



Soil Organic Carbon Stocks and Its Driving Factors Under Different Land-Use Patterns in Semiarid Grasslands of the Loess Plateau, China

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ABSTRACT

Fencing for grazing exclusion and grazing are common land-use methods in the semi-arid areas of the Loess Plateau in China, which have been widely found to change grassland soil organic carbon (SOC); however empirical studies that evaluated driving factors of soil carbon (C) stocks under the different land use are still weak. In this study, we investigated soil physicochemical and soil respiration (Rs) in the fenced and grazed grassland, to study the soil C stock variations and the main driving mechanism of soil C accumulation. The results showed that bulk density (BD), soil moisture content (SMC), and soil porosity (SP) had no significant difference between fenced and grazed grassland. Fencing increased the SOC, total nitrogen (TN), and C/N ratio, and significantly increased the aboveground biomass (AGB), belowground biomass (BGB), and the amount of soil large macro-aggregates in the topsoil layer (0-10 cm), and the soil stability was improved. Meanwhile, grazing increased soil temperature (ST) and Rs. The soil C stock in the topsoil layer (0-10 cm) of fenced grassland was significantly higher than that of grazed grassland. The soil C/N ratio, BD, and MWD explained large proportions of the variations in soil C stocks. Our results indicate that fencing can improve the stability of soil structure, and reduce Rs, then increase soil C stocks, which is an effective way to improve soil C stocks of grassland ecological in semi-arid areas of northwest China.

INTRODUCTION

The Intergovernmental Panel on climate change (IPCC) recently issued its special report on the impact of global climate change on global sustainable development. The report points out that without increased and urgent mitigation ambition in the coming years, leading to a sharp decline in greenhouse gas emissions by 2030, global warming will surpass 1.5°C in the following decades, leading to the frequent occurrence of extreme weather and gradual deterioration of global ecological environment (Lin et al. 2019). But it seems at present that global warming has continued unabated (Makarim et al. 2019). Global warming is related to the rising levels of atmospheric CO₂ over the past 23 million years (Cui et al. 2020). These data suggest present-day CO₂ (412 ppm) exceeds the highest levels that Earth experienced at least the past 800000 years (Keeling et al. 2001).

Soil C is the largest terrestrial C reservoir, containing about twice as much C as the atmospheric CO₂ pool globally, and the soil let 98 Pg C to the atmosphere with the soil respiration (Rs) annually, which is ten times of the burning of fossil energy (Shi et al. 2018). Therefore, we can reduce the atmospheric CO₂ concentration through strategies that

both avoid loss of existing soil organic carbon (SOC) stocks and restore stocks in carbon (C) depleted soils, leading to mitigate the worsening global climate change (Smith et al. 2008, Thakur & Verma 2019). The role of soil organic matter as a regulator of climate has been recognized by scientists for decades (Bossio et al. 2020). Land-use change is identified as a cause of soil C losses and has been a significant source of atmospheric CO₂ over the last few centuries (Pradhan et al. 2019).

Grasslands cover 30-40% of the terrestrial surface area of the earth approximately and with 10% of the global soil C pool (Li et al. 2019, Zhang et al. 2020), which are the most widely distributed terrestrial ecosystems, play an important role in regional climate change and the global C cycle. In China, approximately 40% of the total land area is covered by grasslands; the grassland areas account for approximately 6-8% of the total global grassland area (Li et al. 2019). Compared to forest ecosystems, grasslands are marked by larger vegetation coverage and shorter vegetative growth cycles, which holds great potential as a C sink. And research has suggested that 47% of the total potential mitigation arises from SOC protection and sequestration in grasslands and agriculture, which is well above 9% of soil C mitigation

potential in the forest (Bossio et al. 2020). Fencing for grazing exclusion and free grazing are two common land-use patterns in the grassland ecosystem. Grazing exclusion is an effective practice for restoring overgrazed grasslands and an efficient grassland restoration management strategy that has been widely applied (Li et al. 2019). Generally, fencing increased SOC stocks and enhanced the capacity of soil functioning as a C sink. These studies show that overgrazing and conversion of freely grazed grassland to cropland lead to an annual average decline of 2.3-2.8% in SOC, but fencing increased the capacity of soil C stocks in china (Wang et al. 2011). Research on degraded grasslands in the arid desert regions of Northwest China suggests that although short-term grazing exclusion (three years) was not beneficial for C sequestration in the desert grassland, it is an effective strategy for improving the productivity of plants (Dong et al. 2020). Previous research by Yuan et al. (2020) had demonstrated that grazing exclusion for 14 years did not significantly affect soil SOC of the alpine meadow on the Tibetan Plateau, and differences in soil SOC were mainly controlled by the heterogeneity of the sites, rather than grazing exclusion (Yuan et al. 2020). Hafner et al. (2012) showed that sustainable moderate grazing is a suitable tool to preserve the high ability of the montane pasture land to store carbon, but fencing has a negative effect on soil surface C pool. In addition, Wang et al. (2009) showed that the Rs of grazed grassland is lower than that of grazing exclusion. Still, the study of soil respiration emission flux in alpine meadow suggested that compared with grazed grassland, grazing exclusion reduces CO₂ emission (Luo et al. 2020), and Rs increases with the increase of stocking rate (Cao et al. 2004).

