Effects of shrub encroachment on soil aggregates and organic carbon vary in different grasslands in Inner Mongolia, China

YANKUN ZHU,1,2 HAIHUA SHEN,1,2† YINPING FENG,1,2 HE LI,1 DAMILARE STEPHEN AKINYEMI,1,2 HUIFENG HU,1 AND JINGYUN FANG1,2,3

1State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing 100093 China
2University of Chinese Academy of Sciences, Beijing 100049 China
3Department of Ecology, College of Urban and Environment, Key Laboratory of Earth Surface Processes of the Ministry of Education, Peking University, Beijing 100871 China

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Abstract. Widespread shrub encroachment in grasslands can lead to changes in soil aggregates and soil organic carbon (SOC), especially in deep soils. Soil aggregates physicochemically protect SOC and thus affect soil carbon sequestration. Characterizing the changes in soil aggregates and their organic carbon associated with shrub encroachment is critical for evaluating the ecological consequences of shrub encroachment in different grasslands. In this study, we investigated soil aggregates of various sizes and their associated organic carbon at maximum soil depths of 5 m in shrub patches and neighboring grass matrix in two types (desert and typical) of grasslands in Inner Mongolia, China. The mean weight diameter (MWD) of the soil aggregates was similar between the shrub patches and grass matrix in both grasslands. However, the proportion of each aggregate size fraction to whole soil in the shrub patches was significantly lower than that in the grass matrix in the desert grasslands, while there was no significant difference between the shrub patches and grass matrix in the typical grasslands. In addition, the organic carbon content of aggregates in the shrub patches was greater than that in the grass matrix in deep soil layers (>50 cm) but was lower in the topsoil (0–10 cm depth) in the desert grasslands. However, the organic carbon content of aggregates at all soil depths was greater in shrub patches than in the grass matrix in typical grasslands. Our results suggest that the effects of shrub encroachment on the soil aggregate proportion and associated organic carbon vary at different soil depths and in different grassland types. The results of this study also suggest that it is necessary to determine the size structure and its relation to carbon content for soil aggregates to accurately predict SOC dynamics with shrub encroachment.

Key words: deep soil; desert grassland; shrub encroachment; soil aggregate; soil organic carbon; typical grassland.

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† E-mail: shen.haihua@ibcas.ac.cn

INTRODUCTION

Shrub encroachment is widespread in arid and semiarid regions and is characterized by an increase in the density and cover of native shrubs in grasslands and open woodlands (Throop and Archer 2008, Eldridge and Soliveres 2014, Maestre et al. 2016). This phenomenon has a profound impact on the structure and function of an ecosystem, especially on soil organic carbon (SOC) dynamics; however, considerable uncertainty of SOC dynamics caused by shrub encroachment exists (Maestre et al. 2009, Eldridge et al. 2011, Li et al. 2019). The influence
of shrub encroachment on SOC is controlled by several factors, such as climate (Brantley and Young 2010), plant community type (Chartier et al. 2013, Blaser et al. 2014), and soil properties (Liao et al. 2006). Among these factors, soil physical structure has the largest effect on the degree of SOC accumulation by shrub encroachment (Creamer et al. 2011, Li et al. 2016).

Soil aggregates, which are key units of soil physical structure, provide physical protection of SOC from decomposition and thus affect soil carbon sequestration (Schmidt et al. 2011, Schweizer et al. 2019). Previous studies have revealed that the SOC content is usually associated with the quantity and stability of soil aggregates (Elliott 1986, Six et al. 2004). The ability of soil aggregates to protect SOC varies by size (Keidel et al. 2018), which is often classified into macroaggregate (>0.25 mm) and microaggregate (0.053–0.25 mm and <0.053 mm) fractions (Rabbi et al. 2014). Six et al. (2000, 2002) demonstrated that the turnover of SOC is more rapid within macroaggregates than in microaggregates. Microaggregates provide long-term protection of SOC, whereas the turnover rate of macroaggregates is a crucial process influencing SOC stabilization (Blanco-Canqui and Lal 2004, Zhong et al. 2019). Soil aggregates are affected by many factors, such as soil texture, soil organic matter (SOM), the soil microorganism communities, and the plant root system (Six et al. 2004, Wilson et al. 2009, Rillig et al. 2015). Previous studies have shown that shrub encroachment can lead to changes in physical and chemical soil properties, root traits, and microorganism community (Liao et al. 2006, Hollister et al. 2010, Li et al. 2017, Zhou et al. 2017a, b). These factors can influence soil aggregates and their associated organic carbon, which in turn affect SOC changes under shrub encroachment.

