Grazing decreases net ecosystem carbon exchange by decreasing shrub and semi-shrub biomass in a desert steppe

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Abstract

Livestock grazing can strongly determine how grasslands function and their role in carbon cycle. However, how ecosystem carbon exchange responds to grazing and the underlying mechanisms remain unclear. We measured ecosystem carbon fluxes to explore the changes in carbon exchange and their driving mechanisms in a 16-year long term experiment with different grazing intensities in a desert steppe grassland. We found that grazing intensity influenced above- and belowground biomass during the peak growing season, primarily by decreasing shrubs and semi-shrubs and perennial forbs. Furthermore, alter patterns of net ecosystem exchange primarily via their negative influence on the biomass of shrub and semi-shrub. In addition, grazing-induced reduction belowground biomass, as well as in total plant nitrogen and soil ammonium nitrogen, can strongly influence ecosystem carbon exchange and soil respiration. When nitrogen is lost from the soil due to grazing, plants reallocate resources belowground to maintain growth and development, thus promoting photosynthesis and respiration. Our study indicates that soil available nitrogen and shrubs and semi-shrubs are important factors in regulating ecosystem carbon exchange under grazing disturbance in the desert steppe, which provide a basis for grazing management.

Running title: Grazing effects on ecosystem carbon exchange

Grazing decreases net ecosystem carbon exchange by decreasing shrub and semi-shrub biomass in a desert steppe

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Abstract

Livestock grazing can strongly determine how grasslands function and their role in carbon cycle. However, how ecosystem carbon exchange responds to grazing and the underlying mechanisms remain unclear. We measured ecosystem carbon fluxes to explore the changes in carbon exchange and their driving mechanisms in a 16-year long term experiment with different grazing intensities in a desert steppe grassland. We found that grazing intensity influenced above- and belowground biomass during the peak growing season, primarily by decreasing shrubs and semi-shrubs and perennial forbs. Furthermore, alter patterns of net ecosystem exchange primarily via their negative influence on the biomass of shrub and semi-shrub. In addition, grazing-induced reduction belowground biomass, as well as in total plant nitrogen and soil ammonium nitrogen, can strongly influence ecosystem carbon exchange and soil respiration. When nitrogen is lost from the soil due to grazing, plants reallocate resources belowground to maintain growth and development, thus promoting photosynthesis and respiration. Our study indicates that soil available nitrogen and shrubs and semi-shrubs are important factors in regulating ecosystem carbon exchange under grazing disturbance in the desert steppe, which provide a basis for grazing management.

Keywords Grazing intensity, Net ecosystem carbon exchange, Ecosystem respiration, Soil respiration, *Stipa breviflora* desert steppe

Introduction

Grassland ecosystems cover a large proportion of the arid and semi-arid regions of the world, playing an important role in the global carbon cycle (Scurlock and Hall 1998; Schuman *et al.* 2002; Zhou *et al.* 2019; Lei *et al.* 2020). The degree to which terrestrial ecosystems serve as net carbon sinks or sources depends on the balance between the carbon fixed by plant photosynthesis and the carbon released into the atmosphere by plant and soil respiration (Peng *et al* . 2014; Li *et al.* 2017b; Jin *et al.* 2023). While numerous evidence has addressed the degree to which forested systems serve as net carbon sinks or sources (Martens *et al.* 2004; Rebane *et al.* 2020), the status of grassland ecosystems as sources or sinks can be highly variable (Dai *et al.* 2014; Smith 2014; Chang *et al.* 2021). Grasslands can be an important carbon sink in some places or times (Hafner *et al.* 2012; Sha *et al.* 2020), but in other places and times, grassland carbon fluxes can be in equilibrium (neither a source or sink) (Hao *et al.* 2017), a net carbon source (Kuzyakov and Gavrichkova 2010), or fluctuate between states (Dai*et al.* 2014; Zhang *et al.* 2020).

