

SOIL ORGANIC CARBON AND INORGANIC CARBON ACCUMULATION ALONG A 30-YEAR GRASSLAND RESTORATION CHRONOSEQUENCE IN SEMI-ARID REGIONS (CHINA)

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ABSTRACT

Carbon accumulation is an important research topic for grassland restoration. It is requisite to determine the dynamics of the soil carbon pools [soil organic carbon (SOC) and soil inorganic carbon (SIC)] for understanding regional carbon budgets. In this study, we chose a grassland restoration chronosequence (cropland, 0 years; grasslands restored for 5, 15 and 30 years, i.e. RG5, RG15 and RG30, respectively) to compare the SOC and SIC pools in different soil profiles. Our results showed that SOC stock in the 0- to 100-cm soil layer showed an initial decrease in RG5 and then an increase to net C gains in RG15 and RG30. Because of a decrease in the SIC stock, the percentage of SOC stock in the total soil C pool increased across the chronosequence. The SIC stock decreased at a rate of $0.75 \text{ Mg hm}^{-2} \text{ y}^{-1}$. The change of SOC was higher in the surface (0–10 cm, $0.40 \text{ Mg hm}^{-2} \text{ y}^{-1}$) than in the deeper soil (10–100 cm, $0.33 \text{ Mg hm}^{-2} \text{ y}^{-1}$) in RG5. The accumulation of C commenced >5 years after cropland conversion. Although the SIC content decreased, the SIC stock still represented a larger percentage of the soil C pool. Moreover, the soil total carbon showed an increasing trend during grassland restoration. Our results indicated that the soil C sequestration featured an increase in SOC, offsetting the decrease in SIC at the depth of 0–100 cm in the restored grasslands. Therefore, we suggest that both SOC and SIC should be considered during grassland restoration in semi-arid regions. Copyright © 2016 John Wiley & Sons, Ltd.

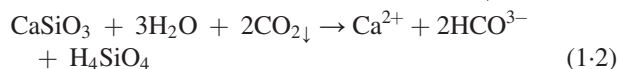
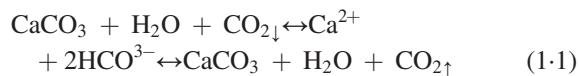
KEY WORDS: soil carbon pool; soil inorganic carbon; profile distribution; grassland restoration; semi-arid regions

INTRODUCTION

Global warming has become an important topic throughout the world. Finding biotic and abiotic approaches to mitigating the greenhouse gas concentrations in the atmosphere is an urgent scientific problem (Wang *et al.*, 2013). Terrestrial ecosystems have been considered as an immense potential to mitigate the rising global atmospheric CO₂ concentrations (Kicklighter *et al.*, 2008; Reich *et al.*, 2013). The global soil C pool contains 2,500 Gt C (1 Gt = 10⁹ t), which includes approximately 62% soil organic carbon (SOC) and 38% soil inorganic carbon (SIC), and is approximately 3.3 and 4.5 times the size of the atmospheric and biotic C pool, respectively (Lal 2004). Therefore, to estimate the soil C pool in terrestrial ecosystems, both SOC and SIC should be considered. Moreover, the mechanisms controlling the soil C pool along grassland restoration in semi-arid regions are still poorly understood.

The formation of SOC mainly comes from the decomposition of biotic residues. In recent decades, most previous studies have focused on SOC mainly because of its rapid turnover response to the effects of climate and land use

changes (Jobbágy & Jackson 2000; Degryze *et al.*, 2004; Axel *et al.*, 2011; Deng *et al.*, 2014b; Deng *et al.*, 2014a; Köchy *et al.*, 2015). Additionally, SOC represents a greater fraction of the C content than SIC, and SOC influences the soil adsorption of CO₂ and soil density distribution (Tan *et al.*, 2014). Compared with SOC, SIC has a long turnover time and it is the dominant form of C in arid and semi-arid climates (Mielnick *et al.*, 2005; Mi *et al.*, 2008; He *et al.*, 2016). Most SIC accumulates as carbonate minerals, predominantly calcium carbonate (CaCO₃) and dolomite (MgCO₃), in arid and semi-arid climates conditions (Schlesinger 2002) and plays an essential role in regional C budgets. The SIC is divided into primary carbonates (lithogenic inorganic C from the soil parent material with no chemical changes) and secondary deposited carbonate (pedogenic inorganic C formed through the dissolution and precipitation of carbonate parent material and derived from the weathering of calcium silicate) (Tan *et al.*, 2014). The formation of pedogenic inorganic C can be expressed through a series of chemical equations:



Atmospheric CO₂ is consumed when carbonate is dissolved and an equal amount of CO₂ is released when

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carbonate is re-deposited (Equation 1-1) (Wu *et al.*, 2009). The pedogenic inorganic C originates from the weathering of calcium silicate dissolution fixes two units of atmospheric CO₂ (Equation 1-2) and releases one unit of CO₂ through secondary carbonate deposition (Equation 1-1). Therefore, this process can sequester atmospheric CO₂ in the soil.

