Isotope analysis reveals differential impacts of artificial and natural afforestation on soil organic carbon dynamics in abandoned farmland

Dong-Rui Di · Guang-Wei Huang

Abstract

Background A multitude of studies analyzed the dynamics of soil organic carbon (SOC), but their methodology does not provide sufficient understanding of the differential impact of artificial and natural afforestation on SOC dynamic.

Methods and aims We investigated the SOC dynamics following artificial (AR) and natural (NQ) afforestation on abandoned farmland (AF) in China’s Loess Plateau in an attempt to evaluate the effects of these afforestation methods. We characterized soil structure and stoichiometry using stable isotope carbon and radiocarbon models. We aim to compare SOC dynamics, clarify SOC sources under different afforestation, examine comparability of the study areas and ascertain how soil aggregate size classes control SOC dynamics.

Results The 0-10 cm and 10-20 cm SOC stocks were significant higher in NQ than AR and AF. At other depths, there is no significant difference among the three land-use systems. Total topsoil SOC stocks, C:N and C:P of differently sized soil aggregates significantly increased following afforestation.

Conclusions Afforestation can lead to SOC accumulation in soil depths up to 1m mainly because the topsoil (0-20 cm) changes significantly. SOC resources are mainly from macroaggregate formation provided by fresh plant residues. The comparability of study sites is validated, so the “space-for-time substitution” method is applicable in this study.

Keywords Soil carbon stock · Vegetation restoration · Soil aggregate · $^{13}$C · $^{14}$C

Introduction

Soil contains vast and dynamic pools of organic carbon that are fundamental for maintaining the balance of atmospheric CO$_2$ concentrations (Lal 2004; Post et al. 1982). The soil organic carbon (SOC) pool and its dynamics are critical ecosystem properties (Schmidt et al. 2011). Many of studies suggest that ecosystem changes significantly affect SOC pool and decomposition rate (Deng et al. 2016; Don et al. 2011; Snell et al. 2015; Wei et al. 2013; Zhang et al. 2015).

The Loess Plateau is the one of the largest geographic units in China. Rapid population growth combining with climate and environmental conditions in the area have resulted in severe soil erosion.
in the Loess Plateau (Shi et al. 2017). Since 1950 s, great attention has been paid to control the severe soil erosion in the Loess Plateau. From the 1950 s to the mid-1960 s, the soil conservation strategies involved terracing on cultivated slopes and planting trees on uncultivated slopes. This strategy shifted in the mid-1960 s to include warp land dam. From the late 1970 s to the late 1990 s, terracing became the first principle soil conservation strategy and natural rehabilitation appeared in some places (Zhou et al. 2013). The Grain for Green project was implemented in 1999 to halt soil erosion and promote ecological restoration in the region. This project promoted both natural and artificial afforestation (Shi et al. 2011).

Human ecosystem interventions such as deforestation and afforestation, can cause rapid and persistent changes in vegetation and soil. Wei et al. (2013) found that SOC stocks decreased most rapidly during the first four years of cropland cultivation after deforestation. Meanwhile, Rytter (2016) reported that SOC pools were generally unchanged after five years of Salicaceae growth during afforestation of former agricultural land. Natural change refers to the dynamic nature of ecosystem succession. Studies indicate that natural regeneration is slow and patchy with low species diversity (Blackham et al. 2014), and factors controlling SOC accumulation differed according to vegetation succession (Liu et al. 2015). Previous studies have applied different methods (e.g. space-for-time substitution, stable isotope analysis) to study SOC dynamics and have successfully answered questions related to soil structure and organic matter dynamics (Marin-Spiotta et al. 2009; Qiu et al. 2015). But, these studies mainly focused on natural ecosystem restoration without considering the effects of artificial restoration. Additionally, although Deng et al. (2016) investigated the SOC turnover during natural succession, SOC dynamics in stable post-succession communities following afforestation remain unstudied.

In this study, we combined (1) a time dependent steady-state box model based on radiocarbon (14 C) to estimate SOC decomposition rates in different land-use systems; (2) a natural abundance stable carbon isotope (13 C) study to quantify old and new carbon turnover during both natural and artificial afforestation; and (3) measures of SOC stock at different depth in relation to the contribution of soil aggregate-associated organic carbon (OC) including macroaggregates-OC, microaggregates-OC and silt & clay-OC to change of total SOC stock following afforestation.

We hypothesized that (1) SOC accumulation under natural afforestation is more effective than artificial afforestation, (2) macroaggregate-OC stock is the main contributing factor to changes in total SOC stock under afforestation, (3) and soil characters in same vegetation/land-use system were not significant different in the study sites.

