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Afforestation of loess soils: Old and new organic carbon in aggregates and density fractions

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ABSTRACT

Afforestation is an important strategy to increase soil organic carbon (SOC) stocks and stabilize soils against degradation and erosion. While the area under afforestation has increased globally during the last 20 years, the dynamics of SOC accumulation due to afforestation are still poorly known, as it cannot be directly compared to that in cropland. The goals of this study were: i) to investigate the dynamics of SOC accumulation after afforestation on the Loess Plateau; ii) to determine SOC contents and distribution in density fractions and aggregates most strongly affected by afforestation; and iii) to analyze the relationships between old and new SOC in relation with afforestation periods. A chronosequence of forest plots aged 1, 5, 12 (with Chinese pine, *Pinus tabulaeformis* Carr.) and 30 years (with white poplar, *Populus alba* L.) were selected within the large scale “Grain for Green” project. A maize field nearby was chosen as a control, which represents the land use prior to afforestation. At each location, soils were sampled at depths of 0–10 and 10–30 cm, respectively and aggregates were fractionated into < 250, 250–2000 and > 2000 μm sizes, which were subsequently separated into light ($\rho < 1.85 \text{ g cm}^{-3}$) and heavy ($\rho > 1.85 \text{ g cm}^{-3}$) density fractions. SOC content was generally higher in afforested soils than in cropland and increased with stand age. The mean SOC accumulation rate was $0.11 \text{ g C kg}^{-1} \text{ year}^{-1}$ for the 0–10 cm layer of the 30-year-old forests, whereas the maximum rate was found for 5-year-old forest at $0.24 \text{ g C kg}^{-1} \text{ year}^{-1}$. For each period of afforestation, the maximum SOC content was recorded in the macroaggregates. The C turnover of the light fraction (calculated based on $\delta^{13}\text{C}$ of SOC) peaked in the large macroaggregates and ranged from 21 to 23 years). Whereas SOC turnover in the heavy fraction, peaked in the microaggregates at a relatively longer period of 46 to 70 years. We conclude that the initial SOC accumulation under afforestation occurs mainly in the macroaggregates, with a faster turnover compared with microaggregates. During the first 30 years of afforestation, the SOC accumulation and stabilization is ongoing mainly in the upper 10 cm, while the C sequestration in 10 to 30 cm depth needs much longer time.

1. Introduction

Soil organic carbon (SOC) is the largest terrestrial carbon (C) sink, consisting of 1500 Pg C, and plays an important role in the global C cycle (Lal, 2004a). Slight SOC fluctuations significantly affect atmospheric CO₂ concentrations (Martens et al., 2004; Houghton et al.,

2012). Thus, increasing C content in terrestrial ecosystems is one of the main approaches to mitigate anthropogenic production of atmospheric CO₂. Afforestation of land previously used for agriculture is one of the most widely used strategies to mitigate increased levels of atmospheric CO₂ (Grünzweig et al., 2007; Post and Kwon, 2000).

Globally, lands under afforestation increased from 618×10^6 ha to

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684×10^6 ha from 2010 to 2015. The greatest expansion occurred in temperate forests, with China recording the highest increase of 1.5×10^6 ha year⁻¹ (Keenan et al., 2015). Increasing the areas under afforestation offers the potential to increase soil C stocks due to increased C input from forest litter coupled with decreasing C loss via erosion, decomposition or tillage (Bashkin and Binkley, 1998; Chang et al., 2011; Lal, 2004b; Kurganova et al., 2014, 2015). The effects of afforestation on SOC stock is dependent on the time after forest establishment, tree species, climatic conditions and soil physico-chemical properties (Laganiere et al., 2010; Zhang et al., 2013; Kurganova et al., 2014). Data from afforestation of the Loess Plateau (the “Grain for Green” project in China) show that the rates of SOC accumulation vary between -0.63 and $+0.37$ t C ha⁻¹ year⁻¹ depending on the length of afforestation period (Chang et al., 2011; Deng et al., 2014; Zhang et al., 2010). This was lower than the rates of SOC accumulation caused by afforestation in Central America (1.6 t C ha⁻¹ year⁻¹) (Martens et al., 2004) or Russia (1.0 t C ha⁻¹ year⁻¹) (Kurganova et al., 2014), respectively. The minimum and maximum changes in the SOC of the upper 20 cm of soil ranged from -30% to $+124\%$ (Song et al., 2014). Post and Kwon (2000) explained that increasing C inputs from forest litter leads to intensive SOC accumulation. In this regard, Bárcena et al. (2014) argued that, SOC loss during the first decades after afforestation is attributable to intensive SOC decomposition caused by increased microbial activity and low litter input by young trees. At 10 to 13 years after afforestation, forest-derived C accumulates in the upper 10 cm of the soil, but a simultaneous C loss may occur in deeper layers (10–55 cm). Thus, the responses of SOC to afforestation relative to new C (derived from new vegetation after afforestation) and old C (initial SOC prior to afforestation) actually occurred in the bulk soil (Bashkin and Binkley, 1998). To better understand SOC dynamics and C sequestration associated with afforestation, it is important to understand changes that take place in both new C and old C within the bulk soil. The ¹³C natural abundances of soil have been proved to be suitable for determining C pathways soil organic matter (SOM) formation (Gunina and Kuzyakov, 2014) as well as investigating changes in new and old C in the bulk soil following afforestation (Deng et al., 2016).

