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### **Research Article**

# Carbon Storage Dynamics in Alfalfa (*Medicago sativa*) Fields in the Hilly-Gully Region of the Loess Plateau, China

Alfalfa (Medicago sativa) has been widely employed in the dryland region of the Loess Plateau, China to improve soil and water conservation and to develop livestock production. Our objective was to study the dynamics of plant and soil organic carbon (SOC) pools following the conversion of sloping farmland to alfalfa fields over a period of 30 years. The succession gradient is composed of seven differently aged alfalfa fields (0, 5, 9, 13, 16, 23, 30 years). The results show that soil C storage (0-100 cm) dynamics were consistent with belowground biomass storage with increased planting years, but C storage always increased with the number of planting years in the 0-5 cm soil layer. Planted perennial alfalfa resulted in a decline in soil C storage in the 0-100 cm soil depth in the early period (nine years). During the late succession stage of alfalfa (13 years) soil C storage tends to recover, and after 16 years, storage values again dropped. However, it had recovered by 30 years at which time alfalfa productivity was very low. Vegetation C storage was mainly decided by the belowground biomass and ecosystem C storage dynamics was consistent with soil C storage. Vegetation biomass, root/shoot ratio, SOC, soil total nitrogen, and total phosphorus were the main factors affecting C storage in the entire alfalfa field ecosystem. The results suggest that C storage in vegetation is directly related to plant productivity, C storage in the soil throughout the entire alfalfa field ecosystem was not only related to plant productivity, but also to SOC and soil nutrients.

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#### 1 Introduction

The Loess Plateau, whose ecosystems have been affected by human activity for thousands of years, is located in the northwest of China [1]. Since the beginning of the twentieth century, accelerated environmental degradation has been taking place as a result of population expansion and excessive land use and development [2, 3]. To reduce soil erosion, maintain land productivity, and improve environmental quality, since 1999, the Chinese government has implemented the "returning degraded or marginal land to forest or grass" program in the Loess Plateau [4–7]. As alfalfa is capable of producing pasture yields of 22–42 Mg ha<sup>-1</sup> year<sup>-1</sup>, the area under alfalfa cultivation has been gradually increased to meet demand for an increasing livestock population and to address the government's environmental objective of preventing soil erosion in the region [8].

Cultivated grassland has the advantage of accelerating vegetation restoration and improving ecological stability [9]. Currently, alfalfa has become one of the most important agricultural forage species and has been evaluated as a nutritious forage crop, primarily because of its high quality and high yield, drought resistance, and is adaptable to various climatic and soil conditions [1]. A great deal of planting has taken place in the Loess Plateau, China [3, 8]. Existing data indicate that about 2.8 million ha of grassland have been sown in alfalfa, which has produced 25 million Mg hay, which is far lower than the actual need [4]. For the Loess Plateau, alfalfa has been widely planted as a sustainable agricultural practice [1]. Gansu and Ningxia are currently China's major production bases for alfalfa forage.

Land use change is bound to affect soil quality, especially soil organic carbon (SOC) content [10], because SOC is the most important indicator of soil quality [4]. Previous studies have demonstrated that converting cropland to pasture or introducing forage legumes into grasslands can improve soil quality [1, 4, 11-13]. Lal and Bruce [14] also report that converting cropland, particularly degraded arable land, into perennial grassland can substantially increase soil C storage. Based on an investigation and research from around the world, Conant et al. [15] report an average rate of C sequestration of 1.01 Mg Cha<sup>-1</sup> year<sup>-1</sup> when converting cultivated land to pasture. In addition, land-use change also affects vegetation productivity. Previous studies show that alfalfa productivity decreases with the number of planting years [1, 8]. Zhang and Cheng [16] report that alfalfa productivity reaches its maximum level in the seventh year, after which its productivity was observed to decrease in the semi-arid region of the Loess Plateau (areas with 450 mm rainfall annually). At present, in the Loess Plateau, most alfalfa fields planted for more than 10 years, some for even more than 20 years, leads to serious land

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Abbreviations: BD, bulk density; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus

degradation and a significant decrease in alfalfa production [1]. Therefore, it is crucial to study the soil C storage dynamics of an ecosystem in relation to the number of years planted, especially for soil C storage. Many studies have reported a short-term or temporary change in either the plant productivity or the SOC dynamics of alfalfa fields [1, 4, 13, 14, 16]. However, few studies have focused on the long-term C sequestration dynamics of whole ecosystems (plant and soil) against alfalfa restoration. These parameters have important implications for both C cycling and ecosystem function.

