Long-term grazing exclusion increases ecosystem carbon stock but decreases nitrogen stock in the karst alpine grassland of China

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Abstract

Grazing management practices are a major factor regulating nutrient cycling and plant growth in grasslands. However, the response of long-term grazing regimes to ecosystem carbon and nitrogen accumulation and plant productivity remains uncertain in karst landscapes. A 17 year-long field experiment, constituting the methods of grazing exclusion (GE), continuous grazing (CG), mowing and grazing (MG), and rotational grazing (RG), was conducted to assess the effects of long-term management measures on plant biomass, ecosystem organic carbon, and nitrogen stocks in a karst alpine grassland of the Yunnan-Guizhou Plateau. Our results showed that grazing significantly reduced the aboveground biomass (CG, MG, and RG) and root biomass (MG and RG) compared to GE, but there were no differences between the results obtained using the various grazing methods. The root/shoot ratio increased by 55.98% in CG and decreased by 52.96% and 37.14% in MG and RG, respectively, compared to that achieved with GE. Soil organic carbon (SOC) of GE was higher than that of CG, MG, and RG in each soil layer, while it was significantly higher at 0–10 cm and 20–30 cm. Grazing promoted the mean total N content with a significant increase in CG and RG, and significantly increased total P content in each soil layer compared to GE. Grazing significantly decreased the C/N and C/P ratios at each soil depth. GE significantly increased the ecosystem organic carbon stocks (EOCs) and decreased the ecosystem total nitrogen stocks (ETNs). Although there were no significant changes among the grazing methods, the EOCs increased by 22.29% (MG) and 16.31% (RG) and ETNs increased by 7.76% (RG) when compared to those obtained with CG. EOSs were positively correlated with SOC, stoichiometry (C: N: P), and aboveground biomass, while being negatively correlated with TP; ETNs were positively correlated with N and P, while being negatively correlated with C/N, C/P, aboveground biomass, and root biomass. Our results indicate that GE can provide significant improvements in plant recovery and ecosystem organic carbon storage, whereas RG is beneficial for promoting both EOCs and ETNs under the condition of pasturing utilisation in karst grasslands.

1. Introduction

Grasslands are one of the major terrestrial ecosystems and account for 40% of the global land surface (Abdalla et al., 2018). As an important part of the global carbon and nitrogen cycle, grasslands accounted for 34% of carbon reserves and 30% of nitrogen reserves in terrestrial ecosystems (Eze, Palmer, & Chapman, 2018; Xu, He, & Yu, 2019). The grazing regime is one of the most widespread management practices in grasslands and is an important factor affecting ecosystem carbon and nitrogen cycling (Abdalla et al., 2018; G. Zhou et al., 2017). However, overgrazing and poor management have detrimental effects on grasslands, such as degradation of plant cover and species diversity (Louhaichi, Ghassali, Salkini, & Petersen, 2012) and changes in plant productivity and nutrient cycles (Ghosh et al., 2022; Wen Li et al., 2017), which in turn affect grassland carbon and nitrogen allocation (Ferlan et al., 2011). Thus, there has been great concern over ways to maintain and improve the ability of grasslands to sequester carbon and nitrogen through reasonable management practices.

Grassland regimes, such as grazing imposition or exclusion, significantly influence grassland carbon and nitrogen storage (G. Zhou et al., 2017). Many studies have been conducted on the effects of management practices on grassland carbon and nitrogen pools, among which the overall major decrease in global grassland carbon stocks is mainly attributed to grazing (Eze et al., 2018). For instance, Soil organic carbon (SOC), total nitrogen(TN), and phosphorus (TP) stocks declined in a five-year grazing period, and an increased stocking rate reduced the SOC content and storage in the Tibetan alpine meadow (D. S. Sun et al., 2011); grazing with moderate to high stocking rates significantly reduced SOC and nitrogen in a semiarid tropical inceptisol (Ghosh et al., 2022). However, optimised grazing has synergistic benefits on soil carbon and nitrogen sequestration in terms of reduced interference with plant-insect interactions, water depletion, and improvement of aboveground biomass (Bossio et al., 2020; Dai, Fu, et al., 2021; de Vries et al., 2012). For example, rotational grazing (RG) could increase soil carbon and nitrogen contents compared to those of hayed pastures (Contosta et al., 2021); soil carbon and nitrogen contents were 13% and 9% higher, respectively, after RG compared to those observed after conventional grazing at the three farms in the northeastern United States (Mosier et al., 2021). However, some studies have reported that heavy grazing has a significant positive effect on soil carbon and nitrogen pools, which are positively correlated with increases in below-ground biomass allocation. Moreover, higher grazing intensity may have a potential positive effect on increasing soil carbon and nitrogen storage (W. Li, Huang, Zhang, & Wu, 2011). Therefore, the effects of grazing practices on grassland carbon and nitrogen pools remain inconclusive.

