

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

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21 **ABSTRACT**

22 Soil organic carbon (SOC) plays a vital role as both a sink for and source of
23 atmospheric carbon. Revegetation of degraded arable land in China is expected to
24 increase soil carbon sequestration, but the role of perennial legumes on soil carbon
25 stocks in semiarid areas has not been quantified. In this study, we assessed the effect
26 of alfalfa (*Medicago sativa* L.) and two locally-adapted forage legumes, bush clover
27 (*Lespedeza davurica* S.) and milk vetch (*Astragalus adsurgens* Pall.) on the SOC
28 concentration and SOC stock accumulated annually over a 2-m soil profile. The
29 results showed that the concentration of SOC of the bare soil decreased slightly over
30 the 7 years, while 7 years of legume growth substantially increased the concentration
31 of SOC over the 0-2.0 m soil depth. Over the 7-year growth period the SOC stocks
32 increased by 24.1 Mg C ha⁻¹, 19.9 Mg C ha⁻¹ and 14.6 Mg C ha⁻¹ under the alfalfa,
33 bush clover and milk vetch stands, respectively, and decreased by 4.2 Mg C ha⁻¹ in the
34 bare soil. The sequestration of SOC in the 1-2 m depth of the soil accounted for 79, 68
35 and 74 % of the SOC sequestered in the 2 m deep soil profile under alfalfa, bush
36 clover and milk vetch, respectively. Conversion of arable land to perennial legume
37 pasture resulted in a significant increase in SOC, particularly at soil depths below 1 m.

38 **Keywords:** Soil organic carbon, SOC stocks, SOC sequestration, perennial legumes
39 pasture.

40 **1. Introduction**

41 Concerns about global warming and increasing atmospheric greenhouse gas
42 concentrations (CO₂, CH₄, and N₂O) have led to questions on the role of soils as a
43 source or sink for carbon. Excluding carbonated rocks, soils constitute the largest
44 surface carbon pool, approximately 1500 Gt, equivalent to almost twice that in the
45 terrestrial biomass and three times that in the atmosphere (IPCC, 2000). Globally,
46 soil cultivation has resulted in the loss of more than 40 Pg C, at a rate of about 1.6 Pg
47 C year⁻¹, to the atmosphere during the 1990s (Smith, 2008). Chinese agricultural soils
48 have also lost 30-50% or more of the soil carbon pool (Lal, 2004a).

49 Soil organic carbon (SOC) is a significant component of the global carbon stocks
50 (Chen et al., 2008). Globally, 24% of the SOC stock has been lost through the
51 conversion of forest to cropland (Murty et al., 2002) and 59% through the conversion
52 of pasture to cropland (Guo and Gifford, 2002). Fortunately, the loss of SOC can be
53 slowed down by implementing crop management practices such as conservation
54 tillage (Lal, 2004b; Puget and Lal, 2005), converting degraded arable land to
55 perennial grassland (Gentile et al., 2005), using diverse rotations, and introducing
56 legume and grass mixtures into the rotation (Lal, 2002, 2004b, c).

57 In the USA, the revegetation of highly-erodible cropland or other environmentally-
58 sensitive areas to resource-conserving vegetation for a period of 10 to 15 years
59 increased the SOC content in the upper 3 m of soil at average rate of 1.1 Mg C ha⁻¹
60 year⁻¹ (Osborn, 1993). This conservation reserve program (CRP) also significantly
61 increased the soil C pool (Staben et al., 1997) and provided multiple benefits both
62 environmentally and economically (Munson et al., 2012; Wu and Lin, 2010). Like the

63 CRP program in USA, a program of soil and water conservation, namely “Grain for
64 Green” was implemented on the Loess Plateau of China in 1999 to alleviate land
65 degradation. The program of eco-environmental revegetation focused on the recovery
66 of damaged ecosystems (Wang et al., 2010) by the use of perennial vegetation to
67 control soil erosion, increase the stocks of SOC and prevent the occurrence of dry
68 layers in the loess soils (Fu et al., 2010). Alfalfa (*Medicago sativa* L.) has been widely
69 grown on the Loess Plateau to increase livestock production and improve water-use
70 efficiency and soil fertility through high forage production, and for its ability to
71 decrease soil erosion and fix atmospheric N (Guan et al., 2013). Additionally,
72 locally-adapted legume species such as bush clover (*Lespedeza davurica* S.) and milk
73 vetch (*Astragalus adsurgens* Pall.) have been widely grown as cover crops or
74 windbreaks to protect the soil from water or wind erosion in arid and semiarid regions
75 of northern China (Wang, 2003; Xu et al., 2006). The “Grain for Green” program has
76 reduced wind and water erosion of marginal arable land and is expected to
77 significantly contribute to soil C sequestration. Recent studies have investigated and
78 estimated the changes in SOC stocks in the top 1-m of soil as a result of revegetation
79 of regional watersheds on the Loess Plateau of China (Fu et al., 2010; Wang et al.,
80 2011; Yan et al., 2007; Zhang et al., 2013). However, deep-rooted perennial legumes
81 may penetrate deeper in the soil profile than 1 m, likely underestimating the SOC-
82 sequestration potential of these forage legumes in northwest China.

