Microbial carbon recycling: an underestimated process controlling soil carbon dynamics. Part II) a C_3-C_4 vegetation change field labelling experiment

A.Basler[1], M. Dippold[2], M.Helfrich[3], J.Dyckmans[1]

[1]{Centre for Stable Isotope Research and Analysis, Büsgen Institute, Georg-August-University Göttingen, Göttingen, Germany}

[2]{Department of Agricultural Soil Science, Georg-August-University Göttingen, Göttingen, Germany}

[3]{Thünen-Institute of Climate-Smart Agriculture, Braunschweig, Germany}

Correspondence to: A.Basler (abasler@gwdg.de)

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 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 ($\leq$1.6 g cm^{-3}; $oPOM_{1.6}$), dense occluded particulate organic matter ($\leq$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 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, MRT of the mainly plant derived xylose were significantly lower than those of mainly microbial derived sugars like galactose, rhamnose, fucose, 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 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 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 Lützow 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 SOM. Although these different underlying mechanisms 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 carbon dynamics in soils and sediments (Takano et al. 2010). However, knowledge about soils especially microbially active topsoils are still missing. Therefore, 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 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 MRT of plant and microbial sugar carbon 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 SOM with different stability and turnover even if the isolated soil fractions are one step towards homogeneity, especially concerning POM fractions. Therefore, we used the term "apparent" 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 about 44 mg C g^{-1} (wheat) and 30 mg C g^{-1} (maize), followed by ara and glc with 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 exC was within the range of the bulk soil, whereas the proportion of maize in 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 MRT for the sugar carbon 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 fractions contained between 2% and 8 % of total carbon and the major part (86%) were found in the Mineral fraction 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 $\rightarrow$ $oPOM_{1.6}$). 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 in both oPOM fractions is comparable to the apparent MRT of the microbial biomass carbon in both soil horizons.

Man, 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 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 MRT for 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 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 compund specific isotope analysis with position-specific labeling help disentangling the processes underlying the carbon recycling (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.

References

Amelung, W., Brodowski, S., Sandhage-Hofmann, A., and Bol, R.: Combining Biomarker with Stable Isotope Analyses for Assesing the Transformation and Turnover of Soil Organic Matter, in: Advances in Agronomy, Vol 100, Adv. Agron., Elsevier Acacemic Press Inc, 155–250, 2008.

Amelung, W., Cheshire, M. V., and Guggenberger, G.: Determination of neutral and acidic sugars in soil by capillary– gas-liquid chromatography after trifluoroacetic acid hydrolysis, Soil Biol. Biochem., 28, 1631–1639, 1996.

Amelung, W. and Zhang, X.: Determination of amino acid enantiomers in soils, Soil Biol. Biochem., 33, 553–562, 2001.

Apostel, C., Dippold, M., and Kuzyakov, Y.: Biochemistry of hexose and pentose transformations in soil analyzed by position-specific labeling and 13C-PLFA, Soil Biol. Biochem., 80, 199–208, doi:10.1016/j.soilbio.2014.09.005, 2015.

Baisden, W. T., Amundson, R., Cook, A. C., and Brenner, D. L.: Turnover and storage of C and N in five density fractions from California annual grassland surface soils, Global Biogeochem. Cy., 16, doi:10.1029/2001GB001822, 2002.

Baldock, J. A., Oades, J. M., Nelson, P. N., Skene, T. M., Golchin, A., and Clarke, P.: Assessing the extent of decomposition of natural organic materials using solid-state 13C NMR spectroscopy, Aust. J. Soil Res., 35, 1061, doi:10.1071/S97004, 1997.

Balesdent, J. and Mariotti, A.: Measurement of soil organic matter turnover using Measurement of soil organic matter turnover using 13C natural abundance, in: Mass spectrometry of soils, Boutton, T. W., Yamasaki, S.-i. (Eds.), Books in soils, plants, and the environment, M. Dekker, New York, 83–111, 1996.

Basler, A., Dippold, M., Helfrich, M., and Dyckmans, J.: Recycling versus Stabilisation of soil sugars– a long-term laboratory incubation experiment, Biogeosciences, submitted.