Grazing exclusion (GE) has been recommended as an effective measure for restoring degraded grasslands (Dai, Fu, et al., 2021). Generally, grassland enclosures can enhance plant production and soil carbon pools worldwide (Su & Xu, 2021). GE facilitates the restoration of soil carbon and nitrogen by increasing the productivity of plants, decreasing the accumulation of surface litter in the soil, and thus minimising the disturbance of vegetation, which in turn increases carbon and nitrogen accumulation (Y. Li et al., 2012). Compared with grazing, soil organic carbon and nitrogen stocks in the 0–100 cm soil layer increased after 25 years of GE (Y. Li et al., 2012), and total soil carbon stocks and nitrogen content were higher after 10 years of GE than those observed after five years of GE and free grazing (Gebregergs, Tessema, Solomon, & Birhane, 2019). However, other studies have arrived at a contrary conclusion; that is, no significant difference is observed in soil organic carbon and nitrogen stocks after GE and light grazing, while markedly higher SOC is observed in areas of heavy grazing than that detected after GE (Reeder, Schuman, Morgan, & Lecain, 2004). Another study (Cui, Dong, Liu, & Sun, 2021) reported that 4–7 years of GE decreased SOC content and SOC stock compared to that obtained after free grazing. These results indicate that the effects of fencing on grassland carbon and nitrogen pools remain uncertain. The different responses to fenced grazing prohibition may result from the diversity of the research regions, topography, vegetation types, grazing histories, enclosure times, and their interactions (Gong et al., 2014; Vivanco & Austin, 2006; Zhang et al., 2018).

Many studies have focused on the effects of grazing management practices on grassland carbon and nitrogen pools in temperate and high-altitude regions, such as the Tibetan, Loess, and Inner Mongolian Plateau located in Northern China (Gong et al., 2014; Tserang Donko Mipam, Chen, Liu, Miehe, & Tian, 2021; Yan et al., 2020). However, grasslands undergoing the transition to forests at the hills and slopes of southwest China have received little attention; these grasslands cover an area of 35.58×10^6 ha and account for 32.59% of the total area of this region (Huangfu, Mao, & Lu, 2012). This situation is more pronounced in case of grasslands in typical karst landscapes. The response of karst grasslands to grazing management practices may not be in line with other regions because of the characteristics of high rock exposure rate, sparse and shallow scattered soils, weak resistance to disturbance, and poor stability (Huang, Cai, & Xing, 2008). To elucidate the responses of plant productivity and soil nutrient sequestration to long-term grazing, it is necessary to evaluate the implications of management practices on the stability of ecosystem carbon and nitrogen storage.

We conducted in situ experiments in an alpine grassland of the Yunnan-Guizhou Plateau after 17 years of grazing impositions and exclusion. The objectives were (1) to assess the effects of long-term GE on the vertical distribution of plant biomass and soil carbon and nitrogen, (2) compare the variations in ecosystem carbon and nitrogen storage under different grazing conditions, and (3) explore the main factors affecting soil carbon and nitrogen accumulation.

2. Materials and methods

2.1 Study area

The experiment was conducted on a karst alpine grassland in the Liangshuigou sheep breeding farm in Guizhou, which is located on the Yunnan-Guizhou Plateau, China (103º36'–104o45'E, 26o36'–27o26'N; 2200 m a.s.l.). It has a subtropical monsoonal humid climate with an annual average temperature of 10–12 degC and precipitation of 962 mm. The grassland is a perennial *Lolium perenne* +*Trifolium repens* grassland. The main plant species are*Lolium perenne* , *Trifolium repens* , *Festucaovina* ,*Poa annua* , *Carex liparocarpos* , and *Potentilla chinensis* . The soil type in the study site is alpine yellow-brown loam (H. Sun, 2014).

2.2 Experimental design

A grassland was established for implementing grazing in 1992. Four methods (GE, continuous grazing (CG), mowing and grazing (MG), and RG) were conducted in 2001, with each method being replicated thrice. GE was implemented by deploying fences to avoid disturbance; CG was performed from April to November each year; MG involved annual mowing in mid-June (with a stubble height of 3 cm) and subsequent grazing till the end of the growing season (November); RG involved grazing for 7 to 10 d per month in each subplot from April to November, with the grass height being maintained at approximately 3 cm after each grazing session. The grazing livestock were 2 to 3 year-old healthy sheep, with a stocking rate of 10 sheep units / hm^2. All grazing methods were performed using 60 $kg*ha^{-1}$ nitrogen fertiliser (urea) and 300 $kg*ha^{-1}$ calcium-magnesium-phosphate fertiliser (superphosphate), which were added in late June and mid-to-late October, respectively (H. Sun, 2014).

2.3 Field sampling and analysis

Plant and soil samples were collected in August, 2017 after a 17-year period. Aboveground biomass was collected from three 50 x 50 cm subplots by clipping the vegetation. Root biomass was collected from soil cores (8 cm in diameter) in the same subplots at the 0–10, 10–20, 20–30, and 30–40 cm soil layers. Soil bulk density was measured at each soil depth simultaneously with a volumetric steel ring (100 cm^3). Soil cores were washed in 2 mm mesh bags to separate roots from the soil. Soil samples were collected from soil cores (5 cm in diameter) of the 0 to 10, 10–20, 20–30, and 30–40 cm soil layers in the same subplot and then mixed into one sample. Each soil sample at 0–10 cm was divided into two sub-samples. One of the sub-samples (approximately 100 g) was brought back to the laboratory and stored at 4 degC to determine dissolved organic carbon (DOC) and labile organic carbon (LOC), and the other sub-samples were air-dried and passed through 10 and 100 mesh sieves to measure pH, soil organic carbon, soil total nitrogen, and phosphorus.

The entire plant biomass was dried for 48 h at 65degC to measure the dry weight. Soil pH was determined for a soil–water ratio of 1:5; SOC was measured using the potassium dichromate oxidation spectrophotometric method; TN concentration was determined using the Kjeldahl method; TP concentration was quantified using the molybdenum antimony anticolorimetric method; LOC content was measured via the potassium permanganate oxidation method; DOC was determined for a soil–water ratio of 1:2.