Shrub encroachment has been very common in the Inner Mongolian grasslands during the past century because of the changing climate and increasing amount of human activities (Chen et al. 2015, Zhou et al. 2019a, b). Previous studies have demonstrated that shrub encroachment can alter the SOC content within 0–500 cm soil layers but varies between typical grasslands and desert grasslands (Li et al. 2019). The differences between typical and desert grasslands are mainly due to their climate and vegetation composition (Liao et al. 1996, Sheng et al. 2010, Liu et al. 2011). Shrub patches in typical grasslands accumulate more SOC than do desert grasslands, which might be attributed to the replacement of carbon decomposition with sufficient vegetation in the former but with insufficient vegetation in the latter (Li et al. 2019, Zhou et al. 2019a, b). However, the dynamics of soil aggregate formation and organic carbon content within aggregate fractions after shrub encroachment remain unclear, hindering the accurate prediction of SOC dynamics in grasslands under shrub encroachment.

Therefore, in this study, we investigated soil aggregates and their associated organic carbon contents at soil depths of up to 5 m in shrub patches and grass matrix in desert grasslands and typical grasslands in Inner Mongolia, China. The aim of this study was to explore how shrub encroachment affects the soil aggregates and their organic carbon contents differently in the two types of grasslands, which is critical for evaluating the ecological consequences of shrub encroachment in different grasslands.

**Materials and Methods**

**Study area and field investigation**

This study was conducted at six sites, of which three are typical grasslands in Zhengxiangbai Banner and three are desert grasslands in Suniteyou Banner, Inner Mongolia, China (Fig. 1). We classified the typical grassland and desert grassland based on the 1:1,000,000 vegetation map of China and in terms of the Ivanov moisture index (Liao et al. 1996, Sheng et al. 2010, Li 2015). The Ivanov moisture index ranged from 0.3 to 0.6 in typical grasslands and from 0.2 to 0.3 in desert grasslands. The mean annual temperature and mean annual precipitation are 5.1°C and 200.7 mm in the desert grasslands and 3.0°C and 300.8 mm in the typical grasslands, respectively. According to the World Reference Base (IUSS Working Group WRB 2015), the soil type is Calcisols for both grassland types. The percentage of vegetation cover in the typical grasslands is approximately 25–40%, and vegetation is dominated by *Leymus chinensis*, *Stipa krylovii*, and *Stipa klemenzii*. The percentage of vegetation cover in the desert grasslands is 15–35%, and vegetation is dominated by *S. klemenzii*, *Stipa glareosa*, and...
*Stipa breviflora* (Li 2015). Both grassland types have been encroached by a shrub species, *Caragana microphylla*. The mean percentage of shrub cover is 15.2% in typical grassland and 9.03% in desert grassland (Zhou et al. 2019b).

Soil sampling was conducted in September 2013, during late growing season. At each site, we selected three shrub patches that presented medium-sized shrub canopies, had diameters >1 m, and had similar species compositions. The distance between the selected shrub patches was more than 5 m. To avoid the effects of heterogeneity, three soil cores within each shrub patch were collected and then combined into a single core sample. Additionally, three cores located at least 3 m from the nearest shrub were collected for the grass matrix that were not yet affected by shrub encroachment. We treated the grass matrix as natural grasslands before shrub encroachment in this study (Jackson et al. 2002, Knapp et al. 2008, Zhou et al. 2018). We used a drilling machine (8 cm diameter, Li et al. 2019) to obtain soil cores from a depth of 0–5 m. Cores were extracted at eight different depths: 0–10, 20–30, 30–50, 70–100, 100–200, 200–300, 300–400, and 400–500 cm. In total, 96 soil samples (2 grassland types × 2 locations [shrub patches vs. grass matrix] × 8 depth increments × 3 sites [replications]) were used for analysis.

