Microbial carbon recycling - an underestimated process controlling soil carbon dynamics. Part I: A long-term laboratory incubation experiment

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Abstract

Independent of its chemical structure carbon (C) persists in soil for several decades, controlled by stabilisation and recycling. To disentangle the importance of the two factors on the turnover dynamics of soil sugars, an important compound of soil organic matter (SOM), a three year incubation experiment was conducted on a silty loam soil under different types of land use (arable land, grassland and forest) by adding ^{13}C-labeled glucose. The compound specific isotope analysis of soil sugars was used to examine the dynamics of different sugars during incubation.

Sugar dynamics were dominated by a pool of high mean residence times (MRT) indicating that recycling plays an important role for sugars. However, this was not substantially affected by soil C content. Six months after label addition the contribution of the label was much higher for microbial biomass than for CO_2 production for all examined land use types, corroborating that substrate recycling was very effective within the microbial biomass. Two different patterns of tracer dynamics could be identified for different sugars: while fucose and mannose showed highest label contribution at the beginning of the incubation with a

subsequent slow decline, galactose and rhamnose were characterised by slow label incorporation with subsequently constant levels, which indicates that recycling is dominating the dynamics of these sugars. This may correspond to a) different microbial growing strategies (r and K-strategist) or b) location within or outside the cell membrane (lipopolysaccharides vs. exopolysaccharides) and thus be subject of different re-use within the microbial food web. Our results show how the microbial community recycles substrate very effectively and that high losses of substrate only occur during initial stages after substrate addition. This study indicates that recycling is one of the major processes explaining the high MRT observed for many SOM fractions and thus is crucial for understanding the global soil C cycle.

1 Introduction

Organic matter that enters the soil is immediately subject to microbial degradation (Fontaine et al., 2003). It has long been assumed that the chemical structure of soil organic matter (SOM) compounds is a key factor controlling decomposition dynamics (Stevenson, 1994). However, in recent years, several studies have shown that carbon (C) compounds are persistent in soil independent of their chemical structure and that mean residence times (MRT) of many compound classes are in the same range (Derrien et al., 2006; Amelung et al., 2008; Gleixner et al., 2002; Kiem and Kögel-Knabner, 2003; Derrien et al., 2007; Schmidt et al., 2011). Two main mechanisms have been discussed to control the C dynamics in soil: on the one hand preservation of SOM due to stabilisation and on the other hand recycling, i.e. the synthesis of C compounds from old C sources (Gleixner et al., 2002; Sauheitl et al., 2005; Six et al., 2002; von Luetzow et al., 2006;Sollins et al., 1996).

The question of stabilisation vs. recycling is particularly imminent for sugars: their high degradability and usability suggest a rapid turnover in soils. In contrast, sugars are characterized by high turnover times, similar to bulk soil C (Gleixner et al., 2002; Derrien et al., 2007). While chemical recalcitrance can be ruled out, it is unknown whether spatial inaccessibility and interactions with surfaces and metal ions on the one hand or recycling on the other hand are predominant for the observed high apparent MRT (where "apparent MRT" refers to the MRT of the compound as opposed to the MRT of the underlying C). Vascular plant-derived carbohydrates are mainly characterised by the pentose sugars arabinose (ara) and xylose (xyl), whereas hexoses (galactose (gal) and manose (man)) and desoxyhexoses (fucose (fuc), rhamnose(rha) are primarily produced by microorganisms (Moers et al., 1990).

Studies that aim to disentangle contribution of recycling and stabilisation to the fate of carbohydrates are rare. Based on exponential decay functions, several studies suggest the existence of different sugar pools in soils (Cheshire et al., 1988; Derrien et al., 2007; Muramaya, 1984). Derrien et al. (2007) and Muramaya (1988) performed glucose incubation experiments with incubation periods up to 1 year, but conclusion about factors controlling the long-term decay kinetics of soil sugars were not possible, presumably due to the short duration of the experiment and a low number of sampling times. The aim of the present study was to investigate the long-term decay of different (plant and microbial derived) sugars in

soil. Therefore, a three year incubation experiment combined with short sampling intervals was set up to evaluate whether sugar pools with different turnover dynamics can be identified in soil during long-term incubation. The incubation was performed on a silty loam under different land use types (and hence soil C concentrations and chemical qualities) to assess the influence of soil C content on microbial recycling. We hypothesize (i) that the high MRT of soil sugars that have often been observed results mainly from microbial recycling and not from stabilisation processes and (ii) that the importance of microbial recycling increases with decreasing soil C content.

2 Material/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 were taken in April 2011 from the following sites and soil depths: (i) the Ap horizon (0-30 cm) and (ii) the E horizon (30-45 cm) of a continuous wheat plot (*Triticum aestivum L.*) established in 1969. Previous vegetation on the wheat plot was grassland. (iii) The Ah horizon (0-10 cm) of a grassland established in 1961 and (iv) the Ah horizon (0-10 cm) of a nearby spruce stand. The soil was classified as a stagnic Luvisol derived from loess (IUSS Working Group WRB, 2014). The soil texture is silty loam. Field moist soil was carefully sieved to 2 mm and stored at 10 °C until use. The soils are described in detail by John et al. (2005) and Helfrich et al.(2006).

2.2 Soil Incubation

For incubation, 1000 g dry weight (dw) soil of the wheat Ap and E horizon and 700 g dw soil of the grassland and forest Ah horizon were placed individually in microcosms, with 3 replicates for each site and depth. The soil was not compacted and equal filling levels of the microcosms resulted for all soils. The soil was amended with 400 mg 99% uniformly-labelled [U-^{13}C] glucose (Euroisotrop, Saint-Aubin, France) equivalent to a C addition of 3, 5, 2 and 1

% of total organic C (C_{org}) in the wheat Ap, E, grassland and forest soil, respectively. The glucose was applied in solution to the soil while adjusting the water holding capacity of 50%, thoroughly mixed and filled in the microcosms. The microcosms were incubated for 34 months at a constant temperature of 10 °C, representing the mean annual soil temperature in Rotthalmünster. The microcosms were kept semi-closed to enable aeration and to reduce drying-out at the same time. Every two weeks approximately 4 g of soil was removed from each microcosm and additionally 20 g after 6, 20 and 34 months of incubation for soil microbial biomass analysis. On these occasions, the complete soil was taken out of the microcosms, thoroughly mixed and carefully rewetted by sprinkling with deionised water to keep fluctuations of soil water content below 10%. The soil samples were stored at -18 °C until extraction. Controls under natural abundance conditions were treated identically.

