14}CO_2$ dynamics methods have shown root respiration contributing 40-50% and microbial respiration contributing 50-60% to total root-derived CO_2 emissions. Rhizodeposit modeling and exudate elution methods have shown that the average contribution of root respiration is 75% and that microbial respiration accounts for approximately 25% of the total CO₂ released in the rhizosphere (Kuzyakov 2002). Based on the estimates of Kuzyakov (2002), the absolute C rates of detectable rhizodeposition $(\Delta C_{rhizodep.})$ and the rhizosphere or belowground respiration ($\Delta C_{below.resp.}$) (Table 7) and the fact that in the present study, the time between labeling and harvesting was 21 days, which is longer than that in most ¹⁴C pulse labeling studies, the two extremes of 40% for root respiration and 60% for microbial respiration were used to estimate the gross rhizodeposition rates in the three developmental stages (first scenario). This calculation revealed that 8.1 mg of C, 16.0 mg of C and 10.7 mg of C in the form of rhizodeposits were built from the C assimilated on the day of labeling at leaf development, inflorescence emergence and development of the fruit, respectively. This means that at each of the three stages, more C was used for rhizodeposition than for root growth.

If we assume that only 25% (second scenario) of the total rhizosphere respiration originates from microbial turnover of rhizodeposits, the gross rhizodeposition should be 4.7 mg of C, 8.5 mg of C and 5.7 mg of C at the three developmental stages. This means that in the second and third experiments, more C was used for rhizodeposition than for root growth. However, in the two scenarios, the amounts of rhizodeposits in relation to root growth seem quite considerable.

This raises the following question: should rhizodeposition be evaluated as a C loss to the plant if rhizodeposition does not serve to cope with stress, attracts pathogenic organisms and promotes nutrient competition between microbes and plants

(mizoaep.)	Amount of C [mg plant ⁻¹ day ⁻¹] and [%] at								
Process	leaf development	[%]	inflorescence emergence	[%]	development of fruit	[%]			
Root growth (ΔC_{real})	6.9	36.7	6.5	20.9	2.9	15.0			
Rhizodeposition ($\Delta C_{rhizoden}$)	2.3	12.2	3.2	10.3	2.1	10.9			
Belowground respiration ($\Delta C_{below, resp}$)	9.6	51.1	21.4	68.8	14.3	74.1			
Sum	18.8	100.0	31.1	100.0	19.3	100.0			

Table 7 Average absolute C amounts associated with root growth rates (ΔC_{root}), rates of detectable rhizodeposition ($\Delta C_{rbinden}$) and rates of rhizosphere or belowground respira-

tion ($\Delta C_{below,resp}$) and their percentages, which were calculated for each of the three developmental stages over all four geno-types (AVATAR, DH-1, DH-2 and VISBY)

(Jones et al. 2004)? Or does rhizodeposition provide a real benefit to the plant, for example, by promoting the mobilization of nutrients (Finzi et al. 2015)?

Furthermore, the estimated gross rhizodeposition and the determined net rhizodeposition rates (Fig. 9) indicate a peak of rhizodeposition during plant development similar to that in spring rye and wheat (Remus and Augustin 2016; Swinnen et al. 1994b). This could explain the effect of plant phenology on rhizosphere-primed soil C loss (Su et al. 2017; Cheng et al. 2003) under the premise that rhizodeposits drive this process.

Conclusions

We found very strong correlations and functional relationships between the relative partitioning of freshly assimilated C into the root tissue and rhizodeposits of all four oilseed rape cultivars, which leads to the conclusion that the relative C amount in rhizodeposition is highly dependent on that of root growth. The facts that oilseed rape is a dicotyledonous plant with a taproot system and that the observed relationship is similar to that found in spring rye (Remus and Augustin 2016), which is a monocotyledonous plant with an adventitious root system, allow us to assume that the relative C partitioning into rhizodeposition also strongly depends on the percentage of recently assimilated C that will be used to build root tissue in other crops.

The increasing ratio of recently assimilated C used for rhizodeposition per unit C used to build root tissue during growth supports the assumption that roots passively release the majority of rhizodeposits into the soil. However, there is also evidence indicating an increase in detached small

root fragments during plant development. Therefore, it can be assumed that not only increased diffusion due to an increase in root surface area but also increasing root turnover are responsible for the significant increase in the ratio of the percentage of freshly assimilated C of rhizodeposits to that used to build root tissue during plant development. The methods we used allowed the detection of not only the significant differences in relative C fluxes but also those in absolute C fluxes. For example, significant differences are found in the absolute rhizodeposition rates of the genotypes in two of the three developmental stages investigated. Since the ranking of the genotypic rhizodeposition rates changes during plant development, it must be concluded that studies with a single sampling time are unsuitable for determining genotypic trends in rhizodeposition. To estimate or model the rhizodeposition of a crop over its growing season, knowledge of its range of genotypic variation is extremely important. Admittedly, measuring rhizodeposition over the entire growing season, e.g., by linking relative C partitioning and absolute C assimilation for each moment of the growing season (Remus and Augustin 2016), is a rather laborious procedure.

Based on our results, we conclude that the root growth rate and temporally changing ratio of the C used for rhizodeposition to the C used for root growth are key factors determining the absolute amount of rhizodeposition in winter oilseed rape. Moreover, our results suggest that the ratio of recently assimilated C used for rhizodeposition to C used for root growth changes monotonically during plant development. If the assumption of a monotonic change (which is easy to model) is confirmed, a less labor-intensive method for modeling rhizodeposition during plant growth could be developed based on the multiple linear model presented here and root growth rates, e.g., from a thermal time-driven root architecture model.

In addition, it should be mentioned that even the conservative estimates of gross rhizodeposition rates suggest that more C flows into rhizodeposition than is used to build the root tissue of winter oilseed rape in two of the three developmental stages studied. This is rather unexpected and necessitates further investigation of the process of rhizodeposition and its role in the terrestrial C cycle.

Acknowledgements We are grateful to Ruth Brose and J. Busse from the Leibniz Center for Agricultural Landscape Research (ZALF) in Müncheberg (Germany) for plant cultivation and sample preparation. We would like to thank Norddeutsche Pflanzenzucht, Hans-Georg Lembke KG (NPZ), Holtsee (Germany) for providing the winter oilseed rape genotypes used here. In addition, we would like to thank the Agency of Renewable Resources (FNR) for providing financial support (FKZ: 22403011).

Author Contributions Rainer Remus and Divya Pandey wrote the manuscript in consultation with Dietmar Lüttschwager. Rainer Remus and Dietmar Lüttschwager were involved in designing the experiment and applying for funding. Rainer Remus supervised the experiments and performed the chemical and statistical analyses

Funding Open Access funding enabled and organized by Projekt DEAL. Part of this study was financially supported by the Agency of Renewable Resources (FNR), project number: FKZ 22403011.

Data Availability The single values on which the conclusions are based are shown in the supplementary material.

Declarations

Ethical approval This manuscript has not been published elsewhere and is not under consideration by any other journal. Furthermore, there are no conflicts with the ethical responsibilities formulated by Springer (https://www.springer.com/journal/11104/submission-guidelines#Instructions for authors_Ethical Responsibilities of Authors)

Consent to participate Not applicable (no human participants and/or animals)

Consent for publication All the authors have approved the submission of the manuscript to Plant and Soil.

Conflict of interest The authors declare they have no financial or nonfinancial conflicts of interest as described, e.g., by

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