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Microbial carbon recycling: an underestimated process controlling soil carbon dynamics

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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 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 ($\leq 1.6\,g\,cm^{-3}$; $oPOM_{1.6}$), 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 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 sugars 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 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.

BGD
12, 9729–9750, 2015

1 Introduction

For several decades, it was assumed that the molecular structure accounts for the rate of decomposition of different organic compounds in soils. However, the use of compound specific isotope analysis provided new understanding of SOM dynamics. As an example, lignin, a compound of high chemical recalcitrance, has shorter 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). Mechanisms for the long persistence of these labile compounds in soil are stabilisation on the one hand, i.e. the reduced accessibility for microorganism caused by physical protection (by mineral interaction or occlusion within soil aggregates) or chemical recalcitrance (Six et al., 2002; Luetzow et al., 2006), and recycling on the other, i.e. the reuse of "old" organic compounds by microorganism (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.

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 high 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, 2007). Sugars in soils are commonly classified according to their main origin into plant (arabinose (ara), xyl) or microbial derived sugars (gal, manose (man), rha, 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 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

BGD
12, 9729–9750, 2015

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 of plant and microbial sugars 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 (48°21′47″ N, 13°11′46″ E). The mean annual temperature is 9.2 °C and the mean annual precipitation is 757 mm. Soil samples (Ap-horizon and 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}

BGD
12, 9729–9750, 2015

sodium polytungstatesolution (SPT, Sometu, Berlin, Germany). 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, Germany) 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 h 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 h. 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 ×8 cation exchange resin (Sigma Aldrich, Steinheim, Germany) and 5 g Serdolit PAD IV adsorption resin (Serva Electrophoresis GmbH, Hei-

BGD
12, 9729–9750, 2015

delberg, 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, Dreieich, Germany) 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 1/2, Maidstone, UK). The dissolved organic carbon was analysed using a TOC analyser multi C/N® 2000 (Analytik Jena, Jena, Germany).

BGD
12, 9729–9750, 2015

For stable isotope measurements freeze-dried aliquots were analysed by EA-IRMS. The isotopic signature of the microbial biomass was calculated as follows:

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

where $\delta^{13}C_{\mathrm{F}}$ and $\delta^{13}C_{\mathrm{nF}}$ 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):

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

where f is the relative proportion of maize-derived carbon, δ_{sample} is the δ^{13}C value of the maize plot sample, $\delta_{\mathrm{refernce}}$ presents the measured ^{13}C value of the corresponding wheat plot sample, 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. Assuming steady state conditions and homogeneous soil fractions which can be described with a single pool model (Six and Jastrow, 2002), the

BGD
12, 9729–9750, 2015

MRT is calculated according to Derrien and Amelung (2011):

$$\mathrm{MRT} = \frac{1}{k}, \tag{3}$$

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

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

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 with different stability and turnover. 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.

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).

BGD
12, 9729–9750, 2015

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. 44 $mg\,C\,g^{-1}$ (wheat) and 30 $mg\,C\,g^{-1}$ (maize), followed by ara and glc with ca. 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 was within the range of the bulk soil, whereas the proportion of

BGD
12, 9729–9750, 2015

maize in microbial biomass was twice as high as in the bulk soil. 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 sugars

BGD
12, 9729–9750, 2015

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 contributed around 2 % to total soil carbon, 4 and 8 % of total carbon were found in the in $oPOM_{1.6}$ and $oPOM_2$, respectively and the major part was located in the Mineral (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 $\rightarrow oPOM_{1.6}$). Due to reduced microbial activity

BGD
12, 9729–9750, 2015

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 sugars in both oPOM fractions is comparable to the apparent MRT of the microbial biomass in both soil horizons.

Mannose, 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 exopolysaccharides 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.

Xylose 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).

BGD
12, 9729–9750, 2015

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., 2015) 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 microbial sugars (influenced by both, stabilisation and substrate recycling), compared to 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.

BGD
12, 9729–9750, 2015

5 Conclusions

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 sugars 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). Ultimately, our findings highlight the importance of recycling processes for SOM dynamics on the molecular as well as the aggregate level.