Afforestation does not only affect SOC accumulation, but also affects the soil structure – the composition of aggregates (Kalinina et al., 2011). Golchin et al. (1998) showed that the dynamics of aggregate formation are closely linked to SOC storage in soils. The termination of plowing leads to an accumulation of macroaggregates, increasing their lifetime in soil and the soil C content (Jones et al., 2005), increasing the amount of particulate organic matter (POM) (Besnard et al., 1996) and stimulating C bounding by clay minerals. In contrast to Jastrow et al. (1996) and John et al. (2005) who found greater SOC content in macroaggregates than in microaggregates, Liao et al. (2015) did not find any significant differences in SOC or particulate organic matter (POM) contents among different aggregate size classes. SOM is a complex entity consisting of different fractions therefore, to elucidate the changes in SOC dynamics in response to afforestation, it is necessary to distinguish between SOC changes occurring in the light and heavy fractions, respectively. Light fraction is considered as labile organic matter in soil (Janzen et al., 1992), while the heavy fraction is considered as the product of microbial necromass, suggesting the SOC in light fraction may be much easier to be decomposed and at a shorter turnover time compared to the heavy fraction (Jastrow et al., 2007; Schwendenmann and Pendall, 2006). However, Gunina and Kuzyakov (2014) found that the C flows are complicated in aggregates and fractions. Therefore, following afforestation, the processes involved in C accumulation regarding different fractions of SOC in the various aggregate size classes remains unclear.

The Loess Plateau in China, covering an area of 6.2×10^5 km², was chosen as the study area because afforestation here started in 1980 to control soil erosion. The goal of the afforestation was to convert approximately 2.04×10^6 ha of croplands with slopes $> 15^\circ$ to woodland and grassland (Chen et al., 2007). The Loess Plateau in China is

therefore, an appropriate research area for estimating the effects of afforestation on the accumulation of SOC at large temporal and spatial scales. Several studies have investigated the spatial-temporal changes in SOC and the controlling factors following the “Grain-for-Green” project (Li et al., 2005; Zhang et al., 2010). For instance, the Ziwuling forest region was frequently chosen as a study area (Chang et al., 2011; Wei et al., 2012). In this region, the aggregation and SOC fractions under different land uses (Liu et al., 2014), the SOC fractions and sequestration across a secondary forest chronosequence (Zhao et al., 2013), and the SOC storage capacity and SOC dynamics (soil new C and old C) following natural vegetation restoration (Deng et al., 2013, 2016) were adequately studied. However, there is no evidence of a systematic analysis of SOC dynamics with respect to size and density fractions combined with stable C isotope analysis (¹³C/¹²C). The ¹³C natural abundance technique combined with the SOM fractionation technique provides an approach to detect the small shifts in SOC that would not be significant in a short term and might not be detected by other conventional methods. The present study used the combined approaches to estimate the effects of afforestation on the dynamics of SOC accumulation in aggregates and density fractions. A 30-year afforestation chronosequence allows us to evaluate the dynamics of SOC accumulation following afforestation. The specific objectives of the study were: i) to quantify the dynamics of SOC accumulation after afforestation and its relation to soil aggregates, ii) to determine which SOC fractions are most strongly affected by afforestation, and iii) to analyze the relationships between old and new C in relation with the period of afforestation.

2. Materials and methods

2.1. Site description and sampling

Soil samples were collected from Huachi County, Gansu Province, China ($36^\circ 17' - 36^\circ 22' N$, $107^\circ 52' - 108^\circ 31' E$) in August 2014. The sampling area is located in the east of the county at the western boundary of the Ziwuling forest region, the largest natural forest region on the Loess Plateau. Here, the continental monsoon climate has an average annual temperature of $7.8^\circ C$, and an annual precipitation of 380–510 mm, with an average of 172 frost-free days. The soil texture in the sampling area is predominantly silt loam with an average clay content of 23.7%. The average bulk density of the soil ranges from 1.28 to 1.32 g cm⁻³ and the pH ranges from 8.28 to 8.72. The high pH value is caused by high soil carbonate content and the dry climate. Farmers have grown corn (*Zea mays*) as the main crop in this area starting since the middle of the 20th century. Afforestation began here during the 1980s with a large area of cropland being converted to white poplar (*Populus alba* L.) forests. In 1999, 31,000 ha of cropland were transformed to Chinese pine (*Pinus tabulaeformis* Carr.) in line with the “Grain for Green” project framework.

The experimental plots included cropland (planted annually with maize) and afforested soils with stand ages of 1, 5, and 12 years planted with Chinese pine, and of 30 years planted mainly with white poplar. It was impossible to find a 30-year Chinese pine dominated forest as only the white poplar species planted in this area was up to 30 years old. Thus the chronosequence was not based on the same vegetation type as the ages of the tree species not the same among the differently aged afforestation projects used in this study. However, $\delta^{13}C$ values of leaves collected from Chinese pine and white poplar were nearly identical (-27.0% vs. -27.3%), indicating that the fraction of new C calculated based on $\delta^{13}C$ was not affected by the differences in species types.

