Replies on referee comments on Van de Broek et al., The soil organic carbon stabilization potential of old and new wheat cultivars: a $^{13}CO2$ labelling study

Replies to reviewer 2

We would like to thank the second reviewer for the comprehensive and detailed comments on our manuscript. These will greatly improve the quality of our manuscript. We present the reviewer comments in *italic*, our replies are formulated in normal font.

General comments

The authors address the question whether the aim of modern plant breeding strategies to maximize grain yield may affect soil C dynamics, because these strategies often have the side effect of reduced root biomass and reduced rooting depth. It is a relevant topic, because optimization of C storage in arable lands can contribute to higher soil C storage and better soil functions. The study has the potential to deduce recommendations for a climate-smart agricultural practice.

The authors conducted a 13C pulse-labelling mesocosm experiment with four wheat cultivars in a closed-chamber greenhouse setting. The elaborate experimental design allows to quantify not only C input, but also soil respiration within a depth profile and is therefore highly suitable to address the question of subsoil C turnover. However, the amount of root biomass in deeper layers was in some cases too low to perform isotopic analyses, which then hampers drawing conclusions on subsoil C input. The thorough experimental set-up comes with the cost of low replication, which may be a reason for the high variability many of the measured parameters exhibited.

Throughout the paper the authors refer to C stabilization, but they actually did not quantify this (rather long-term) process. As in the title, they rather deduce the potential of SOC stabilization from other processes.

Therefore, I suggest referring to the (rather short-term) processes that were actually investigated, which were root growth, rhizodeposition and soil C dynamics. Also, they do not present any results on C stabilization itself (which might be challenging given the duration of the experiment) and do not mention this concept in the introduction extensively. I suggest to adapt the terminology to achieve a more precise and coherent wording in a revised version of this manuscript.

Thanks for this comment. A similar concern was raised by the other reviewer. Therefore, we changed the term ‘carbon stabilization’ to ‘net carbon rhizodeposition’ throughout the text.

Specific comments

Introduction:

The introduction gives a good overview, but could be more concise: Shorten and/or combine paragraphs 2 and 3.

Thanks for this suggestion, we reduced the length of both paragraphs

Lines 61f: Please comment on processes that lead to differences in gross and net rhizodeposition. Are there studies on qualitative differences of rhizodepositions between wheat cultivars?

To clarify the difference between gross and net rhizodeposition, we now complemented this sentence with '[…] , after a portion of gross rhizodeposits are lost from the soil through microbial mineralization or leaching'. The only study we are aware of that has checked for differences in carbon rhizodeposition between different wheat cultivars is Hirte et al. (2018, https://doi.org/10.1016/j.agee.2018.07.010), to which we compare our results in the discussion section.

Lines 74f: A bit vague, which practical limitations do you mean?

Here, we refer to difficulties of continuously labelling agricultural crops in the field. We are aware of the existence of techniques that allow to continuously label the aboveground parts of plants in the field throughout the year (several FACE experiments do so), but this requires a very elaborate set-up with large financial investments. This is, however, not feasible for most studies. To make this clear, we changed this sentence to 'However, the continuous application of 13CO2 or 14CO2 during the course of an entire growing season to plants is often not feasible, as this requires the set-up of open-top chambers while continuously supplying the crops with the isotopic label, which comes at a high financial cost'.

Lines 90f: Hypothesis: Does the experimental design allow to test C stabilization or rather C input/ C balance? Soil C stabilization mechanisms are not assessed, only inferred from other processes (e.g. root growth, rhizodeposition). As far as I understood your study, you did not differentiate between different soil C forms, e.g. mineral associated carbon or labile carbon that are a proxy for C stabilization.

Our experimental set-up did indeed not allow us to check for long-term carbon stabilization through e.g. organo-mineral interactions. Therefore, we adapted our research question to 'do wheat cultivars with shallow roots and lower root biomass lead to less net carbon rhizodeposition compared to wheat cultivars with deeper roots and higher root biomass?' and our hypothesis to '[…] wheat cultivars with shallow roots and lower root biomass would result in less net rhizodeposition over the course of a growing season, compared to cultivars with deeper roots and higher root biomass.' In addition, throughout the manuscript we changed the term 'carbon stabilization' to 'net rhizodeposition', to make clear to the reader that we do not focus on long-term carbon stabilization, but rather on short term (one growing season) carbon rhizodeposition.

Methods:

Well written, good level of detail, mostly easy to follow.

Lines 118f: Why did you choose a cultivar with known high rooting depth? Is this still characteristic for the group of "new" cultivars, or would this be a specific, maybe drought-adapted, cultivar? Since you argue with the two groups of "old" and "new" cultivars later on, I expect your selected cultivars not to be much different from commonly used cultivars.

