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. The results 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 over the 0-2.0 m soil depth. Over the 7-year growth period the SOC stocks increased by 24.1 Mg C ha^{-1}, 19.9 Mg C ha^{-1} and 14.6 Mg C ha^{-1} under the alfalfa, bush clover and milk vetch stands, respectively, and decreased by 4.2 Mg C ha^{-1} in the bare soil. The sequestration of SOC in the 1-2 m depth of the soil accounted for 79, 68 and 74 % of the SOC sequestered in the 2 m deep soil profile 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.

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

1. Introduction

Concerns about global warming and increasing atmospheric greenhouse gas concentrations (CO_2, CH_4, and N_2O) 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 twice that in the terrestrial biomass and three times that in the atmosphere (IPCC, 2000). Globally, soil cultivation has resulted in the loss of more than 40 Pg C, at a rate of about 1.6 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). Globally, 24% of the SOC stock has been lost through the conversion of forest to cropland (Murty et al., 2002) and 59% through the conversion of pasture to cropland (Guo and Gifford, 2002). 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 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 environmentally-sensitive areas to resource-conserving vegetation for a period of 10 to 15 years increased the SOC content in the upper 3 m of soil at average rate of 1.1 Mg C ha^{-1} year^{-1} (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” 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 regions 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 (Smith et al., 2015).

The objective of this research was 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 for 7 years over a 2-m soil profile. The SOC content in the 2-m soil profile was measured at the end of each growing season to 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.

2. Materials and methods

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 level site is located at 1220 m above sea level. The climate is semiarid, with an annual mean temperature of 9.1 ℃ 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 kg N ha^{-1} and 276 kg P_2O_5 ha^{-1} of fertilizer was applied each year before sowing. In 2003, after the winter wheat was harvested, the site remained 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^{-3}, does not change with depth, 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 measurement of aboveground biomass

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 (*Medicago sativa* L.) and bush clover (*Lespedeza davurica* S.), and an unplanted control. Each legume species was grown as an evenly-spaced monoculture (but assumed a more patchy distribution with time) at a seeding density of 25 plants m^{-2}, 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 and the plots were not fertilized. The plants were not inoculated, but relied on the naturally-occurring root nodule bacteria from previous growth of the three species on the experimental station. Nodulation was not observed to be a problem. Treatments were completely randomized in three replicate blocks.

Each year from 2005-2010, measurements of aboveground biomass production for 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 a randomly-selected 1 m × 1 m quadrat within each 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 ℃ for 0.5 h and then further dried at 75 ℃ for 48 h (Guan et al., 2013).

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 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 SOC (in g kg^{-1}) was measured using the wet dichromate oxidation procedure (Moinuddin and Khanna-Chopra, 2004). Briefly, a 0.5 g soil sample was digested with 5 mL of 1N $K_2Cr_2O_7$ and 5 mL of concentrated H_2SO_4 at 150°C for 0.5 h, followed by titration of the digest with standardized $FeSO_4$.

2.4 SOC stock calculation and statistical analyses

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

$$\mathrm{C_{stock}} = SOC \times \rho \times H \times 10 \qquad (1)$$

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

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 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 using Statistical Analysis System (SAS Institute, Cary, NC, version 8.02).

3. Results

3.1 Meteorological conditions

The average monthly mean temperature from June to August, the primary growth period for the legumes, was about 20 ℃. Monthly mean temperatures were about 1 ℃ 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 t ha^{-1} in the first year to 14.3 t ha^{-1} in 2006 and then decreased, alfalfa increased from 2.3 t ha^{-1} in the first year to a maximum of 22.2 t ha^{-1} in 2006 and then decreased, while bush clover increased from 0.2 t ha^{-1} in the first year to 7.8 t ha^{-1} in 2009 and did not decrease significantly thereafter (Table 1). Total aboveground biomass production over the experimental period was highest in alfalfa at 91 t ha^{-1} (equivalent to 45 Mg C ha^{-1} assuming the default C to dry weight ratio of 0.5) compared to 56 t ha^{-1} (28 Mg C ha^{-1}) in milk vetch and 42 t ha^{-1} (21 Mg C ha^{-1}) 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^{-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 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 over whole 2 m soil depth. There were large increases in the concentration of SOC at 0.6-1.0 m, 1.0-1.5 m 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).