**Determination of soil aggregates and other soil properties**

Each soil sample was divided into two aliquots: One aliquot was air-dried in the laboratory, and the other was kept in cold storage at −20°C until analysis. Afterward, the soil samples were sieved with a 2-mm mesh sieve, and the fine roots, seeds, and other plant material were carefully removed before analysis. The bulk soil moisture content (%, SMC) was determined by weight after 10 g of the cold stored soil was dried at 105°C for 24 h. The bulk

![Fig. 1. Sampling site and landscape of desert and typical grasslands in Inner Mongolia.](image-url)
soil total phosphorus (% concentration, TP) content was determined via the molybdenum-blue method, and the soil pH was determined with a pH meter (PHS-3B, Shanghai Optical instrument Factory, Shanghai, China), in which the soil:water ratio was 1:2.5.

Water-stable aggregates were separated via a soil aggregate tester (model XY–100, Beijing Xiangyu Weiye, China) on the basis of a principal similar to that of a Yoder wet sieving apparatus. Three aggregate size classes (>0.25, 0.053–0.25, and <0.053 mm diameter, shown as A1, A2, and A3, respectively) were collected from each sample by wet sieving. Air-dried soil samples (30 g each) were placed on the top sieve of each set of nested sieves, and 2 L of distilled water was rapidly added to each cylinder until the soil sample and top screen were covered with water. The soils were submerged in water for 10 min before the start of the wet sieving action. The oscillation time (10 min), stroke length (4 cm), and frequency (30 cycles/min) were held constant. Floating organic matter (density < 1 g/cm) was removed from the >0.25 mm aggregate size class and almost entirely comprised plant debris. However, organic matter from other size classes was not removed because it was considered to be associated with the aggregates (Wilson et al. 2009). After wet sieving, the material remaining on each sieve was collected and dried at 65 °C for 24 h prior to weighing. The >0.25 mm and 0.053–0.25 mm fractions were mixed with a fivefold volume of a 5 g/L sodium hexametaphosphate solution in an aluminum box for 10 min and then shaken on an orbital shaker at 350 rpm for 15 min. The dispersed aggregates and sand of each sample were collected separately after sieving from the same mesh sieve, washed with distilled water, and then dried at 65 °C for 24 h. The aggregate weights were then recorded to estimate the sand-free correction for calculating the proportions of each aggregate fraction in the bulk soils.

The total carbon (% concentration, TC) content, total nitrogen (% concentration, TN) content, and soil inorganic carbon (% concentration, SIC) content of the bulk soil and aggregates were measured. The TC and TN contents were determined by an elemental analyzer (model PE2400, PerkinElmer, Waltham, Massachusetts, USA), and the SIC was measured with a carbonate content analyzer (Eijkelkamp 08.53, Giesbeek, The Netherlands). The SOC (% concentration) content of the bulk soil and aggregates were obtained by subtracting the SIC from the TC, and the soil bulk density and SOC density (kg C/m³ soil) were measured and estimated according to the method described by Li et al. (2019).

Data analysis

The mean weight diameter (MWD) of the soil aggregates is a statistical index of aggregation and was calculated according to the following formula (van Bavel 1950):

$$MWD = \sum_{i=1}^{n} X_i \times W_i \hspace{1cm} (1)$$

where MWD is the mean weight diameter of the aggregates (mm), $X_i$ is the mean diameter of each size fraction (mm), $W_i$ is the proportion of the corresponding size fraction to the total aggregates, and $n$ is the number of size fractions. The minimum diameter of the soil aggregates in the calculation process is 0 mm, and the maximum value is 2 mm.

All data analyses were performed via R software 3.6.1 and its associated software packages (R Core Team 2019). The Shapiro–Wilk test was used to test for normality and equal variances, and data transformation was carried out when the data were not normally distributed. Repeated measures ANOVA was used to compare the differences in soil aggregates and soil properties among the different soil depths, grassland types, and locations (shrub patches vs. grass matrix), with soil depth used as a repeated measure nested within sites. Furthermore, a t test was used to test the difference between desert grasslands and typical grasslands, and a paired t test was used to test the differences between shrub patches and grass matrix in each grassland type. Moreover, correlation analysis was used to evaluate the relationships between soil aggregates and other soil properties, with whole soil profile values.

