



# Soil organic carbon dynamics following natural vegetation restoration: Evidence from stable carbon isotopes ( $\delta^{13}\text{C}$ )



Lei Deng<sup>a,b</sup>, Kaibo Wang<sup>b,\*</sup>, Zhuangsheng Tang<sup>a</sup>, Zhouping Shangguan<sup>a</sup>

<sup>a</sup> State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A&F University, Yangling, Shaanxi 712100, PR China

<sup>b</sup> State Key Laboratory of Loess and Quaternary Geology, Institute of Earth Environment, Chinese Academy of Sciences, Xi'an, Shaanxi 710075, PR China

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## ABSTRACT

Knowledge of soil carbon (C) dynamics following vegetation restoration is essential for evaluating carbon budgets and cycles at regional and global scales. In this study, we investigated the dynamics of soil organic carbon (OC) following farmland abandonment along with ~160 years of vegetation restoration on the Loess Plateau, China. Our specific objectives were to examine the variation of soil OC decomposition rates, to quantify the changes in the proportion of new and old soil OC, and to explore the factors controlling soil OC stock patterns. The results showed that the rate of new soil OC increase was higher in the early stage (~10 years) after land-use change. The rate of new soil OC increase ranged from 109.17 to 41.88 g m<sup>-2</sup> year<sup>-1</sup> in the early (~10 years) and later stages (~160 years), respectively. It took about 30 years for the amount of new soil OC to reach the same level as old OC in the top 20 cm of soil following farmland abandonment. Also, soil OC decomposition rate was higher (decomposition rate constants = 0.04) in the early stage (~10 years) and showed a non-significant difference after > 30 years of vegetation restoration. Our results suggested that soil C/N is the most factor to effect on soil OC sequestration following vegetation restoration, and the proportions of new soil OC was mainly determined by fine roots, soil OC decomposition rate constants were mainly determined by soil silt content, and the rates of new soil OC increase were mainly determined by soil sand content that these observations were made out of considering many other soil properties.

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## 1. Introduction

Globally, soil contains 1500–2300 Pg of organic carbon (OC), approximately twice as much as the amount in the atmosphere and three times the amount in terrestrial vegetation (Lal, 2004), and thus has a most important role in balancing atmospheric carbon dioxide (CO<sub>2</sub>) concentration (Briones et al., 2006). Whether soil carbon pool acts either as a source or as a sink for atmospheric CO<sub>2</sub> is largely depended on land use and climate conditions (IPCC (Intergovernmental Panel on Climate Change), 2007; Don et al., 2011; Zatta et al., 2013; Deng et al., 2014a). Many studies have reported land use significant affects soil OC pool and decomposition rate (Zatta et al., 2013; Zhang et al., 2015; Deng et al., 2016). Consequently, the dynamics of soil OC and the capacity of soil to accumulate and stabilize OC in response to land use change have become a focus of

research in the scientific debate on global climate change (Knorr et al., 2005; Wick et al., 2009; Wang et al., 2013).

Land use change greatly impacts soil C dynamic by altering C inputs, decomposition, and turnover (Guo and Gifford, 2002; Zatta et al., 2013; Zhang et al., 2015), and thus potentially affects C sequestration and loss (Lal, 2004; Deng et al., 2016). Following the conversion of natural to cultivated vegetation, SOC can be rapidly lost due to enhanced OC decomposition and erosion due to soil disturbance (Van der Werf et al., 2009; Yan et al., 2012; Wei et al., 2014; Guillaume et al., 2015). Globally, 24% of the soil OC stock has been lost through the conversion of forestland to cropland (Murty et al., 2002) and 59% through the conversion of pastureland to cropland (Guo and Gifford, 2002). In contrast, converting cropland into perennial vegetation is found to accumulate SOC by increasing OC derived from the new vegetation, thereby simultaneously decreasing OC loss from decomposition and erosion (Laganière et al., 2010), and SOC is locked up for greater periods of time due to the slower turnover rates associated with natural vegetation (Deng et al., 2013). Generally, soil OC stocks are controlled by the balance

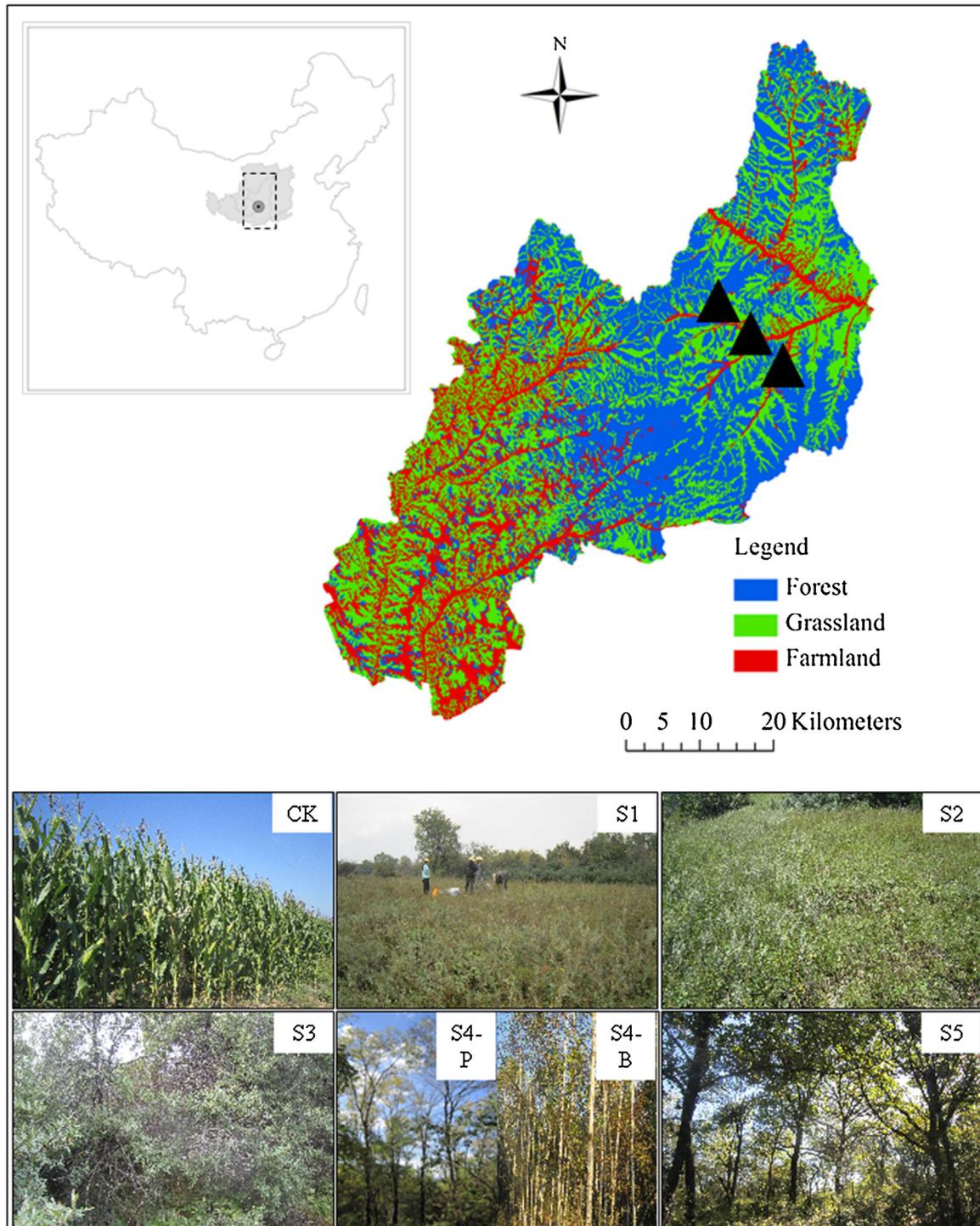
\* Corresponding author.