Basler, A. and Dyckmans, J.: Compound-specific delta C-13 analysis of monosaccharides from soil extracts by high-performance liquid chromatography/isotope ratio mass spectrometry, Rapid Commun. Mass Spectrom., 27, 2546–2550, doi:10.1002/rcm.6717, 2013.

Boschker, H., Moerdijk-Poortvliet, T., van Breugel, P., Houtekamer, M., and Middelburg, J. J.: A versatile method for stable carbon isotope analysis of carbohydrates by high-

performance liquid chromatography/isotope ratio mass spectrometry, Rapid Commun. Mass Spectrom., 22, 3902–3908, 2008.

Bowman, S. M. and Free, S. J.: The structure and synthesis of the fungal cell wall, Bioessays, 28, 799–808, doi:10.1002/bies.20441, 2006.

Brookes, P., Landman, A., Pruden, G., and Jenkinson, D. S.: Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil, Soil Biol. Biochem., 17, 837–842, doi:10.1016/0038-0717(85)90144-0, 1985.

Caesar-Tonthat, T. C.: Soil binding properties of mucilage produced by a basidiomycete fungus in a model system, Mycol Res, 106, 930–937, doi:10.1017/S0953756202006330, 2002.

Chenu, C.: Influence of a fungal polysaccharide, scleroglucan, on clay microstructures, Soil Biol. Biochem., 21, 299–305, doi:10.1016/0038-0717(89)90108-9, 1989.

Cheshire, M. V.: Origins and stability of soil polysaccharide, J. Soil Sci., 28, 1–10, doi:10.1111/j.1365-2389.1977.tb02290.x, 1977.

Cheshire, M. V.: Nature and origin of carbohydrates in soils, Academic Pr, London, 216 pp., 1979.

Coelho, R. R., Linhares, L. F., and Martin, J. P.: Sugars in hydrolysates of fungal melanins and soil humic acids, Plant Soil, 106, 127–133, doi:10.1007/BF02371204, 1988.

Derrien, D. and Amelung, W.: Computing the mean residence time of soil carbon fractions using stable isotopes: impacts of the model framework, Eur. J. Soil Science, 62, 237–252, doi:10.1111/j.1365-2389.2010.01333.x, 2011.

Derrien, D., Marol, C., Balabane, M., and Balesdent, J.: The turnover of carbohydrate carbon in a cultivated soil estimated by 13C natural abundances, Eur. J. Soil Science, 57, 547–557, 2006.

Derrien, D., Marol, C., and Balesdent, J.: The dynamics of neutral sugars in the rhizosphere of wheat. An approach by C-13 pulse-labelling and GC/C/IRMS, Plant Soil, 267, 243–253, 2004.

Derrien, D., Marol, C., and Balesdent, J.: Microbial biosyntheses of individual neutral sugars among sets of substrates and soils, Geoderma, 139, 190–198, 2007.

Dippold, M. and Kuzyakov, Y.: Biogeochemical transformations of amino acids in soil assessed by position-specific labelling, Plant Soil, 373, 385–401, doi:10.1007/s11104-013-1764-3, 2013.

Engelking, B., Flessa, H., and Joergensen, R. G.: Formation and use of microbial residues after adding sugarcane sucrose to a heated soil devoid of soil organic matter, Soil Biol. Biochem., 40, 97–105, doi:10.1016/j.soilbio.2007.07.009, 2008.

Glaser, B., Turrion, M. B., Solomon, D., Ni, A., and Zech, W.: Soil organic matter quantity and quality in mountain soils of the Alay Range, Kyrgyzia, affected by land use change, Biol. Fertil. Soils, 31, 407–413, 2000.

Gleixner, G., Poirier, N., Bol, R., and Balesdent, J.: Molecular dynamics of organic matter in a cultivated soil, Org. Geochem., 33, 357–366, 2002.

Golchin, A., Oades, J. M., Skjemstad, J. O., and Clarke, P.: Soil structure and carbon cycling, Aust. J. Soil Res., 32, 1043–1068, doi:10.1071/SR9941043, 1994a.

Golchin, A., Oades, J. M., Skjemstad, J. O., and Clarke, P.: Study of free and occluded particulate organic-matter in soils by solid-state C-13 CP/MAS NMR-spectroscopy and scanning electron-microscopy, Aust. J. Soil Res., 32, 285–309, doi:10.1071/SR9940285, 1994b.

