



The development of soil organic carbon under young black locust (*Robinia pseudoacacia* L.) trees at a post-mining landscape in eastern Germany

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Abstract

The aim of this study was to evaluate the potential of short rotation alley cropping systems (SRACS) to improve the soil fertility of marginal post-mining sites in Brandenburg, Germany. Therefore, we annually investigated the crop alleys (AC) and black locust hedgerows (ABL) of a SRACS field trail under initial soil conditions to identify the short-term effects of tree planting on the storage of soil organic carbon (SOC) and its degree of stabilization by density fractionation. We detected a significant increase in SOC and hot-water-extractable organic C (HWEOC) at ABL, which was mainly restricted to the uppermost soil layer (0–10 cm). After 6 years, the SOC and HWEOC accumulation rates at ABL were 0.6 Mg and 46 kg ha⁻¹ year⁻¹, which were higher than those in the AC. In addition, comparatively high stocks of approximately 4.6 Mg OC and 182 kg HWEOC ha⁻¹ were stored in the ABL litter layer. Density fractionation of the 0–3 cm soil layer at ABL revealed that the majority of the total SOC (47%) was stored in the free particulate organic matter fraction, which was more than twice that of the AC. At the same time, a higher and steadily increasing amount of SOC was stored in the occluded particulate organic matter fraction at ABL, which indicated a high efficiency for SOC stabilization. Overall, our findings support the suitability of black locust trees for increasing the soil fertility of the reclaimed mining substrate and, consequently, the high potential for SRACS to serve as an effective recultivation measure at marginal sites.

Keywords Density fractionation · SOC sequestration · Agroforestry · Marginal sites

Abbreviations

ABL	Black locust hedgerows
AC	Centre position at the crop alley
AL	Leeward position at the crop alley
AW	Windward position at the crop alley
C _{mic}	Microbial biomass carbon
fPOM	Free particulate organic matter

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HWC	Hot water carbon
HWEOC	Hot water extractable organic carbon
LOI	Loss-on-ignition
Mg	Megagram
MOM	Mineral-associated soil organic matter
OC	Organic carbon
OM	Organic matter
oPOM	Occluded particulate organic matter
SOC	Soil organic carbon
SOM	Soil organic matter
SPT	Sodium polytungstate
SRC	Short rotation coppice
SRACS	Short rotation alley cropping system
TC	Total carbon
TIC	Total inorganic carbon
TN	Total nitrogen
TOC	Total organic carbon

Introduction

Approximately 80% of terrestrial soil organic carbon (SOC) stocks are bound in soils (Blume et al. 2016), and recent estimates indicate that roughly 2060 ± 215 Pg C can be found in the uppermost two metres (Batjes 2016). Therefore, soil contains significantly more carbon than the atmosphere (~ 800 Pg C) and terrestrial vegetation (~ 500 Pg C) (FAO and ITPS 2015) taken together. That means that even small changes in SOC stocks can significantly affect the global C cycle (Walter et al. 2016). Certainly, the C sequestration potential of soils is adversely affected by the degradation caused by land use change and agricultural management, which are globally seen as the major drivers of SOC losses (FAO and ITPS 2015). Consequently, the carbon flows in agricultural ecosystems require urgent optimization in order to reduce anthropogenic CO₂ emissions and thus mitigate the negative effects of climate change (Lorenz and Lal 2014). Agroforestry is of particular interest in this connection, as it represents an agroecosystem in which trees are intercropped with crops or pasture to create ecological and economic benefits (Nair 1993). It combines the potential for increasing above- and below-ground carbon storage by increasing organic matter (OM) inputs (e.g., leaf litter, fine roots) with the maintenance of agricultural production (Cardinael et al. 2015). There are numerous forms of agroforestry systems, but two common examples are shelterbelts and alley cropping systems (ACS). Both consist of linear hedgerow structures, but the hedges in the ACS are planted closer to each other (10 to 100 m), and are therefore more integrated into the agricultural system (Kurz et al. 2011).

In Germany, short rotation alley cropping systems (SRACS), a combination of crops with fast-growing trees planted at a high density and harvested in short rotation periods, are part of the recultivation strategy of lignite post-mining landscapes (Krümmelbein et al. 2012). These sites are predominantly characterized by the unfavourable physical and chemical soil properties of the overburden material; they are relatively free of soil organic matter (SOM) and possess a low nutrient content (Böhm et al. 2011). Consequently, the SOC at post-mining sites is often considered to be a useful indicator of soil quality and ecosystem recovery (Macdonald et al. 2015). In order to reintegrate these sites into agricultural

management and increase their SOM and hence SOC content, numerous and highly labour-intensive reclamation measures are applied. Examples of this include multiple deep ploughing, amelioration with mineral fertilizers and lime, and crop rotations with nitrogen fixing plants such as *Medicago sativa* L. over several years (Krümmelbein et al. 2012). Alternatively, the comparatively less labour-intensive cultivation of fast-growing trees, such as in SRACS, may significantly contribute to the expeditious reintegration as forest or agricultural land, while at the same time improving the microclimate and soil protection. Generally, agroforestry is believed to have a higher potential for sequestering C than pastures or field crops (Nair et al. 2009). This assumption is largely based on field studies performed in tropical climates, but there is also evidence that agroforestry can increase SOC stocks under temperate conditions (Bambrick et al. 2010; Cardinael et al. 2017; Pardon et al. 2017; Peichl et al. 2006; Upson et al. 2016). Nevertheless, these studies investigated comparatively fertile pasture and agricultural sites, with trees that were largely at a moderate age (≥ 13) and where the first soil sampling was carried out several years after tree planting. This is quite reasonable, since changes in the SOC content of agricultural sites under temperate climatic conditions have been reported to have reaction periods of approximately 50 years (Körschens et al. 1990), or at least 10 years after land use change from conventional agricultural system to agroforestry (Oelbermann et al. 2006; Peichl et al. 2006). In order to detect short-term changes in soil carbon stocks, the analysis of readily available C fractions is more suitable than the determination of the SOC content alone. The hot-water extractable carbon, for example, is an indicator of labile decomposable SOM, and is considered to be more sensitive in detecting changes due to varying management practices or land use changes over a short period (Böhm et al. 2010; Ghani et al. 2003; Landgraf et al. 2005). Therefore, this parameter may also allow a better assessment of the impact of trees on soil carbon stock. In addition to the pure quantification of the amount of soil-bound carbon due to land use change, the assessment of the degree of stabilization of SOC in the mineral soil is decisive for the evaluation of long-term humus build-up. A knowledge of the C-distribution among the degradable free particulate organic matter (fPOM), occluded particulate organic matter (oPOM, physically protected by aggregation) and the highly stable mineral-associated soil organic matter (MOM, silt and clay sized) allows for an assessment of accumulation or degradation behavior, and therefore of the source and sink functions of a particular site (Woche and Guggenberger 2012). The fPOM fraction consists mainly of weakly decomposed plant and animal residues with short turnover times of several years, whereas the MOM fraction is considered to be highly stable, with turnover times of decades to centuries (Crow et al. 2007; John et al. 2005). Although several studies investigate the stabilization processes of SOC in temperate agroforestry systems (Cappai et al. 2017; Cardinael et al. 2015; Upson and Burgess 2013), there is still a lack of studies which examine the effects of tree growth on SOC at marginal sites. Moreover, there is uncertainty about the effects of the tree hedgerows grown in SRACS on the development and persistence of SOC in anthropogenic soils.

The objective of the current empirical study was to evaluate the potential of agroforestry land use as a recultivation measure for regional post-mining landscapes in terms of soil carbon sequestration. Therefore, we investigated the SOC and hot water extractable organic carbon (HWEOC) stocks up to a 60 cm soil depth at black locust hedgerows annually. Likewise, we investigated their related crop alleys on a lignite post-mining site during the first years after tree planting. In addition, we also estimated the SOC sequestration rates and gathered knowledge on the stabilization of SOC by density fractionation.

