Isotope Analysis Reveals Differential Impacts of Artificial and Natural Afforestation On Soil Organic Carbon Dynamics in Abandoned Farmland

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Abstract:

Backgrounds A multitude of studies have applied different methods to study the dynamics of soil organic carbon (SOC), but the differential impact of artificial and natural afforestation on SOC dynamic are still poorly understood.

Methods and aims We investigated the SOC dynamics following artificial and natural afforestation in Loess Plateau of China, characterizing soil structure and

stoichiometry using stable isotope carbon and radiocarbon models. We aim to compare SOC dynamics, clarify SOC source under different afforestation, examine comparability of the study areas and find how soil aggregate size classes control SOC dynamics, finally to evaluate effect of reforestation project.

Results The 0-10cm and 10-20 cm SOC stocks were significant higher than other two land-use system. At other depths, there is no significant difference among the three land-use system. Total top soil SOC stocks, C:N and C:P of differently sized soil aggregates significantly increased following afforestation. ^{13}C results and Radiocarbon models indicated that the SOC decomposition rate and new SOC input rate were lower under natural afforestation than artificial afforestation.

Conclusions Afforestation can accumulate SOC in top soils mainly resulting from in topsoil changing. SOC resource is mainly from macroaggregate formation provided by fresh plant residues. SOC loss from soil respiration was derived from microaggregates during afforestation. The"space-for-time substitution" method is suitable for comparability of the study areas.

Keywords: soil carbon stock; vegetation restoration; soil aggregate; soil respiration; ^{13}C; ^{14}C

1. 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 important properties of ecosystems (Schmidt et al. 2011). A multitude of studies report 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 1950s, great attention has been paid to control the severe soil erosion in the Loess Plateau. From 1950s to mid-1960s, the principle soil conservation strategies were terracing on cultivated slopes and planting trees on uncultivated slopes. During the mid-1960s and the late 1970s, warp land dam and terracing became the most popular soil conservation strategies. From late 1970s to late 1990s, 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. As part of this project, both natural and artificial afforestation have been promoted as methods of ecological restoration (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 SOC

66 stocks decreased most rapidly during the first 4 years of cropland cultivation after
67 deforesting, but Rytter (2016) reported that SOC pools were generally unchanged
68 after five years of *Salicaceae* growth during afforestation of former agricultural land.
69 Natural change refers to the dynamic nature of ecosystem succession. Studies indicate
70 that natural regeneration is slow and patchy with low species diversity (Blackham et
71 al. 2014), and factors controlling SOC accumulation differed along vegetation
72 succession (Liu et al. 2015).

73 Previous studies have applied different methods (e.g. space-for-time substitution,
74 stable isotope analysis) to study SOC dynamics and have successfully answered
75 questions related to soil structure and organic matter dynamics (Marin-Spiotta et al.
76 2009; Qiu et al. 2015). However, these previous studies mainly focused on natural
77 ecosystem restoration, without considering the effects of artificial restoration.
78 Furthermore, although Deng et al. (2016) have investigated the SOC turnover during
79 natural succession, SOC dynamics in stable post-succession communities (climax
80 community) following afforestation remain unstudied.

81 In this study, we combined (1) a time dependent steady-state box model based on
82 radiocarbon (^{14}C) to estimate SOC decomposition rates in different land-use systems;
83 (2) a natural abundance stable carbon isotope (^{13}C) study to quantify old and new
84 carbon turnover during both natural and artificial afforestation; (3) measures of
85 change of SOC stock at different depth and soil aggregate-associated organic carbon
86 (OC) & stoichiometry to investigate contribution of SOC change and the sensitivity
87 and efficiency of soil aggregate-associated organic carbon with afforestation; and (4)

long-term field monitoring of soil respiration to assess SOC loss. We aim to compare SOC dynamics under both natural and artificial afforestation, clarify SOC source during afforestation period, examine comparability of the study areas and find how soil aggregate size classes control SOC dynamics, finally to evaluate effect of reforestation project.

2. Materials and methods

2.1. 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; 1245-1395m 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. 2012a). The soils are classified as Calcic Cambisols, which are derived from silt textured loess parent materials (Wei et al. 2013).

2.2. Field investigation and sampling

We obtained estimates of the recovery periods of different communities from vegetation surveys from the 1950s to the early 2000s (Chen 1954; 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). 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 three ecosystems were at least 3km apart. To minimize the effects of area conditions on experimental results, we selected study areas with similar topography, land-use history, and soil type. Five 20m×20m 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 50m apart in each land-use system. Meanwhile, each plot was at least 40m from land-use system boundary to minimize edge effects.

We then dug five 20cm-deep pits for topsoil sampling at the four corners and the center in each plot, and took three soil bulk density (BD) samples. BD was measured at 0-20 cm in each subplot using a stainless steel cutting ring (5×5 cm). The soil cores were dried at 105℃ for 24h. 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 analysis of $\delta^{13}C$, ^{14}C, and aggregate size distribution. Three aggregate-size classes were manually fractionated through 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 inaccurate evaluation of soil organic carbon (Jiang et al. 2014). The subsamples of each aggregate fraction were used for soil organic carbon (SOC), total nitrogen (N), and total phosphorous (P) analysis. Each aggregate fraction was oven dried at 100°C and weighed to determine its proportion of total soil weight.

Meanwhile, SOC stock by investigation in deeper layers (0-1m) in June 2021. Totally, 45 soil profile were obtained for the investigation of 0-1m soil layers: 5pits/ plot × 3 plots/ land-use system × 3 land-use system. Close to every topsoil sampling pits, the soil samples representing depths of 0–10 cm, 10–20 cm, 20–30 cm, 30–50 cm, 50–100 cm were collected using a soil sampling auger (5-cm diameter) considering distribution of root systems (Shi et al. 2011; Tateno et al. 2007). The SOC, TN and bulk density of 0-1 m soil profiles were measured.

Additionally, the paired investigation site was set up in same land-use system with at least 3km distance between the paired sites ((NQ & paired NQ; AR & paired AR; AF & paired AF) for comparing soil characters of over 3km-distance sites in same land-use system in June 2021. The soil samples representing depths of 0–10 cm and 10–20 cm were collected using a soil sampling auger (5-cm diameter) for determining soil characters including soil organic carbon (SOC), total nitrogen (N), total phosphorous (P), fast potassium (K), pH and bulk density (BD).

2.3. Laboratory analysis

Soil organic carbon (SOC), total nitrogen (N), total phosphorous (P) and fast

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

Soil samples for $\delta^{13}C$ analyses were pretreated with excess 1mol 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 100μm meshes. Litters was washed with distilled water, then freeze-dried and ground into fine powder for measurement. The natural abundance of $\delta^{13}C$ 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:

$$\delta(‰) = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000 \qquad (1)$$

Where R_{sample} is the $^{13}C/^{12}C$ ratio of the sample and $R_{standard}$ 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).

2.4. Measurement of soil respiration

Soil respiration was measured using an automated soil CO_2 flux system (LI-8100, LI-COR, USA) equipped with a portable chamber (Model 8100-103). A PVC collar (20.3 cm in diameter and 10 cm in height) was inserted into the forest floor to a depth of 2.5 cm at each sampling point, approximately two months before the first measurements. Small litter and branches were left in the collar while large items were removed. All collars were left at the respective site for the entirety of the study period.

