Geoderma 283 (2016) 101–109

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

Maize root decomposition in subsoil horizons of two silt loams differing in soil organic C accumulation due to colluvial processes

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ARTICLE INFO

Article history:
Received 18 February 2016
Received in revised form 1 June 2016
Accepted 25 July 2016
Available online 1 August 2016

Keywords:
C turnover
Decomposition
Density fractionation
Field experiment
Microbial biomass
Subsoil

ABSTRACT

To analyse mechanisms controlling sequestration of organic C in subsoil, a field experiment was carried out for two years. Soilbags with a mesh size of 100 μm, containing original soil material and maize root residues (C4 plant) were buried at three different depths (35, 45, and 65 cm) at two neighbouring arable sites and were sampled after 12, 18 and 24 months. The sites were a Colluvic Cambisol with high soil organic carbon (SOC) contents in the subsoil (12 mg g^{-1} soil), and the other a Haplic Luvisol with low SOC contents (4 mg g^{-1} soil) below 30 cm depth. We determined the effects of the site, depths, and time on bulk SOC, organic C associated with soil density fractions, and microbial biomass C (MBC) in the soilbags. MBC increased to a similar extent (2.5 fold) from the initial content to its maximum at all sites and depths. This increase relied largely on the added maize root residues, as about 50% of the MBC was maize-derived after two years. However, we detected distinct differences in the substrate use for anabolism compared to catabolism, which decreased with depth and was lower in the Haplic Luvisol than in the Colluvic Cambisol. Freshly added plant material seems to be highly accessible to microorganisms in subsoil, but its metabolic use was determined by the soil properties of the two sites. The addition of plant residues also had an impact on aggregation dynamics, resulting in an almost complete replacement of formerly aggregate occluded material (i.e., occluded light fraction) by maize derived material after 24 months.

1. Introduction

Two-thirds of the terrestrial C is stored in soils, which makes soil an important reservoir in the global carbon cycle (Batjes, 1996). This reservoir can either be a source or sink for CO_2 (Rumpel et al., 2002; Rumpel and Kögel-Knabner, 2011), which is of increasing interest due to climate change concerns (Bailey et al., 2002). The fact that >50% of soil organic C (SOC) is stored at a depth of 30–100 cm (Batjes, 1996; Lal and Kimble, 1997) has directed particular scientific attention towards subsoil. However, there is still limited knowledge regarding the mechanisms that control C sequestration and turnover in subsoil (Sanaullah et al., 2011; Cotrufo et al., 2013).

The high ^{14}C-based mean age found for organic compounds in subsoil (Rumpel et al., 2002; Rumpel and Kögel-Knabner, 2011) leads to the assumption that their mineralization rate is slower than in topsoil. There are strong indications that environmental conditions in subsoil are different from those in topsoil, such as less variation in temperature and reduced nutrient availability (von Lützow et al., 2006), which might lead to reduced substrate mineralization. These environmental factors also have an influence on the microbial community in subsoil, which usually shows smaller biomass and is less fungal dominated compared to topsoil (Fierer et al., 2003; Struecker and Joergensen, 2015). These changes in the microbial community and its functional diversity are presumably among the various factors controlling C sequestration in subsoil.

Another important factor is the limited input of fresh organic matter into subsoil (Fontaine et al., 2007). Only root-derived plant residues play a significant role in subsoils (Rumpel et al., 2002), which contain less labile and therefore less easily degradable compounds than shoot-derived residues (Rasse et al., 2005).

Concerning the fate of plant residues, it is largely unknown to what extent the added substrate is stabilized against microbial decomposition or contributes to the mineralization of stabilized SOC due to priming effects (Fontaine et al., 2007). The latter would be an adverse effect to the desired increase in SOC sequestration. The effects of substrate addition to subsoil have been investigated mostly in laboratory experiments (Kuzyakov, 2010) where not only substrate was added but also other environmental factors (e.g., gas conditions, temperature, moisture) were modified. This impedes the transfer of the results from such experiments to the field or ecosystem scale and underscores the need for more field experiments to analyse the decomposition of plant residues under subsoil conditions. One option is the burial of soilbags in the field (Sanaullah et al., 2011) that mimic hot spots of microbial

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http://dx.doi.org/10.1016/j.geoderma.2016.07.026
0016-7061/

activity (Schrumpf et al., 2013), being similar to naturally occurring hot spots along preferential flow pathways or rooting zones (Chabbi et al., 2009).

Degradation and fate of maize root amendments can be analysed by their incorporation into the microbial biomass but also by density fractionation, providing additional information on the stabilization of SOC (Schrumpf et al., 2013). The free light fraction (fIF) and occluded light fraction (oIF) consist mainly of organic debris present either free and easily available for microorganisms in the soil matrix (fIF) or occluded within aggregates (oIF) (Golchin et al., 1994; Cerli et al., 2012). In contrast to the fIF, the oIF consists of smaller and slightly decomposed organic particles, which are better protected against microbial degradation by occlusion in aggregates. Therefore, the turnover times of oIF are considered to be longer than those of the fIF (Schrumpf et al., 2013). The C in the heavy fraction (HF) is bound to the mineral fraction of the soil matrix (e.g. John et al., 2005) and, therefore, is considered to be stabilized against decomposition with turnover times of decades to centuries (Schrumpf et al., 2013).

In this study, we carried out a field experiment for two years. Soilbags with a mesh size of 100 μm, containing original soil material and maize root residues, were buried at three different depths (35, 45, and 65 cm) at two neighbouring arable sites and were sampled after 12, 18 and 24 months. The sites were a Colluvic Cambisol with high SOC contents in the subsoil (12 mg g^{-1} soil), the other a Haplic Luvisol with low SOC contents (4 mg g^{-1} soil) below 30 cm depth (Struecker and Joergensen, 2015).

The aim of this study was to test the following hypotheses regarding the fate and pathways of freshly added residues from maize roots as a function of soil depth and resource availability under field conditions: (1a) The mean residence times of maize root residues increase with depth and (1b) are lower in the Colluvic Cambisol than in the Haplic Luvisol, due to the higher microbial biomass in the former. (2a) Substrate incorporation into microbial biomass decreases with depth and (2b) is higher in the Colluvic Cambisol than in the Haplic Luvisol, due to higher resource limitations in the Luvisol (Struecker and Joergensen, 2015). (3) Irrespective of site and depth, the maize residues will remain mostly in the fIF, due to limited microbial degradation.

2. Material and methods

2.1. Site

Soil was sampled from two arable fields at the Hessian State Manor of Frankenhausen, northern Hessia, Germany (51°24′ N; 9°25′ E), the experimental farm of the University of Kassel. The area is characterized by a mean annual air temperature of 9.3 °C and a mean annual precipitation of 687 mm. The soils of site I (referred to as Cambisol or Cam) can be characterized as a Colluvic Cambisol according to the WRB (FAO, 2014). The Colluvic horizon of the Colluvic Cambisol covers the original soil surface of a Chernozem by about 70 cm, resulting in an Ap/M/fAh sequence. The soils of site II (referred to as Luvisol or Luv) can be classified as a Haplic Luvisol according to the WRB (FAO, 2014), although the Al horizon was eroded. This results in an Ap/Bt sequence. The soils of the two sites have been developed on loess and are within a distance of 400 m from each other, which means that climatic conditions are equivalent on both sites, although they have different SOC profiles due to erosion and deposition. Land use at both sites was also similar for at least 400 years, during which time both sites were used as grassland first and then as cropland since the early 20th century (Troßbach, 2000). Soil characteristics are shown in Table 1.

Both sites were converted from cropland to cattle pasture using a grass–clover mixture in spring 2013. There was no organic fertilizer added except cattle excretions. There is no evidence for the cultivation of C4 plants in the field history. As the cows did not receive additional fodder during the grazing periods, there was also no input of maize residues during the time of the experiment.

