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 Stefan Karlowsky

We would like to thank Dr. Karlowsky for his 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. The line numbers in our replies refer to the line numbers in the revised manuscript.

General Comments

In the present study, the authors report their findings from a 13CO2 pulse labelling experiment on different wheat cultivars grown in lysimeters filled with agricultural soil (surface and subsoil). The main study objective is to assess how the use of more recent wheat cultivars with lower rooting depths and root biomass alters organic carbon inputs into soil compared to older cultivars from the Swiss wheat breeding program. This research subject is important, because a large share of the global agricultural land is allotted to the cultivation of cereal crops, and it is unclear how the use of mod ern cultivars with altered root traits affect soil organic carbon (SOC) dynamics and consequently SOC stabilisation. Here the authors found no significant effects of different wheat cultivars on SOC in the short term. They conclude that the fate of root biomass after the harvest determines cultivar effects on stabilised SOC pools in the long term. The study is based on a sophisticated methodological approach, including an innovative lysimeter-labelling chamber setup as well as state-of-the-art 13C labelling and analysis techniques. The description of materials and methods used for the study is, in general, detailed enough to follow all steps of the experiment. However, a few things still need clarification (see specific comments). The major limitations of the study are the low number of replicates (probably due to the complex setup) and the fact that root biomass was too low for 13C analysis in many samples. Especially the latter impedes drawing conclusions about the input of plant-derived carbon into soil and its variation between the different cultivars over the growing season. Notably, the authors are well aware of these limitations and discuss them appropriately.

The presentation of results is generally OK but should be modified in order to avoid redundancy between figures and tables. E.g. Fig. 1 and Table 1, both are showing the same values and statistics for aboveground biomass.

The data were presented in the figure for visual interpretation, while we repeated the values in the table so the exact values are available to the reader. In order to reduce further redundancy, we no longer cite these values in the text, but refer to the table.

Furthermore, you do not need to repeat values shown in figures and tables in the text body, neither in the results nor in the discussion section.

These values are now removed from the text.

I would also recommend to change Fig. 1 and Fig. 2, not separating between biomass and delta13C, instead showing aboveground biomass together with its delta 13C in Fig. 1 and the root parameters in Fig. 2 (as it is structured in the text body).

We thank the reviewer for this suggestion, but we think it is more convenient for the reader to see separate figures for OC % and $\delta^{13}C$, although this is discussed in the text differently. This way, for example, we aim to emphasize the important differences in the $\delta^{13}C$ value of above- and belowground biomass in the subsoil between the old and more recent cultivars.

Regarding Fig. 4 and Table 2, I am missing the statistics. These statistics would be necessary to support some of your interpretations from the discussion part.

We fully agree with the reviewer that statistics would aid our interpretation and discussion of the results. As mentioned in the previous version of the manuscript, we did not perform statistics on the data presented in Table 2 and Fig. 4 because missing data points prevented us from doing so. All these missing data points were located in the third soil layer (0.45 – 0.75 m depth), while there was no missing data in the upper two soil layers. Therefore, we decided to limit the results we present with respect to net C rhizodeposition and belowground C allocation to the two uppermost soil layers (0 – 0.45 m depth). We argue that this is reasonable, since the majority of root biomass was situated in these two layers (as shown in Figure 1B). This allowed us to perform the necessary statistics to assess whether net C rhizodeposition and belowground C allocation to these two soil layers were significantly different between these two layers and between cultivars. To make this clear to the reader, the following changes have been made to the manuscript:

- L. 228 – 231 (section 'Net carbon rhizodeposition'): we added the following: 'We note that data on the C concentration and $\delta^{13}C$ value of root biomass could not be obtained from a number of soil layer below 0.45 m depth for certain cultivars, due to the limited root biomass that could be retrieved. Therefore, net C rhizodeposition was only calculated for the two uppermost soil layers (0 – 0.45 m depth), as only for these layers all necessary data to calculate net C rhizodeposition was available for the three replicates of every cultivar.'
- L. 214 – 216 (section Excess 13C calculations): we added the following: 'Calculations of the effect of wheat cultivar on belowground excess ^{13}C were only performed for the upper 0.45 m of the lysimeters, as missing data for deeper soil layers prevented including these layers in the statistical analyses.'
- In Table 2, we adjusted the values to only reflect the results for the two uppermost soil layers, and adjusted the caption accordingly. As we detected no statistically significant differences in belowground C allocation or net C rhizodeposition between any of the cultivars, no letters were added. However, this is now mentioned in the caption.
- Similarly, we adjusted figure 4 to only display the results for the upper 2 soil layers. As the statistical results for differences in total C rhizodeposition and total belowground carbon allocation are already provided in table 2, these were not repeated here. However, we did tested if the amount of net rhizodeposition was different between the two depth layers of each cultivar, and added the appropriate letters to the figure.
- Throughout the result and discussion sections, we adjusted the values of reported net C rhizodeposition and belowground C allocation to only reflect values for the upper two soil layers.

The discussion itself is a bit lengthy and would profit from some restructuring (see also specific comments).

We used your comments below to shorten the discussion

The subsections 4.1 to 4.3 can be shortened, e.g. by excluding the repetition of results and streamlining the remaining text. Maybe it is also better to start the discussion section with the main study object (for non-expert readers), which suddenly comes up in subsection 4.4 now.

Thanks for this suggestion. We now start the discussion by briefly repeating the objective of our study and the main results, for readers who jump to the discussion section at once. Where possible, we shortened sections 4.1 to 4.3, e.g. by removing the first paragraph of section 4.2.

Another possibility to increase readability would be the use of more active and less passive voice, though this is a matter of taste. Overall, the structure of the manuscript is clear and the language is fine. The authors relate their work to a comprehensive set of up-to-date literature and make the data underlying the results available as supplementary material. However, there are a few things in need of improvement and the manuscript will profit from a revision taking into account the addressed points.

Specific Comments

Line25: I think that "net SOC stabilization" is the wrong term. Stabilization implies a long-term effect, which you did not study here (if there was a difference - what about rhizodeposits degraded and respired by microbes off-season?). Therefore, better use "net carbon rhizodeposition" as in the rest of the manuscript.

A similar comment was raised by the second reviewer as well, and we agree with both reviewers. Therefore, we changed the term 'carbon stabilization' to 'net rhizodeposition' throughout the text. However, we did not change the title of the manuscript, as here we talk about 'carbon stabilization potential', and net rhizodeposition and root biomass (which we study) give an indication about the potential to stabilize carbon on the longer term.

Line 85: To my mind, this sentence is unnecessary, because the rationale of the study should be clear from the text above. I suggest starting directly with your research questions and marking them as such.

Thanks for this suggestion. We removed these sentences and explicitly formulated the research question and the hypothesis.

Line 143: Please indicate the approximate time of day when the labelling was carried out.

The labelling was carried out at 2 pm, this has now been added to the text.

Line 146: Was it always the same chamber/cultivar for monitoring CO2 concentrations?

The monitoring was always done at the same chamber and thus cultivar. The monitoring intended to approximate general CO2 uptake within for instance changing chamber volumes and less to adjust for each individual cultivar/chamber. We agree with the reviewer that this is not ideal but we had to consider technical implementations as well as time issues. Therefore, it was decided to only monitor at one chamber. However, given the relatively similar enrichment across all cultivars in aboveground plant biomass we believe that the labeling was done relatively homogeneous. We added to the text that CO_2 concentrations were always measured in the same chamber: 'Throughout the experiment, CO2 concentrations were measured in the same chamber.'.

Line 149: Is there an estimate for the CO2 concentration at the end of the two hours?

No, the CO_2 concentration in the chambers was not measured after these two hours.

Line 158: What does "limited amount of samples" mean – only at the end of the experiment (i.e. data shown in Fig. 3)?

Yes, that is what we meant. We clarified this in the text: 'In addition, the $\delta^{13}C$ value of CO_2 was measured for CO_2 samples collected along the depth profiles on the last sampling date, using a Gasbench II […]'

Line 175: From my own experience, it is better to analyse soil microbial biomass directly from fresh (unfrozen) soil, because the freezing can increase the amount of carbon found in the non-fumigated fraction (probably cell lysis). However, regarding the delta13C values in comparison to SOC, this does not seem to be a problem here.

We are aware of the fact that this would indeed be a better practice. However, due to technical constraints we had to perform the measures on frozen soil samples.

Line 211: Did you use the same value of -28 ‰ for aboveground biomass?

This value was also used for the initial aboveground biomass. We are aware of the fact the roots are often enriched in ^{13}C compared to aboveground biomass by ca. 1 – 2 ‰. However, due to the substantial isotopic enrichment of both roots and aboveground biomass, we argue that it is reasonable to assume this isotopic value for all plant parts. We now added to line 208 that this value was also used for aboveground vegetation.

Lines 217-218: This sentence is unclear. With "some of the input variables", do you mean biomass or delta13C?

This part has now been removed from the manuscript, since we limited the calculations of excess ^{13}C to the top 0.45 m of the soil, so we do not have to deal with missing data for these variables anymore.

Line 225: Please explain why you used the Janzen and Bruisma's equation in addition to excess 13C. If I understand it correctly, Fig. 4A shows the summed values for all soil layers as excess 13C according to Eq. 3 and Fig. 4C shows the data for individual soil layers as rhizodeposition C according to Eq. 4. However, the unit in Fig. 4C (g m-2) rather points to excess 13C. This must be clarified.

That is correct: Eq. 3 was used to calculate the mass of recovered ^{13}C label (g ^{13}C m^{-2}), while Eq. 4 was used to calculate the total amount of net carbon rhizodeposition, using the excess ^{13}C in roots and the soil (g C m^{-2}). To make this more clear in Figure 4, the unit of the label of Fig 4a has been changed to (g ^{13}C m^{-2}), while the unit in the label if Fig 4c has been changed to (g C m^{-2}).

Line 284: Did you find a significant effect for the three blocks? Why did you use the blocks as fixed effects and not as random effects, i.e. error term, in the ANOVA? Please also report the significance levels for the different statistical tests. In general, I would prefer using the Tukey-HSD test, because it also accounts for multiple comparisons (in particular when depth is added as additional factor).

For some of the variables, we did find a significant effect of the blocks (e.g. aboveground biomass), while for other variables this was not the case (e.g. belowground biomass). For the analysis of statistical differences between properties of the cultivars (e.g. aboveground biomass), we used a two-way anova without interactions. This is generally recommended for the analysis of randomized complete block designs (e.g. Dean et al. (eds.), Handbook of design and analysis of experiments, ISBN 978-1-4665-0434-9, or https://stat.ethz.ch/~meier/teaching/anova/block-designs.html). Therefore, block was not treated as a random effect. The significance level for the Tukey's test is now added to this section: '[…] using a significance level of 0.05'.

Lines 296-300: The aboveground biomass values are repeatedly reported in the text, Fig. 1A and Table 1/Table S1. It is sufficient to show the results once, especially since all individual values are available in the supplementary excel file. Remove this redundancy.

Thanks for this suggestion. As stated above, we removed the values for aboveground biomass throughout the test. However, we prefer to show the values for aboveground biomass in Table 1, to give the reader a complete overview of the values of both above- and belowground biomass. We are aware of the fact that these values are shown in Figure 1, but we want the reader to be able to consult the exact values without having to go look for the online supplement.

Line 325: Interpretations/conclusions do not belong to the results section. Delete this sentence.

This sentence has been deleted.

Line 341: Note that the soil microbial biomass was higher in Zinal (Fig. S3), so that excess 13C was probably similar to Mont-Calme 268 (Fig. 4C).

Thanks a lot for this remark, we now included after that sentence: 'However, as the microbial biomass under Zinal was substantially higher compared to under Mont-Calme 268 in this layer, this does not necessarily imply that microbes under Mont-Calme 268 incorporated more excess ^{13}C compared to under Zinal'.

Line 357: Do you mean "statistically significant" with "substantially"? Unfortunately, no statistical information is provided in Fig. 4.

Thanks for pointing this out. After statistics had been applied, we found there were significant differences. 'Varied substantially' has therefore been changed to 'differed significantly'

Lines 364-367: Please improve the sentence structure.

We changed these sentences to: 'The total amount of net carbon rhizodeposition measured at the end of the experiment down to 0.45 m decreased with depth for all wheat cultivars (Figure 4C). The

highest values were observed for Probus (108 ± 34 g C m^{-2}), followed by CH Claro (97 ± 24 g C m^{-2}), Mont-Calme (83 ± 29 g C m^{-2}) and Zinal (62 ± 11 g C m^{-2}). There was thus no clear relationship between the amount of net carbon rhizodeposition and year of release of the wheat cultivars.'

Line 372: Do you have any explanation for the abrupt increase of CO2 concentrations?

We think this was caused by roots growing down to these depths at this moment, although we do not have conclusive evidence for this. For this reason, we do not elaborate on this in the manuscript.

Line 399: How are your results (no differences in root biomass between cultivars) in line the study of Friedli et al. (2019), showing substantially (statistically significant?) higher root biomass in older cultivars than in more recent ones? That is contradictory!

As stated in line 395 – 396, we did find differences in root biomass between old (161 & 205 g m^{-2}) and recent (107 & 97 g m^{-2}) wheat cultivars, although these were not statistically significantly different (due to large variations within cultivars). Friedli et al. (2019) found that cultivars from the Swiss wheat breeding program showed decreasing root biomass with increasing year of cultivar development. Therefore, we state that our results are 'in line' with the results from Friedli et al. However, we have emphasized that are results are not statistically different (L 398-399).

Line 406: To which species does the root:shoot ratio of 0.14 belongs to, is it an average value?

This indeed is the average value for all the cultivars studied by Friedli et al.. This has been clarified in the text: '[…] including the cultivars used in our study (an average value of 0.14 for all cultivars studied by Friedli et al. (2019)).'

Lines 429-444: This paragraph reads like an introduction passage. It is better to move it to delete it from the discussion and combine it with overlapping parts of the introduction.

This comment was also raised by the other reviewer and we agree that this paragraph is redundant here. Therefore, we deleted this paragraph to reduce the length of the discussion, as part of this is covered in the introduction.

Lines 459-471: The repetition of results should be avoided and the two paragraphs streamlined to 2-3 short sentences.

Thanks for this suggestion, these 2 paragraphs have been shortened. We chose to retain the values we provide about the total amount of carbon that is allocated belowground, as this is not reported elsewhere in the manuscript, so we can compare them to literature values.

Line 492: By "assess the effect of wheat cultivars from a century of wheat breeding", do you mean that you assessed the effect of four cultivars representative for changes during a century of wheat breeding?

Yes, that is indeed what we meant. As we now re-stated the aim of our study at the beginning of the discussions (see above), we removed this sentence here, as it is redundant.

Line 494: There is no statistical support for this statement, neither for root biomass nor for belowground carbon allocation. In consequence, it is not surprising that you did not find effects on the SOC pool according to the next sentence.

After having performed the statistical analyses, we added that '[…], although the differences were not statistically significant.'

