

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

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## Research Article

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1 **Title:** Isotope analysis reveals differential impacts of artificial and natural  
2 afforestation on soil organic carbon dynamics in abandoned farmland

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18 **Abstract:**

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

22 *Methods and aims* We investigated the SOC dynamics following artificial and natural

23 afforestation in Loess Plateau of China, characterizing soil structure and  
24 stoichiometry using stable isotope carbon and radiocarbon models. We aim to  
25 compare SOC dynamics under both natural and artificial afforestation and examine  
26 how soil aggregate size classes control SOC dynamics based on stoichiometry and  
27 soil respiration.

28 *Results* Total top soil SOC stocks, C:N and C:P of differently sized soil aggregates  
29 significantly increased following vegetation restoration.  $^{13}\text{C}$  results and Radiocarbon  
30 models indicated that the SOC decomposition rate and new SOC input rate were  
31 lower under natural afforestation than artificial afforestation and revealed the highest  
32 SOC decomposition rate under natural afforestation compared to other two  
33 ecosystems.

34 *Conclusions* Vegetation restorations can accumulate SOC in top soils. Soil aggregates  
35 alternately play a dominant role in SOC accumulation following vegetation  
36 restoration; SOC loss from soil respiration was derived from microaggregates during  
37 afforestation. Recovery time is a key factor for the accumulation of SOC following  
38 afforestation.

39

40 **Keywords:** soil carbon stock; vegetation restoration; soil aggregate; soil respiration;

41  $^{13}\text{C}$ ;  $^{14}\text{C}$

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45 **1. Introduction**

46 Soil contains vast and dynamic pools of organic carbon that are fundamental for  
47 maintaining the balance of atmospheric CO<sub>2</sub> concentrations (Lal 2004; Post et al.  
48 1982).The soil organic carbon (SOC) pool and its dynamics are important properties  
49 of ecosystems (Schmidt et al. 2011). A multitude of studies report ecosystem changes  
50 significantly affect SOC pool and decomposition rate (Deng et al. 2016; Don et al.  
51 2011; Snell et al. 2015; Wei et al. 2013; Zhang et al. 2015).

52 The Loess Plateau is the one of the largest geographic units in China. Rapid  
53 population growth combining with climate and environmental conditions in the area  
54 have resulted in severe soil erosion in the Loess Plateau (Shi et al. 2017). The Grain  
55 for Green project was implemented in 1999 to halt soil erosion and promote  
56 ecological restoration in the region. As part of this project, both natural and artificial  
57 afforestation have been promoted as methods of ecological restoration (Shi et al.  
58 2011).

59 Human ecosystem interventions such as deforestation and afforestation, can  
60 cause rapid and persistent changes in vegetation and soil. Wei et al. (2013) found SOC  
61 stocks decreased most rapidly during the first 4 years of cropland cultivation after  
62 deforesting, but Rytter (2016) reported that SOC pools were generally unchanged  
63 after five years of *Salicaceae* growth during afforestation of former agricultural land.  
64 Natural change refers to the dynamic nature of ecosystem succession. Studies indicate  
65 that natural regeneration is slow and patchy with low species diversity (Blackham et  
66 al. 2014), and factors controlling SOC accumulation differed along vegetation

67 succession (Liu et al. 2015).

68 Previous studies have applied different methods (e.g. space-for-time substitution,  
69 stable isotope analysis) to study SOC dynamics and have successfully answered  
70 questions related to soil structure and organic matter dynamics (Marin-Spiotta et al.  
71 2009; Qiu et al. 2015). However, these previous studies mainly focused on natural  
72 ecosystem restoration, without considering the effects of artificial restoration.  
73 Furthermore, although Deng et al. (2016) have investigated the SOC turnover during  
74 natural succession, SOC dynamics in stable post-succession communities (climax  
75 community) following afforestation remain unstudied.

76 In this study, we combined (1) a radiocarbon ( $^{14}\text{C}$ ) model to estimate SOC  
77 decomposition rates in climax stage or long-term unchanged stage; (2) a natural  
78 abundance stable carbon isotope ( $^{13}\text{C}$ ) study to quantify old and new carbon turnover  
79 during both natural and artificial restoration; (3) measures of change of soil  
80 aggregate-associated ecological stoichiometry to investigate the sensitivity and  
81 efficiency of soil aggregate-associated organic carbon; and (4) long-term field  
82 monitoring of soil respiration to assess SOC loss. We aim to compare SOC dynamics  
83 under both natural and artificial afforestation and examine how soil aggregate size  
84 classes control SOC dynamics based on stoichiometry and soil respiration.

85

## 86 **2. Materials and methods**

### 87 ***2.1. Study site***

88 The study site consisted of semi-arid forests located on Mt. Gonglushan, near Yan'an

89 city in Shaanxi province, China (36°25.40"N, 109°31.53"E; 1245-1395m a.s.l.). On  
90 the Loess Plateau, precipitation and forest cover gradually decrease to the northwest,  
91 and our study site is located in the forest–grassland transition zone (Shi et al.  
92 2014). The 40-year averages (1971-2010) of annual precipitation and annual mean air  
93 temperature were 504.7 mm and 10.1 °C, respectively (Shi et al. 2012a). The soils are  
94 classified as Calcic Cambisols, which are derived from silt textured loess parent  
95 materials (Wei et al. 2013).

## 96 **2.2. Field investigation and sampling**

97 We obtained estimates of the recovery periods of different communities from  
98 vegetation surveys from the 1950s to the early 2000s (Chen 1954; Fan et al. 2006;  
99 Zou et al. 2001) and records from local farmers and government (Tateno et al. 2007;  
100 Wang et al. 2010). These results have been accepted and used widely (Deng et al.  
101 2013; Deng et al. 2016; Qiu et al. 2015). The study examined two different types of  
102 vegetation restoration: natural vegetation restoration from abandoned farmland to  
103 natural forest dominated by *Quercus liaotungensis* Koidz (80 years) and artificial  
104 restoration from abandoned farmland to plantation dominated by *Robinia*  
105 *pseudoacacia* L. (40 years). We compared these restoration types to a control site of  
106 abandoned farmland that remained unforested. The three ecosystems were at least  
107 3km apart. To minimize the effects of area conditions on experimental results, we  
108 selected study areas with similar topography, land-use history, and soil type. Five 20  
109 m×20 m plots were established within each ecosystem in April 2010. Each plot was at  
110 least 40 m from ecosystem boundary to minimize edge effects. Plots were spaced

111 approximately 50 m apart in each ecosystem.

112 In each plot, the litter on the ground was first removed and collected for  
113 measurement. We then dug a 20 cm-deep pit in center of the plot and took three soil  
114 bulk density (BD) samples. BD was measured at 0-20 cm in each subplot using a  
115 stainless steel cutting ring (5×5 cm). The soil cores were dried at 105°C for 24h.