Table 1
Organic C, ^{13}C, Total N, and C/N ratio for maize root residues before mixing. Soil organic C (SOC), ^{13}C, Total N, C/N, microbial biomass C (MBC) for original soil material and Organic C, Total N, and C/N ratio for the soil and maize root residue mixture before burial.

Maize root residues								
Organic C (mg g^{-1} roots)		^{13}C δ‰	Total N (mg g^{-1} roots)		C/N	MBC (μg g^{-1} roots)		
276		−12.5	4.3		64	0		
Soil without roots						Soil with roots		
Depth (cm)	SOC (mg g^{-1} soil)	^{13}C δ‰	Total N (mg g^{-1} soil)	C/N	MBC (μg g^{-1} soil)	Organic C (mg g^{-1} soil)	Total N (mg g^{-1} soil)	C/N
Cambisol								
35	14.4	−26.2	1.5	10	216	20.3	1.6	12
45	10.9	−24.2	1.1	10	133	18.5	1.2	15
65	9.0	−25.3	0.9	10	83	12.3	1.0	12
Luvisol								
35	10.8	−28.8	1.2	9.0	161	17.0	1.3	13
45	6.9	−26.6	0.65	11	49	12.8	0.72	18
65	5.4	−26.0	0.52	10	39	9.3	0.57	16

2.2. Soilbag experiment

Samples from both sites were taken at three different subsoil depths (35, 45, and 65 cm) in April 2013. The autochthonous, naturally occurring SOC contents and isotopic signatures unaffected by the added maize roots were determined from these samples. Afterwards each sample was mixed with 1.5 wt.% of dried and shredded maize root residues (1–2 mm), which equals a C addition of 4.2 mg g^{-1} and an N addition of 0.06 mg g^{-1}. All soil samples had the same dry mass (20 g) and were homogeneously mixed with the same amount of maize root residues (300 mg) after sieving of the soil (<2 mm). The mixture was filled into 5 × 5 × 0.5 cm mesh bags (mesh size: 100 μm). This mesh size prevented soil losses from the bags but allowed access by microorganisms. The possible ingrowth of fine roots was accepted as a compromise, allowing sufficient moisture exchange with the surrounding soil. Afterwards they were stored field moist at 4 °C until they were buried in June 2013. The bags were buried on their original field sites at their original depths with 9 field replicates. They were recovered after 12, 18, and 24 months with 3 field replicates per depth. The sampled soilbags were also stored field moist at 4 °C. At the end of the experiment, the soil surrounding the bags at a distance of 15 cm was also sampled, to investigate the effects of the buried soil/root mixture on the characteristics of the soil material in close vicinity to the buried samples. These samples were sieved <2 mm and stored field-moist at 4 °C.

2.3. Microbial biomass C

Soil microbial biomass C (MBC) was analysed by fumigation–extraction (Vance et al., 1987). Two grams of fumigated (24 h with ethanol-free $CHCl_3$ at 25 °C) and non-fumigated soil was extracted with 8 ml of 0.05 M K_2SO_4 (Potthoff et al., 2003) by 30 min horizontal shaking at 200 rev min^{-1} and filtered (hw3, Sartorius Stedim Biotech, Göttingen, Germany). Organic C in the extracts was determined using a multi N/C 2100S automatic analyser (Analytik Jena AG, Jena, Germany). MBC was calculated as E_C/k_{EC}, with E_C = (organic C extracted from fumigated soil) − (organic C extracted from non-fumigated soil) and k_{EC} = 0.45 (Wu et al., 1990).

For the determination of ^{13}C, 4 ml aliquots of 0.05 M K_2SO_4 extracts of fumigated and non-fumigated samples were freeze dried for about 3 days and were analysed by isotope ratio mass spectrometry (Elemental analyser Flash 2000, Thermo Fisher Scientific, Cambridge, UK; Delta V Advantage, Thermo Electron, Bremen, Germany).

Table 2
Main effects of site, depth, and sampling time on autochthonous soil organic C (SOC_a), maize-derived SOC (SOC_m), autochthonous microbial biomass C (MBC_a), maize-derived MBC (MBC_m), and total N. The factors time and depth were considered as repeated measures. The values given for the factor steps of the main effects are the mean value of all results obtained from this factor step irrespective of the other main effects and factor steps.

	SOC_a	SOC_m	MBC_a	MBC_m	Total N
Main effects	(mg g^{-1} soil)		(μg g^{-1} soil)		(mg g^{-1} soil)
Cambisol	17.4	1.15	156	116	1.16
Luvisol	13.6	1.13	105	81	0.80
35 cm	19.0	1.02	214	132	1.29
45 cm	15.0	1.19	91	83	0.90
65 cm	12.5	1.22	85	80	0.75
12 months	13.3	1.61	176	91	0.97
18 months	14.5	1.42	95	82	0.97
24 months	18.6	0.40	121	123	0.98
Site	0.03	n.s.	0.04	0.01	<0.01
Depth	0.02*	n.s.	0.01*	<0.01*	<0.01*
Time	<0.01*	<0.01*	<0.01*	0.05*	n.s.
Site × depth	n.s.	n.s.	n.s.	n.s.	0.04*
Site × time	n.s.	0.08*	0.04*	0.07	n.s.
Time × depth	n.s.	n.s.	0.04*	0.10	n.s.
Site × depth × time	n.s.	n.s.	0.08*	n.s.	n.s.
CV (± %)	34	71	72	54	30

CV = mean coefficient of variation between replicate samples within site and depth ($n = 3$).
* p-Values after Greenhouse–Geisser correction.

2.4. Density fractionation

Density fractionation was conducted with control and soilbag samples using the method of John et al. (2005) as modified by Cerli et al. (2012), Griepentrog and Schmidt (2013), and Kaiser and Berhe (2014). We conducted a series of pre-tests to define the most suitable experimental setting with respect to the density cut offs used to separate the flF and the olF and the amount of ultrasonic energy applied to disperse aggregates before the olF was separated (Cerli et al., 2012). Soil of 10 g dry weight was dispersed in 50 ml of a 1.6 g cm^{-3} sodium polytungstate (SPT) solution. After 1 h, the sample was centrifuged at 4000*g* for 30 min. After another 30 min, the free light fraction (flF) was decanted on a filter and washed with 1.5 l of distilled water. The solid residue was mixed with 50 ml of a 1.6 g cm^{-3} SPT again and dispersed with an ultrasonic probe at 300 J cm^{-3}. After 1 h, the sample was centrifuged again at 4000*g* for 30 min. After another 30 min for stabilization, the occluded light fraction (olF) was decanted on a filter and rinsed with 1.5 l of distilled water. The residual heavy fraction (HF) was also washed with distilled water until the conductivity of the washing water was below 50 μS cm^{-1}.

The mass of the flF recovered in this procedure equates to the mass of the recovered maize root residues, as >90% of the flF consisted of maize roots.

In the three density fractions and in the bulk soil samples, total C and N as well as $\delta^{13}C$ were measured using an elemental analyser (NA1110, CE-Instruments, Rodano Milan, Italy) with interface (Conflo III, Finnigan MAT, Bremen, Germany) and isotopic ratio mass spectrometry (Delta Plus, Finnigan MAT, Bremen Germany). As the samples did not contain any carbonate, total C equals soil organic C (SOC).

