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Interactive comment on "Recycling vs. stabilisation of soil sugars - a long-term laboratory incubation experiment" *by* A. Basler et al.

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We would like to thank the reviewers for their helpful comments and suggestions, which have greatly improved our manuscript. We hope that our response answers all their concerns.

Anonymous Referee #2

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General Comments. In the present investigation, the authors address the fate of neutral sugars as an important part of SOM in a three year incubation study. Hereby, the main aim is to disentangle the importance of stabilization vs. recycling for the sugar dynamics in soil. This is done by means of application of 13C enriched glucose to

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three different soil and land use types followed by extraction and compound specific isotope analysis of microbial sugars at various time steps together with CO_2 fluxes and measurements of microbial biomass. The authors found evidence, that after an initial phase of high metabolization rates and thus sugar derived C losses in the form of CO_2, recycling by the microbial community of sugar-derived C becomes very effective. Though in general sugar dynamics in the long term were dominated by a pool showing high mean residence times, there were differences between two groups of microbial sugars in the incorporation dynamic of glucose derived 13C. These findings were not affected by the C content of the investigated soils. The study gives valuable information about the importance of recycling of SOM via the sugar pool in soil. My main points of criticism are that the authors use the term MRT though the unknown rate of sugar synthesis is not known and thus the criteria for MRT calculation are not met. Answer: We agree with this comment. However, we are referring to the MRT of the carbon allocated to sugars, but not the sugars themselves, as this is the only information we can derive from our measurements. This was clarified in the introduction (second paragraph). Second, while there are really strong arguments that sugar dynamics are dominated by recycling, the authors do not discuss that they cannot rule out that the differentiation into a fast and a slow reacting sugar pool could also be caused by stabilization mechanisms.

Answer: We agree with the reviewer that we cannot present a final proof to exclude stabilization as underlying mechanism, although we believe that the basis for our argumentation is strong enough.

To finally prove the recycling the application of position-specifically labelled substances followed by a position-specific isotope detection would be necessary. However, the measurement techniques for this kind of studies does not (yet) exist.Finally the authors fail to draw more implications of their finding e.g. on the interpretation of data from foregoing investigations on the persistence of SOM compounds, where high MRT was found, irrespective of the chemical structure.

Answer: We fully agree here and added a respective comment in the conclusion. Nevertheless, after these points and a number of more detailed suggestions have been implemented into the recent manuscript, I suggest to resubmit and publish the manuscript.

Specific Comments:

p.3 l. 4: While in this paragraph it is stressed that recalzitrance is an inadequate model to explain decomposition dynamics, you later on (p. 3 l. 15) define sugars as an easy to degrade compound. This perfectly shows that neither recalcitrance, nor other stabilizing factors can completely explain or predict the fate of certain compounds or compound classes in soil. I would suggest to reorganize these first two paragraphs in a way that shows these contradicting views and thus makes clear the importance of disentangling stabilization vs. recycling.

Answer: We changed this section to more clearly focus on the main points here.

p.3 l.16: how is the term "apparent" defined? If you want to express, that the turnover times have been determined by means of 14C dating and could thus by biased by the synthesis of sugars from old carbon sources, you should explicitly say so. However, in this case stabilization mechanisms like sorption or inclusion (p.3 l.18) would include truly old sugars, thus not contributing to apparent high mean residence times as you write.

Answer: "apparent MRT" here means that these are the MRT that one would get if recycling would be excluded. The term has been used before (e.g. Flessa et al., 2008) exactly due the necessary distinction between "true" MRT of sugars (which to our knowledge have not been measured yet in soils) and MRT of carbon in sugars. We also added an explanation in the introduction.

p.4 l.3: Beside the differing concentrations, the more important thing would be differences in the chemical quality or overall usability of C in these systems. This is

discussed later on, but actually it should already be stated here.

Answer: We have taken this into account by mentioning the different C qualities of the investigated soils in the introduction.

p.4 l.26: clarify, if the glucose was equally labeled or if the 99 at% are only valid for a certain C-position. Answer: This was clarified by stating U-13C. p.5 l.4: How do the 4 g fit to the time steps when CFE has been performed or how was the whole incubation system treated after sampling for CFE? In the same way as for 4 g?

Answer: A sentence was introduced in the "soil incubation" section to clarify that soil for Cmic analysis was sampled together with the soil for sugar analysis

p.5 l.17-18: 13C signature of soil derived CO_2 is not measured by the simple difference between the two samplings, but rather by plotting the isotopic composition vs. the reciprocal of the sampling time an then prolonging the linear equation to the cutting point with the y-axis (Keeling Plot).