Soil organic carbon and nitrogen stocks were calculated using the following equations (Gai et al., 2018):

$$S_1 = C \times B \times S \times 10 \qquad (1)$$

$$S_2 = \sum_{i=1}^{k} S_1 \; (k = 1, 2, 3, 4) \qquad (2)$$

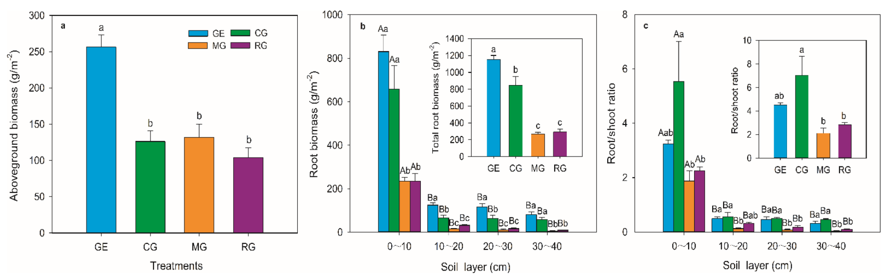
where S_1 is the soil organic carbon and nitrogen storage in the corresponding soil layer ($g \cdot m^{-2}$), C is the thickness of the soil layer (cm), B is the soil bulk density ($g \cdot m^{-3}$), S corresponds to the soil organic carbon and nitrogen content (g·kg^{-1}),S_2 is the sum of soil organic carbon and nitrogen storage (g·m^{-2}), and i represents the number of soil layers (i =1, 2, 3, 4).

2.4 Statistical analysis

One-way analysis of variance (least significance difference method) was used to test for significant differences in biomass, soil physical and chemical indicators, and organic carbon and nitrogen storage between different methods and soil layers. The Pearson correlation coefficient was used to analyse the correlation between plant biomass and nutrient indicators, and all data were analysed using SPSS 21.0 (with a significance level of 0.05). Principal component analysis (PCA) of plant biomass and nutrient indicators was conducted using Origin 2018.

3. Results

3.1 Plant biomass



Grazing significantly decreased aboveground biomass by 48.48%–59.44% compared to GE (P<0.05); however, there were no significant differences between grazing methods (Fig. 1a). The belowground biomass tended to decline with increasing soil depth, and the biomass at the 0–10 cm depth was significantly higher than that of the other soil layers in all methods (Fig. 1b). MG and RG significantly reduced the root biomass at each soil layer at a depth of 0–40 cm, and CG significantly reduced the root biomass at 10–30 cm depth (P<0.05, Fig. 1b). Grazing significantly decreased the total root biomass at 0–40 cm soil depth, while MG and RG decreased the total root biomass significantly compared to the reduction caused by CG (P<0.05, Fig. 1b). The root/shoot ratio (R/S) of 0–10 cm was significantly higher than that of 10–40 cm depth in all methods, and the R/S of CG was significantly higher than that of MG and RG (P<0.05, Fig. 1c). Compared with GE, R/S increased by 55.98% in CG and decreased by 52.96% and 37.14% in MG and RG, respectively (Fig. 1c).

FIGURE 1 Aboveground biomass (a), root biomass in 0–40 soil layers (b), and the root/shoot ratio (c). Note: Different capital letters indicate significant differences (P<0.05) between different soil layers for the same method; different lowercase letters indicate significant differences (P<0.05) between the same soil layers for different methods. Values are represented as mean ± SE.

3.2 Variation of soil

properties

There were no significant differences in soil bulk density between the grazing methods and GE, but soil bulk density in MG was significantly higher than that in CG (P <0.05, Table 1). Soil pH decreased with the deepening of the soil layer in all methods; the mean pH of MG was significantly higher than that observed in the other methods (P <0.05). The SOC of GE was higher than that of all grazing methods in each soil layer, being significantly higher at 0–10 and 20–30 cm (P <0.05). Grazing methods decreased the mean SOC by 41.39%–50.17% compared to that obtained using GE, but there were no differences among the grazing methods (P >0.05, Table 1). Mean DOC values were not significantly different among GE, CG, and RG; however, they were significantly higher than that of MG (P <0.05). LOC obtained with grazing methods was higher than that associated with GE at each soil depth, and the mean LOC of CG was significantly higher than that of GE (P <0.05). Grazing significantly increased the mean TN in CG and RG, as compared to that in GE (P <0.05). TP was highest in the 20–30 cm soil depth, except for RG; grazing significantly increased TP in each soil layer, and there were

TABLE 1 Soil properties in 0-40 cm layers.