E-mail address: [wangkb@ieecas.cn](mailto:wangkb@ieecas.cn) (K. Wang).

of plant input vs. rate of soil OC loss following land-use change (Zhang et al., 2015). Thus, understanding the change in new soil OC (OC derived from new vegetation after land-use change) and old OC (initial soil OC previous to conversion) could provide more information about the dynamic responses of SOC to land-use change (Mendez-Millan et al., 2014).

Worldwide land-use changes involving a conversion of vegetation with different photosynthetic pathways (e.g., C<sub>3</sub> and C<sub>4</sub> vegetation) offer a unique opportunity to quantify the soil C dynamics using the stable carbon isotope technique (Wolf et al.,

2011; Yonekura et al., 2012; Mendez-Millan et al., 2014). Naturally, C<sub>4</sub> ( $\delta^{13}\text{C}$  ca.  $-12\text{‰}$ ) and C<sub>3</sub> ( $\delta^{13}\text{C}$  ca.  $-28\text{‰}$ ) plants could produce detritus with different  $^{13}\text{C}/^{12}\text{C}$  ratios due to their difference in utilizing  $^{13}\text{C}/^{12}\text{C}$  (Marin-Spiotta et al., 2009). Thus, conversion of vegetation with different  $^{13}\text{C}$  signals can affect the  $^{13}\text{C}$  signature of SOC (Smith and Johnson, 2003; Zhang et al., 2015). The isotopic signature of soil organic matter (SOM) after land-use change can be a powerful tool for understanding the biogeochemistry of SOC in different ecosystems (West et al., 2006). The natural abundances of soil  $\delta^{13}\text{C}$  have been used to explore the mechanisms of new and old



**Fig. 1.** Photos of different successional stages in the study sites (black triangles in the map). CK, farmland control; S1, pioneer weeds; S2, herbage; S3, shrub (*H. rhannoides*); S4, early forest (S4-P, *P. davidiana*; S4-B, *B. platyphylla*); and S5, climax forest (*Q. liaotungensis*).

soil OC changes after land use changes (Blagodatskaya et al., 2011; Wang et al., 2013; Mendez-Millan et al., 2014; Wei et al., 2014; Guillaume et al., 2015; Zhang et al., 2015). In particular, this method also makes the data obtained in various individual studies and from different regions comparable (Giardina and Ryan, 2000; Smith and Johnson, 2003; Osher et al., 2003; Wei et al., 2014; Zhang et al., 2015). However, little investigation using the stable carbon isotope technique to explore new and old soil OC dynamics along a long term (~160 year) vegetation restoration chronosequence.

The degree of change in SOC stocks and the timing of the switch between growth and decline of stocks depends on many factors, such as soil properties, methods of site preparation for afforestation, tree species planted, land-use types and environmental conditions (Guo and Gifford, 2002; Arai and Tokuchi, 2010), climate (temperature and precipitation) (Paul et al., 2002; Zhang et al., 2015), previous land use (Högberg and Read, 2006; Laganière et al., 2010), however, a consensus on the relative significance of these factors has yet to be achieved (Deng et al., 2014a). Moreover, soil physical and chemical properties – e.g., soil enzymes, nitrogen (N) nutrients and soil microbial activity – have been extensively used to evaluate soil OC stocks (Zhang et al., 2012). However, soil OC stock patterns cannot be assessed using one property alone but with a variety of soil properties—so far there has been little comprehensive assessment of the soil properties related to soil OC stock changes. Therefore, the selection of indicators that appropriately reflect the overall change of soil OC stocks is important, and understand which factors drive soil OC stock dynamics is crucial to explore soil OC change rate and its temporal patterns following land use change. These parameters have important implications for both C cycling and ecosystem function.

In China, the Loess Plateau has very natural vegetation, it is necessary to understand the process of natural vegetation recovery and its importance to ecological rehabilitation. A fuller appreciation of this process will help guide ongoing vegetation restoration in Western China. Moreover, understanding the carbon sequestration dynamics of ecosystems is important for vegetation restoration, especially when converting farmland to natural restoration grassland or forest. In our study, we investigated SOC dynamics following natural vegetation restoration following farmland abandonment using the stable carbon isotope technique. The vegetation had been converted from adjacent farmland for about 10, 30, 60, 100 and 160 years previously. The objectives of the study were to examine the variation of soil OC decomposition rates, quantify the changes in the proportion of new and old soil OC, and

explore the relationship between the soil OC stock patterns and the controlling factors during vegetation restoration.

## 2. Materials and methods

### 2.1. Study sites

The study was conducted on the Lianjiabian Forest Farm of the Heshui General Forest Farm of Gansu (35°03′–36°37′N, 108°10′–109°18′E, 1211–1453 m a.s.l.), located in the hinterland of the Loess Plateau, in the Ziwuling forest region, covering a total area of 23,000 km<sup>2</sup>. The altitude of the region's hilly and gully landforms averages 1500 m a.s.l., their relative height difference is about 200 m. The area's mean annual temperature is 10 °C and mean annual rainfall is 587 mm (1960–2010) (Deng et al., 2013). The region's soils are largely Cambisols having developed from primitive or secondary loess parent materials according to the FAO classification system (Wei et al., 2014), which are evenly distributed 50–130 m deep above red earth consisting of calcareous cinnamon soil (Jia et al., 2005). The area is covered in species-rich uniform forests with a canopy density in the range of 80–95% (Deng et al., 2013).

### 2.2. Field investigation and sampling

The Ziwuling Mountain region is a scarce place with a complete sequence of natural vegetation succession following farmland abandonment on the Loess Plateau. Natural vegetation with different restoration ages can be observed in this region. The methods adopted to identify the age of the communities were described in our prior studies (Deng et al., 2013, 2014b), and so are not described here in detail.