Lines 506-509: This cannot be generalized, because the activity and substrate preference of microbial communities depends on a variety of factors (e.g. Delgado-Baquerizo et al., 2016: https://doi.org/10.1111/1462-2920.13642). In addition, the preference for recent plant-derived substrates or more stable SOM varies with soil depth (Kramer & Gleixner, 2008: https://doi.org/10.1016/j.soilbio.2007.09.016) and the presence/quality of plant residues is known to alter soil microbial communities (e.g. Bai et al, 2016: http://dx.doi.org/10.1016/j.apsoil.2015.09.009). In this sense, the microbial community can be shifted to more fungi and Gram-positive bacteria in the presence of more complex organic compounds derived from root residues.

We agree with the reviewer that the fate of roots in the subsoil (mineralisation versus stabilization) is more complex than as we stated in the manuscript. Therefore, we shortened this section, as this is not the focus of our study, while briefly also incorporating the remarks raised by the reviewer: 'However, it is not straightforward to make predictions about the amount of root biomass that will be stabilized in the soil in the long term, as this depends on the efficiency with which plant-derived biomass is incorporated in microbial biomass (Cotrufo et al., 2013) and interactions between soil depth, the microbial community composition and its substrate preference (e.g. Kramer and Gleixner, 2008), among other factors.'.

Line 519: There is no statistically significant difference, only a slight trend.

We changed this sentence to: 'In contrast, despite the lack of statistical evidence, we observed differences […]'.

Technical Corrections

Lines 47-49: Please reformulate the two passages with "is also proposed". This is very repetitive, since the term "has been proposed" is already present in Lines 45-46.

Thanks for noticing, we removed two of the three 'is proposed' by an alternative wording.

Line 146: Obviously, this should be 40 g and not 40 mg.

I assume you meant line 176? Here, is should indeed be 40 g, thanks for noticing this.

Line 179: This sentence fits better in the previous subsection at line 172.

This sentence is at this location because the determination of the gravimetric moisture content was necessary to calculate microbial biomass carbon per unit dry soil. To make this clear to the reader, this sentence was changed to: 'To determine microbial biomass carbon per unit of dry soil, the gravimetric soil water content was determined by drying about 10 g of each soil sample at 105 °C and subtracting the weights before and after drying.'. We note that we also determined the gravimetric soil moisture content for bulk soil samples collected from the lysimeters at the end of the experiment. This is mentioned in line 171.

Line 193: Technically, you measured the delta 13C of C-F and C-NF instead of microbial biomass.

Thanks for pointing this out, we changed this in the manuscript: '[…] fumigated and non-fumigated soil (for the determination of microbial biomass C and $\delta^{13}C$) […]'.

Lines 249-250: Separate the "s" and "i-1,i"/"i,i+1" in the formulas (maybe by a semicolon), as it can be confusing otherwise.

Thanks for this suggestion, we changed this accordingly.

Line 350: Replace "showed substantial variation" with "varied".

This has been changed

Line 381: Include "biomass" (Plant biomass, carbon dynamics…).

This has been changed

Line 416 "were respiring CO2 down to greater depths…" -> Reformulate.

This was reformulated to '[…] roots of the old wheat cultivars respired CO_2 at greater depths compared to […]'.

Line 453: The shown references do not include only the same studies.

Thanks for pointing this out. We now shortened and combined both sentences, without mentioning the 'same studies'.

Line 485: Twice "assess/ed"

Thanks, this has been replaced

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. The line numbers in our replies refer to the line numbers in the revised manuscript.

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. 112 - 113), 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 have now performed statistical analyses for the data presented in Table 2 and Figure 4, and removed the sentences you refer to in the revised version of the manuscript.

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. 299 – 300 ('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 ^{13}C 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 are 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. We have also added results from the statistical analyses to this table.

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

The soil organic carbon stabilization potential of old and new wheat cultivars: a $^{13}CO_2$ labelling study

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Abstract. Over the past decades, average global wheat yields have increased by about 250 %, mainly due to the cultivation of high-yielding wheat cultivars. This selection process not only affected aboveground parts of plants, but in some cases also reduced ~~the~~ root biomass, with potentially large consequences for the amount of organic carbon (OC) transferred to the soil. To study the effect of wheat breeding for high-yielding cultivars on subsoil OC dynamics, two old and two new wheat cultivars from the Swiss wheat breeding program were grown for one growing season in 1.5 m-deep lysimeters and pulse-labelled with $^{13}CO_2$, to quantify the amount of assimilated carbon that was transferred belowground and can potentially be stabilized in the soil. The results show that although the old wheat cultivars with higher root biomass transferred more assimilated carbon belowground compared to more recent cultivars, no significant differences in net ~~soil organic carbon (SOC) stabilization~~ rhizodeposition were found between the different cultivars. As a consequence, the long-term effect of wheat cultivar selection on SOC stocks will depend on the amount of root biomass that is stabilized in the soil. Our results suggest that the process of wheat selection for high-yielding cultivars resulted in lower amounts of belowground carbon translocation, with potentially important effects on SOC stocks. Further research is necessary to quantify the long-term importance of this effect.

1 Introduction

Soil management has a large influence on the size of the soil organic carbon (SOC) stock in managed arable soils. This is evident from the large decrease in SOC that is generally observed after soils under natural vegetation are converted to arable

land (Don et al., 2011; Guo and Gifford, 2002; Poeplau et al., 2011). As a consequence, the mineralization of SOC and the loss of forest caused by land use change has contributed about 30 % to the increase in atmospheric CO_2 concentration since the onset of the industrial revolution (Le Quéré et al., 2018). Current contributions of the agricultural sector to global warming have been estimated to be about 11 %, but are mostly in the form of N_2O and CH_4 and not anymore as CO_2 (Tubiello et al., 2015).

The rising awareness that there is potentially an opportunity to increase subsoil organic carbon (OC) stocks (Chen et al., 2018) has led to the proposal that agricultural soils can be a sink of atmospheric CO_2 by applying appropriate climate-smart agricultural practices (Chenu et al., 2018; Minasny et al., 2017; Paustian et al., 2016). Multiple management practices have been shown to increase the OC content of cultivated soils, including the application of organic amendments to soils (Sandén et al., 2018), increasing the amount of crop residues returned to the field (Lehtinen et al., 2014) and planting of cover crops (Kong and Six, 2010; Poeplau and Don, 2015). ~~These mechanisms have been studied intensively over the past decades, with multiple reviews suggesting that these management practices have the potential to increase the SOC content of arable soils (Paustian et al., 1997, 2016; Sandén et al., 2018)~~.

In addition, growing crops with deeper roots and/or higher root biomass has been ~~proposed~~ put forward as a strategy to increase OC sequestration in arable soils (Kell, 2011), while ~~.~~

~~D~~deep rooting ~~is also proposed by breeders to~~can also decrease the effect of drought in climates where deep soil water is available during the main cropping season (Wasson et al., 2012). ~~Increased rooting depth is also proposed as a strategy to mitigate the effects of climate change in temperate climates as exemplified for the Swiss plateau (Friedli et al., 2019). Yet, for improving yield and resource uptake, the proposed root ideotype is steep, cheap and deep (Lynch, 2013) with less biomass and branching in the upper part of the soil (Wasson et al., 2014).~~ However, a direct or marker-assisted selection for root traits is very rare in conventional breeding programs. Accordingly, we have very limited knowledge if and how breeders alter the root system and potentially affect belowground carbon cycling. One way to evaluate the effect of breeder's selection on root characteristics and subsoil carbon cycling is to compare old and new varieties of the same breeding programme. For the Swiss wheat breeding programmes, the selection process reduced the mass and depth of roots under well-watered conditions (Friedli et al., 2019), as has been found for other breeding programs (Aziz et al., 2017), but modern genotypes enhanced root allocation to deep soil layers under drought. However, this pattern ~~(Friedli et al., 2019). The negative trend of rooting depth was also present in other breeding programmes (Aziz et al., 2017), but~~ has not been observed consistently (Cholick et al., 1977; Feil, 1992; Lupton et al., 1974). ~~While our understanding of the indirect effects of breeding on root morphology and architecture is limited, t~~To the best of our knowledge, there is no information about the effect of breeding on changes in subsoil OC dynamics and root respiration.

One reason for the lack of quantitative data about the effects of rooting depth on SOC sequestration is related to difficulties in measuring the amount of carbon transferred from roots to the soil (gross rhizodeposition) and the proportion of carbon that is eventually stabilized there (net rhizodeposition), after a portion of gross rhizodeposits are lost from the soil through microbial mineralization or leaching. The fact that rhizodeposition occurs below the soil surface greatly prevents direct observations of

this '*hidden half of the hidden half'* of the SOC cycle (Pausch and Kuzyakov, 2018). First of all, direct measurements of root exudation rates are hampered by the fact that rhizodeposits are used by rhizosphere microorganisms within a couple of hours after they are released, resulting in very low concentrations of root carbon exudates in the soil (Kuzyakov, 2006). Second, the release of carbon exudates by agricultural crops is not equally divided throughout the growing season, but mainly occurs in the first 1 – 2 months of the growing period and decreases sharply thereafter (Gregory and Atwell, 1991; Keith et al., 1986; Kuzyakov and Domanski, 2000; Pausch and Kuzyakov, 2018; Swinnen et al., 1994). Third, measurements of the effects of rhizodeposits on changes in SOC stocks are further complicated by the priming effect, i.e. their positive effect on the mineralization of native SOC (Fontaine et al., 2007; de Graaff et al., 2009).

To overcome these difficulties, rates of C rhizodeposition can be measured by labelling ~~the~~ plants with $^{13}CO_2$ or $^{14}CO_2$ (Jones et al., 2009; Kuzyakov and Domanski, 2000) and subsequently tracing the amount of photosynthetically assimilated ^{13}C or ^{14}C label in the soil at the end of the growing season (Kong and Six, 2010, 2012). ~~Practical limitations, however, complicate the~~ However, the continuous application of $^{13}CO_2$ or $^{14}CO_2$ 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. Therefore, plants are commonly labelled at fixed time intervals during the growing season (repeated pulse-labelling). This results in reliable estimates of the partitioning of assimilated carbon to different plant compartments, as well as into the soil (Kong and Six, 2010; Kuzyakov and Domanski, 2000; Sun et al., 2018).

In addition, assessing the magnitude of the carbon transfer from roots to the soil is not straightforward, particularly under field conditions. While carbon inputs from crops to the soil are often derived from yield measurements (Keel et al., 2017; Kong et al., 2005; Taghizadeh-Toosi et al., 2016), these quantities are often poorly related to root biomass or the magnitude of root exudates (Hirte et al., 2018; Hu et al., 2018). A better understanding of the factors controlling the rates of carbon rhizodeposition by different agricultural crops is thus necessary to assess how different crops affect SOC cycling and to provide SOC models with reliable rates of carbon inputs to the soil.

~~The lack of an unambiguous relation between wheat yield and root biomass on the one hand, and sufficient knowledge to reliably convert root biomass to rates of subsoil OC sequestration on the other hand underlines the need for additional research on both issues. This is needed to assess (i) differences in the amount of subsoil OC stabilized by different wheat cultivars and (ii) if breeding for high-yielding wheat cultivars with a lower root biomass has resulted in lower amounts of SOC sequestration. Therefore, t~~The present study ~~used four different bread wheat cultivars from a century of Swiss wheat breeding (Fossati and Brabant, 2003; Friedli et al., 2019)~~ addresses the following research question: 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? To address this question, four different bread wheat cultivars from a century of Swiss wheat breeding (Fossati and Brabant, 2003; Friedli et al., 2019) were grown in large mesocosms, which allowed to study the plant-soil system under controlled conditions that closely resemble a field situation. We hypothesized that wheat cultivars with shallow roots and lower root biomass would result in less net carbon rhizodeposition over the course of a growing season, compared to cultivars with deeper roots and higher root biomass. ~~to test the hypothesis that wheat cultivars with shallow rooting systems and lower root biomass~~

~~result in less subsoil OC stabilization over the course of a growing season. The experiments were carried out in large mesocosms, which allowed to study the plant-soil system under controlled conditions that closely resemble a field situation.~~

2. Materials and Methods

2.1 Experimental set-up

2.1.1 ETH mesocosm platform

To assess the effect of wheat root characteristics on ~~subsoil OC stabilization~~net rhizodeposition in a realistic soil environment under controlled ~~environmental~~ conditions, an experiment was set up at the mesocosm platform of the Sustainable Agroecosystems Group at the Research Station for Plant Sciences Lindau (ETH Zürich, Switzerland). The platform was located inside a greenhouse and consisted of 12 cylindrical lysimeters with a diameter of 0.5 m and a height of 1.5 m, constructed using 10 mm wide polyethylene (Figure S1). The lysimeters were equipped with probes installed at five different depths (0.075, 0.30, 0.60, 0.90 and 1.20 m below the surface) to measure the volumetric moisture content at a temporal resolution of 30 min (ECH_2O EC-5, Decagon Devices, US) and to sample soil pore water (Prenart, Frederiksberg, Denmark) and soil pore air (Membrana, Wuppertal, Germany). The lysimeters were filled with mechanically homogenized soil, collected from an agricultural field in Estavayer-Le-Lac, Switzerland. The upper 0.15 m of the lysimeters were filled with topsoil, collected from the A-horizon, while the remainder (0.15 – 1.35 m depth) was filled with subsoil. The bottom 0.15 m of the lysimeters (1.35 – 1.50 m depth) consisted of a layer of gravel (Blähton, Erik Schweizer, Switzerland), to facilitate drainage of soil water through the bottom of the lysimeters. The top and subsoil had a sandy clay loam texture with 21 % silt, 21 % clay, 58 % sand, and top- and subsoil pH values were 7.8 and 7.5, respectively. The OC concentration of the top- and subsoil was 0.77 ± 0.01 % and 0.40 ± 0.01 %, respectively, with a C:N ratio of 6.9 and 5.0, respectively. No carbonates were detected in the soil.

At the top of each lysimeter, pneumatically activated chambers were placed, that were automatically closed when applying the $^{13}CO_2$ label (see section 2.1.3). These chambers were made of stainless steel with fitted Plexiglas panes and covered a rectangular area of 0.5 x 0.5 m with an initial height of 0.1 m. Chamber heights were extended with increasing plant height, using one or two height extensions of 0.5 m each (Figure S1).

2.1.2 Wheat cultivars and growth conditions

Four wheat (*Triticum aestivum* L.) cultivars from the Swiss wheat breeding program (Fossati and Brabant, 2003; Friedli et al., 2019) with different breeding ages were selected: Mont-Calme 268 (introduced in 1926), Probus (1948), Zinal (2003) and CH Claro (2007). Generally, more recent cultivars of this program on average have more shallow roots and lower root biomass under well-watered conditions compared to the older cultivars (Friedli et al., 2019). CH Claro was selected as a modern variety with relatively deep rooting.