2.3 CO_2 respiration

The CO_2 respiration was measured biweekly before soil sampling. At first, microcosms were closed and a headspace sample was taken after approximately 30 minutes of equilibration. With an air tight syringe, 50 mL of synthetic air was pushed into the vessel and the headspace was mixed by pumping the syringe 3 to 4 times. Afterwards 50 mL of the headspace air was taken and transferred to pre-evacuated Exetainers (Labco Limited, Buckinghamshire, UK). A second headspace sample was taken identically after 24 h of CO_2 accumulation in the closed microcosms. The CO_2 concentrations and isotopic values were measured by an IRMS Delta Plus with GP interface and GC-Box (ThermoFisher, Bremen, Germany) and the amount of the produced CO_2 was calculated from the difference in concentration and isotopic composition of the two samplings.

2.4 ^{13}C analysis of individual sugars

2.4.1 Extraction procedure

Carbohydrates were extracted and purified using a modified procedure based on Amelung et al. (1996) as described by Basler and Dyckmans (2013). The sugars were extracted from 500 mg wet soil by hydrolysis with 10 mL 4 M trifluoroacetic acid (TFA) and at 105 °C for four 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). The samples

were re-dissolved with 0.5 mL water and evaporated to dryness 3 times to remove all traces of TFA. After the evaporation process 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. Carbohydrates were eluted from the resin by adding 8 times 2 mL water. The eluate was freeze-dried and stored at -18 °C until analysis. For HPLC/o/IRMS analysis the samples were dissolved in 3 mL water.

The TFA extraction method is known to effectively extract hemi-cellulosic sugars but cellulose is not cleaved by this method (Amelung et al., 1996). The results presented here thus only refer to non-cellulosic sugars.

2.4.2 Sugar analysis

The compound specific analysis of amounts and isotope ratios were 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 LC-Isolink interface (Thermo Scientific, Bremen, Germany) as described by Basler and Dyckmans (2013). Shortly, the chromatographic column (Carbo Pac 20, Dionex, Germering, 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}.

The isotopic values are reported in atm%excess notation:

$$atm\%excess = atm\%_{labelled} - atm\%_{unlabelled} \quad (1)$$

The analysis frequency differed among the different types of land use: To check if short sampling intervals will reveal additional sugar dynamics, all samples (i.e. two-week intervals) from the incubation of the wheat Ap horizon were analysed for the 30 month sampling period. However, as the results did not indicate a multi-pool dynamic, (see Results, Fig.4), the frequency of analysis was reduced for the other sites. From the forest and grassland incubations, samples were analysed in four week intervals over a 24 month period, and from the wheat E horizon, samples were analysed in 8 week intervals for a period of 30 months. Sugar analysis was made from only one microcosm to account for time-dependent dynamics rather than differences among different incubations. To assess the variability among different

microcosms, after 6 and 24 month, all incubation microcosms were analysed for sugar content and isotopic composition. The mean coefficient of variation among the replicates was below 5%, therefore the results of the incubations presented here are taken as representative.

2.5 Chloroform-Fumigation-Extraction

The soil microbial biomass (C_{mic}) was measured before and after 6, 20 and 34 months of incubation by the chloroform-fumigation extraction method (Brookes et al., 1985; Vance et al., 1987). In brief, each sample was divided into two sub-samples of 10 g moist soil. One soil sub-sample was directly extracted as described below. The other sub-sample was placed in a desiccator together with 80 mL of ethanol free CH_3Cl. Desiccators were evacuated and the samples were left at 25 °C for 24 h (fumigation). All samples were extracted by shaking with 60 mL 0.05 M K_2SO_4 (Engelking et al. 2008) for one hour and subsequently filtered (Whatman 595 ½,Maidstone, UK)). The soil extracts were analysed for their C content using a TOC analyser multi C/N® 2000 (Analytik Jena, Jena, Germany). For stable isotope measurements, around 50 mg of the freeze-dried filtrates were filled in tin capsules and analysed by elemental analyser/isotope ratio mass spectrometry (EA/IRMS) using an EuroVector elemental analyser (HEKAtech GmbH, Wegberg, Germany) coupled to a Delta Plus XP isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany). Samples are combusted in a reactor filled with tungsten oxide and silvered cobaltous oxide at 1020 °C.

The isotopic signature of the microbial biomass C was calculated as follows:

$$atm\%excess\,C_{mic} = \frac{(atm\%excess\,C_F \times C_F) - (atm\%excess\,C_{nF} \times C_{nF})}{(C_F - C_{nF})} \quad (2)$$

Where $atm\%excess\,C_F$ and $atm\%excess\,C_{nF}$ are the isotopic composition of the fumigated and non-fumigated extracts and C_F and C_{nF} are the C concentrations in the extracts of the fumigated and non-fumigated soil samples, respectively. For calculation of total microbial biomass-C, a kec factor of 0.45 was used (Joergensen, 1996). Carbon extracted from non-fumigated samples represents the K_2SO_4 extractable C fraction (exC).

2.6 Calculation and statistics

All statistical analyses and modelling were performed with R 3.0.2 (R Core Team, 2013).