Soil samples were collected using a stainless-steel cores (5.0 cm diameter, 65.0 cm length) at depths of 0–10 cm and 10–30 cm, respectively. For each plot, bulk samples were randomly collected at 4 different sites located > 200 m from each other and having similar slope gradient. Each replicate consisted of four individual subsamples which were uniformly mixed after leaf litter, roots, leaves and woody

materials have been removed.

2.2. Aggregate sizes and density fractionations

Soil samples (100 g each) were air-dried at room temperature and dry sieved through wire mesh with sizes ranging from 250 and 2000 μm . Three aggregate size classes were obtained and were defined as large macro- (> 2000 μm), small macro- (250–2000 μm) and microaggregates (< 250 μm) (Tisdall and Oades, 1982). Although the wet sieving method is widely used to obtain water stable aggregates, in this study, dry sieving was used so as to minimize disruption of aggregates, and to obtain aggregates which were more representative of the field situation, where the soil was seldom saturated due to semi-arid climate and the high water permeability of loess soil.

Soil C in each of the 3 aggregate size classes was separated into different density fractions by dry sieving described above. Air-dried aggregates (5 g) were placed into a centrifugation tube and 15 mL of sodium polytungstate solution with a density of 1.85 g cm^{-3} was added. Next, the tube was gently inverted several times. The solution was then centrifuged at 4000 rpm for 1 h. The supernatant with floating particles was filtered and washed with distilled water to obtain a light fraction of organic matter with $\rho < 1.85 \text{ g cm}^{-3}$. The heavy fraction with $\rho > 1.85 \text{ g cm}^{-3}$ was washed four times with 20 mL distilled water each time and dried (Gunina and Kuzyakov, 2014).

2.3. Analysis of C content and $\delta^{13}\text{C}$ values

All aggregate size classes and fractions obtained by density fractionation were subsequently, treated with 12 M HCl for 24 h to remove carbonates (Harris et al., 2001). The HCl was washed off with distilled water and the aggregates were dried at 40 °C, weighed, and ground manually before analysis. The C and N contents of the bulk soils, aggregate size classes, and isolated density fractions were measured by an elemental analyzer (VARIO EL III, Elementar Co., Ltd., Hanau Hessa, Germany).

The natural stable C-isotope composition ($\delta^{13}\text{C}$) of all fractions, bulk soil samples and the plant litter and root samples were measured at Hohai University, using an isotope ratio mass spectrometer (C/N Isotope, Sercon Ltd., Cheshire, UK). The C isotope ratios were expressed relative to the international PDB limestone standard as $\delta^{13}\text{C}$.

2.4. Data analyses

The $\delta^{13}\text{C}$ values of the SOC and its fractions were used to calculate the proportion of new C (f_{new} ; i.e., C derived from the current forest vegetation) and of old C ($f_{\text{old}} = 1 - f_{\text{new}}$; i.e., the C derived from soil organic matter prior to afforestation) for all SOC fractions. This was done using the mass balance equation (Eq. (1)) described by Del Galdo et al. (2003).

$$f_{\text{new}} = \frac{\delta_{\text{new}} - \delta_{\text{old}}}{\delta_{\text{veg}} - \delta_{\text{old}}}, \quad (1)$$

where δ_{new} is the $\delta^{13}\text{C}$ value (‰) of the SOC fraction of the afforested soil, δ_{old} is $\delta^{13}\text{C}$ values (‰) of the SOC fraction in cropped soil (assuming that in the last > 30 years there has been no shift in the ratio between C_3/C_4 input in the cropland soil), and δ_{veg} is the $\delta^{13}\text{C}$ value (‰) of the forest litter. Table 1 shows the $\delta^{13}\text{C}$ values of leaf, wood, root and litter samples for cropland and forest species.

Turnover times (T) of maize C in various aggregate fractions of the forests soils were calculated using the formula described by Yamashita et al. (2006) (Eq. (2)).

$$T = -\frac{t - t_0}{\ln(1 - f_{\text{new}})}, \quad (2)$$

where t is the year of sampling and t_0 is the year when afforestation began.

In the study, data analyses were done using the SPSS 10.0 statistical software. Significant differences in SOC content in between the maize (control) and the afforestation sites, and SOC content and $\delta^{13}\text{C}$ values among the different aggregate sizes and density fractions were tested by one-way ANOVA. The means were separated using a Tukey post hoc test. The significant differences in SOC content and $\delta^{13}\text{C}$ values between the two soil depths (0–10 cm and 10–30 cm, respectively) were also tested using the same method.

3. Results

3.1. Distribution of SOC in bulk soil and fractions

SOC content was generally higher in the afforested lands than in the cropland and increased with stand age (Fig. 1). In the forested soil, SOC content was significantly higher at 0–10 cm depth than at 10–30 cm depth (Fig. 1). In most cases, the large macroaggregates had the highest SOC content in both topsoil and subsoil (Fig. 2). However, the small macroaggregates showed higher SOC content in the topsoils of the 1-year and the 30-year-old forests.