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 ±0.85 Mg C ha^{-1} in the 0.3-0.6 m soil layer to 32 ±0.68 Mg C ha^{-1} 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 Mg C ha^{-1} year^{-1} ($P < 0.05$) in the 1.5-2.0 m layer (Fig. 3d), presumably from the decay and turnover of the wheat roots accumulated over the many years of wheat production prior to the planting of the legumes. 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 Mg C ha^{-1} year^{-1} at a depth of 1.0-1.5 m ($P< 0.001$), and 1.39 Mg C ha^{-1} year^{-1} at a depth of 1.5-2.0 m ($P < 0.001$) (Fig. 3b). The highest accumulation of SOC stock occurred at a depth of 1.0-1.5 m in bush clover where it averaged 1.58 Mg C ha^{-1} year^{-1} ($P < 0.001$) (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 Mg C ha^{-1}, 19.9 Mg C ha^{-1} and 14.6 Mg C ha^{-1} under the alfalfa, bush clover and milk vetch stands, respectively, and decreased by 4.2 Mg C ha^{-1} under bare soil (Fig. 5). In the 1.0-2.0-m soil layer the stocks of SOC increased by 19.1 Mg C ha^{-1}, 13.6 Mg C ha^{-1} and 10.8 Mg C ha^{-1}, 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

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 upper 2 m of the soil at the beginning of the experiment in 2004 was 137 Mg C ha^{-1} and increased to 151, 161 and 157 Mg C ha^{-1} 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^{-1} in milk vetch, alfalfa and bush clover (Fig. 5), respectively, but would have lost 4 Mg C ha^{-1} if the soil had been left unplanted.

The aboveground biomass production over the seven years of the experiment was the highest in alfalfa at 91 t ha^{-1} (45 Mg C ha^{-1}), significantly higher than the biomass production in milk vetch (56 t ha^{-1}, or 28 Mg C ha^{-1}) and bush clover (42 t ha^{-1} or 21 Mg C ha^{-1}) (Table 1). While alfalfa had the highest increase in SOC stocks in the upper 2 m of the soil (24.1 Mg C ha^{-1}), bush clover had a similar increase to alfalfa (19.9 Mg C ha^{-1}) and milk vetch has a significantly smaller increase in SOC (14.6 Mg C ha^{-1}) over the 7-year period (Fig. 5). An increase in the SOC stocks is usually associated with the production of roots and/or their turnover, that is the pattern of root growth and death, particularly the turnover of fine roots (Luo et al., 1995). A high rate of turnover of fine roots and a high rate of exudation of carbon by the roots influences the stability of plant C in soil and the accumulation of SOC (Shahzad et al., 2015). Although the root biomass was not measured in this study, root biomass is usually associated with aboveground biomass. However, the increase in SOC among the three species was not associated simply with aboveground biomass, suggesting that root biomass among the three species was not associated with aboveground biomass, or the increase in SOC was not associated simply with root biomass. Assuming the increase in SOC over the 7 years was all the result of the increase in root biomass, the root mass ratio (root dry weight/total dry weight) would need to be 0.31 in alfalfa, 0.34 in milk vetch and 0.49 in bush clover for the increase in SOC to be associated with the aboveground biomass produced over the 7 years. While the root mass ratios of alfalfa and milk vetch are similar to those reported for alfalfa by Fan et al. (2015), the bush clover would have to produce a much greater root biomass relative to the aboveground biomass. Guan et al. (2013) did not measure root biomass, but showed that the water extraction profile was greater in alfalfa and similar in milk vetch and bush clover below 1.2 m, suggesting 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 milk vetch (Fig. 5). The greater sequestration of SOC by bush clover than milk vetch may indicate the production and turnover of fine roots was greater in bush clover than milk vetch. Sun et al. (2001) reported that the fine roots (root diameter < 0.5 mm) of bush clover accounted for 42% of total root biomass in 0-0.3 m soil layer, while the fine roots of milk vetch were only 25% of total root biomass (Chen and Nie, 1978). If the roots below 1 m are similar to those in the upper soil, this would help to explain why the sequestration of SOC in bush clover was greater than milk vetch. The accumulation of SOC in the upper 0.3 m of the soil was highest in bush clover at 3.4 Mg C ha^{-1}, intermediate in alfalfa at 1.3 Mg C ha^{-1}, and least in milk vetch at 0.8 Mg C ha^{-1}. 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 in this surface layer The sequestration of SOC in the upper 0.3 m of the soil in this study was significantly lower than Zhang et al. (2009) who reported that the SOC stocks in 0 – 0.3 m soil layer had increased by 16

