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# Soil carbon sequestration by three perennial legume pastures is greater in deeper soil layers than in the surface soil

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# Abstract

Soil organic carbon (SOC) plays a vital role as both a sink for and source of atmospheric carbon. Revegetation of degraded arable land in China is expected to increase soil carbon sequestration, but the role of perennial legumes on soil carbon stocks in semiarid areas has not been quantified. In this study, we assessed the effect of alfalfa (Medicago sativa L.) and two locally adapted forage legumes, bush clover (Lespedeza davurica S.) and milk vetch (Astragalus adsurgens Pall.) on the SOC concentration and SOC stock accumulated annually over a 2 m soil profile, and to estimate the longterm potential for SOC sequestration in the soil under the three forage legumes. The results showed that the concentration of SOC of the bare soil decreased slightly over 10 the 7 years, while 7 years of legume growth substantially increased the concentration of SOC over the 0-2.0 m soil depth measured. Over the 7 year growth period the SOC stocks increased by 24.1, 19.9 and 14.6 Mg C ha<sup>-1</sup> under the alfalfa, bush clover and milk vetch stands, respectively, and decreased by 4.2 Mg C ha<sup>-1</sup> under bare soil. The sequestration of SOC in the 1-2 m depth of soil accounted for 79, 68 and 74 % of SOC 15

sequestered through the upper 2 m of soil under alfalfa, bush clover and milk vetch, respectively. Conversion of arable land to perennial legume pasture resulted in a significant increase in SOC, particularly at soil depths below 1 m.

# 1 Introduction

<sup>20</sup> Concerns about global warming and increasing atmospheric greenhouse gas concentrations (CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O) have led to questions on the role of soils as a source or sink for carbon. Excluding carbonated rocks, soils constitute the largest surface carbon pool, approximately 1500 Gt, equivalent to almost three times the quantity stored in the terrestrial biomass and twice the amount stored in the atmosphere (IPCC, 2000).
 <sup>25</sup> Globally, soil cultivation has resulted in the loss of more than 40 PgC, at a rate of about



 $1.6 \text{ Pg C year}^{-1}$ , to the atmosphere during the 1990s (Smith, 2008). Chinese agricultural soils have also lost 30–50 % or more of the soil carbon pool (Lal, 2004a).

Soil organic carbon (SOC) is a significant component of the global carbon stocks (Chen et al., 2008). China has experienced widespread conversion of natural vegeta-

- tion into cultivated arable land to meet the food demand of its rising human population. This conversion has caused a dramatic decrease of 7 Pg of the total SOC pool in the upper 1 m of the soil profile, that is 9.5% of the decrease in SOC worldwide (Wu et al., 2003). Fortunately, the loss of SOC can be slowed down by implementing crop management practices such as conservation tillage (Lal, 2004b; Puget and Lal, 2005), converting degraded arable land to perpendic grapping (Captile et al., 2025), using dis
- <sup>10</sup> converting degraded arable land to perennial grassland (Gentile et al., 2005), using diverse rotations, and introducing legume and grass mixtures into the rotation (Lal, 2002, 2004b, c).

In the USA, the revegetation of highly-erodible cropland or other environmentallysensitive areas to resource-conserving vegetation for a period of 10 to 15 years in-

- <sup>15</sup> creased the SOC content in the upper 3 m of soil at average rate of 1.1 Mg Cha<sup>-1</sup> year<sup>-1</sup> (Osborn, 1993). This conservation reserve program (CRP) also significantly increased the soil C pool (Staben et al., 1997) and provided multiple benefits both environmentally and economically (Munson et al., 2012; Wu and Lin, 2010). Like the CRP program in USA, a program of soil and water conservation, namely "Grain for Green"
- <sup>20</sup> was implemented on the Loess Plateau of China in 1999 to alleviate land degradation. The program of eco-environmental revegetation focused on the recovery of damaged ecosystems (Wang et al., 2010) by the use of perennial vegetation to control soil erosion, increase the stocks of SOC and prevent the occurrence of dry layers in the loess soils (Fu et al., 2010). Alfalfa (*Medicago sativa* L.) has been widely grown on the Loess
- Plateau to increase livestock production and improve water-use efficiency and soil fertility through high forage production, and for its ability to decrease soil erosion and fix atmospheric N (Guan et al., 2013). Additionally, locally-adapted legume species such as bush clover (*Lespedeza davurica* S.) and milk vetch (*Astragalus adsurgens* Pall.) have been widely grown as cover crops or windbreaks to protect the soil from water