Materials and methods

Study site

The study site consisted of semi-arid forests located on Mt. Gonglushan, near Yan’an city in Shaanxi province, China (36°25.40″N, 109°31.53″E; 1245-1395 m a.s.l.). On the Loess Plateau, precipitation and forest cover gradually decrease to the northwest, and our study site is located in the forest–grassland transition zone (Shi et al. 2014). The 40-year averages (1971-2010) of annual precipitation and annual mean air temperature were 504.7 mm and 10.1 °C, respectively (Shi et al. 2012). According to soil classification system of the Food and Agriculture Organization of the United Nations (FAO), the soils are Calclic Cambisols, which are derived from silt textured loess parent materials (Wei et al. 2013).

Field investigation and sampling

We obtained estimates of the recovery periods of different communities from vegetation surveys from the 1950 s to the early 2000 s (Fan et al. 2006; Zou et al. 2001) and records from local farmers and government (Tateno et al. 2007; Wang et al. 2010). These results have been accepted and used widely (Deng et al. 2016; Qiu et al. 2015). Before farmland abandonment, the maize was always cultivated in the farmland of this study field. The study examined two different types of afforestation: natural vegetation restoration from abandoned farmland to natural forest dominated by Quercus liaotungensis Koidz (~80 years) (NQ) and artificial restoration from abandoned farmland to plantation dominated by Robinia pseudoacacia L. (~40 years) (AR). We compared these restoration types to a control site of...
abandoned farmland that remained unforested (AF). The AF in this study was abandoned from maize cultivation for roughly 3-5 years. The three ecosystems were at least 3 km apart. To minimize the effects of external conditions on experimental results, we selected study areas with similar topography, land-use history, and soil type. Five 20 m x 20 m plots were established along the slope within each land use system for avoiding topographic influence: the upper position located on the top of the slope, the upper-middle position located between upper and middle positions, the middle position located on the middle of slope, the bottom-middle position located between bottom and middle positions and the bottom position located in the gully and adjacent area to the slope. Plots were spaced approximately 50 m apart in each land-use system. Meanwhile, each plot was at least 40 m from land-use system boundary to minimize edge effects.

We then dug five 20 cm-deep pits for topsoil sampling at the four corners and the center in each plot, and took five soil bulk density (BD) samples. BD was measured at 0-20 cm in each subplot using a stainless steel cutting ring (5 x 5 cm). The soil cores were dried at 105°C for 24 h. Five soil samples (0-20 cm) were collected from the five pits in each plot, and the collected samples were mixed to form one homogeneous sample for stable carbon isotope and radiocarbon analysis, as well as aggregate size distribution. Three aggregate-size classes were manually fractionated through the dry sieving of fresh soil samples on a series of two sieves (0.25 and 0.053mm) as follows: macroaggregates (>0.25mm), microaggregates (0.25-0.053mm) and silt & clay (<0.053mm). Fresh soil samples were dry sieved because wet sieving compromised the in situ link between the aggregates obtained and their indigenous biota. Furthermore, wet sieving could cause the inaccurate evaluation of soil organic carbon (Jiang et al. 2014). The subsamples of each aggregate fraction were used to analyze SOC, total nitrogen (N) and total phosphorous (P). Each aggregate fraction was oven dried at 100 °C and weighed to determine its proportion of total soil weight.

Meanwhile, we investigate SOC stock in deeper layers (0-1 m) in June 2021. Close to every 20 cm-deep topsoil sampling pits, we obtained 45 soil profiles to investigate the 0-1 m soil layers: 5 pits/plot x 3 plots/land-use system x 3 land-use system. The soil samples were collected in depths of 0–10 cm, 10–20 cm, 20–30 cm, 30–50 cm and 50–100 cm using a soil sampling auger (5-cm diameter) (Shi et al. 2011; Tateno et al. 2007). The SOC, N and bulk density (BD) of 0-1 m soil profiles were measured.

Additionally, we established the paired investigation site in same land-use system with at least 3 km distance between the paired sites (NQ & paired NQ; AR & paired AR; AF & paired AF) for comparing soil characters of paired sites in June 2021. To determine SOC, N, P, rapidly available potassium (K), pH and BD, we collected soil samples in depths of 0–10 cm and 10–20 cm using a soil sampling auger (5-cm diameter).

Laboratory analysis

The SOC, N, P and K were measured using a TOC VWP (Shimadzu, Japan), 2300 kjeltec analyzer unit (FOSS TECATOR, Sweden), and ICP-AES (Spectro, Analytical Instruments, Germany). Soil pH was determined using a soil/water ratio of 1:2.5 (PHSJ-4 A pH acidimeter, Shanghai, China). Soil organic carbon stock was calculated as the product of soil bulk density and SOC concentration.