Soil layer (cm)	Treatments	Bulk density (g·cm^{-3})	pH	SOC (g·kg^{-1})	DOC (mg·l^{-1})	LOC (mg·kg^{-1}
0-10	CK	1.08±0.09Aa	6.87±0.19Aab	53.22±7.94Aa	32.96±1.15Aa	0.51±0.21Aa
	CG	1.18±0.06Aa	6.10±0.76Ab	26.87±2.74Ab	27.34±12.89Aa	0.87±0.04Aa
	MG	1.26±0.05Aa	7.90±0.09Aa	24.99±4.30Ab	18.50±1.99Aa	0.76±0.05Aa
	RG	1.15±0.08Aa	5.74±0.33bA	31.09±2.19Ab	33.02±3.70Aa	0.78±0.04Aa
10-20	CK	1.21±0.09Aab	6.06±0.44Ab	29.35±4.11Ba	17.09±0.40Aa	0.56±0.18Aa
	CG	1.08±0.03Ab	5.94±0.91Ab	15.78±5.85ABa	12.71±1.33Aab	0.86±0.05Aa
	MG	1.38±0.08Aa	7.87±0.13Aa	17.11±5.08Aa	10.45±1.06Bb	0.82±0.08Aa
	RG	1.28±0.05Aab	5.23±0.11ABb	19.15±1.15Ba	15.32±2.15Aa	0.80±0.04Aa
20-30	CK	1.16±0.11Aa	5.91±0.58Ab	23.79±4.17Ba	13.77±0.90Aa	0.43±0.21Ab
	CG	1.04±0.04Aa	5.90±0.94Ab	9.82±0.96Bb	35.03±17.49Aa	0.82±0.03Aa
	MG	1.27±0.09Aa	7.77±0.07Aa	16.21±4.97Aab	9.33±0.44Ba	0.79±0.06Aa
	RG	1.15±0.15Aa	4.90±0.12Bb	13.78±3.86Bab	12.72±2.77Ba	0.71±0.03Aab
30-40	CK	1.11±0.06Aa	5.69±0.56Ab	21.69±3.70Ba	22.17±8.05Aa	0.73±0.08Aa
	CG	1.13±0.04Aa	5.85±0.87Ab	11.32±3.87Ba	15.72±2.86Aa	0.84±0.04Aa
	MG	1.29±0.09Aa	7.70±0.12Aa	13.02±2.59Aa	10.57±1.19Ba	0.78±0.04Aa
	RG	1.07±0.13Aa	4.87±0.09Bb	11.02±3.56Ba	21.98±9.13ABa	0.70±0.01Aa
Average	CK	1.14±0.05ab	6.13±0.35b	32.01±1.44a	21.50±1.01a	0.56±0.15b
	CG	1.11±0.01b	5.95±0.87b	15.95±2.33b	22.70±4.05a	0.85±0.01a
	MG	1.30±0.03a	7.81±0.10a	17.83±3.83b	12.21±0.52b	0.79±0.03ab
	RG	1.16±0.09ab	5.18±0.15b	18.76±2.52b	20.76±2.50a	0.75±0.03ab

Note: Different capital letters indicate significant differences (P<0.05) between different soil layers for the same method; different lowercase letters indicate significant differences (P<0.05) between the same soil layers for different methods. Values are means ± SE.

significant differences among grazing methods: CG>RG>MG (P <0.05). Grazing significantly decreased the C/N and C/P ratios at each soil depth (P <0.05) along with the mean N/P ratio (P >0.05), but there were no significant effects among the grazing methods (Table 1).

3.3 Ecosystem stocks of SOC, TN, and affecting factors

SOC stocks at 0–10 cm were significantly higher than those at 10–40 cm soil depth (P <0.05, Fig. 2a). GE significantly increased SOC stock at 0–40 cm soil depth compared to that observed after implementing the grazing methods, with the maximum increase at 0–10 cm soil depth (P<0.05, Fig. 2a, c, and Fig. 3a). GE decreased the contribution of topsoil (0–10 cm) SOC stock to the total stock compared to that obtained using grazing methods, with contributions of 30.64% (GE), 43.95% (CG), 34.32% (MG), and 41.55% (RG) (Fig. 3a). GE significantly reduced soil N stocks from 20 to 40 cm, with the maximum decrease being observed at 10–20 cm (P <0.05, Fig. 2b, d, and Fig. 3b). GE increased the contribution of topsoil (0–10 cm) TN stock to the total stock with contributions of 47.89% (GE), 37.81% (CG), 34.61% (MG), and 31.11% (RG) (Fig. 3b). Both SOC and TN stocks did not significantly change among grazing methods; however, SOC stocks in RG and MG increased by 20.93% and 27.27%, respectively, and TN stocks in RG increased by 9.06% compared to those observed with CG (Fig. 2c, d).

GE significantly increased the ecosystem organic carbon stocks (EOCs) and decreased ecosystem total nitrogen stocks (ETNs) (P <0.05) (Table 2). There were no significant changes among the results of different grazing methods (P >0.05); however, the EOCs increased by 22.29% (MG) and 16.31% (RG), while ETNs increased by 7.76% (RG) compared to those observed with CG (Table 2). MG and RG significantly increased the contribution of SOC and decreased the contribution of plant carbon to EOCs compared with GE and CG (P <0.05). Implementation of grazing methods significantly increased the contribution of soil N stock and decreased the contribution of plant N stock to ETNs when compared to the contributions associated with GE, and there were significant differences between MG, RG, and CG (P <0.05, Table 2).

