

Answer to Referee 2

Dear referee,

Thank you for taking the time to review the manuscript and for your relevant suggestions. You will find below the answers to your comments.

This study is a convincing original study using sophisticated ^{13}C plant labeling in order to quantify C input to soils via rhizodeposition from different plant species. The study thus addresses a relevant question within the scope of Biogeosciences. The study shows that it is an important C input which affects soils beyond the rhizosphere. The study is conducted well, even though there are analytical limitations due to the small proportion of rhizodeposition as compared to total soil carbon, which is taken into account in the study and discussed.

Thank you for your positive comments.

A key issue is the different state of plant development (phenology) among the 12 plant species and also within plant replicates which makes comparisons between the plants difficult. Maybe plant groups need to be formed with comparable developments.

We do agree that this is a critical point as phenology greatly influences C allocation. However, we wanted to establish a C balance for a situation that aims at reproducing an agronomical practice: the growth of cover crops during two months at the end of the summer. Please see more details on this point in the other comments below. When the crops are destroyed, they may be at different phenological stages. This is not convenient to study the predictors of SOC_{new} but we believe that it facilitates the extrapolation of our results for this specific practice.

Please see below two figures that aim at analysing the importance of the phenology on our results, by grouping the phenological stages as you suggested. By performing an anova, we observed that there was no significant difference for the specific SOC_{new} according to the phenological stage group. For the R:S ratio, we observed a trend (p-value = 0.08) showing that the plants in the last phenological stages allocated less C to their roots. This latter statement is mentioned in the text and we included this figure in the supplementary informations to support our point (L 338-340): **“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).”**

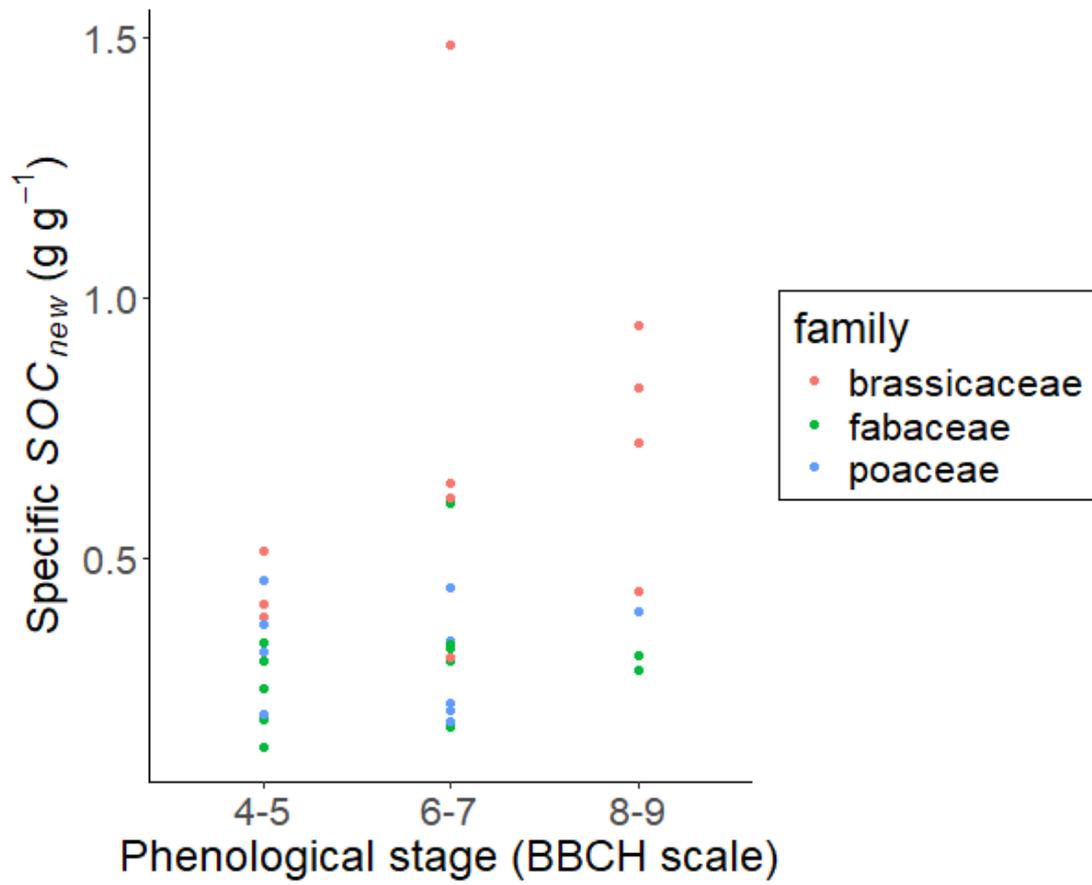


Figure 1: Specific SOC_{new} according to the phenological stage in the BBCH scale (Meier, 2003).