SOC content in the heavy fractions significantly increased with stand age in the upper 0–10 cm, but the light fraction did not show any consistent increase with stand age (Fig. 2). In the upper layers (0–10 cm) of the cropland and the 1-year-old forest soils SOC contents in the heavy fractions were lower than that in the light fractions. The reverse was found in the 12-year and the 30-year-old forest. In the subsoil, the SOC contents in the light fractions were generally greater than in the heavy fractions.

3.2. Natural ^{13}C abundance

Change in land use from cropland to forest significantly affected the $\delta^{13}\text{C}$ values of aggregates and density fractions (Fig. 3) because the vegetation changed from the C_4 (maize) to C_3 (forest) type of photosynthesis (Fig. 3). The least negative $\delta^{13}\text{C}$ values were found in maize cropped soils while the $\delta^{13}\text{C}$ values in afforested soils clearly shifted towards the typical C_3 vegetation signal. The light fraction had the most negative $\delta^{13}\text{C}$ values whereas the heavy fraction showed the least negative $\delta^{13}\text{C}$ values for all afforestation periods and all aggregate fractions. The $\delta^{13}\text{C}$ values in the upper 0–10-cm layer were significantly lighter than the values in the 10–30 cm layer. For the upper 0–10-cm soil layer, the $\delta^{13}\text{C}$ values of aggregates and density fractions shifted towards the C_3 signal starting from the 5-year-old stand forest soil, while for the 10–30 cm layer the shift in signal started from the 1-year-old forest soil (Fig. 3). However, $\delta^{13}\text{C}$ of the heavy fractions in the 10–30 cm layer initially increased in soils of the 1-year- and 5-year-old stands, and remained close to the $\delta^{13}\text{C}$ value of maize roots (Fig. 3; Table 1). Over time, the C_4 signal in the 10–30 cm layer decreased, suggesting that C in the heavy fractions exhibited a longer residence time (Fig. 3).

3.3. Old and new carbon distribution

The proportion of new C (C_3 signature) increased with increasing stand age during the first 12 years in all fractions (Fig. 4) but slightly decreased or became stable afterwards. The proportion of new C in the light fraction increased to > 80% after 12 years of afforestation (Fig. 4). In contrast, the proportion of new C was much lower in the heavy fraction, decreasing to < 50% in the 10–30 cm soil layer. In the forested soils, proportions of new C were much lower in all the aggregate size classes from soil below 10 cm than from the upper 10 cm clearly reflecting the high litter C input at the surface of forested land.

The maximum accumulation rate of new C was found in the upper 10 cm under the 5-year-old forest, whereas the lowest was found in the 30-year-old forest (Table 2). The mineralization rate of old C was highest in the 12-year-old plot and lowest in the oldest (30 years) forest.

Table 1
 $\delta^{13}\text{C}$ (‰) of leaves, wood, roots and litter for cropland and afforested lands. Values represent means \pm standard errors ($n = 3$).

Land use	$\delta^{13}\text{C}$ (‰)			
	Leaves	Wood	Roots	Litter
Cropland (maize)	-14.14 ± 0.46	n.a. ^a	-14.10 ± 0.57	n.a. ^a
Afforestation 1, 5 and 12 years (Chinese pine dominated)	-27.00 ± 0.51	-24.40 ± 0.27	-26.38 ± 0.33	-27.99 ± 0.32
Afforestation 30 years (White poplar dominated)	-27.34 ± 0.29	-28.95 ± 0.27	-27.59 ± 0.45	-27.13 ± 0.63

^a n.a. = not applicable.

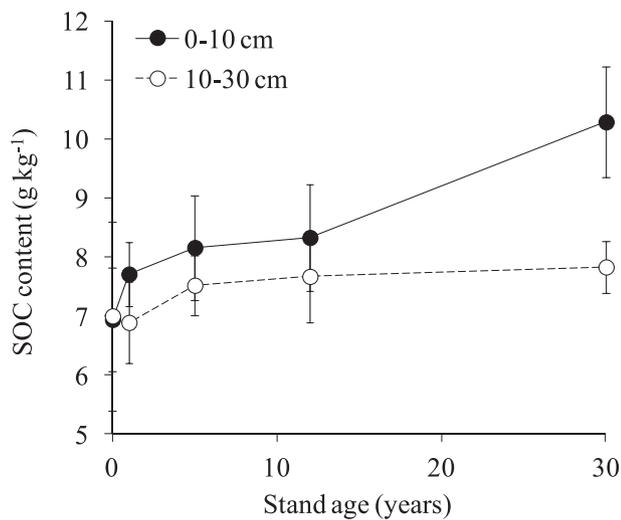


Fig. 1. Dynamics of soil organic carbon (SOC) content depending on stand age.

The rates of new C accumulation as well as old C mineralization were lower in the soil below 10 cm depth, than in the upper 10 cm soil. The maximum values for both rates were found in the 12-year-old forest.

3.4. Turnover time of soil carbon in various fractions

Turnover time of soil C (calculated based on the C_4 -C) in heavy fractions in 10–30 cm depth was much longer than in the upper 10-cm soil layer (Fig. 5). Turnover times for the light fractions in the small macroaggregates were faster than in other aggregates for both depths. The turnover time of the light fraction was 23 and 21 years in large macroaggregates for 0–10 cm and 10–30 cm soil layers, respectively, which was higher than those of the other aggregates. The turnover time of the heavy fraction was 46 years in microaggregates of the 0–10 cm soil layer, which was the longest compared to all other aggregate size classes; while in the 10–30 cm soil layer, the turnover time of the heavy fraction was 73 and 70 years in small macroaggregates and microaggregates.