Many factors affect C pool dynamics in the terrestrial ecosystem. For instance, all global models have predicated a positive feedback between soil C cycling and climate warming (Friedlingstein *et al.*, 2006). According to 11 coupled climate change-carbon cycle models, the additional CO₂ buildup in the atmosphere triggered by the climate-carbon feedback leads to an additional climate warming of 0.1–1.5°C (Luo 2007). Cultivation has led to C loss for more than 40 years, with a total C loss of approximately 51% in the surface 0.1 m of soil in Australian agro-ecosystems (Luo *et al.*, 2010; Mueller *et al.*, 2013). Through differences in allocation, plant functional types help to control soil C distributions with depth in the soil (Jobbágy & Jackson 2000; Mueller *et al.*, 2013; Hassan *et al.*, 2016). The soil C pool is most sensitive to land use, which can change various ecological factors (Parras-Alcántara *et al.*, 2015; Wasak and Drewnik 2015; Hombegowda *et al.*, 2016), such as vegetation productivity (Kukul *et al.*, 2016; Wu *et al.*, 2016), soil physicochemical properties (Degryze *et al.*, 2004; Gelaw *et al.*, 2015; Choudhury *et al.*, 2016), and microbial community properties (Mabuhay *et al.*, 2006; Mukhopadhyay *et al.*, 2016), which directly affect soil C pool. In addition, many studies have come to a consensus that natural vegetation restoration is highly beneficial to soil C sequestration in degraded ecosystems (He *et al.*, 2016). Grazing prohibitions in degraded grasslands and the conversion of cropland to abandoned fields increased the C content by 34% and 62% on average in northern China (Wang *et al.*, 2011). Nevertheless, the net ecosystems exchange of C fluxes in severely degraded grassland was 33–6% lower than in slightly degraded grassland (Peng *et al.*, 2015). The factors affecting soil C are complex and changeable. Therefore, accurately assessing the dynamics and controls of the soil C pool is important for estimating the regional C budget (García-Díaz *et al.*, 2016).

A series of ecological programmes have been launched to control the soil erosion on the Loess Plateau in the last several decades. The Loess Plateau features an arid and semi-arid climate and is known to be suffering from the most serious erosion problems in the world. Many studies have reported the soil pool dynamics, but most of studies have focused only on SOC (Dang *et al.*, 2014; Deng *et al.*, 2014b) or SIC (Mi *et al.*, 2008; Liu *et al.*, 2014; Tan *et al.*, 2014; Wang *et al.*, 2016). Thus, less attention has been devoted to considering both the SOC and SIC in soil profiles on the Loess Plateau (Chang *et al.*, 2012; He *et al.*, 2016). In addition to SOC, the SIC comprises approximately a third of the global C pool in soils (Hirmas *et al.*, 2010). Based on the latest soil profile data the present-day SIC storage in China was estimated at approximately 55.3 ± 10.7 Pg C with an average C content was

6.3 ± 1.2 kg C m⁻² (Wu *et al.*, 2009). This represented approximately 5–8% of all the estimated SIC in the world; meanwhile, the Loess Plateau has the highest SIC content in China (Wu *et al.*, 2009). The mean SIC density is 17.04 kg C m⁻², which is about three times as much as the national average (Wang *et al.*, 2016). Therefore, accurately estimating the dynamics of both the SOC and SIC stocks and understanding the process of C cycling in soils are critical for modelling and regulating the terrestrial C budget on the Loess Plateau. In this study, we evaluated the vertical distribution and soil carbon dynamics along a 30-year grassland restoration chronosequence on the Loess Plateau of China. The aim is to answer the following questions: How are the SOC and SIC stocks distributed in vertical soil profiles along a 30-year grassland restoration chronosequence? What changes occur in the SOC, SIC and STC pools along a grassland restoration chronosequence?

MATERIAL AND METHODS

Study Site

The study area is located in the Wangdonggou watershed (107°41'E, 35°14'N, 1,120 m), at a field station of the Chinese Ecology Research Net in Changwu County, Shaanxi Province, China. This watershed is representative of the gully terrain typical of the Loess Plateau. Based on climate data from 1984 to 2005, the mean annual precipitation is 584 mm, of which nearly 52% occurs between July and September. The mean annual temperature is 9.1°C. The soil is a coarse-textured dark loess soil (Liu *et al.*, 2016).

We verified the restoration time by interviewing local elders. Because there is no historical record of changes in most soil properties because of grassland restoration during the past 30 years, the 'space-for-time' substitution technique has become the main method to study the evolution of ecosystem properties over time. Based on the process of plant succession in the study area, we studied three restoration treatments (RG5, RG15 and RG30) that have been enclosed for 5, 15 and 30 years, respectively, thereby allowing the restoration of natural vegetation. The dominant species in all the communities evaluated was *Agropyron cristatum*, and the main companion species were *Potentilla acaulis* and *Poa subfastigiata* in all restoration grassland (Liu *et al.*, 2016). Another site, 1,000 m from the natural restoration grassland, the cropland planted with crops (maize) represented the initial conditions. The croplands have planted maize for many years (about 30 years by local farmer, Mr. Yucheng Li, Changwu Agro-ecological Experiment Station of the Chinese Academy of Sciences, Changwu County, Shaanxi, NW, China). The seeding period and harvest period of the maize were mid-April and mid-September, respectively. Monitoring of the soil carbon pool along a chronosequence under similar soil and climate conditions is a basic approach studying soil changes over periods of natural restoration (Wang *et al.*, 2015). For minimization

of the effects of varying site locations on the experimental results, all the selected sites had similar topographical conditions in our experiment.

Experimental Design and Sampling

Three blocks (50 × 50 m) were selected for each stage of restoration, three plots (10 × 10 m) were arranged in each block, and five quadrats (1.0 m × 1.0 m) were randomly chosen in each plot in September 2012. The aboveground biomass was determined by clipping the plants to ground level, and litter (dead plant material) was cleared before soil sampling in each quadrat (Liu *et al.*, 2016).

Soil samples were collected with a soil drilling sampler (9 cm i.d.) corer at the four corners and the centre of each quadrat. The samples in soil layer was collected at intervals of 0–5, 5–10, 10–20, 20–30, 30–50, 50–70 and 70–100 cm. The samples from the same layer were mixed to produce one sample in a quadrat. All the soil samples were air dried and sieved through a 2-mm screen, and roots and other debris were removed by hand. The bulk density (BD) of each soil layer was measured using a soil bulk sampler with a 5-cm-diameter and 5-cm-long (100 cm³) stainless steel cutting ring, with three replicates in each quadrat. The samples were oven-dried at 105°C to constant weight and weighted. Soil pH was determined on three subsamples from each depth interval of each quadrat, using a soil/water mass ratio of 1:2.5. The SOC content was measured using the dichromate oxidation method (Walkley and Black 1934). The soil total carbon content was analyzed by the dry combustion (Nelson & Sommers 1982). The SIC content was calculated by the difference between soil total carbon and SOC content. All the analyses in the same soil sampling were repeated five times.