Mg C ha^{-1} ten years after the conversion of a wet reed meadow to an irrigated alfalfa pasture in the Hexi Corridor in north-west China. This suggests that well-managed legume pastures in areas with higher precipitation and with appropriate fertilizer use could sequester significantly more of SOC than in the present unirrigated and unfertilized legumes.

An unexpected result from this study was the greater increase in SOC at soil depths from 1 – 2 m than above 1 m. The water extraction patterns (Guan et al., 2013) do not suggest that there was greater root density or biomass below 1 m than above 1 m and do not provide an explanation for the greater sequestration of SOC at depth. The greater increase in SOC at depth may be associated with a greater proliferation and turnover of fine roots at depth, or alternatively may reflect the movement of SOC down the profile with rainfall. 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 milk vetch, respectively, indicating the importance of deep roots. 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 epidermal 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 led 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.

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

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. 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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Figure Captions

Fig. 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.

Fig. 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).

Fig. 3. Change with stand age in soil organic carbon amount (stock) per hectare at soil 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 milk vetch, alfalfa, bush clover and bare soil (CK). Note the soil layers vary in depth. Data are means ± one standard error of the mean (n = 3) when larger than the symbol. Linear regressions fitted when significant and fitted regressions given.

Fig. 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).

Fig. 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.