Livestock grazing is a major land-use category by which human activities can influence the structure and function of grassland ecosystems, profoundly altering the carbon cycle and stability of grassland productivity (Zhang *et al.* 2023). Grazing directly affects plant productivity and respiration because livestock foraging removes leaves while promoting compensatory growth, and their trampling and excretion redistributes soil organic matter and alters soil respiration (Cao*et al.* 2004; Chen *et al.* 2015; Barthelemy *et al.*2018; Veldhuis *et al.* 2018); Grazing also alters soil nitrogen content and other processes important to the carbon cycle, such as litter decomposition and photosynthate distribution (Xia and Wan 2008). As a result, grazing can moderate the net ecosystem exchange of grasslands and whether they serve as a net carbon sink or source. In some cases, light to moderate levels of grazing can facilitate grasslands being net carbon sinks (Derner *et al.* 2006; Sha*et al.* 2020; Chang *et al.* 2021), while high levels of grazing can accelerate the release of carbon and switch the ecosystem from a carbon sink to a carbon source (Liang *et al.* 2017; Tang*et al.* 2018); In other cases, grazing appears to have little influence on the carbon budget of grassland ecosystems (Fang *et al.* 2010; Piñeiro *et al.* 2010).

Desert steppe is particularly vulnerable to degradation due to livestock grazing and to carbon sinks transitioning to carbon sources (Zhang*et al.* 2020). We assessed ecosystem carbon balances over a sustained 10-year period and explored the influencing factors. We concluded that precipitation patterns and grazing combine to cause changes in the carbon sink function of grasslands (Jin *et al.*2023; Wang *et al.* 2023), but that ecosystem carbon exchange is disturbed by a combination of environmental (soil, climate) and biological (grazing) factors. How environmental and biological factors influence net ecosystem carbon exchange depends on the relationship between carbon uptake via primary productivity and carbon release via plant and soil respiration. Furthermore, there is considerable variability and uncertainty regarding the factors influencing carbon exchange in grassland ecosystems (Liu *et al.* 2015; Sha *et al.* 2020). This is likely because the variability in grassland types is mediated by climate, vegetation and soil (Helfter *et al.* 2015; Hussain *et al.* 2015; Liang *et al.* 2020), as well as by grazing practices (Fang *et al.* 2010; Dai *et al.* 2014).

Thus, simply measuring net ecosystem exchange and aboveground biomass is not enough to fully understand the influence of biotic and abiotic factors on these rates (Li *et al.* 2017a; Bajgain *et al.*2018). It is necessary to more fully identify how carbon exchange and soil respiration are influenced by grazing and background environmental factors, in particular. This information will not only help us better understand the factors influencing the carbon dynamics of these important ecosystems, but it will also help inform the formulation of policies for the sustainable management and conservation of grassland resources. In this study, we measured ecosystem carbon fluxes and their associations in respond to a long-term (16-year) grazer manipulation experiment in a desert steppe grassland in Inner Mongolia, China. We specifically asked (1) how does grazing influence features of the plant community and soil conditions and (2) how those effects influence the parameters of net ecosystem carbon exchange, including gross ecosystem productivity and respiration. On the basis of our previous research, we further measured aboveground and belowground biomass, plant nutrients (carbon and nitrogen content of plant communities) and soil nutrient indexes to analyze the main drivers that influence the changes of CO_2 fluxes in desert steppe ecosystems and their responses to grazing disturbances, and to provide theoretical basis for the adaptive management of desert steppe.

Methods

Study Site

Our study took place within a long-term grazing experiment located in Siziwang Banner (41°46'43 "N, 111°53'42" E, elevation 1456 m) at the comprehensive experiment and demonstration center of the Inner Mongolia Academy of Agriculture and Animal Husbandry Sciences, China. The study site is a typical desert steppe ecosystem dominated by *Stipa breviflora* Griseb., *Artemisia frigida* Willd, and*Cleistogenes songorica* (Roshev.) Ohwi. Subordinate species include *Convolvulus ammannii* Desr., *Kochia prostrata* (L.) Schrad., *Caragana stenophylla* Pojark. and *Caragana microphylla* Lam.. The soil is primarily a sandy loam texture with low nitrogen, phosphorus, and organic matter content, but high potassium. Over the course of the experiment (2004 to 2020), the average annual temperature was 3.4 and the average annual precipitation was 221.7 mm (the majority falling from June to August). We present the air temperature and precipitation during the growing season in which we collected data (2020) in Fig. S1.