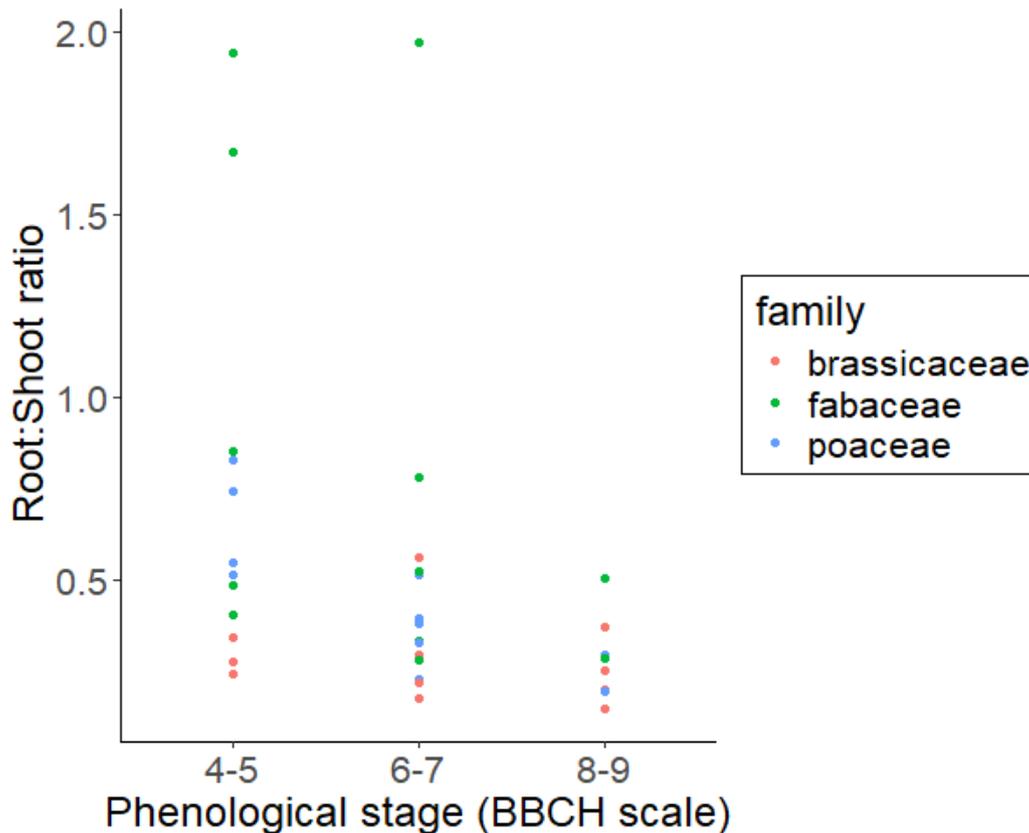


Figure 2: Root:Shoot ratio according to the phenological stage in the BBCH scale (Meier, 2003).

Some more issues listed below should be taken into account to further improve the study. In general, the paper is written well, figures and tables are nice and meaningful and conclusions are substantiated by the presented results. Thus, this study will be an important contribution to a topic that is understudied.

Thank you for your positive comments.

Title: Why is “cover crop” in the title but mentioned only once in the total manuscript? Alfalfa is no cover crop and also barley, rye, oat and oil seed rape are rather used as main crop and not as cover crops. On the other hand: Vetch, white mustard and camelina are typical cover crops species and I do not know them as recently grown main crop. “Plant species” would be a more appropriate term.

We do agree that the title is misleading. We wanted to highlight that our study is mostly valid for a crop that has grown for only two months and that was sown in august. To our knowledge, barley, rye and oats sown at this time of year are only used for intermediate crops, be it to cover the soil or to produce biomass for bioenergy purposes. For rapeseed, this sowing date can also be for a main crop. For alfalfa, this cultivation practice is indeed not representative of continental European agriculture. We selected it for its interesting rooting properties. According to that, we specified in the text that the sowing date is not the most appropriate.