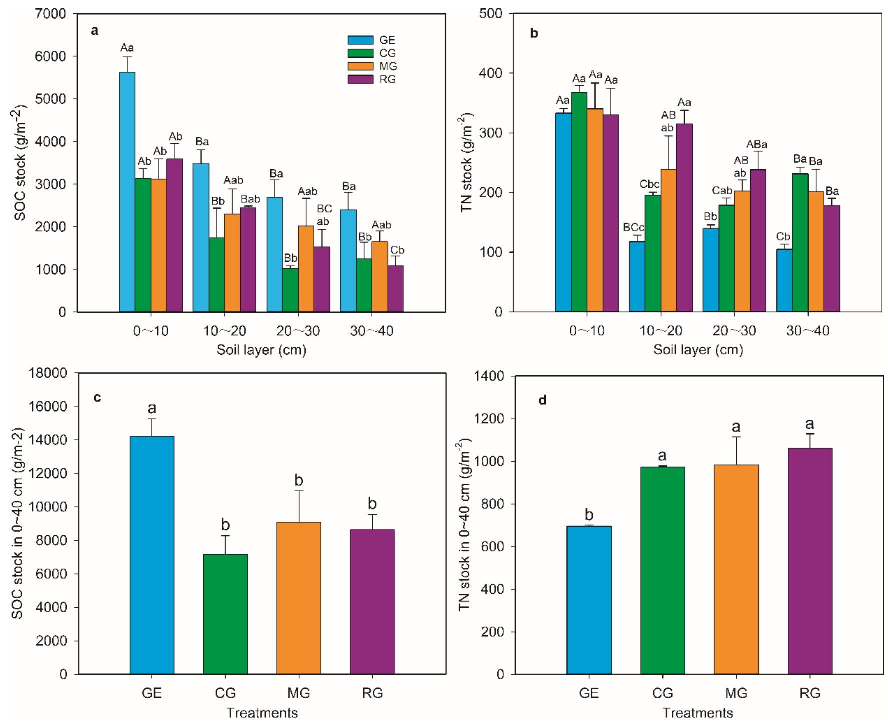


FIGURE 2 Soil organic carbon (a, c) and nitrogen (b, d) stocks in 0–40 cm soil layers.

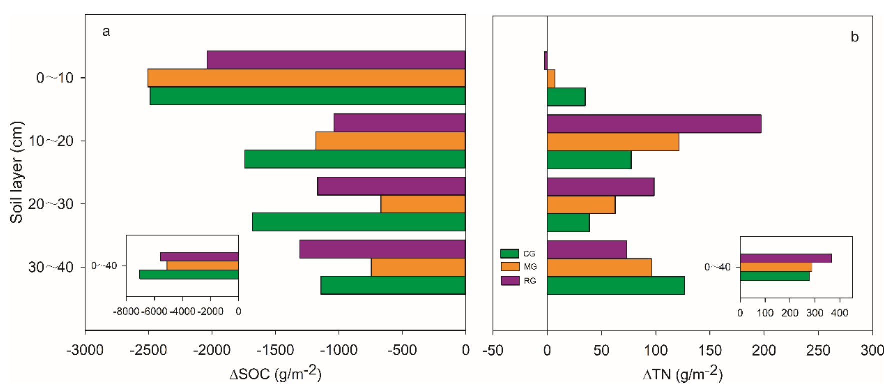
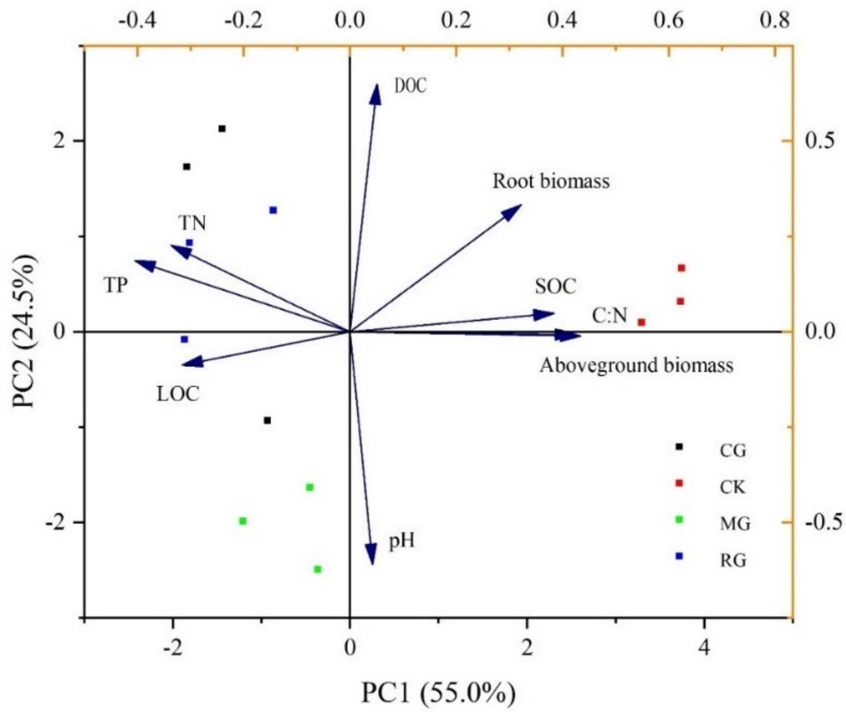


FIGURE 3 Relative changes in soil organic carbon (a) and nitrogen (b) stocks compared to those observed after grazing exclusion.



PCA analysis indicated that PC1 and PC2 together accounted for 79.5% of the variations in soil properties, while their individual contributions were 55.0% and 24.5%, respectively (Fig. 4). There were significant positive correlations of aboveground and belowground biomass, SOC, and C/N and negative correlations of TN, TP, and LOC with PC1, while pH and DOC were significantly correlated with PC2 (Fig. 4). EOSs were positively correlated with SOC, C/N, C/P, N/P, and aboveground biomass and negatively correlated with TP; ETNs were positively correlated with TN and TP and negatively correlated with C/N, C/P, aboveground biomass, and root biomass (P <0.05, Fig. 5).

