Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

p-ISSN: 0972-6268
e-ISSN: 2395-3454

Vol. 17 | No. 1 | pp. 11-20 | 2018

Original Research Paper | **Open Access**

Soil Physico-Chemical Property Dynamics When Continually Growing Alfalfa (*Medicago sativa*) in the Loess Plateau of China

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Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 17-03-2017
Accepted: 23-05-2017

Key Words:
Alfalfa
Soil organic carbon
Soil N storage
Soil structure

ABSTRACT

Continually growing alfalfa (*Medicago sativa*) is very common in the dry land region of the Loess Plateau, China. The objective was to study the dynamics of the soil physico-chemical properties in alfalfa field soils over time. The space-for-time method was used to study a succession gradient of alfalfa, which were seven aged fields (1, 5, 8, 11, 15, 18 and 22 years). The results showed that the soil water content increased between 1 and 11 years, and then significantly decreased at 15 years (6.9%), finally, increased from 15 to 22 years. Soil bulk density continually decreased, and the variability between each layer for a given growing year significantly fell from 1 to 22 years. Long-term (up to 22 years) alfalfa cultivation has an important influence on the soil structure of the upper layer (0-70 cm), but has little effect on the deeper soil layer (70-100 cm). The soil organic carbon and soil carbon storage for the different soil layers in different planting years first decreased (1-11 years) and then increased (11-22 years). Total nitrogen storage (0-100 cm) first increased (1-5 years) and then decreased (after 5 years). The soil available K storage and soil alkali-hydrolyzale N storage, both increased as the number of cultivation years rose.

INTRODUCTION

Alfalfa has been widely grown in the dry land region of the Loess Plateau, China, since 1950s, primarily because of its high quality, yield, drought resistance (Deng et al. 2014) and its adaptability to various climatic and soil conditions (Chang et al. 2012). Since 1998, the alfalfa growing areas have expanded dramatically because a series of new environmental policies have been implemented in the Loess Plateau, such as the "Grain for Green" project, and because of the need to meet the demand for increasing livestock population (Li & Huang 2008). Existing data indicate that about 2.8 million ha of grasslands has been sown with alfalfa, which has produced 25 million Mg hay (Zhang et al. 2009). Gansu and Ningxia are currently China's major production bases for alfalfa forage.

As a perennial, leguminous grass, continuously planted alfalfa may influence the soil physico-chemical properties. Soil organic carbon (SOC) and nitrogen are the key elements of sustainable agriculture and the soil environment (Chang et al. 2012). SOC and total nitrogen (TN) are also the most important indicators of the soil quality. A sustainable soil C pool will mitigate increasing CO_2 levels in the atmosphere and consequently have a substantial influence on the C content of the atmosphere (Lal 2004). How to enhance the potential of the soil C pool to sequester soil carbon, and thus alleviate the rate of increase in CO_2 concentrations in the atmosphere, has become an important research area, globally (Chang et al. 2012). Previous studies have shown that converting forest land and grassland to arable land decreases the SOC content, whereas, converting land under annual crops into perennial grasslands may increase the organic C and N sequestration (Sainju & Lenssen 2011, Zhou et al. 2007). Converting cropland, particularly degraded arable land, into perennial grassland can also substantially increase the soil C storage (Conant et al. 2001).

In addition, after the land is converted from annual crops to perennial grasses, the reduced physical disturbance from ploughing and other agricultural activities improve the soil aggregation (Su et al. 2009). Soil aggregate stability is significantly correlated with SOC due to the binding action of humic substances and other microbial by-products (Goh

2004). SOC can be physically protected from microbial attack within soil aggregates, and contributes to the productivity and structural improvement of soils (Six et al. 1999). Furthermore, soil aggregates play an important role in retaining soil functions, such as soil water movement and retention, soil nutrient recycling, and resistance to soil wind and water erosion (Six et al. 1999). At present, in the Loess Plateau, growing alfalfa in the same fields for more than 10 years lead to serious land degradation and a significant decrease in alfalfa production. Jiang & Xiong (2007) reported that soil quality worsened over the first 9 years, while alfalfa production increased, whereas, soil quality tended to recover over the following years, but alfalfa production decreased. Deng et al. (2014) reported that during the late succession stage of alfalfa (13 years) soil quality tended to recover, and after 16 years, SOC and NT storage values again dropped.

Therefore, it is crucial to study the soil physico-chemical property dynamics of the alfalfa ecosystem in relation to the number of years planted, especially for soil C, N, and K storage, and soil aggregates. Many studies have reported a short-term or temporary change in either plant productivity or the soil properties of alfalfa fields (Bronick & Lal 2005, Fan et al. 2011). However, few studies have focused on soil physical and chemical properties and their relationship to long-term alfalfa growth.

The hypothesis of this study was that differently aged alfalfa fields have the same or similar growing environment, so that the age is the only independent variable. A one-way experiment and a space-for-time method were used to study the physico-chemical properties in the soil around alfalfa vegetation over a 22 year period. The objectives of this study were to evaluate the changes in the physico-chemical properties in alfalfa field soils over time on the Loess Plateau.

MATERIALS AND METHODS

Study site: The study area is located in Guangting Town, Guyuan City, Ningxia Province, China (106°24'-106°26' E, 36°9'-36°12' N, 1667-2148 m a.s.l.) (Table 1), and has a history of long-term alfalfa cultivation. It has a hilly landscape and is located in the middle of the Loess Plateau. There are deeply incised gullies and it is characterized by a sub-arid climate with heavy seasonal rainfall. Alfalfa (*Medicago sativa*) is the most commonly cultivated plant in the area. The study area's soil type is Aeolian soil (silt loam), and the soil pH ranges from 7.9 to 8.1. Average temperature, precipitation, and sunshine hours for the study site over the last 30 years (1984-2013) were 6.9°C, 421.8 mm, and 2583 hours, respectively. In Table 1, the annual mean temperature, precipitation and sunshine hours are the mean values of the growth period for alfalfa.