Soil respiration was measured over the 4-year period from 14 June 2010 to 20June 2014, approximately once every 30 days during April-October (growing season), and once every 45 days in other months (dormant season). The measurements were conducted between 8:30 and 11:30 local time on each sampling day. In each plot, five 5 m×5 m subplots were established at the four corners and the center. The PVC collars were installed in the center of each subplot for measurements of soil CO_2 efflux.

2.5. Data analysis

Soil OC stocks were calculated as follow:

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

Where D is the thinness (cm) of the soil layer, BD is the bulk density (g cm^{-3}), and

198 OC is the soil organic carbon concentrate (g kg^{-1}) at 0-20 cm.

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

200 $$\text{Stocks of } OC_i(kg\ m^{-2}) = \frac{D \times BD \times w_i \times OC_i}{10000} \quad (3)$$

201 Where OC_i is the soil organic carbon concentration of the *i*th aggregate size class (g

202 kg^{-1} aggregate).

203 During the afforestation period, the decomposition rate constants k_1(yr^{-1}) was

204 calculated by these models based on $\delta^{13}C$ method: The proportions of new SOC (f_{new})

205 and old SOC (f_{old}) were estimated based on the mass balance equations (Del Galdo et

206 al. 2003):

207 $$f_{new} = \frac{(\delta_{new} - \delta_{old}) \times 100\%}{\delta_{veg} - \delta_{old}} \quad (4)$$

208 $$f_{old} = 100 - f_{new} \quad (5)$$

209 where δ_{new} is the $\delta^{13}C$ value of the soil sample from current ecosystem, δ_{old} is the$\delta^{13}C$

210 value of the soil sample prior to ecosystem succession and δ_{veg} is the $\delta^{13}C$ value of the

211 mixed litter of current vegetation. Decomposition rate constants (k_1) of soil OC were

212 estimated using the following equations(Marin-Spiotta et al. 2009):

213 $$k_1 = \frac{-ln\ (C_t / C_0)}{t} \quad (6)$$

214 Where C_0 is the initial SOC stock (SOC stock in the reference sites), C_t is initial SOC

215 stock remaining (old C stock) at time t (year) since ecosystem change.

216 For the long-term ecosystem, the decomposition rate constants k_2 (yr^{-1}) was

217 obtained through the bomb-^{14}C model (Cherkinsky and Brovkin 1993; Torn et al.

218 2002; Trumbore 1993). The procedures were as follows (Tan et al. 2013):

^{14}C data (pMC) is defined as

$$\mathrm{pMC}(\%)=\frac{\mathrm{A_{SN}}}{\mathrm{A_{ON}}\times e^{\lambda(y-1950)}}\times 100 \qquad (7)$$

where A_{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_{ON} is the $^{14}C/^{12}C$ ratio of the oxalic acid activity normalized to $\delta^{13}C$ value of −19‰, λ = 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 the can be described by mass balance equation:

$$C_y\times {}^{14}C_y = C_{y\text{-}1}\times {}^{14}C_{y\text{-}1}\times(1-k_2\text{-}\lambda) + I\times {}^{14}C_{atm_{y\text{-}lag}} \qquad (8)$$

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(a^{-1}), λ is the ^{14}C decay constant (1/8267), I is the annual carbon input (g C m^{-2} a^{-1}), $^{14}C_{atm}$is the pMC of the atmosphere CO_2 (%), 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:

$$^{14}C_y = {}^{14}C_{atm_{y\text{-}lag}}\times k_2 + {}^{14}C_{y\text{-}1}\times(1-k_2\text{-}\lambda) \qquad (9)$$

The decomposition rate constant k_2 is obtained by matching the modeled and measured pMC for the year in which the soil was sampled based on $^{14}C_{atm}$ adopt from curve of ^{14}C of atmospheric CO_2.

A method was proposed by Qiu et al. (2012) 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 (F_1) and by changes in the mass of the fraction (F_2). It was also assumed that the mass that 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 F_1 and F_2 to the total change in OC stock within an aggregate fraction as follows:

$$F_1 = \mathrm{M}\times\Delta\mathrm{C} \qquad (11)$$

$$F_2 = \Delta\mathrm{M}\times\mathrm{C} \qquad (12)$$

where F_1 is the change in OC stock (g m^{-2}) within an aggregate fraction due to changes in aggregate-associated OC concentrations, F_2 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.

2.6.Calculation of Annual soil CO_2 emissions

Annual soil CO_2 emissions were estimated by interpolating the average CO_2 flux

rate between sampling dates, and computing the sum of the products of the average flux rate and the time between respective sampling dates for each measurement period(Shi et al. 2014; Shi et al. 2012b; Sims and Bradford 2001)as follows:

$$R = \sum F_{m,n} \Delta t_n \qquad (13)$$

Where $\Delta t_n = t_{n+1} - t_n$, which is the number of days between each field measurement within the year; R is total soil CO_2 emitted in the measurement period, and $F_{m,n}$ is the average CO_2 flux rate over the interval $t_{n+1} - t_n$ recorded by the LI-8100 Soil CO_2 Flux System.

2.7 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 (k_1) and new SOC input rate calculated with a ^{13}C model, and difference of contribution of F_1 and F_2 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, mean annual soil CO_2 emission, SOC decomposition rate constants (k_2) calculated with a ^{14}C model among the three land use systems using Tukeys's HSD test at $p < 0.05$. All differences were evaluated at the 5% significance level.

285 ## 3. Results

286 ### *3.1. The SOC stocks and contents*

287 The Fig 1 showed the soil organic carbon (SOC) stock (a) and soil organic
288 carbon contents at each depth of the three different land-use systems, AF: abandoned
289 farmland; AR: artificial afforestation (plantation of *Robinia pseudoacacia* L); NQ:
290 natural afforestation (natural forest - *Quercus liaotungensis* Koidz). The SOC stock
291 and content in 0-10 cm soil significantly increased with both artificial afforestation
292 dominated by plantation of *Robinia pseudoacacia* L (AR) and natural afforestation
293 dominated by natural forest *Quercus liaotungensis* Koidz (NQ).

294 Nevertheless, SOC stocks in 0-10 cm soil for artificial afforestation was
295 significant lower than for natural afforestation. And the SOC stock and content in
296 10-20cm soil in NQ is significantly higher than other two land-use systems, but there
297 are no significant differences between in AR and AF. At other depths, there are no
298 significant differences among the three land-use system. The results indicated
299 accumulation of 0-1m SOC was mainly from 0-20cm topsoil changes under
300 afforestation and was consistent with previous studies (Guillaume et al. 2015;
301 Hiltbrunner et al. 2013; Song et al. 2016) .

302 ### *3.2. Aggregates organic carbon (OC) stocks, size distribution and C:N & C:P*

303 Fig 2a shows the difference of different aggregate size organic carbon (OC)
304 stocks in the three land-use systems. Under both artificial and natural afforestation,
305 the OC stocks of macroaggregates and microaggregates significantly increased, but
306 the OC stocks of silt & clay were nearly 0 (far lower than for any other fraction), and

could thus be considered a negligible part of total OC stocks. The OC stock of macroaggregates was significant higher than that of microaggregate in AR, but the OC stock of macroaggregate and microaggregate were nearly the same in NQ. Meanwhile, The OC stock of macroaggregate and microaggregate in NQ were respectively significant lower and higher than in AR (Fig. 2a).

