Quantification, spatial distribution and persistence of root-derived carbon for 12 ~~cover crops~~crop species.

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Abstract. Organic carbon derived from roots is a major input fuelling soil organic carbon stocks, especially in agricultural systems, where aboveground biomass might be harvested. However, root sampling for carbon monitoring excludes net rhizodeposition, i.e. the organic compounds released by the roots that have not been rapidly mineralised, and some of the finest root debris, because this fraction of root-derived carbon cannot be directly quantified in the field. To compensate for this shortfall, we set up a two-month experiment with multi-pulse ^{13}C-CO$_2$ labellings of 12 crops to quantify these carbon pools at harvest, operationally grouped under the term SOC_{new}. We also investigated the spatial distribution of belowground carbon inputs within the soil profile. Lastly, in order to follow the fate of this carbon after the plant death, we performed a 524-day litterbag incubation in the field using the labelled material. We found that SOC_{new} accounted for 27 % of belowground carbon inputs at harvest. It was not correlated to carbon amounts of the shoots, but was positively correlated to root carbon (R^2 = 0.14). The vertical distribution of SOC_{new} tended to follow the one of roots. The majority of SOC_{new} was recovered in the bulk soil, rather than solely adhering to the roots, which invites us to consider most of the planted soil as the rhizosphere. We showed that SOC_{new} had a greater persistence time in the soils than roots in the mid-term. However, these findings were marked by high variability because the small quantities of carbon involved make it difficult to assess persistence by isotopic difference. These results suggest that net rhizodeposition and fine root debris should be taken into account in organic carbon management of soils as it refines our estimation of belowground inputs. However, their low predictability, due to the diversity of products and processes, is still a barrier.

1 Introduction

Organic Matter (OM) that originates from roots is a major Carbon (C) pool that fuels Soil Organic Carbon (SOC) stocks (Rasse et al., 2005). In many agricultural systems, this statement is even more valid as a large share of aboveground biomass is exported and therefore does not return to the soil. As a consequence, it is of major importance to quantify accurately root-derived C input, for a monitoring, modelling or even crop selection for additional SOC sequestration purpose. To do so in the field or in mesocosm studies, there is a wide range of methodologies to sample the roots, from using augers to excavating the whole root system or disassembling the mesocosm (Freschet et al., 2021). However, in any case, it is necessary to separate roots from the surrounding soil, generally by hand. This process excludes a large amount of C that also contributes to fuelling the SOC stock. Notably, rhizodeposition, which encompasses the organic compounds released by roots into their surroundings during plant growth ~~(Nguyen, 2009)~~(Nguyen, 2003), is not covered whereas this is a pool that can represent up to 20 % of the photosynthetically fixed C (Hütsch et al., 2002). Besides, fine root debris, such as root hairs or very fine roots, might not be taken into account in the definition of rhizodeposition, although they can also be excluded from root sampling, leaving a gap in the C balance of belowground inputs. This C compounds remaining at harvest form an heterogeneous pool whose operational definition may vary across studies. In recent works, it has been designated as SOC_{new} (Henneron et al., 2020a; Huang et al., 2021), although standardised terminology has to our knowledge not yet been adopted. We chose in this study to focus on SOC_{new}, which is a pool that comprises more C than the compounds strictly coming from rhizodeposition, as defined by Nguyen (2003).

In the last decades, a wide panel of methodologies has emerged to quantify rhizodeposition. Among them, labelling of plant material via an isotopic enrichment of the ambient CO_2 (^{13}C and ^{14}C) has been widely used, as it allows to trace C down into the soil profile. These methodologies have allowed important advances for C rhizodeposition quantification, and have demonstrated the relevance of considering it in C storage strategies. Nevertheless, this task remains challenging as rhizodeposition is composed of a broad variety of ~~carbon~~ C compounds: exudation products like soluble low-molecular-weight organic C compounds; mucilage and border cells; various cell lysates or photosynthates allocated to symbionts (Jones et al., 2009). Their release in the soil ~~, which~~ is governed by a large range of processes: among the most well-documented, we can mention passive diffusion and active transport through the root cell membrane for exudates; secretion through exocytosis for mucilage or enzymes; detachment or lysis of root cells related to root growth or senescence (Jones et al., 2009). Besides, these processes predominate at different times in the plant's life cycle: C allocation to gross rhizodeposition as a whole declines with plant age, as does allocation to roots (Nguyen, 2003). Indeed, C allocation to these two pools is maximal during the first ~~2~~ two months of growth for annual crops (Pausch and Kuzyakov, 2018)~~, is governed by a large range of processes and vary over time~~. On the other side, the senescence-related release of C shows an opposite trend by increasing with plant age (Hirte et al., 2018a). To date, literature reviews have established differences in rhizodeposition quantification between large plant functional groups such as crops, pasture or forest across studies (Li et al., 2024; Pausch and Kuzyakov, 2018). Differentiation between taxa such as families, species or even genotypes has also been achieved (Semchenko et al., 2021; Ndour et al., 2022), but the limited data still prevents generalisation across different studies. For instance, Henneron et al. (2020a) showed for grassland species that legumes yielded more SOC_{new} than other forbs or grasses, which was ~~not confirmed~~ contradicted by another study ~~of Huang et al. (2021). More studies comparing~~ by Huang et al. (2021), who did not found significant differences between families, and who found high a SOC_{new} production for *Artemisia frigida* and *Cleistogenes squarrosa*, a forb and a grass repectively, against a low production for *Medicago ruthenica*, a legume. It is therefore necessary to conduct more comparative studies between different plants under the same conditions~~are therefore needed~~. Beside, they are valuable as they allow to identify predictors of rhizodeposition across taxas. Whereas C allocation to crop roots can be estimated from a large database on root biomass corrected with informations on the environmental conditions and farming systems (Hu et al., 2018; Mattila and Häkkinen, 2025), or from dynamic allocation parameters supported by balanced-growth ecological hypothesis (Kleemola et al., 1996; Shipley and Meziane, 2002), C allocation to SOC_{new} is still poorly understood and requires further data. Promising recent works (Henneron et al., 2020a; Huang et al., 2021; Williams et al., 2022) comparing several species ~~has~~ have led to significant advances that linked plant traits and rhizodeposition~~. They~~ : they demonstrated that the latter is embedded in a roots economic spectrum and that aboveground photosynthetic traits are good predictors of rhizodeposition: acquisitive species, that have high growth rates, tend to allocate more C to rhizodeposition than conservative species~~(Henneron et al., 2020a; Huang et al., 2021; Williams et al., 2022)~~. However, these studies were based on grassland species and it is likely that extrapolation to crops is not straightforward.

Besides the amount of C inputs, their persistence in the soil is also a major factor driving SOC storage (Minasny et al., 2017) . The SOC_{new} ~~C~~ pool, along with ~~root C~~roots, are the main contributor to SOC in the mid-term, even when ~~shoot C is~~ shoots are not harvested (Austin et al., 2017), such as in the case of cover crops, which is a practice that fosters additional SOC sequestration (Poeplau and Don, 2015; Pellerin et al., 2020). Whether it is well documented that roots tend to be more stabilized than aboveground parts due to their chemical and structural composition and their direct release in the soil (Rasse et al., 2005; Bertrand et al., 2006; Freschet et al., 2013; Villarino et al., 2021), there is little literature for rhizodeposition or fine root debris. Reviews suggest that more than half of the rhizodeposition C is lost to respiration within days of his release: around 55 % according to Jones et al. (2009), and around 57 % for crops according to Pausch and Kuzyakov (2018). Nevertheless, the fraction that remains after rapid microbial utilisation, i.e. net rhizodeposition, has been little studied. Some studies assessed the stability of rhizodeposits through size fractionation of organic matter but the results vary greatly from one to another: Islam et al. (2025) and Teixeira et al. (2024) found that more than 70 % of the remaining net rhizodeposition was incorporated into fine fractions, which correlated with its incorporation in microbial biomass. On the other hand, Bicharanloo et al. (2024) and Huang et al. (2021) retrieved more than 70 % in particulate organic matter. Weng et al. (2018) showed more nuanced results with 44 % of the rhizodeposits recovered in particulate organic matter. Fractionation methods are useful to approximate the stability of the remaining litters, but they do not provide information about the amount of rhizodeposits that has been lost since its release in the soil, as would incubations do.

Rhizodeposition is a fresh ~~input of C~~ C input that can alter the cycling of native SOC, already present in the soil, by accelerating or decelerating its mineralisation (Robinson et al., 1989). This phenomenon is often named rhizosphere priming effect (Dormaar, 1990). Across one soil profile, the sensibility of native SOC to this priming might vary vertically: acceleration of native SOC cycling tends to be more pronounced in deeper soils, where mineralisation is slower (Henneron et al., 2022; Schiedung et al., 2023). Beside quantifying rhizodeposition inputs, assessing its vertical distribution is also relevant to apprehend its repercussion on SOC. This is also valuable to predict the fate of root-derived OM itself as its persistence might likewise depend directly on the horizon into which it is released (Berenstecher et al., 2021; Schiedung et al., 2023). Although rhizosphere priming effect generally enhances SOC mineralisation (Huo et al., 2017), it has been shown that an accumulation of rhizodeposits in the close vicinity of the roots may have the opposite effect by protecting SOC through aggregation (Baumert et al., 2018). This aggregation occurs in the rhizosheath (Teixeira et al., 2023), which is a root-adhering soil layer (Watt et al., 1994). As the rhizosheath mass varies across species or genotypes (Ndour et al., 2022; Teixeira et al., 2023), taking it into account and finding out whether SOC_{new} is mostly retrieved there might also help us to apprehend the trend of rhizosphere priming effect.