83 The objective of this research was to assess the effect of alfalfa and two locally-
84 adapted forage legumes, bush clover and milk vetch, on the SOC concentration and

85 SOC stock accumulated annually for 7 years over a 2-m soil profile. The SOC content
86 in the 2-m soil profile was measured at the end of each growing season to quantify the
87 SOC concentration and stock under the locally-adapted forage legumes and alfalfa,
88 and to provide specific information for estimating the SOC sequestration potential of
89 an important agricultural area. The hypothesis tested was that long-term growth of
90 deep-rooted perennial legumes will increase soil organic C, provide a feed source for
91 animals and provide a sink for atmospheric carbon.

92 **2. Materials and methods**

93 **2.1 Experimental site description**

94 The experiment was conducted at the Changwu Agro-ecological Experiment Station
95 on the Loess Plateau (35°12'N, 107°40'E), Shaanxi province, China, from 2004 to
96 2010. The level site is located at 1220 m above sea level. The climate is semiarid,
97 with an annual mean temperature of 9.1 °C and a mean annual precipitation of 579
98 mm (1979-2003) with rainfall concentrated in the period from July to September.
99 Precipitation and temperature data were recorded at the Changwu Meteorological
100 Station, 20 m from the experimental site. The groundwater table is 50 – 80 m below
101 the soil surface, making it unavailable for plant growth. Prior to the establishment of
102 this experiment, the site was planted to winter wheat for many (at least 20) years. For
103 winter wheat production, the site was ploughed to a depth of 0.3 m twice a year, after
104 harvest in early July and again in September before sowing; only wheat stubble was
105 returned to the soil, but 108 kg N ha⁻¹ and 276 kg P₂O₅ ha⁻¹ of fertilizer was applied

106 each year before sowing. In 2003, after the winter wheat was harvested, the site
107 remained fallow for 280 days to allow moisture accumulation over the winter before
108 the legumes were sown in May 2004.

109 Soil at the experimental site belongs to the Loess series. The texture in the top 5 m is
110 a uniform silty clay loam (haplic greyxems, FAO-UNESCO, 1988), with a mean sand,
111 silt, and clay content of 3.5, 65.6, and 30.9%, respectively. The soil physical
112 characteristics do not significantly change in the upper 5 m. The measured average
113 bulk density of the soil in the upper 2 m is 1.31 g cm^{-3} , does not change with depth,
114 and the top 0.3 m contained 1.55% total organic matter, 0.106% nitrogen, and
115 0.095% available phosphate prior to the commencement of the experiment in 2004.

116 **2.2 Treatments and forage yield measurements**

117 Twelve experiment plots, each 4 m by 3 m, were established in early May 2004 with
118 one of three forage legume species, milk vetch (*Astragalus adsurgens* Pall.), alfalfa
119 (*Medicago sativa* L.) and bush clover (*Lespedeza davurica* S.), and an unplanted
120 control. Each legume species was grown as a monoculture at a seeding density of 25
121 plants m^{-2} , weeds were removed from all plots by hand using local farming practice.
122 The plots were adjacent to each other. During the experimental period from 2004 to
123 2010, there was no irrigation or other form of supplementary water. Treatments were
124 completely randomized in three replicate blocks.

125 Each year from 2005-2010, measurements of forage yield of each legume were taken
126 at the end May, July and September (in 2004 only one cut was made in September) by

127 cutting the plants at ground level with hand-held shears in 1 m×1 m quadrats selected
128 randomly within the plot, but avoiding border areas. At the same time, the rest of the
129 plot was also cut at the same height and the forage removed. The oven-dry weight was
130 determined after drying at 105 °C for 0.5 h and then further dried at 75 °C for 48 h
131 (Guan et al., 2013).

132 **2.3 Soil sampling and analysis**

133 Soil samples were taken with a cylindrical steel corer (diameter 40 mm and height
134 200 mm) at two random positions in each plot which were combined into one
135 composite sample per plot before analysis. Each plot was sampled from the surface to
136 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
137 10 May 2004 and at the end of each growing season (29 October) from 2004 to 2010.
138 The soil samples were air-dried, roots and organic debris removed, ground and sieved
139 through a 2 mm sieve, then stored at room temperature before analyzing the SOC.

140 The concentration of SOC (in g kg⁻¹) was measured using the wet dichromate
141 oxidation procedure (Moinuddin and Khanna-Chopra, 2004). Briefly, 0.5 g soil
142 samples were digested with 5 mL of 1N K₂Cr₂O₇ and 5 mL of concentrated H₂SO₄ at
143 150°C for 0.5 h, followed by titration of the digests with standardized FeSO₄.