Materials and methods

Study site, site management, planting design and soil characteristics

The investigations were carried out in the Lower Lusatia region of Brandenburg (Germany). This area is characterized by an average annual precipitation of 568 mm, which is evenly distributed over the year, and a mean annual temperature of 9.6 °C (meteorological station of Cottbus, about 30 km west of the experimental site, for the period 1981–2010; DWD 2016). The site investigated forms part of a broad, mainly flat and comparatively unstructured landscape, and is located in the post-mining area of the lignite opencast mine “Welzow-Süd” (N 51.621161°, E 14.326766°). It is characterized by humus- and nutrient-poor, lignite-free and sand-dominated dump substrates, without any soil structure. The texture of the topsoil layer varies widely and the soil is in an initial stage of development (Table 1). During the course of recultivation, it was meliorated with an average of 27 Mg CaO, 105 kg N, 60 kg P and 65 kg K ha⁻¹, which were worked into the soil to a depth of 50 cm before planting the trees. A 12 ha alley cropping system and a 10 ha short rotation coppice (SRC) were established in Spring 2007 using 1-year old, bare-rooted black locust seedlings (*Robinia pseudoacacia* L.). The tree hedgerows were planted in double rows with a spacing of 0.75 m between the single rows, 1.80 m between the double rows, and 0.85 m within the row, resulting in a plant density of 9227 trees ha⁻¹. The 11 m wide hedgerows included four double rows planted 24 m apart and with a north–south orientation. The trees were managed in short coppicing cycles, and every second tree row of the SRACS and the SRC was harvested in the winter of 2011/12, with the remaining hedges being harvested the following winter. The crop management at the alleys fluctuated between extensive and intensive agriculture (Table 2).

Table 1 Site and soil characteristics (0–30 cm) at the post-mining site (2008). Standard deviation in brackets

Feature	
Soil texture (USDA classification)	Sandy loam (without soil structure) (51–88% sand, 6–18% silt, 5–37% clay)
Soil type (WRB classification)	Regosol
Groundwater table under floor (m)	~40
SOC (Mg ha ⁻¹) (AC/ABL/SRC)	7.6 (±2.0)/7.4 (±2.3)/10.8 (±2.5)
Average tree height (m) winter 2013/14	Black locust: 3.3
Plant density (trees ha ⁻¹ wood area)	Black locust: 9227
Crop alley width (m)	24
Tree harvest rotation (years)	5 to 6
Field size (ha)	22 (SRACS and SRC)
Crop rotation	<i>Medicago sativa</i> L. [2007–2010], <i>Hordeum vulgare</i> L. [2011] <i>Avena sativa</i> L. [2012], <i>Secale cereale</i> [2013], <i>Secale multi- caule</i> [2014]

AC centre of the alley cropping system, ABL black locust hedgerow, SRACS short rotation alley cropping systems, SRC short rotation coppice

Table 2 Crop rotation and field management at the post-mining site during our investigations

Treatment	Action	Date	Fertiliser (ha ⁻¹)
Seeding	<i>Medicago sativa</i> L. (55 kg ha ⁻¹) ^a	04-2007, 09-2008	40 kg N, 60 kg P and 190 kg K
	<i>Hordeum vulgare</i> L. (N/A)	04-2011	50 kg P, 132 kg K
	<i>Avena sativa</i> L. (155 kg ha ⁻¹) ^a	04-2012	45 kg N, 24 kg P, 45 kg K
	<i>Secale multicaule</i> (180 kg ha ⁻¹) ^a	04-2013	54 kg N, 20 kg Ca
Planting	<i>R. pseudoaccacia</i> L. (8715 trees ha ⁻¹)	04-2007	40 kg N, 60 kg P and 190 kg K
Tillage	Disc harrowing, grubber tillage	09-2008,11-2010,03-2012,04-2013	
		Plowing	11-2010, 04-2012
		Seeding	04-2011, 04-2012
	Mulching	08-2008, 07-2009	

N/A not available

^aNot or only partially harvested

Soil and litter layer sampling

The permanent observation area at the experimental site was used to establish sampling transects (Fig. 1), which were extended from the hedgerow through the entire width of the

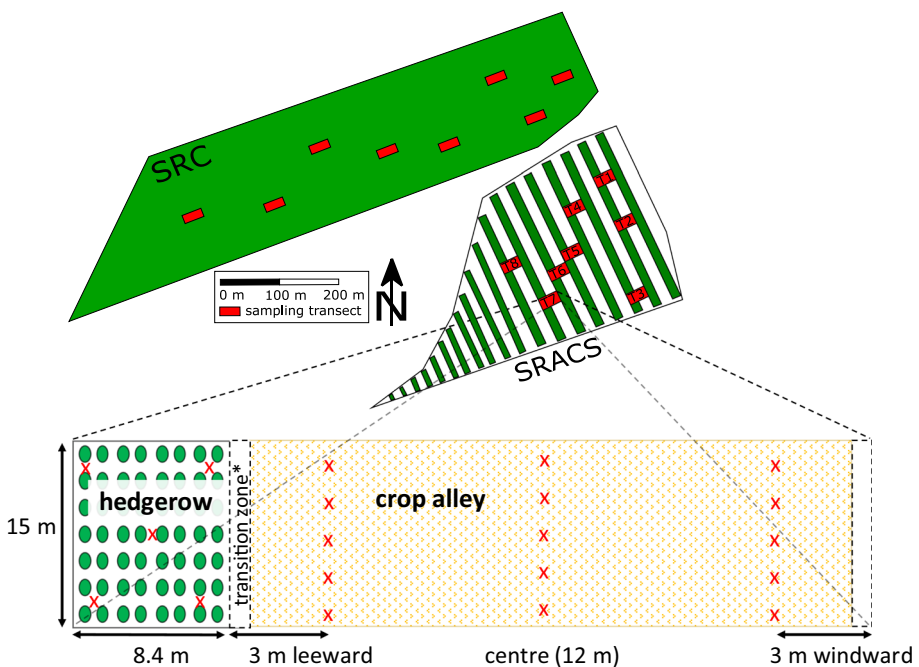


Fig. 1 Location of the sampling transects at the post-mining site and sampling scheme at crop and tree zone (x (red) marks the location of subsamples). *An approximately 1 m wide zone where neither trees nor crops are growing. (Color figure online)

adjacent crop alley. They were selected in order to exclude major soil heterogeneity and carbonaceous hotspot due to a subsequent fine mapping of the substrate. Finally, eight transects were established, each with a length of 35 m and a width of 15 m. They were divided into four different sampling sub-areas. These were (1) the black locust hedgerow, (2) the 3 m leeward position, (3) the 3 m windward position and (4) the centre of the crop alley. The sampling transects at the nearby SRC were sampled analogously to the black locust hedgerow sub-area of the SRACS.

Soil samples were taken annually in mid-April from 2008 to 2014. The litter layer was removed and the soil was sampled manually using an auger (Pürckhauer). In order to obtain sufficient sample material, at least five randomly distributed subsamples were taken on each sub-area. The annual soil sampling depths were 0–3, 3–10 and 10–30 cm. The 30–60 cm soil layer was sampled during the first and fifth years of our investigations. The sample material obtained from the subsamples was pooled to give a composite sample, which was stored in PE bags at $-18\text{ }^{\circ}\text{C}$ until analysis. There were eight replicates per sampling point and depth. In addition, undisturbed soil samples were taken at three soil depths in 2008—0–10, 10–30 and 30–60 cm (each with five replicates)—in order to determine bulk density (BD). They were taken with 100 cm^3 manual short core samplers (Eijkelkamp), which were placed horizontally in the soil profile wall and distributed over the respective soil depth.