The relative specific allocation (RSA) describes the fraction of labelled C relative to total C in a given compartment (Deleens et al., 1994; Dyckmans and Flessa, 2002) and is calculated as follows:

$$RSA = \frac{atom\%_{sample} - atom\%_{control}}{atom\%_{label} - atom\%_{control}} \quad (3)$$

The partitioning (P) describes the proportion of the labelled element in a given soil C compartment relative to the total labelled element in the whole (Deleens et al., 1994; Dyckmans and Flessa, 2002).The partitioning of labelled C was calculated from:

$$P[\%] = \frac{RSA_{fraction} \times A_{fraction}}{RSA_{bulksoil} \times A_{bulksoil}} \quad (4)$$

where A is the amount of the respective fraction.

The following exponential functions were used to analyse decay kinetics for each individual sugar:

mono exponential function

$$y = a \times e^{(-k_1 t)} \quad (5)$$

bi-exponential function

$$y = a \times e^{(-k_1 \times t)} + b \times e^{(-k_2 \times t)} \quad (6)$$

In the equations, y represents the RSA value of individual sugar; k the decay constant of the sugar pool, and a and b represent initial pool sizes.

The MRT of C in the respective sugar pool was calculated according to Derrien and Amelung (2011):

$$MRT = 1/k \quad (7)$$

where k is the decay constant estimated by fitting Eqs. (5) or (6) to the measured values.

Equations (5) and (6) were fitted to the data using R. The best model for each sugar and soil was identified using the Akaike information criterion (AIC;(Akaike)). The AIC is defined as:

$$AIC = 2z - 2\ln(L) \quad (8)$$

where z is the number of parameters in the model and L the maximized value of the likelihood function for the model.

A Pearson correlation test was conducted to determine the relationship between distributions of labelled sugar and total sugar of the organic matter and to test the model efficiency. The statistical significance of the sugar composition, ratios and label derived proportions among different sugars, sampling times were tested by Kruskal–Wallis one-way analysis of variance.

3 Results

3.1 Carbon concentrations and incorporation of the labelled C into soil organic matter fractions and the respired CO_2

Dynamics of added label were monitored in bulk soil, microbial biomass, CO_2 respiration and exC. In general, C_{mic}, showed the highest proportions of label derived C (RSA) followed by CO_2; the lowest RSA were found in exC (Fig. 1).

After 6 months of incubation 1.1, 1.2, 0.9 and 0.3% of the bulk C pool of the wheat Ap, wheat E, grassland Ah and forest Ah, respectively, were derived from labelled C. Between 6 and 34 month of incubation about 30, 20 and 40% of label derived C was lost from the bulk soil C pool in wheat, grassland and forest incubations respectively, while total C concentrations did not change significantly (Fig. 1). The C_{mic} of the wheat Ap, E grassland Ah and forest Ah were 230, 140, 851 and 622 µgC g^{-1}dw soil after 6 months of incubation (Fig. 2). This corresponds to an increase of 8%, 40% and 35% of C_{mic} compared to the initial content before glucose addition in wheat Ap, wheat E and forest Ah, respectively. The grassland Ah lost 8% of C_{mic} after incubation started (Fig. 2) After 6 months, 23, 19, 15 and 21% of the C_{mic} in the wheat Ap, E, grassland and forest incubations were derived from the added label and label contribution decreased during further incubation. Also, total C_{mic} decreased during incubation, with the exception of the forest Ah soil (Fig. 1). The CO_2 emitted from the incubated soils showed similar behaviour, i.e. decreasing production of CO_2 accompanied with decreasing

label contribution. However, the contribution of added label to CO_2 production (4–8 %) was much lower than for microbial biomass (15-25%; Fig. 1). The exC only showed marginal proportions of label-derived C (0.03-0.14%), which also decreased with increasing incubation time.

When regarding the partitioning of labelled C into the different investigated soil fractions (Fig. 3), the bulk soil contained between 26.5 and 42.8% of the added label after 6 months of incubation. The label continually decreased in all treatments with incubation time due to CO_2 losses). The partitioning of labelled C to the sugar pool and Cmic was of comparable size but showed a more pronounced decrease with ongoing incubation time in the Cmic pool as compared to sugars. Less than 1% of the added label was found in the Cex.

3.2 Incorporation of added label into sugars

Around 9% of bulk soil C in the wheat Ap, E and grassland Ah incubations and 5% in forest Ah were attributed to sugars. The relative proportions of the individual sugars were quite similar among the investigated soil horizons (Table 1). The dominant sugar in all types of land use was glucose (glc), followed by the hexoses gal and man and the pentoses ara and xyl. The desoxyhexoses (fuc, rha) showed smallest contributions, with the exception of fuc in forest soil, which occurred in similar proportions as ara. After 6 months of incubation, label-derived C incorporated into all sugars (with the exception of glucose) was 1.9 and 1% in the incubated wheat Ap and E horizons, respectively and this proportion decreased during further incubation (data not shown). In contrast, in the grassland and forest soils, label derived C increased during incubation from 1.2 and 0.6% after 6 months to 1.4 and 0.8%. Apart from glc, label derived microbial sugars were mainly composed of man (~12%) and gal (~9%) and smaller proportions of rha (~6%), fuc, ara and xyl (~3%) (Table 2).

3.3 Dynamics of label-derived C of the individual sugars

Glucose showed highest contribution of labelled C throughout the experiment. Values decreased from 6.4, 6.2, 6.2, and 2.3% after 6 months to 4.2, 3.5, 3.1 and 1.4% in wheat Ap, E, grassland Ah and forest Ah, respectively (data not shown). The trends for the other sugars