Answer: Although a Keeling plot of our data would lead to the same results, we applied a mass and isotopic balance calculation. This was clarified in the text "from the difference in concentration and isotopic composition of the two samplings"

p.6 l.16: The equation uses data from an unlabeled treatment. It was not specified how this treatment was set up; please specify.

Answer: A sentence to clarify this was added in the "Soil incubation" section: "Controls under natural abundance conditions were treated identically."

p.6 l.17: It is rather unclear what you want to state by saying the analysis pattern differed - do you mean a difference in the sampling frequency?

Answer: This sentence was rephrased: "The analysis frequency differed among the different soils: To check if short sampling intervals will reveal additional sugar dynamics. . ."

p.8 l.2: In the equation S(t) is defined as the level of isotopic enrichment. However, in figure 3, where this formula is used, it is not fitted to S(t) but to RSA. Please clarify.

Answer: The section 2.6 was modified. The parameter S of the decay functions was changed to y, were y represents the RSA values of the individual sugar.

p.8 l.19: How can you identify newly synthesized sugars? While it is clear that the amount of label incorporated into microbial sugars represents newly synthesized sugars, it does on the other hand not mean that these are the only freshly synthesized sugars; i.e. you would underestimate the amount of freshly synthesized sugars because whenever old unlabeled carbon is used to synthesize sugars, you would not see, or you would even interpret the following drop of enrichment as a drop in synthesized sugar amount. Though I am aware of the fact, that all tracer studies and especially those that are ran over a longer time period, face this problem and that solutions to overcome this problem are scarce I would suggest to comment on this problem in the text: First of all it should be considered by clearly stating, that newly synthesized sugars are defined as the part of the sugar pool showing incorporation of the label. Second, at some point in your discussion section you should discuss the implications of this problem for your data interpretation.

Answer: We absolutely agree here and consequently rephrased this to "labelled sugar" instead of "newly synthesized sugar"

p.9 l.6: what about RSA in bulk soil?

Answer: We rephrased this, the RSA value of bulk soil is ranked in the arrangement.

p.9 l.8: In the method section it was stated, that the incubation was done for 30 months. Here you say that it was 34 months; please clarify

Answer: We clarified this. The incubation was done for 34months, but sugar analysis was only made for the first 30 months.

p.11 l.1: It is not stated that MRT could frequently not be calculated for a number of

sugars, due to positive k values. Please also note, that for these sugars it is not even correct to define the function as a decay function. Though this fact is already part of the discussion it should also be clearly stated at this point. At this point I would like to stress that the setup of the experiment does not really justify the term MRT. Though the equations are used in the right way, you also have to check if the processes defining e.g. the form of your kinetic functions, are really pure decay processes. Only for this situation it makes sense to speak of MRT. If there is resynthesis of the substance of interest, you would need to correct for the rate of synthesis. However, in your case I see no possibility to get these data. The fact that the recycling of label, i.e. the reincorporation of 13C into newly synthesized sugars impeded the differentiation of several pools (based on the calculated MRT?) is discussed in section 4.3. However, it needs to be stressed, that the calculation of MRT is not just impeded, but that the use of MRT is simply not possibly at this point as the settings simply do not meet the definition of MRT. The actual data set only allows to calculate something that might be defined as a MRT for the label being recycled / circulated through the specific sugars. I feel that this does not really hamper the interpretation of the data - it still enables you to show the importance of recycling of freshly incorporated C into the SOM pool vial sugars and differentiate between different sugars. At this point it might also be usefulto skip the calculation of any residence times and only differentiate by means of the calculated k-values (the smaller the value, the more recycling takes place) - this would enable you to also discuss the role of those sugars having a negative k-value.

Answer: We agree here. However, we wanted to show MRT (where possible) as this is the most commonly used value in soil carbon dynamics. For clarification, we added sentences in the Results and the Discussion sections that decay was not always observed (the implications here of are part of the discussion anyway).

p.12 l.6: It would probably give a more complete picture, if the partitioning of label between the different soil pools would be shown and discussed. Please note that the RSA only gives the proportion of a pool that is made up from incorporated label.

However, it does not show, were most of your label was incorporated.

Answer: We added this information in a new Figure (see below) to draw a more complete picture of the dynamics during the incubation.

p.13 l.18: If glucose (i.e. also labeled glucose) is bound to SOM and is accessible for microorganisms, one should expect significant enrichments in the exC pool. Please discuss this a bit more into depth.

