Answer to reviewers

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. We considered each reviewer individually, with the reviewer's comments in normal font, our answers in italics.

Interactive comment on "Microbial carbon recycling: an underestimated process controlling soil carbon dynamics" by A. Basler et al.
Anonymous Referee #1

Received and published: 22 July 2015

Basler et al. investigate stabilisation and recycling of soil sugars as processes controlling soil carbon dynamics. This is addressed by d13C analyses using HPLC/IRMS of soil sugars in density fractions from a natural 30 year old labelling experiment with wheat-maize vegetation change. Overall, this is a well designed and presented study appropriate for publication in BG. The authors clearly state the motivation/relevance of this study for better understanding turnover dynamics of sugars in the introduction, formulate a clear working hypothesis at the end of the introduction (turnover of plant-derived sugars is ruled by stabilization versus turnover of microbial-derived sugars is ruled by recycling) and provide all necessary information where and how the study was performed in the Material and Method section. The authors found that the contribution of maize-derived carbon in the POM fractions is considerably higher in sugars compared to the bulk fractions, equivalent to mean residence times (MRT) being lower for sugars than for bulk C in these fractions. This is interpreted in terms of aggregate formation being fuelled by microbial activity and fresh organic matter. Concerning the working hypothesis, the authors found that the MRT of xylose is considerably lower than the MRT of the other sugars. The authors argue that xylose (plant-derived) dynamic is primarily dominated by stabilization, whereas the dynamic of the other sugars (microbial-derived) is strongly controlled by recycling. Interestingly, this also holds true for arabinose; this is well highlighted and discussed by the authors. However, as alternative interpretation, I would like to suggest (and the authors may want to include this in their discussion) that the dynamic of arabinose, like that of xylose, is primarily controlled by stabilisation (not by recycling). The arabinose/xylose ratio is close to 1 in the soil fractions, possibly because former vegetation contributed relatively high amounts of arabinose to the soil. The addition of wheat/maize sugars with low ara:xyl ratios (1:6 and 1:5, respectively)

thus resulted in a low admixture of maize-derived arabinose during the last 30 years, while the admixture with maize-derived xylose was much higher.

→ *We agree that the proportions of ara and xyl, as well as the other sugars in soil depends on the vegetation. We still do not think that turnover of ara is substantially influenced by stabilization, as serveral studies (Basler et al., 2015; Coelho et al., 1988;) show that arabinose is substantially influenced by the microbial biomass. We do not entirely exclude stabilization, though the results also of our other study show that in influence by recycling to ara seems to be much higher.*

Furthermore, if I understand right, the authors have a second MS under review in BGD also focussing on stabilisation versus recycling of soil sugars. Hence, in order to increase the impact of their papers, it may be advantageous to publish both papers as companion papers with a) similar titles, e.g.:

1) Recycling vs. stabilisation of soil sugars sugars –: an underestimated process controlling soil carbon dynamics II – I) a natural 30 yrs old labelling field experiment

2) Recycling vs. stabilisation of soil sugars –: an underestimated process controlling soil carbon dynamics II) a long-term laboratory incubation

experiment

b) establishing clear links between these two papers. So far, this is unfortunately not done at all..

→*Thanks for this suggestion, we followed your advice and renamed our manuscripts.*

1) *Microbial carbon recycling: an underestimated process controlling soil carbon dynamics I) a long-term laboratory incubation experiment*

2) *Microbial carbon recycling: an underestimated process controlling soil carbon dynamics II) a C3-C4 vegetation change field labelling experiment*

Minor issues:

- The sugar analyses are performed (in contrast to Amelung et al., 1996) with Serdolit.

Is there a reason why you did not use XAD resin as in the original procedure?

→*At the time we started our experiment the AMBERLITE® XAD-7 resin was not shippable in the laboratory equipment shops and thus we used an alternative product, the Serdolit PADIV, which was advertised as an alternative resin for the XAD7.*

- When emphasizing the importance of recycling dynamics, position-specific d13C differences/methods (co-author M.D. is well known for her excellent expertise on this field) are or will at least soon become of high importance. Hence, the readers will profit from one or two respective sentences and references (maybe in a Conclusion and Outlook chapter)

> *→We included a brief outlook on the perspectives of position-specific labeling in the manuscript.*

- Fig. 1: Please specify what for light/dark grey bars stand for (I guess you mean Ap and E horizons, respectively)

→ We apologize for this error, and we have corrected the figure. The light grey presents the Ap horizon and dark grey the E-horizon.

- Table 1: The carbon contents of the POM fractions seem to be very/too low, please check and correct if necessary.

→The given values for the carbon content are correct according to our measurements. The very low numbers result from the fact that the investigated arable soil generally contains only low amounts of POM (see e.g. John et al. (2005)or Helfrich et al. (2006)). Additionally, sampling had taken place early in spring (April) - the season when plant growth had just started and therefore, amounts of POM were generally low.

Interactie comment on "Microbial carbon recycling: an underestimated process controlling soil carbon dynamics" by A. Basler et al.

Anonymous Referee #4

Received and published: 20 August 2015

For the editor/authors,

This is a review of the manuscript titled "Microbial carbon recycling: an underestimated process controlling soil carbon dynamics". The work presented in this paper nicely compares mean residence time (MRT) and the chemical composition of different fractions of soil organic matter (SOM). The authors present a useful framework for thinking about SOM turnover in terms of stabilization versus recycling processes occurring soils. They demonstrate this framework using sugars. I think this manuscript is ready for publication pending some minor revisions. My comments mainly revolve around how the authors frame their study (in the introduction), and how they synthesize their results (in the discussion).

I would like to see more information in the Introduction that compares and contrasts the authors' stabilization/recycling dynamics with other work that talks about physical protection, microbial access, and chemical recalcitrance as processes controlling SOM turnover.

→ *To our knowledge, there is no attempt to quantify the importance of stabilization or recycling to soil C turnover (and we do not see how it could be done). The basic studies reviewing the mechanisms of C stabilization (von Luetzow et al., 2006; Sollins et al., 1996) do not mention the fact that recycling may affect many studies on stabilization mechanisms and can hardly be distinguished from stabilization of "unmodified" molecules. In addition, most of the work about physical protection focuses on the mechanisms but this is not the scope of our manuscript. Literature proofing the relevance of recycling is – to our knowledge - only available from sediment investigations (e.g. Takano et al. (2010)), which we now cited in the introduction. However, any transfer of these results gained from intact polar lipids in marine sediments on sugar dynamic in topsoils is hardly possible and soil literature on that topic is still absent.*

I think the authors' framework dovetails nicely with existing literature, but this is not clear the way it is written.

Second, I think the authors could do a better job synthesizing their results in both the context of their stated hypotheses, as well as existing theory. I have more detailed comments below.

Abstract

Page 9730, lines 9-11: First word of sentence needs to be capitalized. Also, perhaps I'm missing something here but it seems like this reason doesn't follow if it's a cycle? After reading the rest of the abstract I get what you are saying, but this sentence was rather confusing the first time through.