116 Five soil samples (0-20 cm) were collected from the four corners and the center  
117 of each plot, and the collected samples were mixed to form one homogeneous sample  
118 for analysis of  $\delta^{13}\text{C}$ ,  $^{14}\text{C}$ , and aggregate size distribution. Three aggregate-size classes  
119 were manually fractionated through dry sieving of fresh soil samples on a series of  
120 two sieves (0.25 and 0.053mm) as follows: macroaggregates (>0.25mm),  
121 microaggregates (0.25-0.053mm) and silt & clay (<0.053mm). Fresh soil samples  
122 were dry sieved because wet sieving compromised the *in situ* link between the  
123 aggregates obtained and their indigenous biota. Furthermore, wet sieving could cause  
124 inaccurate evaluation of soil organic carbon (Jiang et al. 2014). The subsamples of  
125 each aggregate fraction were used for soil organic carbon (SOC), total nitrogen (N),  
126 and total phosphorous (P) analysis. Each aggregate fraction was oven dried at 100°C  
127 and weighed to determine its proportion of total soil weight.

### 128 **2.3. Laboratory analysis**

129 Soil organic carbon (SOC), total nitrogen (N), and total phosphorous (P) were  
130 measured using a TOC VWP (Shimadzu, Japan), 2300 kjeltec analyzer unit (FOSS  
131 TECATOR, Sweden), and ICP-AES (Spectro, Analytical Instruments, Germany),  
132 respectively. Soil organic carbon stock was calculated as the product of soil bulk

133 density and SOC concentration.

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

$$144 \quad \delta(\text{‰}) = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \quad (1)$$

145 Where  $R_{\text{sample}}$  is the  $^{13}\text{C}/^{12}\text{C}$  ratio of the sample and  $R_{\text{standard}}$  is the  $^{13}\text{C}/^{12}\text{C}$  ratio in  
146 the PDB standard.

147 The soil samples were pretreated for  $^{14}\text{C}$  analyses as follows. First, the soil  
148 samples were soaked in deionized water overnight, and then transferred to an  
149 ultrasonic bath for 30 min for completely disintegration and homogenization. The wet  
150 samples were sieved using a  $180\mu\text{m}$  mesh to remove root fragments. Then,  $1\text{mol}$   
151  $\text{L}^{-1}\text{HCl}$  was added to the samples, and they were placed in a water bath at  $70^\circ\text{C}$  for 2  
152 hours to remove carbonates. The samples were then rinsed repeatedly until neutral,  
153 then dried overnight in an electric oven at  $60^\circ\text{C}$ . Finally, the desiccated samples were  
154 transferred into glass vials and sealed tightly for subsequent combustion and

155 graphitization (Zhou et al. 1992). Measurement of  $^{14}\text{C}$  in soil organic matter was  
156 carried out at the Xi'an Accelerator Mass Spectrometry Center in the Institute of Earth  
157 Environment, Chinese Academy of Science. Cryogenically purified  $\text{CO}_2$  was  
158 converted to the target using the hydrogen-iron reduction method. This method was  
159 described in detail by Zhu et al. (2010).

160

#### 161 ***2.4. Measurement of soil respiration***

162 Soil respiration was measured using an automated soil  $\text{CO}_2$  flux system (LI-8100,  
163 LI-COR, USA) equipped with a portable chamber (Model 8100-103). A PVC collar  
164 (20.3 cm in diameter and 10 cm in height) was inserted into the forest floor to a depth  
165 of 2.5 cm at each sampling point, approximately two months before the first  
166 measurements. Small litter and branches were left in the collar while large items were  
167 removed. All collars were left at the respective site for the entirety of the study period.

168 Soil respiration was measured over the 4-year period from 14 June 2010 to  
169 20 June 2014, approximately once every 30 days during April-October (growing  
170 season), and once every 45 days in other months (dormant season). The measurements  
171 were conducted between 8:30 and 11:30 local time on each sampling day. In each plot,  
172 five 5 m×5 m subplots were established at the four corners and the center. The PVC  
173 collars were installed in the center of each subplot for measurements of soil  $\text{CO}_2$   
174 efflux.

175

#### 176 ***2.5. Data analysis***

177 Soil OC stocks were calculated as follow:

$$178 \text{ Soil OC stocks (kg m}^{-2}\text{)} = \frac{D \times BD \times OC}{100} \quad (2)$$

179 Where D is the thinness (cm) of the soil layer, BD is the bulk density (g cm<sup>-3</sup>), and  
180 OC is the soil organic carbon concentrate (g kg<sup>-1</sup>) at 0-20 cm.

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

$$182 \text{ Stocks of } OC_i \text{ (kg m}^{-2}\text{)} = \frac{D \times BD \times w_i \times OC_i}{10000} \quad (3)$$

183 Where  $OC_i$  is the soil organic carbon concentration of the  $i$ th aggregate size class (g  
184 kg<sup>-1</sup> aggregate).

185 During the afforestation period, the decomposition rate constants  $k_1$ (yr<sup>-1</sup>) was  
186 calculated by these models based on  $\delta^{13}\text{C}$  method: The proportions of new SOC ( $f_{\text{new}}$ )  
187 and old SOC ( $f_{\text{old}}$ ) were estimated based on the mass balance equations (Del Galdo et  
188 al. 2003):

$$189 f_{\text{new}} = \frac{(\delta_{\text{new}} - \delta_{\text{old}}) \times 100\%}{\delta_{\text{veg}} - \delta_{\text{old}}} \quad (4)$$

$$190 f_{\text{old}} = 100 - f_{\text{new}} \quad (5)$$

191 where  $\delta_{\text{new}}$  is the  $\delta^{13}\text{C}$  value of the soil sample from current ecosystem,  $\delta_{\text{old}}$  is the  $\delta^{13}\text{C}$   
192 value of the soil sample prior to ecosystem succession and  $\delta_{\text{veg}}$  is the  $\delta^{13}\text{C}$  value of the  
193 mixed litter of current vegetation. Decomposition rate constants ( $k_1$ ) of soil OC were  
194 estimated using the following equations (Marin-Spiotta et al. 2009):

$$195 k_1 = \frac{-\ln(C_t/C_0)}{t} \quad (6)$$

196 Where  $C_0$  is the initial SOC stock (SOC stock in the reference sites),  $C_t$  is initial SOC  
197 stock remaining (old C stock) at time t (year) since ecosystem change.