144 **2.4 SOC stock calculation and statistical analyses**

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

$$146 \quad C_{\text{stock}} = SOC \times \rho \times H \times 10 \quad (1)$$

147 where SOC is the SOC concentration (g kg^{-1}) in each soil layer, ρ is the soil bulk
148 density (g cm^{-3}), and H is the depth of soil layers.

149 The data were analyzed by analysis of variance (ANOVA) applied to the data, and
150 means were compared using the LSD at $P < 0.05$ to characterize the differences among
151 treatments. PROC GLM (General Linear Model) were used to assess the temporal
152 changes in SOC stock and the rate and amount of SOC sequestered using Statistical
153 Analysis System (SAS Institute, Cary, NC, version 8.02).

154 **3. Results**

155 **3.1 Meteorological conditions**

156 The average monthly mean temperature from June to August, the primary growth
157 period for the legumes, was about 20 °C. Monthly mean temperatures were about 1 °C
158 warmer than the long-term mean throughout the experimental period (Fig. 1). Over
159 the experimental period, the total annual precipitation varied from 470 mm in 2006 to
160 583 mm in 2010, and was below the long-term mean in all years except 2010 when
161 the rainfall was similar to the long-term mean (Fig. 1). Rainfall from July to
162 September accounted for 55-60% of total annual precipitation, while rainfall in the
163 legume-growing season (from April to October) was about 90% (range from 84% in
164 2009 to 96% in 2005) of the total annual precipitation (Fig. 1).

165 **3.2 Aboveground forage biomass production**

166 The results of the aboveground biomass production over the seven years have been
167 reported by Guan et al. (2013). Briefly, the annual production of milk vetch increased
168 from 2.2 t ha⁻¹ in the first year to 14.3 t ha⁻¹ in 2006 and then decreased, alfalfa
169 increased from 2.3 t ha⁻¹ in the first year to a maximum of 22.2 t ha⁻¹ in 2006 and then
170 decreased, while bush clover increased from 0.2 t ha⁻¹ in the first year to 7.8 t ha⁻¹ in
171 2009 and did not decrease significantly thereafter (Table 1). Total forage yield over
172 the experimental period was highest in alfalfa at 91 t ha⁻¹, compared to 56 t ha⁻¹ in
173 milk vetch and 42 t ha⁻¹ in bush clover (Table 1).

174 **3.3 SOC concentration over the soil profile**

175 The legumes significantly ($P < 0.001$) increased the SOC concentration at each soil
176 depth, and this effect varied with legume species and experimental year (Table 2).
177 The initial concentration of SOC in May 2004 decreased with increasing soil depth
178 (Fig. 2). In the upper 0-0.3 m of soil, the initial SOC concentration was 8.0 ± 0.03 g kg⁻¹
179 ¹, while it was only 3.3 ± 0.27 g kg⁻¹ in the 1.5-2.0 m soil layer (Fig. 2). Comparison of
180 the SOC concentration between the initial values on 10 May 2004 and those at the end
181 of the experimental period in October 2010 showed that the concentration of SOC of
182 the bare soil decreased slightly over the 7 years, while 7 years of legume growth
183 substantially increased the concentration of SOC over whole 2 m soil depth. There
184 were large increases in the concentration of SOC at 0.6-1.0 m, 1.0-1.5 m and 1.5-2.0
185 m soil depth and a small, but significant, increase in the upper 0.3 m of the soil in
186 bush clover, but not in milk vetch and alfalfa. No significant changes were observed
187 after 7 years at the 0.3-0.6 m depth (Fig. 2).

188 3.4 SOC stock over the experimental period

189 SOC stock was calculated by converting SOC concentration to the amount of SOC
190 per soil layer per unit area. The SOC stock in 2004 varied from $20 \pm 0.85 \text{ Mg C ha}^{-1}$ in
191 the 0.3-0.6 m soil layer to $32 \pm 0.68 \text{ Mg C ha}^{-1}$ at 0-0.3 and 1.0-1.5 m depth (Fig.3). In
192 the bare soil, the SOC stock decreased at all depths across the experimental period,
193 but only decreased significantly at $-0.36 \text{ Mg C ha}^{-1} \text{ year}^{-1}$ ($P < 0.05$) in the 1.5-2.0 m
194 layer (Fig. 3d), presumably from the decay and turnover of the wheat roots
195 accumulated over the many years of wheat production prior to the planting of the
196 legumes. In the legume plots, the SOC stock increased linearly with time (2004-2010)
197 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
198 soil layer (Fig. 3). The change in SOC stock over the 7 years was greatest at soil
199 depths below 1.0 m in all three species and was greatest in the alfalfa plots with rates
200 of $1.35 \text{ Mg C ha}^{-1} \text{ year}^{-1}$ at a depth of 1.0-1.5 m ($P < 0.001$), and $1.39 \text{ Mg C ha}^{-1} \text{ year}^{-1}$
201 at a depth of 1.5-2.0 m ($P < 0.001$) (Fig. 3b). The highest accumulation of SOC
202 stock occurred at a depth of 1.0-1.5 m in bush clover where it averaged 1.58 Mg C
203 $\text{ha}^{-1} \text{ year}^{-1}$ ($P < 0.001$) (Fig. 3c).