4. Discussion

4.1. Carbon accumulation rates during afforestation

Afforestation has variable effects on SOC accumulation. Specifically, SOC content can remain constant, decrease or increase with age of afforestation. Although, most studies showed increasing SOC with age of afforestation, some studies reported unstable SOC content during the early stages of afforestation (Kurganova and de Gerenyu, 2008; Deng et al., 2013, 2016). Similarly, decreases in SOC stocks were observed during the early stages of afforestation (< 5 years) in some studies carried out during the “Grain-for-Green” project (Deng et al., 2014; Zhang et al., 2010). Relatively lower SOC stocks occurred during the

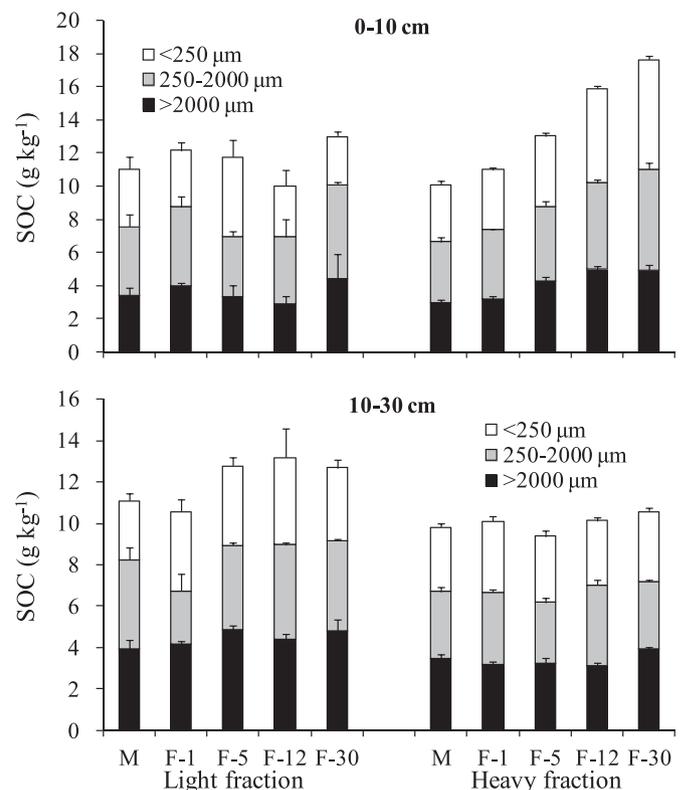


Fig. 2. Soil organic carbon (SOC) content (g C kg^{-1} soil) in aggregate size classes and density fractions separated from the aggregate size classes (M: maize; F-1: forest-1-year; F-5: forest-5-year; F-12: forest-12-year; F-30: forest-30-year).

early stages of afforestation, because the litter produced by trees and root C input into the soil were probably low during the first 2–3 years after afforestation. Additionally, the low SOC found in the early stages of afforestation might be attributed to the effects of the intense soil erosion that often occurs on the Loess Plateau (Zhao et al., 2013). Thus the magnitude of SOC loss due to erosion was greater than SOC added to the soil via mineralization in the initial years of afforestation. In our study, however, SOC contents increased slightly but not significantly during the early years after afforestation. This may be caused by the soil erosion, which occurred during the early years of vegetation restoration (Tang, 2004). The SOC contents in the surface 10 cm of the soil significantly increased after 30 years of afforestation ($P < 0.05$); but no significant differences in SOC were found in the soil at 10–30 cm depth. In addition to the increased biomass input from leaf litter and/or roots, long term afforestation possibly contributed to stable soil aggregates formation and thus facilitated the physical protection of SOC within aggregates (Deng et al., 2016).

The maximum SOC accumulation rate of $0.24 \text{ g kg}^{-1} \text{ year}^{-1}$ was found in the top soil of the 5-year-old forest, and decreased with