2.5. Calculations and statistics

The amount of maize-derived C in the microbial biomass ($^{13}C_{MB}$) was calculated by the following equation (Potthoff et al., 2003):

$$^{13}C_{MB}(\%) = \frac{(^{13}C\text{–}Atm.excess_{fum} \times C_{fum}) - (^{13}C\text{–}Atm.excess_{nfum} \times C_{nfum})}{(C_{fum} - C_{nfum})} \times 100$$

where C_{fum} and C_{nfum} represent the mass of C (mg g^{-1}) extracted from the fumigated and non-fumigated soil, respectively, and ^{13}C-$Atm.excess_{fum}$ and ^{13}C-$Atm.excess_{nfum}$ represent the corresponding ^{13}C atom % excess values calculated from the maize amended and control treatments. The fraction of maize-derived C ($f_{maize\text{-}C}$) was calculated for each individual replicate of all treatments and the fractions derived from the density fractionation from the isotope data according to a two pool-mixing model with the following equation:

$$f_{maize\text{-}C} = \frac{\delta^{13}C_{sample} - \delta^{13}C_{control}}{\delta^{13}C_{maize} - \delta^{13}C_{control}}$$

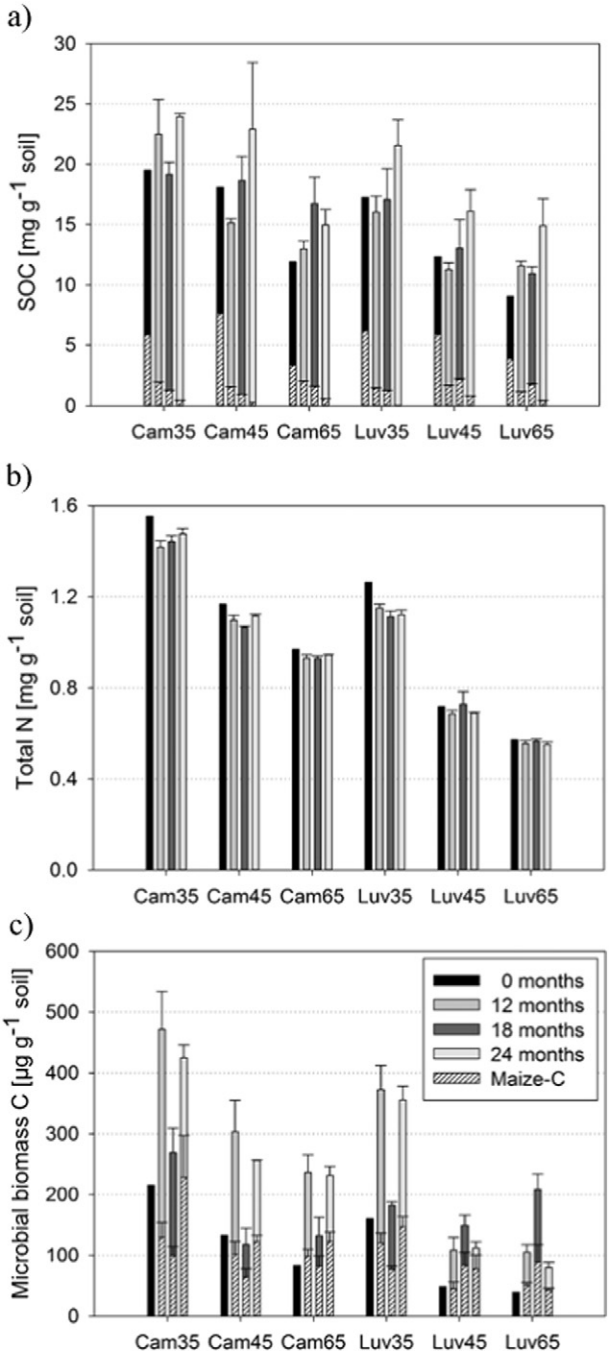

Fig. 1. Contents of the autochthonous a) soil organic carbon (SOC), b) total nitrogen (N), and c) microbial biomass C (MBC) and the proportions of the maize-derived organic C for SOC (a) and MBC (c) at the four sampling dates for the three soil depths of the two sites (soil depth: 35 cm (35), 45 cm (45), and 65 cm (65); sites: Cambisol (Cam) and Luvisol (Luv)). Error bars show one standard error from the three field replicates.

Table 3
Amounts of free light fraction (flF), occluded light fraction (olF), and heavy fraction (HF) with and without maize roots at the beginning of the experiment.

	flF		olF	HF
Depth	− roots	+ roots	− roots	− roots
(cm)	(mg g^{-1} soil)		(mg g^{-1} soil)	(mg g^{-1} soil)
Cambisol				
35	0.1	13.9	6.5	979
45	0.0	12.3	11.0	984
65	0.0	12.4	10.0	983
Luvisol				
35	0.2	12.6	4.5	981
45	0.1	14.1	2.0	984
65	0.0	9.9	2.0	987
CV (± %)	123	12	64	0

CV = mean coefficient of variation between replicate samples within site and depth ($n = 3$).

where $\delta^{13}C_{sample}$ represents the $\delta^{13}C$ value of SOC, MBC and the density fractions (flF, olF, HF); $\delta^{13}C_{control}$ is the average $\delta^{13}C$ value of the non-amended control samples and $\delta^{13}C_{maize}$ is the $\delta^{13}C$ of the maize residues. In accordance with Collins et al. (2000), Ehleringer et al. (2000) and Ekblad et al. (2002), we assumed that no isotopic fractionation occurred during the experimental period, as the added substrate did not show an enrichment in heavy isotopes when comparing the start and end of the experiment.

Assuming a first order decay kinetic (Balesdent and Mariotti, 1996), the degradation can be described by the following equation:

$$SO^{13}C_{ti} = SO^{13}C_{t0} \times e^{-kt} \quad \text{or} \quad -k = \ln\left(SO^{13}C_{ti}/SO^{13}Ct0\right)/t$$

where $SO^{13}C$ equals the maize-derived C content at the sampling date ti, $SO^{13}C_{t0}$ the maize-derived C content before burial, k the decay constant, and t the time between burial and sampling date. Then, mean residence time (MRT) of the maize root residues is $1/k$.

The data were tested for homogeneity of variances, using the Levene test and for normal distribution of residues, using the Shapiro–Wilk test, accompanied by a graphical assessment of histograms and qq-plots. We used Student's *t*-test for pairwise comparisons, because the data for SOM fractions and density fractions were normally distributed. The test for significance of the main effects site, depth, and time as well as their interactions on different measures was conducted with an analysis of variance, where time and depth were considered as repeated measures and site as independent factor, applying a mixed effects model approach using *ezANOVA*. To account for sphericity of the data, Mauchly's test for sphericity was applied and followed by a Greenhouse–Geisser correction. All statistical analyses were performed by R (R Development Core Team, 2010).

3. Results

3.1. SOC and total N

The addition of 1.5 wt.% maize root residues contributed up to 60% to SOC in the soilbags (Table 1). As the maize root residues had a wider C/N ratio (64) than autochthonous SOC (10), N did not strongly increase after substrate addition (Table 1).

The content of maize-derived C significantly decreased during the experiment, without site or depth effects (Table 2). After 12 months, maize-derived SOC in the bulk sample was generally <50% of the initial value (Fig. 1a), which equalled an average mean residence time (MRT) of 355 days for maize root residues for both sites and all depths. After 24 months, maize-derived SOC in the bulk sample was always mostly lost, resulting in an even lower estimated MRT of 291 days. Autochthonous SOC inside the soilbags increased over 24 months, without showing significant site × depth, site × time or time × depth interactions (Table 2, Fig. 1a). Total N showed a slight decrease at 35 cm, but remained stable at all other depths (Fig. 1b).