204 Over the full 0-2.0 m depth, the SOC stock under bare soil decreased slightly over the
205 7 years, but increased under alfalfa, milk vetch, and bush clover (Fig. 4). The SOC
206 stock increased more under the stand of alfalfa than milk vetch, but there was no
207 significant difference between alfalfa and bush clover (Fig. 4). When calculated over
208 the full 2-m soil layer, over the 7-year growth period the SOC stocks increased by
209 $24.1 \text{ Mg C ha}^{-1}$, $19.9 \text{ Mg C ha}^{-1}$ and $14.6 \text{ Mg C ha}^{-1}$ under the alfalfa, bush clover and

210 milk vetch stands, respectively, and decreased by 4.2 Mg C ha^{-1} under bare soil (Fig.
211 5). In the 1.0-2.0-m soil layer the stocks of SOC increased by $19.1 \text{ Mg C ha}^{-1}$, 13.6
212 Mg C ha^{-1} and $10.8 \text{ Mg C ha}^{-1}$, under the alfalfa, bush clover and milk vetch stands,
213 respectively, that is, by 79, 68 and 74% of the increases in the whole soil profile (Fig.
214 5).

215 **4. Discussion**

216 Although highly-productive perennial forage legumes with deep roots have been
217 shown to cause a significant decrease in soil water at depth in semiarid environments
218 (Guan et al., 2013), they are considered to have an important role in sequestering
219 SOC in deep soil layers (Gentile et al., 2005). In the present study, the SOC in the
220 upper 2 m of the soil at the beginning of the experiment in 2004 was 137 Mg C ha^{-1}
221 and increased to 151, 161 and 157 Mg C ha^{-1} under the milk vetch, alfalfa and bush
222 clover stands, respectively, by the end of the experiment in 2010 (Fig. 4). This
223 indicates that as a result of planting the legumes, the SOC sequestered over the 7
224 years was 14, 24 and 20 Mg C ha^{-1} in milk vetch, alfalfa and bush clover (Fig. 5),
225 respectively, but would have lost 4 Mg C ha^{-1} if the soil had been left unplanted.

226 The aboveground biomass production over the seven years of the experiment was the
227 highest in alfalfa at 91 t ha^{-1} , significantly higher than the biomass production in milk
228 vetch (56 t ha^{-1}) and bush clover (42 t ha^{-1}) (Table 1). While alfalfa had the highest
229 increase in SOC stocks in the upper 2 m of the soil ($24.1 \text{ Mg C ha}^{-1}$), bush clover had
230 a similar increase to alfalfa ($19.9 \text{ Mg C ha}^{-1}$) and milk vetch has a significantly

231 smaller increase in SOC ($14.6 \text{ Mg C ha}^{-1}$) over the 7-year period (Fig. 5). An increase
232 in the SOC stocks is usually associated with the production of roots and/or their
233 turnover, that is the pattern of root growth and death, particularly the turnover of fine
234 roots (Luo et al., 1995). A high rate of turnover of fine roots and a high rate of
235 exudation of carbon by the roots influences the stability of plant C in soil and the
236 accumulation of SOC (Shahzad et al., 2015). Although the root biomass was not
237 measured in this study, root biomass is usually associated with aboveground biomass.
238 However, the increase in SOC among the three species was not associated simply
239 with aboveground biomass, suggesting that either root biomass among the three
240 species was not associated with aboveground biomass, or the increase in SOC was not
241 associated simply with root biomass. Guan et al. (2013) did not measure root biomass,
242 but showed that the water extraction profile was greater in alfalfa and similar in milk
243 vetch and bush clover below 1.2 m, suggesting that root biomass did not vary
244 significantly between milk vetch and bush clover and cannot explain the greater
245 accumulation of SOC in the upper 2 m of the soil profile in bush clover than the milk
246 vetch (Fig. 5). Again, while root biomass, root length density, root diameter and root
247 turnover were not measured, the greater sequestration of SOC by bush clover than
248 milk vetch may indicate the production and turnover of fine roots was greater in bush
249 clover than milk vetch. Sun et al. (2001) reported that the fine roots (root diameter <
250 0.5 mm) of bush clover accounted for 42% of total root biomass in 0-0.3 m soil layer,
251 while the fine roots of milk vetch were only 25% of total root biomass (Chen and Nie,
252 1978). If the roots below 1 m are similar to those in the upper soil, this would help to