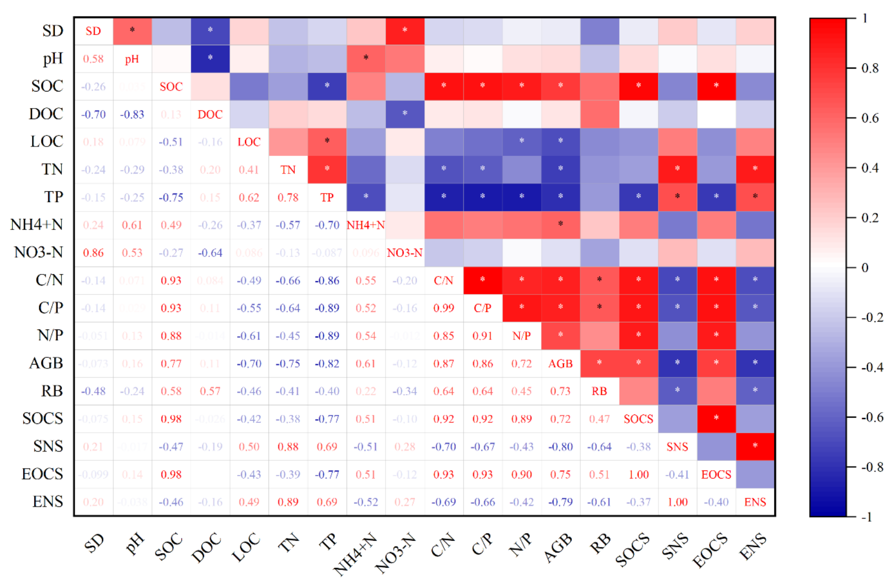


FIGURE 4 Principal component analysis of soil properties.

FIGURE 5 Pearson correlation coefficients among the soil properties, plant biomass, and organic carbon and TN stocks. Red represents positive correlations and blue represents negative correlations. Significance differences are as follows: *** P < 0.001, ** P < 0.01, * P < 0.05. SD, sol bulk density; SOC, soil organic carbon; DOC, dissolved organic carbon; LOC, labile organic carbon; TN, soil total nitrogen; TP, total phosphorus; C/N, SOC to TN ratio; C:P, SOC to TP ratio; N:P, TN to TP ratio; AGB, aboveground biomass; RB, root biomass; R/S, root to shoot ratio; SOCs, soil organic carbon stocks; STNs, soil total nitrogen stocks; EOCs, ecosystem organic carbon stocks; ENs, ecosystem total nitrogen stocks.

TABLE 2 Ecosystem organic carbon and nitrogen stocks and proportion of soil and biomass (%).

Methods	Ecosystem stocks (g·m^{-2})	Ecosystem stocks (g·m^{-2})	EOC stock (%)	EOC stock (%)	EOC stock (%)	ET
	EOC	ETN	Soil	Aboveground	Root	So
GE	14834.24±1048.41a	723.42±3.21b	95.67b	0.79a	3.55a	96
CG	7586.27±1111.83b	992.29±9.95a	93.97b	0.76a	5.27a	98
MG	9277.47±1865.00b	990.58±132,51a	97.90a	0.73a	1.37b	99
RG	8823.39±906.51b	1069.28±67.25a	97.98a	0.53a	1.49b	99

Note: Different letters imply significant differences between methods ($P < 0.05$).

4. Discussion

4.1 Effect of grassland managements on plant biomass

In this study, both aboveground and root biomass were significantly increased after 17 years of GE, which is in agreement with many other studies conducted in alpine meadows (Xiong, Shi, Sun, Wu, & Zhang, 2014) and semiarid grasslands of China (An & Li, 2015; Yu, Sun, & Huang, 2021), highland grasslands of Argentina (Vaieretti et al., 2021), semi-arid grasslands of South Africa (Snyman, 2005), degraded loess farmlands of Israel (Leu, Ben-Eli, & Mor-Mussery, 2021), and temperate grasslands of India (Husain, Geelani, & Bhat, 2021). GE can relieve livestock damage to grassland vegetation, increase vegetation height and cover, alter plant functional groups, and improve photosynthetic material partitioning between aboveground and belowground biomass (Lei Deng, Zhang, & Shangguan, 2014; Gao et al., 2008; Xiong et al., 2014), thus promoting increased productivity of grassland vegetation. Moreover, GE improves soil textures, such as soil water content, bulk density, and erosion (Lei Deng et al., 2014; Feyisa et al., 2017; Y. Li et al., 2012), Yu et al. (2021) found that the increases in AGB and litter were driven by the direct effects of GE and its indirect effects mediated by soil water content. However, few studies have also reported that GE had no effect on total biomass in alpine grasslands (Lu et al., 2015), belowground biomass in desert steppe (Niu et al., 2011), and cause a slight decrease in the aboveground biomass in temperate grasslands (Bork et al., 2019). The mechanism of these differences is not well understood; they may however, occur partly because of the variation in fencing years (Y. Li et al., 2012). It has been shown that aboveground biomass and root biomass tend to increase with increasing years (7, 12, and 25 years) of enclosure (Y. Li et al., 2012), and that grassland species richness index, diversity index, and plant cover reach a maximum after 20 years of GE (Ghorbani et al., 2021).