Relative Calculation

The SOC and SIC storage (Mg hm⁻²) was calculated as follows (Liu *et al.*, 2014):

$$\text{SOC storage} = \sum_{i=1}^n D_i \times B_i \times \text{SOC}_i \quad (2.1)$$

$$\text{SIC storage} = \sum_{i=1}^n D_i \times B_i \times \text{SIC}_i \quad (2.2)$$

Where: n is the number of soil layers, D_i is the soil depth (cm), B_i is the soil bulk density (g cm⁻³), SOC_i and SIC_i are the SOC content and SIC content (%) at the depth of i .

Then, the soil total carbon (STC) storage can be calculated as follows:

$$\text{STC storage} = \text{SOC storage} + \text{SIC storage} \quad (2.3)$$

We calculated the changes in soil C stocks (C change, Mg hm⁻²) as follows:

$$\text{C change} = C_t - C_0 \quad (2.4)$$

Where: C_t represents soil storage in the restored grassland (Mg hm⁻²), and C_0 is the soil storage in the cropland (Mg hm⁻²).

Then, the rate of soil carbon stock change (RSS, Mg hm⁻² y⁻¹) is estimated depending on the changes in the soil C stocks over different restoration times. A linear regression equation between C change and time (Deng *et al.*, 2014a) is presented as follows:

$$\text{C change} = f(\Delta\text{time}) = y_0 + k \times \Delta\text{time} \quad (2.5)$$

Thus, the rate of C stock change is as follows:

$$\text{RSS} = f' \Delta\text{time} = df(\Delta\text{time}) / d \Delta\text{time} = k \quad (2.6)$$

Where: y_0 is a constant; k is the rate of soil C stock change (Mg hm⁻² y⁻¹) and Δtime is the time interval (years) from cropland (0 year) to restored grassland.

Statistical Analyses

A two-way ANOVA followed by the Tukey's HSD test was used to analyze the differences in the BD, SWC, pH, SOC, SIC and stocks among the different restoration times and vertical profiles. The differences were evaluated at the 5% significance level. Linear regression analysis was adopted to determine the relationships between SOC, SIC, total carbon storage and restoration time. All statistical analyses were performed using the software programme SPSS, Version 18.0 (SPSS Inc.).

RESULTS

Soil Bulk Density, Soil Moisture and pH

Results from two-way ANOVA showed that the restoration time and soil depth both significantly affected soil bulk density and soil moisture (Table I). The soil bulk density fluctuated slightly at the soil depth of 0–100 cm across the 30-year grassland restoration chronosequence (Tables I and II). The minimum values of soil bulk density occurred in RG15 at the depths of 0–30 cm (the average is 1.20 g cm⁻³) and the maximum values occurred in RG5 at the depths of 30–100 cm (the average is 1.50 g cm⁻³). The changes in SWC varied between top layers and deep layers (Table II). The SWC in the restored grassland (23.54%) was significantly higher than that in the cropland (21.27%; $p < 0.05$) at the depths of 0–30 cm, whereas the SWC in the cropland (23.13%) was generally higher than that in the restored grasslands (22.27%) at the depths of 30–100 cm. Soil pH decreased significantly with grassland restoration at the soil depth of 0–100 cm, but the changes of pH were not obvious in different soil layers (Table I). Soil pH was 8.07 in cropland, which is significantly higher than 7.67 in RG30 at the depths of 0–100 cm (Table II).

Soil Organic Carbon Content and Soil Inorganic Carbon Stock

The SOC significantly increased (0.49% in cropland and 0.79% in RG30) at the depths of 0–100 cm across the 30-year grassland restoration chronosequence (Table II). Two-way ANOVA showed that the restoration time and soil depth both significantly affected SOC content and stock (Table I).

Table I. Two-way ANOVA F and *p*-values for the effects of restoration time (T), soil depth (D) and the interaction of T and D (T*D) on soil BD (g cm^{-3}), SWC (%), pH, SOC content (%), SIC content (%), SOCS (Mg hm^{-2}) and SICS (Mg hm^{-2})

	BD		SWC		pH		SOC		SIC		SOCS		SICS	
	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>
T	19.72	<0.01	12.51	<0.01	380.55	<0.01	65.5	<0.01	108.12	<0.01	43.54	<0.01	9.88	<0.01
D	13.01	<0.01	5.86	<0.01	2.27	0.04	134.72	<0.01	7.85	<0.01	44.52	<0.01	444.6	<0.01
T*D	3.37	<0.01	2.232	0.01	0.262	0.99	5.787	<0.01	2.122	0.01	4.77	<0.01	0.353	0.98

ANOVA, analysis of variance; BD, bulk density; SWC, soil water content; SOC, soil organic carbon; SIC, soil inorganic carbon; SE, standard error; SOCS, SOC stock; SICS, SIC stock.

SOC showed a decrease with the soil depth in all restoration grassland. The maximal value is 1.12% at the depth of 0–5 cm, and the minimum value is 0.37% at the depth of 70–100 cm. The SOC stock showed a decrease in the soil depths of 0 to 100 cm in RG5 compared with cropland and then significantly increased after 5 years of restoration ($p < 0.05$; Figure 1).

Soil Inorganic Carbon Content and Soil Inorganic Carbon Stock

Compared with cropland (1.64%), the SIC was significantly lower in the restored grasslands (1.29%) at the depths of 0–100 cm ($p < 0.05$; Table II). SIC decreased with the restoration time at the depth of 0–100 cm. Two-way ANOVA also showed that the restoration time and soil depth both significantly affected SIC content and stock (Table I). The SIC stock decreased significantly in the soil layers of 0–30 cm, and fluctuated slightly in the soil layers of 30 to 100 cm, and generally decreased across the 30-year grassland restoration chronosequence ($p < 0.05$; Figure 1).