Soil samples for δ13C analyses were pretreated with excess 1 mol L−1 HCl to remove carbonates at room temperature, then rinsed and freeze dried for at least 24 h and ground into fine powder over 100 µm meshes. Litters was washed with distilled water, then freeze-dried and ground into fine powder for measurement. The natural abundance of δ13C values in the soil organic matter and litter was analyzed with an Elemental Analyser (Eurovector) coupled to an isotope Ratio Mass Spectrometer (Delta plus, Thermo Fisher Scientific, USA) at the State key laboratory of Loess and Quaternary Geology at the Institute of Earth Environment, Chinese Academy of Sciences. Variation in the 13 C/12 C was reported relative to the Vienna PDB standard, and is expressed as:

$$\delta(\%o) = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 100$$  (1)

where $R_{\text{sample}}$ is the $^{13}$C/$^{12}$C ratio of the sample and $R_{\text{standard}}$ is the $^{13}$C/$^{12}$C ratio in the PDB standard.
The soil samples were pretreated for \(^{14}\)C analyses as standardized by Zhou et al. (1992). Measurement of \(^{14}\)C in soil organic matter was carried out at the Xi’an Accelerator Mass Spectrometry Center in the Institute of Earth Environment, Chinese Academy of Science. Cryogenically purified CO\(_2\) was converted to the target using the hydrogen-iron reduction method. This method was described in detail by Zhu et al. (2010).

Data analysis

Soil OC stocks were calculated as follow:

\[
\text{Soil OC stocks (kg m}^{-2}\) = \frac{D \times BD \times OC}{100}
\]  

(2)

where D is the thinness (cm) of the soil layer, BD is the bulk density (g cm\(^{-3}\)), and OC is the soil organic carbon concentrate (g kg\(^{-1}\)) at 0-20 cm.

Stocks of soil OC in each soil aggregate size class were calculated as:

\[
\text{Stocks of OC}_i (kg m}^{-2}\) = \frac{D \times BD \times w_i \times OC_i}{10000}
\]

(3)

where \(OC_i\) is the soil organic carbon concentration of the \(i\)th aggregate size class (g kg\(^{-1}\) aggregate).

During the afforestation period, the decomposition rate constants \(k_i\) (yr\(^{-1}\)) was calculated by these models based on \(\delta^{13}\)C method: The proportions of new SOC (\(f_{\text{new}}\)) and old SOC (\(f_{\text{old}}\)) were estimated based on the mass balance equations (Del Galdo et al. 2003):

\[
f_{\text{new}} = \frac{(\delta_{\text{new}} - \delta_{\text{old}}) \times 100\%}{\delta_{\text{veg}} - \delta_{\text{old}}}
\]

(4)

\[
f_{\text{old}} = 100 - f_{\text{new}}
\]

(5)

where \(\delta_{\text{new}}\) is the \(\delta^{13}\)C value of the soil sample from current land-use type, \(\delta_{\text{old}}\) is the \(\delta^{13}\)C value of the soil sample prior to land-use change and \(\delta_{\text{veg}}\) is the \(\delta^{13}\)C value of the mixed litter of current vegetation. SOC decomposition rate constants \(k_i\) was estimated using the following equations (Marin-Spiotta et al. 2009):

\[
k_1 = \frac{-\ln \left( C_i / C_0 \right)}{t}
\]

(6)

where \(C_0\) is the initial SOC stock (SOC stock in the reference sites), and \(C_i\) is the initial SOC stock remaining (old C stock) at time \(t\) (year) since land-use change.

For ecosystem change, the decomposition rate constants \(k_2\) (yr\(^{-1}\)) was obtained through the bomb-\(^{14}\)C model (Cherkinsky and Broykin 1993; Torn et al. 2002; Trumbore 1993). The procedures were as follows (Tan et al. 2013):

\[
^{14}\text{C data (pMC) is defined as:}
\]

\[
pMC (\%) = \frac{A_{\text{SN}}}{A_{\text{ON}} \times e^{\lambda (y-1950)} \times 100}
\]

(7)

where \(A_{\text{SN}}\) is the \(^{14}\)C/\(^{12}\)C ratio of the sample corrected to a \(\delta^{13}\)C value of \(-25\%\) to account for the assumption that plants discriminate twice as much against \(^{14}\)C as they do against \(^{13}\)C, \(A_{\text{ON}}\) is the \(^{14}\)C/\(^{12}\)C ratio of the oxalic acid activity normalized to \(\delta^{13}\)C value of \(-19\%\), \(\lambda = 1/8267\) is based on the 5730 a half-life, and \(y\) is the year of oxalic measurement.