Before the wheat plants were transplanted to the lysimeters, wheat seeds were germinated in a greenhouse for 2 – 3 days on perforated anti-algae foil laid over 2-mm moistened fleece at a warm temperature (20 °C during day and 18 °C during night) and good light conditions. Next, the seedlings were planted in ~~topsoil filled~~ containers filled with the same topsoil used to fill the lysimeters and transferred to a climate chamber for vernalization for 52 days (Baloch et al., 2003). First, the seedlings were kept 45 days at 4 °C, with 8 hours of light per day and a light intensity of 10 kilolux. During the 3 subsequent days, daylight intensity was increased to 36 kilolux, daytime temperature was increased to 12 °C and night temperature to 10 °C. During the last 4 days, daytime temperature was increased to 16 °C, and night temperature to 12 °C. The relative humidity was maintained at 60 ± 10 % during the entire vernalization period. After vernalization, 70 seedlings were transplanted to every lysimeter, corresponding to a plant density of 387 plants m^{-2}. At the timing of transplanting, the plants were at the onset of tillering.

The experimental set-up consisted of a randomized complete block design. Each of the four wheat cultivars was planted in three lysimeters, i.e. 3 replicates per cultivar, resulting in a total of 12 lysimeters. These were placed in 3 blocks of 4 rows, where each wheat cultivar was planted in one lysimeter in each block. The plants were grown in the greenhouse for about 5 months, between 24 August 2015 and 1 February 2016. Despite uneven maturing of plants within and between the lysimeters, all plants had reached flowering stage at the time of harvest. Fertilizer was applied to the soil lysimeters a first time on 5 October 2015, at a rate of 84 kg N ha^{-1}, 36 kg P_2O_5 ha^{-1}, 48 kg K_2O ha^{-1} and 9 kg Mg ha^{-1}, and a second time on 4 December 2015, at a rate of 56 kg N ha^{-1}, 24 kg P_2O_5 ha^{-1}, 32 kg K_2O ha^{-1} and 6 kg Mg ha^{-1}. The lysimeters were watered manually twice per week with a similar amount of water, to keep soil moisture close to field capacity. Differences in the amount of water used by the different cultivars resulted in differences in the soil water content between the cultivars (Figure S2). The temperature in the greenhouse was set to 20 °C during the day and 15 °C during the night. During the experiment, the average temperature in the greenhouse was 16.9 °C, with a minimum and maximum of 9.3 °C and 29.8 °C respectively. The average humidity was~~of~~ 63.7 %, with a minimum and maximum of 35.3 % and 86.4% respectively.

2.1.3 Repeated ^{13}C pulse-labelling

In order to study carbon allocation within the atmosphere-plant-soil system, a ^{13}C pulse-labelling approach was used. 99% $^{13}CO_2$ (Euriso-top, Saint-Aubin, France) was applied once per week (2 pm on Thursdays) by injecting 15, 56 or 98 mL CO_2 into each chamber depending on the chamber extension used, in order to yield a target $^{13}CO_2$ content of 58 atom%. A weekly labelling frequency has been shown to ensure a sufficient abundance of root-derived ^{13}C in the soil at the end of the experiment (Bromand et al., 2001; Kong and Six, 2010). After chamber closure, CO_2 concentration in one chamber was monitored using a CO_2 analyzer (Li-820, LICOR, Lincoln, US). Throughout the experiment, CO_2 concentrations were measured in the same chamber. After the CO_2 concentration dropped below 200 ppm, another $^{13}CO_2$ pulse was injected to yield a post-label CO_2 concentration of 570 ppm in the chamber headspace. The chamber lids were kept closed for two hours after label injection to achieve sufficient uptake and then re-opened to avoid condensation. On the same day of pulse-labelling, all chambers were closed overnight to recuperate ^{13}C lost through night respiration and allowed to be taken up by the plants in the morning before reopening the chambers.

2.2 Measurements

2.2.1 Belowground CO_2 concentration and $\delta^{13}CO_2$

Soil gas sampling was performed once per week (Wednesdays) by attaching a pre-evacuated 110 mL crimp serum vial to a sampling port at each depth, leaving it equilibrating overnight. For each sample, a 20 mL subsample was transferred to a pre-evacuated Labco exetainer (12 mL), and used to determine the CO_2 concentration ~~and its carbon isotopic composition ($\delta^{13}C$)~~. The CO_2 concentration of each sample was determined using a gas chromatograph equipped with a thermal conductivity detector (Bruker 456-GC, Germany). ~~For a limited amount of samples~~In addition, the $\delta^{13}C$ value of CO_2 was measured for CO_2 samples collected along the depth profiles on the last sampling date, using ~~with~~ a Gasbench II modified as described by Zeeman et al. (2008) coupled to a Delta^{plus}XP isotope ratio mass spectrometer (IRMS, ThermoFisher, Germany). The standard deviation of the measurements was < 0.15 ‰.

2.2.2 Sampling and general soil analyses

At the end of the experiment, the aboveground biomass of the wheat plants was harvested separately for each lysimeter and separated into leaves, ears and stems. Soil from the lysimeters was collected by destructive sampling to analyze bulk density, root biomass and other soil properties. The sampling was done layer by layer. After a soil layer had been sampled, it was removed completely from the lysimeter and the next layer was sampled. From each depth increment (0 – 0.15, 0.15 – 0.45, 0.45 – 0.75, 0.75 – 1.05, 1.05 – 1.35 m depth), five soil cores were collected per lysimeter using a soil core sampler (5.08 cm diameter, Giddings Machine Company Inc., Windsor, CO, US). Three of the five cores per lysimeter and depth increment were used for the determination of root biomass based on a combination of buoyancy and sieving through a 530 µm sieve, using a custom-built root washing station. The remaining two soil cores were sieved at 8 mm, air-dried and stored for further analysis. Prior to air drying, the fresh weight and volume for each core was determined, and a subsample was taken for the determination of gravimetric soil moisture content. Bulk density was calculated based on fresh weight, gravimetric moisture content, and core volume. Soil texture was measured using a particle size analyzer (LS 13 320, Beckman Coulter, Indianapolis, USA). Prior to analysis 0.1g of soil was shaken for 4 h with 4 ml of 10 % Na-hexametaphosphate and sonicated for 1 min.

2.2.3 Soil microbial biomass

Soil microbial biomass was extracted from soil samples that had been frozen at -20 °C for 6 months immediately after sampling. Two subsamples of 40 ~~m~~g were taken from each sieved soil sample. One set was fumigated for 24 hours using chloroform. Next, total dissolved OC was extracted from each fumigated and non-fumigated subsample by shaking it in 200 mL 0.05 M K_2SO_4 for one hour, prior to filtering through a Whatman 42 filter paper. Total OC concentrations in K_2SO_4 extracts were determined using a CN analyzer (multi N/C 2100 S analyser, Analytik Jena, Germany). To determine microbial biomass carbon per unit of dry soil, ~~T~~the gravimetric soil water content was determined by drying about 10 g of each soil sample at 105 °C and

subtracting the weights before and after drying. The carbon content of the soil microbial biomass was calculated according to Vance et al. (1987) as:

$$TOC_{MB} = \frac{TOC_F - TOC_{NF}}{0.45} \qquad \text{(Eq. 1)}$$

Where TOC_F and TOC_{NF} are the total OC in fumigated and non-fumigated samples, respectively. The remainder of the filtered samples was freeze dried in order to analyze the $\delta^{13}C$ value. The $\delta^{13}C$ value of soil microbial biomass was calculated using mass balance according to Ruehr et al. (2009):

$$\delta^{13}C_{MB} = \frac{(\delta^{13}C_F \cdot C_F - \delta^{13}C_{NF} \cdot C_{NF})}{C_F - C_{NF}} \qquad \text{(Eq. 2)}$$

Where C_F and C_{NF} represent total carbon content of the fumigated and non-fumigated samples, respectively.

2.2.4 Organic carbon concentration and isotopic composition of plant material, soil organic carbon and microbes

The OC concentration and isotopic composition ($\delta^{13}C$) of above- and belowground plant material, fumigated and non-fumigated soil (for the determination of microbial biomass C and $\delta^{13}C$) and bulk soil were measured by weighing 2, 4, 80, 80 and 100 mg, respectively, of each sample into Sn capsules (9 x 5 mm, Saentis, CH) for analysis with a Flash EA 1112 Series elemental analyzer (ThermoFisher, Germany) coupled to a Delta^{plus} XP IRMS via a ConFlo III (Brooks et al., 2003; Werner et al., 1999; Werner and Brand, 2001). The measurement precision (SD) of the quality control standards (tyrosine Tyr-Z1, caffeine Caf-Z1), was 0.37 (‰) for above and belowground plant material, microbes and the soil samples.

2.3 Data processing

2.3.1 Excess ^{13}C calculations

The mass of ^{13}C label that was recovered in (i) the aboveground vegetation, (ii) roots of wheat plants and (iii) the soil was calculated following Studer et al. (2014):

$$m^E(^{13}C) = \frac{\chi^E(^{13}C) \cdot m(C) \cdot M(^{13}C)}{\chi(^{12}C) \cdot M(^{12}C) + \chi(^{13}C) \cdot M(^{13}C)} \qquad \text{(Eq. 3)}$$

Where $m^E(^{13}C)$ is the mass of recovered ^{13}C label (g m^{-2}), $\chi^E(^{13}C)$ is the excess atom fraction (unitless, calculated following Coplen (2011)), $m(C)$ is the total mass (g m^{-2}) of C, $M(^{12}C)$ and $M(^{13}C)$ are the molar weight of ^{12}C and ^{13}C (g mol^{-1}), respectively, and $\chi(^{12}C)$ and $\chi(^{13}C)$ are the ^{12}C and ^{13}C atom fraction (unitless), respectively.

To calculate the excess atom fraction ($\chi^E(^{13}C)$) of the soil compartment, the isotopic composition of the soil at the start of the experiment was used as the reference value (-26.45 ± 0.04 ‰ for the topsoil, -25.01 ± 0.13 ‰ for the subsoil). As all lysimeters were labelled with $^{13}CO_2$, no control treatment for the wheat plants was present. Therefore, a $\delta^{13}C$ reference value of -28 ‰ was assumed for the aboveground parts and roots of all wheat plants. The calculation of excess ^{13}C is very sensitive to variability in input parameter values, including the $\delta^{13}C$ value of ~~roots~~ plant biomass and soil. Therefore, a sensitivity analysis was used to show that varying the initial $\delta^{13}C$ value of the wheat plants with +/- 3 ‰, a typical range over which $\delta^{13}C$ values can vary in the field because of e.g. precipitation (Kohn, 2010), led to changes in calculated $m^E(^{13}C)$ in the order of +/- 1 % for aboveground biomass and +/- 1 – 5 % for belowground biomass. The effect of the initial $\delta^{13}C$ value of the biomass on the calculated amount of recovered ^{13}C label in the wheat plants was thus limited. Calculations of the effect of wheat cultivar on belowground excess ^{13}C were only performed for the upper 0.45 m of the lysimeters, as missing data for deeper soil layers prevented including these layers in the statistical analyses. ~~In addition, there was a large variability between the replicate lysimeters of the same cultivar for some of the input variables to calculate excess ^{13}C. Moreover, there were some missing data on the $\delta^{13}C$ value of root biomass for a limited number of depth intervals, due to low recovery of root biomass. Therefore, calculations of excess ^{13}C were performed for each wheat cultivar by combining the average values for the replicates, precluding the application of an analysis of variance to test treatment effects on excess ^{13}C.~~

2.3.2 Net c~~C~~arbon rhizodeposition

The absolute amount of carbon rhizodeposition for the different depth segments in the lysimeters was calculated following Janzen and Bruinsma (1989):

$$Rhizodeposition\ C = \frac{\chi^E(^{13}C)_{soil}}{\chi^E(^{13}C)_{root}} \cdot C_{soil} \qquad \text{(Eq. 4)}$$

Where rhizodeposition C is expressed in g kg^{-1} for the considered layer, $\chi^E(^{13}C)_{soil}$ and $\chi^E(^{13}C)_{root}$ are the excess ^{13}C atom fraction in the soil and roots respectively, calculated as described in section 2.3.1, and C_{soil} is the OC concentration of the considered soil layer (g kg^{-1}). This approach assumes that the isotopic enrichment of rhizodeposits and roots are equal. The absolute amount of carbon rhizodeposition for each soil layer was calculated by multiplying rhizodeposition C (g kg^{-1}) with the carbon content (kg) present in each of the respective layers. We note that data on the C concentration and $\delta^{13}C$ value of root biomass could not be obtained from a number of soil layer below 0.45 m depth for certain cultivars, due to the limited root biomass that could be retrieved. Therefore, net C rhizodeposition was only calculated for the two uppermost soil layers (0 – 0.45 m depth), as only for these layers all necessary data to calculate net C rhizodeposition was available for the three replicates of every cultivar.

2.3.3 Subsoil CO_2 production

Depth profiles of subsoil CO_2 production in the lysimeters were calculated using the weekly measured depth profiles of CO_2 concentration throughout the experiment. To assess the variability among the different lysimeters, these calculations were performed separately for every lysimeter and average CO_2 production depth profiles were calculated for each cultivar. Measurements of CO_2 concentration, soil temperature and soil moisture content were performed at discrete depths (0.075, 0.30, 0.60, 0.90 and 1.20 m depth). Continuous depth profiles of these variables at a vertical resolution of 0.05 m were obtained using linear interpolation. Depth profiles of CO_2 production were calculated using the discretized form of the mass balance equation of CO_2 in a diffusive one-dimensional medium, following Goffin et al. (2014):

$$P(z)_i = \frac{\Delta(\varepsilon_i[CO_2]_i)}{\Delta t} + \frac{F_{top_i} - F_{bot_i}}{\Delta z} \qquad \text{(Eq. 5)}$$

Where $P(z)$ is the CO_2 production in layer i (µmol CO_2 m^{-3} s^{-1}) over timespan Δt, t is the time (s), ε_i is the air-filled porosity in layer i (m^3 m^{-3}), $[CO_2]_i$ is the CO_2 concentration of layer i (µmol CO_2 m^{-3}), F_{top_i} and F_{bot_i} are the CO_2 fluxes transported through the upper and lower boundaries of layer i (µmol CO_2 m^{-2} s^{-1}) during timespan Δt, respectively, and z is the depth (m). The vertical CO_2 fluxes are calculated as (Goffin et al., 2014):

$$F_{top_i} = -\overline{D}_{s;i-1,i} \frac{[CO_2]_{i-1} - [CO_2]_i}{\Delta z} \qquad \text{(Eq. 6)}$$

$$F_{bot_i} = -\overline{D}_{s;i,i+1} \frac{[CO_2]_i - [CO_2]_{i+1}}{\Delta z} \qquad \text{(Eq. 7)}$$

Where $\overline{D}_{si,j}$ is the harmonic average of the effective diffusivity coefficient (D_s) between layers i and j, and Δz is the layer thickness. The effective diffusivity coefficient is calculated using a formula appropriate for repacked soils (Moldrup et al., 2000):

$$D_{s,i} = D_{0,t} \frac{\varepsilon_{i,t}^{2.5}}{\Phi_i} \qquad \text{(Eq. 8)}$$

Where D_0 is the gas diffusion coefficient of CO_2 in free air over timespan Δt (m^2 s^{-1}), ε_i is the air-filled porosity of layer i over timespan Δt (m^3 m^{-3}) and Φ_i is the total soil porosity of layer i (m^3 m^{-3}). The total soil porosity was calculated as $\Phi_i = 1 - \rho_i/\rho_p$ where ρ_i is the soil bulk density (ton m^{-3}) and ρ_p is the particle density (2.65 ton m^{-3}). Due to the large vertical variability in measured bulk density depth profiles, a constant bulk density profile was assumed for the subsoil (below 0.15 m depth), calculated as the average of the measured bulk density values for these layers. The air-filled porosity over timespan Δt was calculated as the difference between the total porosity (m^3 m^{-3}) and the average measured water-filled pore space over timespan

Δt (m^3 m^{-3}). The latter was measured throughout the experiment (see section 2.1.1) and corrected based on differences between these measurements at the end of the experiment and the measured volumetric water content of the sampled soil at the end of the experiment. For this purpose, different correction equations were used for (i) the upper soil layer (0 – 15 cm) and (ii) all deeper layers combined.