Soil samples were collected from three areas in the study region that were approximately 5 km from each other (Fig. 1). Each area included five communities with different restoration stages: about 10 [*Lespedeza dahurica* (Laxm.) Schindl.], 30 [*Bothriochloa ischaemum* (L.) Keng, *Carex lanceolata* Boott or *Potentilla chinensis* (Ser.)], 60 [*Sophora davidii* (Franch.) Skeels, *Hippophae rhamnoides* L. or *Rosa xanthina* Lindl.], 100 (*Populus davidiana* Dode or *Betula platyphylla* Suk.) and 160 years (*Quercus liaotungensis* Koidz.). For comparison, one area of farmland planted with maize (*Zea mays* L.) was selected as a reference site (0 year), because we found the maize is the only crop located in the study area, and we also asked the local elders to know the land use history. The farmlands had

**Table 1**

Summary of features of soil in different successional stages in the study area.  $N=3$ . Values are in the form of mean  $\pm$  SE (standard error).

	Farmland (CK)	S1 (~10 year) pioneer weeds	S2 (~30 year) herbage	S3 (~60 year) shrub	S4 (~100 year) early forest	S5 (~160 year) climax forest
Fine root (g m <sup>-2</sup> )	–	123.2 $\pm$ 6.5	193.4 $\pm$ 12.3	256.9 $\pm$ 23.1	419.8 $\pm$ 32.7	501.1 $\pm$ 19.8
Clay (% <0.002 mm)	9.8 $\pm$ 0.9	11.4 $\pm$ 0.6	14.3 $\pm$ 0.5	13.2 $\pm$ 0.7	14.3 $\pm$ 0.8	15.6 $\pm$ 1.1
Silt (% 0.002–0.02 mm)	30.7 $\pm$ 1.3	31.2 $\pm$ 0.7	33.4 $\pm$ 0.8	34.6 $\pm$ 0.6	37.1 $\pm$ 0.9	37.3 $\pm$ 1.3
Sand (% 0.02–2 mm)	59.5 $\pm$ 0.6	57.4 $\pm$ 0.8	51.7 $\pm$ 0.7	52.2 $\pm$ 0.6	48.6 $\pm$ 0.5	47.1 $\pm$ 0.9
SOM (g kg <sup>-1</sup> )	18.62 $\pm$ 1.1	20.13 $\pm$ 0.9	26.90 $\pm$ 1.8	35.69 $\pm$ 2.1	45.34 $\pm$ 4.6	60.52 $\pm$ 9.7
TN (g kg <sup>-1</sup> )	0.61 $\pm$ 0.2	0.67 $\pm$ 0.3	0.75 $\pm$ 0.5	0.84 $\pm$ 0.4	0.89 $\pm$ 0.6	0.91 $\pm$ 0.5
C/N	18.5 $\pm$ 0.5	17.3 $\pm$ 0.6	20.8 $\pm$ 0.8	24.7 $\pm$ 0.4	29.5 $\pm$ 0.7	38.6 $\pm$ 0.6
SIC (%)	14.5 $\pm$ 0.6	14.9 $\pm$ 0.6	13.1 $\pm$ 0.5	12.6 $\pm$ 0.7	10.1 $\pm$ 0.4	10.2 $\pm$ 0.5
LOC (g kg <sup>-1</sup> )	3.8 $\pm$ 0.4	4.1 $\pm$ 0.5	5.7 $\pm$ 0.6	7.3 $\pm$ 0.8	8.2 $\pm$ 0.7	11.7 $\pm$ 1.4
NLOC (g kg <sup>-1</sup> )	7.0 $\pm$ 0.2	7.5 $\pm$ 0.5	9.9 $\pm$ 0.6	13.4 $\pm$ 1.2	18.1 $\pm$ 2.1	23.4 $\pm$ 4.5
BD (g cm <sup>-3</sup> )	1.22 $\pm$ 0.1	1.30 $\pm$ 0.2	1.18 $\pm$ 0.1	1.09 $\pm$ 0.1	0.87 $\pm$ 0.2	0.82 $\pm$ 0.4
SW (%)	10.1 $\pm$ 0.4	11.5 $\pm$ 0.3	13.3 $\pm$ 0.4	12.7 $\pm$ 0.5	14.5 $\pm$ 0.6	16.7 $\pm$ 1.3
pH	7.7 $\pm$ 0.3	7.5 $\pm$ 0.4	7.5 $\pm$ 0.6	7.3 $\pm$ 0.4	7.2 $\pm$ 0.3	7.2 $\pm$ 0.4
MBC (mg kg <sup>-1</sup> )	184.8 $\pm$ 23.5	74.5 $\pm$ 7.9	96.4 $\pm$ 11.7	100.1 $\pm$ 9.8	112.4 $\pm$ 12.5	102.7 $\pm$ 8.7
MBN (mg kg <sup>-1</sup> )	10.3 $\pm$ 1.5	2.9 $\pm$ 0.7	4.3 $\pm$ 0.6	2.5 $\pm$ 0.3	5.1 $\pm$ 0.5	5.1 $\pm$ 0.4
$\delta^{13}\text{C}$ (‰)	–23.02 $\pm$ 0.8	–24.94 $\pm$ 0.6	–25.23 $\pm$ 0.7	–25.56 $\pm$ 0.9	–25.53 $\pm$ 1.2	–25.86 $\pm$ 0.8

Note: SOM: soil organic matter; TN: soil total nitrogen; SIC: soil inorganic carbon; LOC: labile organic carbon; NLOC: non-labile organic carbon; BD: soil bulk density; SW: soil water content; MBC: microbial biomass C; MBN: microbial biomass N.

more than 200 years of cultivation history. The aboveground biomasses of crops were harvested and removed from the ground each year in the farmlands. Chemical fertilizers have been applied to the farmlands, but the rate of application varied from year to year. Maize was growing on the farmland when the samples were collected. Five plots were established at each community and the farmland site in August 2014. The size of the plots varied with the communities: 20 m × 20 m plots in each forest community; 5 m × 5 m plots in the shrub communities; and 2 m × 2 m plots in the herbaceous communities and farmland plots. To minimize the effects of site conditions on experimental results, all selected sites had a similar slope aspect, slope gradient, elevation, soil type and land-use history. The distance were not more than 50 m between two plots located in each community, and less than 1 km between two communities located in each sample area. The basic soil properties data are shown in Table 1.

Soil samples were collected with a 3-cm inner-diameter corer at five points: the four corners and center of the soil sampling sites in the six restoration stages as described above. The mineral soil layers of 0–20 cm were collected and mixed to make one sample for each layer. All soil samples were air-dried and sieved through a 2-mm screen, and prepared for total OC and  $\delta^{13}\text{C}$  analysis. Soil bulk density (BD) of each soil layer was measured using a soil bulk sampler with a 5-cm diameter and 5-cm-high stainless steel cutting ring with three replicates in each plot. In each plot, the ground litter was first removed and collected for measurement; then, one pit was dug to 20 cm depth in the center of the plot and three soil BD samples taken. The original volume of each soil core and its dry mass after oven-drying at 105 °C for 48 h were measured. To measure roots, soil sampling was repeated three times in 0–20 cm soil layers in the center of each plot using a 9-cm diameter root auger.