RESULTS

Soil aggregate size and their vertical distribution

Typical grasslands had less MWD than desert grasslands ($t = 1.79, P = 0.08; \text{Fig. 2}$). The proportion of total soil aggregates to whole soil in
desert grasslands was significantly lower than that in typical grasslands for shrub patches ($P < 0.01$), but did not differ significantly in the grass matrix ($P = 0.39$). There was a significant interaction effect between grassland type and location on the proportion of total aggregates to whole soil, but there was no significant interaction effect on MWD (Appendix S1: Table S1). In the desert grassland, the proportion of total soil aggregates was significantly lower in shrub patches than in the grass matrix ($P < 0.01$), whereas there was no significant difference in the typical grasslands (Table 1). The proportion of total soil aggregates increased with increasing soil depth in the two grassland types (Fig. 2, Table 1).

Furthermore, the proportion of each aggregate size fraction in the shrub patches was significantly lower than that in the grass matrix in the desert grasslands (Fig. 3a–c; Table 1). Specifically, at depths of 0–10 cm, the A2 proportion of shrub patches was significantly lower than that of the grass matrix (Fig. 3b). Below 300 cm, the A1 proportion of shrub patches was also significantly lower than that of the grass matrix (Fig. 3a). In contrast, there was no significant difference in the proportion of any aggregate size fraction between the shrub patches and grass matrix at all measured depths in the typical grasslands (Fig. 3d–f; Table 1).

**Aggregate organic carbon and its vertical distribution**

There were significant interaction effects of grassland type and location on the organic carbon content and density of A1 (Appendix S1: Table S1). The organic carbon content and density of A1 were significantly greater in the shrub patches than in the grass matrix in the typical grasslands, whereas there was no significant difference in the desert grasslands (Fig. 4; Appendix S1: Fig. S1; Table 1). In addition, in the desert grasslands, the organic carbon content of only A2 was significantly greater in shrub patches than in the grass matrix (Fig. 4a–c; Table 1). The organic carbon content of A1 was significantly greater in the shrub patches than in the grass matrix at >50 cm depth, but there was no significant difference within the 0–50 cm depth (Fig. 4a). Moreover, the organic carbon content of A3 was significantly lower in the shrub patches than in the grass matrix within the 0–10 cm depth but greater at depths >10 cm (Fig. 4c). In the typical grasslands, the organic carbon contents of A1 and A3 were significantly greater in the shrub patches than in the grass matrix but were not significantly greater in A2 (Fig. 4d–f; Table 1). Furthermore, the aggregate organic carbon content and density decreased with increasing soil depth in both grassland types (Fig. 4; Appendix S1: Fig. S1; Table 1).

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**Fig. 2.** Proportion of total aggregates to whole soil and mean weight diameter (MWD) in different grasslands and at different soil depths for shrub patches and grass matrix. Error bar is standard error. * represents that the difference between shrub patch and grass matrix is significant, $P < 0.05$. 

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Table 1. $P$ value of two-way repeated measures ANOVA for the effects of location (shrub patches vs. grass matrix), soil depth and their interaction on soil aggregates, associated organic carbon content and density in desert grassland and typical grassland.

| Source                  | Proportion of aggregates | Organic carbon content | Organic carbon density |
|-------------------------|--------------------------|------------------------|------------------------|
|                         | A1  | A2  | A3  | Total | MWD | A1  | A2  | A3  | A1  | A2  | A3  | A1  | A2  | A3  |
| Desert grassland        |     |     |     |       |     |     |     |     |     |     |     |     |     |     |     |
| Location                | <0.01 | 0.09  | 0.04 | <0.01 | 0.71 | 0.97 | 0.04 | 0.43 | 0.49 | 0.59 | 0.84 |     |     |     |
| Soil depth              | 0.09 | <0.01 | 0.02 | 0.01 | 0.01 | <0.01 | <0.01 | <0.01 | 0.02 | 0.04 | 0.25 |     |     |     |
| Interaction             | 0.01 | 0.85 | 0.72 | 0.32 | 0.66 | 0.82 | 0.76 | 0.5  | 0.94 | 0.55 | 0.43 |     |     |     |
| Typical grassland       |     |     |     |       |     |     |     |     |     |     |     |     |     |     |     |
| Location                | 0.97 | 0.25 | 0.88 | 0.71 | 0.47 | <0.01 | 0.1  | 0.048 | 0.02 | 0.32 | 0.19 |     |     |     |
| Soil depth              | 0.78 | <0.01 | <0.01 | <0.01 | 0.02 | <0.01 | <0.01 | <0.01 | 0.02 | <0.01 | <0.01 |     |     |     |
| Interaction             | 0.38 | 0.31 | 0.97 | 0.99 | 0.09 | 0.29 | 0.34 | 0.55 | 0.49 | 0.76 | 0.9  |     |     |     |