A collectible litter layer was found 3 years after the beginning of our investigation and sampled in a total of 4 years. Litter and soil sampling were carried out in the same period, whereby the litter gathered within a metal frame ($25\text{ cm}\times 25\text{ cm}$) was collected twice per hedgerow plot. The litter was stored in PE bags, transported to the lab and later dried at $40\text{ }^{\circ}\text{C}$ for subsequent analysis. Oven-dry mass was determined after drying the litter for 2 days at $105\text{ }^{\circ}\text{C}$ until weight constancy. The results were then upscaled from a “square centimetre” on a “per hectare” basis.

Laboratory analysis

Preparation of soil and litter layer samples

Unless otherwise stated, the soil analyses were carried out on the fine soil fraction, after homogenization and sieving ($\leq 2\text{ mm}$). Both the fine and the coarse soil fractions were weighed separately to determine their proportions. The determination of soil acidity, carbon and nitrogen fractions were performed on the dry ($40\text{ }^{\circ}\text{C}$) fine soil fraction or the dried ($60\text{ }^{\circ}\text{C}$) and homogenized litter layer. For the analysis of the organic carbon and total nitrogen content, the soil and litter samples were later ground using a vibratory disc mill (FRITSCH, Pulverisette 2). For the determination of bulk density and water content, which was measured gravimetrically, the soil samples were dried at $105\text{ }^{\circ}\text{C}$ until weight constancy was reached.

Physical analysis of the mineral soil

The soil texture was determined by a combination of wet sieving and sedimentation processes (pipet method), based on Köhn (Blume et al. 2011). The bulk density (BD) was calculated by dividing the dry mass of soil by its volume in the soil rings, according to the following equation:

$$\text{bulk density} = \frac{\text{soil dry mass [g]}}{100[\text{cm}^3]} \quad (1)$$

Chemical analysis of the mineral soil and the litter layer

The pH was determined in 0.01 M CaCl₂ extracts in a 1/2.5 (*w/v*) ratio (soil samples) and a 1/10 (*w/v*) ratio (litter samples). The total contents of carbon (TC) and nitrogen (TN) for all the litter and soil samples were measured by means of oxidative combustion using an ELEMENTAR VARIO III elemental analyser. The soil organic matter content was estimated using the loss-on-ignition (LOI) method, after igniting the soil samples at 450 °C for 12 h in a muffle furnace (Bisutti et al. 2007). The carbon was subsequently measured again and the total organic carbon (TOC) was calculated by determining the difference between TC and total inorganic carbon (TIC). The hot-water extracts were obtained according to the method described by Körschens et al. (1990), which involved boiling a 10 g aliquot of an air-dried soil sample (or 5 g for the litter layer) in 100 ml of de-ionized water for 60 min. After it had cooled down to room temperature, 2 ml of a 1 M MgSO₄ solution was added to support the collation of the colloidal particles in the liquid. The suspension was then centrifuged for 10 min at 4000 rpm. The supernatant was separated from the soil by decantation. The TOC concentration in the HWC extracts was determined using a TOC-Analyser (Shimadzu TOC-Vcph und TOC5000).

We performed a density fractionation procedure following the method of Golchin et al. 1994b, but modified it for calcareous soils. Before the fractioning process, calcareous soil samples (50 g, air-dried) were individually prepared for 36 h using 1 M HCl solution in order to destroy the carbonate. The density separation was performed with a solution of sodium polytungstate, Na₆(H₂W₁₂O₄₀) (SPT, SOMETU, Berlin). Three fractions were partitioned: light (fPOM); $\rho < 1.6 \text{ g cm}^{-3}$, medium (oPOM); $\rho = 1.6\text{--}2.2 \text{ g cm}^{-3}$ and heavy (MOM); $\rho > 2.2 \text{ g cm}^{-3}$. Finally, the three fractions were freeze-dried and the C contents were later determined on samples dried at 40 °C using an elemental analyser. The total SOC content of each discrete fraction in each soil layer was considered on the basis of the mass proportion of the bulk soil sample and the SOC content measured.

Microbial analysis

Soil microbial biomass C (C_{mic}) was determined from the fine fraction (<2 mm) of field-fresh soil samples. In addition, they were wetted to 60% of their water holding capacity before the start of the fumigation process. For this, 12.5 g of soil from these subsamples was subjected to a fumigation-incubation process with (ethanol-free) CHCl₃ for 24 h, at room temperature (24 °C), followed by an extraction with 50 ml of a 0.5 M K₂SO₄ solution according to the method of Vance et al. (1987). The extracts were subsequently filtered and the TOC was measured. The soil microbial biomass was then calculated according to the following equation:

$$C_{mic} = EC/k_{EC} \quad (2)$$

where EC = the difference between the TOC in the fumigates and the control samples; k_{EC} = the extractable part of microbial biomass C after fumigation = 0.45 (Joergensen and Brookes 2005).

Estimation of the soil organic carbon stocks and accumulation rates

The SOC stocks of the soil were calculated from the carbon content in the fine soil fraction, the bulk density, the coarse fraction and the depth of the respective soil layer according to the following equation:

$$SOC_s [\text{ha}^{-1}] = SOC_f [\text{mg g}^{-1}] \times BD [\text{g cm}^3] \times sd [\text{m}] \times (1 - (CF[\%]/100)) \times 10 \quad (3)$$

where SOC_s = the SOC storage ha^{-1} ; sd = the soil depth; SOC_f = the SOC fine soil content; CF = the coarse fraction and BD = the bulk density.

The SOC and HWEOC accumulation rates ($\text{Mg C ha}^{-1} \text{ year}^{-1}$) at a given soil depth were expressed as results of the Sens slope estimates of the calculated SOC and HWEOC stocks.

Data analysis

The statistical analysis was performed using SigmaPlot and SYSTAT (Systat Software, Inc., San Jose California USA, Version 12.5 and 13.2) software. All the datasets were tested for normality and equal variance using the Shapiro–Wilk and Levene’s tests. The Dean–Dixon test was used to exclude outliers from the datasets. This test is appropriate for small numbers of observations with little loss of precision (Dean and Dixon 1951). Overall, we eliminated 64 outliers from a total of 3138 observations. A Student’s t test or a one-way ANOVA were used to identify significant differences in the means between two or more groups. Data that were not normally distributed were subsequently log-transformed for this purpose. In the case of significant differences, a two-tailed p value was determined (t test) or a pairwise multiple comparison was conducted (one-way ANOVA, post hoc Holm–Sidak method). Temporal changes between two groups within 2 years were determined using a paired t test. A nonparametric Mann–Kendall-test was performed to evaluate whether the annually measured parameters tend to increase or decrease monotonically during the observation period. A Sens slope estimator that considered a 95% confidence interval was used to calculate the parameter change rate over the observation period. The minimum significance level for rejecting the hypothesis was $p=0.05$. Furthermore, a linear regression analysis was performed in order to determine the possible correlations between different soil carbon fractions.