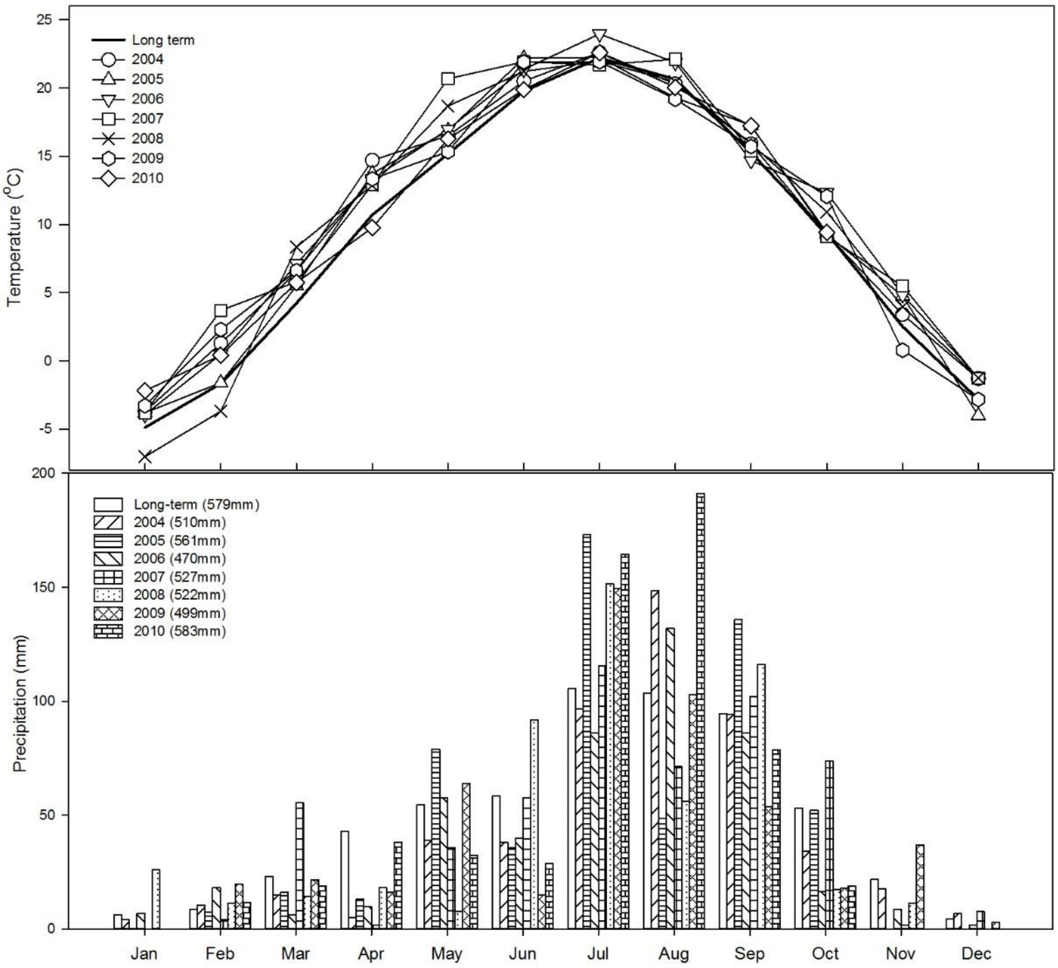

Temperature (°C)
25
20
15
10
5
0
-5
Long term
2004
2005
2006
2007
2008
2009
2010
200
150
100
50
0
Precipitation (mm)
Long-term (579mm)
2004 (510mm)
2005 (561mm)
2006 (470mm)
2007 (527mm)
2008 (522mm)
2009 (499mm)
2010 (583mm)
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec

Figure 1

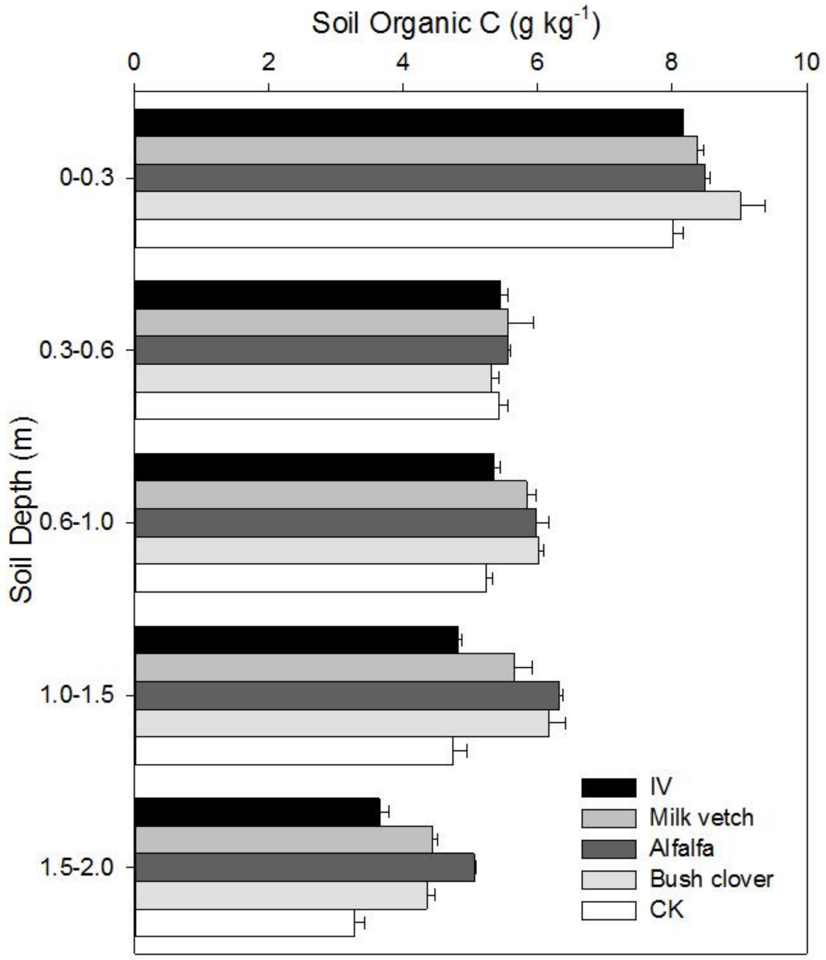

Soil Organic C (g kg^{-1})
0
2
4
6
8
10
Soil Depth (m)
0-0.3
0.3-0.6
0.6-1.0
1.0-1.5
1.5-2.0
IV
Milk vetch
Alfalfa
Bush clover
CK