were quite similar in the different incubated soils (Fig. 4a-d): Man and fuc showed a decreasing trend in label contribution, whereas the label contribution increased in rha and gal during the first weeks of incubation, but did not change afterwards. Mannose and rha showed contributions of labelled C between 0.3 and 1.9% for the different incubations after 6 months, followed by gal and fuc (0.3-1.5%, Fig. 4). The mainly plant-derived sugars ara and xyl showed considerable contribution of label-derived C of about 0.2 and 0.6% after 6 months, although to a lesser extent than the "microbial sugars" (man, gal, rha). The contribution of labelled C to ara slightly increased during the whole incubation time in all but the forest soil, where an initial increase was followed by a decrease. The contributions of labelled C to xyl increased weakly in both wheat soil horizons, whereas it was constant in the grassland and forest soil. Non-linear regression analysis was performed on RSA values to analyse the kinetics of soil sugar turnover. Mono-exponential (Eq.5) as well as bi-exponential (Eq. 6) functions were tested to describe the dynamics of soil sugars. AIC values were used to identify the best fit (Table S1). No clear pattern was observed whether dynamics of individual sugars or of different soils were characterized by mono or bi exponential models (Table S2). Best fits for each sugar and land use are shown in Fig. 4. In the cases where a decaying label contribution was observed, the MRT of the sugar C, calculated from the nonlinear regression analysis with Eqs. (5) and (6), ranged from a few months for the labile pool over several years (1-365 yr), representing an intermediate pool (Table 3). The highest (5957 yr) was calculated for gal in the wheat Ap.

4 Discussion

4.1 Glucose incorporation into soil C and microbial biomass C

Our aim was to investigate the transformation and stabilisation processes of the added labelled C during the first three years after substrate addition. After 6 months of incubation, the bulk soil C pool still contained 25 to 42% of the added label, which is in line with findings of previous studies, where less than 50% of added glucose were recovered after one or two months of incubation (Saggar 1999, Murayama 1988). As an easily accessible C source, glucose stimulates microbial growth in soil and leads to increased initial respiration, especially of newly added C (Schneckenberger et al., 2008). After 6 months, between 15 and

23% of the C_{mic} was derived from the added label and the proportion decreased during further incubation (Fig. 1). As the living microbial biomass actively takes up and incorporates the added glucose, it is expected to have a higher C turnover than the bulk soil C pool. However, the contribution of added label to microbial biomass C was quite high and remained at a high level during the whole incubation (resulting in label contribution of up to 15 % after 30 month, Fig. 1). This could be related to the fact, that some microorganisms, especially K-strategists, are able to store glucose as an intracellular reservoir (as glycogen) (Blagodatskaya et al., 2007, Wilson et al., 2010). This is contradicted by the fact that label partitioning into the sugar pool decreases more slowly than to the C_{mic} pool and that the relative importance of glc as compared to the other sugars remains fairly constant (Fig. 3). Moreover our data on decreasing microbial biomass and decreasing C mineralization over time indicate substrate limitation. It seems unlikely, that high amounts of glucose are stored within the microbial biomass under these conditions. It is more likely that the microbial community maintained their metabolic capacity by feeding on dead microbial biomass as was also shown by Kindler et al. (2006) or Guenet et al. (2011). This would be in line with the slow decline of glucose-derived label contribution in the microbial biomass, which was similarly shown by Gunina et al. (2014). Their data indicate that substrates entering citric acid cycle are preferentially respired whereas substrates, like glucose, entering glycolysis are preferentially incorporated into microbial biomass, i.e. recycled. Corroborating this, our data indicate that considerable amounts of "old" SOM are used for energy gain (mineralization) rather than recent microbial necromass as the RSA of CO_2 is much lower than that of the microbial biomass throughout the experiment. Probably, the constant mixing of the soil during the biweekly sampling events increased the accessibility also of "old" soil C sources. This is in line with Lamparter et al. (2009) and Joergensen and Raubuch (2003) who showed that mixing and rewetting improve the C availability for microbial uptake.

Together with the observed long MRT of sugar C our data indicate that after high initial losses of added C substrate that has often been observed after glucose (Schneckenberger et al., 2008; Saggar et al., 1999), or microbial necromass addition (Miltner et al., 2012; Kindler et al., 2006) the microbial biomass recycled C substrates efficiently and with only minimal C losses.

4.2 Effect of incubation on sugar composition

The relative amounts of the investigated sugars did not differ substantially among the different soils investigated here. Sugars made up around 8% of the C in arable and grassland soils, in the forest soil the proportion was smaller with 6%, corroborating earlier findings (Lowe and Brown, 1975; Rumpel and Dignac, 2006; Guggenberger et al., 1994; Cheshire, 1979). Furthermore, the general sugar distribution pattern did not differ significantly among the investigated types of land use: the dominant sugar was glc, followed by man and gal. The contribution of the plant-derived sugars xyl and ara was somewhat smaller and only minimum proportions of rha and fuc were found. The only variation was observed in the forest soil, where ara was half and fuc was twice of the proportion observed in the other soils. The general distribution of sugars in the arable and grassland soils were concordant with studies by Muramaya (1988), Derrien et al. (2007), Creamer et al. (2012).

There was a close correlation between total and labelled sugar content for the microbial sugars (R =0.69, Data not shown, no correlation for ara and xyl), indicating that the dynamics before and during incubation were basically the same with the exception of plant input. During the incubation highest synthesis rates were observed for man and gal, followed by rha and fuc, whereas new synthesis of xyl and ara was less. These findings are similar to those of Muramaya (1988) and Derrien et al. (2007). The (small) new synthesis of ara and xyl can probably be traced back to fungi and yeast, as shown by Coelho et al. (1988) and Cheshire et al. (1976). As supply by plant debris or root exudates was missing the dynamics of ara and xyl were obviously controlled by the microbial community during the incubation.

Proportions of labelled C ranged between 0.6 to 1.9% of the individual sugars (without glucose) after 6 months of incubation. During further incubation, the proportion of the added label in the sugar pool of both wheat soil incubations decreased very slightly by 5%, whereas it increased in the grassland and forest soil incubations. Additionally, an increase of total sugar amounts occurred in grassland and forest soil incubations, whereas in the wheat soil the amounts decreased by 20% (Data not shown). This suggests that in both wheat soil incubations, due to limited C supply, recycling dominated the sugar C dynamics as the microbial community used all available C-sources. Thus, the contributions of labelled C decreased, as greater amounts of soil organic C (and not only the recently added glucose) were used in microbial metabolism. This showed how effectively the microbial community

converts C compounds and responds to changing conditions. This corresponds with studies by Salomé et al. (2010), Kramer and Gleixner (2006; 2008), Creamer et al. (2014) who showed that microorganisms change their feeding strategies from recent to more old SOM compounds depending on C availability and quality.