As our aim was to assess how wheat cultivars with different rooting depth affect belowground carbon dynamics, we specifically chose wheat cultivars with both low and high biomass. As stated in the manuscript (L. 117 - 118), rooting depth has generally increased through time in the Swiss wheat breeding program (Friedli et al. 2018; https://doi.org/10.1007/s10681-019-2404-7)) as well as in other wheat breeding programs (Aziz et al. 2017; DOI 10.1007/s11104-016-3059-y). For the Swiss wheat breeding program, Friedli et al. have shown that there is a consistent trend of decreased rooting depth with the year of cultivar development under well-watered conditions. Therefore, the

selected wheat cultivars are typical of the 'old' and 'more recent' cultivars developed by this breeding program.

Line 122: Was this the same topsoil as in the lysimeters?

This was indeed the same topsoil. We made this clear in the manuscript: 'Next, the seedlings were planted in containers filled with the same topsoil used to fill the lysimeters and transferred to […]'.

Lines 127f: Did you measure plant biomass per seedling before transplanting/ labelling? What was the phenological stage of the seedlings? Did it differ between individuals and/ or cultivars?

Plant biomass per seedling was measured before transplanting, but we observed no big differences between the cultivars. At the moment of transplanting, the plants were at the onset of tillering. The latter has been added to the manuscript (section 2.1.2): 'At the timing of transplanting, the plants were at the onset of tillering.'.

Line 144: Do you mean CO2 concentration of 58% or 58 atom% 13CO2?

We meant atom%, this has been changed in the manuscript.

Line 158: Please be more specific, what does "limited amount" mean?

We meant that we only performed $\delta^{13}C$ analyses of CO_2 for samples collected on the last sampling day. This has been adjusted in the text to make this clear to the reader: 'In addition, the δ13C value of CO2 was measured for CO2 samples collected along the depth profiles on the last sampling date […]'.

Line 176: 40mg, isn't this very low? Rather 40g, with 200 ml?

Thanks for noticing, this should indeed be 40 g, we changed this in the manuscript

Line 176: Was the chloroform ethanol-free?

Unfortunately, due to some limitations, the chloroform was not ethanol-free. However, since all samples were fumigated with the same chloroform, we think the comparison between different treatments and different depths is valid, as we mostly use these data in a qualitative way (e.g. comparison of microbes under which cultivar incorporated most $^{13}CO_2$)

Lines 217f: Which input variables do you mean specifically?

We meant the $\delta^{13}C$ value of root biomass. To make this clear to the reader, we changed this to: 'In addition, there was a large variability in the $\delta^{13}C$ value root biomass between the replicates of the same cultivar, which complicated the calculation of excess ^{13}C for individual lysimeters.'.

Results:

Lines 295ff/ Section 3.1: Is stem and leaf biomass so much lower in Zinal because of much earlier grain filling? Please include data on phenological states for all cultivars or state more clearly if they have been in the same phenological stage (which I assume they have not). You only mention that they all reached flowering stage, but this does not exclude some been even further developed.

We are not sure why stem and leaf biomass in Zinal was lower compared to the other cultivars. One reason might be that the Zinal cultivars were negatively affected by the growing conditions in the

greenhouse, although this was not the case for the other cultivars. We do not have detailed information about the phenological stage in which the plants were at the moment of harvest, so this can unfortunately not be included in the manuscript. We hope that the sentence in L. 300 – 301 ('It is noted that these data should be interpreted with care, since not all plants reached maturity at the time of harvest, and is potentially not representative for the biomass of the ears of full-grown plants.') will make clear to the reader that there are uncertainties with respect to the aboveground biomass.

Lines 327ff. Do you expect SOC in the initial soil to differ from SOC in the soil in the lysimeters at the begin of the experiment? If so, how?

Given the relative short duration of the experiment (ca. 5 months), we did not expect that the SOC concentration was different compared to the initial soil. Since we did not have a control treatment, we explicitly wanted to emphasize this lack of a difference in SOC concentration, which suggests that the SOC concentration was not affected by the type of cultivar.

Line 345: CO2, not d13CO2 (A value cannot be enriched)

Thanks for noticing this, we corrected this to: 'the CO_2 under the old wheat cultivars was more enriched in 13C compared to […]'

Lines 368ff: What about the d13C of CO2 that was measured in some samples?

In this section (3.5), we only present the calculated depth profiles of CO_2 in the different lysimeters. As we had only limited information about the $\delta^{13}CO_2$ depth profiles, while we did not had sufficient data to separate autotrophic from heterotrophic CO_2 production, we were not able to calculate the $\delta^{13}C$ value of produced CO_2 along the soil profile. Therefore, the $\delta^{13}C$ of CO_2 is not discussed in this section, as this has been done in section 3.3 (L. 343 – 347).