253 explain why the sequestration of SOC in bush clover was greater than milk vetch. The
254 accumulation of SOC in the upper 0.3 m of the soil was highest in bush clover at 3.4
255 Mg C ha⁻¹, intermediate in alfalfa at 1.3 Mg C ha⁻¹, and least in milk vetch at 0.8 Mg
256 C ha⁻¹. The accumulation of SOC in the upper soil layer may be attributed to the high
257 accumulation of legume residues and litter (Zhou et al., 2006), or due to the
258 proliferation and turnover of roots in this surface layer The sequestration of SOC in
259 the upper 0.3 m of the soil in this study was significantly lower than Zhang et al.
260 (2009) who reported that the SOC stocks in 0 – 0.3 m soil layer had increased by 16
261 Mg C ha⁻¹ ten years after the conversion of a wet reed meadow to an irrigated alfalfa
262 pasture in the Hexi Corridor in north-west China. This suggests that well-managed
263 legume pastures in areas with higher precipitation and with appropriate fertilizer use
264 could sequester significant amounts of SOC.

265 An unexpected result from this study was the greater increase in SOC at soil depths
266 from 1 – 2 m than above 1 m. The water extraction patterns (Guan et al., 2013) do not
267 suggest that there was greater root density or biomass below 1 m than above 1 m and
268 do not provide an explanation for the greater sequestration of SOC at depth. The
269 greater increase in SOC at depth may be associated with a greater proliferation and
270 turnover of fine roots at depth, or alternatively may reflect the movement of SOC
271 down the profile with rainfall. The sequestration of SOC in the 1 – 2 m depth of soil
272 accounted for 79, 68 and 74% of SOC sequestered through the whole top 2 m of soil
273 under alfalfa, bush clover and milk vetch, respectively, indicating the importance of
274 deep roots. This was consistent with K äterer et al. (2011) who found that root-

275 derived carbon was about 2.3 times higher than that from above-ground residue-
276 derived C from a long-term field experiment in Sweden. Rasse et al. (2005) and
277 Johnson et al. (2006) attributed the SOC increase in the rhizosphere to the C from root
278 turnover and cells sloughing off the epidermal root tissues during the growing season,
279 and to soluble C compounds released from the roots by exudation. With the water
280 table at a depth of 20–300 m on the Loess Plateau of China, crop and pasture
281 production is reliant on precipitation as its major source of water supply. Low rainfall
282 and high legume water use led to soil water depletion in the 1 – 3 m root zone (Chen
283 et al., 2008), possibly accelerating root turnover and death and increasing the SOC
284 stock at soil depths from 1 – 2 m.

285 The high SOC stocks at soil depths of 1 – 2 m in alfalfa and milk vetch can be
286 attributed to their taproot system that can penetrate to 6.8 and 7.6 m, respectively, in 6
287 years (Cheng et al., 2005; Cheng et al., 2004; Du et al., 2012). The root growth and
288 death along with their penetration would increase SOC stocks in the deep layers. In
289 bush clover the taproot predominates in the 0-0.3 m soil layer with coarse roots (root
290 diameter > 2 mm) accounting for 48% of the total root biomass, and fine roots
291 predominating in the 0.3-1.4 m soil depth, accounting for 60% of the root biomass
292 (Cheng et al., 2005; Cheng et al., 2004; Sun et al., 2001).

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

297 production of aboveground biomass was least in bush clover, the SOC sequestration
298 in the soil profile was not significantly different from alfalfa, indicating that carbon
299 sequestration in the soil is not associated simply with aboveground biomass
300 production in a system in which the forage is removed for animal feed, as in the
301 present study. The root biomass production, turnover of fine roots and exudation of
302 carboxylic acids and other carbon compounds by the roots in the different legume
303 species would be a valuable further step in understanding the differences in carbon
304 sequestration in the three legume species.