Results

Initial soil properties

In 2008 the initial soil pH-values indicate a wide range among the different sampling locations and soil depths, from extremely acid to slightly alkaline (Table 3). The SOM content ranged from 0.8 to 1.7%, with the highest content being detected in the uppermost (0–3 cm) soil layer and without a clear soil depth related trend. The mean TC content varied from 2.8 to 5.2 g kg^{-1} at the different locations and sampling depths, with the mean SOC content ranging from 41 to 55% of the corresponding mean TC content. Both showed

Table 3 Soil characteristics at the beginning of our investigations in 2008. Except for the pH and the soil lime content, the values represent the mean contents and the rates of eight replicates followed by standard deviation in brackets. The different letters indicate significant differences between locations within a soil depth (One-way ANOVA, post hoc Holm–Sidak test)

Plot	sd (cm)	pH (min/max)	SOM (%)	TC (g kg ⁻¹)	TIC (g kg ⁻¹)	TOC/TN ratio
ABL	0–3	5.1/7.4	1.7 (±0.8)a	4.8 (±2.2)a	2.5 (±1.8)a	10.9 (±4)a
	3–10	6.7/7.6	1.5 (±0.9)a	4.6 (±2.8)a	2.8 (±2.2)a	15.0 (±7)a
	10–30	6.5/7.7	1.3 (±0.5)a	3.6 (±2.1)a	2.1 (±1.3)a	6.4 (±3)a
	30–60	6.9/7.6	1.4 (±0.7)a	2.8 (±2.1)a	1.8 (±1.7)a	17.3 (±10)a
AC	0–3	3.8/7.6	0.6 (±0.6)a	3.9 (±1.9)a	2.2 (±1.7)a	11.5 (±5)a
	3–10	4.8/7.6	0.8 (±0.2)a	3.8 (±1.9)a	2.6 (±1.5)a	12.2 (±2)a
	10–30	4.3/7.7	1.0 (±0.6)a	3.1 (±1.6)a	2.7 (±2.5)a	18.6 (±11)a
	30–60	4.3/7.6	1.1 (±0.4)a	2.9 (±2.0)a	1.8 (±1.5)a	12.5 (±6)a
SRC	0–3	7.0/7.6	1.4 (±0.7)a	5.2 (±2.2)a	2.4 (±1.5)a	9.6 (±6)a
	3–10	7.0/7.6	1.4 (±0.8)a	4.9 (±2.4)a	2.8 (±2.0)a	9.2 (±7)a
	10–30	7.0/7.7	1.1 (±0.3)a	4.7 (±2.4)a	2.8 (±1.5)a	11.1 (±9)a
	30–60	7.1/7.5	1.4 (±0.7)a	4.2 (±2.7)a	1.9 (±1.0)a	5.4 (±4)a
AL	0–3	4.7/7.6	1.6 (±0.7)a	4.0 (±2.0)a	2.5 (±1.8)a	10.9 (±4)a
	3–10	3.9/7.6	1.4 (±0.9)a	3.1 (±0.7)a	2.8 (±1.9)a	12.1 (±3)a
	10–30	5.0/7.7	0.8 (±0.3)a	2.7 (±0.6)a	2.1 (±1.3)a	11.0 (±3)a
	30–60	6.1/7.6	1.1 (±0.5)a	2.1 (±0.7)a	1.5 (±0.9)a	11.2 (±9)a
AW	0–3	3.6/7.5	1.6 (±1.0)a	4.3 (±2.0)a	3.0 (±1.4)a	12.0 (±4)a
	3–10	4.2/7.6	1.3 (±0.9)a	4.3 (±2.6)a	3.2 (±1.5)a	11.2 (±5)a
	10–30	4.2/7.7	1.2 (±0.8)a	3.4 (±1.4)a	2.5 (±1.0)a	9.3 (±1)a
	30–60	4.6/7.6	1.1 (±0.5)a	3.3 (±1.9)a	2.4 (±1.5)a	11.2 (±6)a

ABL black locust stripes, AC centre of the crop alley, AL 3 m leeward to the hedgerow, AW 3 m windward to the hedgerow, SOM soil organic matter, TC total carbon in bulk soil, TOC total organic carbon, TIC total inorganic carbon, sd soil depth

a clear downward gradient with soil depth at all the locations investigated. The average inorganic carbon contents (TIC) varied from 1.5 to 3.0 g kg⁻¹, while the mean TOC/TN ratio ranged from 5.4 to 18.6, and neither had a clear soil depth or location-related trend.

Development of the SOC and HWEOC stocks in the topsoil layer under trees and crops

The highest mean TOC and HWEOC stocks in the litter samples under black locust short rotation coppice (SRC) and hedgerows (ABL) were detected in 2014 and amounted to 4.6 and 0.18 Mg ha⁻¹, respectively (Fig. 2a). At 0–3 cm soil depth, the mean SOC stocks at the locations investigated developed roughly comparably until 2012, when SRC and ABL increased to 2.7 and 2.6 Mg ha⁻¹. These values were significantly higher ($p < 0.05$ to 0.001) than at the leeward (AL), windward (AW) and centre (AC) locations (Fig. 2b).

Until 2014, the SOC stocks for ABL and SRC increased to 5.0 and 5.8 Mg ha⁻¹, which was significantly higher ($p < 0.001$ and $p < 0.01$) than the 2.3 Mg ha⁻¹ detected at the AC. Soil sampling at the AL and AW locations was omitted in 2014, as no significant differences to the AC were detected in the previous years. Generally, the HWEOC stocks at the

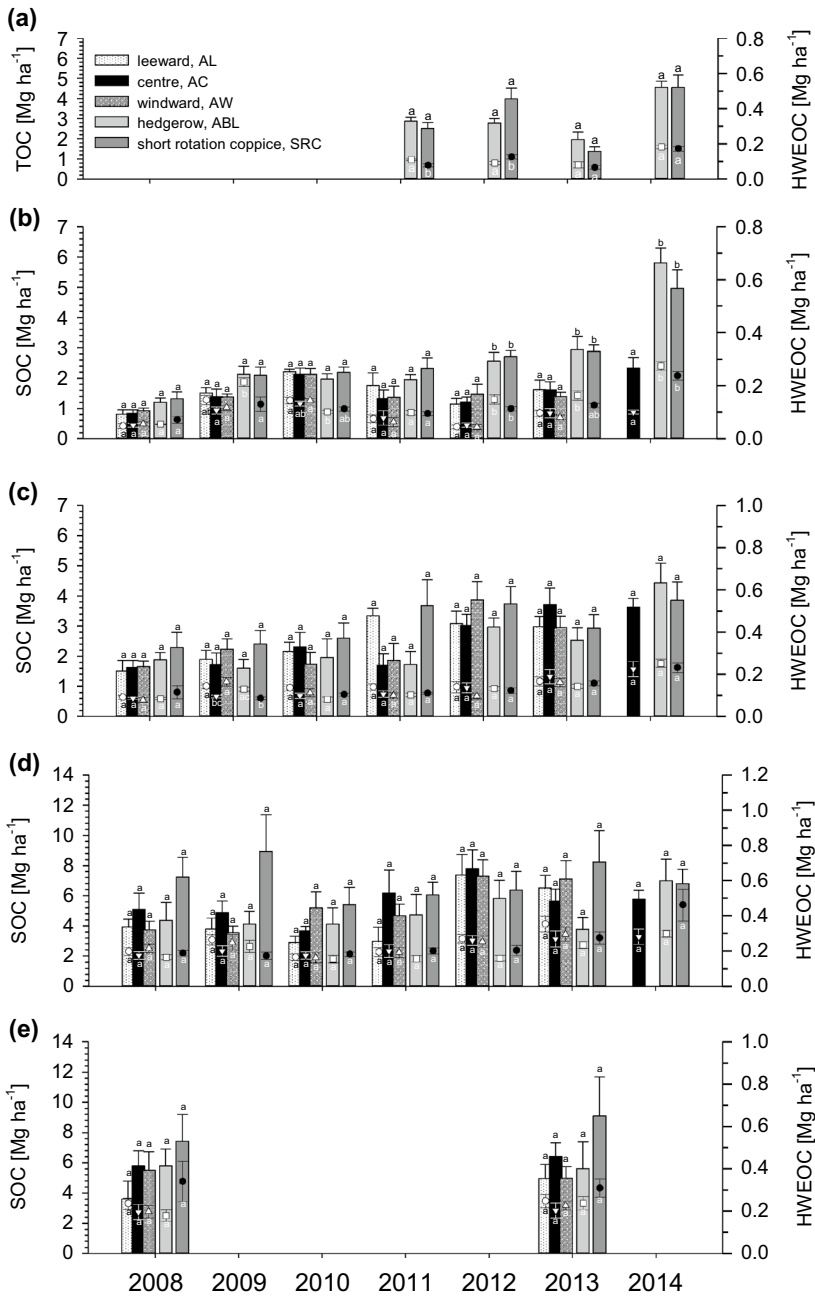


Fig. 2 Mean stocks of OC (bars, $n=8$) and HWOC (symbols in bars), including the standard error of means at the five locations investigated at the post-mining site in the **a** litter layer, **b** 0–3 cm, **c** 3–10 cm, **d** 10–30 and **e** 30–60 cm soil layer. Different letters indicate significant differences among the locations within a sampling year ($p \leq 0.05$, one-way ANOVA with post hoc Holm-Sidak method)