### 2.3. Sample analysis

SOC and  $\delta^{13}\text{C}$  values (in ‰ of Vienna PDB) were measured on ground and air-dried soil and litter samples. Root residues were carefully removed before grinding. SOC content was assayed by dichromate oxidation (Nelson and Sommers, 1982). The natural abundance of  $\delta^{13}\text{C}$  in the SOM was analyzed with an Elemental Analyser (Eurovector) coupled to an isotope ratio mass spectrometer (Delta plus, Thermo Fisher) at the State Key Laboratory of Loess and Quaternary Geology in the Institute of Earth Environment, Chinese Academy of Sciences. Two acetanilide standards were measured every 12 samples. Variations in the  $^{13}\text{C}/^{12}\text{C}$  ratios are reported relative to the Vienna PDB standard. Isotopic composition is expressed as:

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

where  $R$  is the molar ratio of  $^{13}\text{C}$  to  $^{12}\text{C}$  of the sample or the international PDB reference, respectively.

Soil labile organic carbon (LOC) was determined following the method of Vieira et al. (2007), and the concentration of non-labile organic carbon (NLOC) was calculated from the difference between total SOC and LOC concentrations (Zhao et al., 2015). Soil total nitrogen (TN) was assayed using the Kjeldahl method (Bremner, 1996). Soil water content (SW) was measured gravimetrically and expressed as a percentage of soil water to dry soil weight. Soil pH was determined using a soil:water ratio of 1:2.5 (PHSJ-4A pH acidometer, Shanghai, China). The soil particle sizes (clay, silt and sand content) were determined using the MasterSizer 2000 method (Malvern MasterSizer 2000, Worcestershire, UK). The roots at 0–20 cm were also sampled and oven-dried to measure root mass density (RD). Soil inorganic carbon (SIC) was analyzed using the

CM140 Total Inorganic Carbon Analyzer (UIC Inc., Rockdale, Illinois, USA). Microbial biomass C (MBC) and microbial biomass N (MBN) were measured by the fumigation extraction method (Vance et al., 1987).

### 2.4. Data calculation

The proportions of new soil OC ( $f_{\text{new}}$ ) and old soil OC ( $f_{\text{old}}$ ) were estimated based on the mass balance equations (Del Galdo et al., 2003):

$$f_{\text{new}} = \frac{(\delta_{\text{new}} - \delta_{\text{old}}) \times 100\%}{(\delta_{\text{veg}} - \delta_{\text{old}})} \quad (2)$$

$$f_{\text{old}} = 100 - f_{\text{new}} \quad (3)$$

where  $\delta_{\text{new}}$  is the  $\delta^{13}\text{C}$  value of the soil sample from current land use,  $\delta_{\text{old}}$  is the  $\delta^{13}\text{C}$  values of the soil sample previous to land-use change (or soil samples from the paired 'control' sites) and  $\delta_{\text{veg}}$  is the  $\delta^{13}\text{C}$  value of the mixed litter of current vegetation. Decomposition rate constants ( $k$ ) of soil OC were estimated using the following equations (Marin-Spiotta et al., 2009):

$$k = \frac{-\ln(C_t/C_0)}{t} \quad (4)$$

where  $C_0$  is the initial soil OC stock (soil OC stock in the reference sites),  $C_t$  is initial soil OC stock remaining (old C stock) at time  $t$  (year) since land-use change.

Although the rate of increase in new soil C and total SOC may not be constant over time since land-use change, the mean rate of increase could be calculated using the following equation (Li et al., 2012):

$$\text{Rate of increase in new soil C (or total SOC)} (\text{g m}^{-2} \text{ yr}^{-1}) = \frac{\Delta X}{\Delta t} \quad (5)$$

where  $\Delta X$  is the change of new soil OC (or total SOC) stocks following land-use change, and  $\Delta t$  represents years since conversion (year). The new soil OC stocks in deforestation and reforestation sites were calculated through multiplying the current total SOC stocks by the corresponding proportion of new soil OC.

The soil OC stocks were calculated based on the SOC concentration, soil thickness, and bulk density at each site (Guo and Gifford, 2002). The soil bulk density often changes after land-use change, therefore, many researchers have emphasized that the equivalent soil mass (ESM) approach for correcting bulk density changes should be made before comparing soil OC stocks in forest and cultivated soils (Murty et al., 2002; Lee et al., 2009; Don et al., 2011; Poeplau et al., 2011).

The corrected soil OC stocks were calculated in this study using the following equation (Lee et al., 2009; Poeplau et al., 2011):

$$\begin{aligned} \text{Soil OC}_{\text{corr}} \text{ stock} (\text{g m}^{-2}) &= \text{OC stock} \times \frac{\text{BD}_C}{\text{BD}_F} \times 10 \\ &= \text{BD}_C \times \text{SOC} \times D \times 10 \end{aligned} \quad (6)$$

where soil  $\text{OC}_{\text{corr}}$  stock is the corrected soil OC stock based on the equivalent soil mass at each site, OC stock is the uncorrected soil OC stock at each site,  $\text{BD}_C$  and  $\text{BD}_F$  are the bulk densities ( $\text{g cm}^{-3}$ ) of cultivated soils and each site of vegetation restoration, respectively,  $D$  is soil thickness (cm), and SOC is the SOC concentration in each site ( $\text{g kg}^{-1}$ ).

The soil OC sequestration was estimated using the following equation (Deng et al., 2014a):

$$\text{Carbon sequestration} (\text{Mg ha}^{-1}) : \Delta C_s = C_{\text{LU}_n} - C_{\text{LU}_0} \quad (7)$$

where  $C_{LU_n}$  is represent soil OC stocks at each vegetation restoration stage ( $\text{g m}^{-2}$ ), and  $C_{LU_0}$  is soil OC stocks at the initial stage of farmland (Maize lands in the study).

### 2.5. Statistical analysis

Differences between mean values were examined by a one-way analysis of variance (ANOVA), and before conducting the ANOVA procedure, all the data was performed the assumptions of normality and homogeneity of variance. Comparison among means was made using the least significant difference multiple range test, calculated at  $P < 0.05$ . Regression analysis was performed to estimate soil OC stocks and sequestrations, the proportions of new and old soil OC changes, the rates of total soil OC stocks increase,  $k$  and rates of new soil OC increase with restoration age after farmland abandonment. The linear regressions were fitted by either a linear, natural logarithm or exponential functions. The best fit was chosen according to the smallest residual mean square with significance level of  $P < 0.05$  (Yang et al., 2011). Correlation analysis was used to study the correlations among the different soil properties. Stepwise regressions using measured soil variables were performed to build empirical models and to identify the best independent factors affecting soil C sequestration, the proportion of new OC or old OC, soil C decomposition rates and rates of soil OC increase.