Here, we propose to establish a thorough C balance of belowground C inputs across 12 crop species~~from 3 families that exhibit different features relative to nutrient acquisition and root structure~~. We set up a mesocosm experiment in a climate chamber under a ^{13}C-CO$_2$-enriched atmosphere ~~. We used multi-pulse labelling~~ to be able to trace root-derived products~~: net rhizodeposition and fine root debris. We grouped these 2 pools under the term SOC_{new}. Plants were chosen so that they could be candidates for intermediate cropping in temperate farming systems. We had 3~~ . We had three main objectives: 1) quantifying SOC_{new} and linking its release to root and shoot C; 2) disentangle ~~its spatial distribution~~ the spatial distribution of SOC_{new} by assessing its vertical distribution and its vicinity to the roots; 3) assessing ~~its~~ SOC_{new} persistence in the soil after harvest of the aboveground biomass through a following field incubation experiment.

2 Materials and methods

2.1 Climate ~~simulation~~ and labelling

12 plant species were grown in mesocosms for ~~2 months in 3~~ two months in three closed climate simulators in an ecolab system simulation (Verdier et al., 2014). Ecolab systems allow a realistic simulation of climate and ecosystem conditions within a large range of parameters. Here, ~~We~~ we simulated a continental temperate summer climate by averaging ~~6~~ six years of meteorological data from 15 August to 10 October, recorded on the CEREEP Ecotron IdF site (N 48°16'56.5", E 2°40'15.9") (Fig. S1). Temperature and relative humidity were programmed to vary within a 10 minutes resolution. Photosynthetic Photon Flux Density (PPFD) was also simulated on a 10 minutes resolution step and discretized into 10 steps over a day, from 0 % to 100 % of the measured intensity. The maximum (100 %) average PPFD was equal to 1181 μmol of photons s^{-1} m^{-2} at the top of the mesocosms (soil surface). When light intensity meteorological data were higher than this maximum value, light intensity was set to 100 %. CO$_2$ was set at 415 ppm during daytime and at 487 ppm during nighttime, corresponding to on-site measurements. Mesocosms were irrigated with ~~osmosis~~ deionized water by drippers and received in total 83 mm of water, which corresponds to the cumulative precipitation data over 56 days. Soil moisture was measured every 15 minutes in 12 separate mesocosms that were not used for C analysis and each contained one of the 12 plant species. We used two sensors (METER Teros 12) per mesocosm, one at the surface and one at 30 cm depth. As mesocosms soils generally dried faster than soils in the field, the irrigation frequency was smoothed over time to avoid the soil surface water content to decrease below ~~0.5~~ 0.05 cm^3 cm^{-3} to ensure plant survival. If the soil moisture level dropped below this value for any mesocosm, regardless of the species, irrigation was launched for all mesocosms. At day 16, the plants were fertilised with an ammonium nitrate solution, at a dose equivalent to 40 kg of Nitrogen (N) per hectare to ensure growth and to simulate mineral residual soil N following a crop harvest.

To label plant material, we performed multiple injections of ^{13}C-CO$_2$ (99 %). Whereas to assess the dynamic transfer of recent photoassimilates, a single pulse of enriched CO$_2$ is well suited, continuous labelling is preferred to ensure that all root-derived products are labelled and the rhizodeposition can be quantified (Studer et al., 2014). Nonetheless, multi-pulse is a robust alternative if pulses are injected very regularly within a short period of time (Warembourg and Estelrich, 2000). To do so, the δ^{13}C isotopic signature of the air in the chambers was measured on line with a cavity ring-down spectroscopy CO_2 analyser (PICARRO G2201-i) coupled to a multiplexer switching between the different chambers during the whole experiment, in order to control the duration and number of injections (Fig. S2). ^{13}C-CO_2 signature and atmospheric CO_2 concentration were controlled separately.

2.2 Plant and soil material

The 12 plant species, listed in Table 1, are crops from ~~3~~ three families (*Fabaceae; Poaceae and Brassicaceae*). They were selected to cover a wide range of plant traits and for their ability to be grown in the targeted period. Except for *Medicago sativa*, which is perennial, each species could potentially be selected for ~~a short summer intercropping~~ an intermediate cropping in temperate regions. The growth period of 56 days was chosen as it is the minimum period permitted before the crop can be destroyed, according to French regulations in areas vulnerable to nitrate pollution. They were sown in the mesocosms at a different time to target a simultaneous plant emergence. ~~In the two following~~ We sowed twice the amount of seeds recommended by French technical institutes for a pure intermediate crop (Table S2) (ARVALIS, 2022). In the first two weeks, some seedlings have been removed ~~to achieve a realistic plant density as in the field~~if the seed germination rate exceeded 50 %. The number of seeds ranged from two for faba bean to 64 for Alfalfa. The number of plants that have been maintained is available in Table 1.

Polyvinyl chloride mesocosms of 20 cm diameter were filled with 13 litres of sandy soil (6.9 % clay, 19.0 % silt, 74.1 % sand for the upper horizon) (Agapit et al., 2018). This soil was excavated from a semi-open habitat (grassland, shrubs and individual trees) of the research station by distinguishing ~~2~~ two operational horizons and was sieved at 1 cm. Soil was then packed in the mesocosms to recreate the ~~2~~ two horizons, after homogenisation. The upper horizon was 20 cm deep, and the lower one 25 cm deep. Both were packed at a density of 1.2, corresponding to realistic values found on site. C concentrations and δ^{13}C isotopic signatures are 7.2 gC kg^{-1} and -27.4 ‰ for the upper horizon and 4.3 gC kg^{-1} and -26.2 ‰ for the lower horizon, respectively. This design of 12 mesocosms was replicated ~~across 3~~ three times across three climate chambers simulating the same atmospheric conditions.

2.3 Sample collection, preparation and analysis

After 56 days, mesocosms were unpacked with a minimal destruction of the structure, by extracting the entire soil core from the pot. Standing aboveground biomass and dead leaves, if any, were collected after cutting the stems at the soil surface. The phenological stage was recorded according to the BBCH scale (Meier, 2003). Root were at first extracted manually. The soil was then sieved at 2 mm with a minimal shaking, so that remaining fine roots could be sampled with tweezers. All roots were then washed with tap water. We distinguished roots from the upper and lower horizon. Soil was collected in both horizons in several places to obtain a composite sample of 500 grams. Out of each planted mesocosm, the root system of one plant was carefully isolated, and the rhizosheath, which is the soil adhering to the roots after root extraction and shaking (Brown et al., 2017), was collected in both horizons when possible with a paintbrush for this specific plant, after a gentle shaking of the root system. The rhizosheath and its corresponding roots were weighted. It allowed us to obtain a Rhizosheath:Root ratio and therefore to estimate the whole rhizosheath soil mass for each mesocosm with Eq. (1).

$$m_{rhizosheath} = m_{roots} \times \frac{isolated.m_{rhizosheath}}{isolated.m_{roots}} \tag{1}$$

m_{roots} is the whole mesocosm root mass and $isolated.m_{rhizosheath}$ and $isolated.m_{roots}$ are the masses of rhizosheath and roots subsamples respectively. More rhizosheath soil was collected on the roots from the other plants of the mesocosm, but without associating it to a root mass. Soil samples of both horizons and of both locations (bulk vs rhizosheath) were dried at 40 °C, sieved at 2 mm, milled and analyzed for total C and isotopic signature $\delta^{13}C$ with cavity ring-down spectroscopy (PICARRO G2201-i / COSTECH). For every soil compartment, a humidity correction was applied after heating a soil subsample at 105 °C, to obtain a soil mass. Rhizosheath soil was analysed only when more than 13 g of sample was collected in one horizon. Plant material was dried at 40°C, weighted, milled and was analyzed for C and $\delta^{13}C$, as well as for total N (Thermo Fisher Scientific FlashHT).

2.4 Root-derived carbon (SOC_{new}) calculation

We grouped net rhizodeposition and non-collected fine root debris under the term SOC_{new}, which is in our case the labelled ~~soil organic carbon~~ SOC remaining after roots extraction and sieving at 2 mm. We calculated this amount (kg) of root-derived C with Eq. (2) in ~~4~~ four compartments (2 horizons * 2 localisations: bulk soil vs. rhizosheath soil). We obtained the total SOC_{new} of one mesocosm by summing all the compartments.

$$SOC_{new} = \frac{\delta^{13}\mathrm{C}_{soil} - \delta^{13}\mathrm{C}_{reference}}{\delta^{13}\mathrm{C}_{root} - \delta^{13}\mathrm{C}_{reference}} \times OC_{sample} \tag{2}$$

With $\delta^{13}\mathrm{C}_{soil}$ and $\delta^{13}\mathrm{C}_{root}$ being the δ^{13}C isotopic signature (‰) of the soil sample and of the corresponding labelled roots, respectively. $\delta^{13}\mathrm{C}_{reference}$ is the mean δ^{13}C of the soil before the plants were sown, at the corresponding horizon (n=20). OC_{sample} is the mass of OC contained in the soil compartment (kg), obtained with the OC concentration of the sample. This equation assumes that SOC_{new} has an isotopic signature similar to that of roots. As this is a strong assumption, we also provide a quantification of SOC_{new} calculated with the δ^{13}C of the shoots instead of $\delta^{13}\mathrm{C}_{root}$ for comparison (Fig. S4). We calculated specific SOC_{new} (kg kg^{-1}) by dividing SOC_{new} by root C. We expressed C quantities per hectare (kg ha^{-1}) in Fig. 1 ~~, S4 and S5~~ and S4 by doing a cross product with the surface of the mesocosms.