The gas diffusion coefficient in free air was corrected for the individual lysimeters for variations in temperature and soil moisture throughout the experiment (Massman, 1998), as:

$$D_0 = D_{0,stp}\,\frac{p_0}{p}\left(\frac{T}{T_0}\right)^{\alpha} \qquad \text{(Eq. 9)}$$

Where $D_{0,stp}$ is the gas diffusion coefficient for CO_2 in free air under standard temperature (0 °C) and pressure (1 atm) ($1.385 \cdot 10^{-5}$ m^2 s^{-1} (Massman, 1998)) and α is a coefficient (1.81; Massman, 1998)). Semi-continuous measurements of soil temperature in every lysimeter were used to calculate D_0 values throughout the experiment, while a constant atmospheric pressure of 1 atm throughout the experiment was assumed.

To obtain depth profiles of the total amount of CO_2 produced by the different wheat cultivars during the experiment (expressed as g CO_2 m^{-2}), the calculated CO_2 production rates between all measurement days ($P(z)$) were summed for the timespan of the experiment and converted to g CO_2 m^{-2} using the molecular mass of CO_2 (44.01 g mol^{-1}). We applied the boundary condition of the absence of a flux of CO_2 at the bottom of the lysimeters. It is noted that these calculations do not make a distinction between the source of CO_2 of production, thereby combining both autotrophic and heterotrophic CO_2 production (total soil respiration). For more information about these methods, reference is made to Goffin et al. (2014).

2.4 Statistics

To account for the three blocks in the randomized complete block design, statistically significant differences between aboveground characteristics of different cultivars ~~(summed for the different depth layers)~~ were checked using a two-way analysis of variance (anova) without interactions (Dean et al., 2015), followed by a Tukey's test, based on the values obtained for the individual replicates (n = 3 for every cultivar) using a significance level of 0.05. This was done after checking for homogeneity of variance (Levene's test) and normality (Shapiro-Wilk test) using a confidence level of 0.05. These analyses were performed in Matlab®. The effect of cultivar and depth on soil bulk density ~~and~~ , belowground biomass, belowground C allocation and net C rhizodeposition was assessed using a ~~three-way anova~~linear mixed-effects model, with cultivar and depth being fixed effects and blocks being treated as a random effect (*lmer* function in R (R Core Team~~,~~ (2019)). ~~In addition~~Next, a pair-wise comparison ~~of the results of the three-way anova were~~was used to check for statistically significant differences ~~in belowground biomass and bulk density~~ between the cultivars (*emmeans* package in R)~~, when accounting for the effect of depth~~. Belowground biomass was log-transformed to increase normality and homogeneity of variances for the latter analysis. ~~Statistical analyses were performed in Matlab®.~~ Uncertainties on reported variables are expressed as standard errors (n = 3).

3. Results

3.1 Aboveground biomass

The aboveground biomass produced at the end of the experiment was significantly different between Zinal ~~(710 ± 114 g m^{-2})~~ and Probus ~~(1154 ± 220 g m^{-2})~~, while the aboveground biomass of CH Claro ~~(1064 ± 207 g m^{-2})~~ and Mont-Calme 268 ~~(1119 ± 174 g m^{-2})~~ was not significantly different from any other cultivar (Figure 1, Table 1). The biomass of the ears was significantly higher for Zinal ~~(333 ± 68 g m^{-2})~~, compared to CH Claro ~~(92 ± 33 g m^{-2})~~, Probus ~~(21 ± 12 g m^{-2})~~ and Mont-Calme ~~(13 ± 8 g m^{-2})~~ (Figure 1, Table S1). 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. No significant differences were found between the $\delta^{13}C$ values of aboveground biomass of the different cultivars (Figure 2). The high $\delta^{13}C$ values of the aboveground biomass of all wheat cultivars (266 ‰ on average) showed that a substantial amount of the $^{13}CO_2$ tracer was incorporated by all wheat plants (Figure 2).

3.2 Belowground biomass

The average root biomass was highest in the topsoil and significantly lower in the subsoil layers of all four wheat cultivars (Figure 1B). ~~Significant differences between the root biomass of the different cultivars were analyzed (i) using total root biomass as the dependent variable (using a two-way anova without interactions, with cultivar and block as fixed effects) and (ii) using the root biomass per depth layer (using a three-way anova, with cultivar and depth as fixed effects, and block as random effect, after log transformation of the data). When using total root biomass summed for the different depths, the average total root biomass was not significantly different between the cultivars, but was on average largest for the older wheat cultivars (205 ± 67 g m^{-2} for Mont-Calme 268 and 161 ± 54 g m^{-2} for Probus) and lowest for the more recent cultivars (97 ± 20 g m^{-2} for Zinal and 107 ± 28 g m^{-2} for CH Claro) (Table 1). In contrast, when depth was included as an independent variable, the r~~Root biomass of Zinal was significantly lower compared to the root biomass of Probus and Mont-Calme 268, while the root biomass of CH Claro was not significantly different from any of the other cultivars (Figure1B). These differences were mostly present in the two uppermost soil layers, while root biomass was not significantly different between different cultivars at any depth, except for Zinal and Mont-Calme 268 between 0.45 – 0.75 m depth (Figure 1). The root:shoot ratio varied between 0.10 ± 0.02 and 0.19 ± 0.08, and was not significantly different between the different cultivars (Table 1).

The depth profiles of the $\delta^{13}C$ of root biomass were different between the old and more recent wheat cultivars (Figure 2). In the two uppermost soil layers, no significant differences were detected between the $\delta^{13}C$ values of root biomass of the different cultivars. These differences could not be checked for statistical~~ly significant~~ significance ~~differences~~ in deeper soil layers due to a lack of sufficient recovered root biomass in each ~~block~~lysimeter. The $\delta^{13}C$ values of the roots of the old wheat cultivars showed only limited variation with depth, with values between ca. 150 and 200 ‰. In contrast, the $\delta^{13}C$ values of the roots of the more recent wheat cultivars were highest in the two uppermost soil layers (0 – 45 cm) and showed an abrupt decrease with

depth in deeper soil layers. ~~Older wheat cultivars thus allocated more ^{13}C label to their roots compared to the more recent cultivars.~~

3.3 Soil and soil organic carbon characteristics

The SOC concentration in the lysimeters was similar to the OC concentration of the initial soil (Figure 3A). A direct comparison between the SOC concentration before and after the experiment could not be made, as no measurements of the OC concentration of the soil in the lysimeters before the start of the experiment could be made. However, the SOC concentration measured at the different depths in the lysimeters was similar to the OC concentration measured on the soil that was used to fill the lysimeters (Figure 3A). No statistically significant differences in SOC concentration were found between the different cultivars at any depth.

The SOC in the two uppermost soil layers (0 – 45 cm) of all wheat cultivars was enriched in ^{13}C compared to the soil that was used to fill the lysimeters (Figure 3B). Although the δ^{13}C value of SOC was not significantly different at any depth between any of the cultivars, the largest increase in the δ^{13}C value of topsoil OC was observed for Probus and Mont-Calme 268 (Figure 3B), indicating that the soil under the old cultivars incorporated more of the ^{13}C label, compared to the more recent cultivars. The limited difference between (i) the δ^{13}C values of the soil used to fill the lysimeters and (ii) the measurements at the end of the experiment below a depth of 0.45 m, indicates a lower amount of incorporated ^{13}C label in the subsoil. Similarly, the δ^{13}C value of topsoil microbial biomass was more positive compared to deeper soil layers for all cultivars, indicating that microbes utilized more substrate enriched in ^{13}C in the two uppermost soil layers, compared to deeper soil layers (Figure 3C). Statistically significant differences were only detected in the layer between 0.15 and 0.45 m depth, where the δ^{13}C value of microbial biomass under Zinal was significantly lower compared to Mont-Calme 268. However, as the microbial biomass under Zinal was substantially higher compared to under Mont-Calme 268 in this layer (Figure S3), this does not necessarily imply that microbes under Mont-Calme 268 incorporated more excess ^{13}C compared to under Zinal. Depth profiles of microbial biomass carbon were relatively constant (200 – 500 µg C g soil^{-1}) with no consistent differences between different cultivars (Figure S3). The δ^{13}C values of soil CO_2 ($\delta^{13}CO_2$) at the end of the experiment were similar for all wheat cultivars for the two uppermost layers (0 – 0.45 m) (Figure 3D). Deeper down the profile, the ~~δ^{13}~~CO_2 under the old wheat cultivars was more enriched in ^{13}C compared to the more recent cultivars, by an average of ca. 30 ‰. The only statistically significant differences were detected in the lowermost layer, where the δ^{13}C value of CO_2 of Zinal and CH Claro were significantly lower compared to Mont-Calme 268.

There was no significant effect of cultivar on the bulk density of the soil at the end of the experiment ($F_{3,59}$ = 1.9, p = 0.23), while there was a significant effect of depth on bulk density ($F_{4,59}$ = 19.4, p < 0.0005). The average bulk density of all lysimeters was highest in the topsoil (1.67 ± 0.12 Mg m^{-3}) and ~~showed substantial variation~~varied with depth (Figure S4A). The gravimetric moisture content in the lysimeters at the end of the experiment increased with depth for all cultivars, from ca 0.1 g g^{-1} in the top layer to ca. 0.15 g g^{-1} in the bottom layer (Figure S4B), and was only significantly different between Mont-

Calme 268 and Zinal in the uppermost soil layer. The soil moisture content changed relatively little throughout the experiment for all lysimeters, after an initial phase of decreasing soil moisture content at the onset of the experiment (Figure S2).

3.4 Excess ^{13}C and carbon rhizodeposition

The total amount of ^{13}C label that was present in the plant-soil system at the end of the experiment, expressed as excess ^{13}C, ~~varied~~ differed ~~substantially~~ significantly between different wheat cultivars (Figure 4A). When accounting for excess ^{13}C in aboveground biomass and in the soil and roots down to a depth of 0.45 m, ~~The~~ the lowest amount of ^{13}C label was found in the Zinal lysimeters (~~1.28~~1.19 ± 0.11 g m^{-2}), followed by CH Claro (~~1.64~~1.64 ± 0.06 g m^{-2}) and the older wheat cultivars (~~2.14~~2.05 ± 0.09 g m^{-2} for Mont-Calme 268 and ~~2.18~~2.01 ± 0.19 g m^{-2} for Probus), with the majority of ^{13}C tracer in the above-ground biomass (Figure 4A). Despite these differences, the relative distribution of the assimilated ^{13}C between aboveground biomass, roots and soil was similar between the different wheat cultivars (Figure 4B). On average, ~~79.8~~80.7 ± ~~9.4~~1.7 % of the assimilated tracer ended up in aboveground biomass, ~~6.6~~8.4 ± ~~1.2~~1.5 % in root biomass and ~~13.6~~10.9 ± ~~2.6~~1.4 % in the soil. It is noted that root-respired ^{13}C label is not included in this analysis, which may lead to an underestimation of the fraction of ^{13}C label that was allocated belowground.

The total amount of net carbon rhizodeposition ~~carbon~~ measured at the end of the experiment down to 0.45~~75~~ m decreased with depth for all wheat cultivars~~, with the exception of Zinal~~ (Figure 4C)~~,~~, with this difference only being statistically significant for Zinal. ~~with the~~ The highest ~~values~~amount of net carbon rhizodeposition was observed for Probus (~~126~~108 ± ~~57~~34 g C m^{-2}), followed by CH Claro (~~112~~97 ± ~~39~~24 g C m^{-2}), Mont-Calme (83 ± 29 g C m^{-2}) and Zinal (~~100~~62 ± ~~39~~11 g C m^{-2})~~ and Mont-Calme (85 ± 27 g m^{-2})~~. There was thus no clear relationship between the amount of net carbon rhizodeposition ~~C~~ and year of release of the wheat cultivars.

3.5 CO_2 concentration and production

Throughout the experiment, the change in the CO_2 concentration of the two uppermost soil layers was limited, with average values for the topsoil between 470 and 761 ppm for all cultivars (Figure 5). Deeper down the lysimeters, relatively constant CO_2 concentrations were observed during the first 3 weeks of the experiment, ca. 5.000 – 10.000 ppm. After 3 weeks, subsoil CO_2 concentrations abruptly increased and remained high throughout the experiment. These were substantially larger for the older cultivars (with maximum values of ca. 30.000 ppm) compared to the younger cultivars (with maximum values ca. 24.000 ppm).

Despite these high CO_2 concentrations in the subsoil, CO_2 production was mainly taking place in the topsoil, with the highest rates of CO_2 production between 0.10 and 0.20 m depth for all cultivars (Figure 6). For the young cultivars (Zinal and CH Claro), 95 % of CO_2 was produced above a depth of 0.3 m. In contrast, in older cultivars (Probus and Mont-Calme 268) 95 % of CO_2 was produced above a depth of 0.55 and 0.6 m~~ for Probus and Mont Calme 268~~, respectively. Despite these observations, neither the calculated total amount of subsoil CO_2 production or the depth above which 95 % of CO_2 was produced were significantly different between any of the cultivars.

4. Discussion

The aim of the present study was to assess differences in belowground carbon transfer and net rhizodeposition by wheat cultivars with different root biomass and rooting depth. Our results show that although there are marked differences in both the amount of carbon transferred belowground and the timing of belowground carbon transfer, there is no clear relationship between root characteristics and the amount of net rhizodeposition. Therefore, the fate of root biomass might determine the total amount of subsoil carbon stabilization in the long-term.

4.1 Plant biomass carbon dynamics and CO_2 production

No consistent differences in total aboveground biomass between old and new wheat cultivars were observed, ~~although the aboveground biomass of Zinal (710 ± 114 g m^{-2}) was substantially lower compared to the other wheat cultivars (on average 1112 ± 116 g m^{-2})~~. The ~~observed~~ aboveground biomass values were at the high end of reported values for wheat plants in the field (Mathew et al., 2017), while the lack of consistent differences in the biomass of wheat cultivars released over a time span of multiple decades has generally been observed (Brancourt-Hulmel et al., 2003; Feil, 1992; Lupton et al., 1974; Wacker et al., 2002).