## 3. Results

### 3.1. Changes in soil OC stocks and OC sequestrations

Overall, soil OC stocks and OC sequestration in the surface 20 cm of soils were significantly increased along with the vegetation restoration since land-use change ( $P < 0.05$ , Fig. 2). In the first 10 years, soil OC stocks showed non-significant increasing

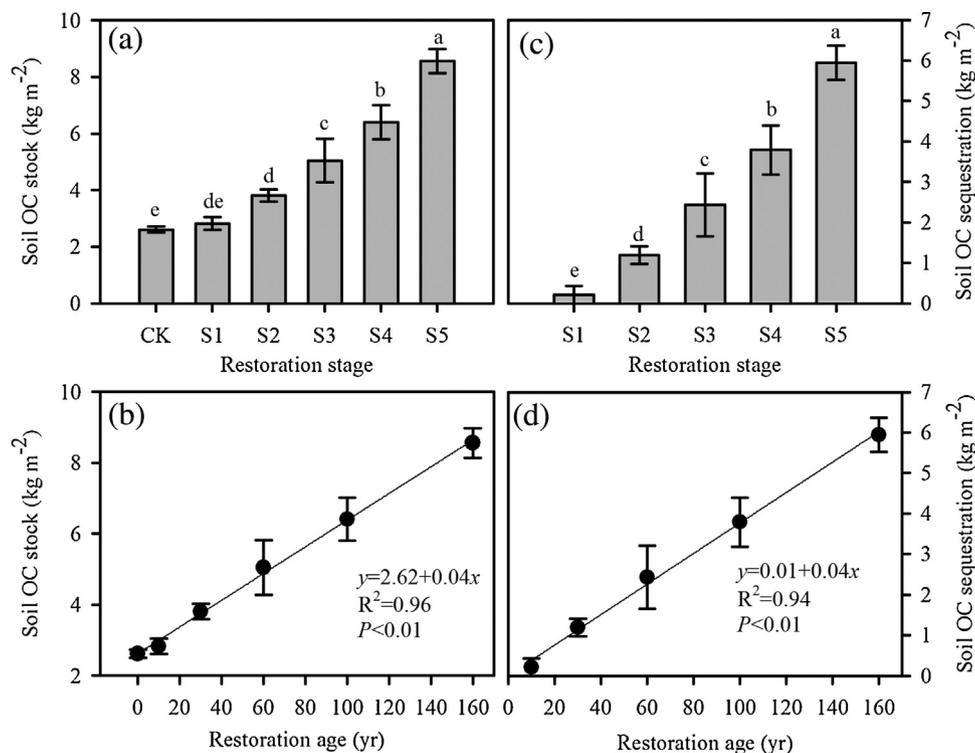
trends and then (>30 year) significantly increased compared to the initial level (farmland) ( $P < 0.05$ , Fig. 2a). Soil OC sequestrations significantly increased after grassland stages [pioneer weeds (S1) and herbage (S2)] (Fig. 2b). Soil OC sequestration achieved  $5.94 \text{ kg C m}^{-2}$  after ~160 years of vegetation restoration since land-use change (Fig. 2b).

The rates of soil OC sequestrations increased in the early 30 years, and then slightly decreased along with vegetation restoration, but the trend was not significant over the restoration age ( $P > 0.05$ , Fig. 3). Among the different restoration stages, the rates showed non-significant differences ( $P > 0.05$ , Fig. 3a), but the values were higher in the early stage (<30 year) of vegetation restoration than the latter (Fig. 3).

### 3.2. Changes in new and old soil OC

The proportions of old soil OC decreased, while the proportions of new soil C increased significantly with time since land-use change ( $P < 0.01$ , Fig. 4). The gain of new OC accounted for 78% of the total SOC stocks in the [Climax forest (S5)] stage (~160 year) after land-use change. The 'switch over' time – that is, years since land-use change when the proportions of new soil OC exceeded the proportions of old soil OC – was estimated from the logarithmic regression equations of the proportions of new and old soil OC vs. years since land-use change. The 'switch over' time was about 30 years (Fig. 4).

Soil OC decomposition rate was higher ( $k=0.04$ ) in the early stage (~10 year) after land-use change (Fig. 5a). It significantly declined along with the vegetation restoration ( $P < 0.01$ ), and soil OC decomposition rate showed a non-significant difference after 30 years of vegetation restoration. Similarly, the rate of new soil OC increase was also higher in the early stage (~10 year) after land-use change (Fig. 5b). It also significantly declined along with the vegetation restoration ( $P < 0.01$ ), but the rate of new soil OC

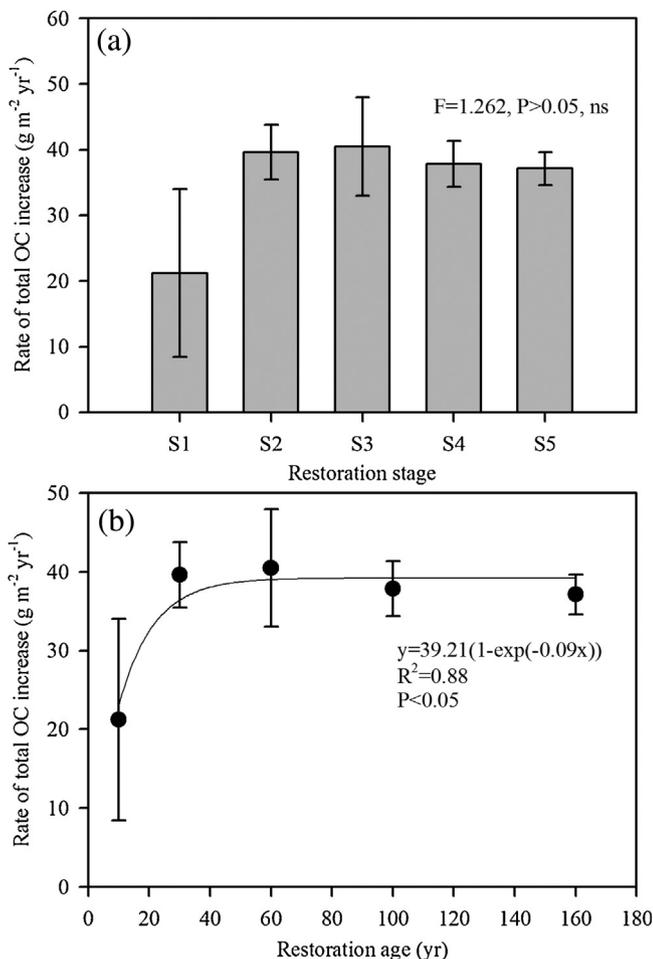


**Fig. 2.** Soil OC stocks (a) and sequestrations (c) in each restoration stage, and soil OC stocks (b) and sequestrations (d) changes over the time since land-use change. The Values represent the means of three area  $\pm$  standard error (SE). Different lower-case letters above the bars indicate significant differences at the different restoration stages ( $P < 0.05$ ). Note: S1, pioneer weeds; S2, herbage; S3, shrub; S4, early forest; S5, climax forest.

increase had significant differences among the different restoration stages. The rate of new soil OC increase ranged from 109.17 to 41.88  $\text{g m}^{-2} \text{ year}^{-1}$  in the early ( $\sim 10$  year) and later stages ( $\sim 160$  year), respectively.