198 For the long-term or unchanged ecosystem, the decomposition rate constants  $k_2$   
 199 ( $\text{yr}^{-1}$ ) was obtained through the bomb- $^{14}\text{C}$  model (Cherkinsky and Brovkin 1993; Torn  
 200 et al. 2002; Trumbore 1993). The procedures were as follows (Tan et al. 2013):

201  $^{14}\text{C}$  data (pMC) is defined as

$$202 \quad \text{pMC}(\%) = \frac{A_{\text{SN}}}{A_{\text{ON}} \times e^{\lambda(y-1950)}} \times 100 \quad (7)$$

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

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

$$211 \quad C_y \times ^{14}C_y = C_{y-1} \times ^{14}C_{y-1} \times (1 - k_2 - \lambda) + I \times ^{14}C_{\text{atm},y-\text{lag}} \quad (8)$$

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

$$219 \quad ^{14}C_y = ^{14}C_{\text{atm},y-\text{lag}} \times k_2 + ^{14}C_{y-1} \times (1 - k_2 - \lambda) \quad (9)$$

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

223 A method was proposed by Qiu et al. (2012) for assessing the relative  
224 contribution of changes in aggregate amount and aggregate-associated OC  
225 concentrations to the total changes in OC stocks within each aggregate fraction. It was  
226 assumed that changes in OC stock within any particular aggregate fraction were  
227 caused both by changes in OC concentration in the fraction ( $F_1$ ) and by changes in the  
228 mass of the fraction ( $F_2$ ). It was also assumed that the mass that was gained or lost  
229 from an aggregate fraction due to ecosystem change had the same OC concentration  
230 as the rest of that fraction after ecosystem change. We therefore calculated the  
231 contribution of  $F_1$  and  $F_2$  to the total change in OC stock within an aggregate fraction  
232 as follows:

$$233 \quad F_1 = M \times \Delta C \quad (11)$$

$$234 \quad F_2 = \Delta M \times C \quad (12)$$

235 where  $F_1$  is the change in OC stock ( $g\ m^{-2}$ ) within an aggregate fraction due to  
236 changes in aggregate-associated OC concentrations,  $F_2$  is the change in the OC stock  
237 ( $gm^{-2}$ ) within an aggregate fraction due to changes in the mass of the aggregate  
238 fraction,  $\Delta M$  is the change in the mass of a particular fraction ( $kg\ m^{-2}$ ),  $M$  is the initial  
239 mass of the aggregate fraction ( $kg\ m^{-2}$ ) before ecosystem change,  $C$  is the final OC  
240 concentration of the aggregate fraction ( $g\ kg^{-1}$ ) after ecosystem change and  $\Delta C$  is the  
241 change in the OC concentration of the aggregate fraction ( $g\ kg^{-1}$ ) due to ecosystem

242 change.

## 243 **2.6. Calculation of Annual soil CO<sub>2</sub> emissions**

244 Annual soil CO<sub>2</sub> emissions were estimated by interpolating the average CO<sub>2</sub> flux  
245 rate between sampling dates, and computing the sum of the products of the average  
246 flux rate and the time between respective sampling dates for each measurement  
247 period (Shi et al. 2014; Shi et al. 2012b; Sims and Bradford 2001) as follows:

$$248 \quad R = \sum F_{m,n} \Delta t_n \quad (13)$$

249 Where  $\Delta t_n = t_{n+1} - t_n$ , which is the number of days between each field measurement  
250 within the year;  $R$  is total soil CO<sub>2</sub> emitted in the measurement period, and  $F_{m,n}$  is the  
251 average CO<sub>2</sub> flux rate over the interval  $t_{n+1} - t_n$  recorded by the LI-8100 Soil CO<sub>2</sub> Flux  
252 System.

## 253 **2.7 Statistical analyses**

254 One-way Analysis of Variation (ANOVA) with Pearson's test was performed to  
255 examine the difference between abandoned farmland and artificial afforestation or  
256 abandoned farmland and natural afforestation in OC stocks of total soil and different  
257 soil aggregate size classes, soil aggregate size class distributions, C:N and C:P of  
258 different soil aggregate size classes, mean annual soil CO<sub>2</sub> emission. ANOVA also  
259 was applied to examine the difference between period of artificial and natural  
260 afforestation in SOC decomposition rate constants ( $k_1$ ) and new SOC input rate  
261 calculated with a <sup>13</sup>C model. Repeated measures ANOVA (RMANOVA) was applied  
262 to test the significance of SOC decomposition rate constants ( $k_2$ ) calculated with a <sup>14</sup>C  
263 model among the three land use types using Tukey's HSD test at  $p < 0.05$ . All

264 differences were evaluated at the 5% significance level.

265

### 266 **3. Results**

#### 267 ***3.1. Total and size class aggregate OC stocks***

268 Fig. 1 shows the change of total and different aggregate size OC stocks. Total OC  
269 stocks in top soil (0-20 cm) significantly increased with both artificial afforestation  
270 dominated by plantation of *Robinia pseudoacacia* L (AR) and natural afforestation  
271 dominated by natural forest *Quercus liaotungensis* Koidz (NQ). Nevertheless, OC  
272 sequestration following artificial afforestation was significant lower than for natural  
273 afforestation. Under both artificial and natural afforestation, the OC stocks of  
274 macroaggregates and microaggregates significantly increased , but the OC stocks of  
275 silt & clay were nearly 0 (far lower than for any other fraction), and could thus be  
276 considered a negligible part of total OC stocks (Fig 1). The OC stock of  
277 macroaggregates was significant higher than that of microaggregate in AR (Fig. 1a),  
278 but the OC stock of macroaggregate and microaggregate were nearly the same in NQ  
279 (Fig. 1b).

#### 280 ***3.2. Aggregate size distribution and C:N & C:P in different size aggregates***

281 Macroaggregates and microaggregates accounted for approximate 99 % of the  
282 dry soil weight in the abandoned farmland. Under both artificial and natural  
283 restoration, the amount of soil in macroaggregates significantly increased, and  
284 microaggreagtes significantly decreased (Fig 2). Furthermore, for both types of  
285 restorations, the amount of soils in silt & clay was less than 1% and did not

286 significantly change.

287 Both C:N and C:P of different aggregate classes significantly increased with both  
288 types of vegetation restoration. Compared with other size aggregates, C:N and C:P of  
289 microaggregates become higher after restoration (Fig 3).

### 290 ***3.3. Turnover of SOC under artificial and natural afforestation***

291 Over the vegetation restoration period, the SOC decomposition rate ( $k_1$ ), and  
292 new SOC input rate under artificial restoration were higher than natural restoration  
293 (Fig 4). For climax or long-term unchanged stage, the SOC decomposition rate ( $k_2$ )  
294 was highest ( $2.6 \times 10^{-3}$ ) in the natural forest ecosystem (*Quercus liaotungensis* Koidz).  
295 The  $k_2$  of abandoned farmland and plantation (*Robinia pseudoacacia* L.) were similar  
296 (Fig 5).