→ *We rephrased the sentence.*

Page 9730, Line 15: Be more specific here, what kinds of sugars?

→*We specified the sugars as "neutral".*

Introduction

I do like casting this issue in terms of stabilization versus recycling of OSM. However, there are lots of hypotheses out there that use different language/words but are in essential agreement. I feel like you could do little more to put stabilization/recycling in context. Talking about physical protection, chemical recalcitrance, and accessibility is good start, but I think you need to expand on this topic a bit.

→*We added some more details to the first paragraph in the introduction as suggested by the referee.*

Page 9731, Lines 1-2: You need some literature references here if you are going to establish this as a paradigm in your narrative.

→*We added a respective reference.*

In the last paragraph of the introduction it seems like you are defining a system where plant-derived sugars are not subject to recycling. Therefore, by definition almost, microbial-derived sugars will be more affected by recycling processes. You need to clarify what, if any pathways exist for recycling of plant-derived sugars. My apologies if this information is there and I just missed it.

→*We use the term plant derived sugar in the sense that these sugars are synthesized by plants. This is opposed to microbial sugars that are synthesized by the microbial biomass. If microbial biomass takes up plant sugars and reuses these (altered or unaltered) they would be counted as microbial sugars.*

Results

Page 9737, Lines 9-11: These data on sugar-C related to total C in oPOM seem to figure prominently in the abstract, they should be presented explicitly, in some fashion, in this section (putting data not shown is not acceptable)

.→*In the abstract we primarily focus on the MRT of sugars and bulk carbon in the oPOM fractions and these data are shown in Table 2. The contribution of sugar C to total C in the respective fractions is of lesser interest, therefore we decided not to shown the data in detail.*

Page 9737, Lines 14-18: I'm not an expert on sugars in plants and soils, so it's not clear to me that there is a standard set of sugars that are only found in plants and not microbes. Could you add some information on what sugars are typically used to differentiate between plant and microbial inputs, as well as how you determined, in your system, which sugars were plant-derived and vice-versa?

→ *Soil sugars are commonly divided in plant and microbial derived sugars; we mentioned that point in the second paragraph in our introduction. In general, arabinose and xylose are plant derived and fucose, rhamnose, galactose and mannose*

are microbial derived sugars. However, this classification should be considered with caution, as our results indicate. Several studies (Basler et al., 2015; Coelho et al., 1988; Muramaya, 1988; Cheshire et al., 1976) show that arabinose and xylose could be synthesized by microorganism. In addition, this point is also part of our discussion.

Page 9738, Lines 25-27: I don't see the data on the contribution of maize to the extractable C anywhere in the paper. Perhaps I missed it?

→We apologize; we missed to add a reference to Figure 2. We changed this, to facilitate a better traceability of our data.

Discussion

Restructure the discussion so that you are synthesizing, not just repeating, results. This happens throughout this section, but is particularly evident in the first part of the first paragraph of this section. Also, simply stating that your findings agree with those of others is not adequate synthesis.

→ We fully agree that pure repetition is not nice writing style for the discussion. However, we think it is helpful to repeat the data, especially if putting them into context with other studies. Showing concordance with results of other studies may convince the reader that this work is no singularity and will enable to bring our data into a larger context. Therefore, we restructured and shortened these sentences, but did not delete the data comparison with literature. .

There seems to be differences in how sugars are referred to throughout the paper. In some places abbreviations are used, but not in others. For those not familiar with the abbreviated names of these sugars, using the full name would reduce confusion.

→ We apologize for the inconsistency. We now use the abbreviations throughout the paper, which are first explained in the introduction.

Page 9740: I would like to see more discussion on how SOM fraction quantity and MRT support existing aggregate hierarchy hypothesis. You present these two findings separately in the discussion, but they actually complement one another quite well, and if discussed together would present a nice synthesis.

→We do agree with the reviewer that the data on SOM fraction quantiy supports the aggregate hierarchy hypothesis. This is also made clear in the discussion, however we would prefer not to dwell on this too much as it has been shown before (e.g. John et al. (2005)and we cannot add any novel information to this. Our novel contribution is the fact that the sugar and microbial biomass dynamics also are in concordance with this concept and therefore we prefer to focus on this.

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Interactive comment on "Microbial carbon recycling: an underestimated process controlling soil carbon dynamics" by A. Basler et al.

Anonymous Referee #3

Received and published: 21 August 2015

The submitted manuscript addresses the age of C in sugar, and discuss it as a consequence of microbial recycling and stabilisation, depending on sugar nature. This topic is very interesting and within the scope of the Journal. The authors benefit from a nice experimental device to address their question and realised a lot of demanding analyses. But at this stage, I consider that the MS is not acceptable for publication.

The first major issue is to clarify what is the MRT of sugar. A mean residence time is the average time during which something resides in a pool. The authors indicate they want to assess the MRT of sugar (presumably in soil or soil fraction). However, this is cannot be achieved based on a C3C4 device!

The obtained data can only help to assess the MRT of C in a given pool, not the MRT of individual molecule in any pool. In addition, the choice of a single pool model only allows estimating the MRT of C in bulk soil, or in plant fraction (fPOM). To assess MRT in aggregate fraction, it is necessary to take into account the delay prior to incorporation C in the fraction, when it resides in another fraction. I recommend that the authors rework their rational and focus on the proportion of new C instead of making an attempt to provide non-rigorous and incorrect MRT values. A study inspecting new C incorporation in so many fractions would provide great results to the community!

> → *We agree with this comment. We have to apologize that we were not explicit enough to stress that we always refer (and discuss) the MRT of sugar C, not the MRT of the sugar molecules. This was clarified throughout the text and explicitly in the M&M section 2.6*

The second major issue is to related to the lack of methodological details and to the fact that raw results of C isotope composition in individual molecule are not provided.

> → *The mean isotope values of all sugar measurement are given in the supplement.*

Sugar 13C analysis in a soil matrix by HPLC-IRMS is very challenging, results are generally associated with a 1 permil uncertainty. I recommend the authors to prepare a table with the bulk data set, including uncertainty.

> →*In Basler and Dyckmans (2013), we could show that the HPLC -IRMS method yields more accurate results than the GC-C-IRMS methods. The uncertainty are <0.66‰ if the amounts are >2.5nM. Due to their small occurrence in soil especially fuc and rha show higher uncertainties. The mean isotopic values and uncertainties are given in the supplement Table1.*

In the M&M section, they should write the equation of errors propagation in the calculation of maize derived C.

> →*We added information on the magnitude of the error for maize contribution but we do not think that the equation to compute these is of interest, as it can easily be derived.*

The data from the control treatment that are used to compute the proportion of new C should also be provided, and possibly discussed as interesting findings may arise from them.(values of individual sugar in individual fractions for the C3 control plot).

→*We agree that the data of the control treatment might be interesting and worth a discussion. However, this is not the scope of this manuscript and would probably make the story unfocussed and lengthy.*

The third major issue is related to the discussion on sugar recycling or stabilisation. It cannot be done without considering the plant input: the study should provide the wheat and maize molecular composition.