The increasing contribution of label C to the sugar pool in the SOM-rich grassland soil can be related to the fact that a considerably larger soil C-pool was initially accessible for microbial metabolism. In the grassland soil, this corresponds with less label-derived C in microbial biomass and CO_2 as compared to the soils under other land use. However, with increasing incubation time more labelled C was incorporated into the sugar pool because the amount of accessible "old" C decreased continuously and thus glucose, bound to SOM is successively used. In the forest soil, microbial biomass and CO_2 contained more label derived C as compared to the grassland soil. This suggests that the added labelled C source was predominantly used by the microbial biomass because most of the "old" C was not accessible for metabolism, i.e. was stabilised. Waldrop and Firestone (2004) found that the microbial community preferentially incorporated added easily degradable C compounds in low quality SOM soils. Forest litter is enriched in aromatic, phenolic and alkyl-C, which might be less attractive for microorganisms (Kögel-Knabner, 2002; Nierop et al., 2001; Helfrich et al., 2006). Therefore, the added glucose provided an easily utilisable C source compared to the SOM in the forest soil and was preferentially used by the microbial community as reflected by the high label contribution to C_{mic} and CO_2 in relation to the bulk soil label contribution (Fig.1 and Fig.3). Additionally, in the acid forest soil, decomposition occurred mainly in the humus layer, whereas in arable or grassland, decomposition occurred directly in mineral soil (Kögel-Knabner et al., 1988; Helfrich et al., 2006; Guggenberger and Zech, 1994). Therefore, the C incorporation seems to be lower than in arable and grassland soils, especially for easily utilisable compounds. Together with litter quality the reduced microbial activity promote the effect of SOM stabilisation in forest soils. Summing up, the accessibility of C compounds control the effect of recycling and stabilisation: Both recycling and stabilisation are important processes in forest soils. However, for arable soils and grassland, recycling seems to dominate the C dynamics.

4.3 Sugar dynamics

Several studies aimed at differentiating different sugar pools, based on incubations for up to 1 year (Muramaya, 1988; Derrien et al., 2007), but conclusions about factors controlling the long term decay kinetics of soil sugars were not possible, presumably due to the short duration of the experiment and a low number of sampling times. Hence, the intended target of our study was to investigate the long-term dynamics of sugars, based on highly frequent sampling during 3 years of incubation to identify multiple decay pools. However, the apparent high importance of recycling, which was shown by increasing label incorporation (Fig.4) and as a consequence positive k-values (Table S2), impeded the differentiation of several pools of the investigated sugars. This displays the drawback of the experiment, as recycling of the added C substrate influenced the decay dynamics. Ara and xyl, as supposedly plant-derived sugars, showed a considerable de-novo synthesis by microorganisms and thus the differentiation into plant derived sugars subject only to stabilisation and microbial sugars, subject to stabilisation and recycling was difficult. In our study the sugar dynamics were described by mono and bi-exponential functions. An incubation study by Derrien et al. (2007), however, used bi-exponential decay functions to describe sugar decay dynamics with a constant pool (k=0) as it apparently remained undecomposed throughout the incubation. However, in our experiment, the contribution of labelled C to the individual sugars changed throughout the incubation even though very slightly, thus the assumption of a constant pool would not correspond to our data. A labile pool could be determined for rha and gal in the wheat Ap; for xyl, fuc and man in wheat E and for ara, xyl, fuc and gal in the forest Ah (Table 3). The MRT ranged between a few days and up to two months, depending on the different investigated soils (Table 3). These data agree well with the study by Derrien et al.(2007). They reported MRT of 17 days for the labile sugar pool. The MRT of the stable microbial-derived sugars ranged up to 365 years. The highest MRT was estimated for gal in the wheat Ap with 5957 years. This is even more surprising because interactions of sugars with the soil matrix are reported as less important for their fate (Gunina and Kuzyakov, 2015) supporting the idea of recycling and not stabilization as dominant process. Therefore, such high values can only be explained by a high contribution of substrate recycling and corresponds with the observed high proportions of labelled C in the microbial biomass throughout the experiment. From pure culture studies it is known that 5% of the dry weight of prokaryotic cells consist of

polysaccharides (Madigan et al., 2003). Thus, the label contribution of the soil sugars to microbial biomass is relatively low and turnover of microbial biomass thus masks changes in sugars over time. Additionally, with chloroform-fumigation extraction mainly C of the cytoplasm is determined, whereas more complex structures in cell walls are probably hardly extracted (Joergensen, 1996; Apostel et al., 2015). This may lead to an overestimation of the dynamics of labelled C in microbial biomass as cell walls probably are neither strongly labelled at the beginning of the experiment, nor do they cycle as fast as the other pools of the microbial biomass (Glaser and Gross, 2005; Miltner et al., 2009; Malik et al., 2013).

Apart from long term label incorporation trends (discussed below), all sugars show small sinus like fluctuations (Fig. 4, most pronounced for man). One could speculate that this phenomenon might be related to shifts in the microbial community, which in turn increased resource availability, in which extracellular enzymes metabolites or lysed cells of one functional group increase substrates for another (Blagodatskaya and Kuzyakov, 2008; Mau et al., 2015).

More importantly, the similar behaviour of fuc and man on the one hand and gal and rha on the other is of interest (Fig. 4). While fuc and man showed highest label contribution directly at the beginning of the experiment and exhibited remarkable decline afterwards, label contribution in rha and gal increased during the first weeks of the experiment and reached steady state after 4 months. These different dynamics could be related with different strategies of microbial groups: fuc and man could be representative for r-strategist that quickly acquire new substrates but are forced into dormancy if nutrient supply becomes limited, whereas K-strategists could be represented by the dynamics of gal and rha: These groups only slowly profit from the added labelled nutrients, but are able to live on these resources for very long times. One could speculate whether the slow increase in gal and rha is due to recycling of starving r-strategist or results from the use of stored glucose (Blagodatskaya et al., 2007) acquired at the beginning of the experiment.