The area of the grassland ecosystem accounts for about 1/3 of the land area of the Loess Plateau and 7.4% of the grassland area of China. With the large-scale implementation of the policy of converting slope farmland into forest and grassland, vegetation cover on the Loess Plateau has increased from 31.6% in 1999 to 59.6% in 2013, then up to 65.2% in 2017. Fencing for grazing exclusion and free grazing are two common land-use patterns in the grassland ecosystem on the Loess plateau (Li et al. 2019, Zhang et al. 2020). Different land-use patterns directly change the physical properties and structural stability of the soil, which is crucial for assessing the impact of enclosure and grazing on soil C stocks and surface flux and also helps us to understand the C sequestration potential of soil under different land-use patterns.

In this study, grazing exclusion grassland for eight years and free grazed grassland in the Yunwu Mountain grassland of the Loess Plateau were selected to measure soil C stocks and soil respiration along with physicochemical and water-stable aggregate. The objectives of this study were as

follows: (1) to compare differences in soil C stocks between grazing exclusion and free grazing grasslands, and (2) to explore the effects of Rs, soil physicochemical and distribution characteristics of soil water-stable aggregates on soil C stocks between grazing exclusion and free grazed grasslands.

MATERIALS AND METHODS

Study Sites

The studied site was located in the Yunwu Mountain National Nature Reserve (36°10'-36°17'N, 106°21'-106°27'E), 45 km northeast of Guyuan City, Ningxia Autonomous Region, China in the southwest of the Loess Plateau at an altitude of 1,700-2,148 m. The soil type is dark loessial soil and mountain grey cinnamon soil, the average depth of the soil is 50 m. The underground water exists approximately 100 m below the land surface, and atmospheric precipitation mainly replenishes water resources. The experiment site has a temperate continental monsoon semi-arid climate with a mean annual temperature of 6.8°C and means annual precipitation of 410mm (1960-2010). Approximately 65%-85% of the total precipitation falls from July to September. The region's dominant plants are *Stipa bungeana*, *Stipa grandis*, *Thymus mongolicus*, *Artemisia stechmanniana*, and *Potentilla acaulis*. Since 1984, the regional government has implemented several mountain closure and grazing prohibition measures gradually from the center of Yunwu Mountain to the periphery, closing plots to livestock grazing and making them available for study. In this study, the fenced and grazed grasslands located in the periphery of Yunwu Mountain National Nature Reserve were selected with an interval of 1 km. Fenced grassland was built in 2011, and before the fencing was placed for grazing exclusion, the grasslands were used as grazed land, and the site's original condition (plant diversity and soil properties) was almost the same in both the grazed and fenced grasslands.

Experimental Design and Soil Sampling

The study was performed in October 2019. A single-factor (two levels, fenced grassland (FG) and grazed grassland (GG)) experiment was designed to investigate the differences between FG and GG. Three plots of 1 × 1 m were set up in each level, with an interval of 20 m. The livestock in GG included goats, and the average stocking rate was 2.5 goats.ha⁻¹.

Before sampling, grass, litter, or any other material on the soil surface were removed. Vertical soil profiles were dug in the sampling plots, and soil samples were collected from three soil layers (0-10, 10-20, 20-30 cm) using a ring knife (volume of 100 cm³) and a spade for digging undisturbed soil weighing about a kilo. Soil samples were collected from the bottom to

the top of the soil profiles to avoid pollution and packed into Ziploc bags on-site for processing in the laboratory.

Measurements and Analysis Methods

We used a Li-8100 soil CO₂ flux system (LI-COR Inc., Lincoln, NE, USA) to measure Rs. A portable temperature probe connected to the Li-8100 was used to measure soil temperature (ST) at 5 cm depth, close to the PVC collar. The experimental measurement time is from 10:00 to 14:00 (local time). In the laboratory, the soil samples collected with the ring knife were used to measure the soil bulk density (BD, g.cm⁻³), Soil moisture content (SMC, %) and soil porosity (SP, %) by the drying method in the laboratory. The sample remainders were air-dried, and each sample was split into two parts: one part passed through a two mm sieve to remove mixed litter and roots, then used for analyzing of soil organic carbon (SOC, g.kg⁻¹), total phosphorus (TP, g.kg⁻¹) and total nitrogen (TN, g.kg⁻¹), and the other portion of the air-dried soil samples were used to determine the soil aggregate composition. Aggregates of six size classes were separated by wet sieving using Elliott's method (Elliott 1986). The aggregates were then split into three fractions: >2 mm (large macroaggregates), 0.25-2 mm (small macroaggregates), and <0.25 mm (microaggregates). The weight of aggregates for each class is used to calculate the mean weight diameter (MWD, mm). MWD was calculated as:

$$MWD = \sum W_i \times X_i$$

Where *i* is each aggregate fraction collected, *W_i* is the average diameter of fraction *i* and *X_i* is the dry mass of fraction *i* relative to the total soil mass.