Nevertheless, our study study focuses on intermediate crops by choosing mid august as a sowing date, and by choosing a growth period of 56 days, which is the minimum period permitted before the crop can be destroyed, according to French regulations in areas vulnerable to nitrate pollution. We do not guarantee that our results can be extrapolated to a main crop cultivation of the same species.

We propose to accept your title suggestion, and to emphasise this point in section 2.2 (L 125-128): **“Except for *Medicago sativa*, which is perennial, each species could potentially be selected for 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.”**

1.26-28: There maybe definition problems with the term “rhizodeposition” if some authors do not include root debris and root hairs under this term. However, rhizodeposition is defined mostly as “the total carbon transfer from plant roots to soil” (see Nguyen, 2003). Thus, this sentence distracts from the key problems. Its not a definition problem but an analytical problem. I recommend to remove the sentence.

This is a critical point of our study. We indeed believe that this is an analytical issue. The quantification method should be chosen according to the research question. In our case, we intended to quantify all the fresh carbon that was not taken into account into the roots pool. This also includes very fine roots, that could in some cases be seen with the bare eye. According to our interpretation of the article from Nguyen (2003), this does not fit into its definition of rhizodeposition, as carbon from root senescence is restrained to root epidermis in its work. In our case, other root cells, not comprised in the epidermis, might have been taken into account due to the sampling strategy. Nevertheless, the latter are also important to establish an accurate carbon balance. We could have used an other sampling method to be more accurate in our quantification of root-derived carbon, such as a water bath to isolate particulate root debris from finer rhizodeposition products, but that would have prevented the analysis of the rhizosheath for instance. To clearly state that our study focuses on a carbon pool that is larger than rhizodeposition as defined by several previous articles, we believe that this sentence is necessary to justify our analytical choice. For more clarity, we added the following sentence (L 31-32): **“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).”**

1.42: “other forbs” as compared to legumes that are also forbs.

We added your suggestion to the text.

1.49: Citation is missing for the “promising recent works” you referred to.

We have reformulated it so that the citations appear earlier (L 57-61): **“Promising recent works (Henneron et al., 2020a; Huang et al., 2021; Williams et al., 2022) comparing several species have led to significant advances that linked plant traits and rhizodeposition: 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.”**

1.82-86: This is a method section in the introduction. I recommend to shorten it or remove it, since it distracts from getting to the objectives of this study.

We do agree and shortened the section (L 91-95): **“Here, we propose to establish a thorough C balance of belowground C inputs across 12 crop species. We set up a mesocosm experiment in a climate chamber under a ¹³C-CO₂-enriched atmosphere to be able to trace root-derived products. We had 3 main objectives: 1) quantifying SOC_{new} and linking its release to root and**

shoot C; 2) disentangle the spatial distribution of SOC_{new} by assessing its vertical distribution and its vicinity to the roots; 3) assessing SOC_{new} persistence in the soil after harvest of the aboveground biomass through a following field incubation experiment.”

l.87: Disentangling the spatial distribution of what?

We have changed to “Disentangle the spatial distribution of SOC_{new} by assessing its vertical distribution and its vicinity to the roots” (L 93-94).

l. 88: “assessing SOC_{new} persistence...after harvest of the above ground biomass through...”

We included your suggestion.

l.91: Please delete “simulation”

We deleted “simulation”.

Throughout the manuscript I learned: Numbers up to twelve should be in letters not in numbers and numbers and do not use numbers or abbreviations at the start of a sentence.

Thank you, we checked throughout the manuscript. However, Biogeoscience recommendations are the following: “For items other than units of time or measure, use words for cardinal numbers less than 10; use numerals for 10 and above (e.g. three flasks, seven trees, 6 m, 9 d, 10 desks). “

l.93: “ Here, we...”

Thank you.

l.100: Use “deionized water” instead of “osmosis water”.

We changed.

l.102: A water content of 0.5 cm³/cm³ means saturated water conditions in most soils (total pore volume of 50% is water filled). Please check and revise. Please also write how you controlled soil moisture. How regular? Using which method? How did you adjust soil moisture? Individual for each plant species or mesocosm?

Indeed, we did a mistake: our threshold was 0.05 cm³·cm⁻³. We completed the description (L 108-113): “Soil moisture was measured every 15 minutes in 12 separate mesocosms that were not used for carbon 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 0.05 cm³·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.”

l.104: “following a crop harvest”

We changed.