Experimental design and sampling: We selected a succession sequence of relatively homogeneous alfalfa fields that have been growing alfalfa for 5 to 22 years prior to the experiment, and one comparison where alfalfa had only been grown for 1 year (actually planted for 4 months). There were six alfalfa planting year intervals: 5, 8, 11, 15, 18 and 22 years. No fertilizer or manure was applied to the soils when the fields were planted with alfalfa, and the history of the sites was determined through interviews with local farmers. Three 10 m × 10 m plots were established for each age class in August 2014. Three sampling sites were separately chosen in the opposite corners and center of each plot. In total, we surveyed three plots with nine sampling sites in each age class, and there were 21 plots with 63 sampling sites for the six alfalfa age classes and the one comparison site in our study.

In each sampling site, 0-100 cm soil core samples were taken. First, we removed the litter layer before soil sampling, and dug a soil profile to take samples from six soil layers: 0-10, 10-20, 20-30, 30-50, 50-70 and 70-100 cm. We then mixed the same layers of the three samples together to make one soil sample for each layer per plot. The roots and other debris in the samples 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 ($g.cm^{-3}$) of the different soil layers was measured using a soil bulk sampler, which was 5 cm diameter and had a 5 cm high stainless steel cutting ring. Samples were taken at points adjacent to the soil sampling plots. The original volume of each soil core and its dry mass after oven-drying at 105°C were measured.

Measurements and analysis methods: Soil water content was measured gravimetrically and expressed as percentage of the soil water to dry soil weight (Jia et al. 2005). The bulk density (BD) calculation was based on the inner diameter of the core sampler, sampling depth, and the oven dried weight of the composite soil samples (Jia et al. 2012). SOC was measured by a K_2CrO_7-H_2SO_4 oxidation procedure, TN by the Kjeldahl method, and soil particle size by a laser particle size analyser (S3500 Microtrac Company, USA). Available K was determined by extraction with ammonium acetate, soil alkali-hydrolyzale N was determined by the alkaline hydrolysis diffusion method. SOC storage was calculated using the following equation (Guo & Gifford 2002):

$$C_S = \frac{BD \times SOC \times D}{10} \quad ...(1)$$

Where, C_s is soil C storage ($Mg.ha^{-1}$); BD is soil bulk density ($g.cm^{-3}$); SOC is soil organic carbon content ($g.kg^{-1}$); and D is soil thickness (cm). There were no stones in the study area, so there was no need to sieve the sample soils. The

Table 1: Location of the study sites and some meteorological parameters.

Life (year)	Latitude and Longitude	Altitude (m)	In the growth period of Alfalfa		
			Annual mean temperature (°C)	Annual mean precipitation (mm)	Hours of sunshine (hours)
1	36°09'43"N, 06°24'49"E	1667	7.9	455.5	2468.5
5	36°10'03"N, 06°25'09"E	1698	7.6	430.5	2464.4
8	36°10'05"N, 06°24'59"E	1714	7.7	404.3	2476.6
11	36°11'48"N, 06°23'42"E	1787	7.8	401.6	2509.4
15	36°08'50"N, 06°26'07"E	1733	7.7	415.7	2534.5
18	36°10'01"N, 06°25'05"E	1695	7.6	412.1	2561.2
22	36°10'50"N, 06°24'41"E	1756	7.4	422.4	2560.9

equations for total N storage, available K storage, and alkali-hydrolyzale N storage were the same as the SOC equation after substituting soil N, available K and alkali-hydrolyzale N contents for SOC content.

One-way ANOVA was used to analyse the means of the same soil layer physico-chemical properties among the different growth years at the $p < 0.05$ level, the factors were the mean values for the six soil layers (0-10, 10-20, 20-30, 30-50, 50-70 and 70-100 cm), the Pearson's correlation was used to analyse the correlation of different factors. The analysis was carried out using SAS (version 9.2).

RESULTS

Soil water content and BD: Fig. 1 shows that the upper layers had higher water contents than the lower layers. The water contents of the different layers first increased, then decreased, and then increased again, and were lowest at 15 years. Average soil water contents increased from 1 to 11 years ($p > 0.05$), and then significantly decreased to their lowest level at 15 years (6.9%). Average water content then significantly increased from 15 to 22 years ($p < 0.05$) to reach 10.4% at 22 years. Fig. 2 shows that soil BD continually decreased, as did the variability between each layer for the same growing year from 1 to 22 years, except for the 18th year. The decrease, as the number of planting years rose, indicated that the continuous alfalfa grassland in this poor soil may increase the soil internal porosity, which decreased BD variation in the different soil layers.