The SOC stock was calculated using the following equation (Li et al. 2019):

$$C_s = \frac{BD \times SOC \times D}{10}$$

Where *C_s*, *BD*, *SOC*, and *D* are soil C stocks (Mg.hm⁻²), soil bulk density (g.cm⁻³), soil organic carbon (g.kg⁻¹), and soil depth (cm), respectively.

Statistical Analyses

All data were expressed as the mean ± standard deviation (SD). One-way ANOVA with Duncan test was performed to determine the differences in soil C stock and other soil properties were examined across the different soil depths, and a *t*-test was applied to determine the differences in the means of soil properties between GG and FG. Significant differences were assessed at the level of *P* < 0.05. We used redundancy analysis (RDA), a constrained ordination method, to determine the proportions of variability in soil C stock and *R_s* explained by environmental factors, using Canoco 5.0 software. The eigenvalues were proportional to the total variance explained for each axis, and were extracted from every variable as linear combinations of environmental attributes. In addition, the relationships of environmental factors with soil C stock were determined using Pearson's correlation analysis.

RESULTS

Variations in Soil Properties under Fenced Grassland and Grazed Grassland

Soil physicochemical properties: The soil bulk density (BD), moisture content (SMC), and porosity (SP) in the 0-30 cm soil layer were not significantly different between FG and GG, except the fencing significantly improved the SP in the soil layer of 20 to 30 cm of FG and led to significant differences between FG and GG (*P* < 0.05) (Table 1). As shown in Table 1, BD increased gradually with an increase in soil depth, whereas SMC decreased stepwise. FG and GG 20-30 cm soil layer BD was significantly higher than the 0-20 cm soil layer, and 0-10 cm SMC is significantly higher than 10-

Table 1: Soil physicochemical in fenced grassland (FG) and grazed grassland (GG).

Variables	FG			GG		
	0-10	10-20	20-30	0-10	10-20	20-30
Soil layer(cm)	0-10	10-20	20-30	0-10	10-20	20-30
Bulk density (g.cm ⁻³)	1.04±0.05Ab	1.09±0.02Aab	1.15±0.02Aa	1.08±0.01Ab	1.08±0.01Ab	1.18±0.07Aa
Soil moisture (%)	21.03±0.10Aa	20.53±1.41Aab	18.22±1.25Ab	22.22±0.87Aa	19.92±0.41Ab	19.96±0.67Ab
Soil porosity (%)	54.23±2.86Aa	55.55±2.68Aa	54.39±0.13Aa	54.46±2.29Aa	56.39±1.39Aa	49.64±1.46Bb
Soil organic carbon(g.kg ⁻¹)	17.71±1.79Aa	14.98±1.31Aab	14.10±0.82Ab	14.22±0.61Aa	14.77±0.70Aa	15.5±1.4Aa
Total nitrogen (g.kg ⁻¹)	2.02±0.04Aa	1.93±0.08Aab	1.81±0.11Ab	1.83±0.06Ba	1.89±0.08Aa	1.9±0.04Aa
Total phosphorus(g.kg ⁻¹)	0.66±0.02Aa	0.64±0.03Aa	0.67±0.01Aa	0.65±0.05Aa	0.67±0.01Aa	0.68±0.01Aa
Soil pH	8.24±0.47Aa	8.49±0.05Aa	8.56±0.06Aa	8.09±0.69Aa	8.47±0.04Aa	8.44±0.03Ba
C/N ratio	8.77±0.65Aa	7.76±0.41Aa	7.92±0.08Aa	7.78±0.57Aa	7.83±0.25Aa	8.15±0.75Aa

Note: Data represent the average of three replicates ± standard deviations. Different capital letters indicate significant differences (*P* < 0.05) among the grassland utilization ways. Different lower case letters indicate significant differences (*P* < 0.05) among the different soil layers, the same below.

30 cm. The SP of soil layers in FG changed little with depth, but the SP of 0-10 cm, 10-20 cm in GG was significantly higher than 20-30 cm. FG had greater SOC content, TN content, and C/N than GG in the 0-10 cm soil layer. The SOC content, TN content, and C/N in FG decreased gradually as soil depth deeper, and the SOC content and TN content of 0-10 cm were significantly higher than 20-30 cm. However, SOC content, TN content, and C/N in GG increased gradually as soil deeper. There was no significant difference in the TP content between FG and GG, and the distribution of TP content was relatively uniform between the vertical layers of the soil. The mean pH value in the topsoil layer (0-10 cm) of FG and GG was lower than 10-20 cm; their values are 8.24 and 8.09, respectively. The pH value of 20-30 cm in FG was significantly higher than that in GG.

Soil respiration and soil temperature: The Rs and ST of GG were higher than that of FG, the difference was not significant. The Rs of GG increased by 5.58%, and the average soil temperature increased by 1.2°C, compared with FG (Fig. 1).

Water-stable aggregate distribution and MWD: In the 0-10 cm soil layer, the amount of large macroaggregates (>2 mm) was significantly higher in FG (50.67%) than GG (36.69%), but the amount of small macroaggregates (0.25-2 mm) was significantly higher in GG (29.27%) than FG (13.13%)(Table 2). The amount of microaggregates (<0.25 mm) in each soil layer was higher in FG than GG, but not significantly.