The SOC turnover times were estimated using a time dependent steady-state box model. This assumes that variation in \(^{14}\)C in a soil with time follows a first-order kinetic law, which can be described by the mass balance equation:

\[
C_y \times ^{14}\text{C}_y = C_{y-1} \times ^{14}\text{C}_{y-1} \times (1 - k_2 - \lambda) + I \times ^{14}\text{C}_{\text{atm-lag}}
\]

(8)

where \(C\) is the organic carbon inventory of a soil sample (g C m\(^{-2}\)), \(^{14}\)C is the pMC of a soil sample (%), \(k\) is the first order decomposition constant for homogeneous C pools (yr\(^{-1}\)), \(\lambda\) is the \(^{14}\)C decay constant (1/8267), \(I\) is the annual carbon input (g C m\(^{-2}\) yr\(^{-1}\)), \(^{14}\text{C}_{\text{atm-lag}}\) is the pMC of the atmosphere CO\(_2\) (%), and lag is the average number of years that atmospheric CO\(_2\) is retained in plant tissue before becoming part of the soil organic matter pool. At steady state, \(C_y = C_{y-1}\) and \(I = kC, Eq. (7) can be transformed into:

\[
^{14}\text{C}_y = ^{14}\text{C}_{\text{atm-lag}} - k_2 \times ^{14}\text{C}_{y-1} \times (1 - k_2 - \lambda)
\]

(9)

The decomposition rate constants \(k_2\) is obtained by matching the modeled and measured pMC for the year in which the soil was sampled based on \(^{14}\text{C}_{\text{atm}}\) adopted from curve of \(^{14}\)C of atmospheric CO\(_2\).

Qiu et al. (2012) proposed a method for assessing the relative contribution of changes in aggregate amount and aggregate-associated OC concentrations to the total changes in OC stocks within each aggregate fraction. It was assumed that changes in
OC stock within any particular aggregate fraction were caused both by changes in OC concentration in the fraction (F₁) and by changes in the mass of the fraction (F₂). It was also assumed that mass was gained or lost from an aggregate fraction due to ecosystem change had the same OC concentration as the rest of that fraction after ecosystem change. We therefore calculated the contribution of F₁ and F₂ to the total change in OC stock within an aggregate fraction as follows:

\[ F_1 = M \times \Delta C \]  
\[ F_2 = \Delta M \times C \]

where F₁ is the change in OC stock (g m⁻²) within an aggregate fraction due to changes in aggregate-associated OC concentrations, F₂ is the change in the OC stock (gm⁻²) within an aggregate fraction due to changes in the mass of the aggregate fraction, ΔM is the change in the mass of a particular fraction (kg m⁻²), M is the initial mass of the aggregate fraction (kg m⁻²) before ecosystem change, C is the final OC concentration of the aggregate fraction (g kg⁻¹) after ecosystem change and ΔC is the change in the OC concentration of the aggregate fraction (g kg⁻¹) due to ecosystem change.

Statistical analyses

One-way Analysis of Variation (ANOVA) with Pearson’s test was performed to examine the difference between period of artificial and natural afforestation in topsoil SOC decomposition rate constants (k₁) and new SOC input rate calculated with a $^{13}$C model, and difference of contribution of F₁ and F₂ to the total change in SOC stock within an aggregate fraction between artificial and natural afforestation. Repeated measures ANOVA (RMANOVA) was applied to test the significance of SOC stocks at different soil depth, SOC in different soil aggregate size classes, soil aggregate size class distributions, C:N and C:P of different soil aggregate size classes, SOC decomposition rate constants (k₂) calculated with a $^{14}$C model among the three land use systems using Tukey’s HSD test at p < 0.05. All differences were evaluated at the 5% significance level.

Results

The SOC stocks and contents

The Fig. 1 shows the SOC stock and contents at each depth of the three different land-use systems, and the SOC stock and content in 0-10 cm soil significantly
increased with both artificial afforestation and natural afforestation.

Nevertheless, SOC stock in 0-10 cm soil in AR was significantly lower than for NQ. The SOC stock and content in 10-20 cm soil in NQ was significantly higher than other two land-use systems, but there were no significant differences between in AR and AF. At other depths, there were no significant differences among the three land-use systems. The results indicated the accumulation of 0-1 m SOC was mainly from 0 to 20 cm topsoil changes under afforestation and was consistent with previous studies (Guillaume et al. 2015; Hiltbrunner et al. 2013; Song et al. 2016).

Aggregates OC stocks, size distribution and C:N & C:P

Under both artificial and natural afforestation, the macroaggregates-OC and microaggregates-OC stocks significantly increased, but of silt & clay-OC stocks were nearly 0—a value far lower than that of any other fraction. This suggests that silt & clay could be considered a negligible part of total OC stocks. The macroaggregates-OC stock was significantly higher than that of microaggregate in AR, but macroaggregates-OC and microaggregates-OC stocks were nearly the same in NQ. The macroaggregates-OC stock was significantly lower in NQ when compared to AR while the microaggregates-OC stock in NQ were significantly higher than in AR (Fig. 2a).

Macroaggregates and microaggregates accounted for approximately 99% of the dry soil weight in the abandoned farmland. Under both artificial and natural restoration, the amount of soil in macroaggregates significantly increased, and microaggregates significantly decreased. There are no significant differences in the proportion of different size aggregates between NQ and AR (Fig. 2b). Furthermore, for both types of restorations, the amount of silt & clay accounted for less than 1% of total soil.