Experimental Design

A grazing manipulation experiment was established in June 2004 in a natural grassland (˜50 ha) with relatively flat terrain and relatively homogeneous vegetation and soil types. The plots were divided into three experimental blocks which each received one of four grazing treatments, control (no grazing), light grazing, medium grazing and heavy grazing (thus, there were three replicates for each treatment). Each experimental plot was 4.4 ha and constructed with iron wire fencing material. The stocking rates in each treatment were 0 (control/ no grazing), 0.91 (light grazing), 1.82 (moderate grazing, MG) and 2.71 (heavy grazing) sheep unit * $(hm^2A^{-1})^{-1}$. Each grazing plot was grazed by adult sheep from June 1 to October 1 each year. During the grazing season, the sheep were driven into the grazing area at 6:00 every day and left to forage freely until their return to the corral at 18:00.

Measurement of aboveground biomass and belowground biomass

We measured aboveground biomass of plants from June to September 2020. In each month, we randomly selected three (1 m^2) quadrats (108 quadrats in total) near the other sampling locations in each plot to record the community characteristics of plants. In each quadrat, we clipped all aboveground biomass and separated them to species. We then dried plants at 65 for 48 h and weighed them. We categorized species into four functional groups (perennial grass, shrub and semi-shrub, perennial forb, annual and biennial plants) based on their life type (Table S1).

We measured belowground biomass in August 2020. To do so, we selected six points near the other sampling locations and collected samples from the 0-10 cm layer with a root auger (7 cm diameter). We took two samples at each point and combined them for analyses. We picked roots from the soil, washed them and dried and weighed them as above.

Measurement of plant total nitrogen and carbon content

We measured total carbon and total nitrogen content from three of the aboveground sampling quadrats in each plot. After weighing, we ground tissues using a ball mill and measured powder samples using an elemental analyzer (Elementar Vario MACRO CUBE).

Measurement of soil properties

We determined several soil physical and chemical properties in August 2020 by collecting soil samples from the 0-10 cm layer. We selected six points in each plot near the other sampling points and collected soil at each point from 0-10 cm using a soil auger (3 cm diameter). At each point, we collected two soil samples, combined them, and passed soil through a 2 mm sieve for determining the physical and chemical properties of the soil in the laboratory.

For each soil sample, we determined total carbon and total nitrogen content in the soil using an elemental analyzer (Elementar Vario MACRO CUBE); total phosphorus content using an ultraviolet spectrophotometer (UV-1800, Mapada, Shanghai, China) with the sodium hydroxide fusion method; organic carbon content using the potassium dichromate external heating method; nitrate (NO_3^--N) and ammonium ($NH4^+$-N) by extraction using KCl (2 mol*L^{-1}) with a flow analyzer; available phosphorus content using the sodium bicarbonate molybdenum antimony anti-colorimetric method; and microbial biomass carbon and microbial biomass nitrogen using the chloroform fumigation extraction method.

Measurement of ecosystem CO_2 exchange

We measured net ecosystem CO_2 exchange and ecosystem respiration monthly during the growing season (June to October) in 2020. To do so, we used a Li-6400 portable photosynthetic (Li-COR, USA) instrument with the static chamber method. We collected measurements between 8:00 a.m. to 12:00 p.m (Niu *et al.* 2008; Wu *et al.*2021). For measurements, we choose a clear, cloudless and windless day as much as possible, at least three days after a rainfall. For measurements we connected a leaf chamber (50 x 50 x 50cm^3 transparent plexiglass box) to the portable photosynthetic instrument and installed small fan in each diagonal direction at the upper end of the glass box so that gas was fully mixed. We place the glass box on one of three aluminum sink frames (50 x 50cm^2) placed randomly within each plot to ensure a smooth and airtight seal. We repeated measurements on each of the three frames.

At each sample point, we collected measurements for 120 s and CO_2 concentration and water exchange flux values were automatically recorded every 10 s. After these measurements, we ventilated the leaf chamber to ensure it was filled with convection-exchanged air, covered it with a black cloth to ensure no light transmission and repeated the above procedure to determine ecosystem respiration.