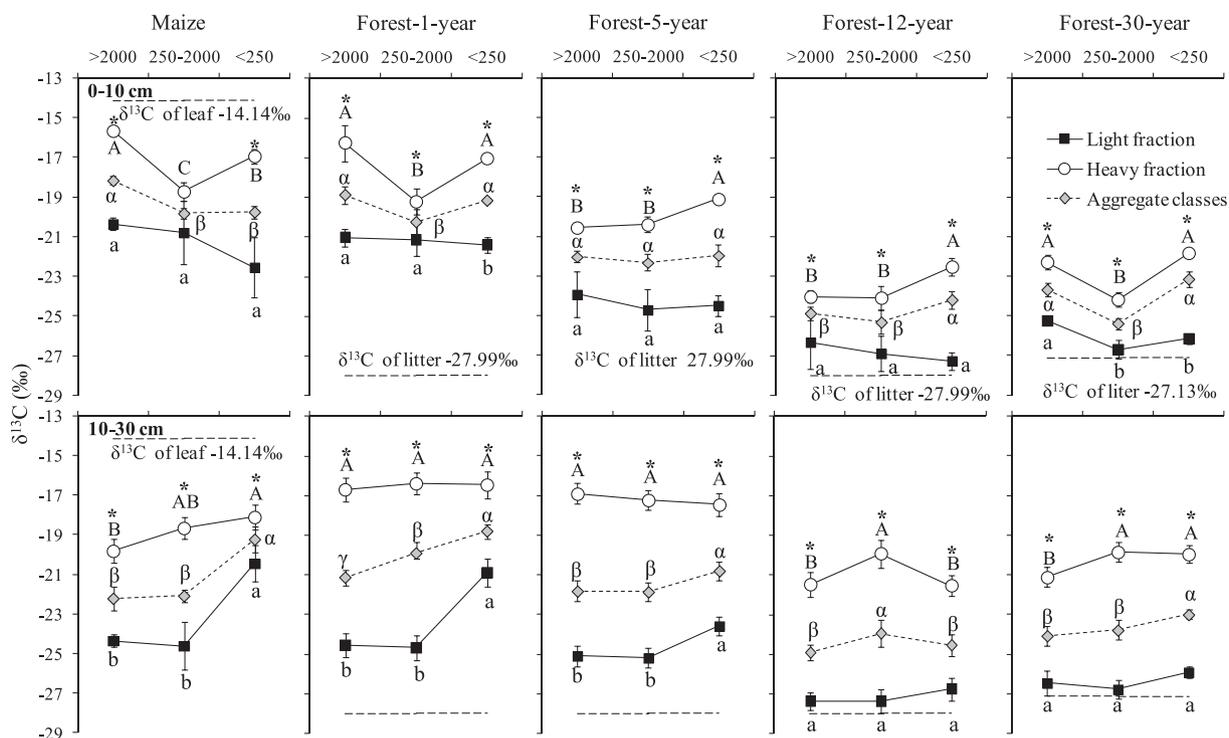


Fig. 3. $\delta^{13}\text{C}$ values (‰) for aggregate size classes and density fractions separated from aggregate size classes. Capital and lowercase Latin letters, and Greek letters indicate significant differences among various aggregates ($P < 0.05$). * indicates significant differences between light and heavy fractions within each aggregate size ($P < 0.05$).

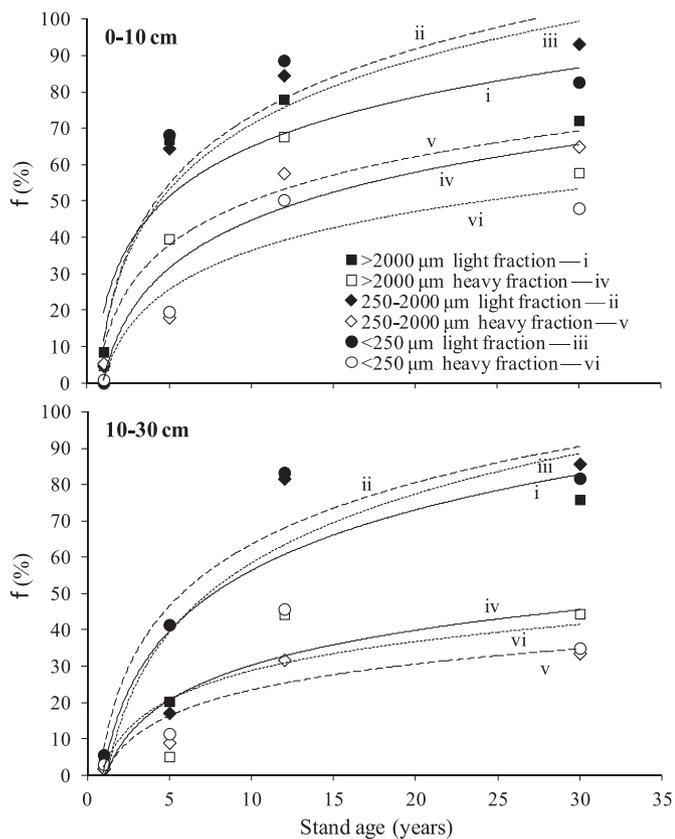


Fig. 4. Increase of the new C proportion (f) in soil density fractions (% from total organic C) depending on stand age.

Table 2

Accumulation rate of new carbon (C ; $\text{g C kg}^{-1} \text{ year}^{-1}$) from forest vegetation and decomposition rate of old C ($\text{g C kg}^{-1} \text{ year}^{-1}$) from maize for various stand ages.

Years after afforestation		1	5	12	30
0–10 cm	New C ^a	0.21	0.54	0.44	0.19
	Old C ^b	–	–0.30	–0.32	–0.08
	Total SOC ^c	–	0.24	0.12	0.11
10–30 cm	New C	–	0.06	0.31	0.09
	Old C	–	–	–0.26	–0.06
	Total SOC	–	–	0.05	0.03

^a New C = organic carbon derived from forest after afforestation.

^b Old C = organic carbon derived from maize before afforestation.

^c SOC = soil organic carbon.