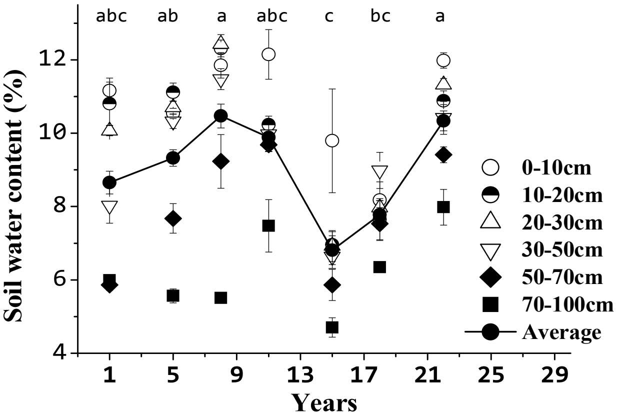

Fig. 1: Soil water content of seven fields that have had alfalfa growing in them for different lengths of time. Different letters indicate significant differences among the seven lengths of time ($p < 0.05$), and the same letters indicate no significant differences among the seven lengths of time ($p > 0.05$). The error bars are SE.

Soil particle size distribution: The soil particle size distributions of the different soil layers varied over the years (Table 2). The coarse sand and fine sand content at 0-10 cm increased as the number of growing years rose, but the clay content decreased. The coarse sand content in the first 7 years of alfalfa cultivation significantly increased compared with the values after 11 years of alfalfa growth ($p < 0.05$). After 7 years, the fine sand content in the 0-10 cm soil layer had significantly increased compared with younger alfalfa farmland ($p < 0.05$), and fine sand content in the 0-10 cm soil layer increased to its highest at 11 years growth (81.2%). However, the clay content in the 0-10 cm soil layer significantly decreased after 7 years of alfalfa cultivation ($p < 0.05$). The coarse sand and fine sand contents in the 10-20 cm and 20-30 cm soil layers have a wave-like, increasing trend, but

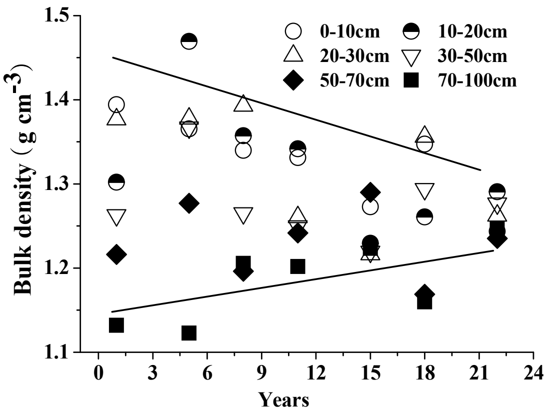

Fig. 2: Bulk density of seven alfalfa fields for each planting year.

Table 2: Particle size distribution of the soils (% vol) in the different soil layers of seven fields that have had alfalfa growing in them for different lengths of time.

Soil depth(cm)	Type	Years of cultivation (year) 1	5	8	11	15	18	22
0-10	Coarse sand	0.8±0.6c	0.7±0.10c	0.7±0.07c	0.8±0.03c	2.0±0.35b	2.0±0.3b	2.9±0.8a
	Fine sand	79.4±0.3bc	74.3±5.45c	77.5±5.08bc	80.9±2.29ab	81.2±2.63a	80.5±1.57abc	79.9±2.38abc
	Clay	19.8±0.3ab	25.1±5.39a	21.7±5.08ab	18.3±2.26b	16.8±2.51bc	17.5±1.36bc	17.2±1.85bc
	Silt	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10-20	Coarse sand	0.56±0.09c	0.5±0.16c	0.6±0.14c	0.9±0.14bc	1.9±0.18a	1.8±0.72a	1.4±0.67ab
	Fine sand	76.77±3.96b	79.0±5.44b	81.7±1.53ab	79.4±4.24b	82.8±1.08a	82.0±2.05ab	80.4±2.11b
	Clay	22.67±4.04a	20.5±5.6a	17.6±1.40a	19.7±4.48a	15.3±1.25b	16.1±1.74ab	18.2±2.42a
	Silt	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20-30	Coarse sand	0.6±0.2c	0.6±0.12c	0.7±0.03c	0.8±0.21bc	1.2±0.12a	0.8±0.22bc	1.0±0.16ab
	Fine sand	78.5±4.0c	81.4±0.95bc	88.5±1.24a	81.9±0.81bc	85.0±2.7ab	85.5±1.16ab	82.2±2.74bc
	Clay	20.9±4.1a	18.0±0.94ab	10.9±1.21c	17.4±0.9ab	13.8±2.79bc	13.7±1.25bc	16.8±2.6ab
	Silt	0.0	0.0	0.0	0.0	0.0	0.0	0.0
30-50	Coarse sand	1.0±0.2ab	0.7±0.37bc	0.7±0.11bc	0.7±0.23bc	1.1±2.4a	0.5±0.1c	0.5±0.07c
	Fine sand	83.8±0.7bc	82.2±1.24cd	87.4±0.05a	82.4±1.82bcd	84.7±1.73b	83.3±1.08bc	80.7±1.23d
	Clay	15.2±0.6bc	17.1±1.26ab	11.9±0.13d	16.9±1.81ab	14.2±1.79c	16.2±1.09bc	18.7±1.29a
	Silt	0.0	0.0	0.0	0.0	0.0	0.0	0.0
50-70	Coarse sand	1.5±0.3a	1.3±0.24ab	0.8±0.11cd	0.9±0.43bcd	1.1±0.21abc	0.6±0.18d	0.8±0.14bcd
	Fine sand	79.9±2.6d	82.1±1.67bc	88.7±0.71a	83.3±2.24bc	86.9±0.62a	85.7±1.46ab	85.8±1.34ab
	Clay	18.6±2.3a	16.6±1.58ab	10.4±1.26d	15.8±2.65ab	12.0±0.52cd	13.8±1.64bc	13.5±1.3bcd
	Silt	0.0	0.0	0.0	0.0	0.0	0.0	0.0
70-100	Coarse sand	1.3±0.5b	2.1±0.87a	1.0±0.17b	0.7±0.05b	0.7±0.27 b	0.5±0.08b	0.7±0.15b
	Fine sand	85.7±1.1abc	86.6±1.92ab	87.3±0.85a	84.2±1.55bc	87.2±1.4a	85.6±1.07abc	83.4±1.75c
	Clay	13.0±0.6bcd	11.3±1.05d	11.7±0.77cd	15.1±1.52ab	12.1±1.67cd	13.9±1.15abc	15.9±1.86a
	Silt	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Note: The particle size distribution of soils was determined in terms of sand 2-0.02 mm (coarse sand 2-0.2 mm, fine sand 0.2-0.02 mm), silt (0.02-0.002 mm) and clay (< 0.002 mm) as per ISSS (1930). Different letters indicate significant differences among the seven lengths of time ($p < 0.05$), and the same letters indicate no significant differences among the seven lengths of time ($p > 0.05$).