Macroaggregates and microaggregates accounted for approximate 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 microaggreagtes significantly decreased. There are no significant differences on the proportion of different size aggregate between in NQ and AR (Fig 2b). Furthermore, for both types of restorations, the amount of soils in silt & clay was less than 1% and did not significantly change.

Both C:N and C:P of different aggregate classes significantly increased with both types of afforestation. Compared with other size aggregates, C:N and C:P of microaggregates become higher after afforestation. Additionally, C:N and C:P in NQ were significantly higher than AR, except for C:N ratio of macroaggregates in NQ comparing with AR (Fig 3).

3.3. Turnover of SOC and soil CO_2 emissions

Over the afforestation period, the SOC decomposition rate (k_1), and new SOC input rate (k_2) under artificial restoration were higher than natural restoration (Fig 4). For the three land-use systems, the SOC decomposition rate (k_2) was highest (2.6×10^{-3}) in the natural forest ecosystem (*Quercus liaotungensis* Koidz). The k_2 of

abandoned farmland and plantation (*Robinia pseudoacacia* L.) were similar (Fig 5).

The amount of soil CO_2 emissions across ecosystems was calculated from continuous measurements over 4 years. Both natural and artificial restoration significantly increased the amount of soil CO_2 emissions, and the increased of amount of soil CO_2 emissions was higher under natural restoration (NQ) than artificial restoration (AR) (Fig 6).

3.4. Contribution of mass and OC content in different soil aggregate size

The aggregate-model (F_1 & F_2) results show that the changes in F_1 & F_2 under artificial and natural restoration were nearly the same, and F_1 and F_2 significantly increased and decreased respectively, for macroaggregate-OC after afforestation, but changes in F_1 & F_2 for macroaggregate-OC were more significant under natural afforestation (Fig 7 a, b). In addition, F_1 significantly increased and F_2 slightly decreased for microaggregate-OC (Fig 7 c, 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 7). The contribution of macroaggregate-OC concentration increases under natural afforestation was more significant than under artificial, but microaggregate-OC changes under natural and artificial afforestation were similar. Therefore, we concluded that macroaggregate-OC stock is the main contributing factor to changes in total OC stock.

4. Discussion

4.1 Afforestation evaluation for SOC accumulation

Restoration effect is the most important for evaluating reforestation project. Actually this is very difficult to find primeval forest as undisturbed site for direct comparison, because in this region there is no primary forest due to more than 2000-years agricultural activity and semiarid climate. Until now the oldest secondary forest is about 200 years which is located in semi-humid region of Loess Plateau (Qiu et al. 2015). In this study, our results not only compared with other reforestation projects but also compared with 200-years secondary forest as "control" undisturbed sites for evaluating SOC accumulation under afforestation.

Laganiére et al. (2010) summarize afforestation project data for investigating the influence of afforestation in agricultural soils on SOC stocks by 120 sites and 189 observations globally. The study found that afforestation resulted in an increase in SOC stocks of 26% on average. In subtropical region, natural afforestation (secondary forest) and artificial afforestation in bare land increase ~190% and ~95% of 0-60 cm SOC stock respectively, but the afforestation still did not lead SOC stock to recover to level of undisturbed sites (primeval forest). The level of SOC stock in natural afforestation was only reach to ~60% of primeval forest (Wang et al. 2017). In sub-alpine region, afforestation in pasture resulted in 13 % increase of 0-80cm total SOC stocks, but the 60 % of increase was resulted from 0-10 cm topsoil (Hiltbrunner et al. 2013). Oppositely, the previous studies on deforestation as reference also can used to evaluate recovery of SOC stock after afforestation. Guillaume et al. (2018)

globally synthesize the impacts on SOC stock of rainforest conversion to tree plantations on carbon stocks and dynamics and found plantations lead to SOC stocks (0-50 cm) decreased by 10%-32%; van Straaten et al. (2015) indicated tropical deforestation for tree plantations decreased 0-300cm 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 not when deeper layers were included.

In this study, natural afforestation (NQ) and artificial afforestation (AR) in abandoned farmland land increase 43% and 14% of total SOC stock (0-100 cm) respectively, and the 80 % of increase was resulted from 0-20 cm topsoil which was consistent with previous studies (Guillaume et al. 2015; Hiltbrunner et al. 2013). Comparing with 200-years secondary forest SOC stock (~33 Mg ha^{-1} in 0-10 cm; ~16 Mg ha^{-1} in 10-20 cm) in semi-humid region of loess plateau (Qiu et al. 2015), natural afforestation in this study is reach up to the similar level. Additionally, our study also indicated SOC stocks (0-50 cm) in artificial afforestation (AR) was 30% lower than natural afforestation (NQ). 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 could conclude that afforestation in study area is effective for SOC accumulation, and natural afforestation is more significant than artificial.

4.2 SOC source analysis based on aggregates and carbon isotope in topsoil

A multitude of studies found that afforestation can lead to the accumulation of

SOC mainly resulting from increase of SOC in topsoil, and 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; Laganiére 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.

Stable isotope techniques provide an ideal tool for investigating the sources and dynamic of SOC based on the theory: the increase in proportion of new OC in soils could be attributed to the OC inputs from the new vegetation producing organic matter with different $^{13}C/^{12}C$ ratio (Marin-Spiotta et al. 2009). And Richter et al. (1999) reported that the rates of new soil OC 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. Deng et al. (2016) summarize that sources of SOC resulting from vegetation restoration: (1) 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 (Laganiére et al. 2010; Zhao et al. 2015); (2) 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).

In our study, although it could be determined that new SOC resource is from vegetation biomass resulting from aboveground leaf litter and belowground roots due to afforestation in no-plant abandoned farmland, but our ^{13}C result indicated old C decomposition and new C inputs during natural afforestation were significantly lower than during artificial afforestation (Fig 4); Meanwhile, the period of natural afforestation (~80 years) was twice as long as the period of artificial afforestation (~40 years). Though the new SOC input rate under natural afforestation was lower compared to artificial afforestation, the decomposition rate under natural afforestation was also lower, which may indicate higher SOC accumulation under natural afforestation is not attributable only to higher net increases in the difference between new SOC input and old SOC decomposition.

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

In our study, the 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 7), and could conclude that macroaggregate-OC stock is the main contributing factor to changes in total SOC stock. Previous studies obtained a similar results (Liu et al. 2018b; Qiu et al. 2015). Combining with our and previous results, the studies confirms that SOC resource is mainly from macroaggregate formation provided by fresh plant residues. These results are also in accordance with previous observations

4.3 SOC turnover and soil CO_2 emission

time since land use change has not been considered as a key parameter for the accumulation of SOC. In this study, the period of natural afforestation was twice as long as the period of artificial afforestation. Though the new SOC input rate under natural afforestation was lower compared to artificial afforestation, the decomposition rate under natural afforestation was also lower, which may indicate higher SOC accumulation under natural afforestation is not attributable only to higher net increases in the difference between new SOC input and old SOC decomposition. We could conclude that time since land use change is a more important factor determining the proportions of new and old OC in soil. Previous studies lend support to this hypothesis.