→*The requested data is given in Table 1.*

Especially, mannose could be important in mannan hemicellulose. The authors could also again discuss what they expect as cellulosic glucose contribution and how its C may be stabilised in the different fractions.

→*We used the method of Amelung et al. (1996) for sugar extraction which is most suitable for non-cellulosic sugars. Cellulose, in contrast is more efficiently extracted by the H2SO4-method. As our focus were the non-cellulosic sugars, we refrain from discussing amounts of glucose in any pool.*

Minor comments

Indicate in the M&M section that you sample two horizons.

→*In the section 2.1 study site we mentioned that we sample the Ap and E-horizon.*

Explain the colours in Fig1

→*Thank you, we have corrected the figure.*

In Tab1, the amount of total C in the first column should be given in the same unit than sugar C (per g of fraction)

→*We believe that the data given (although different units are used in the Table) are the most suitable to enable the reader to gain an overview on carbon distribution in the soil and soil fractions. We therefore prefer to leave the table unchanged.*

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Microbial carbon recycling: an underestimated process controlling soil carbon dynamics. Part II) ~~a natural 30 yrs old labelling fielda~~ C_3-C_4 vegetation change field labelling experiment

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Abstract

The mean residence times (MRT) of different compound classes of soil organic matter (SOM) do not match their inherent recalcitrance to decomposition. One reason for this is the stabilisation within the soil matrix, but recycling, i.e. the reuse of "old" organic material to form new biomass may also play a role as it uncouples the residence times of organic matter from the lifetime of discrete molecules in soil.

We analysed soil sugar dynamics in a natural 30 years old labelling experiment after a wheat-maize vegetation change to determine the extent of recycling and stabilisation by assessing differences in turnover dynamics between ~~in~~ plant and microbial derived sugars: While plant derived sugars are only affected by stabilisation processes, microbial sugars may be subject to both, stabilisation and recycling. To disentangle the dynamics of soil sugars, we separated different density fractions (free particulate organic matter (fPOM), light occluded particulate organic matter (≤1.6 g cm^{-3}; $oPOM_{1.6}$), dense occluded particulate organic matter (≤2 g cm^{-3}; $oPOM_2$) and mineral-associated organic matter (>2 g cm^{-3}; Mineral)) of a silty loam under long term wheat and maize cultivation. The isotopic signature of neutral sugars was measured by high pressure liquid chromatography coupled to isotope ratio mass spectrometry (HPLC/IRMS), after hydrolysis with 4 M Trifluoroacetic acid (TFA).

While apparent mean residence times (MRT) of sugars were comparable to total organic carbon in the bulk soil and mineral fraction, the apparent MRT of sugar carbon~~s~~ in the oPOM fractions were considerably lower than those of the total carbon of these fractions. This indicates that oPOM formation was fuelled by microbial activity feeding on new plant input. In the bulk soil, ~~mean residence times~~MRT of the mainly plant derived xylose ~~(xyl)~~ were significantly lower than those of mainly microbial derived sugars like galactose ~~(gal)~~, rhamnose ~~(rha)~~, fucose ~~(fuc)~~, indicating that recycling of organic matter is an important factor regulating organic matter dynamics in soil.

1 Introduction

For several decades, it was assumed that the molecular structure accounts for the rate of decomposition of different organic compounds in soils~~)~~, i.e. compounds of high chemical recalcitrance were assumed to be selectively preserved (Stevenson, 1994)~~)~~. However, the use of compound specific isotope analysis provided new understanding of soil organic matter (SOM) dynamics. As an example, lignin, a compound of high chemical recalcitrance, has shorter mean residence times (MRT) than labile compounds like sugars or proteins (Amelung et al., 2008; Gleixner et al., 2002; Kiem and Kögel-Knabner, 2003; Schmidt et al., 2011). The main ~~M~~mechanisms for the long persistence of these labile compounds in soil are stabilisation on the one hand, i.e. protection of organic matter from mineralization either by ~~the~~ reduced accessibility for microorganisms caused by physical protection (by mineral interaction or occlusion within soil aggregates) or chemical recalcitrance (Six et al., 2002; Sollins et al., 1996; von L~~ue~~tzow et al., 2006), and microbial recycling on the other, i.e. the reuse of "old" organic compounds by microorganisms (Gleixner et al., 2002; Sauheitl et al., 2005). The latter leads to an underestimation of the actual turnover dynamics but overestimates the persistence of single molecules as a whole within the ~~soil organic matter~~SOM. Although these different underlying mechanisms ~~underlying the long MRT~~ have been proposed quite a while ago, their relevance in different soils and soil horizons, especially concerning the importance of stabilisation versus microbial recycling, still remain unclear. First studies on polar membrane lipids of microorganisms in marine sediments suggest a strong underestimation of recycling in our current view on ~~C~~carbon dynamics in soils and sediments (Takano et al. 2010). However, knowledge about soils especially microbially active topsoils are still missing.

Therefore, ~~A~~assessing the importance of stabilisation and recycling for the persistence of organic matter in soil will improve the understanding of the carbon cycle and close an important knowledge gap.

However, the pool of SOM is highly complex and intractable to analyse as a whole. Thus, we examined the fate of sugars; an important compound class of the SOM that is involved in almost all biological processes in soils, the MRT of which do not match their low biochemical recalcitrance (Gleixner et al., 2002; Derrien et al., 2006; Derrien et al., 2007). Sugars in soils are commonly classified according to their main origin into plant (arabinose (ara), xylose(xyl)) or microbial derived sugars (galactose (gal), mannose (man), rhamnose (rha), fucose (fuc)) (Oades, 1984; Moers et al., 1990). While turnover dynamics of plant derived

sugars should mainly be governed by stabilisation processes, the turnover dynamics of microbial sugars may be influenced by both, stabilisation and recycling.

The MRT of ~~sugars and bulk~~bulk and sugar carbon were examined in density fractions to elucidate turnover dynamics in SOM pools with different degrees of degradation and protection. While free particulate organic matter (fPOM) represents an only partly degraded SOM pool with fast turnover, occluded particulate organic matter (oPOM) and mineral associated organic matter correspond to pools that are more preserved from microbial attacks and show slow turnover (John et al., 2005; Golchin et al., 1994b). The study was made on a field experiment located in Rotthalmünster with natural ^{13}C labelling by a vegetation change from C3 (wheat) to C4 vegetation (maize).

We hypothesise that ~~mean residence times~~MRT of plant and microbial sugar ~~C~~carbon~~s~~ will be different as the mechanisms controlling their turnover dynamics are different: turnover of microbial derived sugars should be mainly ruled by recycling whereas the turnover of plant derived sugars is ruled by stabilisation.

2 Materials and Methods

2.1 Study Site

Soil samples were collected from the long-term field experiment at "Höhere Landbauschule" Rotthalmünster, Bavaria, Germany (N 48° 21' 47'', E 13° 11' 46''). The mean annual temperature is 9.2 °C and the mean annual precipitation is 757 mm. Soil samples (Ap-horizon & E-horizon) were taken in April 2011 from (i) a continuous maize plot (*Zea mays L.*) established in 1979 on a former grassland plot until 1970 followed by wheat cultivation until 1978 and (ii) a continuous wheat plot (*Triticum aestivum L.*) established in 1969. Previous vegetation on the wheat plot was grassland. The soil at the two sites was classified as a stagnic Luvisol (IUSS Working Group WRB, 2014), derived from loess. Soil texture is silty loam (11% sand, 73% silt, 16% clay). More details about the soil properties can be found in John et al. (2005) and Ludwig et al. (2005).