2.5 Incubation experiment

Following the first experiment, we used the labelled material to ~~to~~ perform a litterbag incubation in the field. Out of the 12 species, ~~6~~ six species (2 per family) were chosen to be incubated: *Vicia sativa*; *Vicia faba*; *Secale cereale*; *Avena sativa*; *Brassica napus* and *Raphanus sativus var. longipinnatus*. Plant and soil material was placed in 100 mm*100 mm nylon bags with a mesh size of 50 microns. This mesh size only enables microfauna to access the fresh material, but limits the diffusion of SOC_{new} particles out of the bags.

The design comprises two general treatments, applied to each of the ~~6~~ six plants and their corresponding soil: 1) To assess SOC_{new} decomposition, 50 g of labelled soil was directly placed in the bags. 2) To assess root decomposition, 50 mg of dry labelled roots were mixed with around 50 g of sieved unlabelled soil, corresponding to the soil used in the first labelling experiment. This represented from 0.9 % to 10.3 % of the C in the bags B_{roots} at the start of the incubation. The roots were fine roots (≤2mm), except for *Raphanus sativus*, where some of the taproot was mixed with fine roots due to a lack of material. In the following, we will use B_{SOCnew} and B_{roots} to refer to the bags of these two treatments. Each combination of treatment and plants was replicated ~~3~~ three times, using plants and soils coming from different mesocosms. Besides, ~~3~~ three bags were filled with control soil. This whole design was replicated ~~4~~ four times, to allow ~~4~~ four sampling dates.

Incubation started on 20 December 2023. The bags were buried at a 10 cm depth in the soil of a *Poaceae*-dominated grassland of the research station CEREEP-Ecotron IDF and recovered by means of a wire emerging from the soil. For year 2024, the mean annual temperature was 13.1 °C and cumulative rainfall was 844.7 mm. The incubation site was in the direct vicinity from the excavation site of the soil inside the litterbags. We consider that they are similar. ~~4~~ Four sets of bags were recovered after 124, 195, 330 and 524 days. The whole content of each bag was dried at 40°C, milled and analyzed for total C and δ^{13}C. When retrieving the bags, attention was paid to preserve clods of soil and thus vegetation above the bags. After 124 days, plant cover (*Vicia sativa; Sinapis alba; Secale cereale*) was sown to maximise vegetation recovery.

2.6 Carbon loss calculation during the litter bag incubation

We first calculated the proportion of C originating from the plant for each bag, F_{plant}, be it SOC_{new} or roots, with Eq. (3). This was done for all collected bags and for the initial labelled soil of B_{SOCnew}, before the incubation (t_0).

$$F_{plant} = \frac{\delta^{13}\mathrm{C}_{bag} - \delta^{13}\mathrm{C}_{control}}{\delta^{13}\mathrm{C}_{root} - \delta^{13}\mathrm{C}_{control}} \quad (3)$$

$\delta^{13}\mathrm{C}_{bag}$, $\delta^{13}\mathrm{C}_{control}$, and $\delta^{13}C_{root}$ are the C isotopic signatures of the labelled and control soil in the bag and the corresponding roots respectively. For bags B_{roots}, $\delta^{13}\mathrm{C}_{\mathrm{root}}$ was measured independently of the first experiment, to account more precisely for the roots subsample specifically used for the incubation. We used the mean of all controls for $\delta^{13}\mathrm{C}_{control}$, irrespective of the collection time, for bags B_{roots}, whereas for bags B_{SOCnew}, we used -27.4 ‰, in accordance with the previous labelling experiment.

We then calculated the concentration of plant-derived products, $[SOC_{plant}]$ (gC kg^{-1}), with Eq. (4).

$$[SOC_{plant}] = F_{plant} \times [OC_{sample}] \quad (4)$$

$[OC_{sample}]$ is the OC concentration of the sample (gC kg^{-1}).

To assess SOC_{new} loss, we calculated its remaining proportion, $SOCnew_{remaining}$ (%), by dividing $[SOC_{plant}]$ of the bags B_{SOCnew} at day t_i by the initial $[SOC_{plant}]$ at harvest (t_0) with Eq. (5).

$$SOCnew_{remaining}t_i = \frac{[SOC_{plant}]t_i}{[SOC_{plant}]t_0} \times 100 \quad (5)$$

If $SOCnew_{remaining}$ was above 100 % or below 0 %, we chose to assign it 100 % or 0 % respectively, instead of removing the data point.

To estimate the C loss of roots $Roots_{remaining}t_i$ (%), we devide the root C remaining at the sampling date t_i, estimated with F_{plant}, by the original amount of root C added in the bag, according to Eq. 6.

$$Roots_{remaining}t_i = \frac{mass_{soil}t_0 \times [OC_{sample}] \times F_{plant}}{mass_{roots}t_0 \times [OC_{roots}]} \times 100 \tag{6}$$

$mass_{soil}t_0$ and $mass_{roots}t_0$ are the initial masses (kg) of the control soil and the roots mixed in the bags and $[OC_{root}]$ the respective root C concentrations (gC kg^{-1}).

2.7 Statistical analysis

We used mesocosms as the statistical unit for our analyses. Consequently, we had 35 data points, corresponding to 12 species replicated three times each, except for mustard, where plant growth failed in one mesocosm. One mesocosm may contain one single plant or several plants, according to the sowing density (Table 1). For the latter case, quantitative values, C amounts or Root:Shoot (R:S) ratios for instance, stand for the entire mesocosm. In the text, the error associated with means is ± the standard deviation. For the figures, this is specified in the caption. All statistical analysis were performed with R language ~~(?)~~(R Core Team, 2021). For p-values of models or models parameters, we set the significance threshold at 0.05. However, in certain cases that are specified in the text, we report a trend when p-values < 0.1.

Comparisons between species (n = 3) were performed using one-way analysis of variance. Posthoc comparisons were performed with Tukey HSD tests. To compare families (n = 12), we adopted a linear mixed-effects model with the species as a random effect to account for the hierarchical structure, with the package *lme4* (Bates et al., 2015). Only the intercept was allowed to vary. We then performed an anova on the model with the package *car* ~~(Fox and Weisberg, 2019)~~ (?) and a multiple comparison with the package *emmeans* that uses the marginal means (Searle et al., 1980).

To test for linear correlations between quantitative variables, we also selected linear mixed-effects model with the species as a random effect to let the intercept vary. For these models, we report the marginal R^2, that accounts for the variability explained by the fixed effects, calculated according to the recommendations of Nakagawa and Schielzeth (2013) with the package *Performance* (Lüdecke et al., 2021).

For the incubation experiment, we did not fit any decomposition models given the low number of sampling dates. To compare decomposition status of SOC_{new} and roots, we performed paired Student's t-tests for each sampling date. To compare ~~plants~~species, we performed one-way analysis of variance followed by Tukey HSD tests. The list and results of statistical analysis are available at Hulin et al. (2025).

3 Results~~and discussion~~

3.1 Quantification of the inputs

3.1.1 Shoot and root C quantification

After 56 days of growth, the spectrum of phenological stages was spanning from the development of harvestable vegetative plant to senescence in certain cases (Table 1). ~~C allocation is dependant on phenology: for annual plants, relative allocation to roots decreases in favour of supporting tissues and reproductive organs with plant age (Hegazy et al., 2005). Therefore, our results on relative C allocation could have evolved with a longer growth period.~~ We observed the highest net C production in shoots and roots for *Poaceae* ~~. They~~ (Fig. 1). The latter exhibited the highest shoot C values with a mean of 2513 ± 613 kgC ha^{-1}, but also high root C values ~~,~~ with a mean of 1065 ± 408 kgC ha^{-1} except for oat (~~Fig. 1~~636 ± 80 kg or root C ha^{-1}). R:S ratios varied from 0.2 to 1.5 across all species with no significant differences between species (Table 1). As expected, the only perennial crop, alfalfa, exhibited the highest ~~value~~ R:S ratio (1.5 ± 0.5). ~~Comparing our results with studies that report C inputs is challenging as the varieties, the duration time, the methods and the pedoclimatic context may greatly influence biomass production. We can nevertheless compare to relative data, such as comparisons between plants or R:S ratios. A field study that shared 3 of our species also found that rye had a higher aboveground and belowground biomass than *Fabaceae* (Sainju et al., 1998). A comparison to a greenhouse experiment (1 m^3 mesocosms) showed similar results: a higher biomass for *Poaceae* than for *Fabaceae*. However, they also observed the highest biomass for *Brassicaceae*, unlike our study (Hudek et al., 2022). *Brassicaceae* are commonly sown at a lower density than *Fabaceae* and *Poaceae*: we had only one plant per mesocosm in our experiment. We believe that the surface area of our mesocosms (285 cm^2) was a limiting factor for low-density crops, and in this way, the biomass of brassicaeae could be underestimated in our study. Regarding the R:S ratio, we noted deviations from the literature with a high ratio for alfalfa (Bolinder et al., 2002) and a low ratio for oat (Bolinder et al., 1997). The other crops exhibited values that were in line with the literature. For instance, Bolinder et al. (1997) compiled several field data in western Canada. They reported R:S ratios from 0.4 to 0.6 for barley, against 0.4 in our case. A review from Ahmadi et al. (2025) reported values from 0.08 to 1 for rapeseed, against 0.3 in our case. We suggest that our root and shoot biomass data, obtained in mesocosms under controlled conditions, are of the same order of magnitude as field data, even though extrapolation is limited in certain cases, such as for *Brassicaceae* for instance.~~