### 297 ***3.4. Amount of soil CO<sub>2</sub> emissions***

298 The amount of soil CO<sub>2</sub> emissions across ecosystems was calculated from  
299 continuous measurements over 4 years. Both natural and artificial restoration  
300 significantly increased the amount of soil CO<sub>2</sub> emissions, and the increased of amount  
301 of soil CO<sub>2</sub> emissions was higher under natural restoration than artificial restoration  
302 (Fig 6).

303

## 304 **4. Discussion**

305 A multitude of studies found that vegetation restoration can lead to the  
306 accumulation of SOC, and the effects of land use change during vegetation restoration  
307 on soil OC are mainly due to changes in OC input and C mineralization (Deng et al.

308 2016; Laganière et al. 2010). The OC in soil physical fractions responds more  
309 sensitively and rapidly to land use change than the OC in bulk soils (Qiu et al. 2012;  
310 Wei et al. 2013); therefore, changes in aggregate-associated OC are regarded as  
311 fundamental processes for understanding the effects of vegetation restoration on SOC.

312 In this study, we also found that both types of restorations could accumulate SOC  
313 in top soil (Fig 1). Our study showed that changes in  $F_1$  &  $F_2$  under artificial and  
314 natural restoration were nearly the same, and  $F_1$  and  $F_2$  significantly increased and  
315 decreased respectively, for macroaggregate-OC after afforestation, but changes in  $F_1$   
316 &  $F_2$  for macroaggregate-OC were more significant under natural afforestation (Fig 7  
317 a, b). In addition,  $F_1$  significantly increased and  $F_2$  slightly decreased for  
318 microaggregate-OC (Fig 7 c, d).

319 The model results indicated that increases in SOC stocks in macroaggregates and  
320 microaggregates under artificial and natural afforestation were mainly due to  
321 increases in SOC concentration rather than mass (Fig 7). The contribution of  
322 macroaggregate-OC concentration increases under natural afforestation was more  
323 significant than under artificial, but microaggregate-OC changes under natural and  
324 artificial afforestation were similar. Therefore, we concluded that macroaggregate-OC  
325 stock is the main contributing factor to changes in total OC stock. Qiu et al. (2015)  
326 obtained a similar result. Nevertheless, under artificial afforestation we found that OC  
327 stocks in macroaggregates and microaggregates equally contributed to the dynamics  
328 of total OC stocks.

329 Meanwhile, we found that soil respiration was the main pathway of soil OC loss.

330 We found that long-term soil CO<sub>2</sub> emission during afforestation promoted SOC loss  
331 via soil respiration. Spohn and Chodak (2015) demonstrated that microorganisms  
332 increase their respiration rate with an increase in soil C/P ratio and C concentration. In  
333 this study, we found that C/N and C/P ratios in microaggregates were consistently  
334 higher than in other fractions under afforestation (Fig 3). Therefore, it was concluded  
335 that SOC loss from soil respiration was mainly originated from microaggregate  
336 resulting from higher C/P ratio after afforestation, supporting the previous inference in  
337 terms of relationship between ecological stoichiometry and soil respiration.

338 Moreover, although our study found higher SOC accumulation under natural  
339 afforestation than under artificial afforestation (Fig 1), and the increase in proportion  
340 of new OC in soils could be attributed to the OC inputs from the recent afforestation,  
341 which produced organic matter with different <sup>13</sup>C/<sup>12</sup>C ratios (Marin-Spiotta et al.  
342 2009), based on analysis of <sup>13</sup>C we found old C decomposition and new C inputs  
343 during natural afforestation were significantly lower than during artificial  
344 afforestation (Fig 4). Previous studies focused on the process of old C decomposition  
345 and new C input: Zhang et al. (2015) proposed the relationship between SOC  
346 decomposition and new & old litter input, and Richter et al. (1999) reported that the  
347 rates of new soil OC increase represented the net effect of new OC input and output to  
348 soil. However, time since land use change has not been considered as a key parameter  
349 for the accumulation of SOC. In this study, the period of natural afforestation was  
350 twice as long as the period of artificial afforestation. Though the new SOC input rate  
351 under natural afforestation was lower compared to artificial afforestation, the

352 decomposition rate under natural afforestation was also lower, which may indicate  
353 higher SOC accumulation under natural afforestation is not attributable only to higher  
354 net increases in the difference between new SOC input and old SOC decomposition.  
355 We could conclude that time since land use change is a more important factor  
356 determining the proportions of new and old OC in soil. Previous studies lend support  
357 to this hypothesis (Deng et al. 2016; Marin-Spiotta et al. 2009)

358 Not only climax forest but also other types of ecosystems in semiarid or arid  
359 region often maintain in stable stage for a long time (Fan et al. 2006) and the  
360 dynamics of SOC in the ecosystem could be considered as an ecosystem property  
361 (Schmidt et al. 2011).  $^{14}\text{C}$  dating has been proven to be an ideal tool to study the  
362 dynamics of SOC from decadal to millennial timescales, which provides a direct  
363 measure of the time elapsed since carbon in organic matter was fixed from the  
364 atmosphere (Trumbore 1993). In this study, the steady-state box model of  $^{14}\text{C}$  of SOC  
365 was applied to estimate the mean residence time (MRT) of SOC for the long-term or  
366 unchanged ecosystem, providing a sensitive indicator to quantify the proportion of  
367 SOC derived from the atmosphere in recent years to centuries based on the rate of  
368 incorporation of the nuclear explosion before 1960s carbon tracer (Laskar et al. 2016;  
369 Torn et al. 2009). In our study, the result of the  $^{14}\text{C}$  model suggested that the carbon  
370 decomposition rate was the lowest in the plantation (*Robinia pseudoacacia* L.)  
371 ecosystem, and was the same as abandoned farmland. The carbon turnover was  
372 highest in the natural forest ecosystem (*Quercus liaotungensis* Koidz) (Fig 5). The  
373 model provided a direct evidence that higher accumulation of SOC under natural

374 afforestation can be attributed to its longer restoration period rather than more rapid  
375 carbon turnover under natural afforestation compared to artificial afforestation.  
376 Therefore, our study suggests that longer restoration time could be more important for  
377 SOC accumulation following afforestation than any particular method of  
378 afforestation.

379

380

## 381 **5. Conclusions**

382 In conclusion, we found that both artificial and natural afforestation promoted  
383 the accumulation of SOC in top soil, and accumulation of SOC under natural  
384 afforestation was higher than under artificial afforestation. The SOC concentration of  
385 soil aggregates played a dominant role in determining the dynamics of SOC  
386 accumulation following vegetation restoration. According to analysis of soil  
387 aggregates, soil respiration, and aggregate stoichiometry, we concluded that SOC loss  
388 from soil respiration mainly originated from microaggregates during restoration.  $^{13}\text{C}$   
389 and  $^{14}\text{C}$  models proved effective tools that showed that recovery time is a key factor  
390 determining the accumulation of SOC following afforestation.