Therefore, to maintain the sustainability of alfalfa field ecosystems, it is necessary to understand the long-term C sequestration dynamics of whole ecosystems (plant and soil) against alfalfa restoration. In this study, we used a space-for-time method to study the C storage dynamics of alfalfa vegetation over a 30 year period in the hilly-gully areas of the Loess Plateau in China. The objective of this study was to estimate the dynamics of C storage in the alfalfa field ecosystem, including three parts of vegetation, soil and the whole ecosystem, and to analyze those factors affecting the C storage patterns of the alfalfa field ecosystem.

#### 2 Materials and methods

#### 2.1 Study area

The study area is located in Yunwu Mountain, Guyuan City, Ningxia Province, China (106°16′–106°24′E, 36°13′–36°19′N, 1700–2148 m a.s.l.). It is a hilly landscape in the middle of the Loess Plateau with deeply incised gullies and is characterized by a sub-arid climate with heavy seasonal rainfall resulting in periodic local flooding and drought (Fig. 1). In the region studied, 90% of the land area is hilly, 4% is occupied by villages and rivers, and only 6% of the area is

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considered suitable for intensive agriculture. Most of the land is at an altitude of 1700-2000 m and is closely dissected by steep and very steep gulleys. The main herbaceous plants are Stipa bungeana, Thymus mongolicus, Artemisia sacrorum, Potentilla acaulis, Stipa grandis, Androsace erecta, Heteropappus altaicus, Artemisia capillaries, Artemisia frigid, etc., in which the Stipa bungeana community has the most extensive distribution. Alfalfa (Medicago sativa) is the most common cultivated plant in the area. The study area's soil type is Aeolian soil (silt loam), and soil pH ranges from 7.99 to 8.20. The study area receives a mean annual precipitation of approximately 410 mm (1960-2010) (Fig. 2a), which is distributed for the most part between July and September. The area's semi-arid temperate continental monsoon climate produces a mean annual temperature of 6.7°C (1960-2010) (Fig. 2b), a mean annual total of 2518 sunshine hours, a mean annual evaporation of 1600 mm, and 137 frost-free days per year on average.

#### 2.2 Experimental design and sampling

Mean

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We selected a succession sequence in relatively homogeneous alfalfa fields characterized by planting years ranging from 5 to 30 years prior to the experiment. Six alfalfa planting year intervals, 5, 9, 13, 16, 23, and 30 years, are situated close to the same bedrock and parent material (loess) and topography. The history of the sites was determined through interviews with local farmers and village elders. Experimental fields used in this study were taken from within the same valley. The conventional rotation in this area before soil samples were collected was spring wheat (*Triticum aestivum*) – potato (*Solanum tuberosum*). The fields were all cropped with potato in rotation before seeding in the study. So, we selected five potato cultivated sites for comparison. The average amount of the base



Figure 1. Location of the study site.

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800

600

400

200

 $0 \\ 9$ 

8

6

5

4

1960

1970

1980

Years

temperature (b) at the experimental site from 1960 to 2010.

Annual mean temperature (<sup>0</sup>C)

Annual mean precipitation (mm)



2000

2010

1990

Figure 2. Distribution of mean annual precipitation (a) and mean annual

(a)

fertilizer applied before potato seeding was  $225-300 \text{ kg ha}^{-1}$  of sheep manure. No fertilizer or manure was applied to soils when the fields were planted with alfalfa. The alfalfa fields were managed in accordance with conventional cultivation management techniques (mowed at the soil surface twice a year except in the first year when the alfalfa was seeded). Mowing was usually carried out in July and October. Each alfalfa field is more than 667 m<sup>2</sup> in area.

Five  $20 \text{ m} \times 20 \text{ m}$  plots were established for each age class in August 2011 when the grassland community biomass peaked. These plots were considered to be true replicates as the distance among them exceeded the spatial dependence (<13.5 m) of most soil chemical and microbial variables [17]. Indeed, the plots we selected in the study were from 200 to 500 m apart from each other. Five quadrats (1 m × 1 m) were separately chosen in the four corners and center of each plot. In total, we surveyed five plots with 25 quadrats in each age class, and 30 plots with 150 quadrats for six age class of alfalfa fields in our study. In each quadrat, the community cover, height, number of species (species richness) and alfalfa density (individual plants per quadrat) (Fig. 3), above- and belowground biomass, litter accumulation (Fig. 4), and soil samples in the 0–100 cm soil cores were observed.