ABL and SRC locations increased up to 0.28 Mg ha^{-1} in 2014, which corresponds respectively to four- and threefold of the initial pool. Hence, both locations showed significantly higher ($p < 0.001$) stocks than at the AC. Below a soil depth of 3 cm, both the SOC and HWEOC stocks showed a low variability at the locations investigated during these years, with no significant differences in the mean. Overall, the mean SOC stocks at the AC, ABL and SRC in the 0–3 cm soil layer corresponded to a 20, 34 and 32% share of the respective SOC stocks accumulated in the 3–30 cm soil layers in 2014.

The median HWEOC/SOC ratio ranged from 4 to 10% in both uppermost soil layers, and lower ratios from 2 to 7% were found in the 10–30 cm soil layer. With the exception of 2009 and 2013, no major deviations were observed between the groups and study years (Fig. 3c). A moderate linear relationship was found between HWEOC and SOC contents for the uppermost soil layer under ABL and AC, while both parameters correlated strongly ($R^2 = 0.78$) for SRC (Fig. 3e). However, those relations became weaker with increasing soil depth and decreasing SOC content (Fig. 3f, g).

Accumulation rates of SOC and HWEOC

The SOC accumulation rates at the different locations increased in the majority of the litter and topsoil layers investigated (Fig. 3a). Significant monotonously increasing SOC rates of 0.37 and $0.43 \text{ Mg ha}^{-1} \text{ year}^{-1}$ were detected in the 0–3 cm soil layer at the ABL and SRC, respectively. In the 3–10 cm soil layer, the SOC rates increased monotonously at all the locations, and the AC showed the highest mean with $0.42 \text{ Mg ha}^{-1} \text{ year}^{-1}$. Below this soil depth, a mean SOC accumulation of 0.34 and $0.39 \text{ Mg ha}^{-1} \text{ year}^{-1}$ was calculated for the ABL and AC, while the rate for the SRC decreased by $0.16 \text{ Mg ha}^{-1} \text{ year}^{-1}$. However, no monotonic trend was found for any of these locations.

The HWEOC stocks increased with age at all the locations and in all the layers, but were stronger up to 10 cm soil depth at the ABL and SRC, and in the 10–30 cm soil layer at the AC (Fig. 3b). However, only the SRC showed a significant ($p < 0.01$) monotonic trend in the 0–3 cm soil layer, with $16 \text{ kg HWEOC ha}^{-1} \text{ year}^{-1}$. With the exception of the ABL, the HWEOC stocks in the remaining topsoil layer increased monotonously at all the remaining locations, with rates in the range of 15 (AC, 3–10 cm) to $26 \text{ kg ha}^{-1} \text{ year}^{-1}$ (ABL, 3–10 cm).

Microbial biomass carbon

The average microbial-biomass C (C_{mic}) in the 0–3 cm soil layer ranged from 49 (SRC) to 282 (ABL) mg kg^{-1} soil, but without any significant differences between the locations (Fig. 3d). The C_{mic} content decreased significantly ($p < 0.05$ for ABL and AC, $p < 0.01$ for SRC) below a soil depth of 3 cm soil depth at all the locations. With the exception of the AC and ABL locations in 2008, the average C_{mic} contents in the 3–30 cm soil layer were well below 100 mg kg^{-1} soil, and no spatial or temporal trend could be identified. In an analogous way to the C_{mic} contents, the $C_{\text{mic}}/\text{SOC}$ ratios were also subject to stronger temporal fluctuations. Therefore, this ratio was occasionally more than 6% in the 0–3 cm soil layer at the ABL and AC locations. However, it was under 3% in over 72% of the cases considered. The lowest $C_{\text{mic}}/\text{SOC}$ ratios were observed in the 3–30 cm soil layer at the SRC location, where they were below 1% in over 33% of the cases. No significant correlation was found between C_{mic} and SOC.

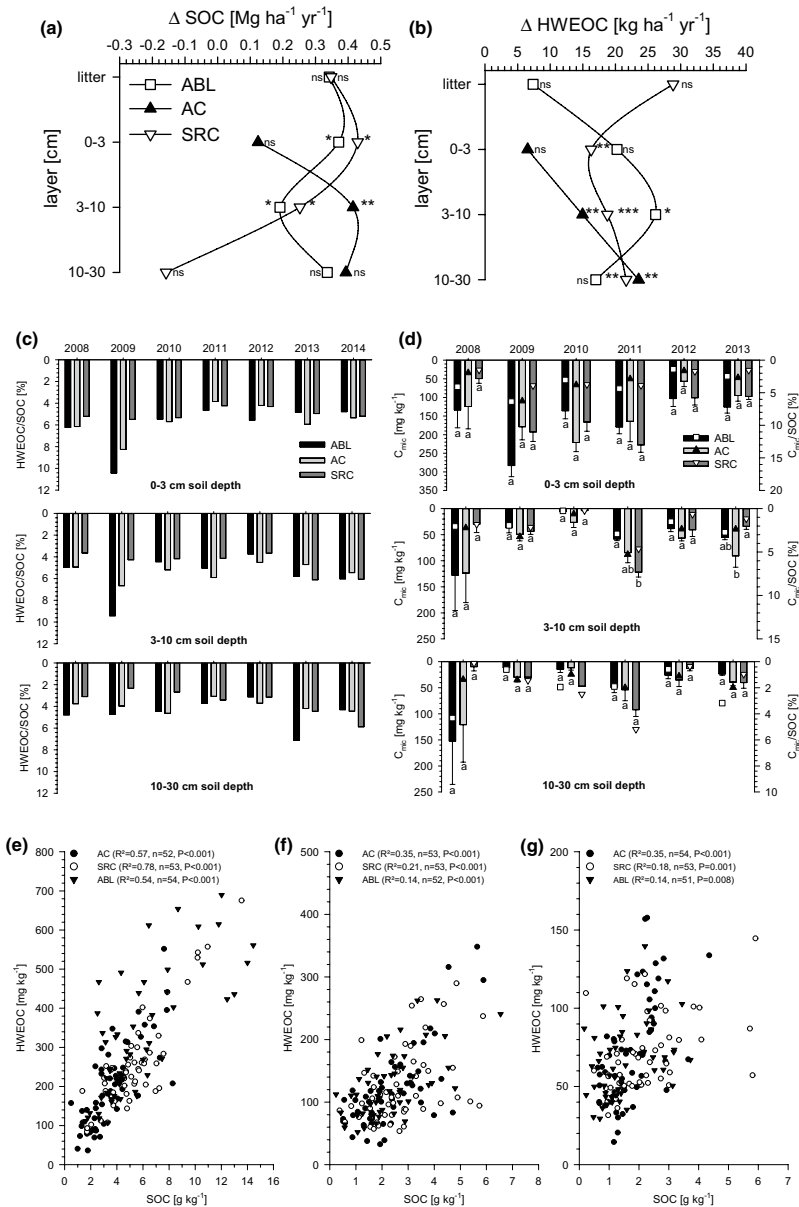


Fig. 3 **a** SOC and **b** HWEOC accumulation rate in litter and topsoil layer, **c** the median HWEOC ratio and **d** the average C_{mic} contents ($n = 1-8$) including standard error of mean, as well as the median C_{mic}/SOC ratio in topsoil at the different locations during a 6-year period. The SOC and HWEOC accumulation data represent the results from Sens slope estimate during the investigation period 2008–2014. **e-g** shows the relationships between SOC and HWEOC content in **e** 0–3, **f** 3–10 and **g** 10–30 cm soil layer among different locations. Symbols (***) for $p \leq 0.001$, ** for $p \leq 0.01$, * for $p \leq 0.05$, ns for $p > 0.05$) indicate significance of a monotonic trend (Mann–Kendall test). The various letters indicate significant differences between the sampling locations within a year ($p \leq 0.05$, one-way ANOVA with post hoc Holm–Sidak method)