Not only climax forest but also other types of ecosystems in semiarid or arid region often maintain in stable stage for a long time (Fan et al. 2006) and the dynamics of SOC in the ecosystem could be considered as an ecosystem property (Schmidt et al. 2011). ^{14}C dating has been proven to be an ideal tool to study the

460 dynamics of SOC from decadal to millennial timescales, which provides a direct
461 measure of the time elapsed since carbon in organic matter was fixed from the
462 atmosphere (Trumbore 1993). In this study, the steady-state box model of ^{14}C of SOC
463 was applied to estimate the mean residence time (MRT) of SOC for the long-term
464 ecosystem, providing a sensitive indicator to quantify the proportion of SOC derived
465 from the atmosphere in recent years to centuries based on the rate of incorporation of
466 the nuclear explosion before 1960s carbon tracer (Laskar et al. 2016; Torn et al. 2009).
467 In our study, the result of the ^{14}C model suggested that the carbon decomposition rate
468 was the lowest in the plantation (*Robinia pseudoacacia* L.) ecosystem, and was the
469 same as abandoned farmland. The carbon decomposition rate was highest in the
470 natural forest ecosystem (*Quercus liaotungensis* Koidz) (Fig 5). The model provided a
471 direct evidence that higher accumulation of SOC under natural afforestation can be
472 attributed to its longer restoration period rather than more rapid carbon turnover under
473 natural afforestation compared to artificial afforestation. Therefore, our study suggests
474 that longer recovery time could be more important for SOC accumulation following
475 afforestation than any particular method of afforestation.

476 Additionally, soil respiration was always regarded as a kind of main pathway of
477 soil OC loss (Shi et al. 2011). In this study, the result indicated that long-term soil
478 CO_2 emission during afforestation promoted SOC loss via soil respiration, especially
479 for natural afforestation (Fig 6). Meanwhile, the analysis of soil stoichiometry in the
480 study suggested that C/N and C/P ratios in microaggregates were consistently higher
481 than in other fractions under afforestation (Fig 3). And Spohn and Chodak (2015)

found that microorganisms increase their respiration rate with an increase in soil C/P ratio and C concentration. Therefore, combining our results with previous studies, it was concluded that SOC loss from soil respiration was mainly originated from microaggregate resulting from higher C/P ratio after afforestation, supporting the previous inference in terms of relationship between ecological stoichiometry and soil respiration (Spohn and Chodak 2015).

4.4 Comparability of the study areas

The comparability of the study areas is the central question in this study. Previous studies in this area also demonstrate topography greatly influence soil characters due to the hilly-gully topography (Shi et al. 2017; Tateno et al. 2017; Zhang et al. 2013). The studies found that the SOC, TN and other characters were significantly different along the hill slope. Therefore, in this study five 20 m×20 m plots were established along the slope within each land use system for avoiding topographic influence (detailed description of method in *2.2. Field investigation and sampling*).

Additionally, the study area located in semiarid Loess Plateau, and rainfed agricultural areas are mainly distributed in the study areas and agricultural lands in this area are mostly on slopes (An et al. 2014). A lot of studies and documents reported that the semiarid region is always regarded as an entirety by reforestation project due to the same agriculture model, soil type, geological and climate condition of this region (He et al. 2014; Yamamoto and Endo 2014; Yamanaka et al. 2014) , and which is resulted in same land-use history. Base on high-density forest resources

504 inventory data and field measurements, Cui et al. (2015) and Li et al. (2017) analyses
505 the carbon stock distribution patterns of forest ecosystems in Shaanxi Province of
506 China （this study area located in the center of the province） and found spatial
507 heterogeneity of soil characters and forest structure is not significant under the same
508 forest age and tree species in semiarid region.

509 For a direct evidence, an invagination on characterization of the soils in the areas
510 was carried out. The paired investigation site was set up in same land-use system with
511 at least 3-km distance between the paired sites for comparing soil characters of over
512 3km-distance sites in same land-use system. The characterization of the soils was
513 presented in table 1 including SOC, TN, TP, fast K. pH and BD. The results showed
514 soil characters between paired sites such as NQ & paired NQ, AR & paired AR, and
515 AF & paired AF were not significant different, and then indicated homogeneity of soil
516 characters in this area. The found was agreed with previous observations

517 In this study area, some studies using “space-for-time substitution” method has
518 been published. The table 2 summarize the related information. All the previous
519 studies more focused on edge effects of ecological transitional zone and topographic
520 influence without spatial heterogeneity due to the high homogeneity of soil under
521 same vegetation in this area. Therefore, in published studies the distances among
522 different vegetation/ land-use types was required a reasonable long distance such as at
523 least 3km for minimizing edge effects of ecological transitional zone. Therefore, we
524 could conclude that the method especially for ~3km distance among different
525 land-use systems is suitable for comparability of the study areas.

5. Conclusions

In conclusion, afforestation in study area is effective for SOC accumulation, and natural afforestation is more significant than artificial, which is mainly resulting from in topsoil changing. Carbon isotope and soil aggregates models analyzed that SOC resource is mainly from macroaggregate formation provided by fresh plant residues. The SOC concentration of soil aggregates played a dominant role in determining the dynamics of SOC accumulation during afforestation period. Based on the analysis of soil aggregates, soil respiration, and aggregate stoichiometry, we concluded that SOC loss from soil respiration mainly originated from microaggregates during afforestation. ^{13}C and ^{14}C models proved effective tools that showed that recovery time is a key factor determining the accumulation of SOC following afforestation. Especially, the study confirm that“space-for-time substitution” method is suitable for comparability of the study areas.

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767

768 Table 1 The characters of soil at 0-10cm and 20-20cm depth of the three land-use systems (AF, AR and NQ) in semiarid Loess Plateau of
769 China[a]
770

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

771 a. AF: abandoned farmland; AR: black locust plantation; QF: natural oak forest.

772 b.The distance between paired sites (NQ & paired NQ; AR & paired AR; AF & paired AF) in same land-use system is over 3km for comparing both of soil characters
773 in the semiarid region.

774 Table 2 The information on study sites of published studies [a]

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

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

778 Figure captions

779 Fig 1 Soil organic carbon (SOC) stock (a) and soil organic carbon contents (b) at each
780 depth (0-10cm, 10-20cm, 20-30cm, 30-50cm and 50-100cm) of the three different
781 land-use systems. Error bars represent the standard error of the mean. Significant
782 differences are indicated by the different letter ($p < 0.05$); AF: abandoned farmland;
783 AR: artificial afforestation (plantation of *Robinia pseudoacacia* L); NQ: natural
784 afforestation (natural forest - *Quercus liaotungensis* Koidz).

785

786 Fig 2 The different aggregate size OC stocks (a) and size class distributions (b) in the
787 three land-use systems. Macroaggregates were >0.25 mm; microaggregatew were
788 between0.25 and 0.053 mm; and silt & clay were <0.053 mm. Error bars represent the
789 standard error of the mean. Significant differences are indicated by different letter (p
790 < 0.05); AF: abandoned farmland; AR: artificial afforestation (plantation of *Robinia*
791 *pseudoacacia* L); NQ: natural afforestation (natural forest - *Quercus liaotungensis*
792 Koidz).