Answer: The first time we measured the exC is after 6 month, at this time the proportion of e glucose derived C is negligible (a high contribution would only be expected immediately after addition. We included this data now in a new figure

p.14 l.13-15: Please also discuss the sinus like fluctuations for instance in the case of manose - this could be an interesting point in showing that there are also short time dynamics present. Probably this could also be the starting point to investigate the short term dynamics of the microbial community in a long term experiment - i.e. the switching between times of degradation of old SOM and the recycling of C from dead and rel.young microbial biomass. I would encourage you to at least discuss this aspect, as these fluctuations are really striking.

Answer: Some sentence about this aspect was included in the section 4.3.

p.14 l.15-18: You note, that due to a de novo synthesis of plant derived sugars by microbes, it was not possible to differentiate between a sugar pool that is only affected by stabilization (plant derived sugars) and another one that is also affected by recycling.While this is true, I do not understand, how it could have helped you, if there was no de novo synthesis of Ara and Xyl. In that case both would have not been labeled and thus it would not have been possible to calculated degradation kinetics. To be able to do so, you would have needed to add labeled Ara and Xyl to the same or a parallel experiment. Thus, this part is confusing and you should clarify this, because I do not really understand, how you were going to disentangle stabilization vs. recycling based

on this approach even if you would not have synthesis of plant derived sugars – please clarify.

Answer: Although the original idea of the study was to find different dynamics for sugars of microbial origin vs. sugars of plant origin we had to acknowledge that all sugar dynamics were dominated by microbial production (and not only influenced in case of the "plant derived sugars", as we hypothesised). This is why the original idea did not work out.

p.16 l.5-8: It is stated that the high MRT indicate that recycling dominates sugar dynamics. However, from a mechanistic point of view this straightforward interpretation is not justified as it is not considering, that the stabilization of microbial sugars would also lead to high MRT and would also end in a steady state in the end of the experiment. Though I agree that due to a bundle of reasons it is much more likely that recycling plays the dominant role, this is not discussed enough in detail in the discussion section. Clearly speaking, the pros and cons for recycling or stabilization are not always clearly named and are not weighed up against each other. However, this is very important, as the experiment itself does not investigate stabilization, e.g. there are no data on the desorption of sugars or other stabilizing mechanisms that are named in the introduction; even if there are few / no studies on stabilization of sugars in soil, the possibility of e.g. sorption to different surfaces in soil should be considered and discussed, based on the chemical characteristics of sugars.

Answer: We do agree here, this why we added further arguments considering recent literature in (on sorption). However, we do not conclude that recycling dominates the dynamics solely on the long calculated MRT. More important is the microbial biomass, especially the high labelling after the long time and the pronounced difference to the produced CO_2

Technical Comments: p.3 l.25: missing space between Derrien et al. and following brackets p.5 l.19: Superscribe 13 in the word 13C p.5 l.25: Use a small "a" in hPa

p.6 l.12: space between author and year p.6 l.16: leave space before and after the mathematical operators p.7 l.7: space between mL and 0.05 p.7 l.11: use "filtrates" rather than "salts"

Answer: We apologize for these errors, and we have corrected the text as suggested.

p.7 l.11: please at least give the brand of your instrument and the temperature/reactor filling at which the analysis in the EA has been done

Answer: The reactor is filled with tungsten oxide and silvered cobaltous oxide. This information was added in the Materials/Methods section.

p.7 l.15-16: use the presence instead of the past as you define the variable of a mathematical function

Answer: We changed this as suggested.

p.7 l.18: kec factor is not defined - it is under discussion, whether this factor is really applicable for all ecosystems, i.e. if it stays constant. As it would anyway not alter the rel. differences between your different soils, I would rather suggest to leave away the factor and define the value as the "extractable microbial biomass".

Answer: We are aware of this discussion concerning the kec factor. However, we decided to provide these data due to the comparableness with other studies.

p.8 l.7: enumeration of this equation and the following ones is incorrect. Answer: The section 2.6 was modified. We kept this point in mind during the new structuring.

p.9 l.12: missing space between μg and C p.10 l.24: kinetics describe reactions but not a soil pool; thus you should rather say kinetics for soil sugar turnover. Please rephrase. p.13 l.31-32: use "incorporation" instead of "input" and "especially for easily" instead of "especially in easily" Table 3: move "wheat Ap to the top of the first section so that the structure is the same for all sections. Also you should increase the distance between the section to get the separation more clear. Table1: The spacing between the different

rows in "Distribution of sugars [%]" is too small and makes the table difficult to read.

Answer: Thank you, we have followed these recommendations.