Both C:N and C:P of different aggregate classes significantly increased with both types of afforestation. The C:N and C:P of microaggregates proportions increased after afforestation—moreso than macroaggregates proportions did. Additionally, C:N and C:P in NQ were significantly higher than in AR, except the C:N ratio of macroaggregates (Fig. 3).

Turnover of SOC

Over the afforestation period, the SOC decomposition rate ($k_1$), and new SOC input rate ($k_2$) under artificial restoration exceeded that of natural restoration (Fig. 4). For the three land-use systems, the SOC

![Fig. 2](image)

**Fig. 2** The different aggregate size OC stocks (a) and size class distributions (b) in the three land-use systems. Macroaggregates were >0.25 mm; microaggregates were between0.25 and 0.053 mm; and silt & clay were <0.053 mm. Error bars represent the standard error of the mean. Significant differences are indicated by different letter ($p < 0.05$); AF: abandoned farmland; AR: artificial afforestation (plantation of *Robinia pseudoacacia* L); NQ: natural afforestation (natural forest - *Quercus liaotungensis* Koidz).
decomposition rate ($k_2$) was highest ($2.6 \times 10^{-3}$) in the natural forest ecosystem (*Quercus liaotungensis* Koidz). The $k_2$ of abandoned farmland and plantation (*Robinia pseudoacacia* L.) were similar (Fig. 5).

**Contribution of mass and OC content in different soil aggregate size**

The aggregate-model ($F_1$ & $F_2$) results showed that the changes in $F_1$ & $F_2$ under artificial and natural restoration were nearly the same. $F_1$ and $F_2$ significantly increased and decreased respectively for macroaggregate-OC after afforestation, but changes in $F_1$ & $F_2$ were more significant under natural afforestation (Fig. 6a, b). In addition, $F_1$ significantly increased and $F_2$ slightly decreased for microaggregate-OC (Fig. 6c, d). The aggregate-model ($F_1$ & $F_2$) results indicated that increases in SOC stocks in macroaggregates and microaggregates under artificial and natural afforestation were mainly due to increases in SOC concentration rather than mass (Fig. 6). Under natural afforestation, the contribution of macroaggregates-OC concentration increased more significant than under artificial, but microaggregates-OC changes were similar regardless of afforestation type. We concluded that...
macroaggregates-OC stock is the main contributing factor to changes in total OC stock.

**Discussion**

Afforestation evaluation for SOC accumulation

Restoration effect is the most important for evaluating reforestation projects. It is challenging to find a primeval forest as an undisturbed site for direct comparison, because of more than 2000-years agricultural activity combined with fragile semiarid climate in this region. The oldest secondary forest is around 200 years old and is located in the study region of the Loess Plateau (Qiu et al. 2015). In this study, our results not only were compared with other reforestation projects but also with 200-years secondary forest as a “control” undisturbed sites for evaluating SOC accumulation under afforestation.

Laganière et al. (2010) summarized afforestation project data from global 120 sites and 189 observations to investigate the influence of afforestation

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**Fig. 5** SOC decomposition rate constants ($k_d$) calculated with a $^{14}$C model for different land use types. Error bars represent the standard error of the mean. Significant differences are indicated by different letters ($p < 0.05$). AF: abandoned farmland; AR: artificial afforestation (plantation of *Robinia pseudoacacia* L); NQ: natural afforestation (natural forest - *Quercus liaotungensis* Koidz)

**Fig. 6** Changes in SOC stocks in macroaggregates and microaggregates with afforestation under (a-b) artificial and (c-d) natural restoration. Macroaggregates were >0.25 mm; microaggregate were between 0.25 and 0.053 mm; and silt & clay were <0.053 mm. Error bars represent the standard error of the mean. Significant differences are indicated by the asterisk symbol (*$p < 0.05$); ns: no significant. AF: abandoned farmland; AR: artificial afforestation (plantation of *Robinia pseudoacacia* L); NQ: natural afforestation (natural forest - *Quercus liaotungensis* Koidz)
in agricultural soils on SOC stocks. The study found that afforestation resulted in a 26% increase in SOC stocks on average. In subtropical regions, natural afforestation (secondary forest) and artificial afforestation in bare land increase SOC stock in 0-60 cm soil by ~190% and ~95%, respectively. Afforestation did not allow SOC stock to recover to predisturbed or undisturbed levels (primeval forest). The level of SOC stock in natural afforestation was only ~60% of primeval forest (Wang et al. 2017). In sub-alpine regions, afforestation in pasture resulted in 13% increase of 0-80 cm total SOC stocks, and the 60% of increase was resulted from 0 to 10 cm topsoil (Hiltbrunner et al. 2013). The previous studies on deforestation also can be used to evaluate SOC stock recovery after afforestation. Guillaume et al. (2018) synthesized the impacts of converting rainforests to tree plantations on SOC stock, finding that plantations decreased SOC stocks (0-50 cm) by 10-32%; van Straaten et al. (2015) indicated replacing tropical forests with tree plantations decreased 0-300 cm SOC stocks by up to 50%; Guillaume et al. (2015) showed SOC stocks (0-60 cm) decreased by 24-42% under different plantations compared to the forest. The SOC stock reduction in plantations was significant down to topsoil (0-30 cm), but deeper soil layers displayed no such reduction.