793

794 Fig 3 The C:N and C:P of different soil aggregate size classes in the three land-use
795 systems. Macroaggregates were >0.25 mm; microaggregatew were between 0.25 and
796 0.053 mm; and silt & clay were <0.053 mm. Error bars represent the standard error of
797 the mean. Significant differences are indicated by the different letter ($p < 0.05$); AF:
798 abandoned farmland; AR: artificial afforestation (plantation of *Robinia pseudoacacia*
799 L); NQ: natural afforestation (natural forest - *Quercus liaotungensis* Koidz).

800 Fig 4 SOC decomposition rate constants (k_1) and new SOC input rate (kg m^{-2} yr^{-1})
801 calculated with a ^{13}C model under (a) artificial and (b) natural afforestation. Error bars
802 represent the standard error of the mean. Significant differences are indicated by the
803 asterisk symbol (*$p < 0.05$).
804
805 Fig 5 SOC decomposition rate constants (k_2) calculated with a ^{14}C model for different
806 land use types. Error bars represent the standard error of the mean. Significant
807 differences are indicated by different letters ($p < 0.05$). AF: abandoned farmland; AR:
808 artificial afforestation (plantation of *Robinia pseudoacacia* L); NQ: natural
809 afforestation (natural forest - *Quercus liaotungensis* Koidz).
810
811 Fig 6 The mean annual soil CO_2 emission over 4 years in the three land-use systems.
812 Error bars represent the standard error of the mean. Significant differences are
813 indicated by the different letter ($p < 0.05$); AR: artificial afforestation (plantation of
814 *Robinia pseudoacacia* L); NQ: natural afforestation (natural forest - *Quercus*
815 *liaotungensis* Koidz).
816
817 Fig 7 Changes in SOC stocks in macroaggregates and microaggregates with
818 afforestation under (a-b) artificial and (c-d) natural restoration. Macroaggregates
819 were >0.25 mm; microaggregatew were between0.25 and 0.053 mm; and silt & clay
820 were <0.053 mm. Error bars represent the standard error of the mean. Significant
821 differences are indicated by the asterisk symbol (*$p < 0.05$); ns: no significant. AF:

822 abandoned farmland; AR: artificial afforestation (plantation of *Robinia pseudoacacia*

823 L); NQ: natural afforestation (natural forest - *Quercus liaotungensis* Koidz).

824

Conflict of Interest

- All authors declare no conflict of interest.

Figures

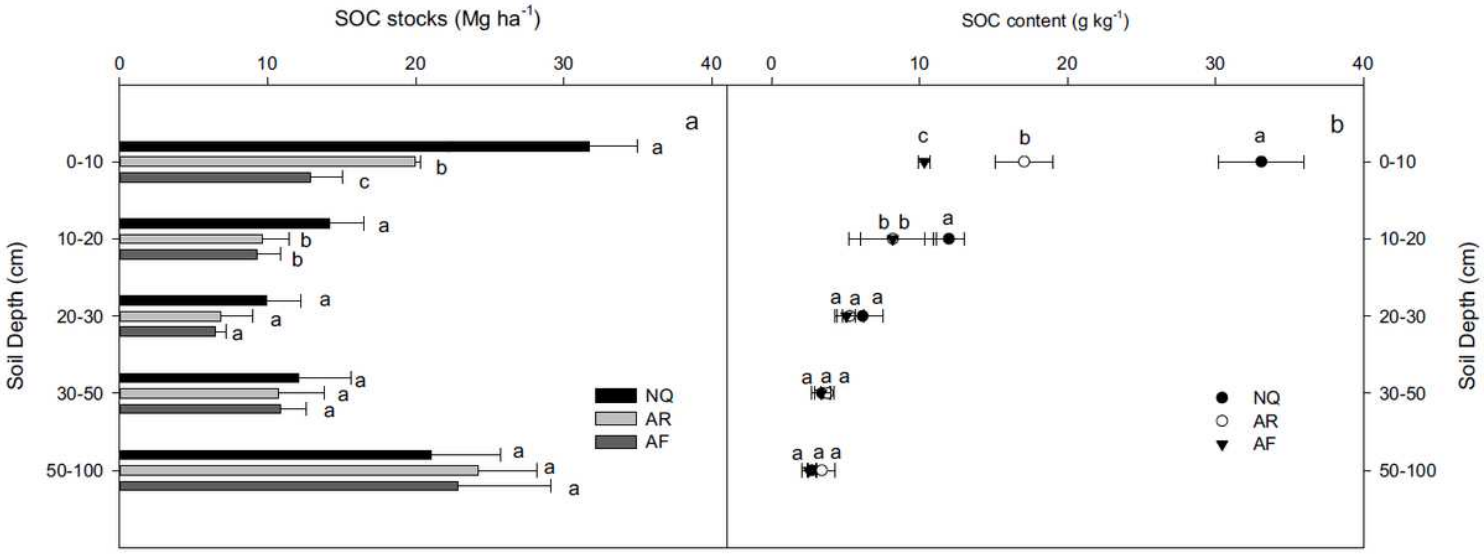

Figure 1

Soil organic carbon (SOC) stock (a) and soil organic carbon contents (b) at each depth (0-10cm, 10-20cm, 20-30cm, 30-50cm and 50-100cm) of the three different land-use systems. Error bars represent the standard error of the mean. Significant differences are indicated by the different letter ($p < 0.05$); AF: abandoned farmland; AR: artificial afforestation (plantation of Robinia pseudoacacia L); NQ: natural afforestation (natural forest - Quercus liaotungensis Koidz).