Stabilization of SOC

During the 5 year period, the average SOC content in the fPOM fraction of ABL increased from 0.5 to 3.4 g kg⁻¹ soil, which was significantly more ($p < 0.01$) than at the AC. In contrast, the AC showed comparatively large variations of SOC in the fPOM fraction, which was most pronounced from the years 2009 to 2012 (Fig. 4a). During 2012 and 2013, more SOC was stored in the oPOM fraction at the ABL in comparison to at the AC, but it was only in 2012 that the difference was significant ($p < 0.01$). With respect to the SOC content in the MOM fraction, no significant differences between the locations were detected. In 2013, the sum of all three fractions at the ABL and AC accounted for a total SOC of 7.3 and 4.5 g kg⁻¹ soil, as the major proportion of 3.4 g kg⁻¹ (47%) and 1.6 g kg⁻¹ (36%) was accumulated in the fPOM and MOM fraction, respectively.

The TOC/TN ratios in the 0–3 cm soil layer ranged from 6 to 48, with the widest ratios detected in the fPOM and oPOM fractions in 2008 (Fig. 4b). In general, the TOC/TN ratios decreased in the order fPOM > oPOM > MOM. The results from the trend analysis revealed that the SOC at both the ABL and AC locations mainly accumulated with age in the fPOM and oPOM fraction (Fig. 4c). The estimated annual SOC accumulation rates in the fPOM and oPOM fractions at the ABL were approximately 0.4 and 0.3 g kg⁻¹ year⁻¹, and were thus three and two times higher than those at the AC. By contrast, an annual decrease of more than 0.1 g kg⁻¹ SOC year⁻¹ was calculated for the MOM fraction at both locations.

For density fractionation of the 3–30 cm soil layer, we concentrated on those three transects with markedly different soil texture in the topsoil layer. These were the T1, which showed the highest sand (84%) and the lowest clay (6%) and silt (10%) content, the T3, which had a moderate sand (46%) and the highest clay (36%) and silt (18%) content, and the T4, which had 66% sand, 20% clay and 14% silt. The texture of the remaining five disregarded transects corresponded roughly to the T1 transect.

The mean SOC content of the fPOM and oPOM fractions in the 3–10 cm soil layer tended to increase with increasing age at both locations, and the highest content was detected in the T1 and T4 transects (Fig. 5a, c). By contrast, the SOC content dynamic in the MOM fraction, which contained the largest amount of SOC at both locations, showed no apparent pattern or texture-related trend. In 2013, the MOM fraction accounted for the highest share of SOC, with an average of 46 and 45% at the ABL and AC, respectively. Analogously to the overlaying soil layer, the SOC content in the 10–30 cm layer at the ABL was the highest in all fractions considered, with values of up to 2.1 g kg⁻¹ in the sand-dominated T1 transect (Fig. 5b). In comparison to the AC, the mean SOC content in the fPOM and oPOM fractions remained largely unchanged, or tended to decrease with increasing age (Fig. 5d). At the AC, the highest SOC content (up to 2.5 g kg⁻¹) was determined at the T4 transect, and was detected in the MOM fraction. On average, the total SOC (the sum of all the fractions) at the ABL and AC was 2 and 3 g kg⁻¹ soil, respectively, in 2013. With a share of 55 and 45% the MOM fraction contained the largest amount of SOC.

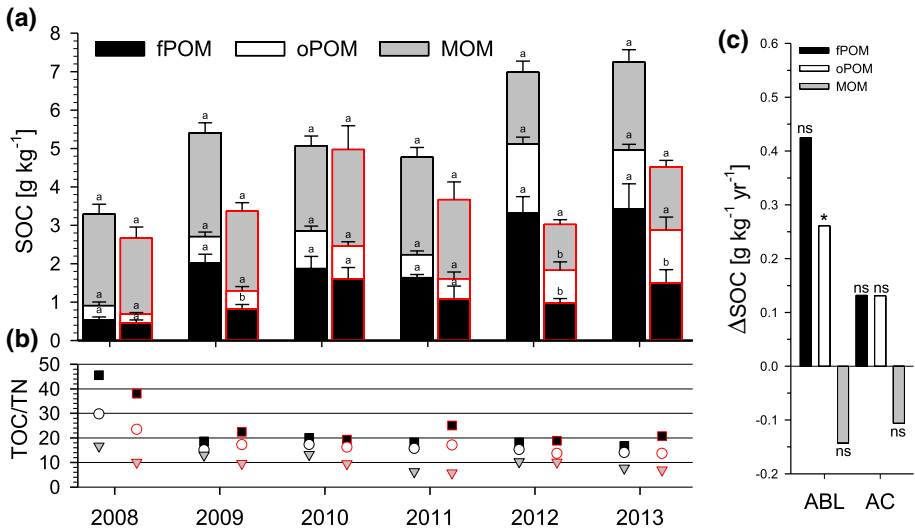


Fig. 4 a Annual mean content of soil organic carbon, b mean TOC/TN ratio ($n=8$, including standard error of mean) and c accumulation rate (MK-test with Sens slope, * for $p \leq 0.05$, ns for $p > 0.05$) in the bulk samples in three different fractions in the 0–3 cm soil layer under black locust hedgerows (ABL, black frame) and at the centre of the crop alleys (AC, red frame) during the 5 year period. The different letters indicate significant differences between the sampling locations within a year ($p \leq 0.05$, Student's t test). (Color figure online)

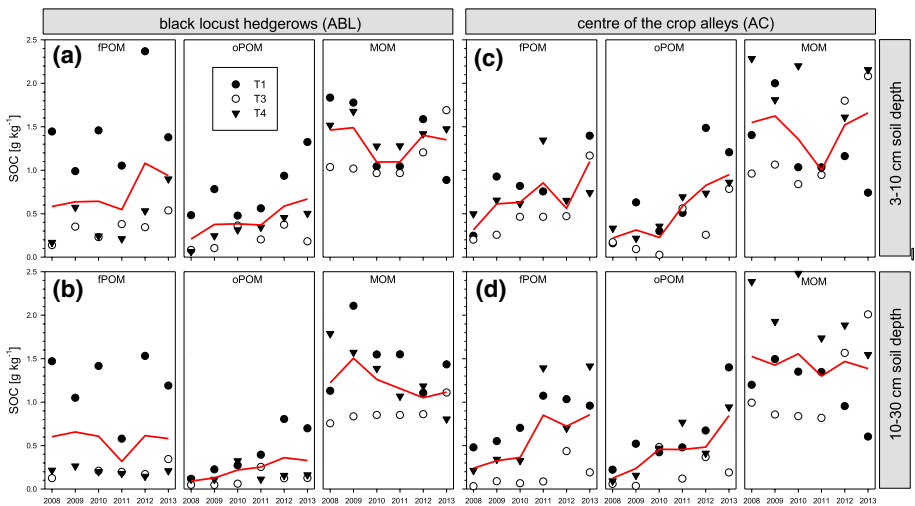


Fig. 5 Development of SOC at bulk sample in the three investigated fractions in a 3–10 and b 10–30 cm soil depth under black locust hedgerows and c 3–10 cm and d 10–30 cm soil depth under crop alleys, with a consideration of three different sampling transects with divergent soil texture (red line = the mean values). (Color figure online)