In each quadrat, all the aboveground parts of the green plants were cut, collected and put into envelops and tagged, as was all litter. To measure belowground biomass soil sampling was done once using a 9 cm diameter root corer to sample soil at a depth of 0–100 cm in each quadrat; the same layers were then mixed together to make one sample. The majority of the roots were found in the soil samples thus obtained and then isolated using a 2 mm sieve. The remaining fine roots taken from the soil samples were isolated by spreading the samples in shallow trays, overfilling the trays with water

and allowing the outflow from the trays to pass through a 0.5 mm mesh sieve. No attempts were made to distinguish between living and dead roots. All the isolated roots were oven-dried at 65°C and weighed. Due to the size of the aboveground biomass samples, they were weighed fresh and then a part of each sample was dried and weighed. The aboveground biomass of the samples was calculated by multiplying the ratio of the dry weight/fresh weight ratio by the fresh weight.

Soil samples were taken at five points in the quadrats of each plot. These were the four corners and the center of the biomass sampling sites as described above. The litter layer was removed before soil sampling. Soil sampling, using a soil drilling sampler (9 cm id), was done in seven soil layers, 0-5, 5-10, 10-20, 20-30, 30-50, 50-70, and 70-100 cm. We then mixed the same layers together to make one sample. All samples were sieved through a 2 mm screen, and the roots and other debris were removed. Each sample was air-dried and stored at room temperature for the determination of soil physical and chemical properties. The soil bulk density (g cm<sup>-3</sup>) of the different soil layers (0-5, 5-10, 10-20, 20-30, 30-50, 50-70, and 70-100 cm) was measured using a soil bulk sampler with a 5 cm diameter and a 5 cm high stainless steel cutting ring at points adjacent to the soil sampling plots. The original volume of each soil core and its dry mass after oven-drying at  $105^{\circ}$ C were measured.

#### 2.3 Physical and Chemical Analysis

Soil water content was measured gravimetrically and expressed as a percentage of soil water to dry soil weight [3]. Soil bulk density (BD) was calculated depending on the inner diameter of the core sampler, sampling depth and the oven dried weight of the composite soil samples [18]. Plant biomass C content and SOC were assayed by



Figure 3. Plant community traits of alfalfa fields during the six planting year intervals. The error bars are SE.





Figure 4. Aboveground biomass (a), litter biomass (b), 0–100 cm belowground biomass (c), total biomass (d), and the distribution of the belowground biomass (e) of six alfalfa fields of planting year intervals. The error bars are SE.

dichromate oxidation [19], soil total nitrogen (TN) was assayed using the Kjeldahl method [20], soil total phosphorus (TP) was determined after digestion of soil with  $HClO_4/H_2SO_4$  [21]. Each analysis was done in two replicates. The physical and chemical properties of the soil samples are presented in Fig. 5.

#### 2.4 Calculation of C storage

#### 2.4.1 Vegetation C storage

The study used the following equation to calculate the vegetation C storage [22]:

$$C_{\rm v} = \frac{BC_{\rm f}}{100} \tag{1}$$

where  $C_v$  is the vegetation carbon storage (Mg ha<sup>-1</sup>), *B* is the vegetation biomass (g m<sup>-2</sup>), and  $C_f$  is the plant biomass *C* content.

#### 2.4.2 Soil C storage

Because there was no stone in the study area and consequently, the sample soils, we did not sieve the soil. Therefore, the study used the following equation to calculate SOC storage ( $C_s$ ) [10]:

 $C_{\rm s} = {\rm BD} \times {\rm SOC} \times D$  (2) where  $C_{\rm s}$  is soil C storage (Mgha<sup>-1</sup>); BD is soil bulk density (g cm<sup>-3</sup>); SOC is SOC content (%); and D is soil thickness (cm).

#### 2.5 Statistical analysis

One-way ANOVA was used to analyze the means of the same soil layers among the different growth years and the means of plant C storage and ecosystem C storage among the different growth years. Differences were evaluated at the 0.05 significance level. When significance was observed at the p < 0.05 level, Tukey's post hoc test was used to carry out the multiple comparisons. The study employed



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Figure 5. 0–100 cm soil physical and chemical properties of slope farmland and six alfalfa fields of planting year intervals; (a) soil BD; (b) soil water content; (c) SOC; (d) soil TN; (e) soil TP.

Spearman correlation analysis to examine the correlations between ecosystem C storage and the factors affecting them (aboveground biomass, belowground biomass, root/shoot ratio, litter biomass, total biomass, aboveground biomass C content, litter biomass C content, belowground biomass C content, species richness, cover, height, alfalfa density, SOC, soil TN, soil TP, soil bulk density, and soil water content) following the growth years. Among the factors, SOC, Soil TN, soil TP, soil bulk density, and soil water content are the mean value of seven soil layers (0–5, 5–10, 10–20, 20–30, 30–50, 50–70, and 70–100 cm).