or wind erosion in arid and semiarid region of northern China (Wang, 2003; Xu et al., 2006). The "Grain for Green" program has reduced wind and water erosion of marginal arable land and is expected to significantly contribute to soil C sequestration. Recent studies have investigated and estimated the changes in SOC stocks in the top 1 m of

- soil as a result of revegetation of regional watersheds on the Loess Plateau of China (Fu et al., 2010; Wang et al., 2011; Yan et al., 2007; Zhang et al., 2013). However, deep-rooted perennial legumes may penetrate deeper in the soil profile than 1 m, likely underestimating the SOC-sequestration potential of these forage legumes in northwest China.
- The objectives of this research were: (i) to assess the effect of alfalfa and two locally adapted forage legumes, bush clover and milk vetch, on the SOC concentration and SOC stock accumulated annually over a 2 m soil profile, (ii) to estimate the long-term potential for SOC sequestration in the soil under the three forage legumes. The SOC content in the 2 m soil profile was measured at the end of each growing season to guantify the SOC concentration and stock under the locally-adapted forage legumes
- quantify the SOC concentration and stock under the locally-adapted forage legumes and alfalfa, and to provide specific information for estimating the SOC sequestration potential of an important agricultural area. The hypothesis tested was that long-term growth of deep-rooted perennial legumes will increase soil organic C, provide a feed source for animals and provide a sink for atmospheric carbon.

#### 20 2 Materials and methods

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#### 2.1 Experimental site description

The experiment was conducted at the Changwu Agro-ecological Experiment Station on the Loess Plateau (35°12′ N, 107°40′ E), Shaanxi province, China, from 2004 to 2010. The site is level and located at 1220 ma.s.l. The climate is semiarid, with an annual mean temperature of 9.1 °C and a mean annual precipitation of 579 mm (1979– 2003) with rainfall concentrated in the period from July to September. Precipitation and



temperature data were recorded at the Changwu Meteorological Station, 20 m from the experimental site. The groundwater table is 50–80 m below the soil surface, making it unavailable for plant growth. Prior to the establishment of this experiment, the site was planted to winter wheat for many (at least 20) years. For winter wheat production, the site was ploughed to a depth of 0.3 m twice a year, after harvest in early July and again in September before sowing; only wheat stubble was returned to the soil, but  $108 \text{ kg} \text{ N} \text{ m}^{-2}$  and  $276 \text{ kg} \text{ P}_2 \text{ O}_5 \text{ m}^{-2}$  of fertilizer was applied each year before sowing. In 2003, after the winter wheat was harvested, the site lay fallow for 280 days to allow

- moisture accumulation over the winter before the legumes were sown in May 2004.
   Soil at the experimental site belongs to the Loess series. The texture in the top 5 m is a uniform silty clay loam (haplic greyxems, FAO-UNESCO, 1988), with a mean sand, silt, and clay content of 3.5, 65.6, and 30.9%, respectively. The soil physical characteristics do not significantly change in the upper 5 m. The measured average bulk density of the soil in the upper 2 m is 1.31 g cm<sup>-3</sup> and the top 0.3 m contained 1.55% total organic matter, 0.106% nitrogen, and 0.095% available phosphate prior
- to the commencement of the experiment in 2004.