### 3.3. Factors effects on soil OC stock patterns

Pearson's correlation analysis showed that soil OC sequestration were significantly correlated with roots, soil clay content, soil sand content, SOM, TN, C/N, SIC, LOC, NLOC, BD, SW and pH; the proportions of new and old soil OC were significantly correlated with roots, soil clay content, soil sand content, SOM, TN, C/N, SIC, LOC, NLOC, BD, SW and pH; soil OC decomposition rate constants were significantly correlated with soil fractions (clay, silt and sand); and the rates of new soil OC increase were significantly correlated with soil fractions (clay, silt and sand), TN, SIC, LOC, BD and MBC (Fig. 6). Moreover,  $\delta^{13}\text{C}$  were significantly correlated with soil OC sequestration, the proportions of new and old soil OC,  $k$  and the rates of new soil OC (Fig. 6). In addition, stepwise regression analysis showed that soil OC sequestration was mainly determined by C/N, the proportions of new and old soil OC were mainly determined by fine roots,  $k$  was mainly determined by soil silt content, and the rates of new soil OC increase were mainly determined by soil sand content (Table 2).



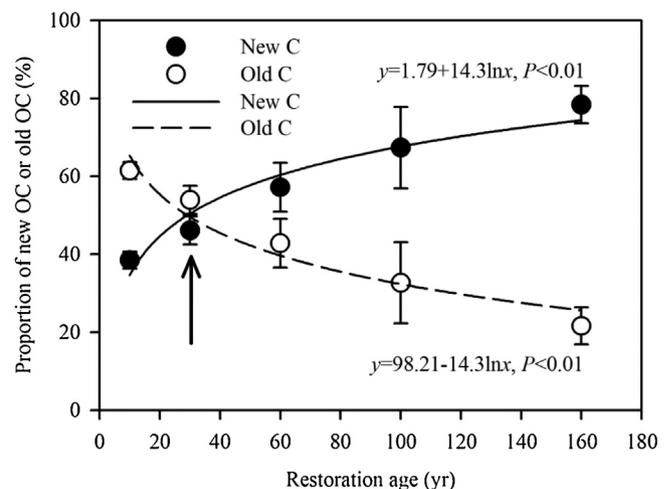
**Fig. 3.** Rates of total soil OC stocks increase in each restoration stage (a) and changes over time (b) since land-use change. The Values represent the means of three area  $\pm$  SE. ns, indicates no significant difference at the different restoration stages ( $P>0.05$ ). Note: S1, pioneer weeds; S2, herbage; S3, shrub; S4, early forest; S5, climax forest.

## 4. Discussion

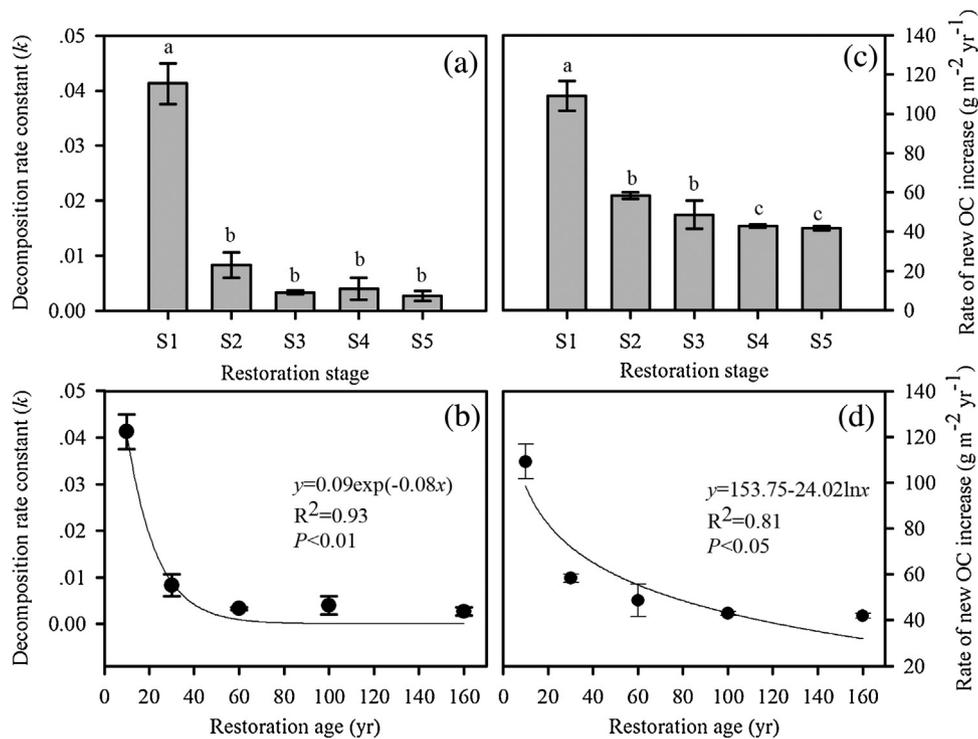
### 4.1. Dynamic patterns of soil OC stock changes

The plant community affects soil processes, which are correlated with successional plant dynamics (Woods, 2000). It is generally accepted that SOC increases over the period of succession (Yang et al., 2011; Deng et al., 2013; Zhao et al., 2015), although a few studies show limited OC change (Bonet, 2004). In our study, soil OC stocks and OC sequestrations in the surface 20 cm of soils significantly increased along with the natural vegetation restoration following land-use change ( $P<0.05$ , Fig. 2). This observation was consistent with a previous study (Deng et al., 2013). This is probably because: (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 (Laganière 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 (Blanco-Canqui and Lal, 2004); and (3) the lower SOC concentrations of farmland under conventional tillage may be due to OC loss resulting from soil erosion, higher organic matter decomposition associated with aggregate disruption and/or OC input reduction caused by continuous removal of crop residues (Saha et al., 2014).

In addition, we found that the rates of total soil OC increase decreased along with vegetation restoration (Fig. 3). In other research in the Loess Plateau, An et al. (2009) found that soil nutrients and microbial properties all increased very quickly in the earlier vegetation restoration stage lasting as long as 23 years, and were stable without significant fluctuation in later years. Soil microorganisms increase following the availability of increased organic inputs from revegetation (Jangid et al., 2011). Soil nutrients and organic matter probably increase following increases in soil microbes and may explain the observed changes in soil carbon sequestration rates. Zhang et al. (2015) also reported the OC sequestration rate was greatest in earlier stages of restoration. The possible mechanism was because the soil carbon stock of farmland is very low, thus when farmland was abandoned and vegetation began to grow, the input of new carbon increased significantly which has a higher weight in soil OC stock. However, as vegetation succession advances, the weight of new carbon input decreases gradually. Thus SOM input and output reached a balance, and so



**Fig. 4.** Changes in the proportions of new and old soil OC in soils with time since land-use change. The values represent the means of three area  $\pm$  SE. The arrows indicated the 'switch over' time.