3.2. Density fractions

Initially, all soil samples (without residue addition) contained no or negligible amounts of the free light fraction (flF). Hence, almost all flF recovered after root residue addition was maize-derived (Table 3). Before substrate addition, the Cambisol samples contained less occluded light fraction (olF) at 35 cm, than at 45 and 65 cm, while the Luvisol contained generally less olF, but with a contrasting distribution (Table 3). Regarding the contribution of each fraction to SOC, the HF contained by far the largest C amounts, despite the low C contents (0.3–1.0%) compared with flF or olF (35–45%), even after substrate addition (Fig. 2). HF-C usually increased over the 24 months, accompanied by a decrease in flF-C, whereas the proportion of olF-C remained within a similar range throughout the experiment.

In the Cambisol, the recovered maize root residues did not decrease within the first 12 months, followed by a decrease within the next

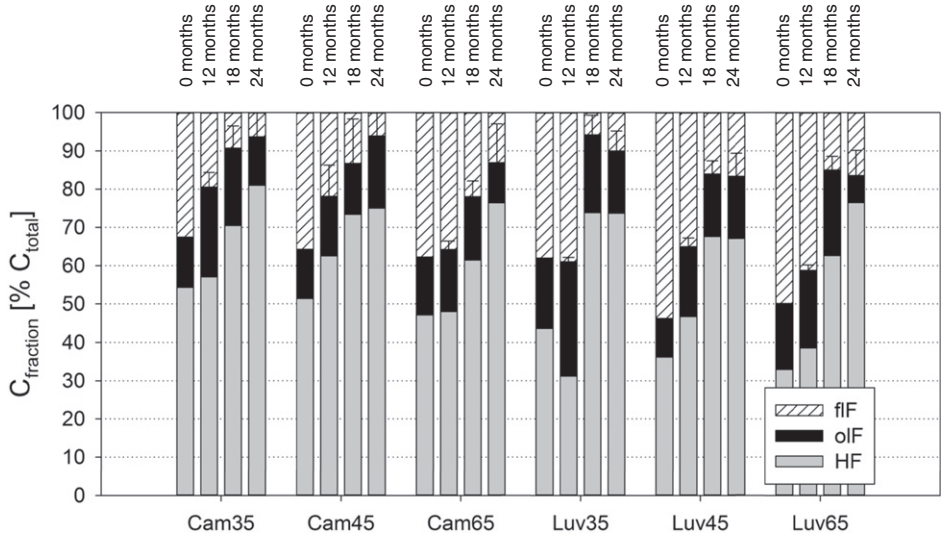

Fig. 2. Contributions of organic C associated with the three density fractions to the bulk soil organic C after 0, 12, 18, and 24 months (bars per group from left to right) for the three soil depths of the two sites (soil depth: 35 cm (35), 45 cm (45), and 65 cm (65); sites: Cambisol (Cam) and Luvisol (Luv)). Error bars show one standard error from the three field replicates.

6 months, which was stronger at 35 cm depth than at 45 and 65 cm depth. Towards the end of the experiment, the recovered amount of maize root residues was similar again at all depths (Fig. 3a). In the Luvisol, the recovered maize root residues already decreased within the first 12 months, followed by a further decrease in the next six months to stable values until the end of the experiment (Fig. 3a). This decrease was also more pronounced at 35 cm than at 45 and 65 cm depth.)

In the Cambisol, the proportion of maize-derived olF-C continuously increased until 18 months (5–15%), followed by an even stronger increase (50–65%) until the end of the experiment, so that 70–90% of olF-C were maize-derived (Fig. 3b). In the Luvisol, there was no continuous increase until 18 months at 35 and 45 cm depth, but also a strong increase (60%) from 18 to 24 months. At 65 cm depth, the Luvisol samples showed a similar development to that of the Cambisol samples, so >95% of olF-C was maize-derived after 24 months (Fig. 3b). The proportion of maize-derived C in HF-C did not show a clear pattern (Fig. 3c). A decrease in maize-derived C in HF-C over time was detected for the 65 cm depth of the Cambisol and all Luvisol depths. The highly variable proportion of maize-derived C in HF-C remained roughly stable at 35 and 45 cm depth of the Cambisol. This resulted in significant time × depth and site × time × depth interactions, partly due to artefacts from the fractionation process.

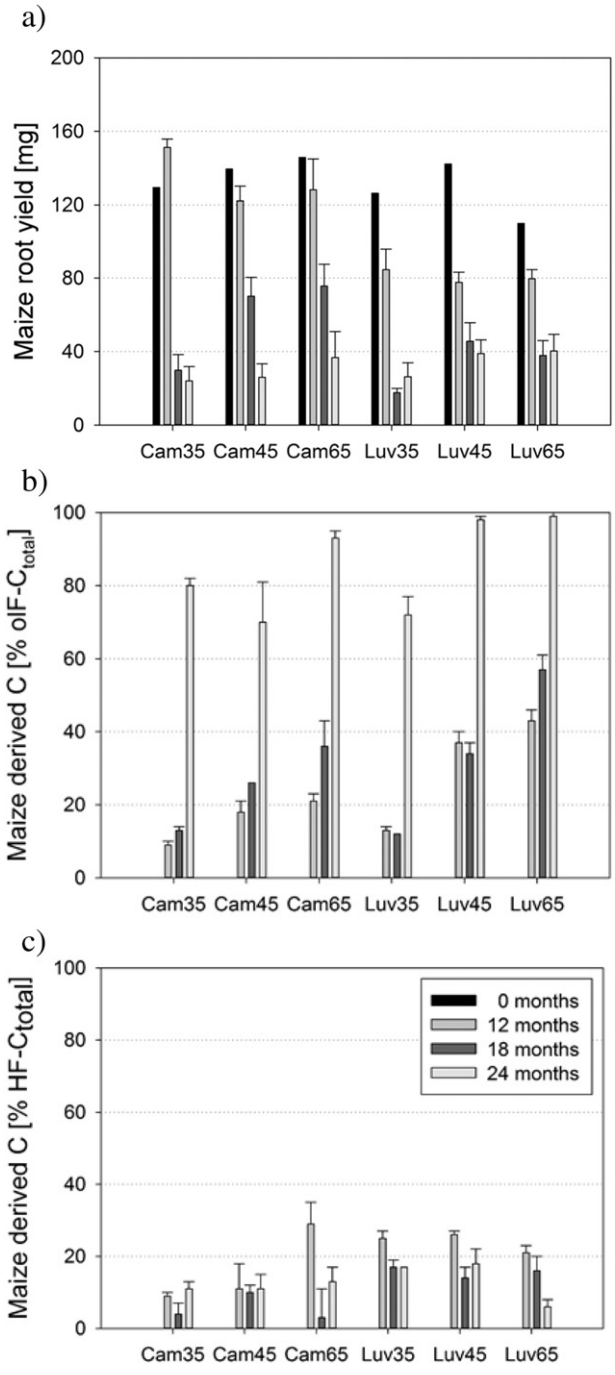

Fig. 3. a) Amount of maize roots recovered in free light fraction (flF), b) maize-derived C in the occluded light fraction (olF), and c) maize-derived C in the heavy fraction (HF) for the three soil depths of the two sites (soil depth: 35 cm (35), 45 cm (45), and 65 cm (65); sites: Cambisol (Cam) and Luvisol (Luv)). Error bars show one standard error from the three field replicates.

The distribution of maize-derived C between the three density fractions showed that large amounts of maize-derived C were lost within the first 12 months (Figs. 1a, 3). Thereafter, the largest proportion of the remaining maize-derived C was still in the flF. However, up to 5% of the initially added maize-derived C was transferred to olF in the first 12 months (Fig. 4). Afterwards, the proportion of initially added maize C in the flF decreased towards the end of the experiment. The proportion of initially added maize C in the olF remained roughly stable between 5 and 10% (Fig. 4).