Figure 2

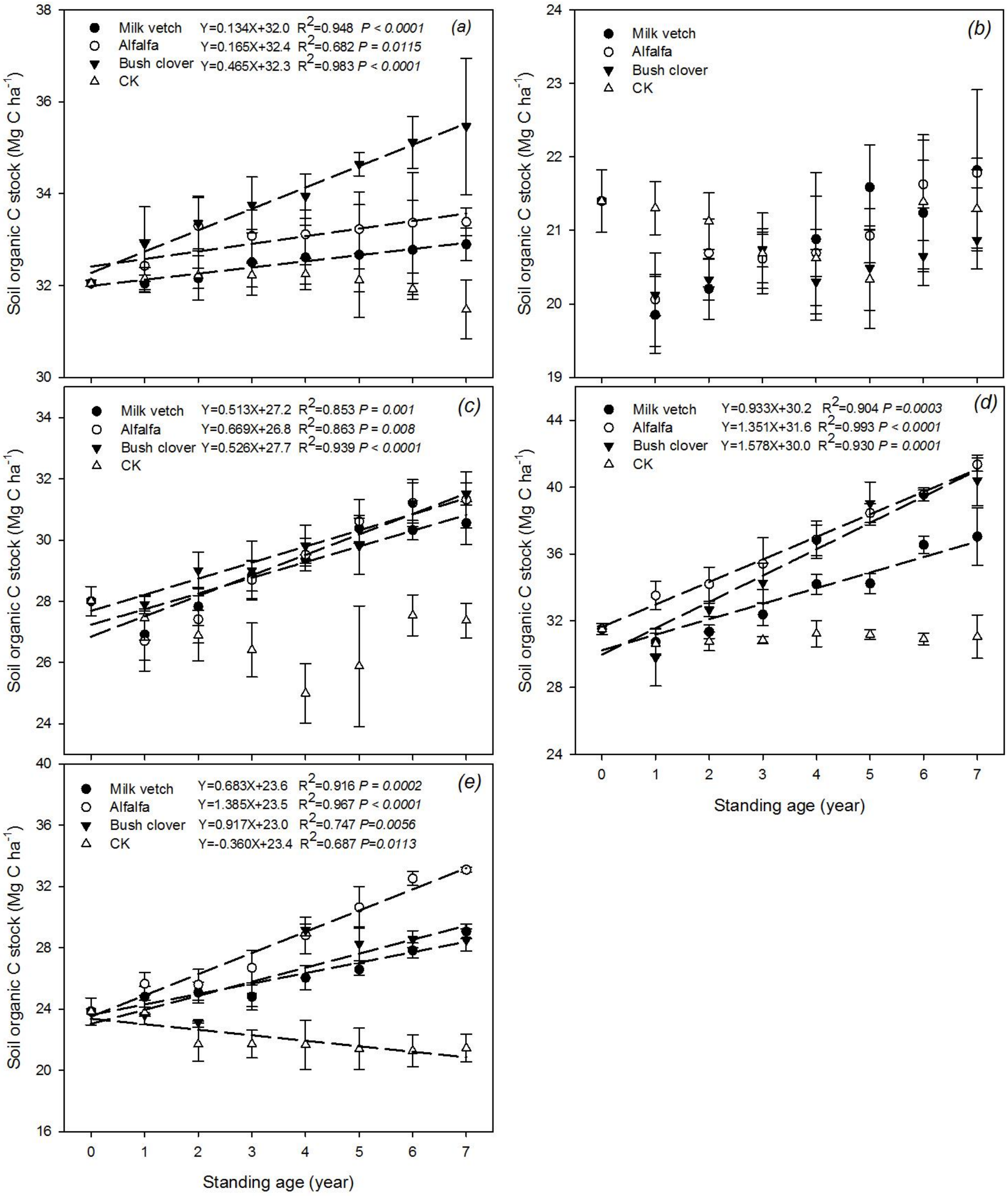

(a)
Milk vetch Y=0.134X+32.0 R²=0.948 P < 0.0001
Alfalfa Y=0.165X+32.4 R²=0.682 P = 0.0115
Bush clover Y=0.465X+32.3 R²=0.983 P < 0.0001
CK
Soil organic C stock (Mg C ha⁻¹)
30
32
34
36
38
(b)
Milk vetch
Alfalfa
Bush clover
CK
Soil organic C stock (Mg C ha⁻¹)
19
20
21
22
23
24
(c)
Milk vetch Y=0.513X+27.2 R²=0.853 P = 0.001
Alfalfa Y=0.669X+26.8 R²=0.863 P = 0.008
Bush clover Y=0.526X+27.7 R²=0.939 P < 0.0001
CK
Soil organic C stock (Mg C ha⁻¹)
24
26
28
30
32
34
(d)
Milk vetch Y=0.933X+30.2 R²=0.904 P =0.0003
Alfalfa Y=1.351X+31.6 R²=0.993 P < 0.0001
Bush clover Y=1.578X+30.0 R²=0.930 P = 0.0001
CK
Soil organic C stock (Mg C ha⁻¹)
24
28
32
36
40
44
0
1
2
3
4
5
6
7
Standing age (year)
(e)
Milk vetch Y=0.683X+23.6 R²=0.916 P = 0.0002
Alfalfa Y=1.385X+23.5 R²=0.967 P < 0.0001
Bush clover Y=0.917X+23.0 R²=0.747 P=0.0056
CK Y=-0.360X+23.4 R²=0.687 P=0.0113
Soil organic C stock (Mg C ha⁻¹)
16
20
24
28
32
36
40
0
1
2
3
4
5
6
7
Standing age (year)