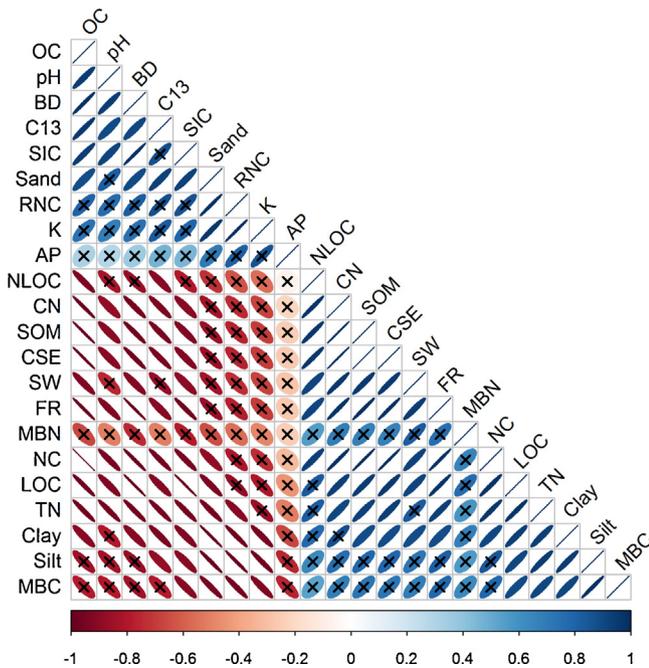
**Fig. 5.** Soil OC decomposition rate constants ( $k$ ) and rates of new soil C increase ( $\text{g m}^{-2} \text{ year}^{-1}$ ) in each restoration stage and changes over time since land-use change. The values represent the means of three area  $\pm$  SE. Different lower-case letters above the bars indicate significant differences at the different restoration stages ( $P < 0.05$ ). Note: S1, pioneer weeds; S2, herbage; S3, shrub; S4, early forest; S5, climax forest.

soil OC sequestration rate declined compared to the early stage of vegetation restoration.

#### 4.2. Changes in proportions of new and old soil OC following land-use change

The alteration in proportions of new and old soil OC showed the dynamics of the source of organic OC in soils, and hence could provide useful information about SOC dynamics after land-use change (Zhang et al., 2015). In our study, the proportion of new soil OC increased following land-use change after farmland abandonment, while old soil OC showed an opposite trend (Fig. 4), and the proportions of new and old soil OC vs. years since land-use change showed a significant logarithmic relationship ( $P < 0.01$ ) (Fig. 4). This indicated that time since land-use change was an important factor determining the proportions of new and old OC in soils (Marin-Spiotta et al., 2009; Zhang et al., 2015). The increase in proportion of new OC in soils could be attributed to the OC inputs from the new vegetation, which produced organic matter with different <sup>13</sup>C/<sup>12</sup>C ratio (Marin-Spiotta et al., 2009). Following land-use change, the litter input from the former vegetation ceased and was replaced by litter from new vegetation, while soil OC derived from the former litter would be decomposed and mineralized by microbes and soil enzymes (Zhang et al., 2015). Also Richter et al. (1999) reported that the rates of new soil OC increase represented the net effect of new OC input (three main processes: litterfall, rhizo-deposition and hydrological leaching of dissolved OC) and output (organic matter mineralization) to soils.

Our logarithmic regression models suggested that the proportion of old OC in soils after land-use change declined rapidly in the initial decades (S1, <10 year), followed by a relatively slow decline (Fig. 4). The rapid decrease of proportion old OC in soils at early stages could be attributed to the rapid loss of old OC or rapid gain of new OC (Zhang et al., 2015). Moreover, in our study, we found that soil OC decomposition rate was higher ( $k = 0.04$ ) in the early stage



**Fig. 6.** Correlation matrix among the different properties determined.  $N = 5$ . Note: ‘x’ indicates correlation is non-significant ( $P > 0.05$ ); blue indicates positive correlations and red indicates negative.  $k$ , soil OC decomposition rate constants; RNC, rates of new soil OC increase ( $\text{g m}^{-2} \text{ year}^{-1}$ ); Sand, soil sand content (%); SIC, soil inorganic carbon ( $\text{g kg}^{-1}$ ); C13,  $\delta^{13}\text{C}$ ; BD, soil bulk density ( $\text{g cm}^{-3}$ ); pH, soil pH; OC, the proportions of old soil OC (%); MBC, microbial biomass carbon ( $\text{mg kg}^{-1}$ ); TN, soil total N ( $\text{g kg}^{-1}$ ); LOC, labile organic carbon ( $\text{g kg}^{-1}$ ); CSE, soil OC sequestration ( $\text{kg m}^{-2}$ ); MBN, microbial biomass N ( $\text{mg kg}^{-1}$ ); NC, the proportions of new soil OC (%); FR, fine root ( $\text{g m}^{-2}$ ); SW, soil water content (%); SOM, soil organic matter ( $\text{g kg}^{-1}$ ); CN, C/N ratio; NLOC, non-labile organic carbon ( $\text{g kg}^{-1}$ ).

**Table 2**  
Summary of stepwise regression models of measured soil variables with determining factors following vegetation restoration.

	Models	R <sup>2</sup>	Sig. (P)	N
Soil OC sequestration (kg m <sup>-2</sup> )	$\Delta C_s = 0.27 \times C/N - 4.38$	0.951	0.000***	5
New OC (%)	$NC = 0.10 \times FR + 27.48$	0.977	0.001**	5
Old OC (%)	$OC = 0.10 \times FR + 72.58$	0.977	0.001**	5
OC decomposition rate constant (k)	$k = -0.01 \times \text{silt} + 0.73$	0.901	0.009**	5
Rate of new OC increase (g m <sup>-2</sup> year <sup>-1</sup> )	$RNC = 6.69 \times \text{sand} - 42.30$	0.963	0.002**	5

Note:  $\Delta C_s$ : soil C sequestration; NC: the proportions of new soil OC; OC: the proportions of old soil OC; k: soil OC decomposition rate constants; RNC: rates of new soil OC increase; FR: fine root (g m<sup>-2</sup>). Note: \*\*\* indicates significant at  $P < 0.001$ , and \*\* at  $P < 0.01$ .