391

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397

398

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532

533 Figure captions

534 Fig 1 Changes in OC stocks of total soil and different soil aggregate size classes under  
535 (a) artificial and (b) natural afforestation. Macroaggregates were  $>0.25$  mm;  
536 microaggregates were between 0.25 and 0.053 mm; and silt & clay were  $<0.053$  mm.  
537 Error bars represent the standard error of the mean. Significant differences are  
538 indicated by the asterisk symbol ( $*p < 0.05$ ); ns: no significant; AF: abandoned  
539 farmland; AR: artificial afforestation (plantation of *Robinia pseudoacacia* L); NQ:  
540 natural afforestation (natural forest - *Quercus liaotungensis* Koidz).

541

542 Fig 2 Changes in soil aggregate size class distributions with afforestation under (a)  
543 artificial and (b) natural restoration. Macroaggregates were  $>0.25$  mm;  
544 microaggregates were between 0.25 and 0.053 mm; and silt & clay were  $<0.053$  mm.  
545 Error bars represent the standard error of the mean. Significant differences are  
546 indicated by the asterisk symbol ( $*p < 0.05$ ); ns: no significant; AF: abandoned  
547 farmland; AR: artificial afforestation (plantation of *Robinia pseudoacacia* L); NQ:  
548 natural afforestation (natural forest - *Quercus liaotungensis* Koidz).

549

550 Fig 3 Changes in C:N and C:P of different soil aggregate size classes with  
551 afforestation under (a-b) artificial and (c-d) natural restoration. Macroaggregates  
552 were  $>0.25$  mm; microaggregates were between 0.25 and 0.053 mm; and silt & clay  
553 were  $<0.053$  mm. Error bars represent the standard error of the mean. Significant  
554 differences are indicated by the asterisk symbol ( $*p < 0.05$ ); ns: no significant; AF:

555 abandoned farmland; AR: artificial afforestation (plantation of *Robinia pseudoacacia*  
556 L); NQ: natural afforestation (natural forest - *Quercus liaotungensis* Koidz).

557

558 Fig 4 SOC decomposition rate constants ( $k_1$ ) and new SOC input rate ( $\text{kg m}^{-2} \text{yr}^{-1}$ )  
559 calculated with a  $^{13}\text{C}$  model under (a) artificial and (b) natural afforestation. Error bars  
560 represent the standard error of the mean. Significant differences are indicated by the  
561 asterisk symbol ( $*p < 0.05$ ).

562

563 Fig 5 SOC decomposition rate constants ( $k_2$ ) calculated with a  $^{14}\text{C}$  model for different  
564 land use types. Error bars represent the standard error of the mean. Significant  
565 differences are indicated by different letters ( $p < 0.05$ ). AF: abandoned farmland; AR:  
566 artificial afforestation (plantation of *Robinia pseudoacacia* L); NQ: natural  
567 afforestation (natural forest - *Quercus liaotungensis* Koidz).

568

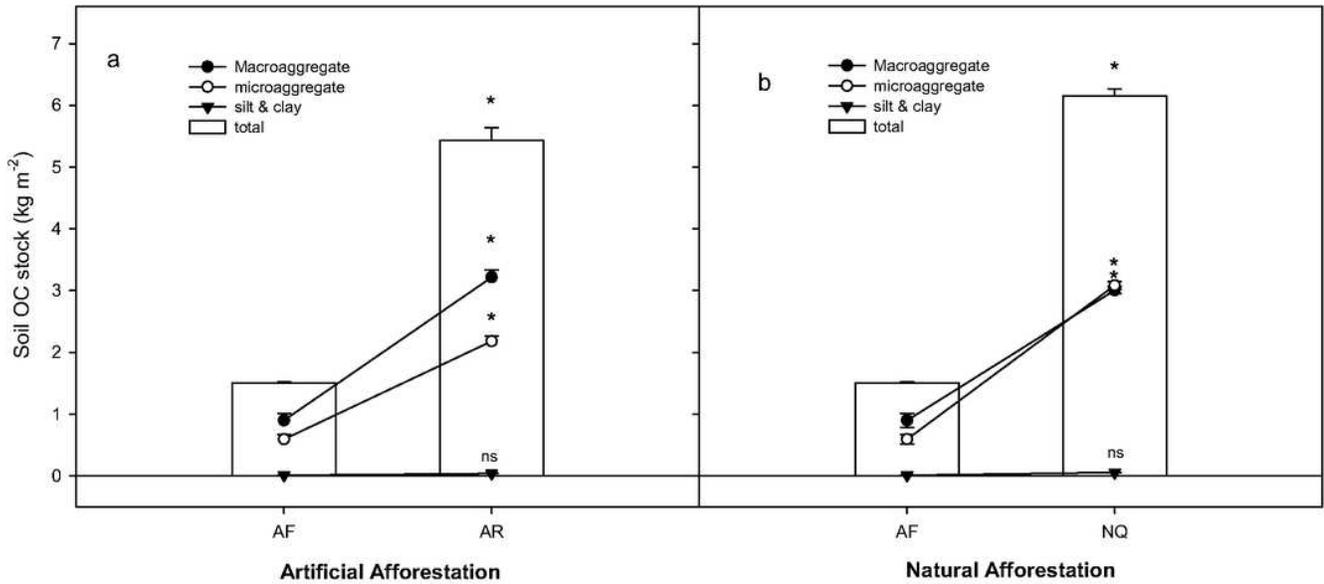
569 Fig 6 Changes in mean annual soil  $\text{CO}_2$  emission over 4 years of vegetation  
570 restoration under (a) artificial and (b) natural afforestation. Error bars represent the  
571 standard error of the mean. Significant differences are indicated by the asterisk  
572 symbol ( $*p < 0.05$ ); ns: no significant; AR: artificial afforestation (plantation of  
573 *Robinia pseudoacacia* L); NQ: natural afforestation (natural forest - *Quercus*  
574 *liaotungensis* Koidz).