3.1.2 SOC_{new} quantification

3.1.3 ~~SOC_{new} quantification~~

~~After 56 days~~At crop harvest, the bulk soil was significantly enriched in ^{13}C compared to the beginning of the experiment ($p > 8.5 \times 10^{-9}$) with a mean δ^{13}C difference of 2.6‰, both horizons together, which allowed us to calculate SOC_{new} in every soil compartment. We found that total SOC_{new} represented between 169 (average for clover) and 441 (average for barley) kgC ha^{-1}, with an average of 284 ± 113 kgC ha^{-1} all species considered (Fig. 1). However, the labelling ~~heterogeneity~~ procedure led

Table 1. List of plant species, plant traits, plant density and phenological stage. R:S ratio is a ratio calculated with C quantities in roots and shoots. For R:S and Carbon:Nitrogen (C:N) ratios, values are averages for species ± their standard deviation (n=3 mesocosms). The letters indicate the results of tukey HSD tests. For the number of plants per mesocosm, each value of each mesocosm is directly reported. ~~The shading differentiates the families.~~ Phenological stages were recorded at harvest according to the BBCH scale (Meier, 2003). 4: Development of harvestable vegetative plant parts or vegetatively propagated organs / booting (main shoot); 5: Inflorescence emergence (main shoot) / heading; 6: Flowering (main shoot); 7: Development of fruit; 8: Ripening or maturity of fruit and seed; 9: Senescence, beginning of dormancy.

Plant species	**Common name**	**Family**	**Root:Shoot**		**Roots C:N**		**Plants mesocosm^{-1}**	**Phenology**
Medicago sativa	Alfalfa	*Fabaceae*	1.5±0.5	a	16.6±2.0	ab	9,8,1	4,6,6
Vicia sativa	Vetch	*Fabaceae*	0.5±0.2	a	19.3±7.7	ab	4,4,4	4,5,8
Trifolium pratense	Red clover	*Fabaceae*	0.3±0.0	a	17.2±3.1	ab	4,7,4	6,4,6
Vicia faba	Faba bean	*Fabaceae*	1.0±0.4	a	14.5±2.9	a	1,1,1	4,6,8
Hordeum vulgare	Barley	*Poaceae*	0.4±0.0	a	26.2±6.2	ab	5,5,5	5,7,7
Lolium multiflorum	Annual ryegrass	*Poaceae*	0.4±0.1	a	27.1±1.6	ab	11,17,3	7,7,7
Secale cereale	Rye	*Poaceae*	0.7±0.1	a	27.1±3.6	ab	5,5,5	4,4,4
Avena sativa	Oat	*Poaceae*	0.2±0.0	a	28.3±5.1	b	6,6,5	8,7,8
Sinapis alba	White mustard	*Brassicaceae*	0.2±0.0	a	24.3±0.3	ab	2,1	6,7
Camelina sativa	Camelina	*Brassicaceae*	0.2±0.0	a	18.5±4.0	ab	3,3,3	8,8,9
Brassica napus	Rapeseed	*Brassicaceae*	0.3±0.0	a	22.7±1.7	ab	5,4,5	4,4,4
Raphanus sativus	Daïkon radish	*Brassicaceae*	0.4±0.1	a	23.9±5.8	ab	2,2,2	7,6,8

to temporal heterogeneity in the atmospheric δ^{13}C, particularly during the second week of growth, during which the labelling was less pronounced (Fig. S2). This resulted in discrepancies between the δ^{13}C of roots and shoots, especially for *Poaceae* and *Brassicaceae* that exhibited a higher root labelling (Fig. ~~S2,~~S3). As a result, using the δ^{13}C of roots as the plant end-member in Eq. (2) is a strong assumption. Therefore, we also calculated SOC_{new} with δ^{13}C of shoots as the plant end-member and found amounts of C that are lower by 18 % across all species and by 26.8; 27.0 and 7.4 % for *Brassicaceae*, *Poaceae* and *Fabaceae* respectively (Fig. S4). As belowground allocation of recently fixed C is a fast process (Pausch and Kuzyakov, 2018), some rhizodeposition products depend on shoot growth and therefore, our SOC_{new} estimations based on root isotopic signature, that we use throughout this manuscript, might be slightly overestimated.

In terms of relative allocation, SOC_{new} accounted for 9.9 ± 3.5% of all ~~3~~ three C pools and 27.8 ± 10.3 % of belowground C pools (roots + SOC_{new}), all species comprised (Fig. 1). *Brassicaceae*, with a mean at 38 ± 10.6 %, had the highest relative belowground C allocation to SOC_{new} ($p = 1 \times 10^{-3}$). For instance, mustard and camelina showed high specific SOC_{new}, along with a low R:S ratio. ~~Our resultsare in line~~ Indeed, we observed that specific SOC_{new} was negatively correlated to root C amounts ($R^2 = 0.20$) (Fig. 2). Regarding the correlation between the other C pools, we found a positive correlation between SOC_{new} and root C amounts ($R^2 = 0.14$) (Fig. 2) and no correlation between SOC_{new} and shoot C amounts.

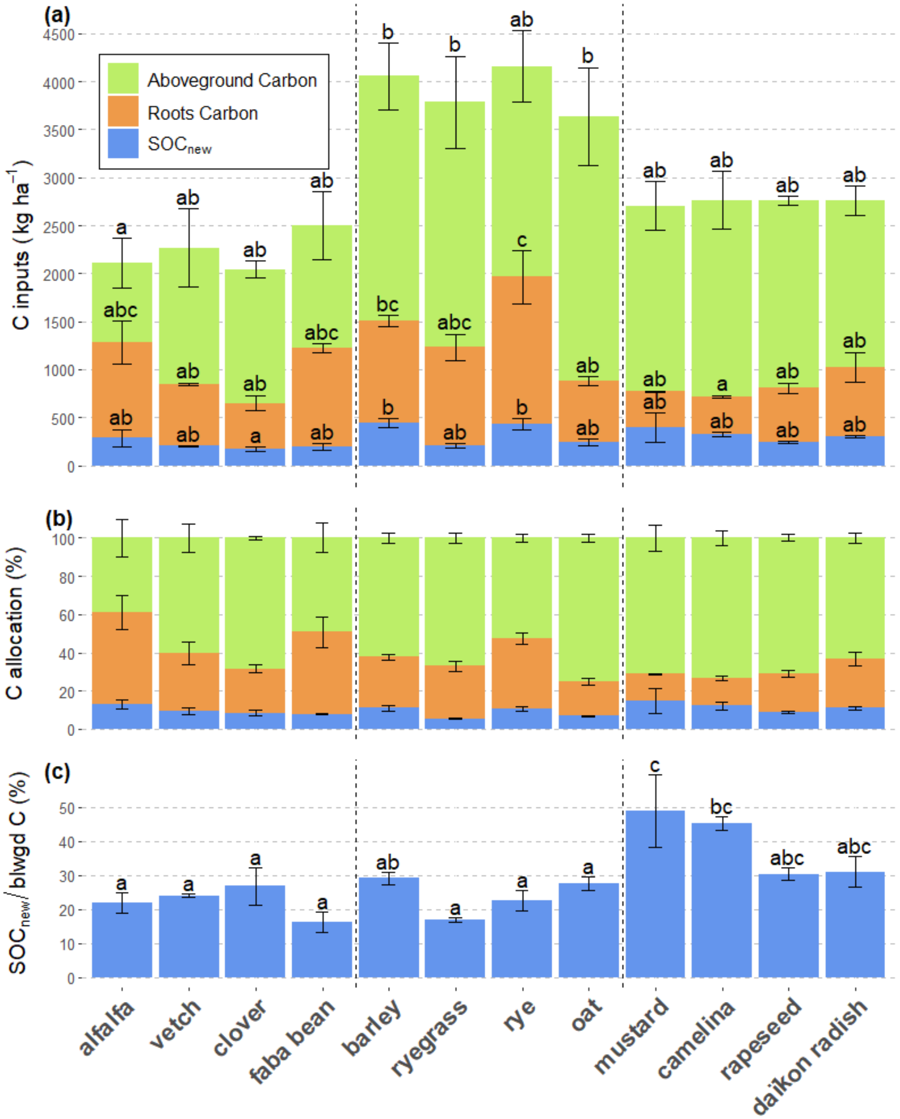

Figure 1. C allocation to different pools for 12 plant species: (a) net primary production, scaled to the hectare with the mesocosm's surfaces; (b) relative C allocation to SOC_{new}, roots and shoots; (c) share of belowground C (*blwgd C*) inputs allocated to SOC_{new}. Values are average values for species and error bars equal two standard errors (n=3 mesocosms). Lowercase letters represent significant differences ($p < 0.05$) between species for the total net primary production (upper panel) and for the SOC_{new}:Belowground C inputs ratio (lower panel). Vertical dashed lines separate plant families displayed in the following order from left to right: *Fabaceae*; *Poaceae*; *Brassicaceae*.