We measured soil respiration using an open circuit Li-8100 soil carbon flux meter (Li-COR, Inc, Lmcoln, NE, USA) at the same time as the net ecosystem exchange measurements. We measured soil respiration within three PVC rings (10.5 cm in diameter and 8 cm in height) that were randomly placed 2 cm above the ground surface in each plot. Prior to measurements, we clipped plants inside the rings flush with the ground and removed debris.

We calculated net ecosystem CO_2 exchange (NEE) and gross ecosystem productivity (GEP) as follows: [?]C' [?]t = INDEX(LINEST(Y1: Y12, A1: A12),1);$NEE = \frac{10VP(1-\frac{W}{1000})}{RS(T+273.15)}\frac{\partial C\prime}{\partial t}$; NEE = GEP - ER.

Where ER is the measured ecosystem respiration. NEE, ER, GEP are in µmol·m^{-2}·s^{-1}, Y1-Y12 is the CO_2 concentration value, A1-A1 is the measurement time, V represents the volume of the box (cm^3), P is the atmospheric pressure inside the box (kpa), W is the water pressure inside the chamber (mmol·mol^{-1}), S is the bottom area of the chamber (cm^2), T is the temperature of the gas inside the chamber (), and R=8.314 J-mol-1-K-1 (constant). We used values of ecosystem CO_2 exchange and soil respiration during the growing season (June-October) to calculated the values of NEE, GEP, ER, SR for each treatment.

Measurement of air temperature and precipitation

We collected meteorological data in 2020 using a small weather station (Gro Weather software version 1.2, Davis instruments corporation, USA). The station recorded temperature and precipitation data automatically at 1 h intervals, which we downloaded and collated at regular intervals.

Measurement of soil temperature and moisture

In parallel with net ecosystem exchange measurements, we measured soil temperature at 10 cm depth in the leaf chamber with two TP3001 electronic thermometers. At the same time, we collected 10 cm soil samples using a 2.5 cm diameter x 10 cm high soil auger, which we collected in an aluminum box, weighed and recorded the wet mass, and then dried at 105 for 24 h to weigh the dry mass and then calculate the mass water content.

Data Analysis

After ensuring data met normality and homogeneity of variance assumptions using the Shapiro-Wilk test, we evaluated the influence of grazing treatment on aboveground and belowground biomass, plant nitrogen and carbon content, plant functional groups and several soil chemical variables, as well as the ecosystem CO_2 exchange and soil respiration. To do so, we used repeated measures ANOVA to test the effects of grazing intensity and sampling month on the aboveground biomass, plant functional group biomass, ecosystem CO_2exchange and soil respiration. We used one-way ANOVA followed by a Duncan test for pairwise comparison to test the effects of grazing intensity on the belowground biomass, plant total carbon, plant total nitrogen and soil nutrient content. A $P < 0.05$ indicated significance in the treatment effects.

We correlated several abiotic factors with ecosystem carbon exchange, including temperature, precipitation, soil temperature, and soil moisture in each treatment using regression analysis.

To investigate the influence of soil and plant factors on ecosystem carbon exchange, we used redundancy analysis to rank the impact of the factors on carbon exchange. Furthermore, we used a generalized linear model (GLM) and structural equation model (SEM) to determine the effects of plant and soil factors on ecosystem CO_2 exchange and soil respiration. To do so, we first calculated the contribution of the plant and soil factors to the ecosystem CO_2 exchange and soil respiration using the GLM, and then we removed insignificant pathways and simplified the SEM model based on the GLM results. We obtained path coefficients using a maximum likelihood estimation technique.

We performed ANOVA, repeated measures ANOVA and the GLM analyses in version R 4.0.3. The SEM analyses were performed using the "piecewise SEM" package (Lefcheck, 2016) in R version 4.0.3. We performed regression and redundancy analyses in Origin 2023 software.