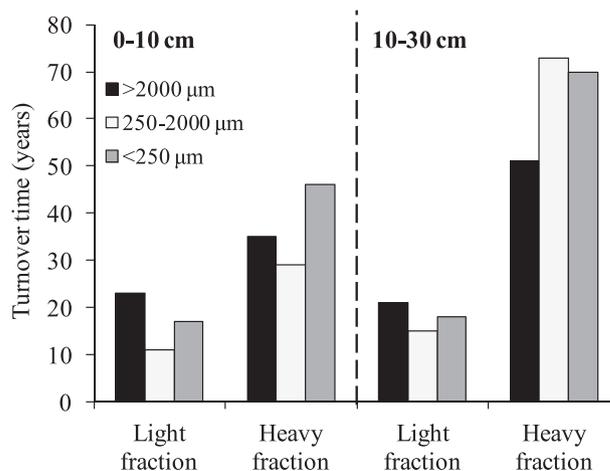


Fig. 5. Turnover time (years) of maize-derived C in aggregate size classes and light and heavy soil fractions.

afforestation age (Table 2). The SOC accumulation rates in the 30-year-old forests in Shaanxi Province ranged between 0.04 g C kg year⁻¹ (in Fuxian County) and 0.37 g C kg year⁻¹ (in the Huanglongshan Forest Area) (Chang et al., 2012; Wei et al., 2012). The rate (0.11 g kg⁻¹ year⁻¹) calculated in our study for the 30-year-old forest falls within these results. The variations in SOC accumulation rates among these studies are attributable to differences in previous land use, afforestation age, precipitation, tree species, soil type and topography, etc. (Burke et al., 1999; Zhang et al., 2013). Most of these factors directly affect the primary production and therefore, the C input into the soil. Several previous studies showed that the annual litter biomass produced by both white poplar and red pine increased from the beginning of afforestation to the seventh year and then became nearly stable, although there are regional differences caused by climatic conditions and nutrients in soil (Song et al., 2010; Yang et al., 2010). Perhaps this explains why we observed an increasing new C accumulation rates during the first 5 years after afforestation. However, in this study, the decreasing SOC accumulation rate observed after 5 years of afforestation, disagreed with the previous findings that the litter biomass contents and hence SOC accumulation rate became stable after 7 years of afforestation (Fig. 1). This may be explained by the high rate of microbial decomposition of old C during early years of afforestation or the low accumulation rate of new C during later years of afforestation (Table 2). Deng et al. (2016) found that the SOC decomposition rate and the rate of new C accumulation were higher in the early 10 years after afforestation and showed no significant differences after 30 years), which were similar to our result. The soil nutrients concentrations and microbial activities were observed to increase very quickly during the initial vegetation restoration stages (An et al., 2009). Increased soil microbial activity often increases the availability of organic inputs from vegetation, but can also increase the microbial decomposition of carbon. The high decomposition rate of old C in the early stage of afforestation could be because the protection of SOM had not yet been restored or well reestablished. The rate of new C increase represented the net effect of new C input as against C output from the soils (Richter et al., 1999). At the beginning of afforestation, the C input from litterfall and rhizo-deposition would increase sharply. However, as the forests age, new carbon input decreases gradually. When SOM input and output reached a balance, the SOC accumulation rate at this steady state declines compared with the early stage of afforestation.

In the current study, the chronosequence was not based on the same vegetation type because Chinese pine was the dominant tree in the 1, 5 and 12 years-forest, while white poplar was the dominant tree in the 30 years-forest. Nevertheless, similar leaf $\delta^{13}\text{C}$ values were found in the white pine and Chinese poplar species. However, increases in the proportions of new C in soil density fractions varied depending on stand age, resulting in several different cumulative curves (Fig. 4), which is indicative that the new C proportions were not affected by the vegetation type. We conclude, therefore, that even though the tree species were different, the results generated from the chronosequence used in our study were reliable.

4.2. Distribution of SOC in aggregates after afforestation

Among the aggregate fractions obtained after dry sieving, the macroaggregates (250–2000 μm and > 2000 μm) had the highest SOC content (Fig. 2). This is in a good agreement with Shaoshan et al. (2010), who showed that aggregates with a size range of 630–1000 μm (wet sieving) had a higher SOC content than microaggregates (< 250 μm) in both forests and abandoned grazing land on the Loess Plateau. Liu et al. (2014) also found that higher SOC contents in the 2–5 and 1–2 mm aggregate fraction (after dry sieving) in forestland and grassland on the Loess Plateau. Additionally, John et al. (2005) also reported that C accumulation was higher in macroaggregates (wet sieving) for old (80 year-old) cultivated forests than in the microaggregates. Thus, results from both dry sieving and wet sieving methods

suggest that C accumulation is ongoing initially in macroaggregates (> 2000 μm) (Six et al., 2004), and macroaggregates accumulated more C than microaggregates. This is because the microaggregates are bound together into macroaggregates by transient binding agents (microbial and plant-derived polysaccharides) and temporary binding agents (fungal hyphae and roots) (Six et al., 2000). Microaggregates are assumed to be stabilized by more persistent SOM; while younger and more labile organic matter (roots and hyphae) is abundant in macroaggregates (Liu et al., 2014).