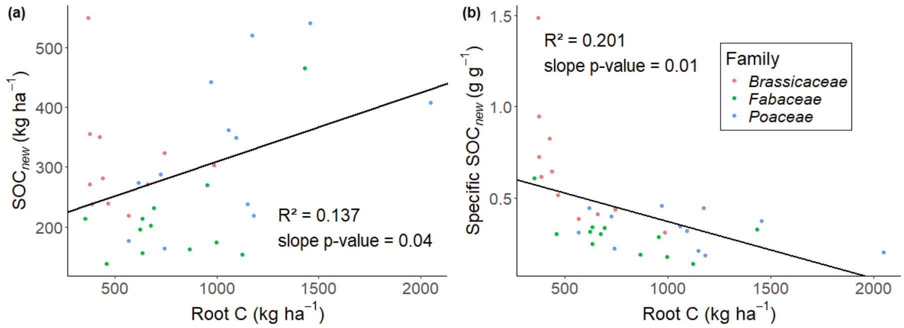

Figure 2. Linear correlations between a) SOC_{new} and Root C amounts, a) specific SOC_{new} and Root C amounts. The slope was obtained with a mixed-effects model with species as a random effect, to let the intercept vary. The R^2 are marginal R^2.

3.2 Spatial distribution of belowground inputs

3.2.1 Vertical distribution

We retrieved 38 ± 13 % of SOC_{new} in the lower horizon in average for all species (Fig. 3). Differences were observed between species. For instance, alfalfa and barley allocated up to 50 and 55 % of SOC_{new} below 20 cm respectively, whereas this value dropped to 19 % for daikon radish, whose root biomass was concentrated in the top soil which contained most of the thick taproot. The distribution of root C follows the same pattern as SOC_{new} with 36 ± 10 % of the C retrieved in the lower horizon (Fig. 3). We observed a weak linear correlation between the distribution of root C and SOC_{new} ($p = 9 \times 10^{-2}$).

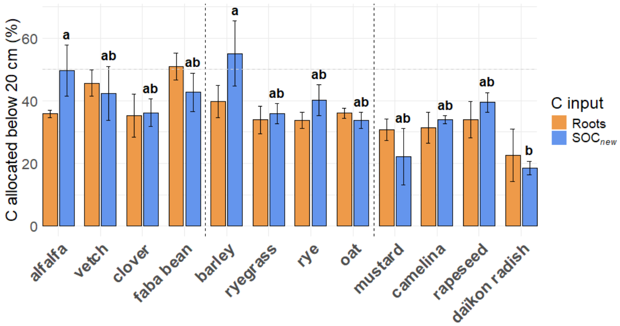

Figure 3. Root C and SOC_{new} retrieved in the soil horizon below 20 cm (20 - 45 cm). The bar heights are the mean for each species and error bars equal two standard errors. The significant letters are only reported for SOC_{new} as no significant differences were observed for roots.

3.2.2 Bulk soil vs. rhizosheath

We also investigated whether SOC_{new} was retrieved in the close vicinity of roots or further away from the rhizosheath. We found that only 14 ± 14 % of SOC_{new} was retrieved in the rhizosheath, i.e. in the soil adhering to the roots when sampling was possible (Fig. 4). In the upper horizon, this accounted for 14 ± 14 % of SOC_{new}, and 18 ± 20 % in the lower horizon, without any significant difference between horizons (paired t-test). We observed high variations between plants, from 40 % (raygrass) to 2 % (daïkon radish) or even no value as too little rhizosheath soil was available for the analysis (mustard), although the differences were not significant with the limited number of data points. This fraction of SOC_{new} was correlated to the mass of rhizosheath ($R^2 = 0.82$), which differed significantly between plants (Fig. 4) ($p = 1.5 \times 10^{-3}$). Rhizosheath mass represented in average 2.0 ± 2.3 % of the soil mass in our experiments, all species comprised (when sampling was possible). The highest rhizosheath values were retrieved for *Poaceae*, which retained the most soil with their arbuscular root system: 4.3 ± 2.9 % of the soil mass. Whereas the amount of SOC_{new} in the rhizosheath is closely linked to the rhizosheath mass, the latter is not correlated to the total amount of SOC_{new} retrieved in the mesocosm (Fig. 4).

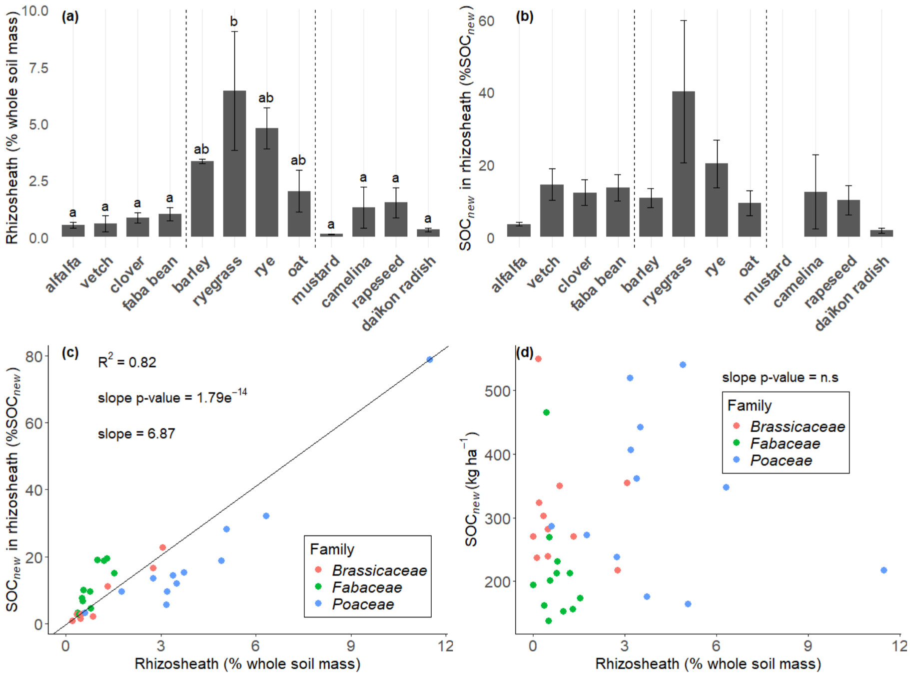

Figure 4. (a) Mass fraction of the soil comprised in the rhizosheath, calculated with Eq. 1. (b) Mass fraction of SOC_{new} comprised in the rhizosheath. The bar heights of the two barplots are the mean for each species and error bars equal two standard errors. The significant letters are only reported for the rhizosheath mass fraction as no significant differences were observed for SOC_{new} mass fraction. (c) Relationship between both mass fractions (SOC_{new} mass fraction and rhizosheath mass fraction). (d) Relationship between total SOC_{new} in the mesocosm and rhizosheath mass fraction. Each point stands for one mecosom. The slope was obtained with a mixed-effects model with species as a random effect, to let the intercept vary. The R^2 is the marginal R^2.

3.3 Persistence of belowground inputs

The root incubation revealed a fast C loss, with 32.7 ± 23.2 % of the root C remaining after 124 days, all plants combined (Fig. 5, Table 2). This tended to reach a plateau as we observed similar values for the other dates of harvest, with 23.7 ± 13.8

% remaining after 524 days. We observed significant differences in mineralisation status between plant species at days 124 and 524 only (p = 4×10^{-3} and 3×10^{-3} respectively). These differences were mostly driven by faba bean that exhibited a slower decomposition that non-legumes species and to a lesser extent by vetch at day 524. However, no significant pattern was detected for other species and sampling dates.

A first important result for SOC_{new} decomposition is that we did not see any significant difference between species. However, a high uncertainty is associated with the results, and the decomposition curves do not show a clear decreasing exponential pattern, as often seen in such experiments (Fig 5). Indeed, its calculation yielded nine incoherent values ($\leq$0 % or $\geq$100 %) out of 65 bags recovered, due to a δ^{13}C lower than the control or to an increase of the δ^{13}C compared to t_0. We attributed this to the fact that fresh SOC_{new} is a very small fraction of SOC (between 0.34 % and 1.71 % at the start of the incubation), resulting in a small δ^{13}C difference with the control, exacerbating errors due to analysis and handling. Indeed, initial δ^{13}C of the bags containing SOC_{new} vary from -21.929 ‰ to -25.9‰, whereas the mean value of native SOC is -27.4‰.