Results

Grazing Intensity Effects on the Plant Functional Group Productivity and Plant Community Carbon and Nitrogen Content

We found that both aboveground (Fig. 1a) and belowground (Fig. 1b) biomass was influenced by the grazing treatment. All grazing treatments had lower aboveground and belowground biomass than the control treatment with no grazing. Aboveground biomass was lowest in the heavy grazing treatment while there were no differences between the two lower grazing intensity treatments (Fig. 1a), belowground biomass was incrementally lower with each increased grazing intensity treatment (Fig. 1b). When we analyzed differences in nutrient content, we found that the total carbon content of the plant community was lowest in the heavy grazing treatment (Fig. 1c), while the total nitrogen content of the plant community was lowest in the moderate grazing treatment (Fig. 1d). Aboveground biomass differed significantly between months, though the difference was not significant for the interaction between month and grazing intensity (Table 1). When we divided plants into functional groups (Fig. 2b-e), we found that most groups strongly declined with increasing grazing, particularly shrubs and semi-shrubs (Fig. 2c), as well as perennial forbs (Fig. 2d). Perennial grasses, in fact, had greater biomass in the moderate grazing treatment (Fig. 2b) We performed repeated measures ANOVAs for different plant functional groups and found that most functional groups differed significantly by month, grazing intensity, and the interaction between month and grazing intensity. This was especially true for the perennial forbs, shrubs and semi-shrubs. Annuals and biennials did not differ significantly by the interaction between month and grazing intensity, and perennial grasses did not significant significantly by month, grazing intensity or the interaction between month and grazing intensity (Table 1, Fig. 2b-e).

Grazing Intensity Effects on Soil Nutrients

Of the soil chemical variables, we found no differences in total carbon (Fig. 3a), total phosphorus (fig. 3c), organic carbon (Fig. 3d), and microbial biomass carbon (Fig. 3g) among grazing intensity treatments. However, we found that total nitrogen (Fig. 3b), ammonium nitrogen (Fig. 3e), microbial biomass nitrogen (Fig. 3i), and available phosphorus (Fig. 3g) content tended to be lower in the highest grazing intensity treatments.

Differences in ecosystem CO_2 exchange under different grazing intensities

During the 2020 growing season, we found that NEE, ER, GEP, and SR showed strong seasonal dynamics consistent with the monthly variation in precipitation (Fig. S2). According to the results of repeated measures ANOVA, NEE, ER, GEP, and SR varied significantly between months. NEE and GEP also varied significantly between grazing intensities and the interaction between month and grazing intensity, but ER and SR did not differ significantly between grazing intensities or the interaction between month and grazing intensity (Table 2). During July, NEE was positive indicating release as a carbon source (Fig. 4a); it was negative, indicating a carbon sink for the rest of the growing season. Both NEE (Fig. 4a) and GEP (Fig. 4c) were lowest in August, while ER (Fig. 4b) and SR (Fig. 4d) were highest in August. When we compared grazing treatments, we found that the rates of NEE (Fig. 4a), ER (Fig. 4b), GEP (Fig. 4c) and SR (Fig. 4d) were all significantly lower than the control plots, with the heavy grazing treatment often having lowest (or highest) values.

plant factors and soil factors on ecosystem CO_2 exchange

We used RDA model to examine the relationship between the explanatory variable (plant and soil factors, blue lines with arrows) and response variable (ecosystem carbon exchange and soil respiration, red lines with arrows) in Fig. 6. We found that plant factors (e.g., above and below ground biomass, plant carbon and nitrogen nutrients) explained 98.10% of the variance of ecosystem CO_2 exchange and soil respiration (Axis 1 explained 71.49 % of the total variance while Axis 2 explained 26.61%; Fig. 6a). Soil factors (e.g., Soil nutrient index) explained 98.20 % of the variance of ecosystem CO_2exchange and soil respiration (Axis 1 explained 73.50 % of the total variance while Axis 2 explained 24.70 %; Fig. 6b). For plant and soil factors, SS (R^2 = 0.36) contributed the highest degree of variance to NEE, and next highest was AGB (R^2= 0.21, Fig. 5c); AGB (R^2 = 0.28) contributed the highest degree of variance to GEP, and next highest was SS (R^2 = 0.22, Fig. 5E); BGB (R^2 = 0.25, R^2 = 0.23) contributed the highest degree of variance to ER and SR (Fig. 5d, Fig. 5f);