Another explanation for the different dynamics could be different provinces of the two pools. For example exopolysaccharides are part of microbial biofilms and are composed mainly of fuc, gal, man and glc (Freitas et al., 2011; Neu and Lawrence, 1997). On the other hand, lipopolysaccharides (LPS) are part of the outer cell membrane and are composed of gal, rha and man monosaccharide units (Lengeler et al., 1999). If the dynamics of fuc and man would

be representative for the dynamics of exopolysaccharides of microbial biofilms, this would indicate that they quickly incorporate available substrate but rely on "old" SOM-derived C when the added substrate is no longer available. Likewise, the gal and rha dynamics could be characteristic for LPS, indicating that these underlie a repeated recycling within the microbial biomass pool: the labelled substrate is only slowly incorporated into the LPS pool but is then retained there for long times.

5 Conclusion

The observed high MRT for sugars indicate that recycling dominates sugar dynamics in soil and that the high importance of recycling is not substantially affected by soil C content. Thus, MRT of substance classes, as stated in many previous studies, has to be taken with care, as they do not necessarily reflect the MRT of these substances but rather the MRT of the pool-derived C, which may be frequently recycled within or outside of this pool.

Further, the persistently higher contribution of added label to microbial biomass as compared to CO_2 production indicates that substrate recycling is very effective in the long term. Two different patterns of tracer dynamics could be identified for different sugars: fuc and man showed highest label contribution at the beginning of the incubation with a subsequent slow decline. Galactose and rha, on the other hand were characterised by slow label incorporation with subsequently constant levels, indicating that the dynamics of these sugars are dominated by substrate recycling.

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Table 1.Sugar composition of the organic matter in the wheat Ap, wheat E, grassland Ah and forest Ah soils before incubation. Means ± standard error, n= 3.

	Depth [cm]	SugarC [%]	Distribution of sugars[%]						
			ara	xyl	fuc	rha	gal	man	glc
wheat Ap	0-30	8±0.1	14±0.9	15±0.3	4±0.2	7±0.1	17±0.1	15±0.2	29±0.6
wheat E	30-45	7±0.8	13±0.2	13±0.5	4±0.3	8±0.1	17±0.0	15±0.1	31±0.5
grassland Ah	0-10	8±0.6	14±0.2	13±0.2	5±0.1	9±0.1	16±0.2	14±0.5	29±0.6
forest Ah	0-10	6±0.3	7±0.2	12±0.2	7±0.3	5±0.2	14±0.7	18±0.3	36±0.6

Table 2. Relative distribution of total label derived sugar C [wt%] among different sugars after 6 and 24 months of incubation (means ± standard error; n=3). Significant differences ($p<0.05$) between the two sampling times are indicated by an asterisk.

	wheat Ap					wheat E					grassland Ah					forest Ah				
Sugar	6m		24m			6m		24m			6m		24m			6m		24m		
fuc	4.0	± 1.1	3.7	± 1.9		2.7	± 0.7	3.0	± 0.4		1.3	± 0.0	1.4	± 0.1		2.1	± 0.1	3.0	± 0.2	*
ara	2.7	± 0.1	3.4	± 0.1	*	3.3	± 0.1	4.2	± 0.0	*	2.5	± 0.2	2.8	± 0.2		0.9	± 0.0	1.4	± 0.0	*
rha	8.7	± 1.2	9.7	± 2.6		7.9	± 1.0	9.8	± 0.6		4.6	± 0.1	5.6	± 0.1	*	1.5	± 0.1	2.3	± 0.1	*
gal	9.9	± 0.1	12.6	± 0.2	*	10.1	± 0.6	13.2	± 0.5	*	6.0	± 0.1	7.6	± 0.0	*	5.1	± 0.3	8.0	± 0.2	*
glc	60.7	± 2.9	54.6	± 5.6		61.0	± 2.8	52.8	± 1.4	*	75.1	± 0.3	70.3	± 1.0	*	78.5	± 1.1	66.8	± 1.3	*
xyl	2.2	± 0.2	2.7	± 0.2	*	2.8	± 0.3	3.1	± 0.1		1.9	± 0.1	2.0	± 0.4		2.5	± 0.2	4.0	± 0.3	*
man	11.8	± 0.7	13.3	± 0.6	*	12.1	± 0.4	14.0	± 0.3	*	8.8	± 0.2	10.3	± 0.3	*	9.5	± 0.5	14.3	± 0.4	*

Table 3. Estimated apparent MRT and pool size of sugars in the wheat Ap, wheat E, grassland Ah and forest Ah incubations. * reflects initial exponential growing pools.

		labile pool		intermediate/stable pool	
		years	pool size [mg g^{-1}]	years	pool size [mg g^{-1}]
wheat Ap	fuc	\	\	44	0.30
	rha	0.02	0.84*	\	\
	gal	0.07	0.17*	5957	0.67
	man	\	\	21	0.82
wheat E	ara	\	\	82	0.16
	xyl	0.2	0.07	\	\
	fuc	0.2	0.11	71	0.07
	man	0.6	0.17	79	0.50
grassland Ah	ara	0.1	0.15*		
	fuc	\	\	79	0.15
	rha	\	\	231	0.54
	gal	0.1	0.32	\	\
	man	0.04	0.25*	15	1.03
forest Ah	ara	1.20	0.26	3	0.37
	xyl	0.05	0.45*	34	0.34
	fuc	0.6	0.24	82	0.06
	rha	\	\	365	0.19
	gal	0.06	0.44*	54	0.66
	man	\	\	45	1.25