Nevertheless, we were able to observe that SOC_{new} tended to have a significantly slower decomposition than roots, at least for three sampling dates (Fig. 5, Table 2). After 524 days, 48.6 ± 35.2 % of SOC_{new} was remaining.

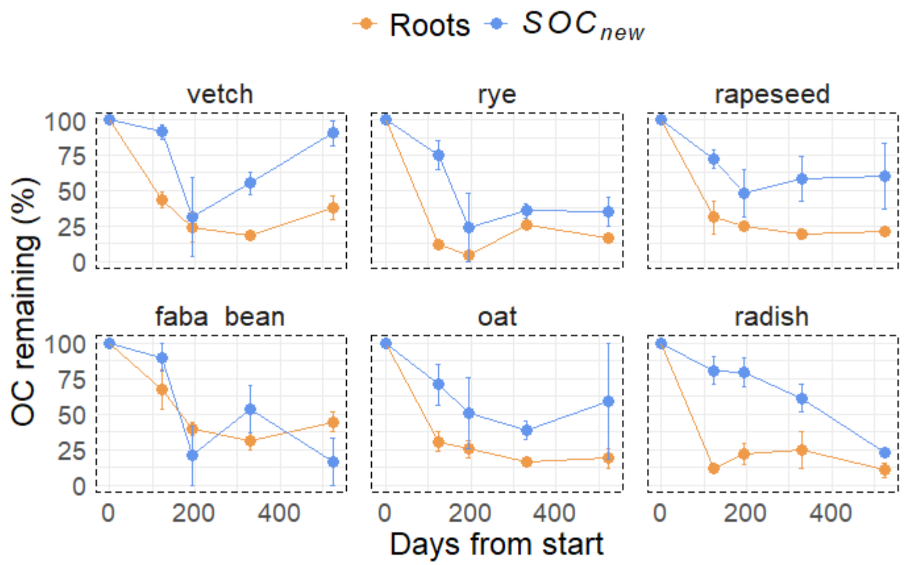

Figure 5. Proportion of OC remaining at the four sampling times, for the six species that were incubated. Day 0 is the incubation start. Values are the averages for plants and error bars equal two standard errors (n = 3).

Table 2. Proportion of the fraction of OC remaining at the four sampling times ± standard deviation (n = 18). The p-value indicate the results of the bilateral paired t-tests comparing SOC_{new} and roots.

Days After Incubation Start	**Remaining SOC_{new} (%)**	**Remaining Roots (%)**	**p-value (bilateral paired t-test, $H_0 =$ true)**
124	79.8 ± 16.5	32.7 ± 23.2	1.3×10^{-7}
195	42.5 ± 35.0	26.7 ± 12.5	ns (5.3×10^{-2})
330	49.9 ± 17.9	25.5 ± 15.4	1.6×10^{-5}
524	48.6 ± 35.2	23.7 ± 13.8	8.2×10^{-3}

4 Discussion

4.1 Quantification of the inputs

4.1.1 Shoot and root C quantification

Comparing our results with studies that report root and shoot C inputs is challenging as the varieties, the duration time, the methods and the pedoclimatic context may greatly influence biomass production. Besides, roots were limited to a depth of 45 cm, unlike in the field. We can nevertheless compare to relative data, such as comparisons between plants or R:S ratios. A field study that shared three of our species also found that rye had a higher aboveground and belowground biomass than *Fabaceae* (Sainju et al., 1998). A greenhouse experiment (1 m^3 mesocosms) showed similar results: a higher aboveground and belowground biomass for *Poaceae* than for *Fabaceae*. However, they also observed the highest aboveground and belowground biomass for *Brassicaceae*, unlike our study (Hudek et al., 2022). *Brassicaceae* are commonly sown at a lower density than *Fabaceae* and *Poaceae*: we had only one plant per mesocosm in our experiment. We believe that the surface area of our mesocosms (285 cm^2) was a limiting factor for low-density crops, and in this way, the biomass of brassicaeae could be underestimated in our study. For many species, we observed a relatively good seedlings emergence rate, equal to or above 50 % in 20 mesocosms out of 35. Considering that we doubled the number of seed compared to recommendations of technical institutes, and considering that emergence rates in the field is generally lower than 50 % in august (ARVALIS, 2025), we may come up with a plant density in our mesocosms that is higher than in a field. As a result, this could lead us to overestimate total plant biomass (roots and shoots) of the whole mesocosm as the latter generally increases with the density. Besides, Root:Shoot ratio may also be influenced as the root mass fraction generally decreases with density (Postma et al., 2021). Regarding the R:S ratio, we noted deviations from the literature: Bolinder et al. (1997) found a ratio of 0.4 for oat, against 0.2 in our case, and Bolinder et al. (2002) found a ratio of 0.7 for alfalfa, against 1.5 in our case. The other crops exhibited values that were in line with the literature. For instance, Bolinder et al. (1997) compiled several field data in western Canada: they reported R:S ratios from 0.4 to 0.6 for barley, against 0.4 in our case. A review from Ahmadi et al. (2025) reported values from 0.08 to 1 for rapeseed, against 0.3 in our case. We suggest that our root and shoot biomass data, obtained in mesocosms under controlled conditions, are of the same order of magnitude as field data, even though extrapolation is limited in certain cases, such as for *Brassicaceae* for instance.

4.1.2 SOC_{new} quantification

Our quantification of SOC_{new} is in line with single pulse labelling studies on crops reviewed in Pausch and Kuzyakov (2018), that found that SOC_{new} represented in average 23 % of belowground C inputs, against 27.8 ± 10.3 % in our study. On the other side, larger scale studies in which labelling was performed during a whole growing season found that SOC_{new} equalled or even exceeded root C ~~(Davenport and Thomas, 1988; Hirte et al., 2018b)~~(Davenport and Thomas, 1988; Hirte et al., 2018a) . One reason for that could be that root senescence and turnover had more time to occur, thus fuelling the SOC_{new} pool.

~~There was a positive correlation~~ We did not observe a statistical relationship between SOC_{new} and ~~root C amounts (R^2 = 0.14) (Fig. S5) and no correlation between SOC_{new} and shoot C amounts~~shoot C production. This latter result contradicts ~~to~~ other studies stating that aboveground photosynthetic traits are good predictors of SOC_{new} (Baptist et al., 2015; Henneron et al., 2020a; Huang et al., 2021). It was indeed proposed by Henneron et al. (2020a) that net rhizodeposition is embedded within a root economics space, with fast-growing species producing high amounts of SOC_{new}. Our contradictory results could be partly explained by the fact that phenological stages ~~, that influence rhizodeposition (Pausch and Kuzyakov, 2018),~~ differed amongst our plants at harvest: after 56 days of growth, the spectrum of phenological stages was spanning from the development of harvestable vegetative plant to senescence in certain cases (Table 1). C allocation does depend on phenology: for annual plants, relative allocation to roots decreases in favour of supporting tissues and reproductive organs with plant age (Hegazy et al., 2005). Here, we could observe it as a non significant trend in our results (Fig. S5). Therefore, our results on relative C allocation could have evolved with a longer growth period. Whereas our experimental design was chosen to simulate an agronomic practice: the destruction of cover crops after two months of growth, it is not optimal to identify the predictors of SOC_{new}. Besides, it is questionable whether plant traits-based theories are suited for our crop species, that likely have undergone an important shift in plant traits due to varietal selection (Veeken et al., 2022). ~~We~~ Lastly, we also have to highlight that SOC_{new} is likely to represent the least reactive portion of gross rhizodeposition. Indeed, soluble compounds have a mean residence time of the order of the hour ~~(?Jones et al., 2009)~~ (Ryan et al., 2001; Jones et al., 2009) whereas mucilage, border cells, root hairs or even fine roots comprise complex molecules which take longer to decompose. Whereas root economics space theory is well suited to study exudation (Wen et al., 2022; Williams et al., 2022), coarser rhizodeposition leading to SOC_{new} might be more related to root growth dynamics, as observed by Atere et al. (2017) for rice. ~~We also observed that~~

On the other side, root C was a better predictor although the linear relationship with SOC_{new} is relatively weak (R^2 = 0.20). Indeed, specific SOC_{new} was negatively correlated to root C amounts (R^2 = 0.20) (Fig. ~~S5~~2), as observed by Baptist et al. (2015). This ~~is~~ can not go hand in hand with the former relationship: considering that the less root is harvested, the more SOC_{new} will be collected relatively (Fig. 2), a fixed allocation coefficient to estimate SOC_{new} is not optimal. This is likely due to the fact that rhizodeposition mostly occurs at the root tip (Nguyen, 2003) and less in the differentiated zone, that might account for a large share of root biomass. It is also legitimate to suppose that it might be to a lesser extent explained by the sampling strategy, as a portion of the finest roots may have been incorporated to the SOC_{new} compartment, thus being at the expense of root biomass for species with fragile roots.