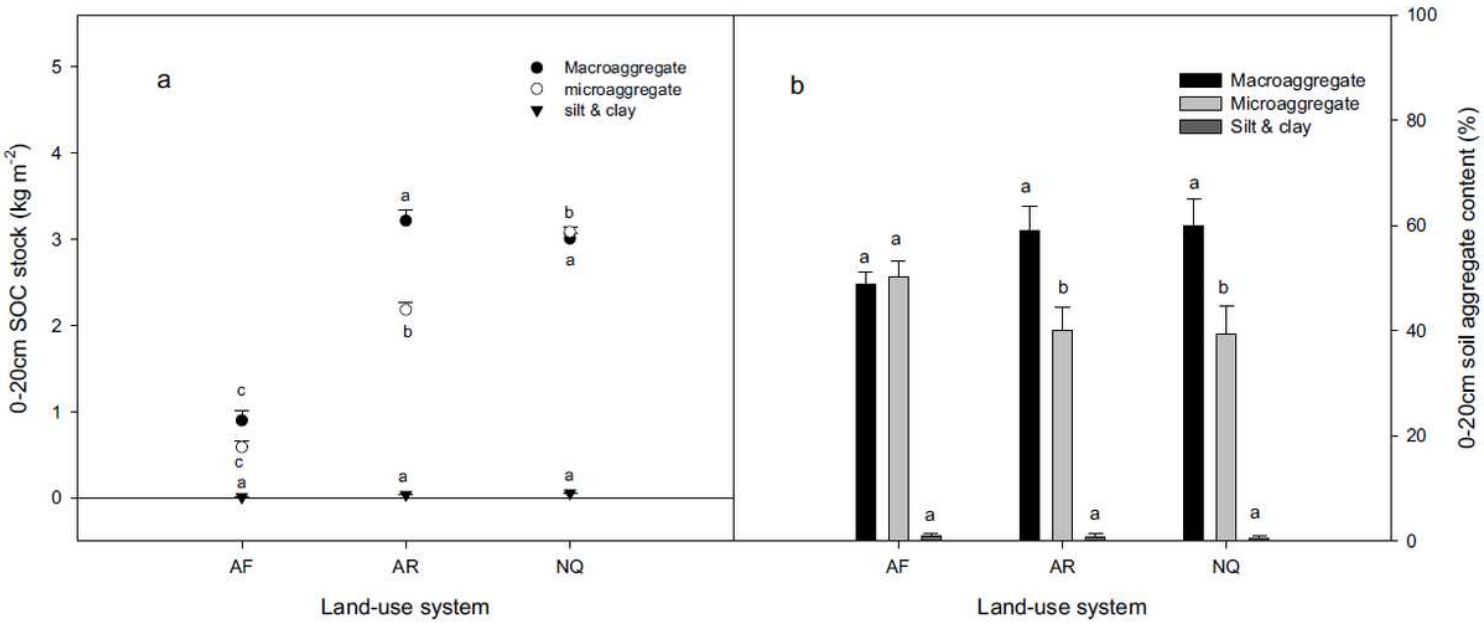

Figure 2

The different aggregate size OC stocks (a) and size class distributions (b) in the three land-use systems. Macroaggregates were >0.25 mm; microaggregatew 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).

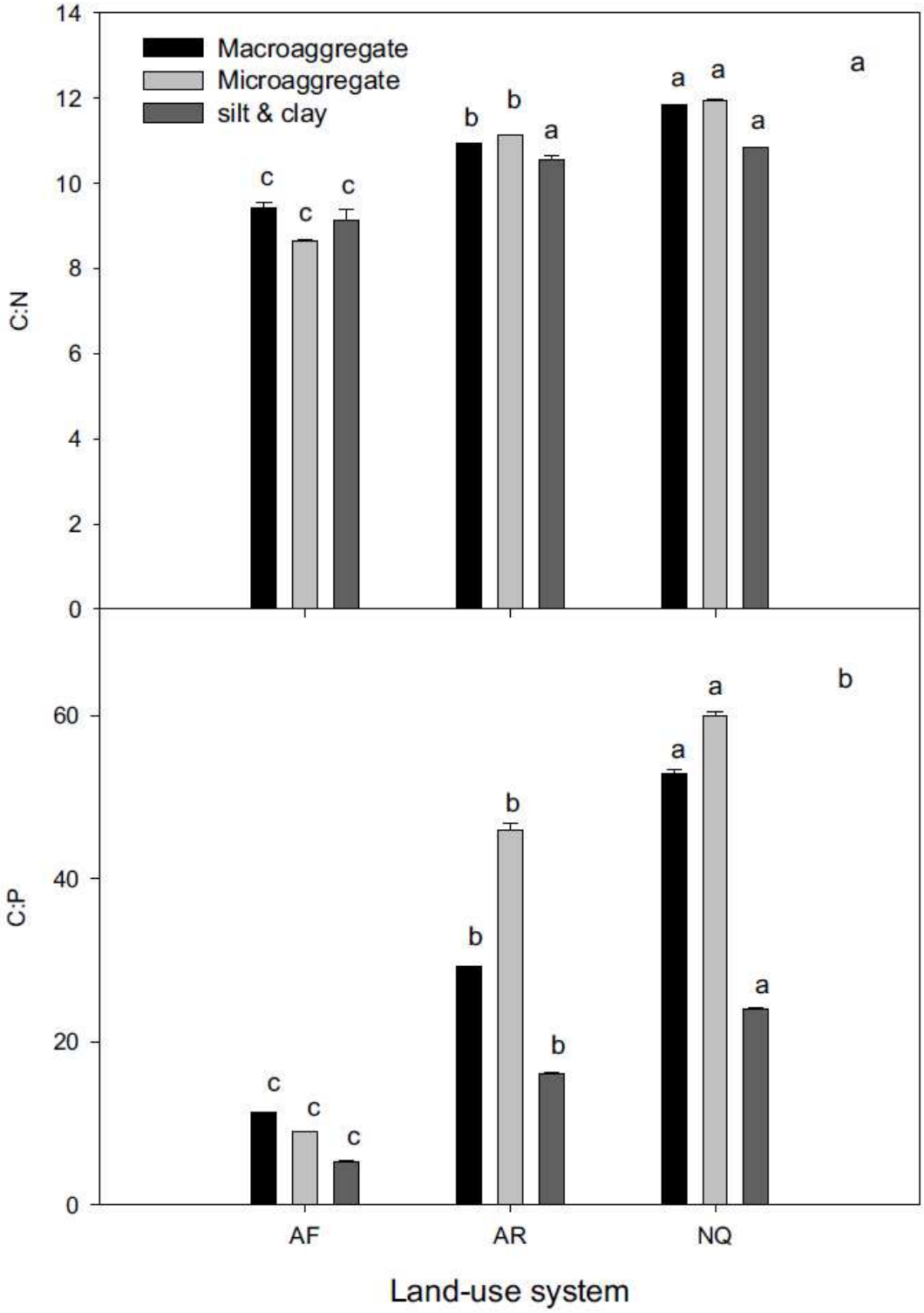

Figure 3

The C:N and C:P of different soil aggregate size classes in the three land-use systems. Macroaggregates were >0.25 mm; microaggregatew 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 different letter ($p < 0.05$); AF: abandoned farmland; AR: artificial afforestation (plantation of Robinia pseudoacacia L); NQ: natural afforestation (natural forest - Quercus liaotungensis Koidz).