575

576 Fig 7 Changes in SOC stocks in macroaggregates and microaggregates with

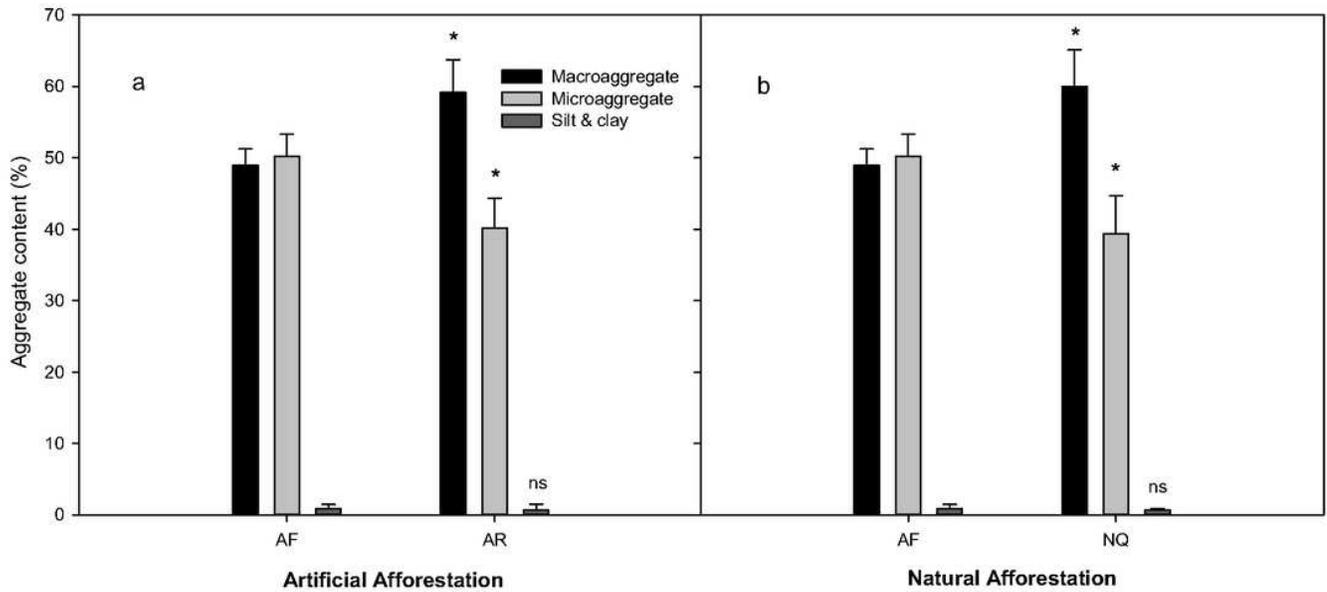
577 afforestation under (a-b) artificial and (c-d) natural restoration. Macroaggregates  
578 were >0.25 mm; microaggregates were between 0.25 and 0.053 mm; and silt & clay  
579 were <0.053 mm. Error bars represent the standard error of the mean. Significant  
580 differences are indicated by the asterisk symbol (\* $p < 0.05$ ). AF: abandoned farmland;  
581 AR: artificial afforestation (plantation of *Robinia pseudoacacia* L); NQ: natural  
582 afforestation (natural forest - *Quercus liaotungensis* Koidz).

# Figures



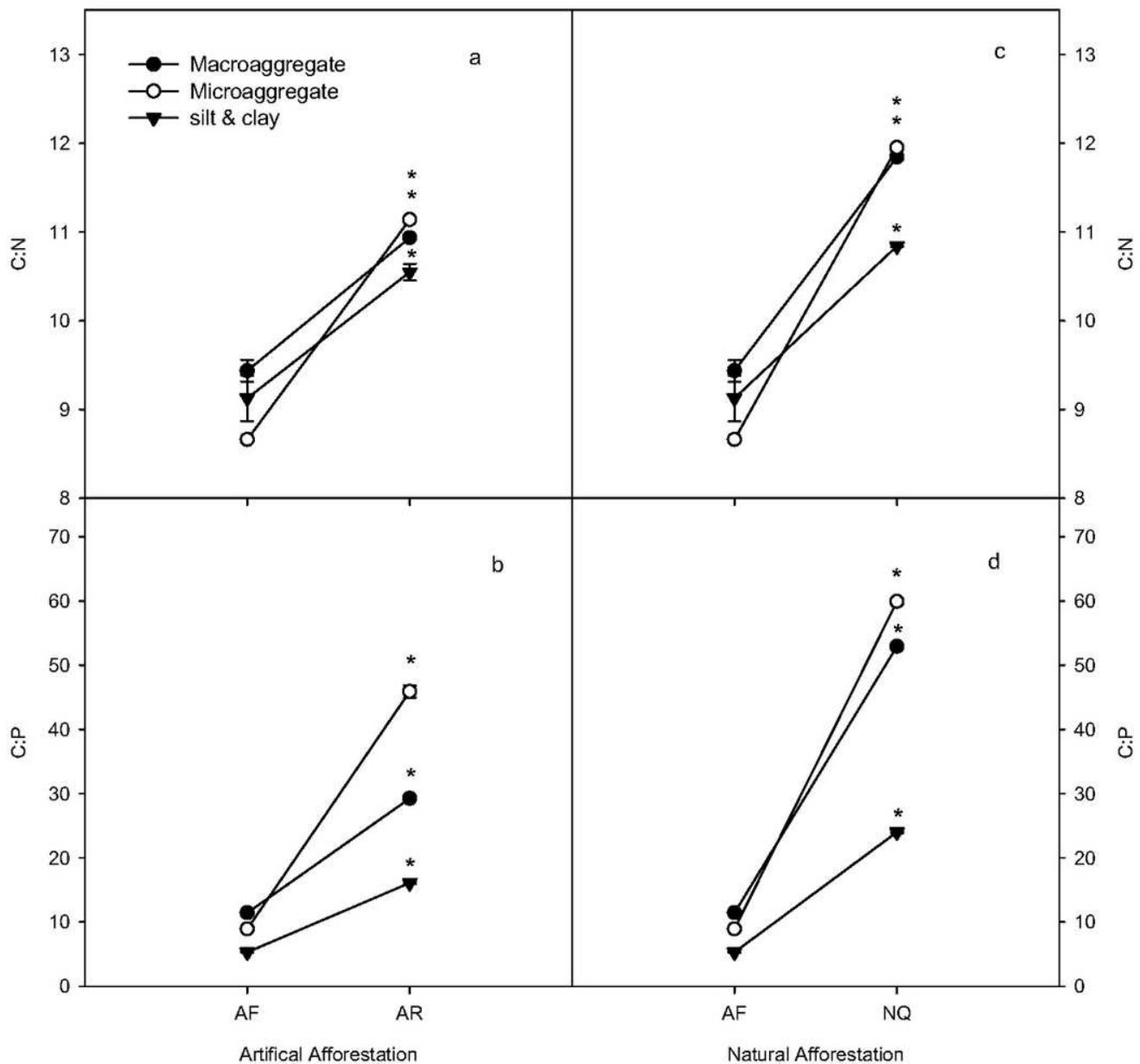
**Figure 1**

Changes in OC stocks of total soil and different soil aggregate size classes under (a) artificial and (b) natural afforestation. Macroaggregates were >0.25 mm; microaggregates 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).