Rate Change in Soil Organic Carbon and Soil Inorganic Carbon

Among the depth segments, the SOC stock increased significantly in the upper 10 cm of soil after 5 years of restoration ($p < 0.05$), which resulted in a carbon storage of approximately 3.97 Mg hm^{-2} at a sequestration rate of $0.40 \text{ Mg hm}^{-2} \text{ y}^{-1}$ (Figures 2a and 3a). The SOC stock strongly decreased below 10 cm depth, which led to a SOC loss of 8.26 Mg hm^{-2} at a rate of $0.33 \text{ Mg hm}^{-2} \text{ y}^{-1}$ at depths of 10–100 cm after 5 years of restoration. Overall, the SOC stocks increased by the total of 11.74 and 30.43 Mg hm^{-2} at the depth of 0- to 100-cm soil after 15 and 30 years, respectively. The SIC concentration decreased at depths of 0–100 cm across the 30-year restoration chronosequence and lost 37.62, 41.21 and 32.69 Mg hm^{-2} after restoration periods of 5, 15 and 30 years, respectively, and resulting in loss rates of 1.07, 0.39 and $0.16 \text{ Mg hm}^{-2} \text{ y}^{-1}$, respectively (Figures 2b and 3b).

Total Carbon Stock Percentages of the Soil Organic Carbon and Soil Inorganic Carbon

The percentage of the soil C pool represented by the SOC stock increased across the chronosequence, with a mean value ranging from 20.59% in cropland to 32.24% in the 30-year treatment (Figure 4). Compared with SOC, the percentage of SIC stock showed the opposite trend. The total C stock and SIC stock did not significantly increase (Figure 5 b, c), but the SOC stock significantly increased ($p < 0.05$) at depths of 0 to 100 cm along the restoration chronosequence (Figure 5d). SOC stock significantly increased ($p < 0.05$) at 0–5 and 30–50 cm, but not for others depths (Figure 5a, b). At soil depths of 0–100 cm across the 30-year grassland restoration, the SOC stock ranged from 55.25 to 85.67 Mg hm^{-2} , and the SIC stock ranged from 212.29 to $179.60 \text{ Mg hm}^{-2}$.

Table II. Soil BD, SWC, SOC and SIC contents (mean \pm SE) at depths of 0–100 cm in the grasslands

	Grassland	BD (g cm ⁻³)	SWC (%)	pH	SOC (%)	SIC (%)
0–5	Cropland	1.31 \pm 0.15aA	21.53 \pm 0.55bCDE	8.07 \pm 0.01aA	0.67 \pm 0.02cA	1.61 \pm 0.08aA
	RG5	1.30 \pm 0.22aCD	24.15 \pm 1.37abA	8.01 \pm 0.03aA	1.20 \pm 0.26bA	1.17 \pm 0.05bB
	RG15	1.27 \pm 0.06aAB	26.87 \pm 1.80abA	7.64 \pm 0.02bA	1.10 \pm 0.11abA	1.20 \pm 0.02bE
	RG30	1.34 \pm 0.09aA	29.71 \pm 7.40aA	7.61 \pm 0.05bA	1.52 \pm 0.24aA	1.18 \pm 0.02bC
5–10	Cropland	1.38 \pm 0.14aA	20.85 \pm 0.56aDE	8.03 \pm 0.02aA	0.65 \pm 0.08cA	1.62 \pm 0.09aA
	RG5	1.28 \pm 0.03abCD	22.70 \pm 0.74aA	7.98 \pm 0.02aA	0.79 \pm 0.09bcB	1.24 \pm 0.06bAB
	RG15	1.19 \pm 0.05bBC	21.28 \pm 5.46aAB	7.61 \pm 0.03bA	0.84 \pm 0.07bB	1.25 \pm 0.01bD
	RG30	1.31 \pm 0.07abA	25.51 \pm 1.30aAB	7.57 \pm 0.03bA	1.05 \pm 0.09aB	1.24 \pm 0.01bBC
10–20	Cropland	1.35 \pm 0.13aA	20.72 \pm 0.35bE	8.08 \pm 0.03aA	0.56 \pm 0.12bAB	1.63 \pm 0.07aA
	RG5	1.24 \pm 0.04abD	21.43 \pm 0.83bA	8.04 \pm 0.01aA	0.59 \pm 0.04abBC	1.28 \pm 0.03bAB
	RG15	1.19 \pm 0.05bBC	20.90 \pm 0.49bB	7.64 \pm 0.04bA	0.74 \pm 0.08aB	1.30 \pm 0.01bC
	RG30	1.30 \pm 0.06abA	23.65 \pm 0.55aB	7.59 \pm 0.03bA	0.73 \pm 0.11abC	1.30 \pm 0.03bABC
20–30	Cropland	1.37 \pm 0.08aA	21.99 \pm 0.77aBCD	8.09 \pm 0.01aA	0.45 \pm 0.11abBC	1.63 \pm 0.05aA
	RG5	1.29 \pm 0.03aCD	21.09 \pm 1.72aA	8.01 \pm 0.02aA	0.45 \pm 0.03abCD	1.30 \pm 0.03bAB
	RG15	1.14 \pm 0.07bC	22.05 \pm 2.15aAB	7.63 \pm 0.03bA	0.57 \pm 0.12abC	1.32 \pm 0.01bBC
	RG30	1.37 \pm 0.03aA	23.17 \pm 1.06aB	7.55 \pm 0.03bA	0.63 \pm 0.10aC	1.36 \pm 0.02bAB
30–50	Cropland	1.24 \pm 0.05cA	24.56 \pm 0.79aA	8.09 \pm 0.04aA	0.36 \pm 0.05bC	1.64 \pm 0.08aA
	RG5	1.42 \pm 0.08aBC	22.84 \pm 2.54aA	8.01 \pm 0.03aA	0.32 \pm 0.04bDE	1.41 \pm 0.12bA
	RG15	1.31 \pm 0.05bcA	21.59 \pm 2.67aAB	7.65 \pm 0.04bA	0.48 \pm 0.07aCD	1.33 \pm 0.02bAB
	RG30	1.35 \pm 0.03abA	22.77 \pm 1.01aB	7.60 \pm 0.04bA	0.56 \pm 0.07aC	1.38 \pm 0.02bAB
50–70	Cropland	1.28 \pm 0.06bA	22.75 \pm 0.55aB	8.01 \pm 0.06aA	0.37 \pm 0.05bC	1.66 \pm 0.11aA
	RG5	1.58 \pm 0.16aA	21.40 \pm 5.10aA	7.98 \pm 0.05aA	0.22 \pm 0.13cDE	1.17 \pm 0.18cB
	RG15	1.37 \pm 0.05bA	20.90 \pm 2.76aB	7.61 \pm 0.03bA	0.41 \pm 0.05abCD	1.35 \pm 0.01bcA
	RG30	1.33 \pm 0.04bA	23.42 \pm 0.69aB	7.55 \pm 0.05bA	0.56 \pm 0.07aC	1.40 \pm 0.02bA
70–100	Cropland	1.27 \pm 0.03cA	22.08 \pm 0.34aBC	8.10 \pm 0.04aA	0.37 \pm 0.05bC	1.66 \pm 0.14aA
	RG5	1.49 \pm 0.04aAB	24.84 \pm 1.53aA	7.97 \pm 0.04aA	0.19 \pm 0.07cE	1.21 \pm 0.21bAB
	RG15	1.34 \pm 0.03bA	17.95 \pm 3.00bB	7.66 \pm 0.03bA	0.39 \pm 0.02bD	1.33 \pm 0.01bAB
	RG30	1.34 \pm 0.05bA	24.72 \pm 1.40aAB	7.60 \pm 0.03bA	0.51 \pm 0.08aC	1.33 \pm 0.18bAB