The C/N ratio of the maize root residues strongly increased from 64 before mixing to 80–115 after mixing them into the soil (Fig. 5a), forming almost all flF. After 12 months, the flF-C/N ratio significantly ($p < 0.01$) decreased again below the initial values of the maize root residues (Fig. 5a). The olF-C/N ratio decreased within the first 12 months, followed by a significant ($p < 0.01$) increase within the next 12 months, exceeding the starting values (Fig. 5b). The visible increase in the HF-C/N ratio was insignificant, due to the high variability of the data (Fig. 5c).

3.3. Microbial biomass C

Total MBC increased within the first 12 months in all samples (Fig. 1c). All Cambisol samples and those at 35 cm depth of the Luvisol (Luv35) showed the lowest MBC content 18 months after burial, while those at lower depths in the Luvisol (Luv45 and Luv65) reached their maximum after burial at the same time. In all samples, MBC was higher after 24 months than the initial contents, but decreased compared with the 12-month content after burial. This resulted in an average turnover time of 624 days for MBC, with the significantly ($p < 0.01$) faster turnover in the Cambisol samples (Cam35 = 418 days; Cam45 = 445 days; Cam65 = 582 days) than in the Luvisol samples (Luv35 = 694 days; Luv45 = 796 days; Luv65 = 808 days). The decrease in turnover times with depth was not significant.

Maize-derived MBC increased within the first 12 months of the experiment in all soilbags, and the proportion of maize-derived MBC compared with autochthonous MBC was even higher after 18 and 24 months than after 12 months (Fig. 1c).

After 24 months, the neighbouring soil contained 11 to 50 μg g^{-1} soil maize-derived MBC, with tendencies to be higher in the Cambisol and to decline with depth (Table 4).

4. Discussion

4.1. SOC and total N

The highly variable increase in SOC in the soilbags was caused by an increase in autochthonous SOC. We observed large amounts of fresh roots growing directly around the bags, which were presumably accompanied by the growth of hyphae from arbuscular mycorrhizal fungi into the bags. Furthermore, the mesh size of 100 μm also allowed the ingrowth of fine roots into the bags, which might have served as SOC input as well. The enhanced root growth was probably supported by the large pores, which were inevitably created during the burial of the soilbags. Planning the experiment, we underestimated the impact of these pores, otherwise control soilbags without maize roots could

have been buried to assess the effect of the bag and the burial itself. The increased nutrient availability due to higher microbial activity at these artificial hotspots presumably also caused a migration of microorganisms towards the soilbags.

The decrease in maize derived C was highly variable within the replicates of the different sample types, suggesting a high spatial heterogeneity for microbial decomposition in subsoil (Jörgensen et al., 2002; Chabbi et al., 2009). However, the means for the three replicates were similar for all sample types, with mean residence times of about 300 days at all depths of both sites. The redistribution of the initially added maize C towards the three density fractions was also similar at all depths of both sites. This suggests that the decomposition kinetics and the following stabilization of plant residues depend rather on very distinct conditions, e.g. small variations in microbial activity or soil moisture, than on more general factors like depth or autochthonous SOC contents.

The effect of the soilbags on the surrounding soil after 24 months differed between 35 cm and the deeper layers. While the SOC contents in the surrounding soil decreased at 35 cm depth, those at 45 and 65 cm depth increased. The increase in the deeper layers of the surrounding soil suggests that substrate addition did not cause enhanced SOC losses in these layers. Hence, there was no evidence of a positive priming effect in the deeper subsoil layers of the surrounding soil. This is contradictory to the findings of Fontaine et al. (2007), who found positive priming effects in subsoil after cellulose addition. The decrease in SOC at 35 cm depth of the surrounding soil was probably supported by enhanced additional substrate input through rhizodeposition (Hütsch et al., 2002; Pausch and Kuzyakov, 2012; Pausch et al., 2013). As rhizodeposition contains soluble and easily degradable substrate (Wichern et al., 2008), they might have provided energy for decomposition of older SOC. To prove this, the same experiment should be conducted including soilbags on a bare fallow field, which would exclude possible effects of root exudates and root ingrowth. Considering these findings, it appears to be crucial to differentiate between different subsoil conditions (e.g., root abundance or resource availability) when assessing the impact of treatments (e.g., substrate addition).

4.2. Density fractions

Similarly to the mean residence time and the redistribution of maize-derived C, the recovered amount of maize root residues suggested that the decomposition of maize roots was similar at all depths of both sites after 24 months of burial. However, there was some variation in the temporal decomposition patterns at different depths, as less maize root residues were recovered at 35 cm depth after 18 months than at lower depths. This might be explained by a larger microbial biomass, measured during the preceding period at this depth, with a higher capability to colonize plant residues. Furthermore, the microbial community is likely better adapted to decomposing plant roots (Sanaullah et al., 2011).

The strong increase in the C/N ratio in the fIF between mixing of soil and maize root residues and the analysis for t_0 (7 days) in comparison with the fresh maize root residues indicates that the microbial community took up large contents of N from the plant material immediately, as >90 % of the fIF consists of maize root residues. This increase in the C/N ratio is typical for the initial decomposition of N-rich substrate in N-limited environments (Ågren et al., 2013). However, the applied residues cannot be considered as a N-rich substrate due to the high C/N ratio of 64 and the metabolization of N from decomposing residues should be coupled to a similar or even higher C loss (mineralization into CO_2 or incorporation into growing microorganisms). An artificial effect due to, for example, the redistribution of material derived from the dissolved OM (i.e., solubilized in the SPT solution) into the fIF during the fractionation procedure thereby increasing the C/N ratios seems to be unlikely. We conclude this from the data of Crow et al. (2007) and Gentsch et al. (2015) who did not find higher C/N ratios for the OM solubilized in the SPT solution than for the fractions recovering organic particles (i.e., light fraction and particulate organic matter fraction). Alternatively, the remaining 10% of the fIF fraction might be the result from a selective accumulation of microbially less attractive material extremely rich in C and poor in N.

After the burial of the soil bags, the C/N ratio in the fIF decreased continuously, following the increase in total N (from 0.40% to 1.17%) in the fIF within the first 18 months of burial, which is typical for the N accumulation in decomposing litter (Moore et al., 2006). Within the last 6 months of burial, C/N ratios remained constant or showed a slight increase again, suggesting that a critical C/N ratio was reached, which had been followed by mobilization of N (Ågren et al., 2013). The decrease of the C/N ratio was caused by the ongoing microbial degradation of the plant material, but the values in the fIF remained well above SOM values, indicating the low degradation state of fIF material (Moore et al., 2006; Sanaullah et al., 2011).

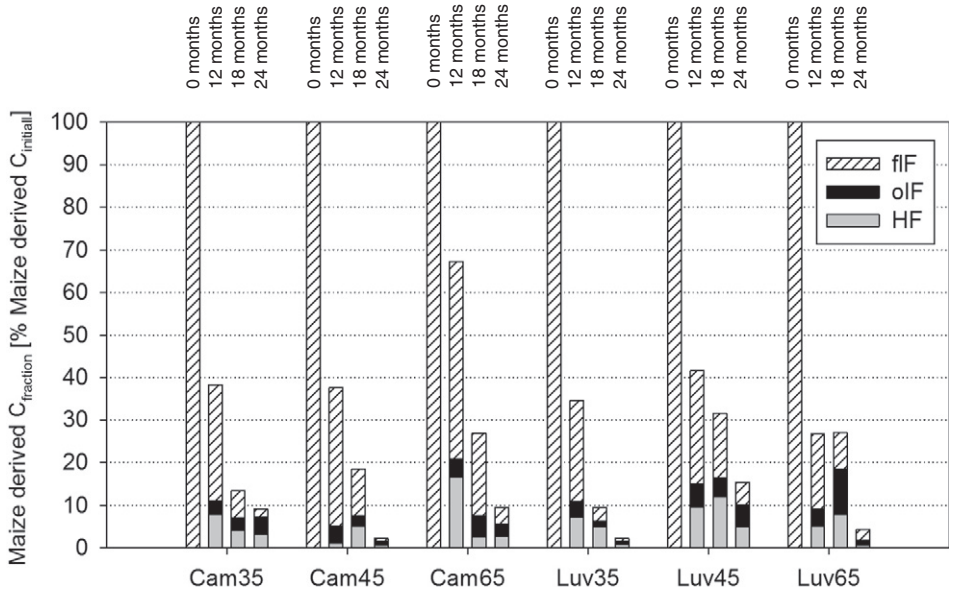

Fig. 4. Distribution of maize derived C within the three density fractions as a percentage of the initial amount of added maize derived C after 0, 12, 18, and 24 months (bars per group from left to right) for the three soil depths of the two sites (soil depth: 35 cm (35), 45 cm (45), and 65 cm (65); sites: Cambisol (Cam) and Luvisol (Luv)).