Discussion

Carbon sequestration following tree planting

Our results indicate that the significant increase in the SOC stocks found several years after tree planting are largely limited to the litter or to the uppermost mineral soil layer (up to 10 cm soil depth) at the post-mining site. According to Kravchenko and Robertson (2011), to determine changes in cumulative SOC stocks, it is important to consider only the soil layers at which significant differences are detected. In our case, this was true for the cumulative 0–10 cm soil layer during the 6-year investigation period. The cumulative SOC accumulation rate there ranged from 0.5 under crops to up to 0.7 Mg ha⁻¹ year⁻¹ under black locust trees. Generally, both the SOC accumulation rates were in keeping with the findings of Nii-Annang et al. (2009). They determined an increase of 0.4 Mg ha⁻¹ year⁻¹ in the 0–10 cm soil layer at the centre of an 18 m wide alley planted with rye (*Secale cereale*), and an increase of 1.3 Mg ha⁻¹ year⁻¹ in the 0–10 cm soil layer of the 9-year-old black locust and poplar hedgerow on a nearby post-mining site. Our results were also comparable to the findings of Cardinael et al. (2017), who observed 0.3 and 0.8 Mg SOC ha⁻¹ year⁻¹ in the same soil layer on a 6-year-old 24 m wide crop alley and hybrid walnut tree rows planted on a Luvisol in France. There is also general consensus that the litter entry in agroforestry systems is an important factor for the above-ground humus structure (Bambrick et al. 2010; Matos et al. 2012; Cardinael et al. 2017; Pardon et al. 2017). Furthermore, the absence of tillage is considered to be another important factor for SOC accumulation, due to reduced soil disturbance and increased soil aggregation (Six et al. 2000). The soil is aerated in tillage systems and organic residues and aggregates are broken up, making the carbon more accessible to microbial decomposition activity (Weil and Brady 2017). Increased mineralization with tillage, together with reduced plant C inputs (Maillard et al. 2018) and a more even distribution of C inputs throughout the plough layer (Six et al. 2002), have also been reported. This is consistent with our findings regarding soil microbial activity (C_{mic}), which was mainly concentrated in the uppermost (0–3 cm) soil layer at the black locust hedgerows (ABL) and decreased sharply with soil depth, indicating an on-going decline of OM with soil depth (Stockfisch et al. 1999). In contrast, the crop alley (AC) showed a higher SOC distribution below a soil depth of 3 cm, especially in the sampling years following the soil tillage and the incorporation of unharvested cereals (2011–2014). Therefore, the regular tillage and ploughing activities at the AC could explain the comparatively lower SOC gains in the uppermost soil layer. Instead, a lack of soil relaxation could have led to comparatively weak or even declining SOC stocks in the deeper ploughing layer (3–30 cm) at the black locust trees. This development is also typical in the lower topsoil layer (10–30 cm) of long-standing fallow land and can be traced back to the formation of year-round vegetation cover, which is accompanied by a lack of loosening (Jahn et al. 1994). When comparing the OC stocks bound in the litter layer of the site investigated, one notes that substantial amounts of up to 4.6 Mg ha⁻¹ year⁻¹ were accumulated, which even correspond to the SOC stocks calculated for both uppermost soil layers. In this context, it is worth noting that the post-mining site was in an initial stage of soil development during our investigations, with extremely low humus stocks and almost no vegetation. Consequently, the organic material of the trees planted (deciduous and root litter) formed a comparatively rich source of humus build up. The comparatively high OC stocks bound in the litter also indicate less favourable turnover

conditions, such as inhibited microbial decomposition processes (Quinkenstein and Kanzler 2018). Although the high C_{mic}/SOC ratios of more than 6% in the 0–3 cm soil layer at the ABL and AC locations suggest a comparably enhanced C turnover, these were largely between 1 and 4%, which is generally within the range of agricultural and forest soils at a neutral pH (Anderson 2003). However, the ratios in deeper soil layers were partially lower than 1%, which indicates a severe restriction of the mineralization processes (Landgraf et al. 2005; Landgraf and Klose 2002).

The initial stage of soil development at the post-mining site is also evident when considering the labile HWEOC fraction. After 6 years, the topsoil contents of the crops and tree plots still ranged from an average of 0.1 to 0.2 g HWEOC kg⁻¹ soil, respectively. According to Körschens and Schulz (1999) values below 0.2 g kg⁻¹ soil for sandy and loamy soils under the climatic conditions of Central Germany mark a threshold of poor soil conditions, as at least 0.3 g kg⁻¹ is required for a sufficient SOM supply. At the end of our investigation, the 0–10 cm soil layer showed higher HWEOC stocks under tree plots, which is primarily related to HWEOC sensitivity to land management (Ghani et al. 2003; Schulz 2002; Weigel et al. 2011). As Hamkalo and Bedernicsek (2014) note with regard to a Ukrainian *Gleyic Albeluvisol*, higher HWEOC contents in forests than in arable plots might be partly related to the extraction of lignin, lignocellulose and microbial biomass by hot water extraction. Landgraf et al. (2005) attribute higher HWEOC contents in litter layers under 40-years-old black locust stands on sandy soil in Saxonia (Germany) to high N-input rates by symbiotic N₂ fixation, which accelerates C and N turnover. However, a management-related effect was largely absent below the litter and the 0–10 cm soil layer of our post mining site and it did not yield additional insight into the C sequestration process in comparison to the determination of SOC. Overall, the median HWEOC/SOC ratios for the entire topsoil layer were largely comparable to the 3 to 5% range, which is reported for arable soils of different soil types in Germany and the Czech Republic (Kubát et al. 2004; Leinweber et al. 1995). Given that the higher ratios, when they occasionally occurred, were most frequent at the ABL location, this suggests that black locust has a stronger ability to provide short-term available OC compounds (Böhm et al. 2010).

Notwithstanding the careful selection of the sampling transects and the use of a comparatively high number of repetitions ($n=8$), the results from the SOC and HWEOC stocks below a soil depth of 10 cm at the post-mining site should be considered with caution, due to site-specific soil heterogeneity. The statistical analysis of these data showed comparatively high standard errors and low statistical power, which commonly indicate the high probability of a type II error. This means that the results are highly depend on sampling and effect size, and are less likely to support a rejection of a wrong null hypothesis (Vanvoorhis and Morgan 2007). This is a widespread problem in studies investigating SOC stocks, as the magnitude of the variation between the sampling points and depths is high in relation to the magnitude of the changes in SOC stock (Upson et al. 2016). We performed a power analysis using an extreme example of a paired t test of SOC stocks in the 30–60 cm soil layer at ABL from 2008 to 2013, and observed that 263 repetitions (in contrast to five repetitions for the 0–3 cm soil layer) were required in order to maintain adequate statistical power ($\beta=0.2$).