BD, bulk density; SWC, soil water content; SOC, soil organic carbon; SIC, soil inorganic carbon; SE, standard error.

RG5, RG15 and RG30 represent the grasslands at different stages of restoration. Different lowercase letters indicate significant differences among the different restored grasslands at $p < 0.05$; different uppercase letters indicate significant differences among the different soil depths at $p < 0.05$.

DISCUSSION

Vertical Distributions of Soil Organic Carbon Along the Grassland Restoration Chronosequence

Our study showed that the SOC stock at 0–5 and 30–50 cm increased significantly across the chronosequence (Figure 5a). But along the grassland restoration SOC lost 7.77% in RG5, and gained 21.24% and 55.08% in RG15 and RG30, respectively (Figure 5d). The SOC stock changes in the 0–100 cm soil layer initially decreased in RG5 then increased to net C gains in RG15 and RG30. The accumulation of C commenced >5 years after cropland conversion on the Loess Plateau. This is agreement with previous studies (Laganiere *et al.*, 2010; Deng *et al.*, 2014a) that observed that initial losses in SOC stocks occur in ‘younger’ plantations, followed by a gradual return of SOC stocks to cropland levels in ‘medium-aged’ plantations and then net C gains in ‘older’ plantations. The SOC decrease phenomenon is probably due to the lower productivity of new vegetation in earlier restoration years and higher C loss from soil disturbance (Don *et al.*, 2009; Zhang *et al.*, 2010).

Our results also revealed that the rate of SOC change was higher in the surface (0–10) than in the deeper soil (10–100) in RG5 (Figure 5a), which was similar to the results of previous research (Guo & Gifford 2002; Deng *et al.*, 2014a; Shang *et al.*, 2014). The main reason can be attributed to

the different C sequestration mechanisms in the surface and deeper soils. The increase in the quantity of C inputs (from aboveground litter and fine root biomass) is accompanied by a new microclimatic regime and enhanced organic matter protection, which also promotes SOC accumulation in the surface soil (Novara *et al.*, 2015). The surface soil sequestered more C from the atmosphere after cropland conversion (Guo & Gifford 2002). Moreover, the cessation of tillage of cropland during the restoration of grassland can lower SOC decomposition and subsequently increase the SOC. Some studies have shown that the addition of nitrogen-fixing species (in this case, *Vicia sepium*, *Medicago ruthenica* and *Oxytropis racemosa*) during restoration can lower the decomposition of old and new C, hence favouring SOC sequestration (Resh *et al.*, 2002). Meanwhile, nitrogen-fixing species may also contribute to additional C sequestration through an input of (biologically fixed) N. In addition, soil physical properties can influence the SOC. For example, the positive effects of the soil clay content on the SOC content and accumulation have been well documented (Paul *et al.*, 2002; Laganiere *et al.*, 2010). Therefore, higher soil clay contents in the topsoil in the restored grassland may partly contribute to the C accumulation (Tan *et al.*, 2004). Moreover, climate may affect soil C accumulation through biotic processes associated with both the vegetation productivity and decomposition of organic matter (Li *et al.*, 2012).