The dominant fractions in the distribution of soil aggregates were the large macroaggregates (>2 mm) and microaggregates fractions (<0.25 mm) in each soil layer of FG and GG, and the large macroaggregates (>2 mm) fractions significantly decreased as soil depth increased. However, there was an inverse trend in the microaggregates (<0.25mm) fractions (Table 2).

The aggregate MWD in the 0-10 cm soil layer was significantly higher in FG than GG, and higher than the other soil layers. Among which, compared with 10-20 cm and 20-30

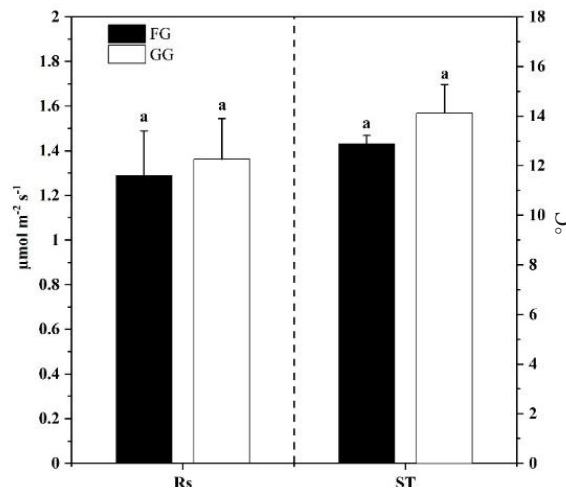


Fig. 1: Soil respiration (Rs) and soil temperature (ST) in fenced grassland (FG) and grazed grassland (GG)

Note: Different lower case letters indicate significant differences ($P < 0.05$) among the grassland utilization ways, the same below.

Table 2: Distribution of aggregate size and mean weight diameter (MWD) in fenced grassland (FG) and grazed grassland (GG)

Variables	Soil layer (cm)	Aggregate proportion in size class (%)			MWD
		>2mm	0.25-2mm	<0.25mm	
FG	0-10 cm	50.67±2.08Aa	13.13±2.93Ba	36.2±1.21Ab	3.78±0.14Aa
	10-20 cm	24.45±8.61Ab	16.89±10.57Aa	58.66±13.33Aa	1.90±0.61Ab
	20-30 cm	22.77±15.05Ab	13.13±5.21Aa	64.1±10.32Aa	2.35±0.26Ab
GG	0-10 cm	36.69±1.02Ba	29.27±7.93Aa	34.03±7.91Ab	2.83±0.25Ba
	10-20 cm	33.89±9.17Aa	15.27±1.65Ab	50.84±7.53Aa	2.57±0.67Aa
	20-30 cm	29.45±5.21Aa	13.12±0.35Ab	57.43±4.97Aa	2.25±0.36Aa

cm, aggregate MWD of 0-10 cm increased 98.9% and 60.6% in FG, respectively, and reach a significant level (Table 2).

Changes in Plant Biomass and Soil C Stocks Under Fenced Grassland and Grazed Grassland

FG had greater aboveground biomass (AGB) ($P < 0.05$) than GG. The belowground biomass (BGB) ($P = 0.0669$) in the underlying soils was not significantly different between FG and GG (Fig. 2). AGB and BGB of FG increased by 193.62% and 154.32% respectively compared with GG. And the biomass of FG and GG are both BGB higher than AGB.

The soil C stock in the 0-30 cm soil layer did not significantly differ between FG and GG. And FG improved by only 8.92%, compared with GG in the 0-20 cm soil layer. However, the C stock in the 0-10 cm soil layer of FG was significantly higher than that of GG, which was $17.57 \text{ Mg} \cdot \text{hm}^{-2}$ and $15.25 \text{ Mg} \cdot \text{hm}^{-2}$, respectively. In addition, the changing trend of C stock in the 0-30 cm soil layer of FG and GG was different with the soil depth increased. Soil C stocks of FG decreased gradually with the increase of soil depth. But the changing trend of C stocks in GG is the opposite of that of FG. Moreover, the C stock in the 20-30 cm soil layer of GG was significantly higher than the other soil layers (Fig. 3).

Factors Affecting Soil C Stocks and Soil Respiration

The RDA showed that soil physicochemical and distribution characteristics of soil aggregates explained 92.70% of the total variation in surface soil C stock and Rs (Table 3). The Monte Carlo permutation test showed that surface soil C stock and Rs variations of FG and GG were explained by the first two axes (Table 3), with the first axis explaining 72.70% ($F = 10.7$, $p = 0.004$) and the second axis explaining 20.00% ($F = 4.6$, $p = 0.016$, Table 3). The C/N ratio and BD were the factors most strongly related to the first axis; the second axis