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BGD

12, 9729–9750, 2015

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BGD
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BGD
12, 9729–9750, 2015

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BGD
12, 9729–9750, 2015

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BGD
12, 9729–9750, 2015

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
	mg C g^{-1} bulk	mg C g^{-1} fraction						
Continious Wheat plot (Ap)								
oPOM$_{1.6}$ ($n = 5$)	0.28 ± 0.01	$0.68 \pm 0.27^{\alpha d}$	$0.91 \pm 0.28^{\alpha cd}$	$2.68 \pm 0.6^{\alpha ab}$	$6.08 \pm 1.4^{\alpha a}$	$7.42 \pm 1.95^{\alpha a}$	$2.24 \pm 0.51^{\alpha bc}$	$2.23 \pm 0.49^{\alpha bcd}$
oPOM$_{2}$ ($n = 5$)	0.71 ± 0.06	$0.37 \pm 0.03^{\alpha c}$	$1.39 \pm 0.5^{\alpha bc}$	$2.70 \pm 0.1^{\alpha ab}$	$5.83 \pm 1.01^{\alpha a}$	$6.37 \pm 2.29^{\alpha a}$	$1.85 \pm 0.56^{\alpha ab}$	$3.19 \pm 0.84^{\alpha ab}$
mineral ($n = 3$)	9.51 ± 1.02	$0.05 \pm 0.01^{\beta d}$	$0.09 \pm 0.01^{\beta cd}$	$0.15 \pm 0.01^{\beta bc}$	$0.15 \pm 0.01^{\beta b}$	$0.26 \pm 0.03^{\beta a}$	$0.18 \pm 0.02^{\beta ab}$	$0.16 \pm 0.05^{\beta b}$
bulk ($n = 3$)	12.06 ± 0.8	$0.03 \pm 0.00^{\beta c}$	$0.07 \pm 0.00^{\beta c}$	$0.13 \pm 0.02^{\beta b}$	$0.14 \pm 0.01^{\beta b}$	$0.27 \pm 0.27^{\beta a}$	$0.16 \pm 0.00^{\beta ab}$	$0.14 \pm 0^{\beta b}$
Continious Wheat plot (E)								
oPOM$_{1.6}$ ($n = 5$)	0.25 ± 0.05	$0.42 \pm 0.19^{\alpha b}$	$0.60 \pm 0.32^{\alpha b}$	$2.38 \pm 0.91^{\alpha ab}$	$3.36 \pm 1.25^{\alpha a}$	$5.38 \pm 1.87^{\alpha a}$	$1.93 \pm 0.85^{\alpha ab}$	$2.06 \pm 0.66^{\alpha ab}$
oPOM$_{2}$ ($n = 5$)	0.29 ± 0.04	$0.40 \pm 0.09^{\alpha c}$	$0.88 \pm 0.23^{\alpha bc}$	$1.90 \pm 0.42^{\alpha abc}$	$2.96 \pm 0.7^{\alpha ab}$	$4.39 \pm 0.76^{\alpha a}$	$1.90 \pm 0.37^{\alpha abc}$	$1.81 \pm 0.34^{\alpha abc}$
mineral ($n = 3$)	5.90 ± 0.01	$0.03 \pm 0.01^{\beta d}$	$0.08 \pm 0.01^{\beta cd}$	$0.07 \pm 0.01^{\beta bc}$	$0.07 \pm 0^{\beta cd}$	$0.14 \pm 0.01^{\beta a}$	$0.10 \pm 0.01^{\beta ab}$	$0.09 \pm 0.01^{\beta b}$