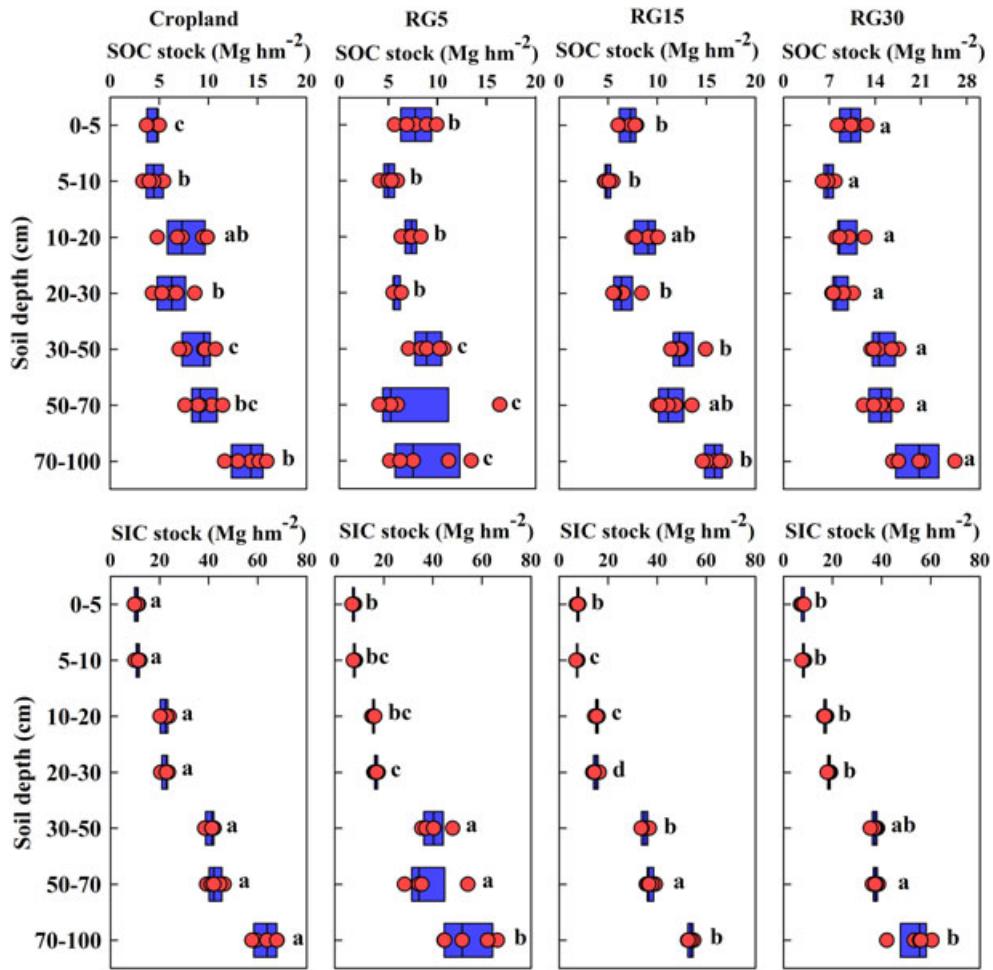


Figure 1. The vertical distribution of soil organic carbon (SOC) and soil inorganic carbon (SIC) stocks for different restored grasslands. Note: Different lowercase letters indicate significant differences among the different restored grasslands at $p < 0.05$. This figure is available in colour online at wileyonlinelibrary.com/journal/ldr.

Deng *et al.*, (2014a) showed that annual average temperature was the main factor influencing the soil C stock in surface soil (0–20 cm) during first 5 years, and temperature and precipitation are the main factors determining soil C stock change in the later stages following cropland conversion on the Loess Plateau. However, the loss of fertilizer and

the limited deeper-soil root system of annuals led to a decrease in SOC in RG5 (Carter & Gregorich 2010). Grassland restoration for 20 years significantly increased belowground biomass, and most of belowground biomass was in the 0- to 20-cm horizon on the Loess Plateau (Cheng *et al.*, 2011). Root biomass enhanced SOC by increasing input and

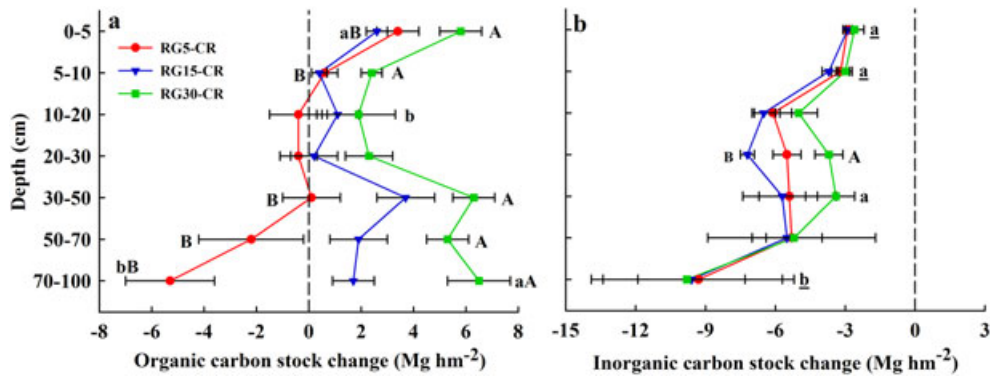


Figure 2. Vertical distributions of gains and losses in soil organic carbon (a) and inorganic carbon (b) stock (restored grassland – cropland) for different restored grasslands. Note: error bars represent standard error. CR, RG5, RG15 and RG30 represent cropland and grasslands restored for 5, 15 and 30 years, respectively. Different uppercase letters indicate significant differences among the different restored grasslands at $p < 0.05$; different lowercase letters indicate significant differences among the different soil depths at $p < 0.05$. The lowercase letters with underlines indicate the same letter in the different restored grasslands. This figure is available in colour online at wileyonlinelibrary.com/journal/ldr.

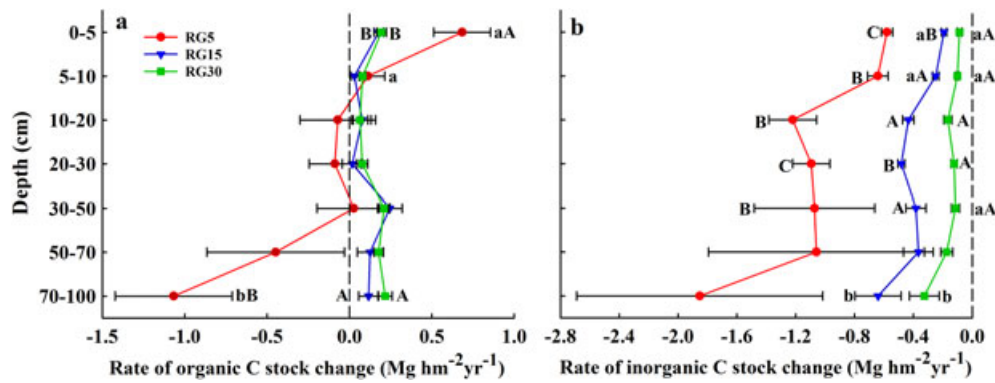


Figure 3. Rates of soil organic carbon (a) and inorganic carbon (b) stock changes for different restored grasslands. Error bars represent standard error. CR, RG5, RG15 and RG30 represent cropland and grasslands restored for 5, 15 and 30 years, respectively. Note: different uppercase letters indicate significant differences among the different restored grasslands at $p < 0.05$; different lowercase letters indicate significant differences among the different soil depths at $p < 0.05$. This figure is available in colour online at wileyonlinelibrary.com/journal/ldr.