the clay did not significantly increase ($p > 0.05$) except at the 11 years stage ($p < 0.05$) and in the 10-20 cm soil layer. The clay contents showed a wave-like trend in the 20-30 cm soil layer. The coarse sand and fine sand content in the 30-50 and 50-70 cm soil layers decreased and the clay content increased as the number of cultivation years rose. However, in the 70-100 cm soil layers, the coarse sand, fine sand and clay contents did not vary much over the years.

SOC and soil C storage: The average SOC (Fig. 3) and the soil C storage values (Fig. 4) of the different soil layers over the cultivation years first decreased and then increased. The average SOC value decreased to its lowest at 11 years (4.86 g.kg^{-1}), and then increased to its highest at 22 years (8.9 g.kg^{-1}). The 0-10 cm soil C storage values first decreased and then increased as the number of cultivation years rose. Its lowest level was at 8 years and reached a maximum at 18 years after alfalfa planting (9.66 Mg.ha^{-2} and 18.74 Mg.ha^{-2} respectively) (Fig. 4a). At 15 years after alfalfa planting, C storage in the 10-20 cm soil layers significantly increased to a maximum of 12.7 Mg.ha^{-2} ($p < 0.05$) (Fig. 4b). The 20-30 cm soil C storage first decreased to its lowest level at 5 years and reached a maximum at 18 years (Fig. 4c). The 30-50, 50-70, and 70-100 cm soil C storage values first decreased and then gradually increased. Carbon storage was 7.3 Mg.ha^{-2} at 8 years, 1.4 Mg.ha^{-2} at 11 years, and 3.3 Mg.ha^{-2} at 15 years (Fig. 4c-f).

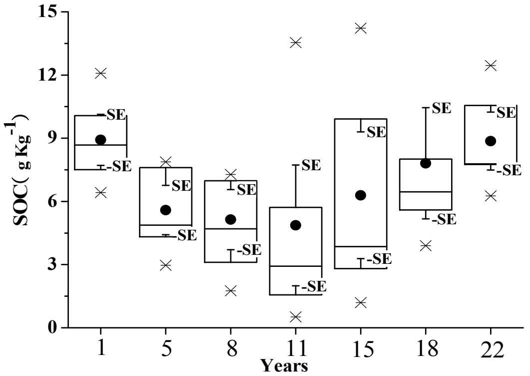

Fig. 3: The average soil organic C contents for the different planting years (g.kg^{-1}). The error bars are SE.

The soil C storage accumulation in the different soil layers also varied over the years (Table 3). The C storage in

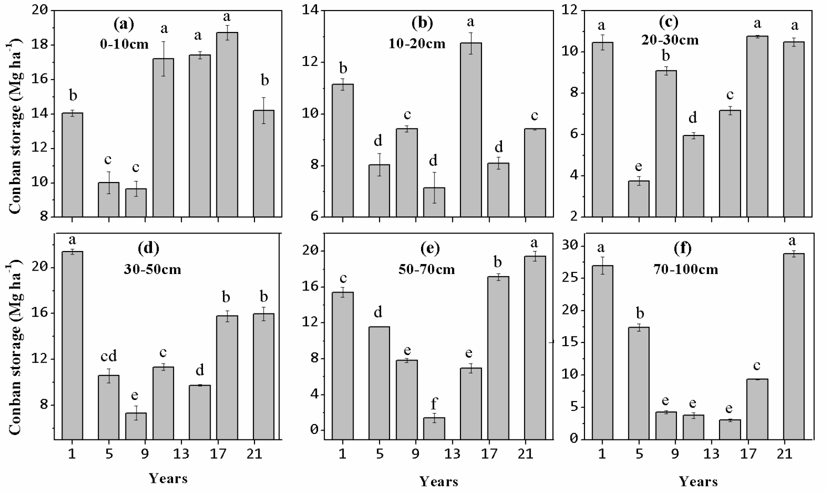

Fig. 4: Soil C storage ($Mg.ha^{-1}$) in the different soil layers of seven fields that have had alfalfa growing in them for different lengths of time. Different letters indicate significant differences among the seven lengths of time ($p < 0.05$), and the same letters indicate no significant differences among the seven lengths of time ($p > 0.05$). The error bars are SE.