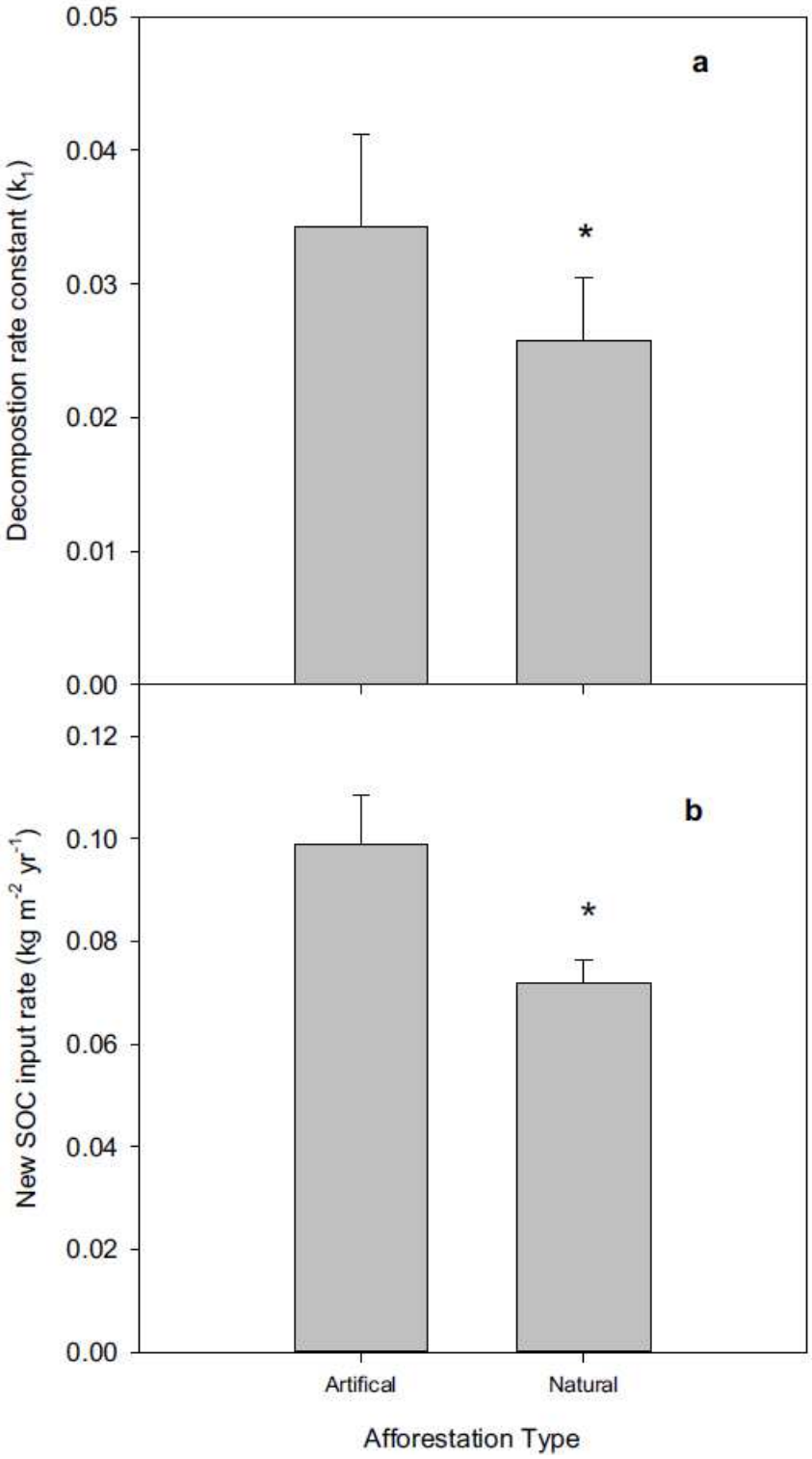

Figure 4

SOC decomposition rate constants (k1) and new SOC input rate (kg m-2 yr-1) calculated with a 13C model under (a) artificial and (b) natural afforestation. Error bars represent the standard error of the mean. Significant differences are indicated by the asterisk symbol (*p < 0.05).

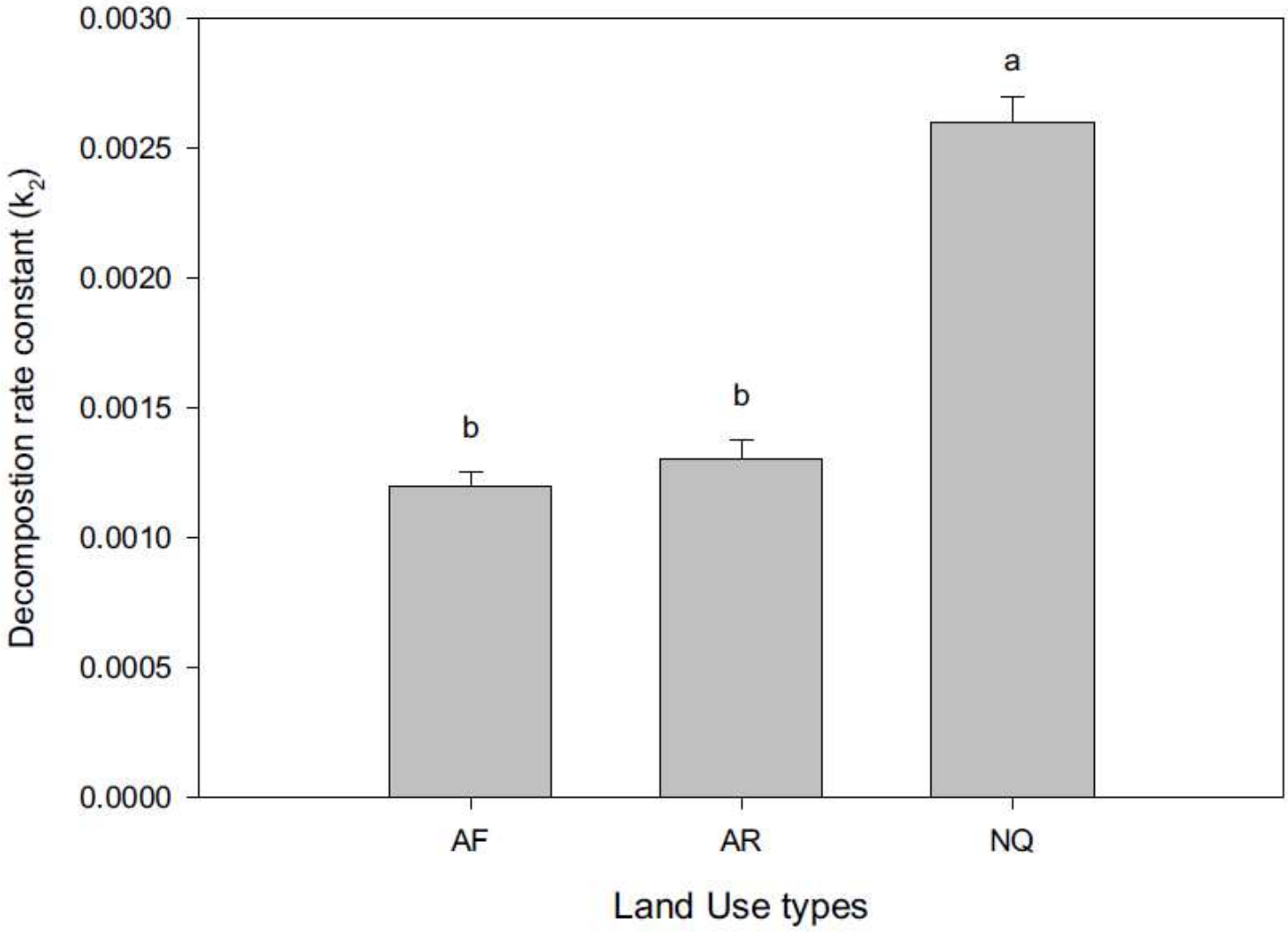

Figure 5

SOC decomposition rate constants (k2) calculated with a 14C 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).