Years

#### 3 Results

#### 3.1 Vegetation C storage

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The total biomass, belowground biomass, and litter biomass C storage of six alfalfa fields showed the same trend with an increase in planting years. They all first increased to the highest value at 13 years, then decreased to the lowest at 23 years and then increased again

(Fig. 6). Total biomass and belowground biomass C storage had significant differences among the six planting years (p < 0.05), but no significant differences for litter biomass C storage (p > 0.05) were observed. The aboveground biomass C storage first decreased and then increased with an increase in planting years (Fig. 6), and showed significant differences among the six planting years (p < 0.05). Belowground biomass C storage values were all higher than aboveground biomass and litter biomass C storage (Fig. 6). The total biomass C storage of six alfalfa fields for the planting years were 1.0, 1.1, 5.0, 4.0, 1.3, and 2.9 Mg ha<sup>-1</sup>, respectively (Fig. 6).

#### 3.2 Soil C storage

The soil C storage of the different soil layers showed that various characteristics increased with the number of planting years (Tab. 1). At 30 years, C storage in the surface soil (0–5 cm) had gradually increased. Thirty years after planting alfalfa, the 0–5 cm soil C storage had significantly increased (p < 0.05). On sloping farmland, C storage was  $4.16 \pm 0.28 \text{ Mg ha}^{-1}$  and it increased to  $9.58 \pm 0.39 \text{ Mg ha}^{-1}$ .



**Figure 6.** Plant C storages of six alfalfa fields of planting years. Note: NS means no significant differences among the six planting year intervals at the 0.05 level (p > 0.05). \* means significant differences among the six planting year intervals at the 0.05 level (p < 0.05). The error bars are SE.

The 5–10 cm soil C storage first decreased and then increased. It was lowest after 9 years. At 16 years after alfalfa planting, C storage in the 10–20 and 20–30 cm soil layers first decreased to the lowest value at the 9 year mark, and then increased to its highest at 16 years. The 30–50 cm soil C storage first decreased to its lowest level at 5 years, and then increased to its highest at 16 years. The 50–70 and 70–100 cm soil C storage values gradually increased. From 16 to

30 years, soil C storage in the 10–70 cm soils had decreased at 23 years and then increased at 30 years. At 70–100 cm the soil C storage gradually decreased.

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The soil C storage of the different soil depths also showed varied characteristics with the number of planting years (Tab. 2). Thirty years after planting alfalfa, C storage at 0-5, 0-10, 0-20, and 0-30 cm soil depths had significantly increased compared with that of the slope farmland (p < 0.05). However, the 0–50, 0–70, and 0–100 cm soil depth C storage values had not significantly increased compared with that in the slope farmland (p > 0.05). In the slope farmland, 0–100 C storage was  $69.97 \pm 2.36 \,\text{Mg}\,\text{ha}^{-1}$ , and increased to the highest at 16 planting years with  $83.38 \pm 0.88$  Mg ha<sup>-1</sup>. After 30 years of alfalfa cultivation, the 0-100 cm soil depth C storage was  $76.83\pm5.84\,Mg\,ha^{-1}.$  In the 30 years, C storage in the 0–5 cm soil depth gradually increased; 0-10 cm soil depth C storage first decreased and then increased. It was lowest after nine years. Following 16 years of alfalfa cultivation, the C storage in the 0-20, 0-30, 0-50, 0-70 cm soil depths first decreased to the lowest at nine years, and then increased to the highest at 16 years. The 0-100 cm soil depth first decreased to its lowest value at five years, and then increased to the highest at 16 years. From 16 to 30 years, the soil C storage in the 0-20, 0-30, 0-50, 0-70, and 0-100 cm soils depth decreased for 23 years and then had increased at 30 years.

#### 3.3 Ecosystem C storage

Similar to the soil C storage, the C storage of the whole ecosystem first decreased and then increased following 16 years of alfalfa cultivation (Fig. 7a). C storage in the five year alfalfa field  $(66.46 \pm 1.28 \text{ Mg ha}^{-1})$ 

Table 1. C storage (Mg ha<sup>-1</sup>) in different soil layers of slope farmland, six alfalfa fields of planting years