In contrast, the C/N ratio of the olF decreased within the first 12 months of burial. Due to the addition of easily available plant material, the microbial community might have been enabled to access this material occluded in aggregates via excretion of exo-enzymes, causing further degradation of the organic material (Kuzyakov, 2010). The later increase in the C/N ratio of the olF was probably caused by the occlusion of less degraded maize derived compounds. This is supported by the extreme shift from autochthonous C to maize-derived C between 18 and 24 months of burial, which suggests that 75–95% of the olF were maize-derived after 24 months. These findings indicate that freshly added plant residues replaced the formerly occluded material rather quickly and similarly in all subsoil depths. They also show that the aggregate break-up and formation in the subsoil is affected by the addition of plant residues. The added plant residues might stimulate microbial activity and, after being colonized and/or partially decomposed, which is accompanied by an increased reactivity, serve as aggregation cores. At the same time, the stimulated microbial community might also decompose largely the material already occluded in aggregates, leading to their break up and reformation occluding the newly added plant residues. Such a stimulating effect of fresh plant residues on aggregation processes has been already observed in topsoils (Wang et al., 2014). As microbial biomass increased significantly and to a similar maximum at all depths after the addition of the plant residues, this amendment had very similar effects on the formation and break up of aggregates at all depths. This enhanced dynamic in aggregation processes caused the exposure of material to degradation that was protected before. On the one hand, the protection of the added plant residues from degradation is beneficial in terms of reduced C mineralization, but on the other hand the fate of the formerly stabilized and now exposed material is unknown. Therefore, it remains difficult to assess the overall effects of plant residue additions in terms of enhanced C sequestration. However, the increase of SOC in the soilbags provided some evidence that the addition of maize roots has no adverse effects regarding C storage.

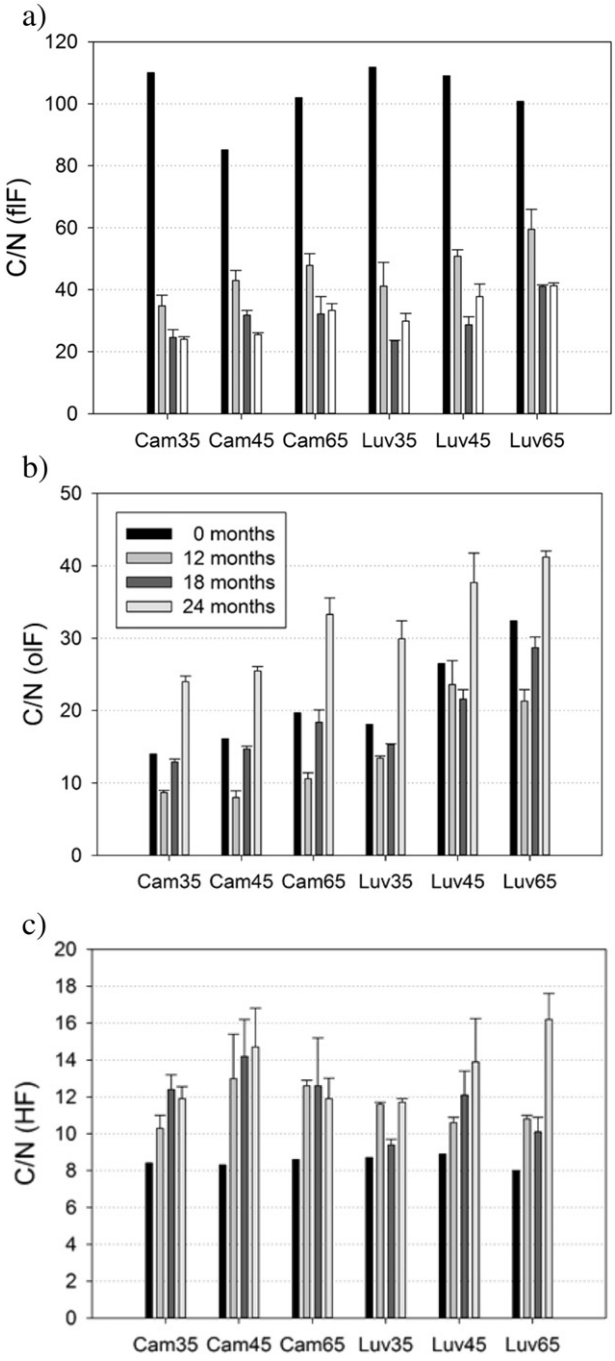

Fig. 5. C to N ratios of the different density fractions during the experiment. a) Free light fraction (flF), b) occluded light fraction (olF), and c) heavy fraction (HF) for the three soil depths of the two sites (soil depth: 35 cm (35), 45 cm (45), 65 cm (65); sites: Cambisol (Cam) and Luvisol (Luv)). Error bars show one standard error from the three field replicates.

The increase in the C/N ratio of the HF showed a high variability. However, the proportion of maize-derived C in the HF remained relatively small and did not explain the whole increase in HF-C. Therefore, the increase in HF-C relied to a certain extent on autochthonous SOC, which would suggest an enhanced autochthonous SOC stabilization in the HF during the experiment. Diochon et al. (2016) found an enhanced stabilization of microbial decomposition by-products in the mineral fraction during an incubation experiment with plant residues. The increase in MBC, the apparent decomposition of maize residues by the microorganisms, the mixture of maize-derived and autochthonous SOC in the HF, as well as the smaller C/N ratios in the HF provided some evidence that the stabilized C in the HF in our experiment is also of microbial origin. Presumably the organic matter formerly protected in aggregates as olF, which was replaced by maize-derived components, was further metabolically processed and thereby partially stabilized in the HF (Cotrufo et al., 2013).

Before substrate addition, Luv35 contained more olF than Luv45 and Luv65. As the total C contents in the Luvisol also decreased with depth, the decrease in olF followed this pattern. The Cambisol samples showed a contrasting pattern, with Cam35 containing less olF than Cam45 and Cam65. As the olF consists mainly of small, slightly degraded plant material particles (Wagai et al., 2009; Schrumpf et al., 2013), which are occluded in aggregates, this pattern was also reflected in a decreasing proportion of SOC metabolically processed by microorganisms in total SOC (Struecker and Joergensen, 2015). The reason for this depth distribution in the Cambisol was probably the ongoing deposition of eroded material containing weakly degraded plant material (Berhe et al., 2008; Berhe, 2012). This organic matter from the eroded material became occluded in aggregates after deposition (Wang et al., 2014) and was therefore protected from further degradation as long as disturbance is kept to a minimum (Berhe et al., 2012).