~~Carbon allocation to different pools for 12 plant species. Values are average values for species (n=3 mesocosms). The upper panel represents net primary production, scaled to the hectare with the mesocosm's surfaces. The 2 lower panels represent relative allocation for the whole plant and for belowground inputs. Lowercase letters represent significant differences ($p < 0.05$) between species for the total net primary production (upper panel) and for the SOC_{new}:Belowground C inputs ratio (lower panel). Vertical dashed lines separate plant families displayed in the following order from left to right : *Fabaceae*; *Poaceae*; *Brassicaceae*.~~

4.2 Spatial distribution of belowground inputs

4.2.1 Vertical distribution

When we consider C sequestration in soils, studying the vertical ditribution of C inputs is of major importance for at least two main reasons: 1) the stabilisation processes of the recent inputs might vary according to the depth, with a preferential accumulation of root C often observed below 20 cm (Gill et al., 1999; Dietzel et al., 2017), possibly explained by a higher physico-chemical protection (Rasse et al., 2005) and 2) the magnitude of the priming effect induced by the inputs also differs between soil horizons, notably because of such differences in OM stability (Bastida et al., 2019; Schiedung et al., 2023). At the beginning of the experiment, the $\delta^{13}C$ of the subsoil (~~0-20~~ 20-45 cm) (-26.2 ± 1.0 ‰) was significantly higher than for the topsoil (~~20-45~~ 0-20 cm) (-27.4 ± 0.5 ‰) (p = 3×10^{-7}). This ^{13}C enrichment with depth is a common observation reflecting an enrichment in microbial-derived products and an increased stability of OM (Schweizer et al., 1999; Boström et al., 2007; Schaub and Alewell, 2009). ~~As the magnitude of priming differs between soil horizons, notably because of such differences in OM stability (Bastida et al., 2019; Schiedung et al., 2023), it therefore makes complete sense to consider the vertical distribution of fresh inputs in our study. We retrieved 38 ± 13 % of SOC_{new} in the lower horizon in average for all species (Fig. 3). Differences were observed between species. For instance, alfalfa and barley allocated up to 50~~ This observation shows that the difference in SOM stability is true in our case, and ~~55 % of SOC_{new} below 20 cm respectively, whereas this value dropped to 19 % for daïkon radish, whose roots were concentrated in the top soil. Indeed, we observed a weak linear correlation between the distribution of root C and SOC_{new} (p = 9×10^{-2}). The distribution of root C follows the same pattern with 36 ± 10 % of the C retrieved in the lower horizon (Fig. 3). We can therefore~~ that considering the vertical distribution of C inputs is relevant even on a profile which is only 45 cm deep. Our results suggest that deep-rooted crops will also lead to a deep release of SOC_{new} ~~, and vice versa,~~ as expected (Farrar et al., 2003). This ~~alignment~~ implies that both C ~~pools~~ inputs might be subject to the stabilisation mechanisms that are inherent to the horizon. ~~Indeed, a preferential accumulation of root C is often observed below 20 cm (Gill et al., 1999; Dietzel et al., 2017), possibly explained by a higher physico-chemical protection (Rasse et al., 2005).~~ Whether SOC_{new} might be preferentially stabilized in deep horizons, its release may also alleviate the bioenergetics constraint that protects deep native SOC, resulting in accelerated mineralisation (Henneron et al., 2022). Both mechanisms should be taken into consideration if one is to account for rooting depths to foster additional C sequestration.

~~Distribution of root C and SOC_{new} between the 2 soil horizons (0 - 20 cm and 20 - 45 cm). The bar heights are the mean for each species and error bars equal 2 standard errors. The significant letters are only reported for SOC_{new} as no significant differences were observed for roots.~~

4.2.2 Bulk soil vs. rhizosheath

~~Besides vertical distribution, we also investigated whether SOC_{new} was retrieved in the close vicinity of roots or further away from the rhizosphere. We found that only 14 ± 14 %~~ Our results highlight that most of SOC_{new} ~~was~~ is retrieved in the ~~rhizosheath, i.e. in the soiladhering to the roots (Fig. 1). We observed high variations between plants, from 40 % (raygrass) to 2 % (daïkon radish) or even no value as too little rhizosheath soil was available for the analysis (mustard), although the differences were not significant with the limited number of data points. This fraction of SOC_{new} was correlated to the mass of rhizosheath ($R^2 = 0.82$), which differed significantly between plants (Fig. 4) ($p = 1.5 \times 10^{-3}$). Rhizosheath mass represented in average 2.0 ± 2.3 % of the soil mass in our experiments, all species comprised (when sampling was possible). The highest rhizosheath values were retrieved for *Poaceae*, which retained the most soil with their arbuscular root system. Whereas the amount of SOC_{new} in the rhizosheath is closely linked to the rhizosheath mass (Fig. 4), the latter is not correlated to the total amount of SOC_{new} retrieved in the mesocosm. As the whole rhizosphere represents the soil under influence of the roots (Hinsinger et al., 2006; York et al., 2016), thus comprising all rhizodeposition compounds, and~~ bulk soil, regardless of the soil horizon. This invites us to consider most of the planted soil as the rhizosphere ~~soil is often sampled in an operational way that in fact solely accounts for the soil rhizosheath (Freschet et al., 2021), we consider important to state here that the rhizosheath mass is not a suitable proxy to estimate the quantity of rhizodeposition, or at least net rhizodeposition.~~

~~Besides, our result highlight that the~~ and it suggests that soil processes inherent to the rhizosphere are not constrained to the vicinity of the roots. For instance, an enhancement of native SOC cycling that would be associated to rhizodeposition release (Huo et al., 2017) ~~is not constrained to the vicinity of the roots. Rather, this invites us to consider~~ may affect most of the ~~planted soil as the rhizosphere with most of the native SOC being subject to positive priming~~soil volume above the maximal rooting depth. On the other side, the deceleration through SOC protection in aggregates (Andrade et al., 1998; Baumert et al., 2018; Li et al., 2020), which is restricted to C accumulation zones near the roots, concerns a lower amount of native SOC and might differ between species as they retain different amounts of soil around their roots.

~~Mass fraction of the soil comprised in the rhizosheath (brown bars), calculated with Eq. 1, and mass fraction of SOC_{new} comprised in the rhizosheath (blue bars). The y axis both indicate the fraction (%), but their scale is different. The bar heights are the mean for each species and error bars equal 2 standard errors. The significant letters are only reported for the rhizosheath~~

~~mass fraction as no significant differences were observed for SOC_{new} mass fraction. The inset shows the linear correlation linking both fractions, by plotting the value of each mesocosm.~~

4.3 ~~Persistence of belowground inputs~~

~~The root incubation revealed a fast carbon loss, with 32.7 ± 23.2 % of the roots C remaining after 124 days, all plants combined~~ We consider important to state here that the rhizosheath mass is not a suitable proxy to estimate the quantity of rhizodeposition, or at least net rhizodeposition (Fig. ~~5, Table 2). This tended to reach a plateau as we observed similar values for the other dates of harvest, with 23.7 ± 13.8 % remaining after 524 days.~~ 4). The rhizosphere represents the soil under influence of the roots (Hinsinger et al., 2006; York et al., 2016), thus comprising all rhizodeposition compounds, and as the rhizosphere soil is often sampled in an operational way that in fact solely accounts for the soil rhizosheath (Freschet et al., 2021), focusing only on the soil layer attached to the roots could lead to the omission of an important component of the C inputs that will influence the biogeochemical cycles of the soil.

4.3 Persistence of belowground inputs

We compared our results on root decomposition with data from literature reviews ~~on root decomposition (Silver and Miya, 2001; Zhang and~~ from which we recalculated a percentage of dry mass presumed to be remaining at day 524 (Table S1). Our decomposition rates for graminoids are in line with literature values reporting 12 to 17 % of the C remaining at day 524 (Silver and Miya, 2001; Zhang and Wang against 17 % in our case (Rye and Oat). However, for forbs, we observed a slower decomposition rate compared to data from See et al. (2019) that report a mean of 1 %, against 27 % in our case (faba bean, vetch, rapeseed and radish). ~~We observed significant differences in mineralisation status between plant species at days 124 and 524 only ($p = 4 \times 10^{-3}$ and 3×10^{-3} respectively). These differences were mostly driven by Faba bean that exhibited a slower decomposition that non-legumes species and to a lesser extent by vetch at day 524.~~ The only differences between species that we observed were for legumes whose roots were less decomposed at two sampling dates. This was unexpected as legumes have the lowest C:N ratios (Table 1). Even though C:N is explaining less than 5 % of the variance of decomposition for fine roots at a global scale, the expected trend is in the opposite direction (Zhang and Wang, 2015). However, no pattern was detected for other species and sampling dates, which assigns a limited role to litter quality as a driver of SOC storage, compared to the quantity of the inputs, in accordance with findings from Pellerin et al. (2020).