2.2 Density fractionation

Density fractionation of soil was performed according to John et al. (2005). Briefly, 10 g of soil were weighed into a 50 mL centrifuge tube and filled with 40 mL 1.6 g cm^{-3} sodium polytungstatesolution (SPT, Sometu, Berlin). The tube was gently shaken 5 times by hand and allowed to settle for 30 min. Afterwards the solution was centrifuged for 40 min at 3700 rpm. The supernatant including floating materials was filtered with polyamide membrane filters (0.45 µm, Sartorius Göttingen) using vacuum and washed with distilled water to gain the fPOM. Residual soil was re-suspended in 25 ml SPT (1.6 g cm^{-3}) and 18 glass pearls (4 mm diameter) were added, the solution was then shaken for 16 hours at 60 movements per minute to break up the aggregates. Subsequently, the solution was centrifuged 40 min at 3700 rpm, vacuum filtered (0.45 µm) and washed with distilled water to obtain the occluded particulate organic matter ($oPOM_{1.6}$). The residual soil was re-suspended with 25 mL SPT using a density of 2 g cm^{-3}, shaken for 10 min at 100 rpm and centrifuged (40 min at 3700 rpm). To obtain the occluded particulate organic matter with a density of 1.6-2 g cm^{-3} ($oPOM_2$), the supernatant was vacuum-filtered and washed with distilled water. The remaining fraction (Mineral) was washed three times with 20 mL water to remove SPT. Each time, the sample was centrifuged and the supernatant discarded. All fractions were dried at 40 °C.

2.3 Sugar analysis

Sugars were extracted and purified using a modified procedure based on Amelung et al. (1996) and Amelung and Zhang; (2001). For extraction, sub-samples containing approximately 0.5-5 µg C (depending on the availability of the respective fraction) were hydrolysed with 10 mL 4 M TFA at 105 °C for 4 hours. Afterwards the samples were filtered through a glass fibre filter (Minisart GF, Sartorius, Göttingen, Germany) and dried by rotary evaporation (40 °C, 50 hPa). In contrast to Amelung et al. (1996), the pre-dried samples were re-dissolved in 0.5 mL water and evaporated to dryness for 3 times to remove all traces of TFA (which impedes chromatographic separation, see Basler and Dyckmans (2013)). Then, the samples were re-dissolved in approximately 3 mL water and passed through 4 g Dowex X8 cation exchange resin (Sigma Aldrich, Steinheim, Germany) and 5 g Serdolit PAD IV adsorption resin (Serva Electrophoresis GmbH, Heidelberg, Germany) for purification. Sugars were eluted by adding 8 times 2 mL water. The eluate was freeze-dried and stored at -18 °C

until analysis. For HPLC-IRMS analysis the samples were dissolved in 3 mL water and transferred into measurement vials.

The TFA extraction method is known to effectively extract hemi-cellulosic sugars (Amelung et al., 1996) but cellulose is not cleaved by this method. The results presented here thus only refer to non-cellulosic sugars and substantially underestimate the total sugar contribution of plants SOM.

2.4 Isotopic analysis

Isotopic composition and total carbon content of plant material, bulk soil and density fractions was analysed by EA-IRMS. The compound specific isotope analysis of the monosaccharides was performed using a high-pressure liquid chromatography system (Sykam, Fürstenfeldbruck, Germany) coupled to an isotope ratio mass spectrometer (Delta V Advantage, Thermo Scientific, Bremen, Germany) via an interface (LC-Isolink, Thermo Scientific, Bremen, Germany) as described by Basler and Dyckmans (2013). Shortly, the chromatographic column (Carbo Pac 20, Dionex) was held at 10 °C and a 0.25 mM NaOH solution was used as mobile phase at a flow rate of 250 µL min^{-1}.

2.5 Chloroform-Fumigation-Extraction

Microbial Biomass (C_{mic}) was determined by the fumigation extraction method (Brookes et al., 1985; Vance et al., 1987). K_2SO_4 concentrations were adapted for isotopic analysis (Engelking et al., 2008). Briefly, a sub-sample of 20 g moist soil was separated into two portions of 10 g. One soil sub-sample was directly extracted as described below. One portion was placed in a desiccator with ethanol free CH_3Cl at 25 °C for 24 h. For extraction, soil samples were shaken with 60 mL 0.05 M K_2SO_4 for one hour and subsequently filtered (Whatman 595 ½). The dissolved organic carbon was analysed using a TOC analyser multi C/N® 2000 (Analytik Jena, Jena, Germany). For stable isotope measurements freeze-dried aliquots were analysed by EA-IRMS. The isotopic signature of the microbial biomass was calculated as follows:

$$\delta^{13}C_{mic} = \frac{(\delta^{13}C_F \cdot C_F) - (\delta^{13}C_{nF} \cdot C_{nF})}{(C_F - C_{nF})} \quad (1)$$

where $\delta^{13}C_F$ and $\delta^{13}C_{nF}$ are the isotopic signatures of the fumigated and non-fumigated extracts and C_F and C_{nF} are the extracted carbon content [mg kg^{-1}] of the fumigated and non-fumigated soil samples. Carbon extracted from non-fumigated samples represents the K_2SO_4 extractable C fraction (exC).

2.6 Estimations of maize-derived carbon and turnover times

Under the assumption that the maize and wheat sites have a similar history and similar C dynamics and fractionation during decomposition is comparable for wheat and maize plant material., the proportion of maize-derived carbon in bulk soil and density fractions was calculated according to (Balesdent and Mariotti, 1996; Derrien et al. 2006):

$$f = \frac{(\delta_{sample} - \delta_{reference})}{(\delta_{maize} - \delta_{wheat})} \quad (2)$$

where f is the relative proportion of maize-derived carbon, δ_{sample} is the $\delta^{13}C$ value of the maize plot sample, $\delta_{refernce}$ presents the measured ^{13}C value of the corresponding wheat plot samples, and δ_{maize} and δ_{wheat} are the ^{13}C values of the crop residues of maize (-13.2‰) and wheat (-27.5‰). The resulting difference of 14.3 between wheat and maize plants was used for all fractions (bulk material and individual sugars) because the determination especially of mainly microbial derived sugars in plant material was very difficult.

The error of maize contribution percentage calculated from error propagation was below 10 % for all samples, which is in the range of the standard error calculated from the replications.

Assuming steady state conditions and homogeneous soil fractions which can be described with a single pool model (Six and Jastrow, 2002), the MRT is calculated according to Derrien and Amelung (2011):

$$MRT = \frac{1}{k} \quad (3),$$

Where the time constant *k* is calculated from the following equation:

$$f = 1 - \exp^{(-kt)} \quad (4)$$

where t is the time of maize cultivation.