I.118: What is “a realistic plant density”?

We added a table in the supplementary informations reporting the sowing density and changed the text to “**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 if the seed germination rate exceeded 50 %. The number of seeds ranged from 2 for faba bean to 64 for Alfalfa. The number of plants that have been maintained is available in Table 1**” (L 129-132).

I.125: “Replicated three times across three climate chambers...”

We changed.

I.131: How much soil was collected as composite sample out of the 13 liters soil?

We precised in the text (L 146-147): “**Soil was collected in both horizons in several places to obtain a composite sample of 500 grams.**”

I.148: The net rhizodeposition that you measured was accumulated during the 56 days of plant growth, likely with much more input during the last days of the experiment when the roots were well developed. There might be also some rhizodeposits that were recent (some hours before the experiment was harvested). Are those also net rhizodeposition? Some parts of rhizodeposition is already decomposed and mineralized and thus not captured. How did you account for this flux of rhizodeposition?

To our knowledge, there is no time threshold that has been defined in literature to distinguish between net rhizodeposition and “fresh” rhizodeposition. Pausch and Kuzyakov (2018) wrote: “net rhizodeposition is defined as the part of the C that remained in the soil after microbial utilization and partial decomposition to CO₂”. Indeed, some rhizodeposits are likely recent and mixed with “proper net rhizodeposition”. We did not find a good idea to take this into account. We could have let the soil at room temperature after having separated the roots, so that the fresh rhizodeposits could decompose, but we decided that it would not be representative of realistic decomposition conditions. Besides, our incubation experiment partially allows to take this into account as if we sampled fresh rhizodeposits that would have been quickly mineralised, the latter were likely mineralised in the first days of the incubation. Therefore, the combination of the two experiments helps to obtain a final result, here a remaining quantity of SOC_{new} after two months of growth and 524 days of incubation, which is valid.

Regarding the fraction of rhizodeposition that was lost as CO₂ during growth, we indeed did not take it into account. From an experimental point of view, it would have required a significant effort. This C must be of major importance regarding microbial activity, priming effect or even nutrient acquisition. However, to simply establish a quantification of the C remaining in the soil after crop harvest, as we did, we decided that this was not unavoidable, and we chose as a compromise not to take it into account.

I.206: There was large variability in the number of plants per mesocosm and plant species but also among the plant species. How does plant density affect the results? Is plant density a major predictor for root biomass and maybe also allocation (R:S ratios and SOC new)?

As shown in Figure 3, the variability in plant density is very pronounced for alfalfa and ryegrass. However, for the other species, the homogeneity is satisfactory. To test whether plant density affects the results, we believe that it would be best to test at an intra-species level, rather than at an inter-species level. However, only alfalfa and ryegrass would allow that, which is in the end not enough data. We nevertheless tested whether there was a correlation between plant density (plant number mesocosm⁻¹) and Root C, SOC_{new}, and Root:Shoot ratio with a mixed model, by using all the species together. We did not find any relationship between plant number and root C, neither with SOC_{new}. We observe a weak positive correlation between plant number and R:S ratio though (slope p-value = 0.03 and marginal R² = 0.10). Nevertheless, this relationship is driven by a few extreme values (Figure 4).