~~A first important result for~~ The fact that SOC_{new} ~~decomposition is that we did not see any significant difference between species. However, a high uncertainty is associated with the results, and the decomposition curves do not show a clear decreasing exponential pattern, as often seen in such experiments (Fig 5). Indeed, its calculation yielded 9 incoherent values (≤0 % or ≥100 %) out of 65 bags recovered, due to a δ^{13}C lower than the control or to an increase of the δ^{13}C compared to t_0. We attributed this to the fact that fresh SOC_{new} is a very small fraction of SOC (between 0.34 % and 1.71 % at the start of the incubation), resulting in a small δ^{13}C difference with the control, exacerbating errors due to analysis and handling. Indeed, initial δ^{13}C of the bags containing SOC_{new} vary from -21.929 ‰ to -25.9‰, whereas the mean value of native SOC is -27.4‰.~~

~~Proportion of OC remaining at the 4 sampling times, for the 6 species that were incubated. Day 0 is the incubation start. Values are the averages for plants and error bars equal 2 standard errors (n = 3).~~

~~Nevertheless, we were able to observe that SOC_{new} tended to~~ tends to have a significantly slower decomposition than roots ~~, at least for three sampling dates (Fig. 5, Table 2). After 524 days, 48.6 ± 35.2 % of the fresh OC was remaining~~may seem counterintuitive as rhizodeposition is often consider as a labile pool, notably because it is made of small molecules. Two hypotheses could explain this result. First, the fraction of SOC_{new} that remains at harvest may have already been depleted of labile compounds. As a result, we compared the decomposition of roots, that have not undergone any transformation and therefore contain easily decomposable products, and of SOC_{new} which has undergone a first decomposition during the growth of the plants. Secondly, another hypothesis is proposed by the Microbial Efficiency-Matrix Stabilization framework (Cotrufo et al., 2013): SOC_{new} would have decomposed more slowly precisely because gross rhizodeposition may contain in proportion more labile compounds than roots. These compounds would have been preferentially incorporated in the microbial loop and would thus have had a greater likelihood of forming associations with mineral. At the start of the incubation, SOC_{new} would then already contain a portion of relatively stable OM. Results of studies tracing labelled C in stable OM fractions support this assumption (Villarino et al., 2021). These ~~2~~ two hypotheses rely on ~~2~~ two different concepts: recalcitrance versus C use efficiency associated to stabilization mechanisms. Nevertheless, they are not contradictory, but complementary. There is little incubation data available in the literature for comparison. Van der Krift et al. (2001) found that after 69 days of incubation, 80 to 57 % of net rhizodeposition from perennial grasses was remaining (against 74 to 89 % for the roots). For rice, Lu et al. (2003) found that 54 % was remaining after 240 days (against 58.1 % for the roots). These ~~2~~ two studies, together with our results show that net rhizodeposition is a sub compartment of gross rhizodeposition which stands out for its longer decomposition time. Whereas more than the half of gross rhizodeposition is respired within 15 days (Jones et al., 2009; Pausch and Kuzyakov, 2018; Weng et al., 2018), about half of the fraction remaining at harvest, here embedded in SOC_{new}, is not mineralised after 524 days (Table 2). Nevertheless, the high variability in our results for SOC_{new} puts the strength of our conclusions into perspective and encourages further research to confirm them.

~~Proportion of the fraction of OC remaining at the 4 sampling times ± standard deviation (n = 18). The p-value indicate the results of the bilateral paired t-tests comparing SOC_{new} and roots. **Days After Incubation Start Remaining SOC_{new} (%) Remaining Roots (%) p-value (bilateral paired t-test, H_0 = true)** 124 79.8 ± 16.5 32.7 ± 23.2 1.3×10^{-7} 195 42.5 ± 35.0 26.7 ± 12.5 ns (5.3×10^{-2})330 49.9 ± 17.9 25.5 ± 15.4 1.6×10^{-5} 524 48.6 ± 35.2 23.7 ± 13.8 8.2×10^{-3}~~

4.4 Implications for SOC storage strategies

We propose that SOC_{new}, as defined in our study and in other continuous labelling studies (Henneron et al., 2020a; Huang et al., 2021) is not a C pool that derives from a physiological process, as it encompasses a broad variety of compounds (Jones et al., 2009) and it depends on root sampling, which is soil and operator dependant (Freschet et al., 2021). But rather, it is an operational parameter which is valuable to refine estimations of C inputs for annual crops as it allows an assessment of all the inputs that are not mineralized at the harvest of the crop. Our results showed that it is a significant input of C ~~,~~ with a mid-term persistence in soils ~~comparable~~ greater to that of roots, even though previous incubations studies moderate the latter statement (Van der Krift et al., 2001; Lu et al., 2003). This makes it a relevant and a necessary pool to consider when reasoning SOC sequestration. For instance, our observation of inter-species differences in specific SOC_{new} reveals that a fixed allocation coefficient is not suitable for SOC_{new} quantification from root C. In our case, the belowground C inputs of *Brassicaceae*, that exhibited high specific SOC_{new} would be relatively underestimated with such an approach.

~~However, considering~~

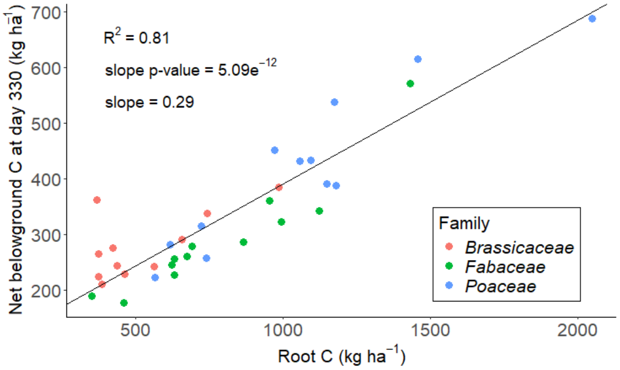

Figure 6. Relationship between belowground C inputs (SOC_{new} and root C) remaining after 330 days of incubation and root C inputs. The remaining C at day 330 was obtained by multiplying the belowground inputs and their respective average fraction remaining at day 330: 0.499 for SOC_{new} and 0.255 for root C (Table 2). The slope was obtained with a mixed-effects model with species as a random effect, to let the intercept vary. The R^2 is the marginal R^2.

However, including SOC_{new} in C assessments requires ~~to be able to estimate it from the crops~~ estimating it from crop traits or other C pools~~as its~~ , as its direct quantification is costly. We highlighted here that the task remains challenging for annual crops, and it this is likely to be exacerbated if we try to extrapolate our results to a complex cultivation system. Nevertheless the positive correlation with root C allows to have a first guidance. Crop selection favouring high root inputs to the soil ~~has already proven to be efficient~~ seems to be a promising practice to sequester additional C ~~,~~ in certain pedoclimatic contexts without being at the expense of the yield (Heinemann et al., 2023). Considering that there is more knowledge on root selection than on SOC_{new} and considering that there is a coupling between the two pools, in terms of quantity and vertical distribution, adjusting the reasoning of additional SOC sequestration on root inputs is likely not to be contradictory with a reasoning based on SOC_{new}. Moreover, we highlighted that root inputs are the main contributors of the fresh root-derived C pool which persists in the soil in the medium term (Fig. 6): even though SOC_{new} tends to remain longer in the soil based on our result, its low net production compared to that of roots (27.8 % of net belowground C production) restrains its relevance as a lever to sequester additional SOC.

~~Relationship between belowground C inputs (SOC_{new} and root C) remaining after 330 days of incubation and root C inputs. The remaining C at day 330 was obtained by multiplying the belowground inputs and their respective average fraction remaining at day 330: 0.499 for SOC_{new} and 0.255 for root C (Table 2). The slope was obtained with a mixed-effects model with species as a random effect, to let the intercept vary. The R^2 is the marginal R^2.~~

A missing link of our analysis is the priming effect. This process can greatly restrict C sequestration (Guenet et al., 2018) and should therefore be taken into account. We can nonetheless note that priming effect is mostly driven by aboveground biomass or by rhizodeposition and less by roots (Huo et al., 2017; Henneron et al., 2020a). This further reinforces the previous statement that belowground inputs, roots like SOC_{new}, are decisive to increase SOC stocks. Moreover, the rhizosphere priming effect is closely linked to nutrient acquisition (Henneron et al., 2020b) and may, therefore, be more of a return on investment associated with biomass production rather than a net C loss.

5 Conclusions

In this study, we attempted to establish a thorough ~~carbon~~ C balance of belowground C inputs by quantifying net rhizodeposition and root debris that are not taken into account in traditional root sampling. We found that this C pool represents 27 % of belowground C inputs, making it an essential input to consider. Although the rhisoheath is a hotspot of root-derived C accumulation, most of it was retrieved in the bulk soil, which invites us to consider most of the planted soil as the rhizosphere. We also highlighted through an incubation experiment that its residence time is comparable, or even greater in our case to that of roots. Nevertheless, results were highly variable as SOC_{new} represents less than 1.5 % of the SOC, which exacerbated the errors. Although differences in terms of quantity emerge between species, the complexity of this group of compounds makes it difficult to predict for annual crops. ~~The~~ Nonetheless, the positive correlation with root C ~~is nonetheless a valuable first step to account for~~ indicates that additional SOC sequestration through high root C inputs is compatible with high SOC_{new} ~~in SOC storage strategies~~inputs.

Code and data availability. Raw data, metadata, calculations and their results have been made open access (Hulin et al., 2025). A list of the statistical tests is also provided. Any additional data or metadata is available upon reasonable request.

Author contributions. BH conducted the experiment and the data analysis, and wrote the original draft. SA leaded the project and the fundraising, and supervised the writing. FM, SC, FD and SP participated in conducting the experiments and helped finalise the draft.

Competing interests. The authors declare that they have no conflict of interest.

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