Since conditions like fertilization and C contents of the soil remained about the same after the change from C3 to C4 vegetation, and the conversion was from other cereal crops (wheat) to maize, which are very similar with respect to biochemical nature, soil inputs, location of soil inputs, decay rates and decay products, the system approximates a steady-state system (Balesdent and Mariotti, 1996) as required. It is well known that the assumption that MRT of soil organic carbon can be described by a single pool model is a rough simplification since it is a complex mixture of ~~soil organic matter~~SOM with different stability and turnover even if~~.~~ ~~However,~~ the isolated soil fractions are one step towards homogeneity, especially concerning POM fractions. ~~Still, the Mineral remains heterogeneous.~~ Therefore, we used the term "apparent" MRT ~~instead of (actual) MRT~~. In addition it has to be noted that we refer to the MRT of the carbon in individual molecules and not of the intact molecules as a whole.

2.7 Statistical analysis

Analysis of Variance (ANOVA) with ensuing Post-hoc test (Tukey) were conducted to detect differences among the sugars within a soil fraction (bulk soil, density fractions) and among individual sugars of different soil fractions. Statistical analysis were made using R 3.0.2 (R Core Team, 2013).

3 Results

3.1 Carbon and sugar content in soil, density fractionations and plant material

The recovery of carbon after density fractionation of the wheat and maize plots was about 90% in the Ap-horizon and about 86% in the E-horizon. Between 79 and 89% of total recovered carbon was found in the Mineral in the investigated soils (Fig. 1). The $oPOM_2$ fraction accounted for 7 and 10% of the carbon found in the Ap-horizon and for 4 and 9% in the E-horizon of the wheat and maize plot, respectively. Less carbon was found in the $oPOM_{1.6}$ fractions (between 3 and 5%) and the free particulate organic matter (fPOM; 2-3%). The contribution of sugar carbon to total carbon in $oPOM_{1.6}$ was between 5 and 8%. Higher contributions were observed in the $oPOM_2$ with 11 to 15% (data not shown). The general sugar distribution in the bulk soil fraction was glc>gal>man=ara=xyl>rha>fuc and was slightly different in the POM fractions, where ara and xyl occurred in higher proportions than gal and man (Table 1).

In the plant, sugars were dominated by xyl with ~~ca.~~about 44 mg C g^{-1} (wheat) and 30 mg C g^{-1} (maize), followed by ara and glc with ~~ca.~~about 8 mg C g^{-1} (wheat) and 6 mg C g^{-1} (Table 1). The other sugars each contributed 4 mg C g^{-1} or less. The extracted sugars accounted for 20% and 8% of total carbon and in the wheat and maize plants, respectively.

3.2 Contribution of maize-derived Carbon to the sugars in different soil fractions

In general, the contribution of maize-derived carbon in the varying density fractions decreased in the order fPOM>oPOM$_2$>Mineral>oPOM$_{1.6}$. The proportion of maize-derived carbon in bulk soil was around 40% in the Ap and 30% in the E-horizon (Fig. 2). The apparent MRT of carbon calculated from this data ranged between 25 (fPOM, Ap) and 119 (oPOM$_{1.6}$, E) years (Table 2). The contribution of maize to the ~~extractable carbon~~exC was within the range of the bulk soil, whereas the proportion of maize in ~~microbial biomass~~Cmic was twice as high as in the bulk soil (Fig. 2). The proportions of maize-derived carbon in individual sugars showed a distinct pattern (Fig.2): In the bulk soil, the highest proportion of maize-derived carbon was observed in xyl (~70% in Ap, 56% in E). The other sugars showed maize-derived carbon proportions in the range of the bulk soil of about 37% in Ap and 30% in E with the exception of ara, fuc and gal in E with only 25% maize contribution. Bulk fPOM had maize contributions of 88 and 78% in the Ap and E-horizon, respectively. Maize contribution for all sugars in both horizons was close to 100% and thus the fPOM fraction was not evaluated further. In the oPOM$_{1.6}$ fraction, the proportions of maize-derived carbon of individual sugars were two or three times higher than for total carbon in this fraction (Fig. 2A and B). In the oPOM$_{1.6}$ fraction of Ap, xyl and man showed the highest percentages (~85%) of maize-derived carbon, followed by glc (77%) and ara, rha and gal (about 50%). The lowest percentage of maize-derived carbon was found for fuc (~30%) in the Ap-horizon. In the E horizon, all sugars contained about 55% maize-derived C and showed no significant differences ($p<0.05$), but there was still a trend towards higher percentages of maize-derived carbon in xyl and man as compared to the other sugars.

In the oPOM$_2$ fraction, the highest percentages of maize-derived carbon in the sugars of all fractions were observed with about 77% and 65% in the Ap and E-horizon. In the oPOM$_2$ fraction no significant difference in maize contribution among the sugars was observed ($p<0.05$) in both horizons, but a trend of higher values for xyl (88%) and lower values for rha (58%) were found for the Ap-horizon (Fig. 2).

In the Mineral, the percentages of maize-derived carbon in the Ap-horizon showed no significant difference to the bulk soil fraction and amounted about 52% of maize-derived carbon. Xylose showed the highest values with 66% and man and ara showed the smallest percentages (44%). In the Mineral of the E-horizon, the maize percentages were about 37% and showed no significant difference to the bulk soil (Fig.2). Xyl and man showed the highest percentages (~50%) of maize-derived carbon, followed by ara, glc, fuc and gal with about 25%. The calculated ~~apparent~~ MRT for the sugar carbon~~s~~ in density fractions (Table 2) showed values from 14 years (xyl in $oPOM_2$ Ap-horizon) to 152 years (man, in bulk soil E-horizon).

4 Discussion

Carbon content increased with decreasing density of the fractions concomitant with decreasing organo-mineral associations, similar to earlier findings on the same (John et al., 2005) and other soils (Baisden et al., 2002; Golchin et al., 1994a). The fPOM fraction~~s~~ contained ~~contributed around~~ between 2% and 8 % of total carbon and the major part (86%) were found in the Mineral fraction ~~to total soil carbon, 4% and 8% of total carbon were found in the in $oPOM_{1.6}$ and $oPOM_2$, respectively. and t~~The major part ~~of soil organic carbon was located in the Mineral fraction (86%).~~ The relative contribution of sugars to bulk carbon was 8% in the Ap-horizon and around 7% in the E horizon in agreement with values reported by Cheshire (1979), Derrien et al. (2006) and Guggenberger et al. (1994). The proportions of sugar carbon in the POM fractions decreased in the order $oPOM_2$>fPOM>$oPOM_{1.6}$ in both horizons. This corroborates the ^{13}C NMR analysis on the same soil, which revealed decreasing O-alkyl carbon content (representing e.g. sugars) in $oPOM_{1.6}$ as compared to $oPOM_2$, whereas alky-carbon content (representing lipids, fatty acids, plant aliphatic polymers) increased (Helfrich et al., 2006). The ratio of alkyl to O-alkyl carbon has been reported to provide an indicator of decomposition, as O-alkyl carbon rich substances are more easily accessible and thus preferentially decomposed and more recalcitrant compounds accumulate (Golchin et al., 1994b; Baldock et al., 1997). Consequently, the higher sugar contribution in $oPOM_2$ as compared to $oPOM_{1.6}$ probably indicates a higher degree of decomposition in the $oPOM_{1.6}$ fraction. This supports the concept of Golchin et al. (1994a), who suggest that the fresh, carbohydrate rich POM is utilised by microorganisms with concurrent increase of organo-mineral associations ($\rightarrow$ $oPOM_2$) and the formation of

aggregates. Within the aggregates, decomposition proceeds and labile compounds become more and more depleted. In turn, microbial activity decreases and less binding agents are produced and binding to mineral particles is decreased (decreased density → $oPOM_{1.6}$). Due to reduced microbial activity and decreasing production of binding agents the aggregates become unstable and finally disrupt, and new aggregates may develop if fresh plant or microbial debris is available to fuel microbial activity.