# 2.2 Treatments and forage yield measurements

Twelve experiment plots, each 4 m by 3 m, were established in early May 2004 with one of three forage legume species, milk vetch (*Astragalus adsurgens* Pall.), alfalfa (*Med-icago sativa* L.) and bush clover (*Lespedeza davurica* S.), and an unplanted control. Each legume species was grown as a monoculture at a seeding density of 25 plants m<sup>-2</sup>, weeds were removed from all plots by hand using local farming practice. The plots were adjacent to each other. During the experimental period from 2004 to 2010, there was no irrigation or other form of supplementary water. Treatments were arranged in completely randomized in three replicate blocks.

Each year from 2005–2010, measurements of forage yield of each legume were taken at the end May, July and September (in 2004 only one cut was made in September) by cutting the plants at ground level with hand-held shears in  $1 \text{ m} \times 1 \text{ m}$  quadrats



selected randomly within the plot, but avoiding border areas. At the same time, the rest of the plot was also cut at the same height and the forage removed. The oven-dry weight was determined after drying at  $105^{\circ}$ C for 0.5 h and then further dried at  $75^{\circ}$ C for 48 h (Guan et al., 2013).

#### **5 2.3 Soil sampling and analysis**

Soil samples were taken with a cylindrical steel corer (diameter 40 mm and height 200 mm) at two random positions in each plot which were combined into one composite sample per plot before analysis. Each plot was sampled from the surface to 2 m deep at depths of 0–0.3, 0.3–0.6, 0.6–1.0, 1.0–1.5 and 1.5–2.0 m before sowing on 10 May 2004 and at the end of each legume growing season (29 October) from 2004 to 2010. The soil samples were air-dried, roots and organic debris removed, ground and sieved through a 2 mm sieve, then stored at room temperature before analyzing the SOC.

The concentration of soil organic carbon (in  $gkg^{-1}$ ) was measured using the wet dichromate oxidation procedure (Nelson and Sommers, 1996). Briefly, 0.5 g soil samples were digested with 5 mL of 1N K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and 5 mL of concentrated H<sub>2</sub>SO<sub>4</sub> at 150 °C for 0.5 h, followed by titration of the digests with standardized FeSO<sub>4</sub>.

# 2.4 SOC stock calculation and statistical analyses

Soil organic C stock was calculated as Eq (1):

<sup>20</sup>  $C_{\text{stock}} = \text{SOC} \times \rho \times H \times 10$ 

where SOC is the SOC concentration  $(gkg^{-1})$  in each soil layer,  $\rho$  is the soil bulk density  $(gcm^{-3})$ , and H is the depth of soil layers.

The data were analyzed by analysis of variance (ANOVA) applied to the data, and means were compared using the LSD at P < 0.05 to characterize the differences

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(1)

among treatments. PROC GLM (General Linear Model) were used to assess the temporal changes in SOC stock and the rate and amount of SOC sequestered by Statistical Analysis System (SAS Institute, Cary, NC, version 8.02).

## 3 Results

# 5 3.1 Meteorological conditions

The average monthly mean temperature from June to August, the primary growth period for the legumes, was about 20 °C. Monthly mean temperatures were about 1 °C warmer than the long-term mean throughout the experimental period (Fig. 1). Over the experimental period, the total annual precipitation varied from 470 mm in 2006 to 583 mm in 2010, and was below the long-term mean in all years except 2010 when the rainfall was similar to the long-term mean (Fig. 1). Rainfall from July to September accounted for 55–60 % of total annual precipitation, while rainfall in the legume-growing season (from April to October) was about 90 % (range from 84 % in 2009 to 96 % in 2005) of the total annual precipitation (Fig. 1).

# **3.2** Aboveground forage biomass production

The results of the aboveground biomass production over the seven years have been reported by Guan et al. (2013). Briefly, the annual production of milk vetch increased from 2.2 tha<sup>-1</sup> in the first year to 14.3 tha<sup>-1</sup> in 2006 and then decreased, alfalfa increased from 2.3 tha<sup>-1</sup> in the first year to a maximum of 22.2 tha<sup>-1</sup> in 2006 and then 2.0 decreased, while bush clover increased from 0.2 tha<sup>-1</sup> in the first year to 7.8 tha<sup>-1</sup> in 2009 and did not decrease significantly thereafter (Table 1). Total forage yield over the experimental period was highest in alfalfa at 91 tha<sup>-1</sup>, compared to 56 tha<sup>-1</sup> in milk vetch and 42 tha<sup>-1</sup> in bush clover (Table 1).