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414

415 **Figure Captions**

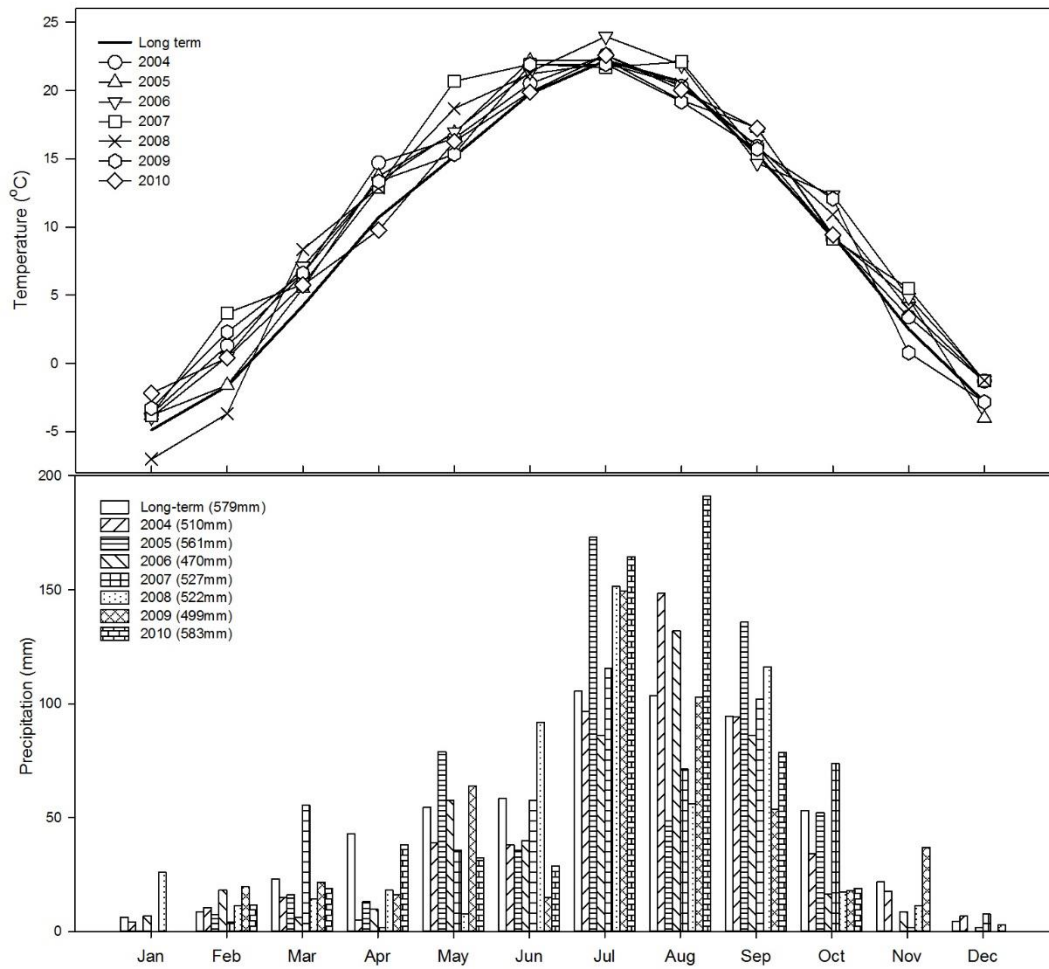
416 **Fig. 1.** Mean monthly temperature and precipitation from 2004-2010 and the long
417 term mean at the experimental site at Changwu Agricultural Research Station,
418 Shaanxi Province, China.

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

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

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

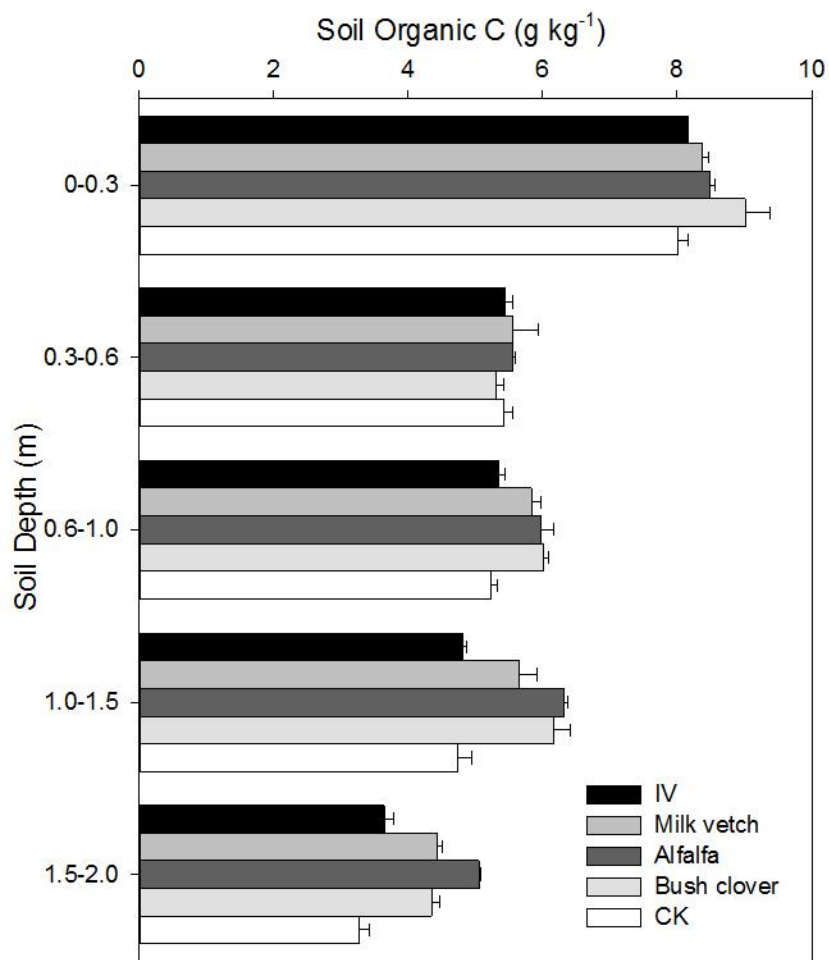
432 **Fig. 5.** Change in soil organic carbon amount (stock) in different soil layers under
433 milk vetch, alfalfa, bush clover and bare soil (CK) from May 2004 to October 2010.
434 Different letters indicate significant differences ($P < 0.05$) between total carbon stocks.