Figure 1: Using the same colors for different groups is confusing (e.g. leaves in 1A vs. Zinal in 1B). I don't find the inset in 1B useful, the statistics could be included in the main figure.

Thanks for pointing this out, we changed the colors in Fig. 1A. However, we prefer to keep the inset, as otherwise the values, and (lack of) differences between the root biomass at different depths would not be clear to the reader. That is also the reason why we put the letters indicating significant differences in the inset, so the reader can visually inspect the actual values behind the statistics, which would not be possible in the main figure.

Figure 2, 3: Please use your color coding also for error bars.

We would prefer to keep the error bars in black, because colored error bars would be much more difficult to differentiate from the data points, and will overlap with some of the individual data points that are plotted, which would make their colors less distinguishable.

Figure 3A: Use an x-axis range that fits the data, starting higher than 0.

To not give the impression that the organic carbon concentrations are very low, we prefer to keep starting the x-axis at 0.

Table 1 is redundant, except for root:shoot ratios.

Also the OC % of aboveground biomass, the total biomass and OC% of roots is not presented in the figures, so we would like to keep these numbers here so they can be consulted by the reader. To decrease redundancy, we no longer repeat these numbers in the text.

Discussion:

Please do not repeat values, except for comparisons with other studies, where you name their values explicitly. Also, please do not only repeat results.

Thanks for pointing this out, we no longer repeat these numbers in the text, except when we compare them to other studies, and we removed sections where we repeat the results while this was not necessary.

Line 399: Which differences in root architecture do you have in mind?

We meant mostly differences in root biomass. To clarify this to the reader, we changed this to: '(ii) actual differences in root biomass'.

Lines 424ff: d13C in roots, variation with depth and cultivar: Why would the $\delta^{13}C$ signal of plant carbon change throughout the experiment, given that all plants received 13CO2 in regular time periods and equal amounts. Do you expect seedling biomass at the time of transplanting/ before the first labelling to differ and therefore causing these differences? If you started with equal plant biomass and equal amounts of 13C, I would not expect these strong differences in plant d13C.

Our results indeed suggest that differences in timing of C allocation to deep roots (only shortly after planting for more recent cultivars versus throughout the growing season for old cultivars) cause the difference in the $\delta^{13}C$ value of roots. However, this does not need to imply that the $\delta^{13}C$ of plant biomass changed throughout the experiment. We interpreted this as follows: as more recent cultivars grew deep roots in the beginning of the experiment, less total ^{13}C had been assimilated by the plants over the period when root biomass was constructed. The old cultivars, which created root biomass throughout the experiment, therefore assimilated much more ^{13}C during the period when roots were build, therefore leading to higher $\delta^{13}C$ values of roots along the entire soil profile.

To make this clear to the reader, we changed part of this section to: 'This suggests that both old and more recent wheat cultivars grew roots down to depths of > 1 m in the beginning of the experiment (when the total amount of ^{13}C assimilated by the plants was limited), while only the old cultivars kept on allocating carbon down to deep roots (> 0.45 m) throughout the experiment (thus having assimilated more ^{13}C over the period of root growth compared to the more recent cultivars).'.

Lines 431-444: This paragraph is appropriate as part of the introduction, rather than the discussion.

Thanks for pointing this out, we now removed this paragraph.

Lines 503ff: Could you test this hypothesis with your data for short-term relationships, independently from cultivar development time, e.g. by looking for relationships between SOC concentration and root biomass or d13C in soil and SOC concentrations?

Testing this hypothesis would be difficult, given the relative short duration of our experiment. We suggest that the fate of decayed root biomass (thus upon incorporation in microbial biomass) will determine how much of this C will remain in the soil, mainly as stabilized microbial necromass. As we found no differences in SOC concentration between the cultivars, we do not expect to find relationships between root biomass and SOC concentration. Testing the hypothesis that higher root

biomass would lead to larger amounts of stabilized SOC was, however, outside the scope of our study.

Lines 531f: Please also mention the share of croplands in total landmass and the share of SOC of croplands in global SOC to give a comprehensive perspective.

Thanks for this suggestion, this has now been added to this section: '[…] and cereal crops are grown on ca. 20 % of croplands globally (Leff et al., 2004) (covering ca. 12 % of global land mass and storing ca. 10 % of global SOC in the upper meter of soil (Govers et al., 2013)) […]'.

Technical corrections

Line 786: Error bar (or bars), not bard

Thanks for noticing, this has been corrected