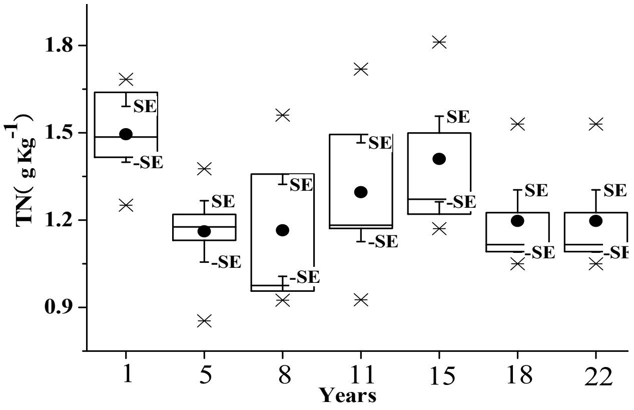

Fig. 5: The average soil total N values for the different planting years ($g.kg^{-1}$). The error bars are SE.

the 0-10 cm soil layers had significantly decreased at 5 and 8 years after planting compared to the first year value ($p < 0.05$), and significantly increased at 11, 15, and 18 years ($p < 0.05$). However, the 0-20 cm soil layer C storage values fluctuated. At 15 years after alfalfa planting, C storage, significantly increased and was higher than the other years ($p < 0.05$) at 30.2 $Mg.ha^{-2}$. Over 22 years of alfalfa cultivation, C storage in the 0-30 and 0-50 cm soil layers first decreased to its lowest at 8 years, and then increased dramatically. The C storage in the 0-30 cm soil layers reached a maximum at 18 years, and C storage in the 0-50 cm soil layer increased, but not in the first few years. The C storage in the 0-70 cm soil layer decreased to its lowest value at 11 years (39.1 $Mg.ha^{-2}$). The 0-100 cm soil layer first decreased to its lowest value at 11 years (48.2 $Mg.ha^{-2}$), and then increased to reach a maximum at 22 years (98.4 $Mg.ha^{-2}$), which was not significantly different to the first year of alfalfa cultivation ($p < 0.05$).

TN and soil N storage: The average value for soil TN (Fig. 5) and total N storage (Fig. 6) in the different soil layers first decreased, then increased, and then decreased again as the number of cultivation years rose. From 5 to 22 years, the average TN value increased and reached a maximum at 15 years (1.17 $g.kg^{-1}$), and then decreased to a minimum at 22 years (1.25 $g.kg^{-1}$). In the 0-10 cm soil layer, the total N storage first decreased and then increased to its highest level at 15 years. However, after 22 years, the total N storage value dropped to its lowest level (Fig. 6a). After 5 years of alfalfa cultivation (Fig. 6a-b), the total N storage in the 10-20 cm and 20-30 cm soil layers significantly increased to their highest value compared to the other layers ($p < 0.05$). The values then fluctuated until they reached their lowest at 22 years (1.27 $Mg.ha^{-2}$ and 1.47 $Mg.ha^{-2}$ respectively). The total N storage in the 30-50 cm and 50-70 cm soil layers had a similar trend to the 20-30 cm soil layer (Fig. 6c-e). However, N storage in 70-100 cm layer first decreased and then increased to its maximum at 22 years (Fig. 6f).

Table 4 shows the total N storage accumulation by the different soil layers after 22 years. It also shows the variation over the years. The total N storage after 22 years of cultivation significantly decreased compared to the first year in a number of the soil layers ($p < 0.05$), but the total N storage at 18 years was similar to the first year's value for all soil layers ($p > 0.05$) except for the 0-50 cm soil depth ($p < 0.05$). It seems that after alfalfa planting, soil N storage first decreases, then increases, and then decreases again.

Soil available K and soil available K storage: The average value for soil available K content showed a wave-like

Table 3: Carbon storage (Mg.ha^{-1}) in the different soil layers of seven fields that have had alfalfa growing in them for different lengths of time.

Years	Soil depth (cm)					
	0-10	0-20	0-30	0-50	0-70	0-100
1	14.1±0.18 b	25.2±0.35 bc	35.7±0.69 ab	57.1±0.72 a	72.5±0.94 a	99.5±1.81 a
5	10.0±0.64 c	18.1±1.07 d	21.8±1.03 d	32.4±0.88 f	44.0±0.90 e	61.4±1.40 c
8	9.7±0.44 c	19.1±0.32 d	28.2±0.46 c	37.6±0.87 e	47.3±0.41 d	51.6±0.44 e
11	17.2±1.01 a	24.4±1.17 c	30.3±1.31 c	39.5±0.42 e	39.1±0.83 f	42.8±0.93 f
15	17.4±0.22 a	30.2±0.55 a	37.4±0.56 a	47.1±0.51 d	54.0±0.91 c	57.1±1.11 d
18	18.7±0.43 a	26.8±0.31 b	37.6±0.25 a	53.4±0.73 b	70.5±0.62 ab	79.9±0.61 b
22	14.2±0.76 b	23.6±0.74 c	34.1±0.95 b	50.1±1.31 c	69.5±1.03 b	98.4±1.18 a

Note: Different letters indicate significant differences among the seven lengths of time ($p < 0.05$), and the same letters indicate no significant differences among the seven lengths of time ($p > 0.05$).

Table 4: Total nitrogen storage (Mg.ha^{-1}) in the different soil layers of seven fields that have had alfalfa growing in them for different lengths of time.