The turnover time of C in the upper 10 cm of soil increased with decreasing aggregate size for the heavy fraction (Fig. 5). Similarly, Rabbi et al. (2013) and John et al. (2005) also reported that C turnover time increased with decreasing aggregate size. Dorodnikov et al. (2009) indicated that the contribution of fungi to the C turnover was much faster in macroaggregates than in microaggregates. In addition, macroaggregates have more labile C than microaggregates (Bronick and Lal, 2005). Therefore, the heavy fraction of SOC in soil macroaggregates was more easily decomposed and, thus, had a shorter turnover time than in microaggregates. Our result showed that the turnover time of C in heavy fractions can be explained by the aggregate formation theory of Six et al. (2000), which is similar to Gunina and Kuzyakov's (2014) conclusion, that the C flows are mainly directed from macro- to microaggregates. However, for the light fractions, the turnover time of C was shorter in small macroaggregates and longest in large macroaggregates (Fig. 5). Gunina and Kuzyakov (2014) studied C flows in soil aggregates and density fractions by ^{13}C natural abundance. They concluded that C flows were mainly directed from small macroaggregates to microaggregates in light fractions, and then from microaggregates in light fractions to large macroaggregates and microaggregates in heavy fractions.

Deng et al. (2016) showed that SOC stocks were negatively correlated with soil pH following vegetation restoration in the Ziwluling forest region. The high soil pH values (8.28–8.72) found in this study could have affected the SOC turnover time. However, there is a paucity of information regarding the effects of soil pH on SOC accumulation. Rousk et al. (2009) showed that bacterial growth increased fivefold with a soil pH increase from 4.5 to 8.3. A similar result was reported by Blagodatskaya and Anderson (1998). The increase in bacteria growth along with pH increase was also demonstrated by Haynes and Naidu (1998) in a liming experiment. In agreement with the above-mentioned authors, the SOC turnover times we observed in this study may have been shorter than they will be in soils with pH lower than 8 due to the increased bacterial growth, which would have resulted in higher microbial C decomposition rates. John et al. (2005) reported C turnover time of 86 years in a lower Rottal soil with pH lower than 7, which was longer than what we found in the relatively higher pH soils used in our study. These observations suggest that C turnover time might increase with decreasing soil pH, but this requires further investigations, particularly, in the Ziwluling forest regions using soils with different pH values.

4.3. Isotopic composition of aggregates and density fractions

The $\delta^{13}\text{C}$ values of SOC in light fractions were less than those in heavy fractions in all the forests and stand ages. These results were in agreement with previous reports (Gunina and Kuzyakov, 2014; Sollins et al., 2009; Throop et al., 2013), who attributed this trend mainly to the incorporation into SOM of forest C with lower $\delta^{13}\text{C}$ than maize.

The $\delta^{13}\text{C}$ values of SOC were heavier in the upper 10-cm layer than those in the 10–30 cm soil layer. This illustrates the contributions of aboveground litter input under forest compared to that under maize and the absence of plowing (John et al., 2005; Wynn et al., 2006). At the same time, $\delta^{13}\text{C}$ values of the light and heavy fractions decreased with age, showing an increase in the portion of plant residues from C₃ plants.

Microaggregates and small macroaggregates had lighter $\delta^{13}\text{C}$ values

in the upper 10-cm soil layer under maize than large macroaggregates. On the contrary, the $\delta^{13}\text{C}$ value in microaggregates was heavier than that in large macroaggregates and small macroaggregates after 12 years of afforestation (Fig. 2). The natural ^{13}C abundance in SOC was used to distinguish between old (crop-derived) and new (forest-derived) C (Wei et al., 2012). The proportion of new C in the upper layer was higher than in the subsoil after afforestation in all the fractions indicating a strong contribution of new C input from the surface layer common in all forests.

The proportion of new C in all fractions increased at a high rate during the first 12 years of afforestation, but the rate of increase became slower afterwards (Fig. 5). Wei et al. (2012) reported a similar growth pattern in the proportion of new C in the Loess Plateau. In afforested soils, the proportion of new C in the density fractions increased non-linearly with stand age and the light fractions always showed a higher proportion of new C than heavy fractions (Fig. 4). This finding indicates that the light fractions were close to the $\delta^{13}\text{C}$ values of the C input (i.e. current vegetation), whereas the heavy fractions had a long residence C time (i.e. maize root).

5. Conclusions

The SOC accumulation was studied within the “Grain for Green” project area of the Loess Plateau in a chronosequence after 1, 5, 12 and 30 years of afforestation and compared with continuously cropped maize. The mean rate of SOC accumulation was $0.11 \text{ g C kg}^{-1} \text{ year}^{-1}$ in the 0- to 10-cm soil layer of 30-year-old forests, while the maximum rate was found in the 5-year-old forest ($0.24 \text{ g kg}^{-1} \text{ year}^{-1}$). The maximum C amount was accumulated in the macroaggregates in all plots. The C turnover of the light fraction was fastest in the large macroaggregates and ranged from 21 to 23 years depending on the soil depth. In contrast, C turnover in the heavy fraction of microaggregates was longer and ranged from 46 to 70 years, and was longer in the 10–30 cm soil layer than in the 0–10 cm layer. We conclude that the initial accumulation of SOC is ongoing mainly in the macroaggregates, having a faster turnover rate than in microaggregates. Afforestation contributes to the SOC accumulation and stabilization within the upper 10 cm during > 30 years.

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