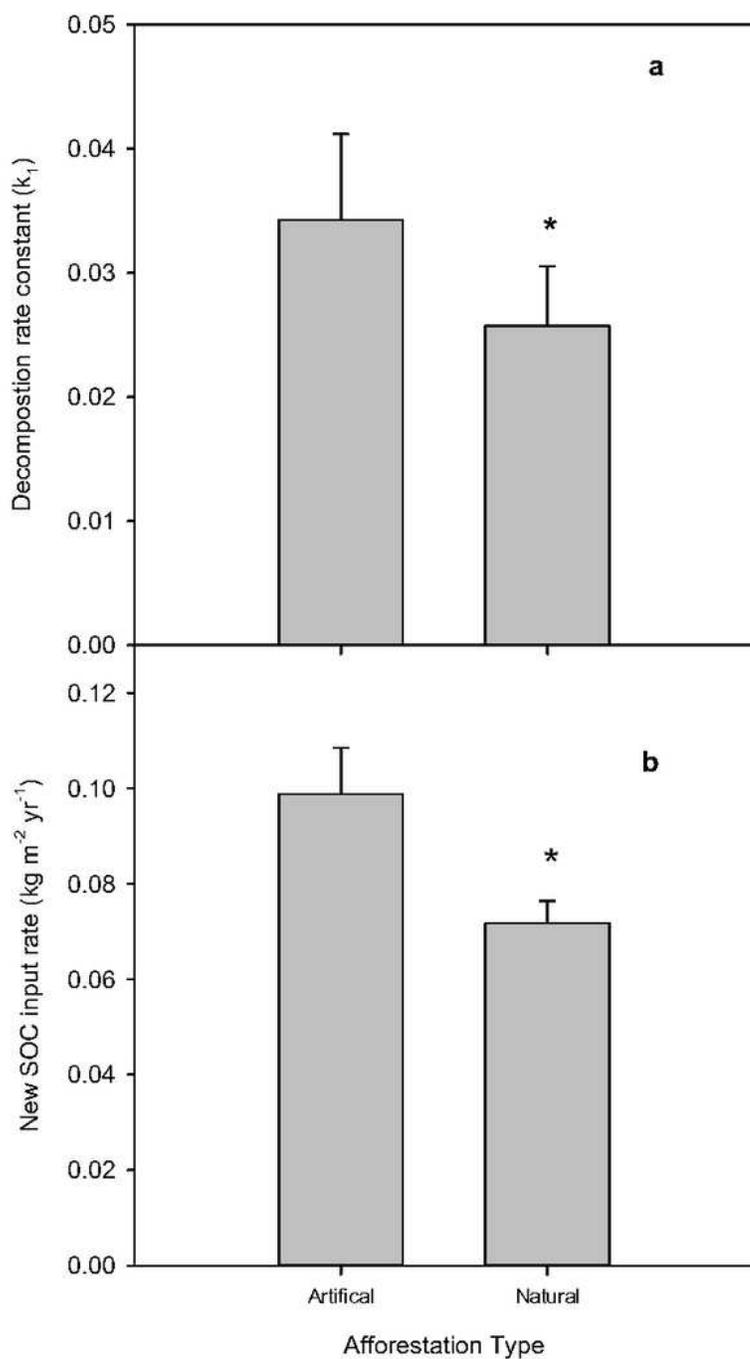
**Figure 2**

Changes in soil aggregate size class distributions with afforestation under (a) artificial and (b) natural restoration. Macroaggregates were >0.25 mm; microaggregates 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).



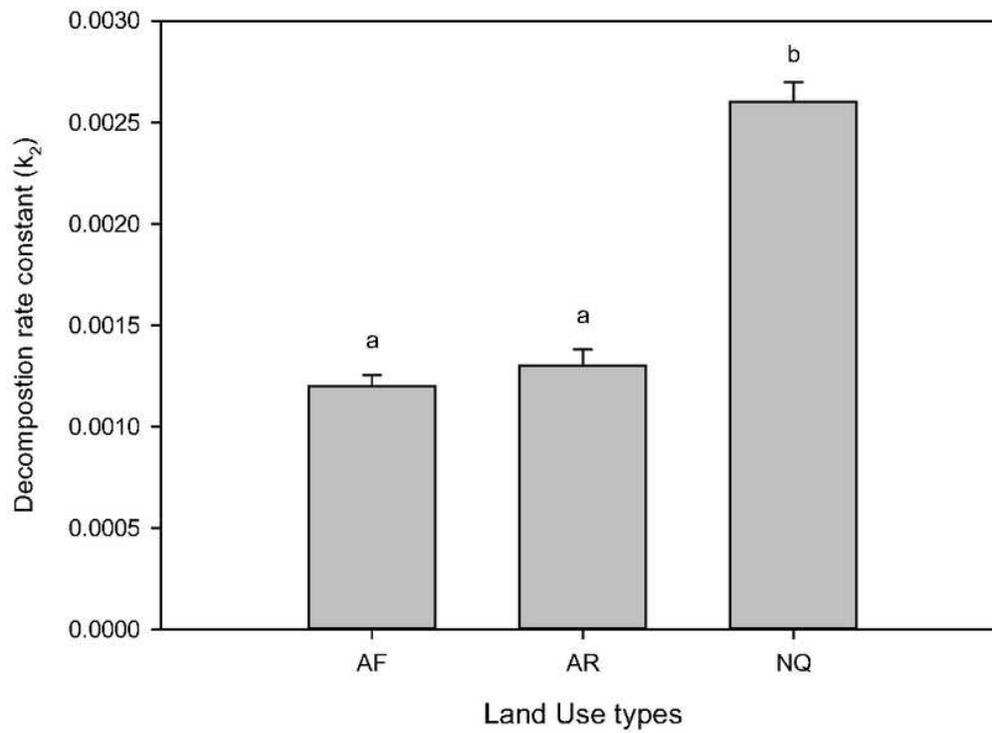
**Figure 3**

Changes in C:N and C:P of different soil aggregate size classes with afforestation under (a-b) artificial and (c-d) natural restoration. Macroaggregates were >0.25 mm; microaggregates 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).