#### 3.3 SOC concentration over the soil profile

The legumes significantly (P < 0.001) increased the SOC concentration at each soil depth, and this effect varied with legume species and experimental year (Table 2). The initial concentration of SOC in May 2004 decreased with increasing soil depth (Fig. 2).

In the upper 0–0.3 m of soil, the initial SOC concentration was 8.0 ± 0.03 g kg<sup>-1</sup>, while it was only 3.3 ± 0.27 g kg<sup>-1</sup> in the 1.5–2.0 m soil layer (Fig. 2). Comparison of the SOC concentration between the initial values on 10 May 2004 and those at the end of the experimental period in October 2010 showed that the concentration of SOC of the bare soil decreased slightly over the 7 years, while 7 years of legume growth substantially increased the concentration of SOC at 0.6–1.0, 1.0–1.5 and 1.5–2.0 m soil depth and a small, but significant, increase in the upper 0.3 m of the soil in bush clover, but not in milk vetch and alfalfa. No significant changes were observed after 7 years at the 0.3–0.6 m depth (Fig. 2).

#### 15 3.4 SOC stock over the experimental period

SOC stock was calculated by converting SOC concentration to the amount of SOC per soil layer per unit area. The SOC stock in 2004 varied from  $20 \pm 0.85$  MgC ha<sup>-1</sup> in the 0.3–0.6 m soil layer to  $32 \pm 0.68$  MgC ha<sup>-1</sup> at 0–0.3 and 1.0–1.5 m depth (Fig. 3). In the bare soil, the SOC stock decreased at all depths across the experimental period, but only decreased significantly at –0.36 MgC ha<sup>-1</sup> year<sup>-1</sup> (P < 0.05) in the 1.5–2.0 m layer (Fig. 3d). In the legume plots, the SOC stock increased linearly with time (2004–2010) in the 0–0.3 m, 0.6–1.0 m, 1.0–1.5 m and 1.5–2.0 m soil layers, but not in the 0.3– 0.6 m soil layer (Fig. 3). The change in SOC stock over the 7 years was greatest at soil depths below 1.0 m in all three species and was greatest in the alfalfa plots with rates of 1.35 MgC ha<sup>-1</sup> year<sup>-1</sup> at a depth of 1.0–1.5 m (P < 0.001), and 1.39 MgC ha<sup>-1</sup> year<sup>-1</sup> at a depth of 1.5–2.0 m (P < 0.001) (Fig. 3b). The highest accumulation of SOC stock oc-



curred at a depth of 1.0–1.5 m in bush clover where it averaged 1.58 Mg C ha<sup>-1</sup> year<sup>-1</sup> (P < 0.001) (Fig. 3c).

Over the full 0–2.0 m depth, the SOC stock under bare soil decreased slightly over the 7 years, but increased under alfalfa, milk vetch, and bush clover (Fig. 4). The SOC
stock increased more under the stand of alfalfa than milk vetch, but there was no significant difference between alfalfa and bush clover (Fig. 4). When calculated over the full 2 m soil layer, over the 7 year growth period the SOC stocks increased by 24.1, 19.9 and 14.6 Mg C ha<sup>-1</sup> under the alfalfa, bush clover and milk vetch stands, respectively, and decreased by 4.2 Mg C ha<sup>-1</sup> under bare soil (Fig. 5). In the 1.0–2.0 m soil layer the stocks of SOC increased by 19.1, 13.6 and 10.8 Mg C ha<sup>-1</sup>, under the alfalfa, bush clover and milk vetch stands, respectively, that is, by 79, 68 and 74 % of the increases in the whole soil profile (Fig. 5).