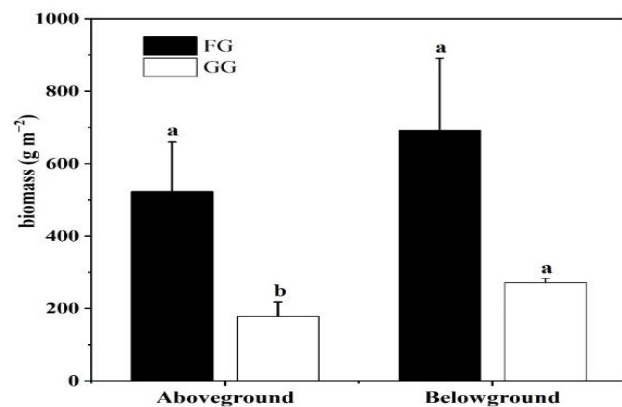


Fig. 2: Aboveground and belowground biomass in fenced grassland (FG) and grazed grassland (GG)

was closely related to BGB and AGB (Table 3). The forward selection was conducted on the environmental variables, in turn until there was no obvious explanatory variable in the RDA ordinations, it indicated that surface soil C stock and Rs were mainly affected by the C/N ratio, TN, BD, and BGB (Table 3 and Fig. 4). The surface soil C stock was primarily affected by C/N, BD, and MWD while the Rs was primarily affected by the BGB and ST (Table 4). In addition, there is a positive correlation between Rs and soil C stocks under different land uses. The bivariate correlation analyses were used to evaluate the impact factors on soil C stocks (Fig. 5), the results show that there were large differences in the correlations on environmental factors and soil C stocks between different land uses. Surface soil C stock of FG and GG was positively correlated with SOC content and C/N ratio. And soil C stocks of GG had a significant positive relationship with BD alone, in addition to the SOC content and C/N ratio. However, surface soil C stock of FG was positively correlated with Rs, large macroaggregates, microaggregates, AGB, SMC, ST, and MWD (Fig. 5).

DISCUSSION

Soil is the most important component of the grassland ecosystem and the core of the ecosystem structure and function. The change of soil condition greatly affects the grassland ecosystem and closely related to human survival. Therefore, soil change caused by land-use change has always been a research hotspot in the field of the ecosystem. Normally, in the ways of traditional grazing, the soil compaction caused by animal weight is distributed vertically through the hoof

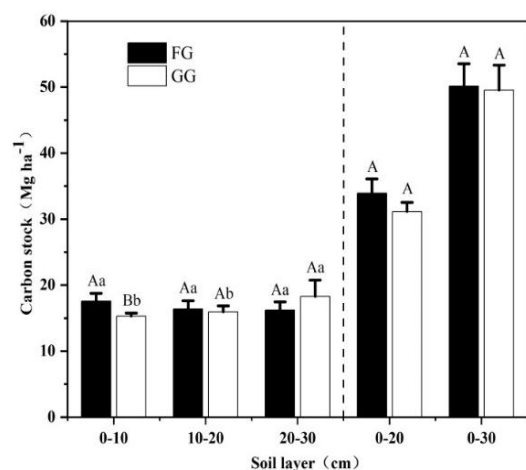


Fig. 3: Soil C stocks in fenced grassland (FG) and grazed grassland (GG).

Note: Different capital letters indicate significant differences ($P < 0.05$) among the grassland utilization ways. Different lower case letters indicate significant differences ($P < 0.05$) among the different soil layers.

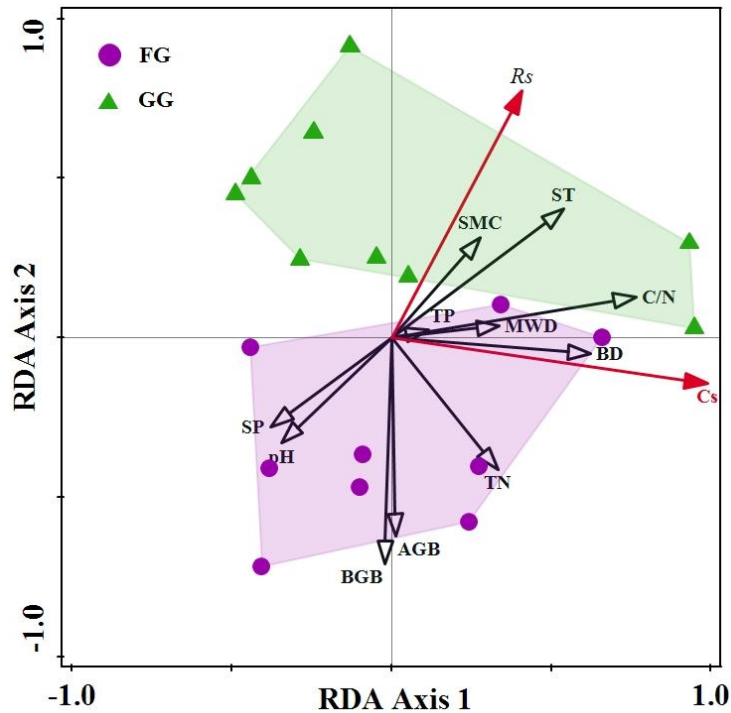


Fig. 4: Redundancy analysis (RDA) ordination diagram for soil carbon stock (Cs) and soil respiration (Rs) with environmental variables in fenced grassland (FG) and grazed grassland (GG).

Table 3: Statistic summary and canonical coefficients of Soil physicochemical and bio-mass characteristics for the first two axes of the RDA in fenced grassland (FG) and grazed grassland (GG).