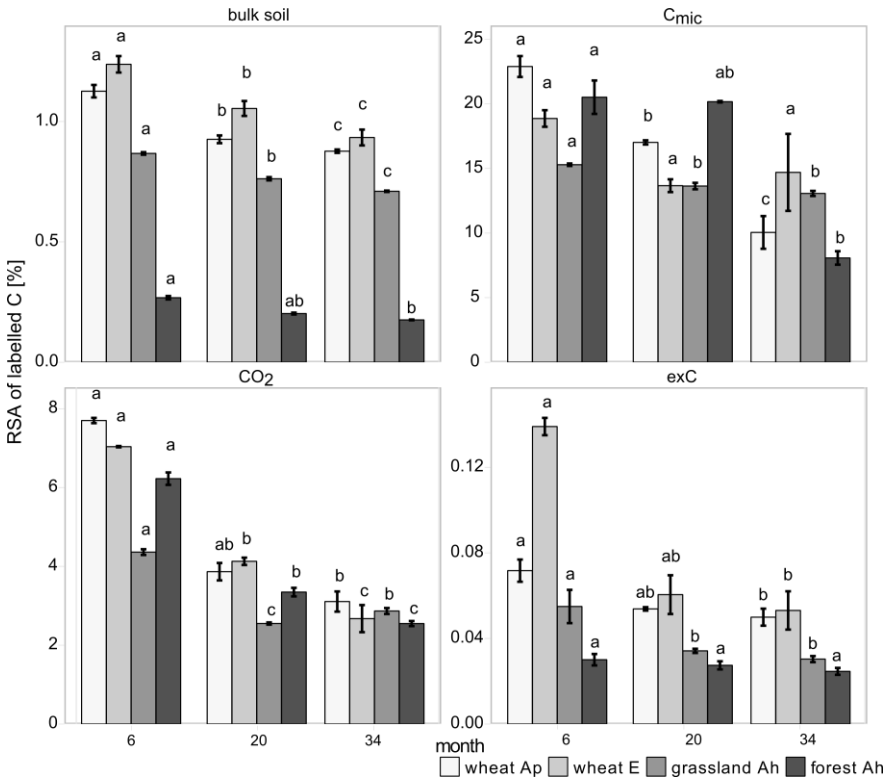

Figure 1. Fraction of labelled C in total C of bulk soil C, microbial biomass (C_{mic}), respired CO_2,and K_2SO_4-extractable carbon (exC) of the wheat Ap and E, grassland Ah and forest Ah after 6, 20 and 34 months of incubation. Different letters indicate significant differences ($p<0.05$) within one treatment over time. Means ± standard error (n=3).

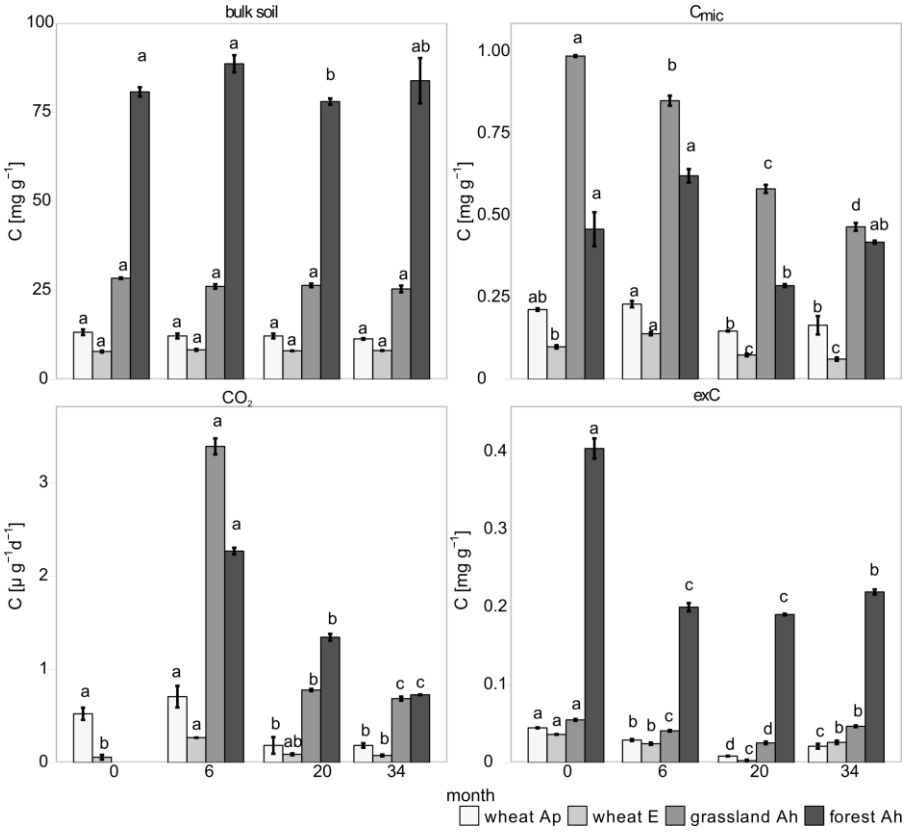

Figure 2. Concentrations of bulk soil C, microbial biomass C (C_{mic}), respired CO_2 and K_2SO_4-extractable carbon (exC) in wheat Ap and E, grassland Ah and forest Ah before (0) and after 6, 20 and 34 months of incubation. Different letters indicate significant differences ($p<0.05$) within one treatment over time. Mean and standard error (n=3).