In the density fractions the apparent MRT of bulk carbon increased in the order fPOM<$oPOM_2$<Mineral<$oPOM_{1.6}$ in both soil depths, which is in line with studies by John et al. (2005) and Rethemeyer et al. (2005) on the same soil and corroborates the concept of Golchin et al. (1994a) of the aggregate hierarchy described above. Although the $oPOM_{1.6}$ fraction had the highest proportion of C3 carbon, the sugars in the $oPOM_{1.6}$ fractions were much younger than the bulk fraction, but in range with the $oPOM_2$ fraction and the microbial biomass. This indicates that the microbial activity leading to aggregate formation also in the "old" $oPOM_{1.6}$ fraction is fuelled from relatively fresh assimilates and shows the importance of microbial activity to form binding agents, as mentioned before by Oades (1984). Corroborating, the apparent MRT of sugar carbon~~s~~ in both oPOM fractions is comparable to the apparent MRT of the microbial biomass ~~C~~carbon in both soil horizons.

Man~~nose~~, as a microbial derived sugar showed considerably higher incorporation of maize-derived carbon similar to xyl in the oPOM fractions although the contribution of man by plants was very little. A possible explanation could be fungal activity, as it is known that fungi feed mainly on the recent vegetation (Hobbie et al., 2002; Kramer and Gleixner, 2006). Additionally, mannan, a mannose polymer, is abundant in exo-polysaccharides and cell walls of fungi (Osaku et al., 2002; Stribley and Read, 1974; Bowman and Free, 2006) and the involvement of fungal activity in soil aggregate formation was highlighted in several studies (Chenu, 1989; Caesar-Tonthat, 2002; Tisdall and Oades, 1982). In the oPOM fractions of the E-horizon (especially $oPOM_{1.6}$) man was much less influenced by maize-derived carbon compared to Ap; this may indicate a reduced importance of fungal activity to oPOM formation in the subsoil or at least no distinct allocation of maize-derived carbon through the hyphal network to the subsoil.

Xyl~~ose~~ had the highest percentages of maize-derived carbon in all soil fractions and depths, owing to the high input of xyl from plant material (mainly from hemicellulose). Additionally, root exudates provided a further small source of xyl as shown by Derrien et al. (2004) and, in

turn, roots and their exudates promote aggregate formation (Six et al., 2004; Oades, 1984). In contrast ara, which has also been described as mainly plant derived (Oades, 1984), showed smaller percentages of maize-derived carbon in all density fractions compared to xyl. One could assume that ara and xyl, as sugars of the same origin, were subject to the same dynamics, and more specifically, a similar mole ratio of ara to xyl in plants and oPOM was expected. In the oPOM fractions, however, the ratio of ara to xyl increased (as compared to the plants) and, in addition, the percentages of maize-derived carbon of ara were not significantly different from sugars derived mainly from microorganisms (fuc, rha and gal). This indicates that in our soil, ara and xyl dynamics are not ruled by the same factors. It has been shown that both sugars are highly abundant in plant material at a molar ratio of 1:3 (ara:xyl) or higher (Boschker et al., 2008; Glaser et al., 2000; Moers et al., 1990; Oades, 1984), however we found much less contribution of ara than xyl by both wheat and maize plants with a molar ratio of 1:5 (Table 1). On the other hand, both ara and xyl are produced by microbial biomass (Muramaya, 1988; Cheshire, 1977; Coelho et al., 1988; Basler et al.) and we therefore assume that in this study, ara was much more influenced by microbial production than xyl and its high mean age in $oPOM_{1.6}$ and $oPOM_2$ (28 to 48 years), was considerably influenced by microbial activity (and substrate recycling). This also indicates that the formation of oPOM fractions is predominantly based on microbial activity and not plant input in the first place. In contrast to ara, the dynamics of xyl were dominated by plant input and recycling seems to play a minor role.

Taken together the finding of substantially higher ~~apparent~~ MRT for ~~C~~carbon of microbial sugars (influenced by both, stabilisation and substrate recycling), compared to that of plant derived sugars (the turnover dynamics of which are dominated by stabilisation processes) indicates, that the mean age of SOM is strongly influenced by substrate recycling and that stabilisation processes do not play a dominant role for SOM dynamics.

5 Conclusion

This study provides new insight in the dynamics of soil sugars, as an important compound of SOM. Our data show that the reuse of organic matter is of high importance for soil sugar dynamics and is largely responsible for high MRT of sugar carbon~~s~~ in soil. Stabilisation processes on the other hand seem to play only a minor role for the persistence of sugars in soil, as only xyl dynamics were dominated by stabilisation. Moreover, we could show that

microbial activity fuelled by fresh organic matter plays an important role in aggregate formation, corroborate the concept of Golchin et al. (1994a). However, the mechanisms of recycling i.e. intact re-utilization versus intensive metabolization and incorporation in modified compounds remain unclear based on compound specific isotope analysis only. However, combining ~~CSIA~~compund specific isotope analysis with ~~the unique properties of~~ position-specific labeling ~~will enable~~ help disentangling the processes underlying the carbon recycling ~~of C~~ (Apostel et al. 2015, Dippold and Kuzyakov 2013~~,~~). Ultimately, our findings highlight the importance of recycling processes for SOM dynamics on the molecular as well as the aggregate level.

Acknowledgements

This research was funded by the Deutsche Forschungsgemeinschaft (DFG). We gratefully thank Reinhard Langel for his technical assistance and Iris Ficht and Viola Lauenstein for assistance in the laboratory.

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Table 1. Carbon content [mg C g $^{-1}$(dw)] and sugar content [mg C g^{-1}(dw)] in bulk soil, soil density fractions and wheat and maize plants. Latin letters (a-e) within one row indicate significant differences ($p<0.05$) among the different sugars within one fraction. Greek letters (α-δ) within one column indicate significant differences among different fractions for individual sugars. Means and standard error.