The fraction of biomass in the grain-bearing ears was, however, much larger for the modern wheat cultivars (on average 9 and 47 % of total aboveground biomass for CH Claro and Zinal respectively) compared to the old wheat cultivars (on average 1 and 2 % for Mont-Calme 268 and Probus respectively). While an increase in the fraction of biomass allocated to grains is generally observed ~~when~~ in old ~~and~~ versus modern wheat cultivars ~~are compared~~ (Brancourt-Hulmel et al., 2003; Feil, 1992; Shearman et al., 2005), mostly as a consequence of the introduction of reduced height genes (Tester and Langridge, 2010), the harvest index reported here for the old cultivars might have been ~~slightly~~ underestimated because older cultivars where not yet fully mature at plant harvest.

The total root biomass of the older wheat cultivars was substantially larger compared to the more recent cultivars, although these differences were not consistently ~~statistically~~ significant between all modern and old varieties ~~due to the large variation among different replicates~~ (Table 1). These differences were mostly apparent in the top 0.45 m of the lysimeters (Figure 1). It is not clear if the lack of statistically significant differences in the root biomass within the deeper soil layers was due to (i) inability to collect all fine roots from the soil or (ii) actual differences in root ~~architecture~~biomass. These results are in line with a recent study on the biomass of roots of different wheat cultivars of the Swiss wheat breeding program, including the cultivars used in our experiment~~s~~ (Friedli et al., 2019). This study showed that under well-watered conditions, older wheat cultivars had a substantially higher root biomass compared to the more recently released wheat cultivars. Similar results have been obtained for wheat cultivars released in e.g. Australia (Aziz et al., 2017) and other countries around the world (Waines and Ehdaie, 2007). The root:shoot ratio of the wheat cultivars in our study (0.10 ± 0.2 – 0.19 ± 0.08, Table 1) were at the low end of reported values for wheat plants globally (Mathew et al., 2017), but in line with reported values for wheat cultivars of

the Swiss wheat breeding program, including the cultivars used in our study (an average value of 0.14 for all cultivars studied by, as measured by Friedli et al. (2019)).

The maximum rooting depth was similar between the old and recent wheat cultivars (Figure 1B). This is in contrast with the results from Friedli et al. (2019), who found that the older wheat cultivars had deeper roots (the depth above which 95 % of roots were found (D_{95}) was on average 101 cm) compared to the more recent cultivars included in the present study (average D_{95} of 85 cm). These differences might partly arise from the different set-up used in both studies. Both experiments were carried out in a controlled greenhouse environment, but Friedli et al. (2019) used soil columns with a diameter of 0.11 m, while in our study lysimeters with a diameter of 0.5 m were used. Additional information about subsoil root dynamics could be obtained from the measured depth profiles of the CO_2 concentration and $^{13}CO_2$, with the latter only being measured in the last phase of the experiment. The calculated depth profiles of CO_2 production showed that CO_2 was being produced down to greater depths under the old wheat cultivars (Figure 6). Combined with the higher $\delta^{13}C$ values of subsoil CO_2 of the lysimeters under the old wheat cultivars at the end of the experiment (Figure 3D), this suggests that the roots of the old wheat cultivars were respiring respired CO_2 down toat greater depths compared to the recent wheat cultivars. It is noted that subsoil CO_2 was not partitioned between CO_2 originating from (i) root respiration and the heterotrophic respiration of root derived OC by microorganisms and (ii) heterotrophic respiration of native SOC by microorganisms due to lack of data.

In addition, tThe $\delta^{13}C$ values of root biomass suggest that the temporal root carbon dynamics of the old and recent wheat cultivars differed substantially (Figure 2B). The root biomass of the old wheat cultivars had a high $\delta^{13}C$ value at all measured depths, indicating that the $^{13}CO_2$ label was allocated to the roots at all depths throughout the experiment. In contrast, the root biomass of the recent wheat cultivars was greatly enriched in ^{13}C in the top 0.45 m, while deeper roots were much less enriched in ^{13}C. 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). The similar $\delta^{13}C$ value of the aboveground biomass of all wheat cultivars (Figure 2A) suggests that the differences in $\delta^{13}C$ values of the root biomass are unlikely to be caused by differences in the relative amount of $^{13}CO_2$ assimilated by the plants, relative to unlabeled CO_2. Thus, these results corroborate the hypothesissuggest that old wheat cultivars allocate photosynthates down to their roots throughout a substantial part of the plant growth phase, while this is not the case for more recent cultivars.

4.2 Carbon allocation by wheat plants

Subsoils receive the largest portion of OC inputs through dead roots and rhizodeposition (Jones et al., 2009; Nguyen, 2003). To correctly simulate subsoil carbon dynamics, it is therefore important to reliably estimate the fraction of assimilated plant carbon that is transferred to roots and eventually released to the soil. However, estimations of the magnitude of these carbon inputs to the soil are prone to large uncertainties (Oburger and Jones, 2018). A first reason for this is a lack of knowledge on the root biomass of crops. Although the root:shoot ratio of most common crops is well known (e.g. Bolinder et al. (2007),

~~Mathew et al. (2017)), it has recently been shown that calculating root biomass based on aboveground plant biomass generally leads to erroneous results (Hirte et al., 2018; Hu et al., 2018; Taghizadeh-Toosi et al., 2016). Secondly, although the partitioning of belowground carbon inputs into root biomass, root respiration and rhizodeposition is relatively constant among different species (if the same growth period is considered (Kuzyakov and Domanski, 2000)), this knowledge is based on a limited number of available studies (e.g. 20 studies for crops in Pausch and Kuzyakov (2018)). In addition, qualitative and quantitative information about root exudates can be greatly influenced by the methodology used, adding to the uncertainty of available data (Oburger and Jones, 2018). Since uncertainties about the magnitude of carbon inputs to the soil have a great effect on simulations of SOC dynamics (Keel et al., 2017), there is a great need for additional data collection, both in lab environments as well as in the field.~~

The partitioning of the ^{13}C label was very similar between the different wheat cultivars (Figure 4B). It is noted that the amount of rhizosphere-respired $^{13}CO_2$ could not be included in these calculations, although this typically accounts for ca. 7 - 14 % of assimilated carbon in crops, or 40 % of total belowground C allocation (Kuzyakov and Domanski, 2000; Pausch and Kuzyakov, 2018). The fraction of assimilated carbon that is transferred belowground reported here is therefore underestimated. The belowground transfer of ca. 20 % of assimilated C for all cultivars is in line with previous studies, which have reported fractions of similar magnitude for wheat plants, when not accounting for rhizosphere CO_2 respiration: 18 – 25 % (Hirte et al., 2018), 18 % (as reviewed by Kuzyakov and Domanski (2000)), 15 % (Keith et al., 1986), 17 % (Gregory and Atwell, 1991) and 31 % (Sun et al., 2018). In contrast, reported values of the partitioning of belowground translocated carbon by wheat plants to (i) roots and/or (ii) net rhizodeposition are much more variable, with ~~. The same studies~~ reported net rhizodeposition carbon as a percentage of total belowground carbon (root carbon and net rhizodeposition carbon combined) for wheat plants ~~to be~~ between 23 % (as summarized by Kuzyakov and Domanski (2000)) and 72 % (Sun et al., 2018). The results obtained here (68 %) are thus at the high end of reported values. However, they were similar to results from a field study in Switzerland which used two modern Swiss wheat cultivars, among which CH Claro (58 %; Hirte et al. (2018)).

4.3 Rates of net carbon rhizodeposition

The total amount of carbon assimilated by the wheat cultivars that was transferred to roots and soil in the top 0.45~~75~~ m at the end of the experiment ranged between 97 ± 14 g m^{-2} (Zinal) and 164 ± 38 g m^{-2} (Probus) ~~was highest for the older wheat cultivars (159 ± 37 g m^{-2} for Mont-Calme 268, 184 ± 60 g m^{-2} for Probus) and lowest for the more recent cultivars (135 ± 40 g m^{-2} for Zinal and 147 ± 40 g m^{-2} for CH Claro)~~ (Table 2). ~~It is evident~~ It is noted that the total amount of belowground carbon translocation by the wheat plants ~~was higher than these values~~is underestimated, as rhizosphere respiration could not be included in our calculations. These numbers are in the range of reported values for wheat plants of 94 – 295 g m^{-2} (as summarized by Keith et al. (1986)) ~~, but higher than the~~and the value reported by Kuzyakov and Domanski (2000) (150 g m^{-2}), as well as the reported amount ~~by~~for two recent wheat cultivars of the Swiss wheat breeding program (including CH Claro) of 110 – 134 g m^{-2} (Hirte et al., 2018).

In contrast to the total amount of carbon translocated below ~~the soil surface~~ground, the amount of net carbon rhizodeposition was not consistently different between the old and more recent wheat cultivars (62 ± 11 – 108 ± 34 g m^{-2}). ~~For the top 0.75 m, this was 85 ± 27 g m^{-2} for Mont-Calme 268, 126 ± 57 g m^{-2} for Probus, 100 ± 39 g m^{-2} for Zinal and 112 ± 39 g m^{-2} for CH Claro~~ (Figure 4, Table 2). These values are higher compared to values calculated by Pausch and Kuzyakov (2018) (18 – 34 g m^{-2}, depth unknown) and Hirte et al. (2018) (63 – 73 g m^{-2}; down to 0.75 m depth).

A large uncertainty associated with calculated values of subsoil carbon sequestration using isotopic labelling approaches is related to the assumption that the isotopic enrichment of roots and rhizodeposits are similar (eq. 4). ~~Under this assumption, the $\delta^{13}C$ value of roots is used as a proxy for the $\delta^{13}C$ of the rhizodeposits.~~ This simplification is made because of the difficulties in measuring quantitative characteristics of rhizodeposits in a soil medium (Oburger and Jones, 2018), but leads to erroneous calculations of the amount of carbon rhizodeposition when this assumption is violated (Stevenel et al., 2019). ~~The latter is likely to be the case for two main reasons. First, since a large portion of rhizodeposits is derived from recently assimilated photosynthates (Ekblad and Högberg, 2001; Kuzyakov and Gavrichkova, 2010), the $\delta^{13}C$ values of rhizodeposits are likely to depend on the time after the last labelling pulse had been applied, with more ^{13}C-enriched rhizodeposits being produced shortly after labelling (Kuzyakov, 2006; Kuzyakov and Gavrichkova, 2010). Second, the fact that most carbon is allocated to belowground plant parts during the first 1 to 2 months after growth initiation (Pausch and Kuzyakov, 2018) likely causes an important temporal variation in the ^{13}C enrichment of roots. Recently, Stevenel et al. (2019) have shown that the $\delta^{13}C$ value of roots of isotopically labeled *Canavalia brasiliensis* differed both temporally and spatially, as different parts of the root had a different isotopic value at the end of their 22-day experiment.~~

To assess the uncertainty of calculated values of subsoil carbon sequestration, we ~~assessed~~ calculated how these values differ when the value of root $\delta^{13}C$ is varied with +/- 25 % (Figure S5). This results in calculated values of total carbon rhizodeposition, down to a depth of 0.~~75~~45 m, of ~~68~~69 – ~~101~~105 g m^{-2} for Mont-Calme 268, ~~103~~88 – ~~154~~138 g m^{-2} for Probus, ~~76~~51 – ~~106~~78 g m^{-2} for Zinal and ~~87~~81 – ~~124~~121 g m^{-2} for CH Claro, or uncertainties in the amount of carbon rhizodeposits between –~~14~~18 and + ~~28~~28 %. Further research on the effect of the assumption of using root $\delta^{13}C$ values as a proxy for carbon rhizodeposits is thus necessary to better quantify the effect on estimates of carbon sequestration.

4.4 The effect of old and recent wheat cultivars on ~~soil organic carbon stabilization~~net carbon rhizodeposition

~~The aim of this study was to assess the effect of wheat cultivars from a century of wheat breeding in Switzerland on belowground carbon allocation and SOC stabilization. We found~~Our results indicate that the old wheat cultivars, with deeper active roots throughout the experiment and larger root biomass, allocated more assimilated carbon belowground, although the differences were not statistically significant (Figure 4C, Table 2). However, we found no evidence that wheat cultivars with larger root biomass lead to ~~additional~~ higher ~~SOC stabilization~~net carbon rhizodeposition~~, as both the lowest (85 ± 27 g m^{-2} by Mont-Calme 268) and highest (126 ± 57 g m^{-2} by Probus) amounts of carbon stabilization in the soil were observed for the wheat cultivars with the highest root biomass~~ (Table 2). Our hypothesis, which stated that wheat cultivars with larger root

biomass and deeper roots would lead to larger amounts of net carbon rhizodeposition~~carbon stabilization~~, could therefore not be confirmed ~~in the short term~~.

The total amount of OC that will be stabilized in the soil by the studied wheat cultivars will therefore depend on the long-term fate of the root biomass ~~in the long term~~. The root biomass was higher for the old wheat cultivars, although these differences were mainly limited to the upper 0.45 m of the soil. Due to the destructive sampling of vegetation and soil at the end of the experiment, the fate of root biomass after harvest could not be assessed. Based on the results, one could therefore hypothesize that the higher root biomass of old wheat cultivars would lead to larger rates of carbon sequestration in the long-term. Similarly, Mathew et al. (2017) suggested that growing grasses and maize plants would lead to larger SOC stocks because these plants have the highest total and root biomass, compared to growing crops with a lower biomass. However, it is not straightforward to make predictions about the amount of root biomass that will be stabilized in the soil in the long term, as this depends on the efficiency with which plant-derived biomass is incorporated in microbial biomass (Cotrufo et al., 2013) and interactions between soil depth, the microbial community composition and its substrate preference (e.g. Kramer and Gleixner, 2008), among other factors. ~~However, higher carbon stocks in plant biomass do not necessarily lead to higher rates of SOC sequestration, as it has been suggested that easily decomposable organic compounds, such as root exudates, lead to higher rates or OC sequestration compared to more complex organic compounds, such as root biomass (Castellano et al., 2015; Cotrufo et al., 2013). High-quality carbon inputs, such as root exudates, are more easily incorporated into the microbial biomass, of which the necromass is an important precursor of stabilized SOC (Denef et al., 2010; Kallenbach et al., 2016; Kästner and Miltner, 2018; Kögel-Knabner, 2002). Therefore, the portion of root biomass that is eventually stabilized in soils through interactions with minerals (Hemingway et al., 2019; Kleber et al., 2015) or incorporated in soil aggregates (Six et al., 2000) will determine how much of this carbon is sequestered over the long term.~~

During the past century, there has been a continuing increase in the importance of wheat cultivars with smaller root biomass ~~The cultivars of wheat that are grown around the world have gone through considerable changes throughout the past century, with a continuing increase in the importance of wheat cultivars with smaller root biomass~~ (Fossati and Brabant, 2003; Friedli et al., 2019; Waines and Ehdaie, 2007). This can have profound implications for OC stocks of soils under wheat cultivation, as rhizodeposition and root-derived carbon are the most important inputs of OC to the soil (Kong and Six, 2010). ~~Our results show that over the course of one growing season, the amount of net rhizodeposition of the old cultivars (on average 105 ± 63 g m^{-2}) was similar to the recent cultivars (on average 106 ± 55 g m^{-2}). In contrast, we observed differences in the total amount of belowground carbon allocation to the roots and rhizodeposition combined between old (on average 171 ± 35 g m^{-2}) and recent (on average 141 ± 28 g m^{-2}) cultivars. This suggests that in the long-term, more recently developed wheat cultivars might lead to less SOC sequestration compared to older cultivars due to the lower root biomass of the former.~~ Testing the long-term effect of the gradual change in wheat cultivars on OC inputs to the soil would thus require experiments that run over multiple growing seasons, and allow the quantification of the amount of root carbon that is eventually ~~sequestered~~ stabilized in the soil.