decomposition of litter on the Loess Plateau (Zhu *et al.*, 2014). Otherwise, some studies have suggested that fine roots are primarily responsible for the accumulation of sub-soil SOC in other places (Rasse *et al.*, 2005; Hooker & Compton 2008).

Vertical Distributions of Soil Inorganic Carbon Along the Grassland Restoration Chronosequence

The SIC content and stock in the 0- to 100-cm soil layer decreased across the grassland restoration chronosequence. According to the vertical characteristics of the SIC content (Table II), our results showed that the maximum values of SIC content appeared at depths of 30–50 cm in RG5 (1.41%) and at depths of 50–70 in cropland (1.66%), RG15 (1.35%) and RG30 (1.40%). These results were accordance with previous studies that showed the SIC content was peak at the intermediate depth of the top 1 m in grassland (Mi *et al.*, 2008; Liu *et al.*, 2014). However, the peaks did not influence the general trend of SIC stocks in the 0- to 100-cm soil layer across the grassland restoration chronosequence. This result agrees with previous studies that vegetation restoration has a significant effect on SIC

redistribution throughout the soil profile (Wang *et al.*, 2016). For example, on the Loess Plateau, Wang *et al.*, (2016) found that the SIC stocks in the top 10 cm gradually decreased along the vegetation restoration chronosequence, but it was basically unchanged in the subsoil (10–100 cm). Chang *et al.* (2012) demonstrated that the SIC level decrease in forest topsoil was offset by an increase in the subsoil (60–100 cm). This offset may be attributed to the process of dissolution of carbonates in the upper sections and their subsequent precipitation into lower sections, which resulted in calcic horizons transferred from topsoil to subsoil (Wu *et al.*, 2009). Liu *et al.*, (2014) reported a significant decrease in both the SIC content and stock at the depths of 0–80 cm during the conversion of cropland to grassland. The vertical mixing of SIC during tillage in the cropland and the dissolution and leaching of carbonate from the topsoil to deeper layers may play the primary and significant roles in leading to the lower SIC in the topsoil during grassland restoration. A higher SOC pool often results in a looser soil structure, higher SWC content and higher soil permeability in the topsoil, which could increase the dissolution and leaching of carbonate from the topsoil. Moreover, vegetation restoration could also affect the SIC stock, but the exact extent of the impact of vegetation could not be determined because of the effects of multiple factors acting on the SIC in the soil profile (Wang *et al.*, 2016). Soil moisture, pH, CO₂ partial pressure, and Ca²⁺ and HCO₃⁻ concentrations are the direct factors that influence SIC; thus, a change in any factor would cause an effect on SIC. SIC stock in the 0- to 100-cm layer decreased at a rate of 0.75 Mg hm⁻² yr⁻¹, but no significant decreasing trend was observed over the restoration time (Figure 4b). This indicated that the dissolution and leaching of SIC made little contribution to the loss of SIC stock during the restoration of grassland from cropland. The rate of SIC stock decrease in the different restoration treatments was as follows: RG5 > RG15 > RG30 (Figure 3b). One of the main reasons for this result is the decrease in surface runoff reducing the SIC loss after vegetation restoration. Moreover, the rapidly increasing plant biomass resulted in considerable Ca²⁺ accumulated in plant tissues and led to the decline of soil Ca²⁺ in

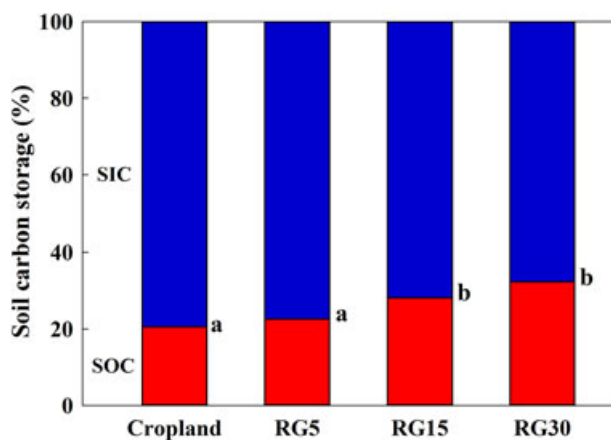


Figure 4. Percentages of soil organic carbon (SOC) and inorganic carbon (SIC) stocks (a) at soil depths of 0–100 cm for grasslands with different restoration ages. Note: different lowercase letters indicate significant differences among the different restored grasslands at $p < 0.05$. This figure is available in colour online at wileyonlinelibrary.com/journal/ldr.