bulk ($n = 3$)	7.86 ± 0.26	$0.03 \pm 0.00^{\beta e}$	$0.04 \pm 0.01^{\beta de}$	$0.07 \pm 0.01^{\beta bcd}$	$0.06 \pm 0.02^{\beta cd}$	$0.16 \pm 0.02^{\beta a}$	$0.10 \pm 0.02^{\beta ab}$	$0.08 \pm 0.03^{\beta bc}$
Continious Maize plot (Ap)								
oPOM$_{1.6}$ ($n = 5$)	0.49 ± 0.02	$0.76 \pm 0.3^{\alpha e}$	$1.17 \pm 0.28^{\alpha c}$	$3.22 \pm 0.67^{\alpha b}$	$5.85 \pm 1^{\alpha a}$	$8.61 \pm 0.67^{\alpha a}$	$3.04 \pm 0.56^{\alpha a}$	$2.90 \pm 0.34^{\alpha b}$
oPOM$_{2}$ ($n = 5$)	1.15 ± 0.09	$0.50 \pm 0.02^{\alpha c}$	$0.86 \pm 0.09^{\alpha cd}$	$2.61 \pm 0.36^{\alpha b}$	$5.39 \pm 1.17^{\alpha ab}$	$6.00 \pm 0.33^{\beta a}$	$2.56 \pm 0.22^{\alpha b}$	$2.28 \pm 0.23^{\alpha bc}$
mineral ($n = 3$)	9.31 ± 1.51	$0.03 \pm 0.00^{\beta e}$	$0.09 \pm 0.02^{\beta bc}$	$0.13 \pm 0.03^{\beta abc}$	$0.13 \pm 0.02^{\beta ab}$	$0.27 \pm 0.05^{\gamma a}$	$0.17 \pm 0.04^{\beta ab}$	$0.16 \pm 0.03^{\beta ab}$
bulk ($n = 3$)	12.51 ± 0.38	$0.04 \pm 0.00^{\beta f}$	$0.09 \pm 0.01^{\beta d}$	$0.15 \pm 0.01^{\beta c}$	$0.16 \pm 0.02^{\beta bc}$	$0.36 \pm 0.02^{\gamma a}$	$0.20 \pm 0.01^{\beta ab}$	$0.17 \pm 0.01^{\beta b}$
Continious Maize plot (E)								
oPOM$_{1.6}$ ($n = 5$)	0.42 ± 0.02	$0.46 \pm 0.09^{\alpha c}$	$0.92 \pm 0.09^{\alpha e}$	$3.63 \pm 0.21^{\alpha bc}$	$5.76 \pm 0.93^{\alpha ab}$	$9.44 \pm 0.51^{\alpha a}$	$3.25 \pm 0.24^{\alpha cd}$	$3.03 \pm 0.17^{\alpha d}$
oPOM$_{2}$ ($n = 5$)	0.82 ± 0.04	$0.48 \pm 0.02^{\alpha d}$	$0.82 \pm 0.08^{\alpha c}$	$2.48 \pm 0.34^{\beta b}$	$4.56 \pm 0.9^{\alpha ab}$	$5.42 \pm 0.36^{\beta a}$	$2.47 \pm 0.23^{\beta b}$	$2.09 \pm 0.21^{\beta b}$
mineral ($n = 3$)	7.75 ± 0	$0.03 \pm 0.00^{\beta c}$	$0.08 \pm 0.01^{\beta d}$	$0.07 \pm 0^{\gamma cd}$	$0.10 \pm 0.01^{\beta dc}$	$0.14 \pm 0.01^{\gamma a}$	$0.07 \pm 0.01^{\gamma ab}$	$0.09 \pm 0.01^{\gamma bc}$
bulk ($n = 3$)	11.10 ± 0.24	$0.04 \pm 0.00^{\beta d}$	$0.07 \pm 0.00^{\beta ef}$	$0.11 \pm 0.01^{\delta cd}$	$0.10 \pm 0.01^{\beta dc}$	$0.25 \pm 0.02^{\delta a}$	$0.16 \pm 0.02^{\delta a}$	$0.13 \pm 0.02^{\delta bc}$
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.