435

436 Figure 1

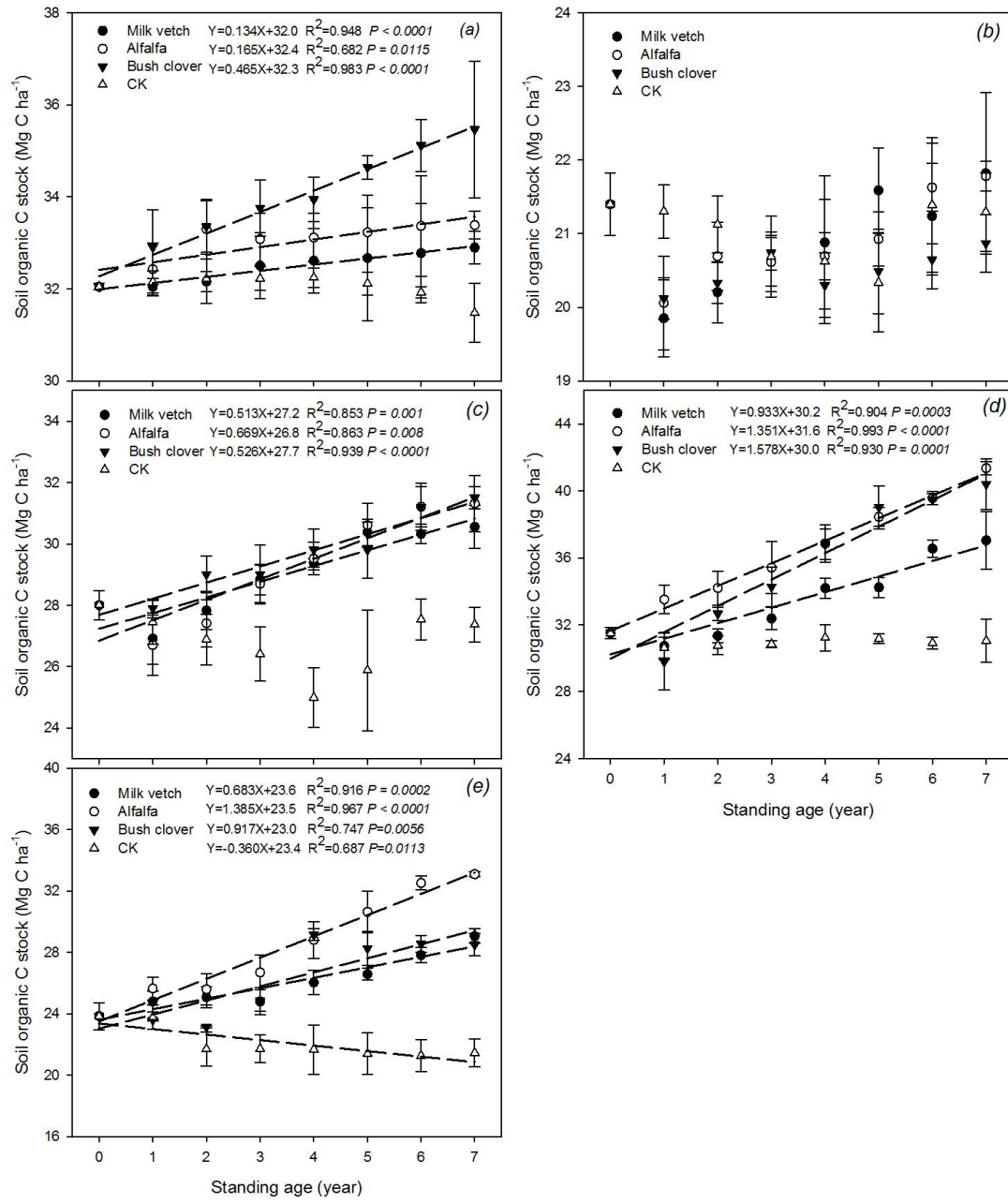
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439 Figure 2