Correct knowledge on the amount of OC that is transferred belowground by plants is necessary to reliably model SOC dynamics. However, this knowledge is currently limited and changes in belowground carbon allocation due to the cultivation of different cultivars is generally not considered in SOC models. Moreover, it has recently been shown that accounting for changes in belowground carbon allocation by relating this to changes in aboveground biomass does not improve model results (Taghizadeh-Toosi et al., 2016). Rather, it has been suggested that more reliable model results are obtained when crop-specific amounts of belowground carbon allocation are used, independent of aboveground biomass production (Taghizadeh-Toosi et al., 2016). Since model results are very sensitive to the amount of carbon inputs (Keel et al., 2017), 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)) ~~(Leff et al., 2004)~~, a correct assessment of a potential decrease in belowground carbon inputs by wheat plants over the past century through the cultivation of different cultivars will have important implications for the simulation of changes in SOC on the global scale.

Assessing the overall impact of the past evolution of wheat cultivars on SOC stocks also requires taking into account the amount of land needed to produce sufficient food. For example, if future research would show that more recent wheat cultivars lead to less SOC stabilization compared to older cultivars, this does not necessarily imply a net loss of SOC as a consequence of the historical shift to planting recently developed wheat cultivars. ~~Indeed, i~~If the aim is to increase overall SOC stocks, it might be more favorable to grow high-yield wheat cultivars that sequester less OC per unit area compared to a low-yielding cultivar, if this results in a larger area of arable land that can be taken out of cultivation. This land can be put under native vegetation, such as forest or grassland, which ~~is known to~~ stores substantially more SOC compared to arable land (Jobbágy and Jackson, 2000). ~~In addition, high-yield recent wheat cultivars often have a higher N use efficiency, which might have positive effects on reducing N losses (Aziz et al., 2017; Brancourt-Hulmel et al., 2003; Voss-Fels et al., 2019).~~

5. Conclusion

In this study, four different wheat cultivars were grown in lysimeters and labeled with $^{13}CO_2$ using repeated pulse-labelling to quantify the effect of rooting depth and root biomass on net carbon rhizodeposition ~~and stabilization~~. Our results show that there is no clear trend between the time of cultivar development and the amount of net carbon rhizodeposition ~~carbon inputs into the soil that is stabilized. Our results confirmed Friedli et al. (2019), who showed that older wheat cultivars from the Swiss breeding program had a higher root biomass and rooted deeper compared to more recently released wheat cultivars. However, all wheat cultivars resulted in similar amounts of net carbon rhizodeposition,~~ with large variabilities being observed between replicates of the same cultivars. Based on these results, the hypothesis that wheat cultivars with a larger root biomass and deeper roots would promote carbon ~~stabilization~~net carbon rhizodeposition, was rejected. An important remaining uncertainty is related to the fate of root biomass after harvest, which might contribute to the stabilized SOC pool over the long-term.

Data availability

Additional figures and tables can be found in the supplementary information. The data associated with this manuscript is available in the online version of this manuscript.

Author contribution

CDK, SA, AH, CF and JS conceived the idea for the study

CDK set up the lysimeter and labelling experiments and collected the data

SG, CDK, SA and RW performed lab analyses

MVdB, SG, CDK and JS analysed and interpreted the data and performed the statistics

MVdB and SG wrote the manuscript, with contributions from CDK, AH, SA, JS, CF and RW

MVdB and SG contributed equally to this manuscript

Conflict of interest

The authors declare that they have no conflict of interest.

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References

Aziz, M. M., Palta, J. A., Siddique, K. H. M. and Sadras, V. O.: Five decades of selection for yield reduced root length density and increased nitrogen uptake per unit root length in Australian wheat varieties, Plant Soil, 413, 181–192, doi:10.1007/s11104-016-3059-y, 2017.

Baloch, D. M., Karow, R. S., Marx, E., Kling, J. G. and Witt, M. D.: Vernalization Studies with Pacific Northwest Wheat,

Agron. J., 95, 1201–1208, doi:10.2134/agronj2003.1201, 2003.

Brancourt-Hulmel, M., Doussinault, G., Lecomte, C., Bérard, P., Le Buanec, B. and Trottet, M.: Genetic improvement of agronomic traits of winter wheat cultivars released in France from 1946 to 1992, Crop Sci., 43, 37–45, 2003.

Bromand, S., Whalen, J. K., Janzen, H. H., Schjoerring, J. K. and Ellert, B. H.: A pulse-labelling method to generate 13C-enriched plant materials, Plant Soil, 235, 253–257, doi:10.1023/A:1011922103323, 2001.

Brooks, P. D., Geilmann, H., Werner, R. A. and Brand, W. A.: Improved precision of coupled δ13C and δ15N measurements from single samples using an elemental analyzer/isotope ratio mass spectrometer combination with a post-column six-port valve and selective CO2 trapping; improved halide robustness of the combustion, Rapid Commun. Mass Spectrom., 17, 1924–1926, doi:10.1002/rcm.1134, 2003.

Chen, S., Martin, M. P., Saby, N. P. A., Walter, C., Angers, D. A. and Arrouays, D.: Fine resolution map of top- and subsoil carbon sequestration potential in France, Sci. Total Environ., 630, 389–400, doi:10.1016/j.scitotenv.2018.02.209, 2018.

Chenu, C., Angers, D. A., Barré, P., Derrien, D., Arrouays, D. and Balesdent, J.: Increasing organic stocks in agricultural soils: Knowledge gaps and potential innovations, Soil Tillage Res., doi:10.1016/j.still.2018.04.011, 2018.

Cholick, F. A., Welsh, J. R. and Cole, C. V.: Rooting Patterns of Semi-dwarf and Tall Winter Wheat Cultivars under Dryland Field Conditions, Crop Sci., 17, 637–639, 1977.

Coplen, T. B.: Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results, Rapid Commun. Mass Spectrom., 25, 2538–2560, doi:10.1002/rcm.5129, 2011.

Cotrufo, M. F., Wallenstein, M. D., Boot, C. M., Denef, K. and Paul, E.: The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: Do labile plant inputs form stable soil organic matter?, Glob. Chang. Biol., 19, 988–995, doi:10.1111/gcb.12113, 2013.

Dean, A., Morris, M., Stufken, J. and Bingham, D., Eds.: Handbook of design and analysis of experiments, CRC Press., 2015.

Don, A., Schu, J. and Freibauer, A.: Impact of tropical land-use change on soil organic carbon stocks - a meta-analysis, Glob. Chang. Biol., 17(4), 1658–1670, doi:10.1111/j.1365-2486.2010.02336.x, 2011.

Feil, B.: Breeding Progress in Small Grain Cereals — A Comparison of Old and Modern Cultivars, Plant Breed., 108, 1–11, 1992.

Fontaine, S., Barot, S., Barré, P., Bdioui, N., Mary, B. and Rumpel, C.: Stability of organic carbon in deep soil layers controlled by fresh carbon supply, Nature, 450, 277–280, doi:10.1038/nature06275, 2007.

Fossati, D. and Brabant, C.: La sélection du blé en Suisse Le programme des stations fédérales Une progression impressionnante, Rev. Suisse Agric., 35(4), 169–180, 2003.

Friedli, C. N., Abiven, S., Fossati, D. and Hund, A.: Modern wheat semi-dwarfs root deep on demand: response of rooting depth to drought in a set of Swiss era wheats covering 100 years of breeding, Euphytica, 215(85), 1–15, doi:10.1007/s10681-019-2404-7, 2019.

Goffin, S., Aubinet, M., Maier, M., Plain, C., Schack-Kirchner, H. and Longdoz, B.: Characterization of the soil CO2 production and its carbon isotope composition in forest soil layers using the flux-gradient approach, Agric. For. Meteorol., 188, 45–57, doi:10.1016/j.agrformet.2013.11.005, 2014.

Govers, G., Merckx, R., Van Oost, K. and van Wesemael, B.: Managing Soil Organic Carbon for Global Benefits: A STAP Technical Report, Global Environmental Facility, Washington, D.C., 2013.

de Graaff, M.-A., Van Kessel, C. and Six, J.: Rhizodeposition-induced decomposition increases N availability to wild and cultivated wheat genotypes under elevated CO2, Soil Biol. Biochem., 41, 1094–1103, doi:10.1016/j.soilbio.2009.02.015, 2009.

Gregory, P. J. and Atwell, B. J.: The fate of carbon in pulse-labelled crops of barley and wheat, Plant Soil, 136, 205–213, doi:10.1007/BF02150051, 1991.

Guo, L. B. and Gifford, R. M.: Soil carbon stocks and land use change: a meta analysis, Glob. Chang. Biol., 8, 345–360, doi:10.1046/j.1354-1013.2002.00486.x, 2002.

Hirte, J., Leifeld, J., Abiven, S., Oberholzer, H.-R. and Mayer, J.: Below ground carbon inputs to soil via root biomass and rhizodeposition of field-grown maize and wheat at harvest are independent of net primary productivity, Agric. Ecosyst. Environ., 265, 556–566, doi:10.1016/j.agee.2018.07.010, 2018.

Hu, T., Sørensen, P., Wahlström, E. M., Chirinda, N., Sharif, B., Li, X. and Olesen, J. E.: Root biomass in cereals, catch crops and weeds can be reliably estimated without considering aboveground biomass, Agric. Ecosyst. Environ., 251, 141–148, doi:10.1016/j.agee.2017.09.024, 2018.

Janzen, H. H. and Bruinsma, Y.: Methodology for the quantification of root and rhizosphere nitrogen dynamics by exposure of shoots to 15N-labelled ammonia, Soil Biol. Biochem., 21(2), 189–196, doi:10.1016/0038-0717(89)90094-1, 1989.

Jobbágy, E. G. and Jackson, R. B.: The vertical distribution of soil organic carbon and its relation to climate and vegetation, Ecol. Appl., 10(2), 423–436, doi:10.1890/1051-0761(2000)010[0423:TVDOSO]2.0.CO;2, 2000.

Jones, D. L., Nguyen, C. and Finlay, R. D.: Carbon flow in the rhizosphere: carbon trading at the soil–root interface, Plant Soil, 321, 5–33, doi:10.1007/s11104-009-9925-0, 2009.

Keel, S. G., Leifeld, J., Mayer, J., Taghizadeh-Toosi, A. and Olesen, J. E.: Large uncertainty in soil carbon modelling related to method of calculation of plant carbon input in agricultural systems, Eur. J. Soil Sci., 68(6), 953–963, doi:10.1111/ejss.12454, 2017.

Keith, H., Oades, J, M. and Martin, J, K.: Input of carbon to soil from wheat plants, Soil Biol. Biochem., 18(4), 445–449, 1986.

Kell, D. B.: Breeding crop plants with deep roots: their role in sustainable carbon, nutrient and water sequestration, Ann. Bot., 108, 407–418, doi:10.1093/aob/mcr175, 2011.

Kohn, M. J.: Carbon isotope compositions of terrestrial C3 plants as indicators of (paleo)ecology and (paleo)climate, Proc. Natl. Acad. Sci. U. S. A., 107(46), 19691–19695, doi:10.1073/pnas.1004933107, 2010.

Kong, A. Y. Y. and Six, J.: Tracing Root vs. Residue Carbon into Soils from Conventional and Alternative Cropping Systems, Soil Sci. Soc. Am. J., 74(4), 1201, doi:10.2136/sssaj2009.0346, 2010.

Kong, A. Y. Y. and Six, J.: Microbial community assimilation of cover crop rhizodeposition within soil microenvironments in alternative and conventional cropping systems, Plant Soil, 356, 315–330, doi:10.1007/s11104-011-1120-4, 2012.

Kong, A. Y. Y., Six, J., Bryant, D. C., Denison, R. F. and van Kessel, C.: The Relationship between Carbon Input, Aggregation, and Soil Organic Carbon Stabilization in Sustainable Cropping Systems, Soil Sci. Soc. Am. J., 69, 1078–1085, doi:10.2136/sssaj2004.0215, 2005.

Kramer, C. and Gleixner, G.: Soil organic matter in soil depth profiles: Distinct carbon preferences of microbial groups during carbon transformation, Soil Biol. Biochem., 40, 425–433, doi:10.1016/j.soilbio.2007.09.016, 2008.

Kuzyakov, Y.: Sources of CO2 efflux from soil and review of partitioning methods, Soil Biol. Biochem., 38, 425–448, doi:10.1016/j.soilbio.2005.08.020, 2006.

Kuzyakov, Y. and Domanski, G.: Carbon Input By Plants in the Soil. Review, J. Plant Nutr. Soil Sci., 163, 421–431, doi:10.1002/1522- 2624(200008)163:4<421::AID-JPLN421>3.0.CO;2-R, 2000.

Leff, B., Ramankutty, N. and Foley, J. A.: Geographic distribution of major crops across the world, Global Biogeochem. Cycles, 18, 1–27, doi:10.1029/2003GB002108, 2004.

Lehtinen, T., Schlatter, N., Baumgarten, A., Bechini, L., Krüger, J., Grignani, C., Zavattaro, L., Costamagna, C. and Spiegel, H.: Effect of crop residue incorporation on soil organic carbon and greenhouse gas emissions in European agricultural soils, Soil Use Manag., 30(4), 524–538, doi:10.1111/sum.12151, 2014.

Lupton, F. G. H., Oliver, R. H., Ellis, F. B., Barnes, B. T., Howse, K. R., Welbank, P. J. and Taylor, P. J.: Root and shoot

growth of semi-dwarf and taller winter wheats, Ann. Appl. Biol., 77, 129–144, 1974.

Massman, W. J.: A review of the molecular diffusivities of H2O, CO2, CH4, CO, O3, SO2, NH3, N2O, NO, and NO2 in air, O2 and N2 near STP, Atmos. Environ., 32(6), 1111–1127, doi:10.1016/S1352-2310(97)00391-9, 1998.

Mathew, I., Shimelis, H., Mutema, M. and Chaplot, V.: What crop type for atmospheric carbon sequestration: Results from a global data analysis, Agric. Ecosyst. Environ., 243, 34–46, doi:10.1016/j.agee.2017.04.008, 2017.

Minasny, B., Malone, B. P., McBratney, A. B., Angers, D. A., Arrouays, D., Chambers, A., Chaplot, V., Chen, Z.-S., Cheng, K., Das, B. S., Field, D. J., Gimona, A., Hedley, C. B., Hong, S. Y., Mandal, B., Marchant, B. P., Martin, M., McConkey, B. G., Mulder, V. L., O'Rourke, S., Richer-de-Forges, A. C., Odeh, I., Padarian, J., Paustian, K., Pan, G., Poggio, L., Savin, I., Stolbovoy, V., Stockmann, U., Sulaeman, Y., Tsui, C.-C., Vågen, T.-G., van Wesemael, B. and Winowiecki, L.: Soil carbon 4 per mille, Geoderma, 292, 59–86, doi:10.1016/j.geoderma.2017.01.002, 2017.