Years of cultivation (year)	Soil depth (cm)					
	0-10	0-20	0-30	0-50	0-70	0-100
1	2.1±0.09 a	4.0±0.13 ab	5.6±0.12 b	8.5±0.15 b	12.0±0.20 b	15.4±0.18 b
5	1.9±0.09 ab	4.2±0.16 a	6.0±0.12 a	9.1±0.04 a	12.9±0.08 a	16.3±0.16 a
8	1.8±0.11 b	3.4±0.12 c	4.9±0.07 c	7.8±0.11 c	10.8±0.13 c	12.9±0.16 d
11	2.0±0.08 ab	3.7±0.10 bc	5.1±0.14 c	7.4±0.12 d	10.1±0.11 d	12.4±0.05 d
15	2.1±0.05 a	4.0±0.10 ab	5.5±0.06 b	8.7±0.06 b	11.0±0.03 c	14.0±0.13 c
18	2.1±0.03 ab	4.0±0.05 ab	5.7±0.08 ab	9.1±0.05 a	12.9±0.11 a	15.7±0.17 b
22	1.5±0.06 c	2.8±0.10 d	4.2±0.10 d	7.2±0.13 d	10.1±0.20 d	13.6±0.26 c

Note: Different letters indicate significant differences among the seven lengths of time ($p < 0.05$), and the same letters indicate no significant differences among the seven lengths of time ($p > 0.05$).

trend (Fig. 7). After 15 years of cultivation, the average soil available K content value had non-significantly decreased ($p > 0.05$), and from 15 years to 22 years, the soil available K content increased significantly compared to 15 years ($p < 0.05$), which meant that the soil available K content returned to its original level. Similar to the average value for soil available K content, the total soil available K storage in the different soil layers first dramatically decreased and reached their lowest values after 8 years and 15 years ($p < 0.05$), and then significantly increased to their highest values at 22 years (Fig. 8).

Soil alkali-hydrolyzale N and soil available N storage: The average soil alkali-hydrolyzale N content values showed an increasing trend (Fig. 9). After 11 years of cultivation, the average soil alkali-hydrolyzale N content value had significantly increased ($p < 0.05$), and had reached its highest value (43 mg.kg^{-1}). From 11 years to 22 years, the soil alkali-hydrolyzale N content decreased non-significantly ($p > 0.05$). The variation in the alkali-hydrolyzale N content in different layers for the same year increased at first and then decreased (Fig. 10). Similar to the average value for soil alkali-hydrolyzale N content, the total soil alkali-hydrolyzale N storage in the different soil layers first dramatically increased to their highest value at 11 years ($p < 0.05$), and then decreased non-significantly ($p > 0.05$), except for the 0-10 and 0-30 cm layers where the total soil alkali-hydrolyzale N storage decreased significantly ($p < 0.05$) (Fig. 10).

DISCUSSION

Growing Leguminosae plants tend to maintain organic C levels, compared to bare soil by continuously supplying C from the roots and by stimulating microbial growth in the rhizosphere, which tends to decrease SOC (Sanchez et al. 2002). It is generally accepted that organic C increases along a succession timeline (Brye & Kucharik 2003), and some studies have shown limited changes to organic C (Chang et al. 2012, Ghimire et al. 2014). In this study, C storage in the different soil layers first decreased and then increased after 22 years of alfalfa cultivation (Fig. 4). In the first 10 years of succession, soil C storage decreased dramatically, mainly because the early cropland had accumulated SOC from fertilization by long-term natural organic and inorganic fertilizer applications during the farmland stage. Furthermore, underground root C storage also substantially increased (Deng et al. 2014). In addition, because of the low vegetation cover, soil erosion is seen as serious in the early periods of grassland establishment in the central Loess Plateau

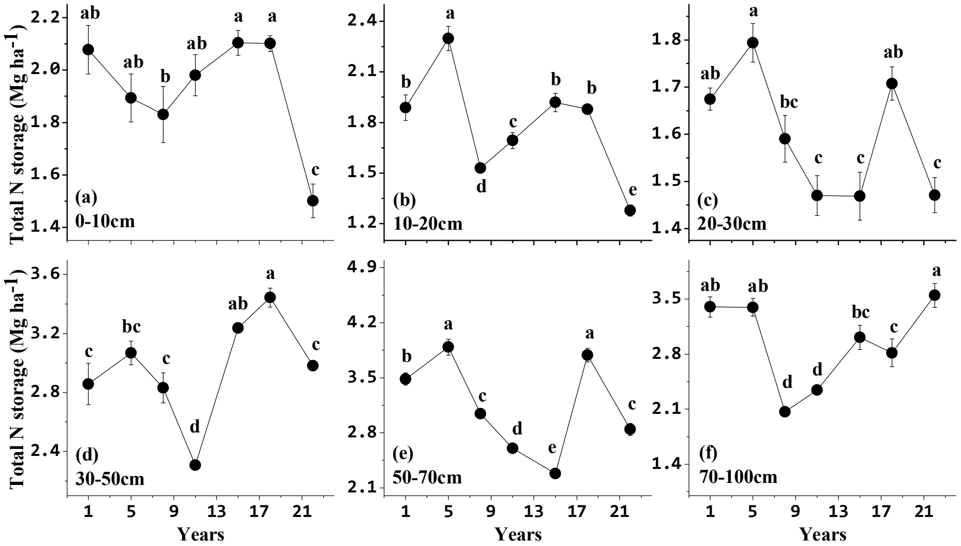

Fig. 6: Total N storage (Mg.ha^{-1}) in different soil layers of seven fields that have had alfalfa growing in them for different lengths of time. Different letters indicate significant differences among the seven lengths of time ($p < 0.05$), and the same letters indicate no significant differences among the seven lengths of time ($p > 0.05$). The error bars are SE.