#### 4 Discussion

The aboveground biomass production over the seven years of the experiment was highest in alfalfa at 91 tha<sup>-1</sup>, significantly higher than the biomass production in milk vetch 15 (56 tha<sup>-1</sup>) and bush clover (42 tha<sup>-1</sup>) (Table 1). While alfalfa had the highest increase in SOC stocks in the upper 2 m of the soil (24.1 MgCha<sup>-1</sup>), bush clover had a similar increase to alfalfa (19.9 MgCha<sup>-1</sup>) and milk vetch has a significantly smaller increase in SOC (14.6 Mg C ha<sup>-1</sup>) over the 7 year period (Fig. 5). The study by Guan et al. (2013) showed that alfalfa extracted more water from the soil below 1.2 m than milk vetch and 20 bush clover, but there was no difference in water extraction between milk vetch and bush clover at any depth in the 5 m profile measured. While Guan et al. (2013) did not measure the root biomass, the water extraction profile suggests that root biomass did not vary significantly between milk vetch and bush clover and cannot explain the greater accumulation of SOC in the upper 2 m of the soil profile in bush clover than the 25 milk vetch, particularly in the 1.0-1.5 m soil depth (Fig. 5).



Although highly-productive perennial forage legumes with deep roots have been shown to cause a significant decrease in soil water at depth in semiarid environments (Guan et al., 2013), they are considered to have an important role in sequestering SOC in deep soil layers (Gentile et al., 2005). In the present study, the SOC in the <sup>5</sup> upper 2 m of the soil at the beginning of the experiment in 2004 was 137 Mg C ha<sup>-1</sup> and increased to 151, 161 and 157 Mg C ha<sup>-1</sup> under the milk vetch, alfalfa and bush clover stands, respectively, by the end of the experiment in 2010 (Fig. 4). This indicates that as a result of planting the legumes, the SOC sequestered over the 7 years was 14, 24 and 20 Mg C ha<sup>-1</sup> in milk vetch, alfalfa and bush clover (Fig. 5), respectively, but would have lost 4 Mg C ha<sup>-1</sup> if the soil had been left unplanted. The accumulation of SOC in the upper 0.3 m of the soil was highest in bush clover at 3.4 Mg C ha<sup>-1</sup>. The accumulation of SOC in the upper soil layer may be attributed to the high accumulation of legume residues and litter (Zhou et al., 2006) or due to the proliferation and turnover of roots

- <sup>15</sup> in this surface layer, suggesting that bush clover had the greatest turnover of leaf litter or the greatest density and turnover of roots in the surface layer. Our results for the sequestration of SOC in the upper 0.3 m of the soil were significantly lower than Zhang et al. (2009) who reported that the SOC stock in 0–0.3 m soil layer had increased by 16 Mg Cha<sup>-1</sup> ten years after the conversion of a wet reed meadow to an alfalfa pasture
- <sup>20</sup> under irrigated condition at Hexi Corridor in northwestern China. This suggests that well-managed legume pastures in areas with higher precipitation and with appropriate fertilizer use could sequester significant amounts of SOC.

The sequestration of SOC in the 1–2 m depth of soil accounted for 79, 68 and 74% of SOC sequestered through the whole top 2 m of soil under alfalfa, bush clover and <sup>25</sup> milk vetch, respectively, indicating the importance of deep roots (Fig. 5). This was consistent with Kätterer et al. (2011) who found that root-derived carbon was about 2.3 times higher than that from above-ground residue-derived C from a long-term field experiment in Sweden. Rasse et al. (2005) and Johnson et al. (2006) attributed the SOC increase in the rhizosphere to the C from root turnover and cells sloughing off the epi-



dermal root tissues during the growing season, and to soluble C compounds released from the roots by exudation. With the water table at a depth of 20–300 m on the Loess Plateau of China, crop and pasture production is reliant on precipitation as its major source of water supply. Low rainfall and high legume water use lead to soil water depletion in the 1–3 m root zone (Chen et al., 2008), possibly accelerating root turnover and death and increasing the SOC stock at soil depths from 1–2 m.