Guggenberger, G., Christensen, B. T., and Zech, W.: Land-use effects on the composition of organic matter in particle-size separates of soil: I. Lignin and carbohydrate signature, Eur. J. Soil Science, 45, 449–458, doi:10.1111/j.1365-2389.1994.tb00530.x, 1994.

Helfrich, M., Ludwig, B., Buurman, P., and Flessa, H.: Effect of land use on the composition of soil organic matter in density and aggregate fractions as revealed by solid-state C-13 NMR spectroscopy, Geoderma, 136, 331–341, 2006.

Hobbie, E. A., Weber, N. S., Trappe, J. M., and van Klinken, Gert J.: Using radiocarbon to determine the mycorrhizal status of fungi, New Phytol, 156, 129–136, doi:10.1046/j.1469-8137.2002.00496.x, 2002.

IUSS Working Group WRB: World reference base for soil resources 2014: International soil classification system for naming soils and creating legends for soil maps, World soil resources reports, FAO, Rome, Online-Ressource, 2014.

John, B., Yamashita, T., Ludwig, B., and Flessa, H.: Storage of organic carbon in aggregate and density fractions of silty soils under different types of land use, Geoderma, 128, 63–79, doi:10.1016/j.geoderma.2004.12.013, 2005.

Kiem, R. and Kögel-Knabner, I.: Contribution of lignin and polysaccharides to the refractory carbon pool in C-depleted arable soils, Soil Biol. Biochem., 35, 101–118, 2003.

Kramer, C. and Gleixner, G.: Variable use of plant- and soil-derived carbon by microorganisms in agricultural soils, Soil Biol. Biochem., 38, 3267–3278, doi:10.1016/j.soilbio.2006.04.006, 2006.

Ludwig, B., Helfrich, M., and Flessa, H.: Modelling the Long-Term Stabilization of Carbon from Maize in a Silty Soil, Plant Soil, 278, 315–325, doi:10.1007/s11104-005-8808-2, 2005.

Moers, M. E. C., Baas, M., Deleeuw, J. W., Boon, J. J., and Schenck, P. A.: Occurrence and origin of carbohydrates in peat samples from a red mangrove environment as reflected by abundances of neutral monosaccharides, Geochim. Cosmochim. Ac., 54, 2463–2472, 1990.

Muramaya, S.: Microbial synthesis of saccharides in soils incubated with 13C-labelled glucose, Soil Biol. Biochem., 20, 193–199, doi:10.1016/0038-0717(88)90036-3, 1988.

Oades, J. M.: Soil organic matter and structural stability: mechanisms and implications for management, Plant Soil, 76, 319–337, 1984.

Osaku, C. A., Sassaki, G. L., Zancan, G. T., and Iacomini, M.: Studies on neutral exopolysaccharides produced by the ectomycorrhiza Thelephora terrestris, FEMS Microbiol Lett, 216, 145–149, doi:10.1111/j.1574-6968.2002.tb11428.x, 2002.

R Core Team: R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2013.

Rethemeyer, J., Kramer, C., Gleixner, G., John, B., Yamashita, T., Flessa, H., Andersen, N., Nadeau, M.-J., and Grootes, P. M.: Transformation of organic matter in agricultural soils: radiocarbon concentration versus soil depth, Geoderma, 128, 94–105, doi:10.1016/j.geoderma.2004.12.017, 2005.

Sauheitl, L., Glaser, B., and Bol, R.: Short-term dynamics of slurry-derived plant and microbial sugars in a temperate grassland soil as assessed by compound-specific delta C-13 analyses, Rapid Commun. Mass Spectrom., 19, 1437–1446, 2005.

Schmidt, M. W., Torn, M. S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, David A C, Nannipieri, P., Rasse, D. P., Weiner, S., and Trumbore, S. E.: Persistence of soil organic matter as an ecosystem property, Nature, 478, 49–56, doi:10.1038/nature10386, 2011.

Six, J., Bossuyt, H., Degryze, S., and Denef, K.: A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics, Soil Till. Res., 79, 7–31, doi:10.1016/j.still.2004.03.008, 2004.