Variables	Axis 1	Axis 2	$\lambda-1$	$\lambda-A$	<i>P</i> -value	F ratio
C/N	0.7652	0.1288	42.9	42.9	0.002	10.5
TN	0.3321	-0.4132	11.4	16	0.006	5.1
BD	0.6234	-0.0494	28.3	16.4	0.002	7.9
BGB	-0.0214	-0.7066	10	8.5	0.04	5.7
SMC	0.2787	0.316	7.6	5	0.054	4.5
TP	0.1147	0.0255	1	1.1	0.35	1
MWD	0.3375	0.0385	8.3	1.8	0.248	1.7
AGB	0.0126	-0.6217	7.7	0.6	0.484	0.6
ST	0.5397	0.4049	24.5	0.3	0.696	0.2
pH	-0.3445	-0.3304	10.8	0.1	0.782	<0.1
SP	-0.3772	-0.2778	11.9	<0.1	0.89	<0.1
Eigenvalues	72.70	20.00				
P value	0.004	0.016				
F ratio	10.7	4.6				

Note: C/N, C/N ratio; TN, total nitrogen; BD, bulk density; BGB, belowground biomass; SMC, soil moisture content; TP, total phosphorus; MWD, mean weight diameter; AGB, aboveground biomass; ST, soil temperature; pH, pH value; SP, soil porosity. $\lambda-1$: the variance when the variable is used as the only factor. $\lambda-A$: the additional variance of each variable explain when it is included in the model. *P*-value indicates the significance of $\lambda-A$.

area. This determines an increased BD and thus a reduction in SP and vertical water permeability. However, fencing for grazing exclusion is known to increase plant biomass and litter input to the soil, and consequently the quantity and quality of SOC. SOC plays a potential beneficial effect role in forming and stabilizing soil structure, enhancing soil physical properties. This may, in turn, increase the accumulation of organic matter on the soil surface that may reduce the volume, velocity, and erosive capacity of surface run-off (Yimer et al. 2015). Our results show that FG had greater AGB ($P < 0.05$) and BGB ($P = 0.0669$) than GG, but grazing exclusion had weak effects on BGB in comparison to that in GG. This was similar to previous studies showing that AGB and BGB in long-term fenced and overgrazed temperate grasslands in northwest China, in that, fencing can enhance plant cover

and biomass because it protects the soil seed bank and increases species composition recovery (Li et al. 2019). There was little difference in the soil physical properties under two soil-use patterns, in both FG and GG, such as BD, SMC, and SP. This was similar to previous studies showing that both BD and SMC did not vary with land-use types at the Central Rift Valley area of Ethiopia (Yimer et al. 2015). However, in contrast to the results from other studies, the SMC of the 0-10 cm layer of FG was lower than that of GG in our study. This is because vegetation restoration provided a favorable environment with rich moisture and moderate temperature, which facilitated the development of biocrusts at the early stage of grazing exclusion. The surface soil moisture was increased after eight years on fencing for grazing exclusion because biocrusts decreased the amount and depth of rain-