Note: A1, A2, A3, and Total indicate the $>$0.25 mm, 0.053–0.25 mm, $<$0.053 mm, and total aggregates, respectively. MWD is mean weight diameter of soil aggregates. Bold values indicates the significance of $P < 0.05$.

Fig. 3. Proportion of aggregates to whole soil in different grasslands and at different soil depths for shrub patches and grass matrix. A1, A2, and A3 represent the $>$0.25 mm, 0.053–0.25 mm, and $<$0.053 mm aggregates, respectively. Error bar is standard error. * indicates that the difference between shrub patches and grass matrix is significant, $P < 0.05$. 

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**Relationships between soil aggregates and other soil properties**

The SOC density of the shrub patches was significantly greater than that of the grass matrix in the typical grasslands \( (P < 0.05) \), but was similar between the grass matrix and shrub patches in the desert grasslands \( (P = 0.29; \) Appendix S1: Fig. S2). The soil TN, soil TP, and SMC of the grass matrix were significantly greater than those of the shrub patches in the desert grasslands \( (P < 0.05) \), but there was no significant difference in the typical grasslands (Appendix S1: Fig. S3). The MWD of the soil aggregates was positively correlated with the SOC but negatively correlated with TC, TP, SIC, and SMC (Fig. 5). In addition, the proportion of total aggregates and each size fraction to whole soil was positively correlated with TC, TP, SIC, and SMC (Fig. 5).

**DISCUSSION**

The results of our study showed that the effects of shrub encroachment on the proportion of soil aggregates to whole soil and the associated organic carbon content vary with grassland type. There are several possible explanations for
the lower proportions of total aggregates and each size aggregate to whole soil recorded in the shrub patches compared with the grass matrix in the desert grasslands, but no significant difference was recorded in the typical grasslands. First, the lower proportion of total aggregates to whole soil in the shrub patches compared with the grass matrix could be attributed to the large amount of sand intercepted by the shrub patches in the desert grasslands (Wang et al. 2012). Second, there were different responses to shrub encroachment in part because of the smaller soil aggregate size in the typical grasslands compared with the desert grasslands. Previous studies have shown that the stability of aggregates increases with decreasing diameter (Totsche et al. 2018), and root penetration can decrease the proportion of macroaggregates, which are relatively unstable (Six et al. 2004). Therefore, the soil aggregates of the desert grasslands were more susceptible to shrub root penetration than those of the typical grasslands. Third, other soil properties could also affect soil aggregation. Our study showed that soil nutrients and moisture in the shrub patches were significantly lower than those in the grass matrix in desert grasslands, but this significant difference was not recorded in the typical grasslands (Appendix S1: Fig. S3). These differences in soil nutrients and moisture contents could partly explain the different responses of soil aggregation to shrub encroachment between the typical and desert grasslands. For example, previous studies have shown that a decrease in SMC and the resulting reduced vegetation can result in decreased soil structural development and aggregation (Bronick and Lal 2005, Cosentino et al. 2006). Increased soil nutrient contents can also promote soil aggregation (Chen et al. 2017, Wang et al. 2018). In our study, the proportion of total aggregates and each size fraction was also highly positively influenced by the soil nutrient and moisture contents (Fig. 5).