Years of cultivation (year)	Soil layer (cm)								
	0-5	5-10	10-20	20-30	30-50	50-70	70-100		
Slope farmland Alfalfa	$4.16\pm0.28^c$	$4.82 \pm 0.31^{abc}$	$9.07.3 \pm 0.61^{bc}$	$9.16 \pm 0.48^{a}$	$15.86 \pm 0.80^{ab}$	$11.21.0 \pm 0.41^{\rm b}$	$15.68.5 \pm 0.92^{a}$		
5	$4.44\pm0.09^{bc}$	$3.98\pm0.08^{\rm bc}$	$8.49.5\pm0.13^{\rm c}$	$7.29\pm0.12^{\rm a}$	$12.41\pm0.17^{\rm b}$	$11.55.9 \pm 0.45^{\rm b}$	$17.30.4 \pm 0.61^{\rm a}$		
9	$4.55\pm0.08^{\rm bc}$	$3.27\pm0.02^{\rm c}$	$7.55.6 \pm 0.23^{\rm c}$	$6.82\pm0.21^{\rm a}$	$13.36\pm0.28^{ab}$	$12.33.2 \pm 0.82^{ab}$	$19.11.9 \pm 0.39^{\rm a}$		
13	$5.18\pm0.13^{\rm bc}$	$4.30\pm0.48^{\rm bc}$	$9.26.9 \pm 0.14^{ m bc}$	$8.06\pm0.13^{\rm a}$	$15.26 \pm 0.34^{\rm ab}$	$13.51.0 \pm 0.45^{\rm ab}$	$19.51.3 \pm 0.41^{\rm a}$		
16	$5.84\pm0.24^{\rm bc}$	$5.43\pm0.13^{\rm ab}$	$11.11.8 \pm 0.15^{\rm a}$	$9.34\pm0.18^{\rm a}$	$17.13\pm0.60^{\rm a}$	$14.65.8 \pm 0.10^{\rm c}$	$19.85.8 \pm 0.59^{\rm a}$		
23	$6.07\pm0.45^{\rm b}$	$5.45\pm0.27^{\rm ab}$	$8.00.7\pm0.37^{\rm c}$	$7.11\pm0.38^{\rm a}$	$11.91\pm0.60^{\rm b}$	$12.00.4 \pm 0.52^{\rm ab}$	$16.46.6 \pm 0.76^{\rm a}$		
30	$9.58\pm0.39^{\rm a}$	$6.46\pm0.18^{\rm a}$	$10.45.2 \pm 0.37^{ab}$	$10.25\pm2.63^{\rm a}$	$14.96\pm1.62^{ab}$	$12.18.6 \pm 1.10^{ab}$	$12.93.5 \pm 1.50^{\rm a}$		

Different letters indicates significant differences in the same soil layers at the 0.05 level (p < 0.05), and the same letters indicate no significant differences in the same soil layers at the 0.05 level (p > 0.05). The values are mean  $\pm$  SE.

Table 2. C storage (Mg ha<sup>-1</sup>) in different soil depth of slope farmland, six alfalfa fields of planting years

Years of cultivation (year)	Soil depth (cm)								
	0-5	0-10	0-20	0-30	0-50	0-70	0-100		
Slope farmland Alfalfa	$4.16.2 \pm 0.28^{c}$	$8.98.2 \pm 0.58^{bcd}$	$18.05.5 \pm 1.17^{cd}$	$27.21.3 \pm 1.52^{bc}$	$43.07.4 \pm 2.12^{ab}$	$54.28.4 \pm 2.42^{ab}$	$69.96.9 \pm 2.36^{ab}$		
5 9	$\begin{array}{c} 4.43.9 \pm 0.09^{bc} \\ 4.54.7 \pm 0.08^{bc} \end{array}$	$\begin{array}{c} 8.42.3 \pm 0.11^{cd} \\ 7.82.0 \pm 0.89^{d} \end{array}$	$\begin{array}{c} 16.91.9 \pm 0.21^{cd} \\ 15.37.6 \pm 0.98^{d} \end{array}$	$\begin{array}{c} 24.21.0 \pm 0.25^c \\ 22.19.1 \pm 1.09^c \end{array}$	$\begin{array}{c} 36.61.6 \pm 0.38^{b} \\ 35.54.8 \pm 1.20^{b} \end{array}$	$\begin{array}{c} 48.17.6 \pm 0.71^{b} \\ 47.88.0 \pm 1.65^{b} \end{array}$	$\begin{array}{c} 65.48.0 \pm 1.23^{b} \\ 67.00.0 \pm 1.93^{ab} \end{array}$		
13 16	$\begin{array}{c} 5.18.0 \pm 0.13^{bc} \\ 5.84.4 \pm 0.24^{bc} \end{array}$	$\begin{array}{c} 9.47.7 \pm 0.60^{bcd} \\ 11.27.5 \pm 0.35^{bc} \end{array}$	$\frac{18.74.6\pm0.64^{bcd}}{22.39.2\pm0.43^{ab}}$	$\begin{array}{c} 26.80.3 \pm 0.70^{bc} \\ 31.73.3 \pm 0.48^{ab} \end{array}$	$\begin{array}{c} 42.06.6\pm0.78^{ab} \\ 48.86.6\pm0.92^{a} \end{array}$	$\begin{array}{c} 55.57.5 \pm 0.967^{ab} \\ 63.52.4 \pm 0.94^{a} \end{array}$	$\begin{array}{c} 75.08.8 \pm 1.03^{ab} \\ 83.38.2 \pm 0.88^{a} \end{array}$		
23 30	$\begin{array}{c} 6.07.4 \pm 0.45^{\text{b}} \\ 9.58.0 \pm 0.39^{\text{a}} \end{array}$	$\begin{array}{c} 11.52.0 \pm 0.38^{\text{b}} \\ 16.04.0 \pm 0.50^{\text{a}} \end{array}$	$\frac{19.52.7\pm0.36^{bc}}{26.49.3\pm0.74^{a}}$	$\begin{array}{c} 26.63.9 \pm 0.52^{bc} \\ 36.74.3 \pm 2.32^{a} \end{array}$	$\begin{array}{c} 38.55.2 \pm 0.89^{\rm b} \\ 51.70.4 \pm 3.77^{\rm a} \end{array}$	$\begin{array}{c} 50.55.7 \pm 1.37^{\rm b} \\ 63.89.1 \pm 4.57^{\rm a} \end{array}$	$\begin{array}{c} 67.02.3 \pm 2.12^{ab} \\ 76.82.6 \pm 5.84^{ab} \end{array}$		