Based on the results of the redundancy and GLM analyses, we developed structural equation models to better explain the driving mechanisms of ecosystem carbon exchange and soil respiration. Our SEM analysis showed that grazing had a direct negative effect on NEE and GEP. Specifically, grazing reduced NEE and GEP by reducing aboveground biomass, especially through the indirect reduction of NEE due to lower shrub and semi-shrub biomass (Fig. 6a and c). However, the lower soil nutrient content in the grazing treatment was not associated with NEE and GEP (Fig. 6e and g). In contrast, grazing and aboveground biomass did not directly affect ER and SR (Fig. 6b and d), but they did directly affect belowground biomass and indirectly reduce belowground biomass by decreasing ammonium N. This came to affect the rate of SR as well (Fig. 7f and h).

Discussion

Grazing by livestock influences the productivity and stability of grassland ecosystems, which in turn generates feedback mechanisms on the carbon cycle. However, the factors underlying the complex changes of vegetation and soil as a result of grazing and their impact on the processes underlying carbon cycling remain poorly understood (Yuan*et al.* 2011; Hussain *et al.* 2015; Oram *et al.* 2023; Zaret *et al.* 2023). Our results from a 16-year long grazing intensity manipulation study in a desert steppe grassland show that grazers alter patterns of net ecosystem exchange primarily via their negative influence on the biomass of shrub and semi-shrub, which play a prominent role in ecosystem functions.

Effect of grazing intensity on net ecosystem CO_2exchange

Grazing can shift the balance between vegetation being a carbon source or sink in steppe grasslands (de la Motte *et al.* 2018; Ondier*et al.* 2021). Our finding that NEE, ER, and GEP decreased with increasing grazing intensity is consistent with previous results from desert steppe (Jin *et al.* 2023; Wang *et al.* 2023). Our finding that heavy grazing had a stronger inhibitory effect on ER than GEP is consistent with the results of Peng et al. (2007). This may have resulted because livestock feeding reduces the aboveground biomass which cannot be compensated by regrowth (Zhang *et al.* 2018; Zhang*et al.* 2023), such that the effective amount of leaf area available for both photosynthesis and respiration is reduced so that the net CO_2 exchange rate decreases (Oba *et al.* 2000; Shi *et al.* 2022).

Although our finding of the positive correlation between productivity and NEE is consistent with many previous studies (Danielewska *et al.* 2015; Xu *et al.* 2022), we also found a positive correlation between aboveground biomass and NEE, primarily driven by shrubs and semi-shrubs and perennial forbs, which is consistent with previous work (Zhang *et al.* 2023). Our finding that shrubs and semi-shrubs and perennial forbs were strongly influenced by grazing, while grasses were less so is consistent with the idea that shrubs and semi-shrubs and perennial forbs are more palatable and have higher nutritional value than grasses, mainly *stipa breviflora* , which are not preferred by livestock. Shrub roots can reach up to 70 cm deep into the soil layer, allowing them to better utilize deeper water and nutrients to maintain a high carbon fixation capacity and a high net carbon uptake capacity (Niu*et al.* 2023). which can explain why their loss dramatically influenced NEE.

Our finding that plant N content is negatively correlated with net ecosystem CO_2 exchange is inconsistent with previous findings that loss of leaf N attenuates ecosystem carbon cycling (Wang et al. 2014 in Chinese)(Gong *et al.* 2021), This may be because altered plants allocated more N to non-photosynthetic proteins to increase their compensatory growth in response to grazing, but with reduced photosynthetic capacity (Onoda *et al.* 2004), resulting in a decrease in net CO_2 exchange rate (Zhang *et al.*2006). A study by Wu et al. (2021) showed that N addition in desert steppe increased the net CO_2 exchange rate, while You et al. (2016) showed that high levels of N addition inhibited NEE, but moderate levels promoted NEE. This suggests that the changes of nitrogen absorbed and used by plants are complex and require further investigation (Schimel *et al.* 2001).