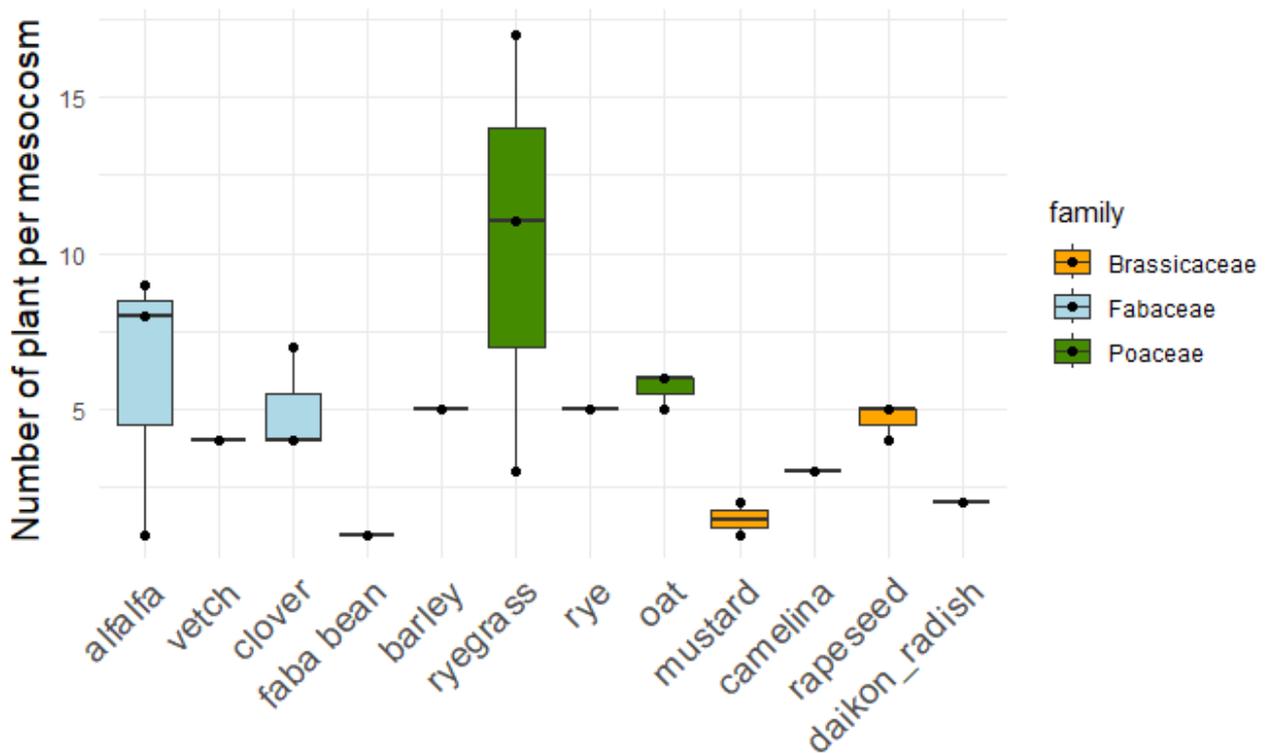


Figure 3: Number of plant per mesocosm

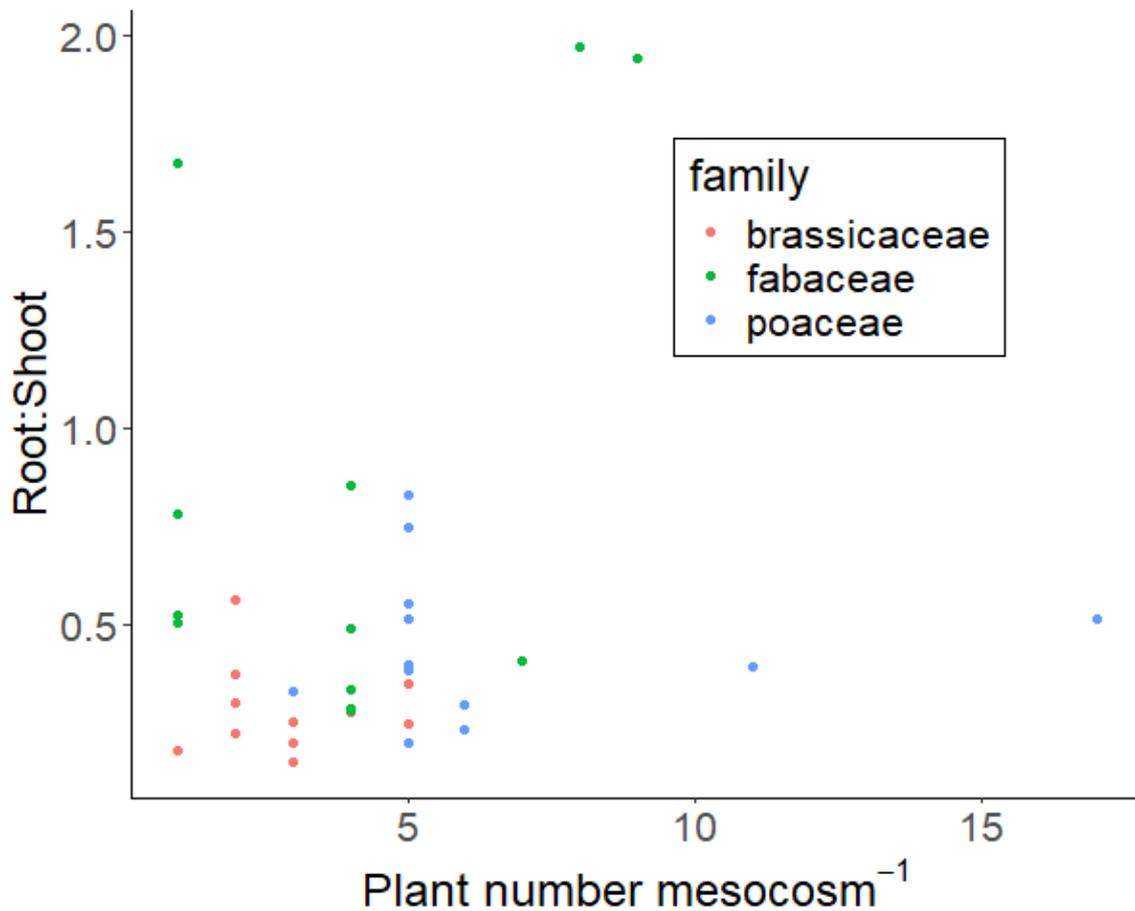


Figure 4: Relationship between plant density and Root:Shoot ratio

How well does the density fits to density of crops when cultivated at field sites?

We included in the supplementary informations the following table that reports the number of seeds that were sown and we introduced the following paragraph in section 4.1.1 (L 313-318): “**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).**”

| Species | Plant number | Seeds sown | Recommended seed number |
|---------------------------|--------------|------------|-------------------------|
| <i>Medicago sativa</i> | 9,8,1 | 64 | 32 |
| <i>Vicia sativa</i> | 4,4,4 | 6 | 3 |
| <i>Trifolium pratense</i> | 4,7,4 | 43 | 22 |
| <i>Vicia faba</i> | 1,1,1 | 2 | 1 |
| <i>Hordeum vulgare</i> | 5,5,5 | 9 | 5 |
| <i>Lolium multiflorum</i> | 11,17,3 | 45 | 23 |
| <i>Secale cereale</i> | 5,5,5 | 10 | 5 |

| | | | |
|-------------------------|-------|----|---|
| <i>Avena sativa</i> | 6,6,5 | 7 | 4 |
| <i>Sinapis alba</i> | 2,1 | 6 | 3 |
| <i>Camelina sativa</i> | 3,3,3 | 14 | 7 |
| <i>Brassica napus</i> | 5,4,5 | 10 | 5 |
| <i>Raphanus sativus</i> | 2,2,2 | 3 | 2 |

l.225: It is not helpful that results and discussion are merged into one section. This dilutes the results of this nice study and makes it more difficult to read. I strongly recommend to rewrite this section and disentangle results and discussion.

We changed according to your suggestion.

l.235-238: Not clear if you refer to above and below ground biomass with the term “biomass”.

We meant both aboveground and belowground biomass. We specified it (L 308-310): “**A greenhouse experiment (1 m³ 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)**”.