Figure 3

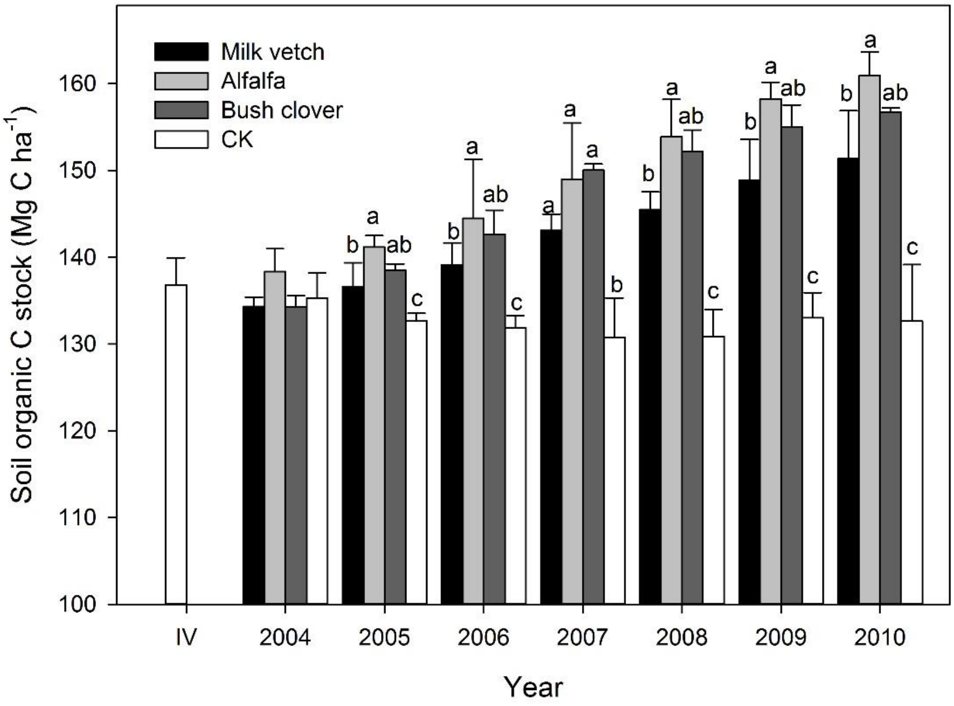
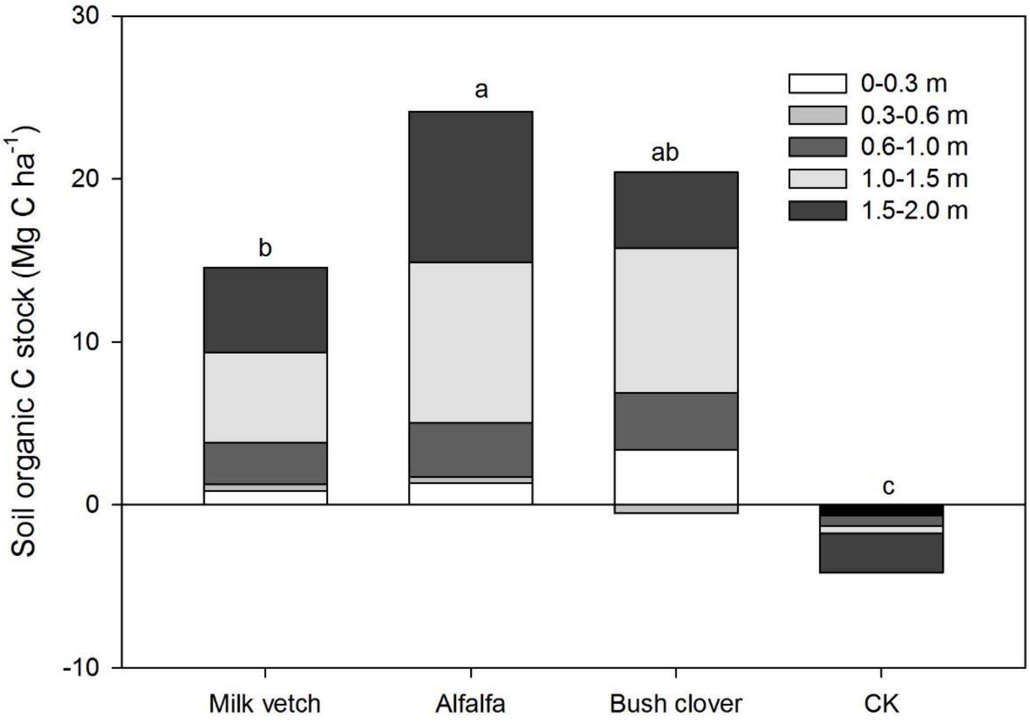

Milk vetch
Alfalfa
Bush clover
CK
Soil organic C stock (Mg C ha^{-1})
100
110
120
130
140
150
160
IV
2004
2005
2006
2007
2008
2009
2010
Year
b
a
ab
c
b
a
ab
c
a
a
a
b
b
a
ab
c
b
a
ab
c
b
a
ab
c

Figure 4



30
20
10
0
-10
Soil organic C stock (Mg C ha^{-1})
a
ab
b
c
0-0.3 m
0.3-0.6 m
0.6-1.0 m
1.0-1.5 m
1.5-2.0 m
Milk vetch
Alfalfa
Bush clover
CK

Figure 5

Table 1. Annual aboveground biomass production of the three legume species, milk vetch, alfalfa and bush clover, from 2004 to 2010. Adopted from (Guan et al. (2013)) and used with permission.

Year	Aboveground biomass production (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

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 ($P < 0.05$).

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	df	*F* value	*Pr* > *F*
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