Although NEE decreased in response to increasing grazing intensity, it is of interest that there was no significant difference in NEE rates between the LG and MG treatments in our study (Fig. 4a, bars), because although short-flowered needlegrass was a well-established species and widely distributed in our experimental sample plots, livestock did not prefer it, resulting in no significant difference in vegetation stock and cover between the LG and MGC treatments and the non-grazed areas, so their net CO_2 exchange rates were not significantly different from those of ck. The net CO_2 exchange rate was not significantly different from that of CK ($p > 0.05$, Fig. S1a).

Although NEE decreased in response to increasing grazing intensity, we found no difference in NEE rates between the light and moderate grazing treatments. This was likely because less preferred grasses dominated both treatments.

Effect of grazing intensity on soil resperation

Desert steppe is sparsely vegetated, so soil respiration is likewise an important determinant of carbon balance in the ecosystem. The rate of SR decreased with grazing intensity (Fig. 4d, bars), and belowground biomass (Fig. S1b), ammonium N (Fig. S2e), and available P (Fig. S2g) also significantly decreased ($p < 0.05$), but the effect of different grazing intensities on soil organic carbon was not significant ($p > 0.05$, Fig. S2d). In this study, belowground biomass, available P, and soil organic carbon were all significantly correlated with SR based on redundancy analysis. We further constructed structural equations and the results showed that grazing did not directly affect SR, but indirectly reduced the rate of SR by decreasing belowground biomass and ammonium N (Fig. 7b).

Belowground biomass is highly correlated with soil respiration (Pregitzer *et al.* 2008; Wu *et al.* 2016; Diao *et al.*2022). Higher CO_2 fluxes may be caused by higher root biomass, which can promote soil respiration by releasing more secretions at the inter-root level and providing a favorable environment for soil microbial respiration (Wu *et al.* 2016). In contrast, heavy grazing reduced above- and belowground biomass, thus reducing the amount of root growth, soil microbial load and soil enzyme activity, which likely led to the inhibition of microbial respiration and ultimately reduced soil respiration rate (Li *et al.* 2013).

In addition, based on the GLM and SEM analyses, we also found that soil ammonium N content correlated with respiration (Fig. 5d and f, Fig. 6f and h), which is consistent with the result that nitrogen addition can stimulate soil respiration in nutrient-poor soil (Smith, 2005). The affinity of dissolved oxygen and aeration tissue for NH_4^+ and NO_3^- in root respiration mainly depends on NH_4^+, which is enhanced when NH_4^+ is absorbed. The enhancement of glutamate dehydrogenase regulation and amino acid metabolic reactions increases root N use efficiency and promotes root growth (Knapp *et al.* 2017). Thus, the change in soil ammonium N content is one of the main factors influencing soil respiration (Onoda *et al.* 2004; LeBauer and Treseder, 2008; Gong *et al.* 2021).

Effects of climate variables on ecosystem carbon exchange and soil respiration

As expected, we found that NEE, ER and GEP were all influenced by precipitation which was highest in July and August (Fig. S1, Fig. S2b). This is likely primarily a result of the influence of aboveground biomass and its influence on productivity and ecosystem carbon exchange, which is strongly influenced by variation in rainfall (Jobbagy *et al.* 2002). We also showed that soil moisture was positively correlated with ER and SR (Fig. S2d), which likely promoted the growth of plant roots to enhance microbial activity and promote organic matter decomposition, leading to an increase soil respiration (Helfter *et al.* 2015; Peng *et al.* 2015). This is consistent with previous studies in desert steppe (Jin *et al.*2023; Wang *et al.* 2023).

Likewise, variation in temperature influences ecosystem carbon exchange mainly by affecting GEP and ER (Luo *et al.* 2001; Ganjurjav*et al.* 2018; Li *et al.* 2019). However, consistent with our results showing a minimal influence of temperature on ecosystem carbon exchange in a desert steppe (Fig. S2a), WU et al. (2021) found similar results in a 12-year study. We did, however, find that variation in soil temperature contributed to ER (Fig. S2c), which was consistent with results from a previous analysis.