Different letters indicates significant differences in the same soil depth at the 0.05 level (p < 0.05), and the same letters indicate no significant differences in the same soil depth at the 0.05 level (p > 0.05). The values are mean  $\pm$  SE.



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**Figure 7.** Ecosystem C storage of slope farmland (no plant C storage) and six alfalfa fields of planting year intervals (a), and ecosystem C sequestration compared with slope farmland in the six alfalfa fields of planting year intervals (b). Different letters indicate significant differences among the seven stages of succession at the 0.05 level (p < 0.05), and the same letters indicate no significant differences among the seven stages of succession at the 0.05 level (p > 0.05). The error bars are SE.

was decreased compared with slope farmland (69.97 ± 2.36 Mg ha<sup>-1</sup>), but did not show a significant difference (p > 0.05), after which, it had significantly increased at 16 years (87.34 ± 2.32 Mg ha<sup>-1</sup>) (p < 0.05) (Fig. 7a). From 16 to 30 years, ecosystem C storage was significantly decreased at 23 years (68.33 ± 2.08 Mg ha<sup>-1</sup>) (p < 0.05) and then increased to the 30 year mark (79.70 ± 5.26 Mg ha<sup>-1</sup>) (p > 0.05) (Fig. 7a). From Fig. 7b, it can be seen that C sequestration for the whole ecosystem in the alfalfa fields was highest at the 16 year mark.

#### 3.4 Relationship between C storage and its factors

Vegetation C storage had significant positive correlations with belowground biomass, root/shoot and litter biomass at the 0.01 level (p < 0.01), and it had significant positive correlations with soil TP at

the 0.05 level (p < 0.05) (Tab. 3). Although it had positive correlations with aboveground biomass, species richness, cover, height, alfalfa density, SOC and soil TN, they were not significant (p > 0.05) (Tab. 3). C storage of vegetation had negative correlations with soil bulk density and soil water content, but they were also not significant (p > 0.05) (Tab. 3). (Tab. 3).

Soil and ecosystem C storage had similar relationships with these factors. They both had significant positive correlations with belowground biomass, root/shoot and litter biomass, SOC, soil TN, soil TP at the 0.05 level (p < 0.05) (Tab. 3). Although it had positive correlations with species richness and soil water content, they were not significant (p > 0.05) (Tab. 3). Soil and ecosystem C storage had negative correlations with aboveground biomass, cover, height, alfalfa density and soil bulk density, which were also not significant (p > 0.05; Tab. 3).