Moldrup, P., Olesen, T., Gamst, J., Schjønning, P., Yamaguchi, T. and Rolston, D. E.: Predicting the Gas Diffusion Coefficient in Repacked Soil, Soil Sci. Soc. Am. J., 64, 1588–1594, doi:10.2136/sssaj2000.6451588x, 2000.

Oburger, E. and Jones, D. L.: Sampling root exudates – Mission impossible?, Rhizosphere, 6, 116–133, doi:10.1016/j.rhisph.2018.06.004, 2018.

Pausch, J. and Kuzyakov, Y.: Carbon input by roots into the soil: Quantification of rhizodeposition from root to ecosystem scale, Glob. Chang. Biol., 24, 1–12, doi:10.1111/gcb.13850, 2018.

Paustian, K., Lehmann, J., Ogle, S., Reay, D., Robertson, G. P. and Smith, P.: Climate-smart soils, Nature, 532, 49–57, doi:10.1038/nature17174, 2016.

Poeplau, C. and Don, A.: Carbon sequestration in agricultural soils via cultivation of cover crops – A meta-analysis, Agric. Ecosyst. Environ., 200, 33–41, doi:10.1016/j.agee.2014.10.024, 2015.

Poeplau, C., Don, A., Vesterdal, L., Le, J., van Wesemael, B., Schumacher, J. and Gensior, A.: Temporal dynamics of soil organic carbon after land-use change in the temperate zone - carbon response functions as a model approach, Glob. Chang. Biol., 17(7), 2415–2427, doi:10.1111/j.1365-2486.2011.02408.x, 2011.

Le Quéré, C., Andrew, R. M., Friedlingstein, P., Sitch, S., Pongratz, J., Manning, A. C., Korsbakken, J. I., Peters, G. P., Canadell, J. G., Jackson, R. B., Boden, T. A., Tans, P. P., Andrews, O. D., Arora, V. K., Bakker, D. C. E., Barbero, L., Becker, M., Betts, R. A., Bopp, L., Chevallier, F., Chini, L. P., Ciais, P., Cosca, C. E., Cross, J., Currie, K., Gasser, T., Harris, I., Hauck, J., Haverd, V., Houghton, R. A., Hunt, C. W., Hurtt, G., Ilyina, T., Jain, A. K., Kato, E., Kautz, M., Keeling, R. F., Klein Goldewijk, K., Körtzinger, A., Landschützer, P., Lefèvre, N., Lenton, A., Lienert, S., Lima, I., Lombardozzi, D., Metzl, N., Millero, F., Monteiro, P. M. S., Munro, D. R., Nabel, J. E. M. S., Nakaoka, S., Nojiri, Y., Padin, X. A., Peregon, A., Pfeil, B., Pierrot, D., Poulter, B., Rehder, G., Reimer, J., Rödenbeck, C., Schwinger, J., Séférian, R., Skjelvan, I., Stocker, B. D., Tian, H., Tilbrook, B., Tubiello, F. N., van der Laan-Luijkx, I. T., van der Werf, G. R., van Heuven, S., Viovy, N., Vuichard, N., Walker, A. P., Watson, A. J., Wiltshire, A. J., Zaehle, S. and Zhu, D.: Global Carbon Budget 2017, Earth Syst. Sci. Data, 10, 405–448, doi:10.5194/essd-10-405-2018, 2018.

R Core Team: R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/., 2019.

Ruehr, N. K., Offermann, C. A., Gessler, A., Winkler, J. B., Ferrio, J. P., Buchmann, N. and Barnard, R. L.: Drought effects on allocation of recent carbon: from beech leaves to soil CO2 efflux, New Phytol., 184, 950–961, doi:10.1111/j.1469-8137.2009.03044.x, 2009.

Sandén, T., Spiegel, H., Stüger, H.-P., Schlatter, N., Haslmayr, H.-P., Zavattaro, L., Grignani, C., Bechini, L., D'Hose, T., Molendijk, L., Pecio, A., Jarosz, Z., Guzmán, G., Vanderlinden, K., Giráldez, J. V., Mallast, J. and ten Berge, H.: European long-term field experiments: knowledge gained about alternative management practices, edited by M. Aitkenhead, Soil Use Manag., 34, 167–176, doi:10.1111/sum.12421, 2018.

Shearman, V. J., Sylvester-bradley, R., Scott, R. K. and Foulkes, M. J.: Physiological Processes Associated with Wheat Yield

Progress in the UK, Crop Sci., 45, 175–185, 2005.

Stevenel, P., Frossard, E., Abiven, S., Rao, I. M., Tamburini, F. and Oberson, A.: Using a Tri-Isotope (13C, 15N, 33P) Labelling Method to Quantify Rhizodeposition, in Methods in Rhizosphere Biology Research, edited by D. Reinhardt and A. K. Sharma, pp. 169–195, Springer, Singapore., 2019.

Studer, M. S., Siegwolf, R. T. W. and Abiven, S.: Carbon transfer, partitioning and residence time in the plant-soil system: a comparison of two ¹³CO₂ labelling techniques, Biogeosciences, 11, 1637–1648, doi:10.5194/bg-11-1637-2014, 2014.

Sun, Z., Chen, Q., Han, X., Bol, R., Qu, B. and Meng, F.: Allocation of photosynthesized carbon in an intensively farmed winter wheat–soil system as revealed by 14CO2 pulse labelling, Sci. Rep., 8(3160), 1–10, doi:10.1038/s41598-018-21547-y, 2018.

Swinnen, J., van Veen, J. A. and Merckx, R.: 14C pulse-labeling of field-grown spring wheat: an evaluation of its use in rhizosphere carbon budget estimations, Soil Biol. Biochem., 26(2), 161–170, 1994.

Taghizadeh-Toosi, A., Christensen, B. T., Glendining, M. and Olesen, J. E.: Consolidating soil carbon turnover models by improved estimates of belowground carbon input, Sci. Rep., 6(32568), 1–7, doi:10.1038/srep32568, 2016.

Tester, M. and Langridge, P.: Breeding technologies to increase crop production in a changing world, Science (80-.)., 327, 818–822, doi:10.1126/science.1183700, 2010.

Tubiello, F. N., Salvatore, M., Ferrara, A. F., House, J., Federici, S., Rossi, S., Biancalani, R., Condor Golec, R. D., Jacobs, H., Flammini, A., Prosperi, P., Cardenas-Galindo, P., Schmidhuber, J., Sanz Sanchez, M. J., Srivastava, N. and Smith, P.: The Contribution of Agriculture, Forestry and other Land Use activities to Global Warming, 1990-2012, Glob. Chang. Biol., 21(7), 2655–2660, doi:10.1111/gcb.12865, 2015.

Vance, E. D., Brookes, P. C. and Jenkinson, D. S.: An extraction method for measuring soil microbial biomass C, Soil Biol. Biochem., 19(6), 703–707, doi:10.1016/0038-0717(87)90052-6, 1987.

Voss-Fels, K. P., Stahl, A., Wittkop, B., Lichthardt, C., Nagler, S., Rose, T., Chen, T.-W., Zetzsche, H., Seddig, S., Majid Baig, M., Ballvora, A., Frisch, M., Ross, E., Hayes, B. J., Hayden, M. J., Ordon, F., Leon, J., Kage, H., Friedt, W., Stützel, H. and Snowdon, R. J.: Breeding improves wheat productivity under contrasting agrochemical input levels, Nat. Plants, 5, 706–714, doi:10.1038/s41477-019-0445-5, 2019.

Wacker, L., Jacomet, S. and Körner, C.: Trends in Biomass Fractionation in Wheat and Barley from Wild Ancestors to Modern Cultivars, Plant Biol., 4, 258–265, doi:10.1055/s-2002-25735, 2002.

Waines, J. G. and Ehdaie, B.: Domestication and Crop Physiology: Roots of Green-Revolution Wheat, Ann. Bot., 100, 991–998, doi:10.1093/aob/mcm180, 2007.

Wasson, A. P., Richards, R. A., Chatrath, R., Misra, S. C., Prasad, S. V. S., Rebetzke, G. J., Kirkegaard, J. A., Christopher, J. and Watt, M.: Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops, J. Exp. Bot., 63(9), 3485–3498, doi:10.1093/jxb/ers111, 2012.

Werner, R. A. and Brand, W. A.: Referencing strategies and techniques in stable isotope ratio analysis, Rapid Commun. Mass Spectrom., 15, 501–519, doi:10.1002/rcm.258, 2001.

Werner, R. A., Bruch, B. A. and Brand, W. A.: ConFlo III - an interface for high precision δ13C and δ15N analysis with an extended dynamic range, Rapid Commun. Mass Spectrom., 13(13), 1237–1241, doi:10.1002/(SICI)1097-0231(19990715)13:13<1237::AID-RCM633>3.0.CO;2-C, 1999.

Zeeman, M. J., Werner, R. A., Eugster, W., Siegwolf, R. T. W., Wehrle, G., Mohn, J. and Buchmann, N.: Optimization of automated gas sample collection and isotope ratio mass spectrometric analysis of δ 13 C of CO 2 in air, Rapid Commun. Mass Spectrom., 22, 3883–3892, doi:10.1002/rcm.3772, 2008.

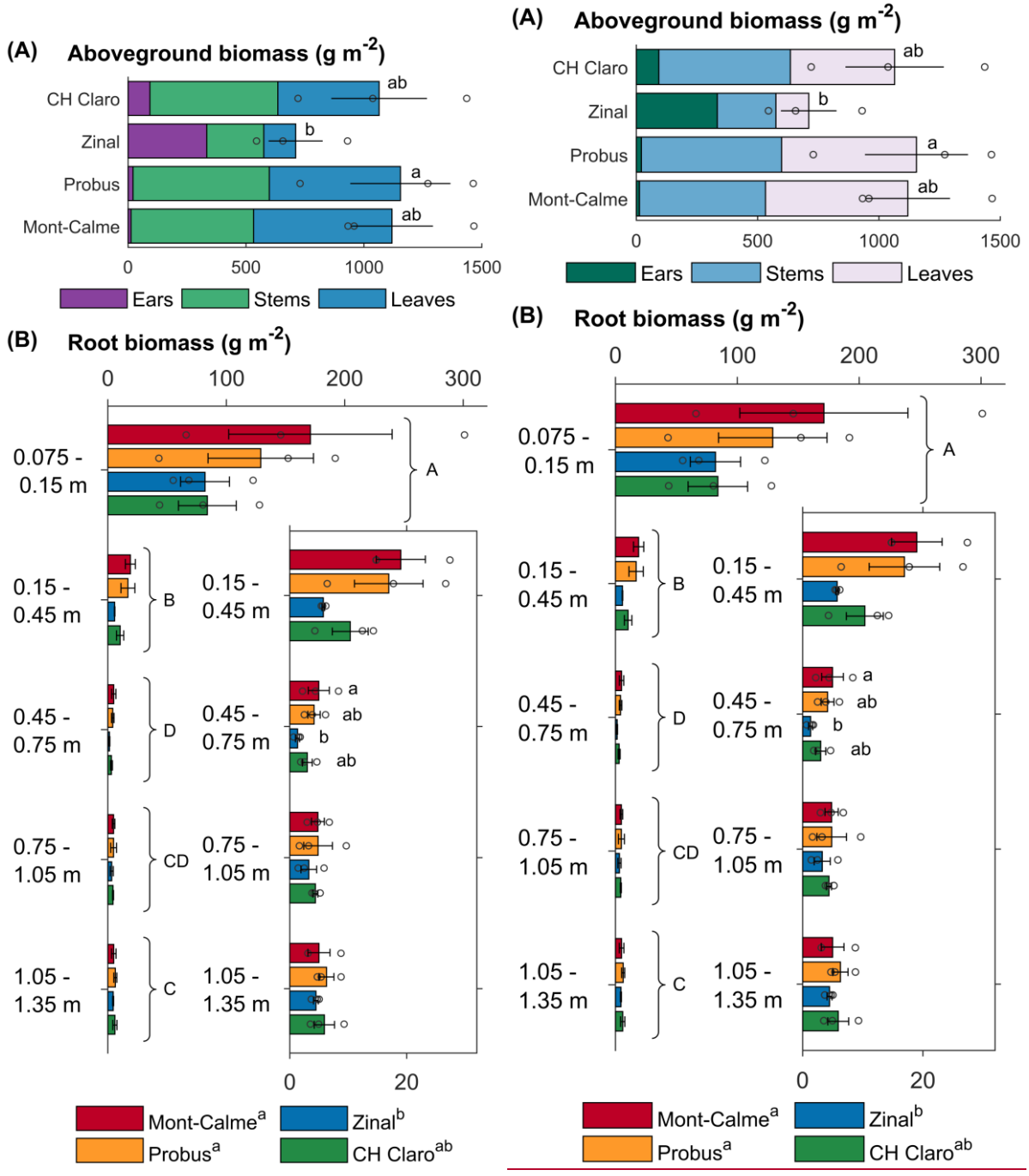

Figure 1 Aboveground (A) and root (B) biomass of the different wheat cultivars at the end of the experiment. Bars represent the average per wheat cultivar, error bars show the standard error (n = 3) and circles show the individual data points. The inset in (B) shows a detail of the subsoil root biomass. If statistically significant differences were present, these are indicated with letters, with variables sharing a letter not being significantly different. For root biomass, statistical analyses were performed using a three-way

analysis of variance, in contrast to the two-way analysis used to calculate~~d~~ significant differences for the total root biomass summed over all depths as reported in Table 1 (see section 2.4).

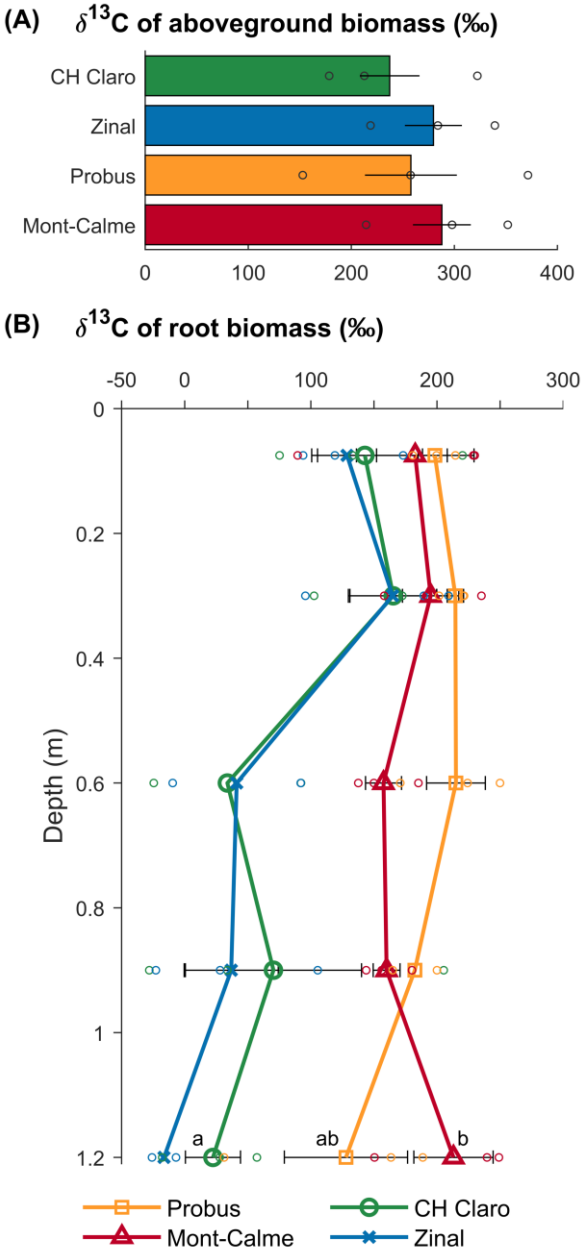

Figure 2 δ^{13}C value~~s~~ of aboveground (A) and belowground (B) biomass for the different wheat cultivars at the end of the experiment. Bars (A) and symbols (B) represent the average per wheat cultivar based on 3 replicates, error bars show the standard error (n = 3), ~~while~~ symbols without error bar~~s~~ indicate samples for which no 3 replicates were available. Circles show the individual data points. If statistically significant differences were present for root biomass at the same depth, these are indicated with letters, with variables sharing a letter not being significantly different and data points without error bars being left out of the analyses.