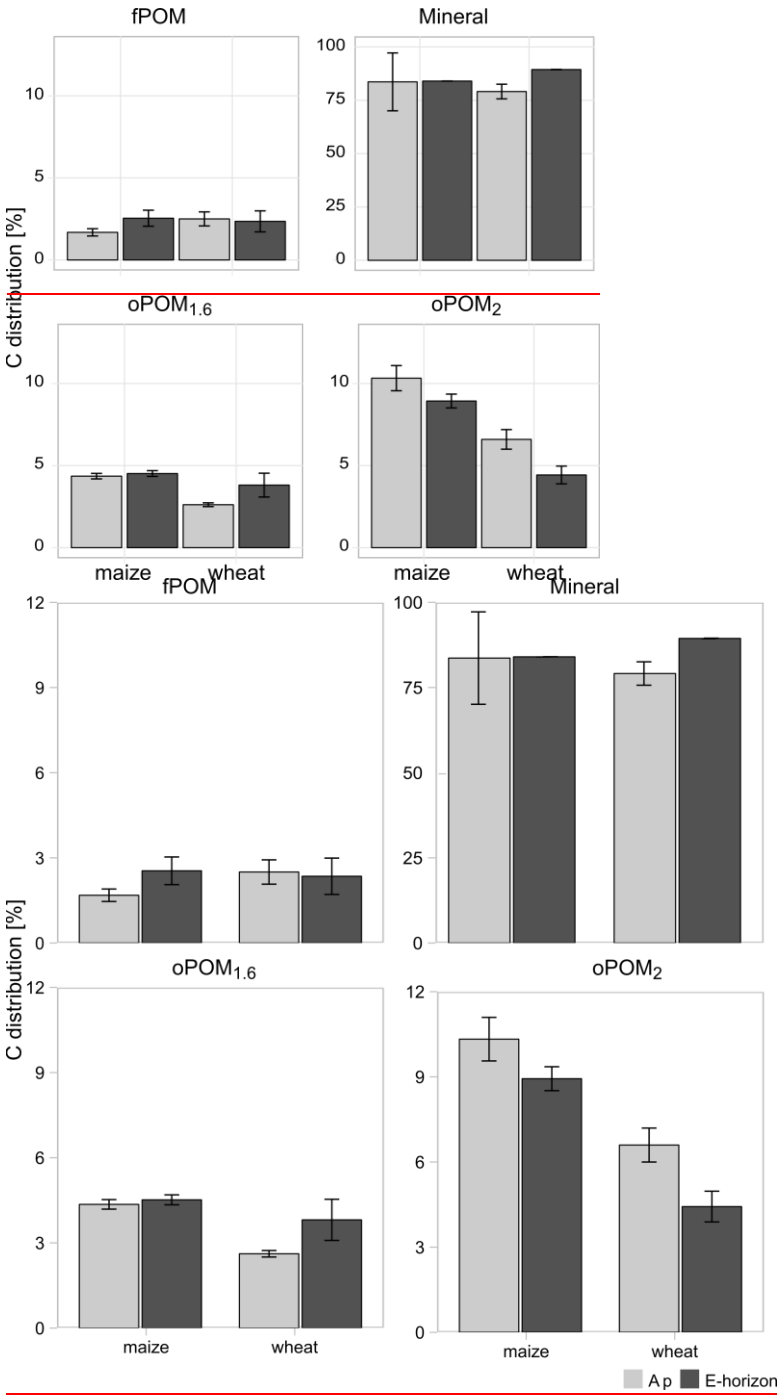
Fraction	Carbon		Fuc			Rha			Ara			Xyl			Glc			Gal			Man		
continious Wheat plot (Ap)	mg Cg^{-1} bulk		mg Cg^{-1} fraction																				
oPOM$_{1.6}$ (n=5)	0.28	±0.01	0.68	±0.27	αd	0.91	±0.28	α cd	2.68	±0.6	α ab	6.08	±1.4	α a	7.42	±1.95	α a	2.24	±0.51	α bc	2.23	±0.49	α bcd
oPOM$_{2}$ (n=5)	0.71	±0.06	0.37	±0.03	αc	1.39	±0.5	α bc	2.70	±0.1	α ab	5.83	±1.01	α a	6.37	±2.29	α a	1.85	±0.56	α ab	3.19	±0.84	α ab
mineral (n=3)	9.51	±1.02	0.05	±0.01	β d	0.09	±0.01	β cd	0.15	±0.01	β bc	0.15	±0.01	β b	0.26	±0.03	β a	0.18	±0.02	β ab	0.16	±0.05	β b
bulk (n=3)	12.06	±0.8	0.03	±0.00	β c	0.07	±0.00	β c	0.13	±0.02	β b	0.14	±0.01	β b	0.27	±0.27	β a	0.16	±0.00	β ab	0.14	±0	β b
continious Wheat plot (E)																							
oPOM$_{1.6}$ (n=5)	0.25	±0.05	0.42	±0.19	αb	0.60	±0.32	α b	2.38	±0.91	α ab	3.36	±1.25	α a	5.38	±1.87	α a	1.93	±0.85	α ab	2.06	±0.66	α ab
oPOM$_{2}$ (n=5)	0.29	±0.04	0.40	±0.09	αc	0.88	±0.23	α bc	1.90	±0.42	α abc	2.96	±0.7	α ab	4.39	±0.76	α a	1.90	±0.37	α abc	1.81	±0.34	α abc
mineral (n=3)	5.90	±0.01	0.03	±0.01	β d	0.08	±0.01	β cd	0.07	±0.01	β bc	0.07	±0	β cd	0.14	±0.01	β a	0.10	±0.01	β ab	0.09	±0.01	β b
bulk (n=3)	7.86	±0.26	0.03	±0.00	β e	0.04	±0.01	β de	0.07	±0.01	β bcd	0.06	±0.02	β cd	0.16	±0.02	βa	0.10	±0.02	β ab	0.08	±0.03	β bc
continious Maize plot (Ap)																							
oPOM$_{1.6}$ (n=5)	0.49	±0.02	0.76	±0.3	α e	1.17	±0.28	α c	3.22	±0.67	α b	5.85	±1	α a	8.61	±0.67	α a	3.04	±0.56	α a	2.90	±0.34	α b
oPOM$_{2}$ (n=5)	1.15	±0.09	0.50	±0.02	α c	0.86	±0.09	α cd	2.61	±0.36	α b	5.39	±1.17	αab	6.00	±0.33	β a	2.56	±0.22	α b	2.28	±0.23	α bc
mineral (n=3)	9.31	±1.51	0.03	±0.00	β e	0.09	±0.02	β bc	0.13	±0.03	β abc	0.13	±0.02	β ab	0.27	±0.05	γ a	0.17	±0.04	β ab	0.16	±0.03	β ab
bulk (n=3)	12.51	±0.38	0.04	±0.00	β f	0.09	±0.01	β d	0.15	±0.01	β c	0.16	±0.02	β bc	0.36	±0.02	γ a	0.20	±0.01	β ab	0.17	±0.01	β b
continious Maize plot (E)																							
oPOM$_{1.6}$ (n=5)	0.42	±0.02	0.46	±0.09	α c	0.92	±0.09	α e	3.63	±0.21	α bc	5.76	±0.93	α ab	9.44	±0.51	α a	3.25	±0.24	α cd	3.03	±0.17	α d
oPOM$_{2}$ (n=5)	0.82	±0.04	0.48	±0.02	α d	0.82	±0.08	α c	2.48	±0.34	β b	4.56	±0.9	α ab	5.42	±0.36	β a	2.47	±0.23	β b	2.09	±0.21	β b
mineral (n=3)	7.75	±0	0.03	±0.00	β c	0.08	±0.01	β d	0.07	±0	γ cd	0.10	±0.01	β dc	0.14	±0.01	γ a	0.07	±0.01	γ ab	0.09	±0.01	γ bc
bulk (n=3)	11.10	±0.24	0.04	±0.00	β d	0.07	±0.00	β ef	0.11	±0.01	δ cd	0.10	±0.01	β dc	0.25	±0.02	δ a	0.16	±0.02	δ a	0.13	±0.02	δ bc
Plants																							
wheat (n=9)	417.09	±28.33	n.d.			1.07	±0.73		7.57	±0.72		44.16	±4.66		8.82	±1.18		3.66	±0.5		1.89	±0.44	