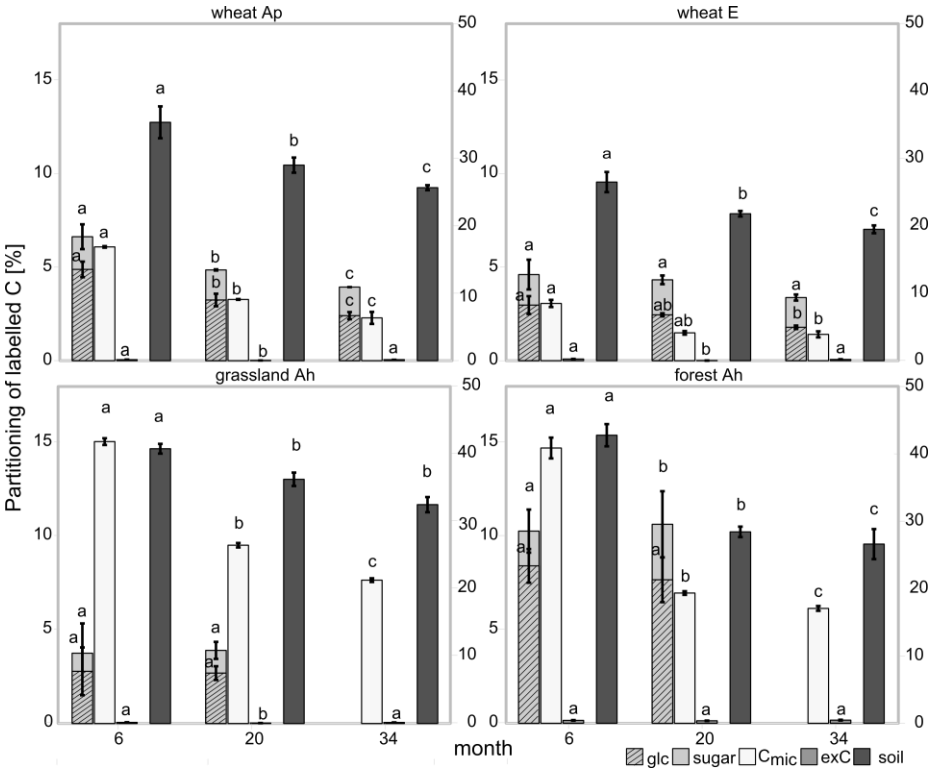

Figure 3. Partitioning of the labelled C into microbial biomass (Cmic), K_2SO_4-extractable carbon (exC), glc and sum of all sugars (left axis) and bulk soil (right axis) in wheat Ap and E, grassland Ah and forest Ah after 6, 20 and 34 months of incubation. Different letters indicate significant differences ($p<0.05$) within one treatment over time. Means ± standard error (n=3).

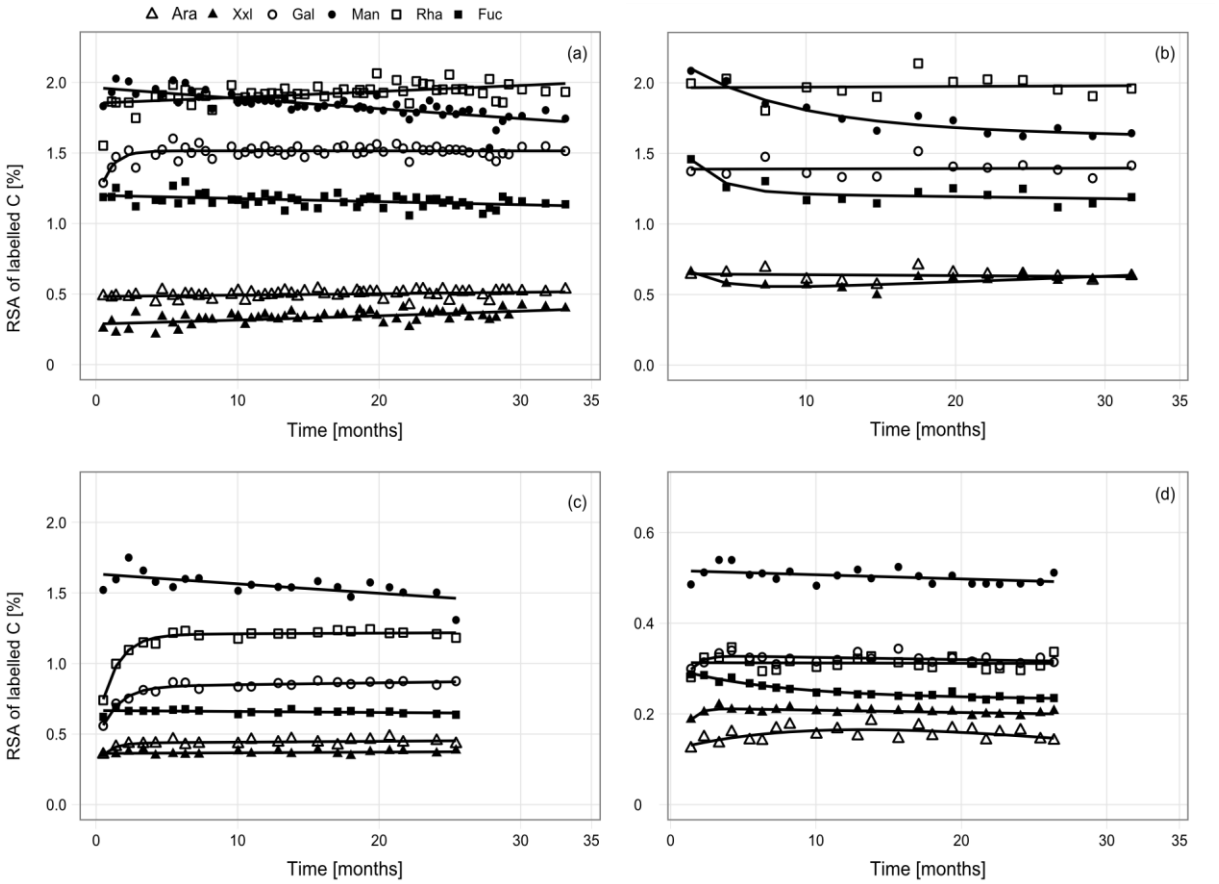

Figure 3. RSA of labelled C of individual sugars in the incubated soil samples. Lines show the fit of the observed data. a) wheat Ap ,b) wheat E horizon of c) grassland and d) forest soil. The parameters of the exponential equations are given in Table S2.