l.250: Need to be better explained what is labelling heterogeneity? Is it a spatial heterogeneity or variability between replicates or is it heterogeneity in the label within the plants due to the pulse labelling?

We precised in the text (L 251-254): “**However, the labelling procedure led to temporal heterogeneity in the atmospheric $\delta^{13}\text{C}$, particularly during the second week of growth, during which the labelling was less pronounced (Fig. S2). This resulted in discrepancies between the $\delta^{13}\text{C}$ of roots and shoots, especially for *Poaceae* and *Brassicaceae* that exhibited a higher root labelling (Fig. S3).**”

l.275: Are there differences in the SOCnew quality (exudates vs mucilage, vs root fragments; differences in compounds and CN ratio) among the different plant species? Thus, how could the gross vs net SOCnew ration vary among plant species? Maybe you could add some discussion on these questions here.

I do agree that this point would be very interesting. However, we do not have such data and I do not see how to introduce a robust discussion about it without them.

l.291: Daikon radish is considered as plant for deep soil melioration (to break up soil compaction) due to the deep reaching tap root. You found the opposite. Please discuss.

We do not think that this result is surprising. Daikon radish indeed has a thick tap root and might therefore be chosen to break up soil compaction. However, its tap root is thickest in the first soil layers. As a result, the root biomass is concentrated in the first soil layers (0 – 20 cm). This is what happened in our study, where daikon radish had only 56 days to grow: the thick part of the tap root was only present in the first layer (Figure 5). This is not inherent to our study. Even though the tap root can penetrate deeply in the soil, a large share of the thick part of the root is always found in the first 20 cm, as we can see in Figure 6, obtained from a study by Fedosiy et al., (2026).