Uncertainties also exist with regard to the potential contamination of the soil substrate with lignite residue. Although as the mining company declared the site investigated to be lignite free, lignite traces, such as dust due to ongoing mining activities, could result in a mistaken determination of recent SOC from plant residues (Quinkenstein et al. 2011). Given that we did not quantify the lignite content, such as that caused by radiocarbon measurements, we are unable to quantitatively assess the proportion of lignite in the SOC

reported in this study. Nevertheless, we assume that any overestimation of our SOC results caused by dust deposition from ongoing mining activities is negligible, which is also supported by the results of Funk et al. (2013). They used several Modified Wilson and Cooke sediment catchers (MWAC, in 45 and 165 cm height) and Suspension Sediment Traps (SUSTRA, in 45 cm height) on a nearby (<5 km) post-mining area in order to collect dust deposits and analyse their chemical composition. They found that the highest carbon content of the transported material was 7.9%, which corresponded to 2.1 kg TC ha⁻¹. This was detected in 2012 over a period of 252 days.

Climate and precipitation have also been described as major factors influencing the SOC dynamic (Jobbágy and Jackson 2000; Post and Kwon 2000). However, although the influence of soil temperature and rainfall interception on SOC due to tree shelter is an interesting field for future investigation, it was beyond the scope of this research.

Short-term land use related effects on SOC stabilization

The isolation of functional fractions revealed that land-use associated effects on SOC distribution in the different fractions were crucial in the uppermost (0–3 cm) soil layer a few years after planting. In this regard, we observed an SOC increment with tree stand age stored in degradable fPOM and oPOM fraction under ABL, which was greater than under the conventional managed AC. However, an early increase of SOC in the fPOM fraction was expected, as this fraction is most influenced by root exudation and above- and below-ground litter input (Cappai et al. 2017). This allowed a major proportion of SOC, accounting for more than 47% of the total, to accumulate in this fraction after a 5-year period, largely without soil disturbance from agricultural management. These results are in contrast with those reported from other studies (Cappai et al. 2017; Crow et al. 2007; Herold et al. 2014). Their authors conclude that the heavy MOM fraction with > 64% contributed the largest proportion of total SOC content to the bulk topsoil under the trees at agro-silvo-pasture and forest sites. Similar amounts in the fPOM fraction are usually only reported in the upper top soil layer of forest soils with lower microbial activity (John et al. 2005), or in young post-industrial calcareous soils under natural succession, and with an imbalance between litter production and decomposition (Grünwald et al. 2006). Nevertheless, the enrichment of SOC in the oPOM fraction at ABL showed a significant monotonic increase with stand age, indicating that stabilization against the microbial decomposition of OM was more efficient than at the corresponding AC. This was probably due to the formation of the first organic–mineral associations, which ensure that OM is occluded within stable soil aggregates (Christensen 2001; Grünwald et al. 2006). Overall, the greater increase in SOC in the fPOM and oPOM fraction in the uppermost soil layer under ABL suggests that this development, together with the higher input of OM, is probably favoured by the fallow period. In particular, in the years after tillage (namely, 2011 and 2012) the mean SOC contents in the fPOM and oPOM fractions at the AC were distinctly lower in comparison to the previous years, and in direct comparison to ABL. It is likely that tillage practices adversely affected a near-surface accumulation at the AC, while C inputs were distributed more evenly throughout the plough layer (Six et al. 2002). In addition, the management at AC from October 2010 to August 2012 switched from extensive (harvest-free) cultivation to conventional management with harvest removal. Consequently, the lower C inputs caused by the removal of crop residues due to harvest and the frequent disruption of soil aggregation in agriculturally managed soil (Six et al. 1998) could also have led

to a preferential loss of OM (Kögel-Knabner et al. 2008). The stronger enrichment of SOC by age across the fPOM and oPOM fractions in the lower soil depth (3–30 cm) at AC in comparison to ABL tends to reflect this well. Although our results from the 0–3 cm soil layer showed a slight decrease of SOC with age in the MOM fraction (more pronounced at AC than at ABL), neither a significant difference across locations nor a monotonic trend was obvious. Our investigation period was probably not long enough to detect recalcitrant associations of plant compounds and the soil mineral phase, or else the microbial biomass was too low to facilitate the formation of mineral-associated complexes in a significant magnitude (Ye et al. 2019). In addition, the SOC stored in the MOM fraction is considered to be more recalcitrant to changes in land use (Christensen 2001; Herold et al. 2014), and therefore a significant management-related effect would not be expected. The OC accumulation in the MOM fraction is mainly influenced by the mineral composition, particularly the amount of clay and effective sorbents like Al and Fe hydrous oxides of the clay fraction (Kaiser et al. 2002). Our results from the density fractionation below a 3 cm soil depth suggest that at least the soil texture at the crop alley had a greater SOC accumulation in the MOM fraction. However, a correlation between the SOC content in any of the three density fractions investigated and the fine particle content (clay and/or silt) did not yield significant linear or non-linear relationships (data not shown), which was probably due to our inadequate data basis and comparatively high soil heterogeneity. Texture analysis was only conducted in the first year of our investigation and only once for each sampling transect. However, a direct relationship between fine particle content and the actual amount of SOM, and consequently SOC cannot generally be established (Christensen 1992; Hassink et al. 1997) without considering other factors such as aggregation of primary particles, bioturbation and anthropogenic influences (Christensen 2001).

We also observed decreasing TOC/TN ratios with increasing density in the order fPOM > oPOM > MOM, which is keeping with findings from other studies on soils with widely divergent textures, locations, mineralogy and management (Cappai et al. 2017; Golchin et al. 1994a; Grünewald et al. 2006; John et al. 2005; Schrumpf et al. 2013; Sollins et al. 2006). These results indicate that increasing density brings increasing resilience against microbial decomposition (Cappai et al. 2017), together with progressive humification and nitrogen enrichment (Golchin et al. 1994a; Grünewald et al. 2006; John et al. 2005). By contrast, the wide TOC/TN ratios of the fPOM fraction indicate a large proportion of carbon from fresh plant residues in an initial stage of decomposition (Grünewald et al. 2006), which is prone to degradation by soil microbes and rather small amounts of TN (<0.1%, data not shown). As the duration of our investigation progressed, the TOC/TN ratios of fPOM and oPOM became hardly distinguishable, especially at ABL. Similar ratios of oPOM and fPOM were observed by Cappai et al. (2017), who explained this observation by the fractionation method, as both pools could include material of a density < 1.6 g cm⁻³, in either free or occluded form.

Conclusions

We determined the OC and HWEOC content of the litter and soil layer annually, and isolated three density fractions in the topsoil layer of a SRACS on an anthropogenic soil over a 6-year period. Generally, the accumulation of significant amounts of OC was largely limited to the litter and the 0–10 cm soil layer under the recently planted

black locust hedgerows. Meanwhile, the investigation into the labile HWEOC fraction revealed significant enrichment up to a 30 cm soil depth, particularly in the 10–30 cm soil layer most noticeable on the crop alleys, which represents a conventional reclamation measure. By contrast, SOC accumulation in the uppermost soil layer of the corresponding hedgerows turned out to be superior. This was largely accompanied by a higher increase in SOC in the labile fPOM and intermediate oPOM fraction, indicating a steady supply of OM derived from the trees and the formation of first organic-mineral associations, respectively. However, with increasing soil depth the SOC and HWEOC gains under the hedgerows became less pronounced in comparison to their related crop alleys. Given that the crops were frequently not harvested and plowed back into the soil, which enhances the distribution of OM into the deeper soil layers in the crop alleys, the question arises of whether one can expect a significant relocation of OM under trees in the future such as due to increasing root growth. Since these results only reflect the initial phase after tree planting, the land use-specific long-term effects need to be further investigated. Nevertheless, our results indicate that black locust trees can significantly increase the soil fertility of overburden material at a reclaimed mining site, even during the first few years. Against the background of climate change, our results also suggest that hedgerows in SRACS could make a valuable contribution as a future carbon sinks at reclaimed sites. Therefore, and due to its comparatively simple adaption to agricultural practices, SRACS is a meaningful recultivation measure for marginal sites in Brandenburg.

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