#### 4 Discussion

In this study, with an increase in planting years, vegetation storage had the same trend with plant biomass, especially with the belowground biomass and litter biomass (Figs. 4 and 6). This study also found C storage of vegetation had significant positive correlations with belowground biomass, root/shoot and litter biomass (p < 0.01), indicating that vegetation biomass is closely related to vegetation C storage [23]. Thus, vegetation C storage can be decided by plant biomass. Our results showed that the aboveground biomass of alfalfa was the highest in the early succession stages (five years) and decreased in the latter (Fig. 4a), which was also reported in the previous study [1, 16]. The aboveground biomass mainly decided the aboveground biomass C storage. Both above- and belowground biomass C storage increased inconsistently (Fig. 6), which mainly related to the changes in their biomass (Fig. 4). This probably related to the root-shoot biomass allocations in different stages of vegetation restoration [24], while harvesting aboveground biomass every year may be another reason. Species compositions have a significant effect on C allocation patterns due to above- and belowground niche complementarily [24] However, the study found vegetation storage had positive correlations with species richness, cover, height, alfalfa density, but were not significant (p > 0.05), which suggests that plant community structure and composition are not the main factors deciding the vegetation C storage in alfalfa fields. After the conversion of slope farmland to grassland, plants would quickly invade competing weakly for unlimited resources, which are the main factors determining plant community composition, plant diversity and succession dynamics [25]. As plant succession is ongoing, plant resources will become limited and plants will reach a relatively stable balance through competition. Therefore,

Table 3. Correlation coefficients of the vegetation, soil, and ecosystem C storage with their factors

Carbon storage	Biomass property			Community property				Soil property					
	Above- ground	Below- ground	Root/shoot	Litter	Species richness	Cover	Height	Alfalfa density	SOC	TN	TP	Bulk density	Soil water content
Vegetation Soil Ecosystem	$0.3 \\ -0.06 \\ -0.38$	1.00** 0.80* 0.90**	0.98** 0.80* 0.89**	0.90** 0.71* 0.91**	0.6 0.48 0.55	0.36 -0.11 -0.35	$0.35 \\ -0.03 \\ -0.36$	$0.01 \\ -0.28 \\ -0.42$	0.55 0.72* 0.71*	0.62 0.71* 0.72*	0.73* 0.93** 0.93**	$-0.32 \\ -0.03 \\ -0.09$	-0.33 0.12 0.02

\*Significant correlation at p < 0.05.

\*\*Significant correlation at p < 0.01.

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the amount of resources available to the plants is a key factor in the quantity of their biomass.

The plant community has an effect on soil processes, which are correlated with succession plant dynamics [26]. In our study, we found that soil C storage dynamics were consistent with belowground biomass storage with increased planting years (Tab. 1, Fig. 4c), because soil changes are associated with increasing belowground plant biomass [27]. Soil C storage had significant positive correlations with belowground biomass (p < 0.05), but no correlations with aboveground biomass also serves to illustrate this point. They indicate that the biomass accumulation was mainly attributed to belowground plant biomass rather than aboveground biomass after the alfalfa fields were established, which led to a higher belowground C input into the soil. It looks like the soil C sequestration dynamics along the chronosequence was synchronized with biomass C sequestration: soil C sequestration occurred at sites 13, 16, and 30, and these sites have the higher biomass C (especially belowground biomass and litter as they replenish SOC). From this, one can conclude that ecosystem net primary production in this ecosystem might be the most important factor controlling C sequestration, which is consistent with other studies [27, 28]. Moreover, soil C storage had significant positive correlations with litter biomass in the study (p < 0.05). Soil C input is mainly derived from the decomposition of litter [28]. In addition, the content of organic C and soil C storage decreased in the early succession stages (five years), when the alfalfa field had a high aboveground biomass and a low belowground biomass (Fig. 4a-c). This might be caused by less vegetation root biomass input into the soil and a high aboveground productivity, which consumed a large amount of soil nutrient in the early period.

It is well documented that farmland to grassland conversion will significantly increase soil C sequestration [6, 29, 30]. It is generally accepted that organic C increases along the succession timeline [6, 31], although a few studies have shown limited changes to organic C [32]. The study obtained similar results in alfalfa fields, i.e. C storage in the different soil layers increased when compared with those of the slope farmland after 30 years of cultivation, especially in the top 0-5 cm soil layer. This agrees with findings from other regions. Mensah et al. [29] found soil C increased by 52.7% (0-5 cm) following the conversion of cropland to grassland for 5-12 years in the eastcentral Saskatchewan region of Canada. Malhi et al. [33] observed a 114% increase in soil C (0-5 cm depth) in a 30-year old alfalfa field compared to an adjacent cultivated field. Su [34] found that farmland to perennial alfalfa conversion can increase soil C. Nelson et al. [30] reported that increased above- and belowground C inputs and decreased erosion resulting from permanent grass vegetation are likely to be the main factors contributing to an increase in soil C sequestration. Moreover, the study showed that SOC at upper soil levels (5-50 cm) first significantly decreased and then increased at the 16 year mark of alfalfa cultivation. Jiang et al. [13] also found similar results, namely that SOC decreases continuously up to 13 years of alfalfa planting in unfertilized and mown alfalfa grassland, only increasing thereafter. This is probably due to the long-term natural organic fertilizer and inorganic fertilizer added to the soil resulting in higher SOC in the farmland stage; immediately after having been abandoned the soil also had a higher level of SOC (Fig. 5c). Also, because the deeper soil did not have many roots due to crop removal after the harvest during the farmland stage, soil C storage in deeper layers always increased after alfalfa cultivation. In addition, because of low vegetation cover (Fig. 3a), soil erosion is seen as serious in