Six, J., Conant, R. T., Paul, E. A., and Paustian, K.: Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils, Plant Soil, 241, 155–176, doi:10.1023/A:1016125726789, 2002.

Six, J. and Jastrow, J. D.: Organic matter turnover, in: Encyclopedia of soil science, Lal, R. (Ed.), Dekker, New York, NY, 936–942, 2002.

Sollins, P., Homann P., and Caldwell BA.: Stabilization and destabilization of soil organic matter: mechanisms and controls, Geoderma, 74, 65–105, doi:10.1016/S0016-7061(96)00036-5, 1996.

Stevenson, F. J.: Humus chemistry: Genesis, composition, reactions, 2nd ed., Wiley, New York, xiii, 496, 1994.

Stribley, D. P. and Read, D. J.: The Biology of Mycorrhiza in the Ericaceae. III. Movement of Carbon-14 from Host to Fungus, New Phytol, 73, 731–741, doi:10.1111/j.1469-8137.1974.tb01301.x, 1974.

Takano, Y., Chikaraishi, Y., Ogawa, N. O., Nomaki, H., Morono, Y., Inagaki, F., Kitazato, H., Hinrichs, K.-U., and Ohkouchi, N.: Sedimentary membrane lipids recycled by deep-sea benthic archaea, Nature Geosci, 3, 858–861, doi:10.1038/ngeo983, 2010.

Tisdall, J. M. and Oades, J. M.: Organic matter and water-stable aggregates in soils, J. Soil Sci., 33, 141–163, doi:10.1111/j.1365-2389.1982.tb01755.x, 1982.

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

von Lützow, M. von, Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., and Flessa, H.: Stabilization of organic matter in temperate soils: mechanisms

and their relevance under different soil conditions - a review, Eur. J. Soil Science, 57, 426–445, doi:10.1111/j.1365-2389.2006.00809.x, 2006.

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 and individual sugar carbon 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

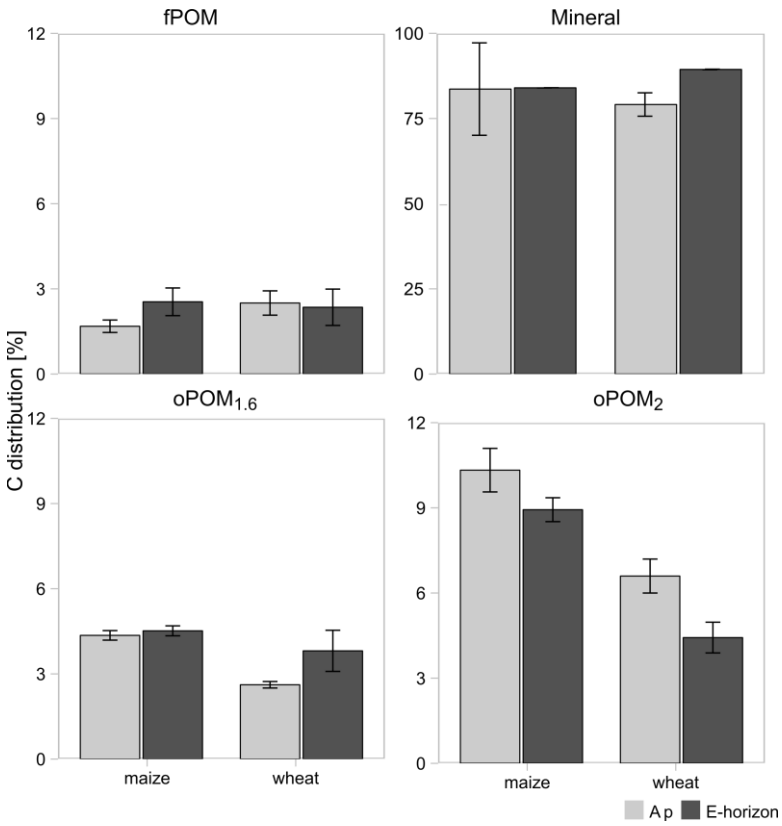

Figure1. Organic Carbon distribution in the investigated density fractions. Means and standard error (n=5).