The organic carbon content of aggregates in shrub patches was greater than that in the grass matrix in the deep soil layer (>50 cm) but was lower in the surface soil layer (0–10 cm depth) in desert grasslands (Fig. 4a–c). However, the organic carbon content of aggregates at all soil
depths was greater in shrub patches than in the grass matrix in the typical grasslands (Fig. 4d–f). Liao and Boutton (2008) reported that variations in the organic carbon content of aggregates were mainly caused by changes in SOM inputs and the decreased ability of soil microbes to decompose this organic matter. In addition to dissolved organic carbon and soil faunal bioturbation, plant roots and root exudates are the primary sources of carbon input in the subsoil (Jobbágy and Jackson 2000, Sokol and Bradford 2019). Plant growth forms differ in their rooting patterns, with shrubs having root systems that are larger and deeper than those of grasses (Schenk 2006, Wang et al. 2017). Moreover, shrub encroachment reduces nutrient limitation and promotes soil carbon sequestration (Blaser et al. 2014, Wang et al. 2019). In deep soil, owing to both the accumulation of nutrients in shrub patches, the so-called fertility island effect, and increases in the underground root biomass and microbial biomass, there is more organic carbon in the cementing material of soil aggregates, which could result in an increase in the aggregate organic carbon content (Schlesinger et al. 1996, Hibbard et al. 2001, Hollister et al. 2010, Rau et al. 2011).

In the surface soil layer, the different responses of the aggregate organic carbon content between the typical and desert grasslands can be explained by the replacement of carbon decomposition with sufficient vegetation in the typical grasslands but with insufficient vegetation in the desert grasslands. Our previous study showed that more soil fungal biomass measured by phospholipid fatty acids was present in the shrub patches than in the grass matrixes in desert grasslands, but this significant difference was not detected in typical grasslands (Li et al. 2017). Thus, these results indicated that there was enhanced decomposition in the shrub patches only in the desert grasslands. In addition, shrubs are associated with increasing plant biomass in humid areas but decreasing it in drier regions (Knapp et al. 2008, Eldridge et al. 2011). In the desert grasslands, soil nutrients and moisture contents were lower in the shrub patches than in the grass matrix (Appendix S1: Fig. S3). This could be attributed to the large amount of sand intercepted by the shrub patches in the desert grasslands (Wang et al. 2012). These intercepted sands, which have low nutrient contents, may counteract the topsoil accumulation effect of shrubs (Li et al. 2019). In addition, the higher soil moisture contents in the grass matrix than in the shrub patches were also attributed to high rainfall interception by the shrub canopy, lower water infiltration, and elevated runoff in the shrub patches than in the grass matrix (Ravi et al. 2007, Li et al. 2013). These factors restrict the growth of shallow herbaceous roots in shrub patches (Zhou et al. 2019), which could decrease SOM inputs and reduce the organic carbon content of surface soil aggregates. In the typical grasslands, the soil TN, soil TP, and SMC were greater than those in the desert grasslands (Appendix S1: Fig. S3), and shrub encroachment could lead to greater biomass carbon input than in desert grasslands (Li et al. 2019).

Our results also indicated that the significantly greater SOC density of the shrub patches than of the grass matrix in the typical grasslands was mainly due to the accumulation of organic carbon in A1, as only the organic carbon density of A1 was significantly greater in the shrub patches than in the grass matrix (Table 1). This result might be due to the rapid turnover of macroaggregates, which can increase SOC storage (Blanco-Canqui and Lal 2004, Six et al. 2004). Liao et al. (2006) reported similar results, showing that the accumulation of new C3-derived organic matter in macroaggregates made an important contribution to the increased soil carbon content. In the desert grasslands, shrub encroachment reduced the proportion of aggregates to whole soil but increased the aggregate organic carbon content, which resulted in no differences in SOC density or organic carbon density in any size aggregates between the shrub patches and the grass matrix.

**CONCLUSION**

Our results showed that the effects of shrub encroachment on soil aggregates and organic carbon content vary in different grasslands. In desert grasslands, shrub encroachment reduced the proportion of aggregates but increased their organic carbon content, which resulted in no significant difference of SOC content between the shrub patches and grass matrix. In contrast, shrub encroachment only increased the organic carbon content in the typical grasslands.
carbon content of the soil aggregates, thereby increasing the SOC content in typical grasslands. The increased SOC content in the typical grasslands was mainly due to the sequestration of organic carbon within A1. These results suggest that shrub encroachment can potentially increase the carbon sink in typical grasslands but that it would have limited impact on the carbon sink in desert grasslands and alter soil structure. The investigation of soil aggregates and associated organic carbon provides insight into the carbon protection dynamics and carbon allocation patterns under shrub encroachment and enhances the accurate prediction of SOC dynamics in grasslands under shrub encroachment.

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