(~10 year) after land-use change, and significantly declined along with the vegetation restoration ( $P < 0.01$ ); similarly, the rate of new soil OC increase was also higher in the early stage (~10 year) after land-use change (Fig. 5). The rapid decreases in old soil OC in the early stage following vegetation restoration could be because the protection of SOM had not yet been restored or well reestablished (Paul et al., 2002; Zhang et al., 2015). Helfrich et al. (2006) found that the organic matter from crops had higher proportions of O-alkyl-C and lower contents of alkyl-C, aryl-C and carbonyl-C compared with organic materials from grass; therefore, the organic matter from maize decomposes much more rapidly than that from grass. In addition, after land-use change, the old soil OC derived from initial soil OC before land use conversion would be gradually broken down by soil microbes, especially in the light fractions of soil OC (Wei et al., 2014; Qiu et al., 2015). Our results showed that it took about 30 years after farmland conversion for the amount of new soil OC to reach the level of the old OC in 0–20 cm of soil (Fig. 4). Zhang et al. (2015) found that the 'switch over' time was 45.4 years for reforestation in the global forest. Through analyzing global reforestation sites, Zhang et al. (2015) revealed a rapid accumulation of new SOC of 139.9, 55.0 and 50.3 g m<sup>-2</sup> year<sup>-1</sup> in tropical, subtropical and temperate regions, respectively. In our study, the rate of new soil C increase ranged from 109.17 to 41.88 g m<sup>-2</sup> year<sup>-1</sup> in the early (~10 year) and later stages, respectively (Fig. 5). These results suggest that new OC derived from plants played important roles in supplementing soil OC after farmland conversion.

#### 4.3. Factors controls over soil C stocks patterns

Soil OC stock is affected by numerous soil chemical and physical properties (Wynn et al., 2006; Zhang et al., 2011; Wang et al., 2013). In the present study, soil OC sequestration was significantly positively correlated with soil clay content, but negatively with soil sand content (Fig. 6). Wang et al. (2013) demonstrated that soil OC storage was positively correlated with silt content and negatively with sand content in 0–40 cm of soil. This confirms that fine-textured soils contain higher soil OC across ecosystems. Generally, organic matter breaks down faster in sandy than in fine-textured soils under similar environmental conditions (Thomsen et al., 1999; Wang et al., 2013). Our study also showed that soil OC decomposition rate constants were significantly positively correlated with soil sand contents, and significantly negatively correlated with fine-textured soil fractions (silt and sand) (Fig. 6). In contrast, the rates of new soil OC increase were significantly positively correlated with soil clay content, and significantly negatively correlated with soil sand content (Fig. 6).

Soil C stocks had close relationships with SOM/SOC and TN in many previous studies (Wynn et al., 2006; Awiti et al., 2008; Zhang et al., 2011; Saha et al., 2014; Zhao et al., 2015) as also found in the present study. Additionally, we found that soil OC sequestration had significant positive correlations with C/N following vegetation

restoration (Fig. 6). This was similar to our previous study in the same study area, in which soil C/N increased with long-term vegetation restoration (Deng et al., 2013). Different soil processes (e.g., soil OC and N cycles) can be influenced by different plant traits and stand properties due to different tree species, and tree species influence the release of nutrients to soil via mineralization (Mueller et al., 2012), which is probably due to the increased influence of forest litter on SOM quality (Ussiri et al., 2006). Wang et al. (2011) reported that significantly increased C/N probably resulted from increased SOC but decreased N, which indicated that accumulated soil OC was less readily broken down once N shortages occurred, and vice versa. Also, in our study, the proportions of new soil OC had significant positive correlations with C/N, indicating that the increase of soil OC may be because the rate of new OC input into soils was much higher than the rate of new N input, because soil OC and N both increased following vegetation restoration (Table 1).

SOC plays a very important role in determining the variation of SIC as shown by significant negative correlations with SIC (Jelinski and Kucharik, 2009; Chang et al., 2012). Moreover, in our study, soil OC sequestration was significantly negatively correlated with SIC following vegetation restoration (Fig. 6). Sartori et al. (2007) reported that the accumulation of SOC in restoration vegetation could induce the increase of carbonic and organic acid production, which reduces the availability of soil calcium through the soil cation exchange—overall, these would increase the dissolution and leaching of carbonate of the topsoil and cause a decrease of SIC. In addition, in our study, soil OC sequestration was significantly positively correlated with LOC and NLOC (Fig. 6), consistent with a previous study of Zhao et al. (2015) in the same area. Zhao et al. (2015) also reported that the LOC displayed higher sensitivity than NLOC and SOC to both successional stage and soil depth. The increases in LOC and NLOC fractions may be related to organic C input from plant litter and roots, respectively (Sierra et al., 2013; Zhao et al., 2015). The contents of lignin and other recalcitrant compounds (e.g., tannins) (Kraus et al., 2003) are generally higher in plant roots than in leaf litter, which contributes to the chemical recalcitrance of SOC (Sierra et al., 2013), so we can conclude that the increase in new soil OC mainly come from the increase in LOC.

Many previous studies found that soil OC stocks were negatively correlated with BD and pH, and positively with SW, following vegetation restoration (Huang et al., 2011; Bach et al., 2012; Deng et al., 2013, 2014c)—consistent with our present study. Moreover, soil OC sequestration and the proportions of new soil OC were both significantly positively correlated with roots biomass in the present study (Fig. 6), indicating that primary productivity was the main driver of soil OC sequestration (De Deyn et al., 2008), and belowground biomass (dead roots, mycorrhizae and exudates) was an important element of soil OC sequestration (Langley and Hungate, 2003). However, they had non-significant correlations with MBC, indicating that soil microbes were not the key contributing factor to soil OC increases, instead plant roots played

a key role in determining the soil OC increase in topsoil. In addition, stepwise regression analysis showed that soil C/N is the most factor to effect on soil OC sequestration following vegetation restoration. Luo et al. (2004, 2006) have reported that N dynamics are a key factor in the regulation of long-term terrestrial C sequestration, and C–N interactions are very important in determining whether the C sink in land ecosystems can be sustained over the long term. Those suggested soil OC sequestration was related to soil C/N following vegetation restoration. Our stepwise regression analysis also showed the proportion of new soil OC was mainly determined by fine roots,  $k$  were mainly determined by soil silt content, and the rates of new soil OC increase were mainly determined by soil sand content that these observations were made out of considering many other soil properties (Table 2).

## 5. Conclusions

In the present study, soil OC stocks and OC sequestrations in the surface 20 cm of soils significantly increased along with the natural vegetation restoration ( $P < 0.05$ ). The rates of total soil OC increase declined along with vegetation restoration. The proportion of new soil OC increased while the old soil OC showed an opposite trend following land-use change after farmland abandonment. Moreover, we found that soil OC decomposition rate was higher ( $k = 0.04$ ) in the early stage ( $\sim 10$  year) after land-use change, and significantly declined along with the vegetation restoration ( $P < 0.01$ ). And the rate of new soil OC increase was also higher in the early stage ( $\sim 10$  year) following land-use change. The rate of new soil OC increase ranged from 110.18 to 28.17  $\text{g m}^{-2} \text{ year}^{-1}$  in the early ( $\sim 10$  year) and later stages ( $\sim 160$  year), respectively. It took about 30 years for the amount of new soil OC to reach the same level as old OC in 0–20 cm of soil after farmland conversion. Our results suggested that soil OC sequestration was mainly determined by C/N, the proportion of new soil OC was mainly determined by fine roots,  $k$  were mainly determined by soil silt content, and the rates of new soil OC increase were mainly determined by soil sand content that these observations were made out of considering many other soil properties.

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