Figure 1: it is not clear, whether the significant differences were found between the different systems but within one time step or throughout the three time steps – please clarify. Also there is an error in the block setting of the figure capture (last line).

Answer: For clarification we rephrased the capture.

Figure 2: Please explain why there is no data for CO_2 fluxes for grassland and forest at time step 0.

Answer: We cannot provide data for the CO_2 for forest and grassland, as we still had some trouble at the beginning with the experiment. Leaky microcosms and high inaccuracies in the measurements due to required dilution of the samples forced us to neglected these values

References

Flessa, H., Amelung, W., Helfrich, M., Wiesenberg, G. L., Gleixner, G., Brodowski, S., Rethemeyer, J., Kramer, C., and Grootes, P. M.: Storage and stability of organic matter and fossil carbon in a Luvisol and Phaeozem with continuous maize cropping: A synthesis, J. Plant Nutr. Soil Sc., 171, 36–51, 2008.

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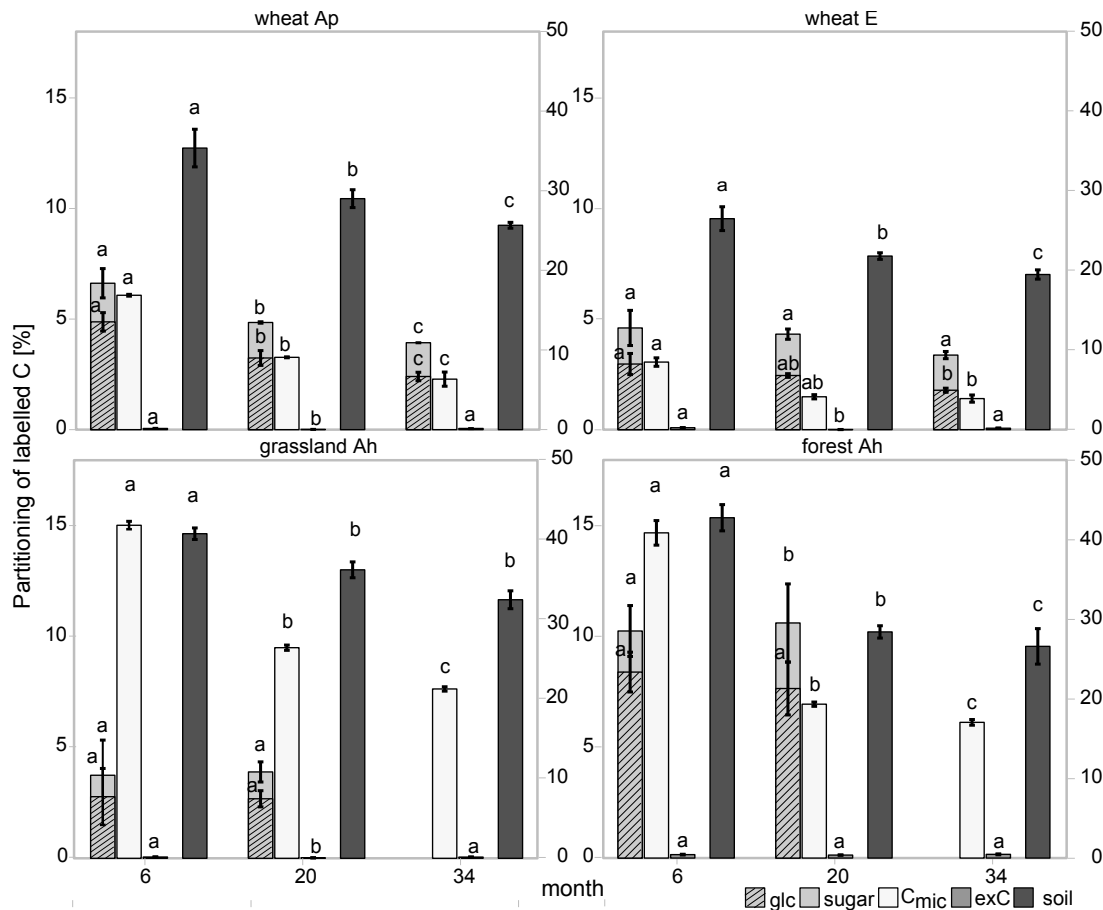Interactive Discussion

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Fig. 1. Partitioning of the labelled C into microbial biomass (Cmic), K2SO4-extractable carbon (exC), glc and sum of all sugars (left axis) and bulk soil (right axis) in wheat Ap & E, grassland Ah and forest