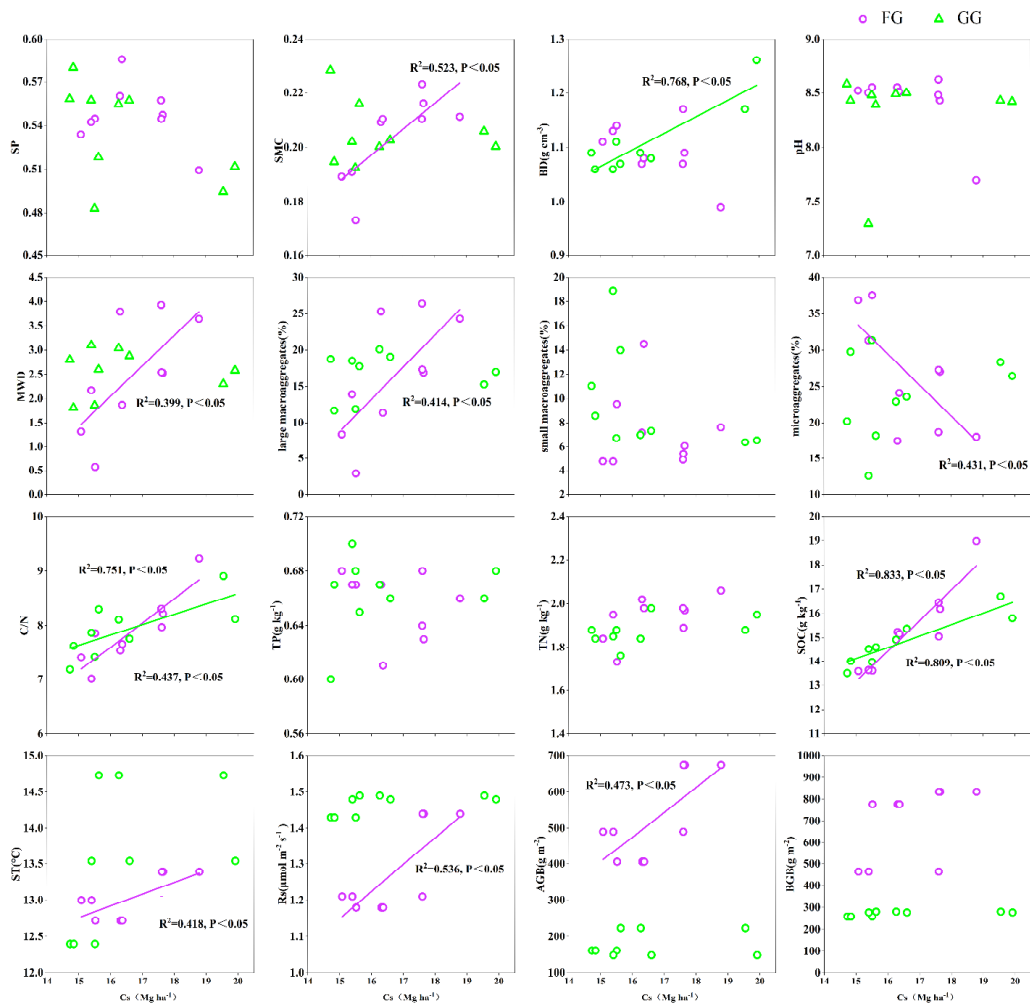


Fig. 5 Relationship of surface (0-30 cm) soil carbon stock with soil physicochemical and Soil aggregate stability of fenced grassland (FG) and grazed grassland (GG).

fall infiltration due to their lower infiltrability and higher water-holding capacity (Xiao et al. 2016).

Our findings indicate that the soil nutrient content showed strong variation under different land-use patterns. The difference was mainly observed topsoil layer (0-10 cm) between GG and FG, and distributions between the vertical layers of the soil. SOC content, TN content, and C/N in the 0-10 cm soil layer of FG are significantly higher than those of GG, and the TN content reaches a significant level ($p < 0.05$). This is in line with previous studies in northern China's grasslands showing that the changes of the nutrient in the 0-10 cm soil layer between the different grasslands management and land use were the most evident (Wang et al. 2011). Our studies on the vertical direction of soil nutrients in grassland suggested that the SOC content, TN content, and C/N in FG decreased gradually as soil depth increased, which revealed the vertical transport of soil nutrients. On the contrary, the vertical direction of soil nutrients in GG increased as soil depth increased. This might be because frequent trampling by animals causes litter on the ground which mixed well with the soil (Carter et al. 2014) and then due to the higher SMC of GG compared with FG which facilitated decomposition and the release of labile-C inputs, it prompted a greater growth and activity of microbial biomass, resulting in the decomposition of both residue-C and native C (the C priming effect) (Shahbaz et al. 2017).

Well-developed soil structures are often seen as reliable indicators of grassland restoration. Soil aggregates are regarded as the basic unit of soil structure (Liu et al. 2020). Their formation and stabilization significantly affect SOC stocks and turnover. Our studies suggested that FG significantly improves the stability of soil aggregates in the 0-10 cm soil layer, compared with GG, and the MWD significantly increases by 33.6% (Table 2). At the same time, the amount of large macroaggregates in FG also increased significantly

(Table 2). This might be because relatively abundant plant roots of FG enhance the formation of large macroaggregates and soil aggregate stability through physical entanglement, and provide root exudates and soil organic compounds as soil particle binders. However, frequent and severe grazing pressure will disturb the large macroaggregates and modify them into more microaggregates, and reduce the stability of soil aggregates. A study in Ghamishloo National Park, Isfahan, central Iran also showed that more proportions of large macroaggregates were observed in the protected area compared to the grazing-free area (Molaeinasab et al. 2018). However, we found that the amount of small macroaggregates in the 0-10 cm soil of FG was significantly lower than that of GG (Table 2), indicating that the soil large macroaggregates after the fencing for grazing exclusion were mainly composed of small macroaggregates. This is different from the view put forward by some scholars in perceptions that microaggregates formed from organic molecules are combined with clay and cations, which in turn are coupled to other microaggregates to form large macroaggregates following the hierarchy arrangements (Kurmi et al. 2020). This is perhaps a result of fencing for grazing exclusion.

Land-use change is identified as a cause of soil C losses and has been a significant source of atmospheric CO₂ over the past few centuries (Wang et al. 2011). Rs is the main way for CO₂ fixed by plants to return to the atmosphere, and it is the main factor affecting the carbon balance of the ecosystem (Hogberg & Read 2006). Changes in land use and management can cause changes in the structure or species composition of plant communities, soil physical and chemical properties, soil microclimate, and ground climate, thereby affecting the rate of Rs. Our study shows indicate that BGB is the primary factor affecting Rs under different land use patterns. Some studies have shown that 50%-93%

Table 4: Total variance of surface soil carbon stock and soil respiration explained by environmental variables based on redundancy analysis.