Grazing has a highly complex effect on plant growth because plant characteristics such as life history, plant height, and life type are significantly responsive to grazing on a global scale (Díaz et al., 2007). Overall, there are direct effects of trampling and feeding on plant leaves and stems and indirect effects of returning livestock manure into the soil, thus interfering with the synthesis and supply of carbohydrates and the accumulation of nutrients, which in turn affect grassland productivity (Dai, Fu, et al., 2021; Snyman, 2005; Wu, Wang, & Sun, 2021). In the present study, all the grazing methods significantly decreased both aboveground and root biomass, which is in contrast to GE. This finding is consistent with the majority of previous studies conducted across grasslands worldwide (Díaz et al., 2007; Yan et al., 2020). Despite the decreased plant biomass observed after implementing all grazing methods, we found that the aboveground biomass was similar for the three grazing methods; however, the root biomass of CG was significantly higher than that of MG and RG. This may partly be because compensatory plant growth depends on the net effect between promotion and inhibition, which is closely related to the grazing method (Dai et al., 2019). Plant growth in CG may not fully compensate for the biomass because the aboveground parts are eaten and trampled consecutively (Schönbach et al., 2011). We found that the root/shoot ratio of CG was significantly higher than that of MG and RG, and a previous study reported that the root/shoot ratio of plants increased significantly with increasing grazing pressure (L. Deng, Sweeney, & Shangguan, 2014; Yan et al., 2020). An increase in the root/shoot ratio implies an increase in the uptake of nutrient elements from the soil (Kiær, Weisbach, & Weiner, 2013). Plants need to absorb more nutrients and water to resume growth of aboveground parts under long-term sustained grazing pressure, and therefore allocate photosynthetic products to the roots for storage and utilisation as much as possible (Bai et al., 2015; Dong, Wu, Zhu, & Shi, 2014; Hafner et al., 2012), leading to an increase in belowground biomass and root/shoot ratio in CG (J. Sun et al., 2014).

4.2 Effect of grassland managements on carbon and nitrogen stocks

The grazing regime alters ecosystem carbon and nitrogen cycles through livestock feeding, trampling, and manure return in grassland ecosystems (Tserang Donko Mipam et al., 2021; G. Zhou et al., 2017). We found that GE significantly increased soil organic carbon storage in this study, which is consistent with other studies in alpine meadows (Xiong et al., 2014), desert steppe (Niu et al., 2011), and arid and semiarid grasslands (Yu et al., 2021). A meta-analysis concluded that GE mostly increased the soil C pool from 78 study sites in the Tibetan alpine grassland (Yu, Chen, Sun, & Huang, 2019) and 164 sites across grasslands worldwide (Abdalla et al., 2018). This positive effect may result from the following mechanisms. First, we found that GE significantly increased the aboveground and root biomass (Fig. 1). A study (Xiong et al., 2014) indicated that the soil organic carbon and nitrogen stocks of fencing grasslands were positively correlated with plant root biomass. Positive feedback may exist between soil organic carbon and plant biomass (Dai, Guo, et al., 2021), because most soil organic carbon comes from root exudates and litter decomposition (Kaiser, 2000; Xiong et al., 2014). Fencing-induced increases in root, litter, and aboveground biomass subsequently increase organic carbon input and promote the contribution of root-derived C to SOC by increasing root carbon content (Su & Xu, 2021; Yang, Wang, & An, 2021), whereas, grazing decreases the organic matter in the soil by consumption of the plant. This was supported by a significant positive correlation between plant biomass and SOC stocks (Fig. 5). Second, soil stoichiometry plays a critical role in the regulation of grassland nutrient cycles (Chen, Wang, & Baoyin, 2021). A previous study indicated that GE promoted nitrogen release from roots and litter, thereby increasing organic carbon (Yu et al., 2019). We found that the C/N ratio was significantly higher after years of GE than that observed after different grazing methods (Table 1), suggesting that the grassland may have nitrogen restrictions without exogenous N input after years of fencing, while a lower C/N ratio of the grazing methods can promote the decomposition rate of soil microorganisms and thus reduce SOC (Yang et al., 2021). Additionally, GE could facilitate soil moisture and temperature and decrease soil degradation by increasing plant cover and preventing livestock trampling, respectively, which in turn stimulates soil microbial biomass C and plant growth and reduces soil carbon leaching (Feyisa et al., 2017; Niu et al., 2011; Xiong et al., 2014). Furthermore, grazing causes a decrease in palatable species, which have faster litter decomposition and nutrient release than unpalatable species, leading to a lower SOC content than GE (Dai, Fu, et al., 2021). However, few studies have found that GE decreases SOC accumulation and storage in subtropical (Wilson, Strickland, Hutchings, Bianchi, & Flory, 2018), semiarid (Chen et al., 2021), alpine grasslands (Wu et al., 2021) and causes no variation in SOC of alpine meadows (Yuan & Jiang, 2021). These different conclusions may be due to variations in belowground allocation, such as root biomass, fine root exudates, and microbial biomass (Wu et al., 2021).