Figure 5: Photograph of the root system of a Daikon radish. Most of the biomass that was recovered is concentrated in the upper soil layer.

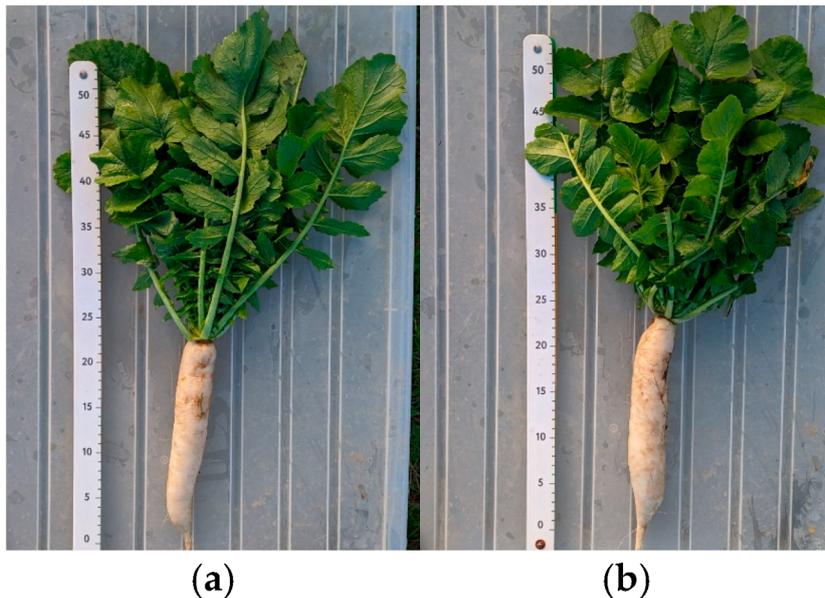


Figure 6: Photograph of two varieties of daikon radish. The image is taken from a study of Fedosiy et al., (2026).

Besides, the ability of daikon radish to alleviate soil compaction is mostly revealed in compacted horizons, where its roots might thrive better than the one from other cover crops. Indeed, Chen and Weil (2010) showed that radish tended to produce more roots down to 50 cm, in terms of number of roots, than rye and to a lesser extent rapeseed in compacted soils. However, in soil that are not compacted, this difference vanishes. In our case, where the soil is not compacted with a density of 1.2, we do not think that there is a reason to find more roots in the lower horizon for radish than for the other species.

Lastly, in our study, roots can reach down only to 45 cm, which was the case for every mesocosm. Therefore, our study is not suited to properly discuss the rooting depths. This is a limit that we did not pointed out. We added a discussion sentence about this in the text in section 4.1.1 (L 305-306): **“Besides, roots were limited to a depth of 45 cm, unlike in the field.”**

Figure 2 is not easy to read with the mix of colors and shading. The orange and blue bars going up to 100% contain no information and could be removed.

Your comment goes hand in hand with the comment by the first referee. We suppressed the background bar. Please see below the new figure.