BGD
12, 9729–9750, 2015

Table 2. Calculated MRT of total carbon and individual sugars 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

BGD
12, 9729–9750, 2015

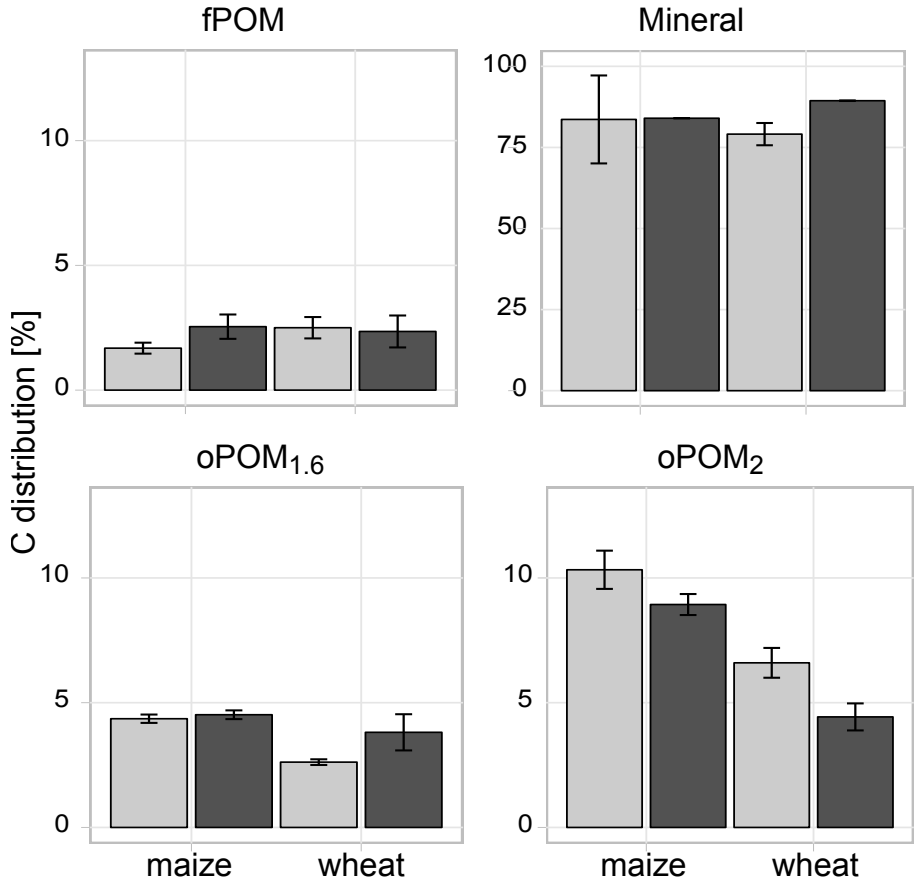

Figure 1. Organic Carbon distribution in the investigated density fractions. Mean and standard error (n = 5).

BGD
12, 9729–9750, 2015

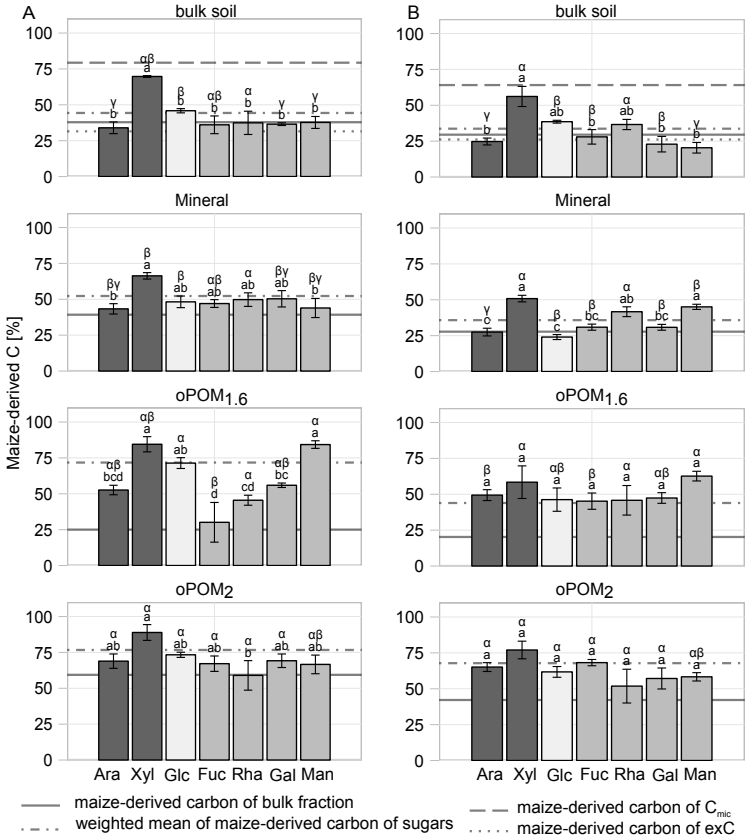

Figure 2. Maize contribution to sugars in bulk soil, Mineral, oPOM$_{1.6}$ and oPOM$_2$ fractions in the **(a)** Ap (0–30 cm) and **(b)** E-horizon (30–45 cm). Latin letters (a–d) indicate significant differences ($p < 0.05$) among the individual sugars within one fraction. Greek letters (α–γ) indicate significant differences among different fractions for individual sugars. Means and standard error ($n = 4$).

BGD
12, 9729–9750, 2015