In this study, natural afforestation and artificial afforestation in abandoned farmland increased 0-100 cm SOC stock by 43% and 14% respectively. And the 80% of increase resulted from 0 to 20 cm topsoil, which was consistent with previous studies (Guillaume et al. 2015; Hiltbrunner et al. 2013). In our study, natural afforestation experimental plots reached level comparable with the 200-year-old secondary forest SOC stock (~33 Mg ha$^{-1}$ in 0-10 cm; ~16 Mg ha$^{-1}$ in 10-20 cm) (Qiu et al. 2015). Additionally, our study indicated SOC stocks (0-50 cm) in artificial afforestation to be 30% lower than in natural afforestation. This result also agrees with previous studies (Guillaume et al. 2015; van Straaten et al. 2015). In view of global afforestation projects or deforestation activities, we conclude that afforestation effectively promotes SOC accumulation, and natural afforestation does so more efficiently than artificial afforestation.

SOC source and turnover based on aggregates and carbon isotope

Many studies found that afforestation can lead to the accumulation of SOC mainly resulting from an increase in topsoil SOC. The effects of land use change during vegetation restoration on SOC are mainly due to changes in OC input and C mineralization (Deng et al. 2016; Laganière et al. 2010). The OC in soil physical fractions responds more sensitively and rapidly to land use change than the OC in bulk soils (Qiu et al. 2012; Wei et al. 2013); Therefore, changes in aggregate-associated OC are regarded as fundamental processes for understanding the effects of vegetation restoration on SOC.

Generally, $C_3$ and $C_4$ plants could produce detritus with different $^{13}C/^{12}C$ ratios due to their difference in utilizing $^{13}C/^{12}C$ (Zhu et al. 2021). Thus, conversion of vegetation with different $^{13}C$ signals can affect the $^{13}C$ signature of SOC (Zhang et al. 2015). In this study, the maize, $C_4$ plants, was cultivated for a long term in the AF before abandonment. And both of Robinia pseudoacacia L and Quercus liaotungensis Koidz as afforestation tree species are $C_3$ plants. Therefore, the stable carbon isotopic signature with land-use change can be a powerful tool for investigating the SOC dynamics in this study. Meanwhile, the increase in proportion of new SOC could be attributed to the OC inputs from the new vegetation which produces organic matter with different $^{13}C/^{12}C$ ratio (Marin-Spiotta et al. 2009; Richter et al. 1999) reported that the rates of new SOC increase represented the net effect of new OC input including litterfall, rhizo-deposition and hydrological leaching of dissolved OC and output with organic matter mineralization to soils. These results, combined with our study, suggest that new SOC resources come from aboveground leaf litter and belowground roots due to afforestation in the abandoned farmland. Our $^{13}C$ results indicated old C decomposition and new C inputs during natural afforestation were significantly lower than artificial afforestation (Fig. 4).

In addition, Deng et al. (2016) summarized sources of SOC resulting from vegetation restoration, including that vegetation restoration facilitated SOC accumulation from biomass input (Tang et al. 2010). Vegetation biomass resulting from aboveground leaf litter and belowground roots is the main source of organic matter input into the soil (Laganière et al. 2010; Zhao...
Vegetation restoration probably contributed to the formation of stable soil aggregates (An et al. 2010), thus facilitating physical protection of SOC within aggregates (Lal 2004). This indicated that the duration of such vegetative restoration was a key parameter. As our study found, although the new SOC input rate and decomposition rate were both lower under natural afforestation than under artificial afforestation (~80 years) was twice as long as the period of artificial afforestation (~40 years), so SOC accumulation under natural afforestation was still significantly higher than artificial afforestation. Therefore, the duration of vegetation restoration should be considered as a key parameter on SOC resources and accumulation.

Aggregate hierarchy theory points out that macroaggregates contained less decomposed organic material and had faster soil C turnover compared to microaggregates (Tisdall and Oades 1982). Some studies using $^{13}$C technology confirm this theory and find the $^{13}$C in aggregates is enriched with decreasing aggregate size in forest and farmland soils. Additionally, these studies also find that SOC in macroaggregates is younger and more labile than SOC in microaggregates (Liu et al. 2018a). This is likely because lower $\delta^{13}$C values have been linked to more recent litter inputs, while higher $\delta^{13}$C values are related to older organic matter (Deng et al. 2016). Such studies concluded that fresh plant residues are the main agent for macroaggregate formation (Six and Paustian 2014).