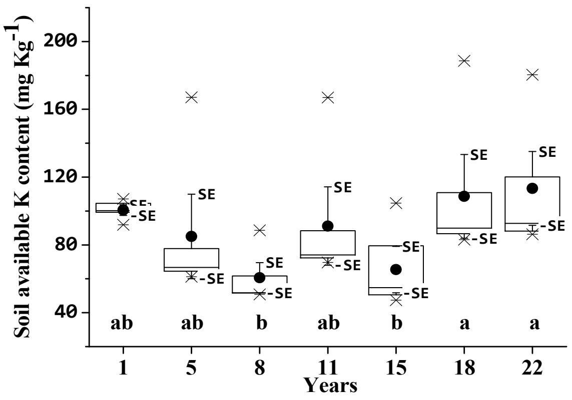

Fig. 7: Soil available K content (mg kg^{-1}) in seven fields that have had alfalfa growing in them for different lengths of time. Different letters indicate significant differences among the seven lengths of time ($p < 0.05$), and the same letters indicate no significant differences among the seven lengths of time ($p > 0.05$). The error bars are SE.

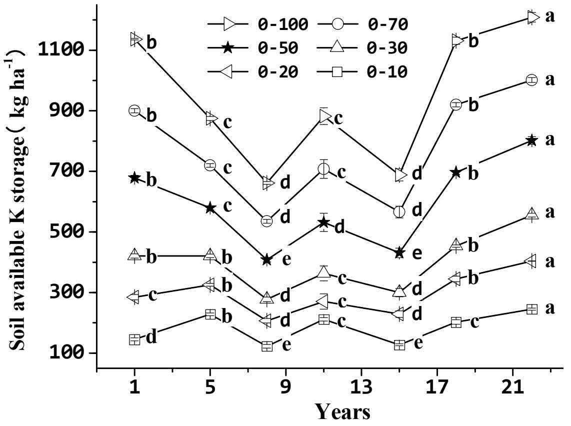

Fig. 8: Soil available K storage in the different soil layers of seven fields that have had alfalfa growing in them for different lengths of time. Different letters indicate significant differences among the seven lengths of time ($p < 0.05$), and the same letters indicate no significant differences among the seven lengths of time ($p > 0.05$). The error bars are SE.

(Chang et al. 2011), so the soil C storage as a C source, and soil C production cannot replace the soil C lost in the early stages of converting farmland to alfalfa grassland. However, after 10 years cultivation of no-tillage alfalfa, the soil C storage increased significantly, especially in the 30-50 cm, 50-70 cm, and 70-100 cm layers, and this agrees with findings from other researchers (Li & Huang 2008). It seems that soil C storage rose in the deeper soil layers as the number of cultivation years increased. Furthermore, because the deeper soil did not have many roots during the early stages of cultivation, soil C storage in the deeper layers always increased after alfalfa roots had reached them. The soil C storage accumulation in the different soil layers also varied with the number of cultivation years (Table 3). After 22 years of alfalfa cultivation, the C storage in the upper soil layers, especially the 0-10, 0-20, and 0-30 cm layers, was smaller than in the 0-50, 0-70 and 0-100 cm layers (Table 3). The change in C storage by the deeper soil layers was larger than in the upper layers, which suggests that the deeper soil layers are the more important C storage layers during long-term alfalfa cultivation. At the same time, soil C storage dynamics

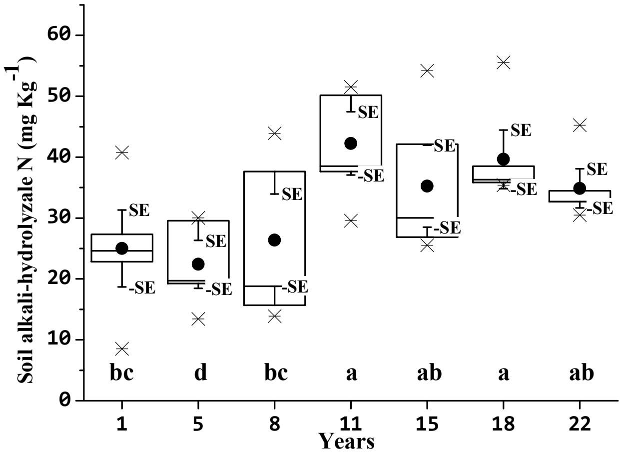

Fig. 9: The average value of soil alkali-hydrolyzale N (mg.kg^{-1}) in seven fields that have had alfalfa growing in them for different lengths of time. Different letters indicate significant differences among the seven lengths of time ($p < 0.05$), and the same letters indicate no significant differences among the seven lengths of time ($p > 0.05$). The error bars are SE.

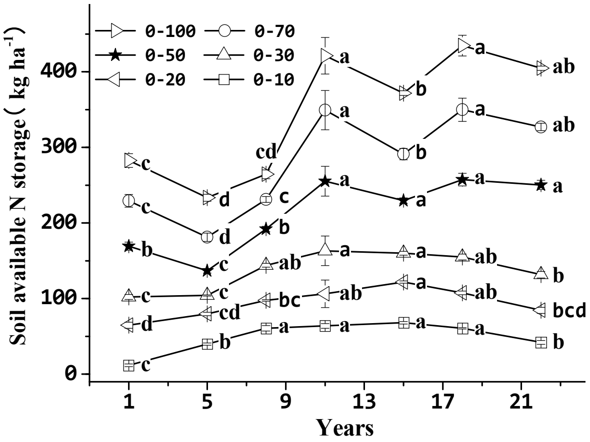

Fig. 10: Soil available N storage in the different soil layers of seven fields that have had alfalfa growing in them for different lengths of time. Different letters indicate significant differences among the seven lengths of time ($p < 0.05$), and the same letters indicate no significant differences among the seven lengths of time ($p > 0.05$). The error bars are SE.

in every layer were consistent with SOC (Figs. 3 and 4) and had a significant correlation with SOC ($r = 0.79$, $n = 7$, $p < 0.05$). Moreover, C storage varies according to the length of time the alfalfa has been cultivated, which may in turn help to determine whether grassland is a CO_2 sink or source during vegetation succession.