the early periods of grassland establishment following sloping farmland in the central Loess Plateau [6]. When alfalfa is planted for >13 years, degradation led to a decrease in plant biomass (Fig. 4) [13]. It also led to weed invasion and the death of large expanses of fibrous roots due to insufficient water content (Fig. 5b). At the same time, the input of SOC into soils increased, thereby enhancing the SOC for up to 16 years, followed by a decrease in the belowground biomass when SOC decreased. So, C storage decreased from the 16 year mark to 23 years after alfalfa planting. Soil C storage dynamics in every layer were consistent with SOC (Tab. 1, Fig. 5c), thus, soil C storage had significant correlations with SOC (p < 0.05) (Tab. 3).

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Piovanelli et al. [35] reported that SOC and TN were highly correlated in different tillage systems. In the present study, C storage had significant positive correlations with soil TN and the TN dynamics in every soil layer was consistent with that of SOC; these findings basically agree with Piovanelli et al. [35] and Zhang et al. [4]. Zhang et al. [4] also reported that intensive tillage and other operations carried out during the process of conversion affected not only SOC but also TN. Alfalfa, which can derive N from symbiotic fixation, would be expected to have a concomitant positive effect on both C and N added to the soil [36]. In addition, C storage also showed significant positive correlation with soil TP. On the Loess Plateau, P deficit in soil is a primary limiting factor to plant production [1]. In the study, C storage of vegetation had significant positive correlations with soil TP (p < 0.05). Moreover, soil TP dynamics, which were consistent with the vegetation aboveground biomass (Figs. 4a and 5e), also confirmed the relationship between soil TP and plant production. SOC also had significant positive correlations with soil TP in this study (p < 0.05). As Li et al. [37] reported, in the Loess Plateau soils of Northwest China, total P content increases linearly with increased organic matter content [37].

In water-limited ecosystems, plant growth, reproduction, and survival depend on the ability to absorb water through the roots [38]. The growth of perennial alfalfa can lead to the desiccation of deep soil layers in the hilly region of the Loess Plateau [38]. Planted perennial alfalfa causes soil desiccation because it takes up large amounts of water for growth, a finding observed in several field studies [8, 13, 38, 39]. Further, Li and Huang [8] found that soil water content decreased with the number of years of alfalfa growth in a long-term field experiment. Li and Huang [8] also found that the reduction in soil water storage resulted in the pasture yield responding more vigorously to variations in annual precipitation, particularly during the latter stages of their study. In our study, we also found that soil water content decreased with increasing years (Fig. 5b). Although C storage showed positive correlations with soil water content, it was not significant (p > 0.05) (Tab. 3). This suggests that soil moisture was not a limiting factor to ecosystem C accumulation in the alfalfa fields in the study area.

The ecosystem C pool is composed of two parts, plant and soil. Both the plant and soil dynamics of an ecosystem influence its structure and function [40]. The study showed that of the alfalfa field ecosystem, both plant and soil C storage increased with the number of years of cultivation, resulting in an increase in the C storage of alfalfa field ecological systems, showing the highest C storage at 16 years after which time the C storage decreased. This shift suggests that alfalfa field ecosystems gradually degrade after 16 years of cultivation, when the SOC, TN, TP, and soil moisture decrease. So, for conventional alfalfa C management in these areas, alfalfa fields



should be plowed under after the alfalfa has been cultivated for more than 16 years. This is important for sustainable ecological agriculture in the area. Jiang et al.'s [1] study in Yuzhong County, Gansu Province on the Loess Plateau reported that alfalfa fields should be plowed after alfalfa has been planted for more than nine years, when soil moisture and fertility conditions are favorable for the production of follow-up crops, and soil quality can be promoted by using water-harvesting technologies, adding organic fertilizers and inorganic compound fertilizers (N and P) to the soil. This finding has guiding significance for the sustainable management of alfalfa fields. Moreover, C storage varies with time according to the length of time the alfalfa has been cultivated, which may in turn help to determine whether grassland is a CO<sub>2</sub> sink or source in the process of vegetation succession [3].

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