Contrary to SOC stocks, GE significantly decreased soil TN stocks compared to grazing methods in the present study (Fig. 2b), indicating that grazing improved the soil N content in this region. This result is in agreement with other studies (Contosta et al., 2021; Wu et al., 2021; Y. Zhou et al., 2020) and a meta-analysis showing that grazing led to a decrease in SOC but an increase in TN stocks at a global scale (Abdalla et al., 2018). Grazing results in higher levels of plant N, efficient use of N uptake, and accelerated N release from roots and plankton, leading to a significant increase in TN during grazing (Dai, Fu, et al., 2021; Yu et al., 2019). A study by Zhu, Liu, Wang, Sun, & Han (2021) found that grazing disturbances could change the activities of nitrogen assimilation-related enzymes which are beneficial for nitrogen assimilation by grassland plants. On the contrary, grazing could increase soil N content by returning excreta from livestock into the soil, as urine and faeces contain a large amount of available N for uptake (Oenema, Oudendag, & Velthof, 2007). Meanwhile, the grazing methods were implemented using 60 kg·ha^{-1} urea and 300 kg·ha^{-1} of superphosphate in late June and mid-to-late October, respectively; this suggests that the grazing grassland contains sufficient nitrogen but lacks C due to plant intake when compared to grassland subjected to GE, which resulted in a lower C/N ratio and in turn inhibited the decomposition of soil organic matter for nitrogen utilisation (Kumar, Kundu, Ghorai, Mitra, & Singh, 2018). We found that soil TN stock was significantly negatively correlated with the C/N ratio (Fig. 4). Furthermore, soil N content may depend on stocking rates because soil N sequestration potential tends to decrease with increasing stocking rates, which is beneficial for soil N sequestration; however, heavy grazing leads to lower N sequestration potential and N loss (Y. Li et al., 2012). The stocking rate in this study was 10 sheep units / hm^2. However, some previous studies have reported that GE increased SOC stocks and TN stocks in alpine meadows (W. Li et al., 2011; Xiong et al., 2014). The meta-analysis of 32 studies reported that grazing significantly decreased both SOC (-20%) and TN (-15%) in alpine grasslands (Yan et al., 2020). As very few meta-analyses have been conducted on the responses of soil SOC and TN stocks to grazing at regional and global scales, the effects and related mechanisms have not been consistently determined due to the complicated effects of grazing on soil-plant systems depending on the regional climate (precipitation gradient, mean annual temperature), altitude, soil depth, stocking rate, fencing years, and preceding grazer type to some degree (Abdalla et al., 2018; Su & Xu, 2021; Yan et al., 2020; G. Zhou et al., 2017). For instance, grassland SOC was enhanced by mean annual temperature, but stocking rates had little effect, regardless of the mean annual temperature (Throop, Munson, Hornslein, & McClaran, 2022).

4.3 Implications for management practices

Ecosystem organic carbon and nitrogen stocks in grasslands are determined by soil, plants, and their interactions. Although there was a decrease in ecosystem nitrogen stock, GE significantly increased the ecosystem organic carbon stock and improved its distribution in the aboveground soil and roots and enhanced plant productivity, as compared to those observed after implementing the grazing methods in this region (Table 2). This suggests that long-term GE is an effective way to improve ecological health and resilience in karst grasslands, regardless of livestock grazing. Although the ecosystem organic carbon and nitrogen stocks were not significantly different among grazing methods, we found that the EOCs and ETNs of MG and RG increased by 16.31% and 7.76%, respectively, while MG increased the EOCs by 22.29% and slightly decreased the ETNs compared with CG (Table 2), indicating that grazing could partly improve the ecosystem C and N stocks. Similar results were found in other studies (Chen et al., 2021; Wen Li et al., 2017). RG can substantially increase livestock stocking levels while improving vegetation, soil carbon, and water infiltration functions (Mosier et al., 2021). According to the moderate disturbance hypothesis, reasonable grazing or mowing causes an increase in grassland productivity and improve soil physicochemical properties, thus maintaining a continuous supply capacity of nutrients and enabling the ecosystem to stay in a healthy and stable condition (T. D. Mipam, Zhong, Liu, Miehe, & Tian, 2019; G. Zhou et al., 2017). Previous studies also found that RG increased SOC and TN stocks (0-1 m) by 13% and 9%, respectively, compared with continuous grazing (Mosier et al., 2021). Mowing significantly increased the SOC and TN stocks in the topsoil layer (0–30 cm) (Chen et al., 2021). Overall, GE is a viable approach to significantly improve vegetation resilience and soil carbon storage, whereas RG should be considered under the condition of pasturing utilisation in karst grasslands (regardless of GE).

5. Conclusion

GE significantly increased the aboveground biomass and root biomass compared to that observed after implementing different grazing methods. Meanwhile, the R/S ratio of GE was higher than that of MG and RG and lower than that of CG. The SOC content of GE was higher than that associated with grazing methods at soil depths of 0–40 cm, while being significantly higher at 0–10 and 20–30 cm; however, the TN content was lower than that observed after implementing the grazing methods, exhibiting a significant decrease when compared to that obtained with CG and RG. GE significantly increased the C/N and C/P ratios at each soil depth and N/P ratio in the topsoil layer. GE significantly increased the ecosystem organic carbon stocks (EOCs) and decreased the ecosystem total nitrogen stocks (ETNs), with the EOCs being 22.29% (MG) and 16.31% (RG) higher and the ETNs being 7.76% (RG) higher than those obtained with CG. There was a significant positive relationship between EOSs and SOC, C/N, C/P, N/P, and aboveground biomass, and a negative relationship with TP; the ETNs were positively correlated with N and P and negatively correlated with C/N, C/P, aboveground biomass, and root biomass. These results indicate that GE can provide significant improvements in plant recovery and ecosystem organic carbon storage, whereas RG is beneficial for promoting both EOCs and ETNs in karst grasslands.

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Conflict interest

The authors declare that there are no conflict of interests.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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