maize (n=9)	431.93	±32.03	n.d.	1.14	±0.27	5.85	±0.52	29.66	±2.95	5.64	±0.41	2.88	±0.35	n.d.

Table 2. Calculated MRT of total ~~carbon~~ and individual sugar carbon ~~s~~ in density fractions and bulk soil. Means and standard error (n=4).

		MRT [years]						
fractions	horizon	bulk C	Ara	Xyl	Fuc	Rha	Gal	Man
$oPOM_{1.6}$	Ap	97 ± 3	44 ± 4	17 ± 3	65 ± 24	54 ± 6	39 ± 2	17 ± 2
	E	119 ± 6	48 ± 5	28 ± 4	55 ± 9	59 ± 15	51 ± 6	33 ± 3
$oPOM_2$	Ap	37 ± 6	28 ±4	14 ± 4	30 ± 4	43 ± 14	28 ± 4	30 ± 5
	E	61 ± 8	31 ± 2	23 ± 5	28 ± 2	37 ± 12	41 ± 9	37 ± 3
Mineral	Ap	64 ± 3	57 ± 6	29 ± 2	51 ± 4	48 ± 7	47 ± 7	58 ± 10
	E	98 ± 3	102 ± 11	45 ± 3	87 ± 7	60 ± 6	88 ± 7	54 ± 3
bulk soil	Ap	68 ± 4	80 ± 13	27 ± 1	77 ± 15	77 ± 22	71 ± 2	70 ± 11
	E	85 ± 4	115 ± 12	41 ± 8	104 ± 19	72 ± 8	138 ± 32	152 ± 32

Figure1. Organic Carbon distribution in the investigated density fractions. Means and standard error (n=5).



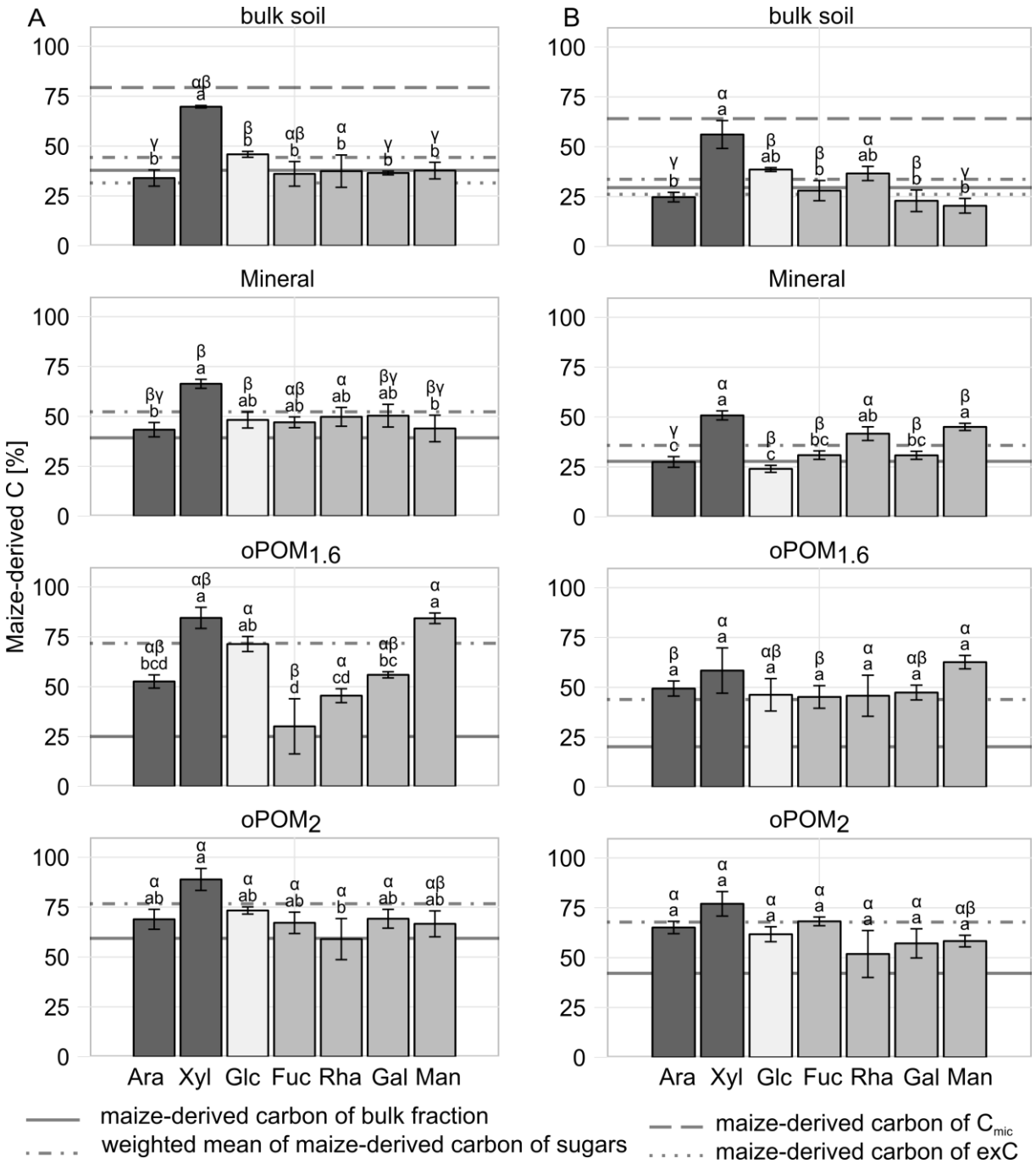


Figure 2. Maize contribution to sugars in bulk soil, Mineral, $oPOM_{1.6}$ and $oPOM_2$ fractions in the (A) Ap (0-30cm) and (B) E-horizon (30-45cm). Latin letters (a-d) indicate significant differences ($p<0.05$) among the individual sugars within one fraction. Greek letters (α-γ) indicate significant differences among different fractions for individual sugars. Means and standard error (n= 4).

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