Our aggregate-model ($F_1$ & $F_2$) results indicated that increases in SOC stocks were mainly due to increases in SOC concentration rather than mass in macroaggregates and microaggregates (Fig. 6). This may suggest that macroaggregates-OC stock is the main factor contributing to changes in total SOC stock. Previous studies obtained similar results (Liu et al. 2018b; Qiu et al. 2015), and suggested that SOC resources come mainly from macroaggregates formation provided by fresh plant residues.

The SOC dynamics are an ecosystem property (Schmidt et al. 2011). $^{14}$C dating has been proven an ideal tool to study the SOC dynamics from decadal to millennial timescales, providing a direct measure of the time elapsed since carbon in organic matter was fixed from the atmosphere (Trumbore 1993). In this study, the steady-state box model of $^{14}$C of SOC was applied to estimate the mean residence time (MRT) of SOC in ecosystem. This allowed us to quantify the proportion of SOC derived from the atmosphere in recent years to centuries based on the rate of incorporation of the nuclear explosion before the 1960 s carbon tracer (Laskar et al. 2016). Our $^{14}$C model results suggested that the carbon decomposition rate was the lowest in the plantation (Robinia pseudoacacia L.) ecosystem, which was the same as in the abandoned farmland. The carbon decomposition rate was highest in the natural forest ecosystem (Quercus liaotungensis Koidz) (Fig. 5). The model provided a direct evidence that higher SOC accumulation under natural afforestation can be attributed to a longer restoration period rather than the more rapid carbon turnover under natural afforestation compared to artificial afforestation. Our study therefore suggests that a prolonged recovery time could be more important for SOC accumulation than any particular method of afforestation.

### Comparability of the study areas

The comparability of the study areas is the central question in this study. We selected study areas in the semi-arid Loess Plateau with rainfed agricultural areas mainly distributed on slopes (An et al. 2014). Previous studies in this area also demonstrate the significant influence of topography on soil characters due to the hilly-gully topography (Shi et al. 2017; Tateno et al. 2017; Zhang et al. 2013). Such studies found that the SOC, TN and other characteristics differed according to slope. In considering this, we established five 20 m×20 m plots along the slope within each land use system for avoiding topographic influence (detailed description of method in 2.2. Field investigation and sampling).

Actually, comparability of the study areas is always approbated, which was from three dimensions. (1) Background: Generally, most of the plateau belongs to semi-arid zone. The physical environment with socio-economic development display a high degree of spatial homogeneity throughout the region. Many studies and documents reported that this semi-arid region is regarded homogeneously by reforestation projects due to the similarities in agricultural model, soil type, geological and climate condition (He et al. 2014; Yamamoto and Endo 2014; Yamanaka et al. 2014). In order to support the growing population in the Loess Plateau, natural forests have been gradually
transformed to farmland. An afforestation project was implemented after 1999 to counter this transformation. The long history of deforestation & reforestation across different periods resulted in same land-use history in the entire area. Regional backgrounds provided us with sufficient history to account for comparability across study sites.

(2) Empirical confirmation: A paired investigation on soil characterization in the area was carried out. The sites were set up in same land-use system with at least 3-km distance between the paired sites for comparing soil characters of over 3 km-distance sites in same land-use system. Our results showed soil characters between paired sites were not significantly different, indicating the homogeneity of soil characteristics in this area (Table 1). Additionally, based on high-density forest resources inventory data and field measurements, Cui et al. (2015) and Li et al. (2017) analyzed the carbon stock distribution patterns of forest ecosystems in Shaanxi Province of China -the center of which is our study location- and found the spatial heterogeneity of soil characters and forest structure is not significant under the same forest age and tree species in semiarid region. In conjunction with previous findings, our data offer an empirical confirmation for the comparability of the study areas, suggesting spatial homogeneity of soil and vegetation.

(3) Practical paradigm: The “space-for-time substitution” method has been widely used in investigating

| Conversion type                        | Land-use system                       | Distance | Reference         |
|---------------------------------------|---------------------------------------|----------|-------------------|
| Deforestation                         | Natural forest; Cropland              | >3 km    | Wei et al. (2013)  |
| Natural restoration                   | Farmland; Grass land; Natural forest  | ~5 km    | Deng et al. (2013) |
| Deforestation                         | Natural forest; Cropland              | >3 km    | Wei et al. (2014)  |
| Natural & artificial restoration      | Natural forest; Plantation            | >1.5 km  | Song et al. (2016) |
| Natural restoration                   | Farmland; Grass land; Natural forest  | >3 km    | Deng et al. (2016) |
| Natural restoration                   | Grass land; Natural forest            | >5 km    | Zhu et al. (2021)  |

a. The published studies were carried out by using “space-for-time substitution” method in the same study area with current study
b. Distance means the distance among different land-use systems