Ranking	Cs				Rs			
	Variables	Explains (%)	P	F	Variables	Explains (%)	P	F
1	C/N	54.5	0.004	16.8	BGB	30.9	0.024	6.3
2	BD	21.9	0.008	12.1	ST	16.4	0.062	4.0
3	MWD	10.7	0.008	10	SMC	10.6	0.108	3.0
4	ST	2.6	0.114	2.8	MWD	5.5	0.224	1.6
5	BGB	1	0.376	1	TP	7.5	0.122	2.6
6	pH	1.6	0.21	1.8	AGB	2.9	0.340	1.0
7	TP	0.3	0.612	0.3	pH	2.1	0.424	0.7
8	SMC	0.3	0.628	0.3	C/N	0.8	0.620	0.2
9	SP	<0.1	0.942	<0.1	TN	0.2	0.846	<0.1
10					SP	0.2	0.86	<0.1

of R_s is produced by plant root respiration in alpine regions (Kuz'yakov & Blagodatskaya 2015), and some studies also show that CO_2 emission in the soil increases with the increase of BGB of plants (Liu et al. 2016). Past studies have shown that a better correlation between R_s and ST as well (Wang et al. 2020). As the soil temperature changes, the number and activity of microorganisms in the soil are enhanced or inhibited, which speeds up or slows down the decomposition rate of organic matter, resulting in a change in the R_s (Shao et al. 2017). Some studies have shown that the global temperature rise of $0.5^\circ C$ will reduce the soil C stock in steady-state by about 6% (Trumbore et al. 1996). We found that the measurement of total CO_2 efflux from soil showed no significant differences between the land use types. This is consistent with the results of previous studies on grassland management in the Tibetan Plateau (Hafner et al. 2012). In addition, the R_s and ST of FG are lower than those of GG in our study. In general, the vegetation coverage and aboveground biomass of GG were significantly lower than that of FG due to the impact of trampling and feeding by livestock, which led to ST being strongly affected by light conditions, so ST was significantly higher than that of FG. And higher soil temperature accelerated the metabolic rate of the soil microbial community, both microbial metabolic quotients and microbial respiration of organic C in soil were also higher in the soils to warmer temperatures (Maranon-Jimenez et al. 2018). Our studies also indicate that soil temperature was an important factor affecting the R_s . We also identified a significant correlation between R_s and ST (Fig. 5).

Understanding the soil variables that affect the C stocks is a key goal for understanding the process of grassland vegetation restoration under different land use. The level of soil C/N ratio will facilitate or limit soil microbial activity to a certain extent, and the change of microbial activity will affect its respiration and ultimately affect soil C stocks. Microbial decomposition is faster in soils with lower C/N ratios (Zhao et al. 2019). In the study, the soil C/N ratio was an important factor affecting the soil C stocks. Our research also found that in FG there is a relatively low rate of R_s compared to GG (Fig. 1), which may cause the C stock in the 0-10 cm soil of FG to be significantly higher than that of GG (Fig. 3). In addition, the R_s showed a significant positive correlation with the C stock of FG (Fig. 5), which indicated that fencing for grazing exclusion promoted vegetation restoration, soil structure was stable, and higher C stock would increase its respiration rate. The C/N of 0-10 cm soil layer in GG was significantly lower than that of FG (Table 1). This explains the results found in our study in which 0-10 cm C stock of GG is significantly lower than that of FG. Soil physical properties and aggregates also significantly affected the C stock (Table 4, Fig. 4). The soil C stocks of FG were positively correlated

with SWC, large macroaggregates, and MWD, but the soil C stocks of GG were positively correlated with BD, consistent with previous findings (Yimer et al. 2015, Molaeinasab et al. 2018, Kurmi et al. 2020). This may be because fencing increased litter (fresh dead organic material) in the grassland ecosystem, and soil microorganism controls the content of soil organic matter and the stability of soil aggregates by directly transforming or physically intertwining secretion of extracellular polymeric substances or by changing the soil hydrophobic property (Tisdall & Oades 2006). SOC can promote the formation of stable soil aggregates, creating a large volume of mesopores and micropores, which hold capillary and hygroscopic water, respectively (Farley et al. 2004). This also explains the positive correlation between SMC and SOC of FG in this study. The soil aggregate contains about 90% of SOC, and the stable composition of soil aggregate can effectively reduce the decomposition of SOC (Jastrow 1996). Therefore, the soil surface C stock of FG is higher than that of GG, which is similar to the results of this study. Frequent and severe grazing pressures in GG disturb macroaggregates and modify them to more microaggregates, which weaken the stability of the surface soil structure and expose SOC to the ground surface (Molaeinasab et al. 2018), thus accelerating the decomposition of SOC. And trampling by livestock can significantly increase the BD of deep soil (Deng et al. 2014), so this study obtained a positive correlation between C stocks and BD.

CONCLUSION

We conclude that fencing for grazing exclusion can effectively increase AGB, BGB, and surface soil C stocks in semi-arid areas of the Loess Plateau. This is mainly due to the vegetation restoration of grassland after fencing for grazing exclusion enhances the formation of large macro-aggregates and soil aggregate stability. R_s may also be an important factor in affecting soil C stocks because the lower vegetation coverage of GG shows that ST was strongly affected by light conditions, and higher soil temperature accelerates the rate of R_s . This may result in lower C stocks on topsoil in GG than FG. In addition, grazing and trampling by livestock in GG may also affect surface soil C stocks. Therefore, further studies on mechanisms of R_s and grazing intensity on soil C stocks in grassland utilization are needed to fully explain how soil properties affect the vertical patterns of C stocks.

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