Zhang et al. (2009) reported that intensive tillage and other operations carried out during the process of conversion affected not only SOC, but also TN. However, in the present study, TN content in the different soil layers significantly decreased compared to the first year of alfalfa cultivation (Fig. 5), mainly because the farmland had accumulated too much artificial nitrogenous fertilizer, which cannot be consumed over a short period of time. In other words, the N that was derived from symbiotic fixation by perennial alfalfa over 22 years does not reach the N value of farmland. Furthermore, TN storage in all soil layers increased slightly. In the 0-10 cm layer, it decreased during the first 5 years after alfalfa planting (Fig. 6a). This was because artificial nitrogenous fertilizer losses were larger than N fixation by perennial alfalfa just after farmland conversion to alfalfa. However, TN storage in the other layers also decreased during the first 5 years after alfalfa planting (Fig. 6b-f). From 5 to 22 years of cultivation, the TN storage in each layer first decreased, then increased, and finally decreased. This was probably also due to an imbalance between TN fixation and loss, and this underground and aboveground bio-nitrogen variation may affect soil TN storage.

Soil structure is a key factor that affects soil function, and its ability to support plant and animal life. It is able to moderate environmental quality, particularly with regards to soil C sequestration and water quality. Aggregate stability is used as an indicator of soil structure (Six et al. 1999, Six et al. 2000). Previous studies have shown that long-term alfalfa planting has positive effects on the proportion of large size fractions (Su et al. 2009, Zhang et al. 2015). This study suggested that the proportion of coarse sand (2-0.2 mm) and fine sand (0.2-0.02 mm) in the 0-10 cm layer had increased as the number of cultivation years rose (Table 2), and the coarse sand and fine sand content in the 10-20 cm and 20-30 cm soil layers had a wave-like increasing trend. The coarse sand and fine sand content in the 30-50, and 50-70 cm soil layers decreased over time, but at 70-100 cm, the coarse sand, fine sand, and clay content remained constant. Therefore, we can say that long-term (up to 22 years) alfalfa cultivation has an important influence on the soil structure of the upper layers (0-70 cm), but has little effect on the deeper soil layer (70-100 cm).

In water-limited ecosystems, plant growth, reproduction, and survival depend on the ability to absorb water through the roots (Fan et al. 2011). Therefore, the growth of perennial alfalfa can lead to drying out of the deep soil layers in the hilly region of the Loess Plateau (Deng et al. 2014, Fan et al. 2011, Jiang et al. 2006). This study found that the upper layers had higher water contents than the lower layers (Fig. 1), which means soil water content decreased as soil depth increased. It seems that deeper alfalfa roots absorb more water than the upper roots. The average water content in the soil layers first increased, then decreased, and then increased again, and reached its lowest value at 15 years

(Fig. 1). We conclude that alfalfa cover increased in the first 8 years of cultivation and that this led to a rise in the water content of the upper soil because there was less water evaporation from the bare soil. From 8 to 15 years, the water content decreased, mainly because in the deeper layers (30-100 cm), more and more soil water was absorbed by roots and then transpired from the plants into the air. Finally, soil water content increased from 15 to 22 years. This was probably because of low transpiration and low productivity by the alfalfa.

The soil bulk density continually decreased (Fig. 2) as the number of years increased, which indicated that continuous alfalfa grassland in this poor soil may potentially improve soil internal porosity, which confirms previous reports (Deng et al. 2014, Jiang et al. 2006).

Available K and alkali-hydrolyzale N play a key role in plant growth and productivity, and are an evaluation index of soil fertility because they are directly taken up by plants (Baptista et al. 2014, Brodsky et al. 2006, Qian & Schoenau 2007). In this study, the average soil available K storage value showed a wave-like, significantly increasing trend as the number of years rose (Fig. 9). The variability in the soil available K content in the different layers also generally increased over the 22 years (Fig. 8). Therefore, we can conclude that long-term planting with alfalfa did not change the soil available K content, but changed the distribution of soil available K in the different layers. Further research is needed to investigate why the soil available K content changed and what factors affected its distribution.

The average value of soil alkali-hydrolyzale N content showed an increasing trend (Fig. 9). The average value of soil alkali-hydrolyzale N content reached its highest value at 11 years, but from 11 years to 22 years, the soil alkali-hydrolyzale N content did not significantly increase ($p > 0.05$) (Fig. 10). The total soil alkali-hydrolyzale N storage had a similar trend to the average soil alkali-hydrolyzale N content. This means that long-term planting with alfalfa can increase the soil alkali-hydrolyzale N content and storage in the first 11 years, and this result is similar to the results reported by Li & Huang (2008) and Su et al. (2009). After 11 years of alfalfa cultivation, it seems that soil alkali-hydrolyzale N levels remain stable. This finding is important for the sustainable management of alfalfa fields.

ACKNOWLEDGEMENT

The study was funded by the National Natural Science Foundation of China (31660143), Ningxia University Top Disciplines Foundation (NXYLXK2017A01) and the Chinese Postdoctoral Science Foundation (2015M580896) .

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