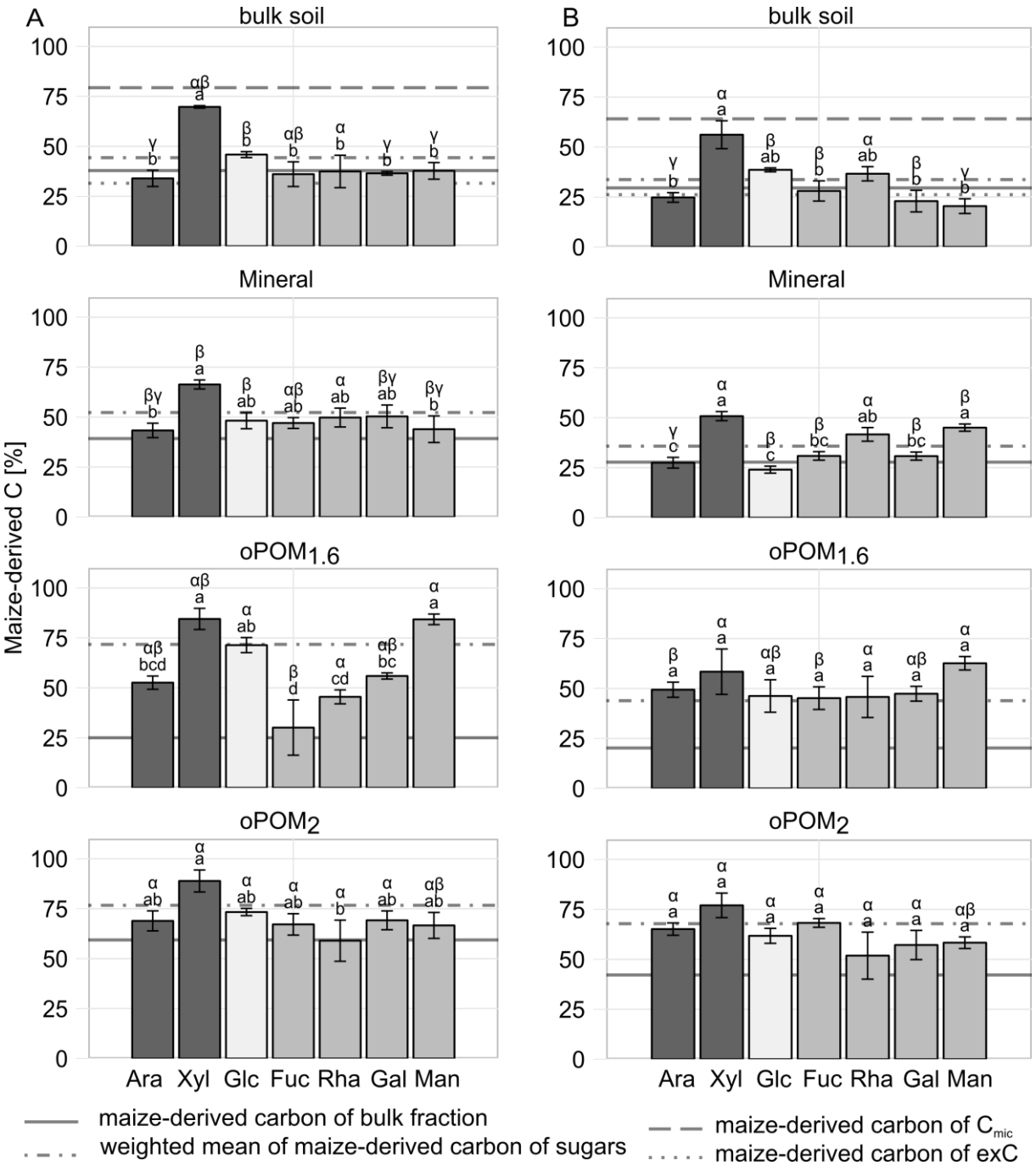

Figure 2. Maize contribution to sugars in bulk soil, Mineral, $oPOM_{1.6}$ and $oPOM_2$ fractions in the (A) Ap (0-30cm) and (B) E-horizon (30-45cm). Latin letters (a-d) indicate significant differences ($p<0.05$) among the individual sugars within one fraction. Greek letters (α-γ) indicate significant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