4.3. Microbial biomass C

The increasing proportion of maize-derived MBC proved that the microbial community utilized the added maize root residues for biomass formation, with the amount of maize-derived C, which is incorporated into microbial biomass, being higher in the Cambisol and declining with depth. However, as only a small proportion of the maize-derived C that was lost from the soilbags was incorporated, the largest amount was presumably respired as described by Rubino et al. (2012) for laboratory incubations. Furthermore, material of the maize root residues added was also lost from the soilbags due to leaching of soluble compounds (Murphy et al., 2000; Chantigny, 2003) or hyphal transfer via

Table 4
Contents of maize-derived microbial biomass C (MBC_m) in soil surrounding the soilbags after 2 years of exposure.

Depth (cm)	MBC_m (μg g^{-1} soil)	MBC_m (% of MBC)
Cambisol		
35	50	20.0
45	43	25.5
65	18	44.8
Luvisol		
35	16	15.8
45	16	21.9
65	11	32.6
CV (± %)	24	

CV = mean coefficient of variation between replicate samples within site and depth ($n = 3$).

soil fungi (Poll et al., 2008; Rottmann et al., 2010), as maize-derived C was detected in the microbial biomass of the surrounding soil at all depths of both sites. Luv45 and Luv65 differed from all other sampling points in terms of MBC development, reaching the highest contents after 18 months. These two sampling points also differ strongly from the other sampling sites in their autochthonous C and N contents, which are lower than at all other sites and they also had the smallest initial amounts of MBC, which indicates that microbial growth might be resource limited (Fierer et al., 2003; Struecker and Joergensen, 2015). Hence, the substrate was presumably used for catabolism first to cover energy demands. Afterwards, the proportion of anabolism increased.

The increase in autochthonous MBC after 24 months provides some evidence that additional autochthonous SOC is used by soil microorganisms, which was not accessible before due to resource limitations. The decrease in maize-derived C and the increase in autochthonous SOC support the view that soil microorganisms preferably degrade fresh substrate compared with autochthonous SOC. The latter is presumably energetically less attractive or no longer contains labile compounds (Salome et al., 2010; Rumpel and Kögel-Knabner, 2011; Dungait et al., 2012; Cotrufo et al., 2013). The fact that at those sampling points containing higher amounts of autochthonous C, no >60% of MBC were maize-derived at any sampling date, indicates a co-use of this C for anabolism, while the strong initial losses of maize-derived C indicate a preferential use of substrate-derived C for catabolism.

5. Conclusions

Maize root residues were similarly decomposed in the soilbags added at all depths and both sites. Hence, the initial differences in MBC, which remain visible throughout the experiment, had no effect on the loss rates. However, these differences became apparent when looking at substrate incorporation, which was lower in soil samples with lower MBC contents, i.e. in the Luvisol in comparison with the Cambisol and at 45 and 65 cm depth in comparison with 35 cm depth. This means that the maize root residues were catabolised largely at sampling points with smaller autochthonous resource stocks, compared to those with larger autochthonous resource stocks. The accessibility of the maize root residues for soil microorganisms was also reflected by the decreasing C/N ratio of the material that remained in the flF, indicating an enhanced degradation of the added maize residues. The addition of maize roots also had an impact on material that was formerly protected within aggregates, as this material was almost completely replaced by maize derived material. However, despite this release of protected material and the energy input through fresh plant material, there was no evidence to suggest enhanced microbial decomposition of autochthonous SOC within and outside the soilbags in the subsoil. Therefore, the addition of relatively complex substrates, e.g. plant residues, seems not to enhance CO_2 emissions from the studied subsoils caused by a positive priming effect.

Acknowledgements

The technical assistance of Gabriele Dormann and Anja Sawallisch is highly appreciated. We would also like to thank Thomas Fricke and Hannes Schulz for their help with the field experiment. This project was supported by the Research Training Group 1397 “Regulation of soil organic matter and nutrient turnover in organic agriculture” of the German Research Foundation (DFG).

References

Ågren, G.I., Hyvönen, R., Berglund, S.L., Hobbie, S.E., 2013. Estimating the critical N:C from litter decomposition data and its relation to soil organic matter stoichiometry. Soil Biol. Biochem 67, 312–318.
Bailey, V.L., Smith, J.L., Bolton, H., 2002. Fungal:bacterial ratios in soils investigated for enhanced C sequestration. Soil Biol. Biochem 34, 997–1007.
Balesdent, J., Mariotti, A., 1996. Measurement of soil organic matter turnover using ^{13}C natural abundance. In: Boutton, T.W., Yamasaki, S. (Eds.), Mass Spectrometry of Soils. Marcel Dekker, New York, pp. 83–111.
Batjes, N.H., 1996. Total carbon and nitrogen in the soils of the world. Eur. J. Soil Sci 47, 151–163.
Berhe, A.A., 2012. Decomposition of organic substrates at eroding vs. depositional landform positions. Plant Soil 350, 261–280.
Berhe, A.A., Harden, J.W., Torn, M.S., Harte, J., 2008. Linking soil organic matter dynamics and erosion-induced terrestrial carbon sequestration at different landform positions. J. Geophys. Res 113, G04039.
Berhe, A.A., Harden, J.W., Torn, M.S., Kleber, M., Burton, S.D., Harte, J., 2012. Persistence of soil organic matter in eroding versus depositional landform positions. J. Geophys. Res 117, G02019.
Cerli, C., Celi, L., Kalbitz, K., Guggenberger, G., Kaiser, K., 2012. Separation of light and heavy organic matter fractions in soil — testing for proper density cut-off and dispersion level. Geoderma 170, 403–416.
Chabbi, A., Kögel-Knabner, I., Rumpel, C., 2009. Stabilized carbon in subsoil horizons is located in spatially distinct parts of the soil profile. Soil Biol. Biochem 41, 256–261.
Chantigny, M.H., 2003. Dissolved and water-extractable organic matter in soils: a review on the influence of land use and management practices. Geoderma 113, 357–380.
Collins, H.P., Elliott, E.T., Paustian, K., Bundy, L.G., Dick, W.A., Huggins, D.R., Smucker, A.J.M., Paul, E.A., 2000. Soil carbon pools and fluxes in long-term corn belt agroecosystems. Soil Biol. Biochem 32, 157–168.
Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K., Paul, E., 2013. The microbial efficiency-matrix stabilization MEMS framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable organic matter? Glob. Chang. Biol 19, 988–995.
Crow, S.E., Swanston, C.W., Lajtha, K., Brooks, J.R., Keirstead, H., 2007. Density fractionation of forest soils: methodological questions and interpretation of incubation results and turnover time in an ecosystem context. Biogeochemistry 85, 69–90.
Diochon, A., Gillespie, A.W., Ellert, B.H., Janzen, H.H., Gregorich, E.G., 2016. Recovery and dynamics of decomposing plant residues in soil: an evaluation of three fractionation methods. Eur. J. Soil Sci http://dx.doi.org/10.1111/ejss.12316.
Dungait, J.A.J., Hopkins, D.W., Gregory, A.S., Whitmore, A.P., 2012. Soil organic matter turnover is governed by accessibility not recalcitrance. Glob. Chang. Biol 18, 1781–1796.
Ehleringer, J.R., Buchmann, N., Flanagan, L.B., 2000. Carbon isotope ratios in belowground carbon cycle processes. Ecol. Appl 10, 412–422.
Ekblad, A., Nyberg, G., Högberg, P., 2002. ^{13}C-discrimination during microbial respiration of added C_3-, C_4- and ^{13}C-labelled sugars to a C_3-forest soil. Oecologia 131, 245–249.
FAO, 2014. World Reference Base for Soil Resources 2014. International Soil Classification System for Naming Soils and Creating Legends for Soil MapsWorld Soil Resources Reports Vol. 106. FAO, Rome.
Fierer, N., Schimel, J.P., Holden, P.A., 2003. Variations in microbial community composition through two soil depth profiles. Soil Biol. Biochem 35, 167–176.
Fontaine, S., Barot, S., Barré, P., Bdioui, N., Mary, B., Rumpel, C., 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Nature 450, 277–281.
Gentsch, N., Mikutta, R., Shibistova, O., Wild, B., Schnecker, J., Richter, A., Urich, T., Gittel, A., Šantrůčková, H., Bárta, J., Lashchinskiy, N., Mueller, C.W., Fuß, R., Guggenberger, G., 2015. Properties and bioavailability of particulate and mineral-associated organic matter in Arctic permafrost soils, Lower Kolyma Region, Russia. Eur. J. Soil Sci 66, 722–734.
Golchin, A., Oades, J.M., Skjemstad, J.O., Clarke, P., 1994. Study of free and occluded particulate organic-matter in soils by solid-state ^{13}C CP/MAS NMR-spectroscopy and scanning electron-microscopy. Aust. J. Soil Res 32, 285–309.
Griepentrog, M., Schmidt, M.W.I., 2013. Discrepancies in utilization of density fractionation along with ultrasonic dispersion to obtain distinct pools of soil organic matter. J. Plant Nutr. Soil Sci 176, 500–504.
Hütsch, B.W., Augustin, J., Merbach, W., 2002. Plant rhizodeposition — an important source for carbon turnover in soils. J. Plant Nutr. Soil Sci 165, 397–407.
John, B., Yamashita, T., Ludwig, B., Flessa, H., 2005. Storage of organic carbon in aggregate and density fractions of silty soils under different types of land use. Geoderma 128, 63–79.
Jörgensen, R.G., Raubuch, M., Brandt, M., 2002. Soil microbial properties down the profile of a black earth buried by colluviums. J. Plant Nutr. Soil Sci 165, 274–280.
Kaiser, M., Berhe, A.A., 2014. How does sonication affect the mineral and organic constituents of soil aggregates? — A review. J. Plant Nutr. Soil Sci 177, 479–495.