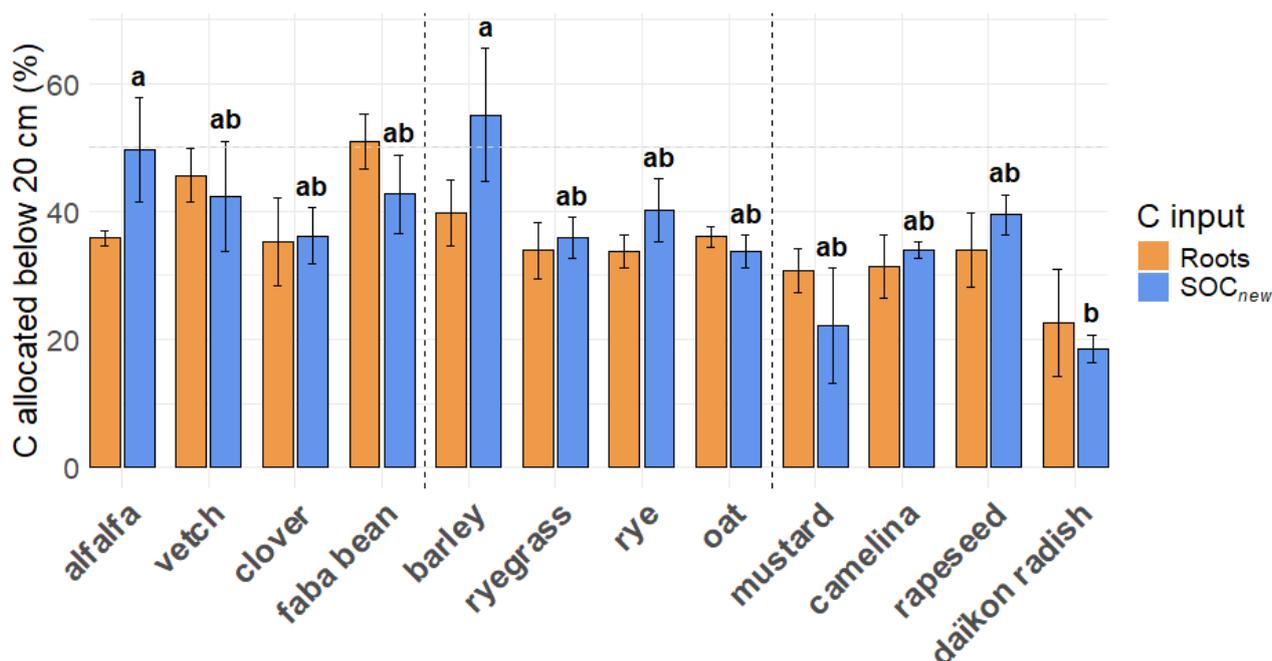


Figure 7: Root C and SOC_{new} retrieved in the soil horizon below 20 cm (20 -45 cm). The bar heights are the mean for each species and error bars equal 2 standard errors. The significant letters are only reported for SOC_{new} as no significant differences were observed for roots.

L.316: This result maybe different in topsoil and subsoil (any data on this to show and discuss?). Please discuss if also subsoils (also undisturbed once outside pot experiments) receive SOC_{new} across the whole soil matrix as you found it. It is in any case an important finding of your paper and may be better considered in the abstract (l. 10): Which volume is rhizosheats compared to bulk soils and which fraction of SOC_{new} is in the rhizosheats and which is outside of them?

Indeed we have data on this that we introduced in the results section (L 280-281): **“Rhizosheath mass represented in average 2.0 ± 2.3 % of the soil mass in our experiments, all species comprised (when sampling was possible)”** and **“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)”** (L 274-277).

We also we reiterated this in the discussion (L 375): **“Our results highlight that most of SOC_{new} is retrieved in the bulk soil, regardless of the soil horizon”**

We introduced in the abstract the following sentence (L 10-11): **“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.”**

It is difficult to obtain the volume of the rhizosphere, as the sampling disaggregates the structure of the soil. However, we reported the mass fractions in the text and Figure 4 of the new manuscript was redesigned to display these results more clearly.

l.368: Above and in the abstract you claim SOC_{new} being more persistent than roots. Here you write about similar persistence. Please rewrite.

We wanted to take into account previous incubations studies with this sentence. Because our results are affected by significant variability, we find delicate to claim that SOC_{new} is definitely more persistent than roots. We therefore nuanced the sentence: **“Our results showed that it is a significant input of C with a mid-term persistence in soils greater to that of roots, even though previous incubations studies moderate the latter statement (Van der Krift et al., 2001; Lu et al., 2003).”**

l.397: This “first step” is common practice in modelling studies of soil C dynamics that need to estimate C inputs including rhizodeposition (e.g. <https://doi.org/10.1371/journal.pone.0256219> or <https://doi.org/10.1007/s11104-022-05438-w>). Pausch and Kuzyakov 2018 generated a valuable estimate for this rhizodeposition that was confirmed in your study. Thus, your study rather supports this common practice than facilitating it.

We wanted to highlight that selecting important root systems to sequester additional SOC is compatible with an increase in SOC_{new} inputs. However we also stated in the text that a fixed allocation coefficient to estimate SOC_{new}, as adopted in the articles you mention, is not appropriate as specific SOC_{new} is negatively correlated to root C. We do not wish to discredit this practice though, as we do not have a much better alternative to offer. Therefore, we changed “appropriate” to “optimal” in the discussion (L 351-352): **“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.”**

To clarify our conclusion point, we changed the sentence to **“Nonetheless, the positive correlation with root C indicates that additional SOC sequestration through high root C inputs is compatible with high SOC_{new} inputs”** (L 455-456).

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