Table 1 The characters of soil at 0-10 cm and 10-20 cm depth of the three land-use systems (AF, AR and NQ) in semiarid Loess Plateau of China

| land-use system | depth | NQ                  | Paired-NQ | AR           | Paired-AR | AF         | Paired-AF |
|-----------------|-------|---------------------|-----------|--------------|-----------|------------|-----------|
| SOC(%)          | 0-10 cm | 3.32±0.42          | 3.38±0.23 | 1.71±0.19    | 1.81±0.15 | 0.99±0.09 | 1.07±0.03 |
|                 | 10-20 cm | 1.27±0.11          | 1.19±0.13 | 0.87±0.12    | 0.92±0.08 | 0.81±0.02 | 0.79±0.05 |
| N(%)            | 0-10 cm | 0.26±0.02           | 0.23±0.03 | 0.18±0.01    | 0.18±0.03 | 0.10±0.01 | 0.11±0.01 |
|                 | 10-20 cm | 0.12±0.02           | 0.11±0.01 | 0.09±0.01    | 0.11±0.02 | 0.09±0.02 | 0.08±0.01 |
| P(g kg⁻¹)       | 0-10 cm | 0.62±0.05           | 0.63±0.03 | 0.69±0.07    | 0.66±0.02 | 0.59±0.05 | 0.61±0.02 |
|                 | 10-20 cm | 0.66±0.01           | 0.62±0.04 | 0.67±0.05    | 0.65±0.03 | 0.61±0.01 | 0.62±0.02 |
| K(mg kg⁻¹)      | 0-10 cm | 142.88±7.76         | 137.53±6.33 | 190.13±10.67 | 197.19±9.86 | 76.20±7.10 | 82.37±5.99 |
|                 | 10-20 cm | 145.91±9.37         | 141.21±8.92 | 186.78±11.56 | 192.18±10.45 | 73.19±8.17 | 80.15±8.23 |
| pH              | 0-10 cm | 8.23±0.11           | 8.19±0.05 | 8.45±0.04    | 8.43±0.02 | 8.48±0.12 | 8.50±0.09 |
|                 | 10-20 cm | 8.09±0.10           | 8.12±0.02 | 8.36±0.09    | 8.30±0.06 | 8.43±0.10 | 8.39±0.11 |
| BD (g mm⁻³)     | 0-10 cm | 0.99±0.04           | 1.02±0.05 | 1.16±0.04    | 1.13±0.05 | 1.30±0.03 | 1.28±0.02 |
|                 | 10-20 cm | 1.18±0.01           | 1.16±0.03 | 1.17±0.02    | 1.15±0.02 | 1.25±0.02 | 1.27±0.04 |

Table 2 The information on study sites of published studies

| Conversion type                        | Land-use system                       | Distance | Reference |
|---------------------------------------|---------------------------------------|----------|-----------|
| Deforestation                         | Natural forest; Cropland              | >3 km    | Wei et al. (2013)  |
| Natural restoration                   | Farmland; Grass land; Natural forest  | ~5 km    | Deng et al. (2013) |
| Deforestation                         | Natural forest; Cropland              | >3 km    | Wei et al. (2014)  |
| Natural & artificial restoration      | Natural forest; Plantation            | >1.5 km  | Song et al. (2016) |
| Natural restoration                   | Farmland; Grass land; Natural forest  | >3 km    | Deng et al. (2016) |
| Natural restoration                   | Grass land; Natural forest            | >5 km    | Zhu et al. (2021)  |

a. The published studies were carried out by using “space-for-time substitution” method in the same study area with current study
b. Distance means the distance among different land-use systems
vegetation/land-use chronological variation as a classic paradigm. Published studies confirm the comparability of results based on this paradigm and indicate different vegetation/land-use types require a long distance (as at least 3 km) to minimize edge effects of ecological transitional zones (Table 2). The studies suggest that such edge effects warrant more focus, while spatial heterogeneity is perhaps less critical given the homogeneity of soil under similar types of vegetation. The practical paradigm establishment proved the comparability of study sites, so the “space-for-time substitution” method is applicable in this study.

Conclusions

Afforestation in our study area is effective for SOC accumulation, and natural afforestation is more effective than artificial afforestation largely due to changes in topsoil. Carbon isotope and soil aggregates models determined that SOC resource come from macroaggregates formation provided by fresh plant residues. The SOC concentration of soil aggregates played a dominant role in determining the dynamics of SOC accumulation during the afforestation period. $^{13}$C and $^{14}$C models proved to be effective tools, highlighting that recovery time is a key factor in determining SOC accumulation following afforestation. Especially, the study confirms that the comparability of study sites, and the “space-for-time substitution” method is applicable in this study.

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Declarations

Conflict of interest All authors declare no conflict of interest.

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