Kuzyakov, Y., 2010. Priming effects: interactions between living and dead organic matter. Soil Biol. Biochem 42, 1363–1371.
Lal, R., Kimble, J.M., 1997. Conservation tillage for carbon sequestration. Nutr. Cycl. Agroecosyst 49, 243–253.
von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Matzner, G., Guggenberger, G., Marschner, B., Flessa, H., 2006. Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions — a review. Eur. J. Soil Sci 57, 426–445.
Moore, T.R., Trofymow, J.A., Prescott, C.E., Fyles, J., Titus, B.D., 2006. Patterns of carbon, nitrogen and phosphorus dynamics in decomposing foliar litter in Canadian forests. Ecosystems 9, 46–62.
Murphy, D.V., Macdonald, A.J., Stockdale, E.A., Goulding, K.W.T., Fortune, S., Gaunt, J.L., Poulton, P.R., Wakefield, J.A., Webster, C.P., Wilmer, W.S., 2000. Soluble organic nitrogen in agricultural soils. Biol. Fertil. Soils 30, 374–387.
Pausch, J., Kuzyakov, Y., 2012. Soil organic carbon decomposition from recently added and older sources estimated by $\delta^{13}C$ values of CO_2 and organic matter. Soil Biol. Biochem 55, 40–47.
Pausch, J., Tian, J., Riederer, M., Kuzyakov, Y., 2013. Estimation of rhizodeposition at field scale: upscaling of a ^{14}C labeling study. Plant Soil 364, 273–285.
Poll, C., Marhan, S., Ingwersen, J., Kandeler, E., 2008. Dynamics of litter carbon turnover and microbial abundance in a rye detritusphere. Soil Biol. Biochem 40, 1306–1321.
Potthoff, M., Loftfield, N., Wick, B., John, B., Buegger, F., Joergensen, R.G., Flessa, H., 2003. The determination of $\delta^{13}C$ in soil microbial biomass using fumigation-extraction. Soil Biol. Biochem 35, 947–954.
R Development Core Team, 2010. R: A Language and Environment for Statistical Computing Vienna, Austria.
Rasse, D.P., Rumpel, C., Dignac, M.-F., 2005. Is soil carbon mostly root carbon? Mechanisms for a specific stabilization. Plant Soil 269, 341–356.
Rottmann, N., Dyckmans, J., Joergensen, R.G., 2010. Microbial use and decomposition of maize leaf straw incubated in packed soil columns at different depths. Eur. J. Soil Biol 46, 27–33.
Rubino, M., Dungait, J.A.J., Evershed, R.P., Bertolini, T., De Angelis, P., D'Onofrio, A., Lagomarsino, A., Lubritto, C., Merola, A., Terrasi, F., Cotrufo, M.F., 2012. Carbon input belowground is the major C flux contributing to leaf litter mass loss: evidences from a ^{13}C labelled-leaf litter experiment. Soil Biol. Biochem 42, 1009–1016.
Rumpel, C., Kögel-Knabner, I., 2011. Deep soil organic matter — a key but poorly understood component of terrestrial C cycle. Plant Soil 338, 143–158.
Rumpel, C., Kögel-Knabner, I., Bruhn, F., 2002. Vertical distribution, age, and chemical composition of organic carbon in two forest soils of different pedogenesis. Org. Geochem 33, 1131–1142.
Salome, C., Nunan, N., Pouteau, V., Lerch, T.Z., Chenu, C., 2010. Carbon dynamics in topsoil and in subsoil may be controlled by different regulatory mechanisms. Glob. Chang. Biol 16, 416–426.
Sanaullah, M., Chabbi, A., Leifeld, J., Bardoux, G., Billou, D., Rumpel, C., 2011. Decomposition and stabilization of root litter in top- and subsoil horizons: what is the difference? Plant Soil 338, 127–141.
Schrumpf, M., Kaiser, K., Guggenberger, G., Persson, T., Kögel-Knabner, I., Schulze, E.-D., 2013. Storage and stability of organic carbon in soils as related to depth, occlusion within aggregates, and attachment to minerals. Biogeosciences 10, 1675–1691.
Struecker, J., Joergensen, R.G., 2015. Microorganisms and their substrate utilization patterns in topsoil and subsoil layers of two silt loams, differing in soil organic C accumulation due to colluvial processes. Soil Biol. Biochem 91, 310–317.
Troßbach, W., 2000. Frankenhausen in der Geschichte landwirtschaftlicher Großbetriebe. Arbeitsergebnisse: Schriftenreihe der Arbeitsgemeinschaft für Ländliche Entwicklung am Fachbereich Stadtplanung, Landschaftsplanung der Gesamthochschule Kassel. Vol. 47, pp. 19–25.
Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem 19, 703–707.
Wagai, R., Mayer, L.M., Kitayama, K., 2009. Nature of the "occluded" low-density fraction in soil organic matter studies: a critical review. Soil Sci. Plant Nutr 55, 13–25.
Wang, X., Cammeraat, E.L.H., Cerli, C., Kalbitz, K., 2014. Soil aggregation and the stabilization of organic carbon as affected by erosion and deposition. Soil Biol. Biochem 72, 55–65.
Wichern, F., Eberhardt, E., Mayer, J., Joergensen, R.G., Müller, T., 2008. Nitrogen rhizodeposition in agricultural crops: methods, estimates and future prospects. Soil Biol. Biochem 40, 30–48.
Wu, J., Joergensen, R.G., Pommerening, B., Chaussod, R., Brookes, P.C., 1990. Measurement of microbial biomass C by fumigation extraction — an automated procedure. Soil Biol. Biochem 22, 1167–1169.