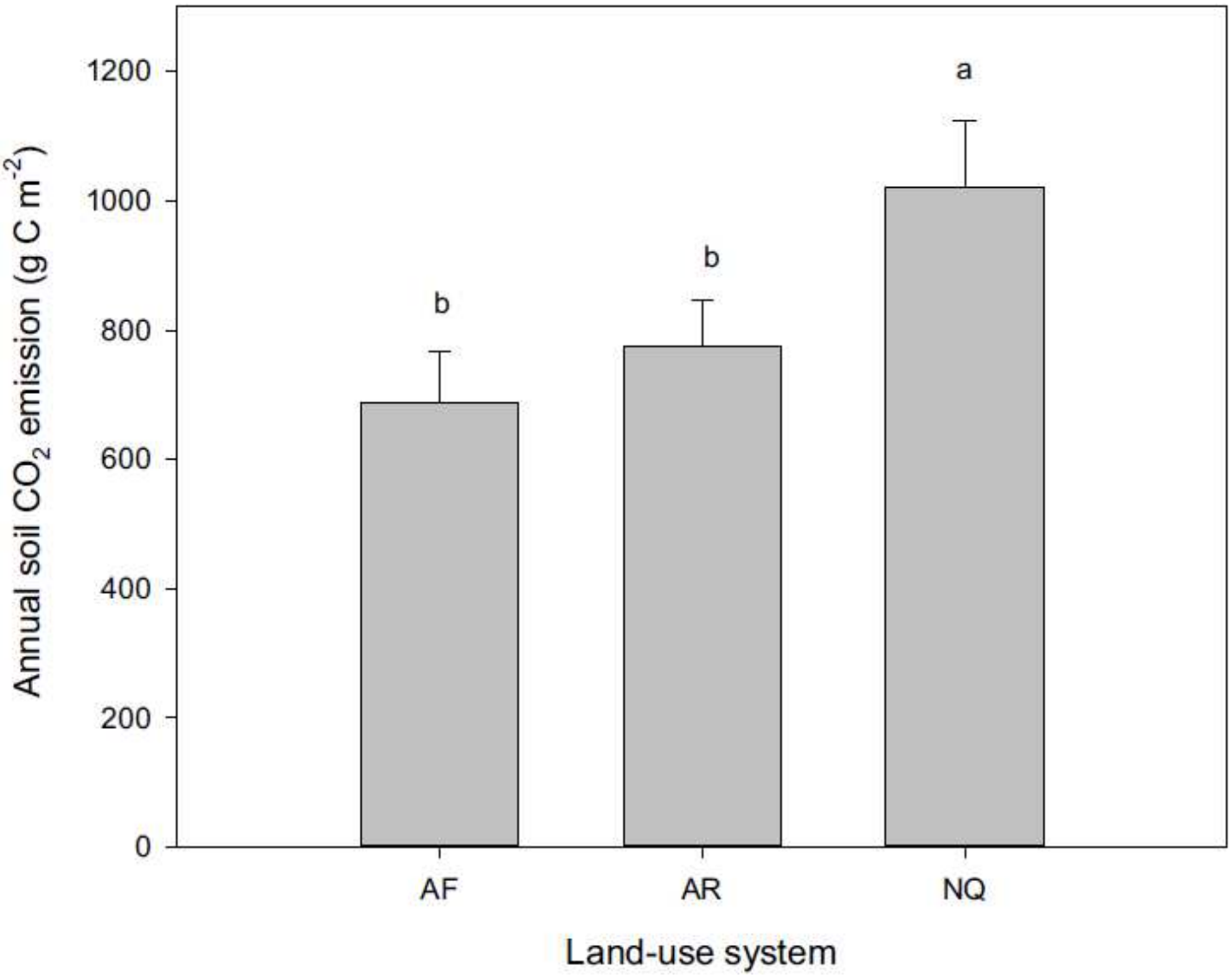

Figure 6

The mean annual soil CO2 emission over 4 years in the three land-use systems. Error bars represent the standard error of the mean. Significant differences are indicated by the different letter ($p < 0.05$); AR: artificial afforestation (plantation of Robinia pseudoacacia L); NQ: natural afforestation (natural forest - Quercus liaotungensis Koidz).

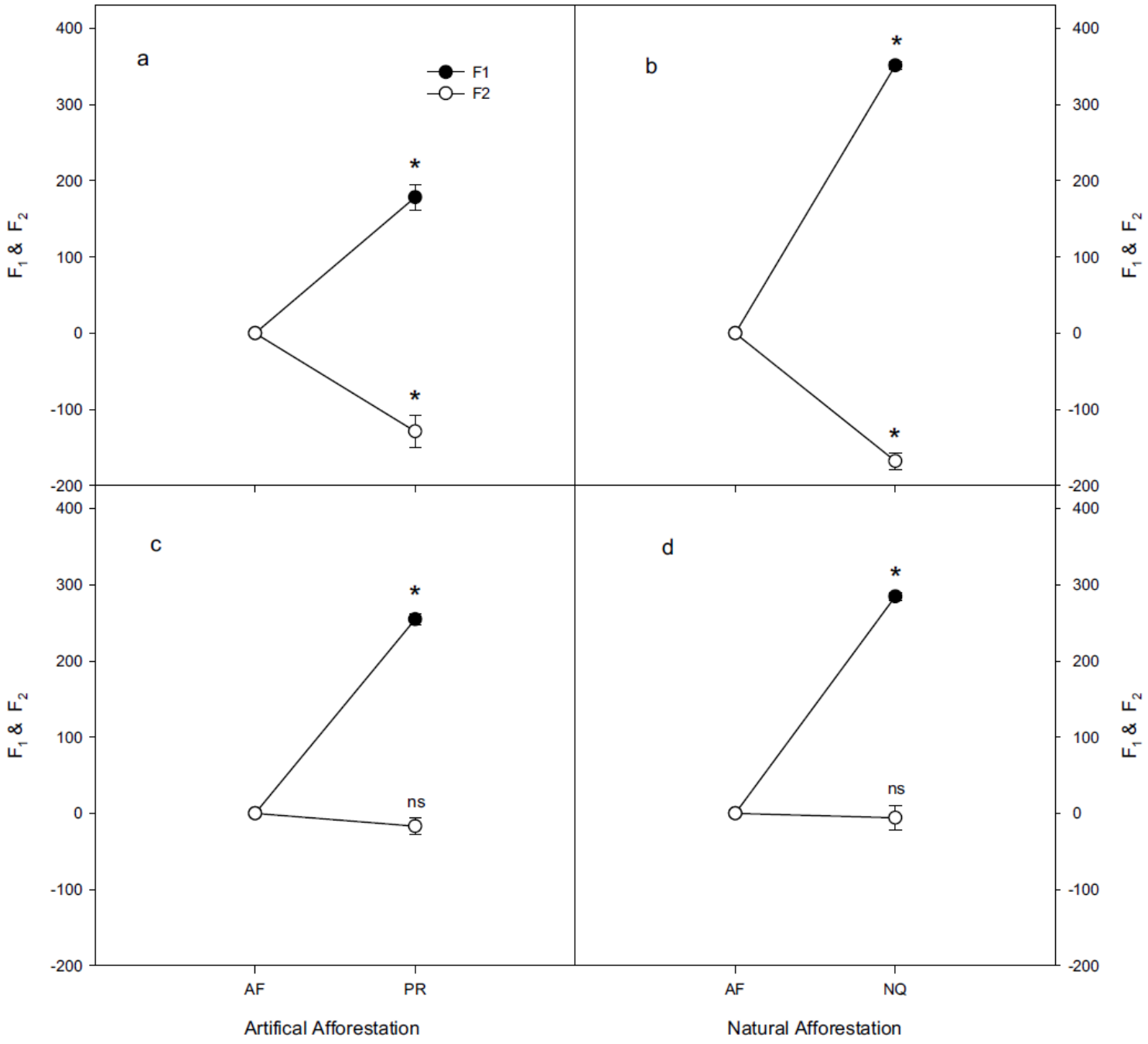

Figure 7

Changes in SOC stocks in macroaggregates and microaggregates with afforestation under (a-b) artificial and (c-d) natural restoration. Macroaggregates were >0.25 mm; microaggregatew 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 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).