Thus, the conversion of arable land that had been growing crops for many years to perennial legume pasture resulted in a significant increase in SOC, particularly at soil depths below 1 m. All three legume species increased the SOC in the top 2 m of the soil profile, but the increase was greatest in alfalfa and least in milk vetch. While the production of aboveground biomass was least in bush clover, the SOC sequestration

- in the soil profile was not significantly different from alfalfa, indicating that carbon sequestration in the soil is not associated simply with aboveground biomass production in a system in which the forage is removed for animal feed, as in the present study.
- <sup>15</sup> The root biomass production, turnover of fine roots and exudation of carboxylic acids and other carbon compounds by the roots in the different legume species would be a valuable further step in understanding the differences in carbon sequestration in the three legume species.

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Table 1. Annual forage	yield of the three legu	me species, milk vetch	1, alfalfa and bush clover,
from 2004 to 2010. Adop	oted from Guan et al. (	2013) and used with p	ermission.

Year	Forage yield (tha <sup>-1</sup> )		
	Milk vetch	Alfalfa	Bush clover
2004	2.2Ac	2.3Ad	0.2Bd
2005	14.1Ba	20.2Aa	5.3Cc
2006	14.3Ba	22.2Aa	7.8Ca
2007	6.8Bb	9.3Ac	6.4Bbc
2008	5.6Bb	13.4Ab	7.3Bab
2009	7.2Bb	12.4Ab	7.8Ba
2010	5.8Bb	10.8Abc	7.4Bab
2004–2010 Mean	8.0B	13.0A	6.0C
2004–2010 Total	56.0B	90.7A	42.1C

Data in each column with a different lower-case letter are significantly different (P < 0.05) and data in each row with a different capital letter are significantly different (P < 0.05).

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**Table 2.** Results of the ANOVA for soil organic carbon concentration as affected by legume species, soil depth and experimental year. The bare soil plot is considered as a legume species in the analysis. GLM model has been applied in the analysis.

Factors	d <i>f</i>	F value	Pr > <i>F</i>
Species	3	38.52	0.0003
Depth	4	1649.40	< 0.0001
Year	7	31.68	< 0.0001
Species · Depth	12	5.65	< 0.0001
Species · Year	21	5.96	< 0.0001
Depth · Year	28	3.20	< 0.0001
Species · Depth · Year	84	0.95	0.6053





**Figure 1.** Mean monthly temperature and precipitation from 2004–2010 and the long term mean at the experimental site at Changwu Agricultural Research Station, Shaanxi Province, China.





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Figure 2. Profile of soil organic carbon (SOC) concentration in May 2004 (IV) and in October 2010 under three forage legumes: milk vetch, alfalfa and bush clover, and bare soil (CK). Bars give + one standard error of the mean (n = 3).



**Figure 3.** Change with stand age in soil organic carbon amount (stock) per hectare at soil depths of 0–0.3 m (a), 0.3–0.6 m (b), 0.6–1.0 m (c), 1.0–1.5 m (d) and 1.5–2.0 m (e) under milk vetch, alfalfa, bush clover and bare soil (CK). Note the soil layers vary in depth. Data are means  $\pm$  one standard error of the mean (n = 3) when larger than the symbol. Linear regressions fitted when significant and fitted regressions given.





**Figure 4.** The soil organic carbon amount (stock) under milk vetch, alfalfa, bush clover and under bare soil (CK) over the upper 2 m of the soil profile. The lower case letters indicate significant differences (P < 0.05) between forage types within a year. IV denotes initial value, the soil organic carbon stock in May 2004. Bars give + one standard error of the mean (n = 3).





**Figure 5.** Change in soil organic carbon amount (stock) in different soil layers under milk vetch, alfalfa, bush clover and bare soil (CK) from May 2004 to October 2010. Different letters indicate significant differences (P < 0.05) between total carbon stocks.