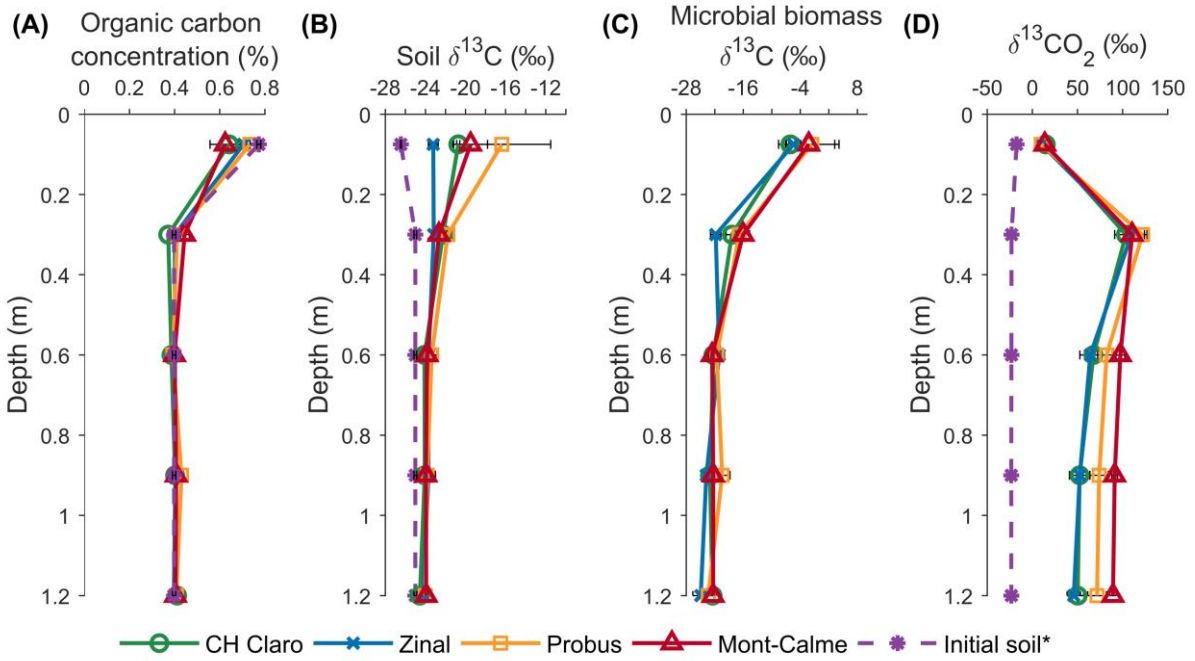

Figure 3 Depth profiles of organic carbon concentration (A), the δ^{13}C value of organic carbon (B), the δ^{13}C value of microbial biomass (C) and the δ^{13}C value of soil CO_2, averaged per wheat cultivar at the end of the experiment. Error bars represent the standard error (n = 3). *The initial soil indicates measurements performed on the soil that was used to fill the lysimeters prior to the experiments.

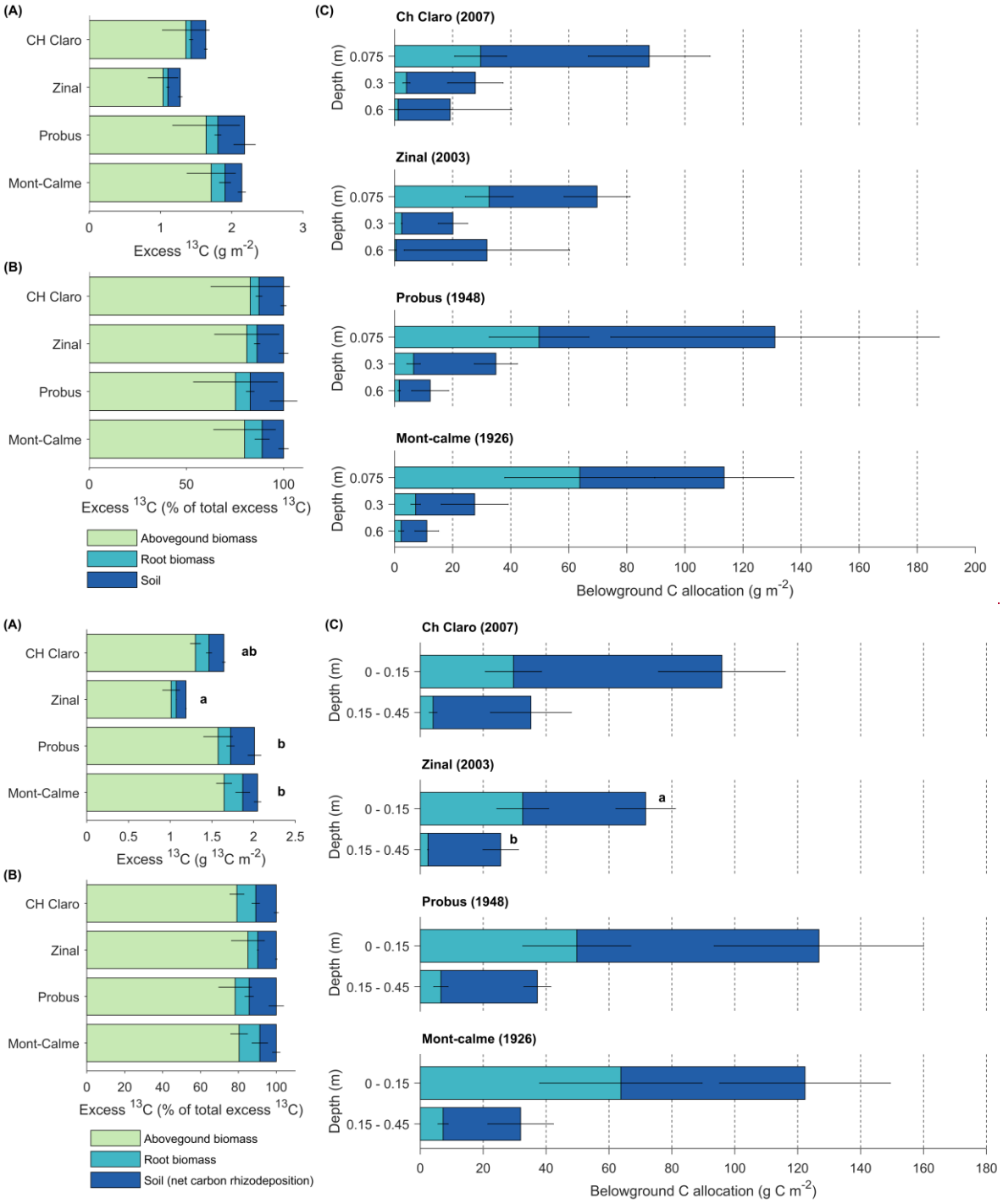

Figure 4 Absolute (A) and relative (B) distribution of excess ^{13}C between aboveground biomass, root biomass and soil for the different wheat cultivars. Soil compartments are calculated down to 0.45 m depth. (C) shows ~~depth profiles of~~ the total carbon rhizodeposition ~~carbon~~ and root carbon for the upper ~~three~~ two soil layers for the different wheat cultivars (0 – 0.45 m depth). Error bars represent

the standard error (n = 3). In (A), letter indicate significant differences between the total amount of excess ^{13}C of the different cultivars. In (C), letters are provided when the total belowground C allocation differed between the different depth layers, which was only the case for Zinal.

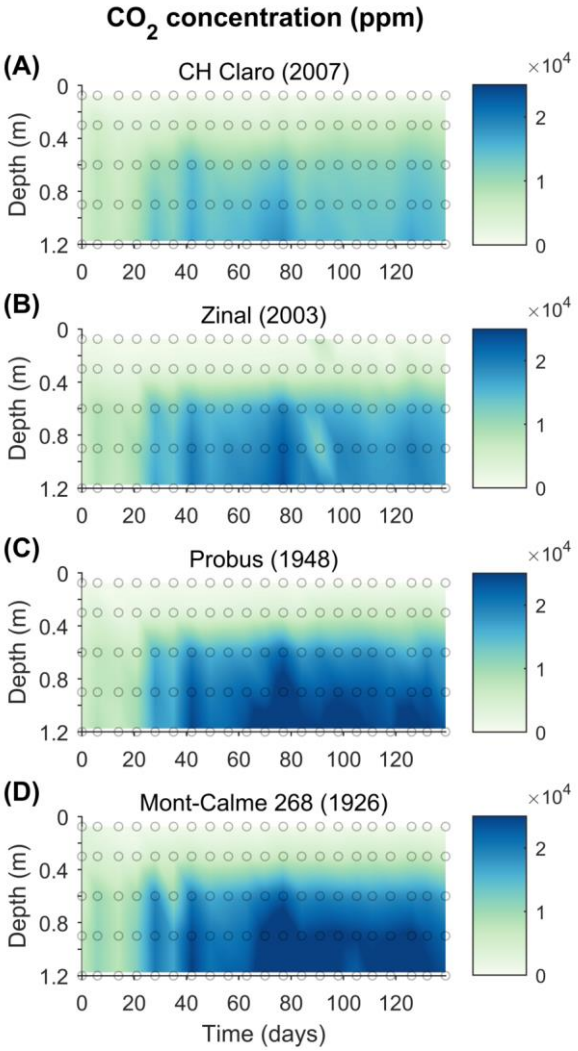

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Figure 5 Changes in the CO_2 concentration (ppm) in the lysimeters throughout the experiment for the four wheat cultivars. The average CO_2 concentration of three replicates are shown (n = 3). Dots indicate the measured data points.

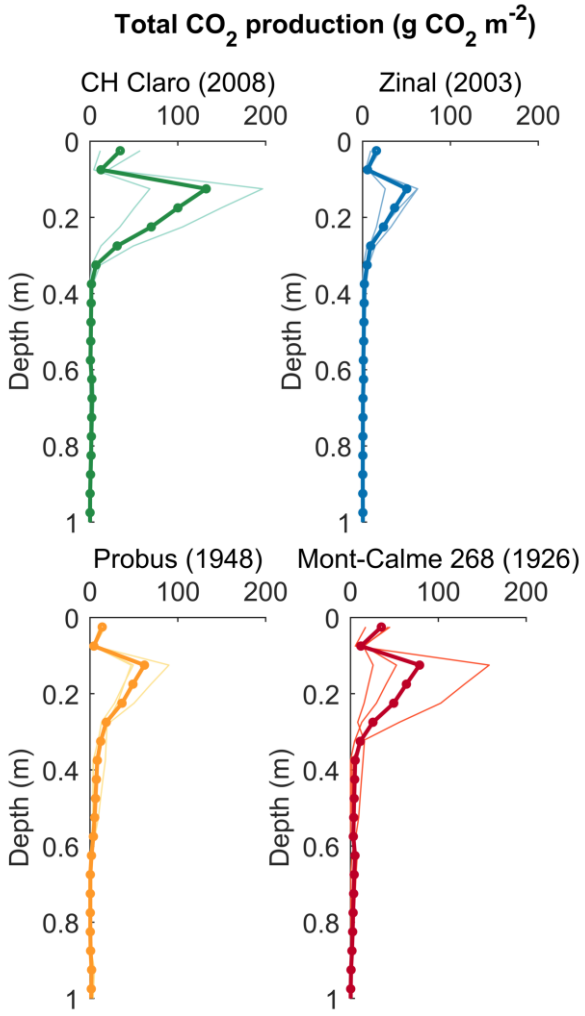

Figure 6 Depth profiles of calculated cumulative CO_2 production (g CO_2 m^{-2} per 0.05 m depth layer). Dots show the calculated production rates, thin lines show the calculated CO_2 production for the individual lysimeters, while the thick lines show the average based on three replicates (two for CH Claro).

Table 1 Characteristics (± standard error, n = 3) of the biomass of the different wheat cultivars at the end of the experiment. ~~No significant differences were found between the belowground biomass (average biomass per cultivar summed over the different depths, using a two-way anova, see section 2.4) or root:shoot ratio of the different cultivars.~~ Values that share a letter in the same column are not significantly different.

Cultivar (year of release)	Aboveground biomass		Root biomass		Root:shoot ratio
	Biomass (g m^{-2})	OC %	Biomass (g m^{-2})	OC %	
CH Claro (2007)	1064 ± 207^{ab}	40.5 ± 0.3^{ab}	107 ± 28^{ab}	38.7 ± 1.9^{a}	0.10 ± 0.02^{a}
Zinal (2003)	710 ± 114^{b}	40.0 ± 0.14^{a}	97 ± ~~20^{a}~~20^{b}	39.1 ± 1.1*	0.14 ± 0.01^{a}
Probus (1948)	1154 ± 220^{a}	41.9 ± 0.2^{b}	161 ± 54^{a}	38.1 ± 0.5^{a}	0.13 ± 0.03^{a}
Mont-Calme 268 (1926)	1119 ± 174^{ab}	40.8 ± 0.4^{ab}	205 ± 67^{a}	36.8 ± 1.3^{a}	0.19 ± 0.08^{a}

* Was excluded from statistical analysis due to missing data

Table 2 Average belowground carbon allocation (net rhizodeposition and root biomass combined) and net carbon rhizodeposition by the different wheat cultivars, calculated down to a depth of 0.~~7~~45 m (variation is reported as the standard error, n = 3). No statistically significant differences in belowground C allocation of net C rhizodeposition between any of the cultivars were detected.

	Belowground C allocation (g m^{-2})	Net ~~carbon~~ C rhizodeposition (g m^{-2})
CH Claro	~~147~~ 131 ± ~~40~~26	97~~112~~ ± ~~39~~24
Zinal	~~135~~ 97 ± ~~40~~14	~~100~~ 62 ± ~~39~~11
Probus	~~184~~ 164 ± ~~60~~38	~~126~~ 108 ± ~~57~~34
Mont-Calme 268	~~159~~ 154 ± ~~37~~39	~~85~~ 83 ± ~~27~~29