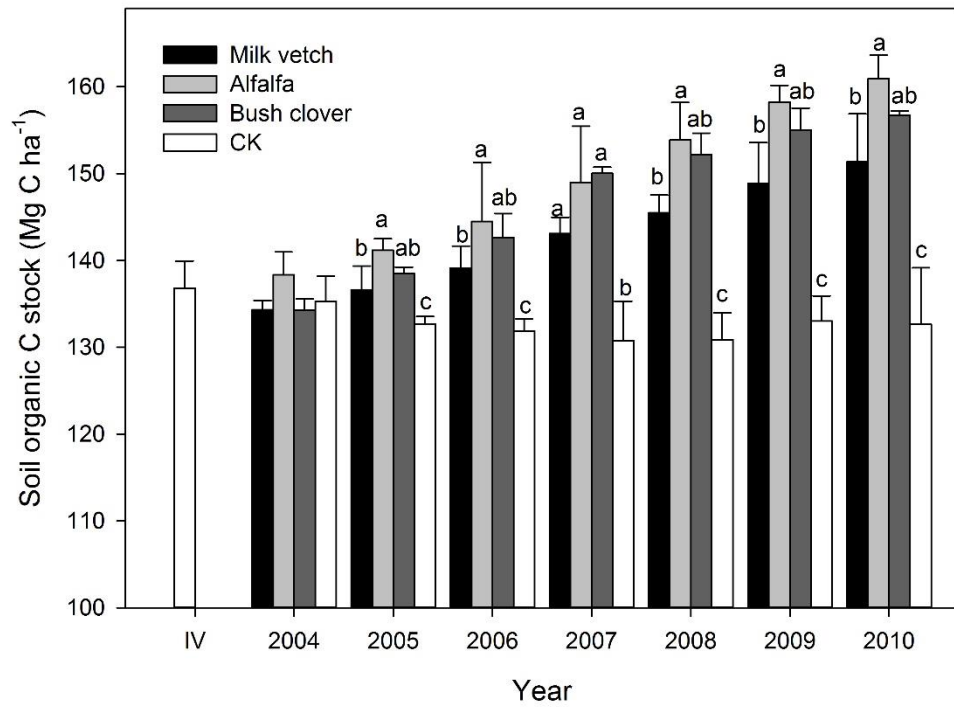
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442 Figure 3

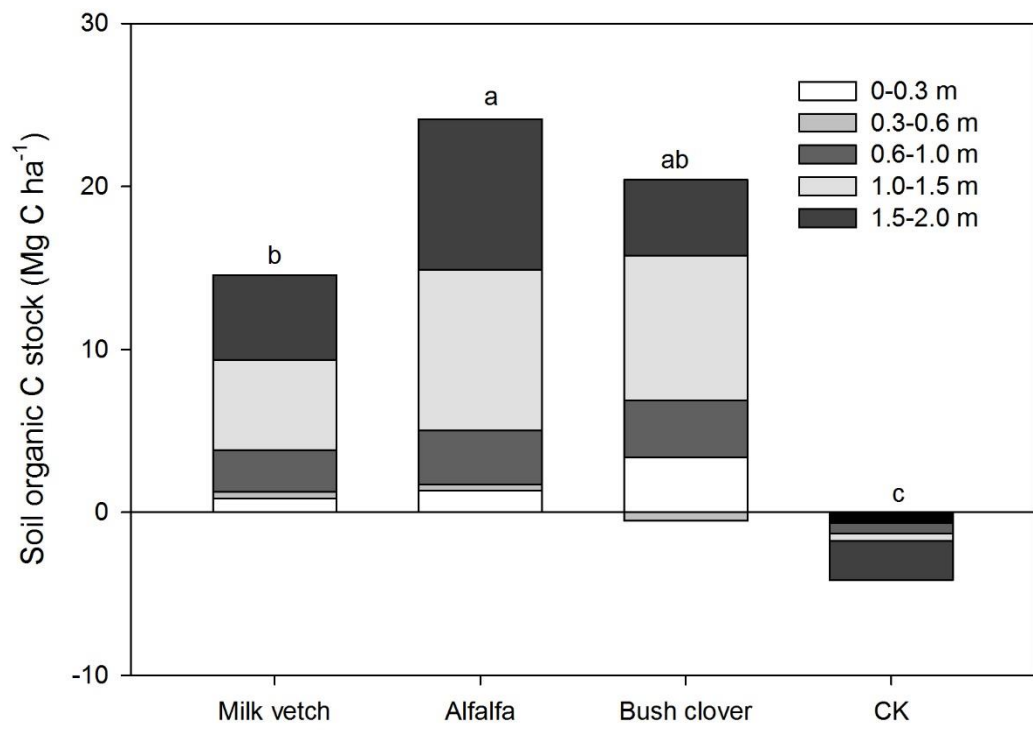
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445 Figure 4

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448 Figure 5

449 **Table 1. Annual forage yield of the three legume species, milk vetch, alfalfa and bush**
 450 **clover, from 2004 to 2010. Adopted from (Guan et al. (2013)) and used with permission.**

| Year | Forage yield (t ha ⁻¹) | | |
|-----------------|---------------------------------------|---------|-------------|
| | 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 |

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

453

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

| Factors | df | <i>F</i> value | <i>Pr</i> > <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 |

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