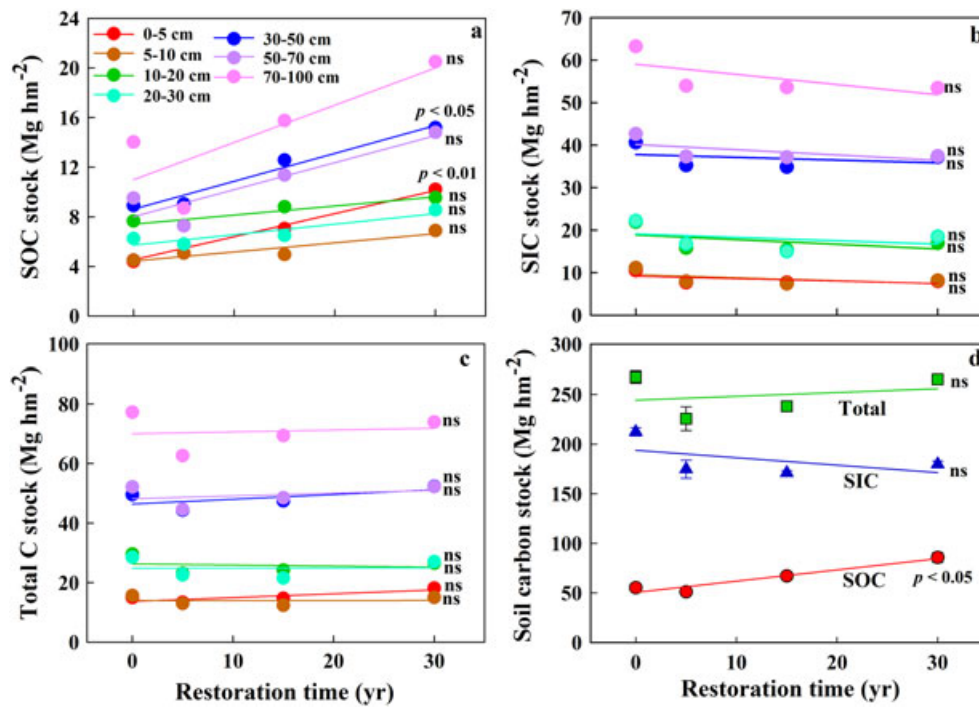


Figure 5. Changes in SOC (a), SIC (b) and total carbon (c) stocks at different soil depths, and soil carbon stock at the depth of 0–100 cm with different restoration time. Note: ns represents that linear regression is not significant. This figure is available in colour online at wileyonlinelibrary.com/journal/ldr.

the restored grasslands (White 2001). The SWC and root biomass increased with grassland restoration and motivated the activity of microorganisms, which will decompose much more organic matter and increase the concentration of soil CO_2 (Liu *et al.*, 2014). The higher SWC in restoration grassland induced the equilibrium in Equation 1.2 towards the right and resulted in higher soluble SIC. In our study, pH decreased with grassland restoration due to the larger amounts of litter input, which produced much more carbonic and organic acids and resulted in decreasing the content of soil carbonate (Jelinski & Kucharik 2009). Additionally, the increase in H^+ and SWC inevitably transform a portion of the SIC to CO_2 (Equation 1.1), which is ultimately released to the atmosphere. However, a portion of the C is temporary because Ca^{2+} is returned to soil by decomposition of organic matter (Liu *et al.*, 2014). Grassland restoration could reduce SIC loss through decreased runoff, which have been well documented on the Loess Plateau (Liu *et al.*, 2014; Wang *et al.*, 2016). In addition, some studies reported that soil texture has an important effect on the formation of inorganic forms of C, as the formation of SIC is more sensitive in coarse-textured soils than in fine-textured soils (Rasmussen 2006; Wang *et al.*, 2013).

Changes in Soil Organic Carbon and Soil Inorganic Carbon Percentages During Grassland Restoration

The vegetation restoration had a significant positive effect on SOC stock, which has been well documented (Deng *et al.*, 2014a). Compared with the SOC stock, the SIC stock represented more than 60% of the soil total carbon. Our study showed a higher percentage of SIC stock in cropland (average of 79.41%) than in RG5, RG15 and RG30

(averages of 77.44%, 71.89% and 67.76%, respectively). Therefore, even if the SIC content decreases with grassland restoration, the SIC stock still represents a very high percentage of soil C pool in the study area. Interestingly, although the SIC stock decreased with vegetation restoration at depths of 0–100 cm, there was no significant decrease in the soil total carbon across the grassland restoration chronosequence. In our study, SOC stock ranged from 55.25 to 85.67 Mg hm^{-2} , and the SIC stock ranged from 212.29 to 179.60 Mg hm^{-2} at soil depth of 0–100 cm across the 30-year grassland restoration (Figure 5). So the decrease in SIC stock was balanced by the increase in SOC, which agrees with the results of previous studies (Jelinski & Kucharik 2009; Chang *et al.*, 2012; Liu *et al.*, 2014). Present study showed SIC lost at a rate of 0.16 $\text{Mg hm}^{-2} \text{y}^{-1}$ after 30-year restoration, which is obviously lower than the rates of 5 and 15 years. So the capacity of carbon sequestration is promotion along long term restoration grassland in the semi-arid regions. The soil total C storage remains stable across the restoration sequence, with redistribution of the dominant forms of the total soil C pool from SIC to SOC (Wang *et al.*, 2016). The inconsistent changes in SOC and SIC illustrate that both SOC and SIC should be considered when assessing soil C sequestration in a restoration chronosequence.

CONCLUSIONS

We examined the changes in the SOC and SIC stocks and changes at depths of 0–100 cm following the conversion of cropland to grassland on the Chinese Loess Plateau. The results showed that the SOC stock changes in the 0–100 cm

soil layer showed an initial decrease in RG5 and then an increase to net C gains in RG15 and RG30. However, because of the decrease in SIC stock, the percentage of the soil C pool represented by the SOC stock increased across the chronosequence. The accumulation of C commenced >5 years after cropland conversion on the Loess Plateau. Although the SIC content decreased with grassland restoration, the SIC stock still represented a higher percentage of the soil C pool. Moreover, the STC showed an increasing trend during grassland restoration in the study area. The soil C sequestration followed the increase in SOC, which offset the decrease in SIC in the restored grasslands. Therefore, we suggest that both SOC and SIC should be considered during grassland restoration on the Loess Plateau, which may provide basic data and direction for the studies on regional carbon cycling.

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CONFLICT OF INTEREST

All the authors declare no conflicts of interest.

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