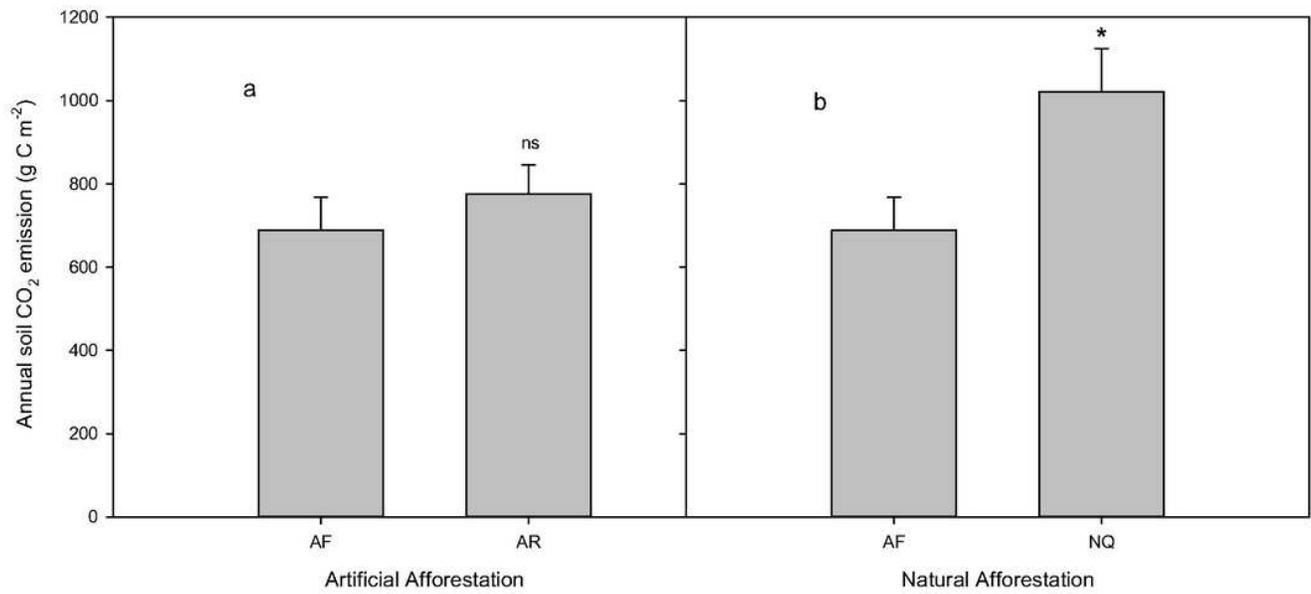
**Figure 4**

SOC decomposition rate constants ( $k_1$ ) and new SOC input rate ( $\text{kg m}^{-2} \text{yr}^{-1}$ ) calculated with a  $^{13}\text{C}$  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 ( $*p < 0.05$ ).



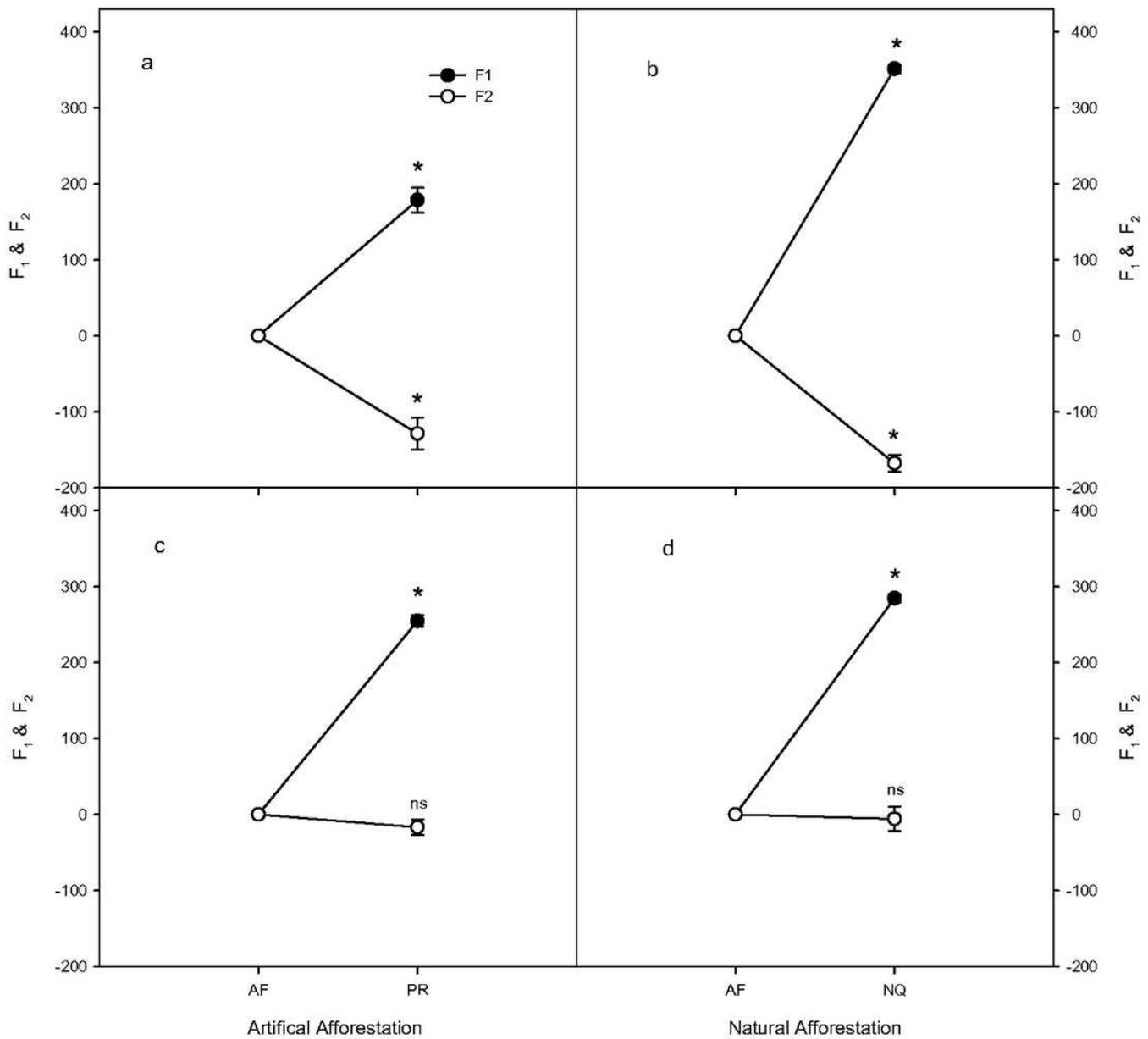
**Figure 5**

SOC decomposition rate constants ( $k_2$ ) calculated with a  $^{14}\text{C}$  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).



**Figure 6**

Changes in mean annual soil CO<sub>2</sub> emission over 4 years of vegetation restoration 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); ns: no significant; 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; microaggregates 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). AF: abandoned farmland; AR: artificial afforestation (plantation of *Robinia pseudoacacia* L); NQ: natural afforestation (natural forest - *Quercus liaotungensis* Koidz).