Conclusions

In this study, we assessed the impact of different levels of grazing intensity, as well as the associated direct and indirect effects, on ecosystem carbon exchange and soil respiration. Over the course of the growing season, we found that the desert steppe remained in a state of carbon uptake (carbon sink) under the conditions of 16 years of continuous grazing. Our study shows that alter patterns of net ecosystem exchange primarily via their negative influence on the biomass of shrubs and semi-shrubs. In addition, grazing-induced reduction belowground biomass, as well as in total plant nitrogen and soil ammonium nitrogen, can strongly influence ecosystem carbon exchange and soil respiration. When nitrogen is lost from the soil due to grazing, plants reallocate resources belowground to maintain growth and development, thus promoting photosynthesis and respiration.

Author contributions

Xin Ju : Data curation (equal); formal analysis (lead); investigation (lead); methodology (lead); resources (equal); software (lead); visualization (lead); writing-original draft (lead); writing-review and editing (lead). **Bingying Wang** and**Xiaojia Zhang** : Data curation (Equal). **Qian Wu** : Conceptualization (lead); funding acquisition (lead); project administration (lead); supervision (lead); validation (lead); Writing-review & editing (Lead). **Guodong Han** : Conceptualization (lead); funding acquisition (lead); project administration (lead); supervision (lead); validation (lead).

Data availability statement

The data that support the findings of this study are available in the Supporting Information of this article.

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Table 1: Repeated-measures ANOVA for above-ground biomass and biomass of plant functional groups.

plant biomass	Month	Month	Grazing intensity	Grazing intensity	Month × Grazing intensity	Month × Grazin
	F value	*P* value	*F* value	*P* value	*F* value	*P* value
AGB($g \cdot m^{-2}$)	6.59	0.002	10.91	0.003	0.63	0.75
PG($g \cdot m^{-2}$)	1.78	0.18	1.77	0.24	0.53	0.83

plant biomass	Month	Month	Grazing intensity	Grazing intensity	Month × Grazing intensity	Month × Grazin
SS(g·m^{-2})	4.22	0.02	10.62	0.004	2.22	0.05
PF(g·m^{-2})	9.74	¡0.001	8.28	0.008	3.96	0.003
AB(g·m^{-2})	19.62	¡0.001	4.66	0.025	0.97	0.49

The F values are presented together with their levels of significance. AGB, PG, SS, PF and AB represent aboveground biomass, perennial grass biomass, shrub and semi-shrub biomass, perennial forb biomass and annual and biennial plant biomass.

Table 2: Repeated-measures ANOVA for ecosystem carbon fluxes and soil respiration.

ecosystem fluxes	Month	Month	Grazing intensity	Grazing intensity	Month × Grazing intensity	Month × G
	F value	P value	F value	P value	F value	P value
NEE(μmol·m-2·s-1)	1280.10	¡0.001	32.56	0.004	7.59	¡0.001
ER(μmol·m-2·s-1)	190.52	¡0.001	2.28	0.16	3.64	0.06
GEP(μmol·m-2·s-1)	1082.33	¡0.001	40.77	¡0.001	8.02	¡0.001
SR(μmol·m-2·s-1)	48.76	¡0.001	1.98	0.2	1.42	0.21

The F values are presented together with their levels of significance. NEE, ER, GEP and SR represent net exchange of ecosystem CO_2, ecosystem respiration, gross ecosystem productivity and soil respiration.

Figure legends

Figure 1: The effects of grazing intensity on plant aboveground biomass, belowground biomass, plant community carbon and nitrogen content. Different lowercase letters indicate significant differences between means at $p < 0.05$. Error bards are ± SE, Codes of different treatments are as follows: CK, control/no grazing; LG, light grazing; MG, moderate grazing; HG, heavy grazing.

Figure 2: The effects of grazing intensity and month on plant aboveground biomass and biomass of plant functional groups. Each panel represents a different grouping of plant biomass. Different lowercase letters indicate significant differences between means at $p< 0.05$. Error bards are ± SE, and the lines in panels b-e show the biomass of each plant functional group during the 2020 growing season. Codes of different treatments are as follows: CK, control/no grazing; LG, light grazing; MG, moderate grazing; HG, heavy grazing.

Figure 3: The effects of grazing intensity on soil nutrients. Different lowercase letters indicate significant differences between means at $p < 0.05$. Error bars are ± SE**.** Codes of different treatments are as follows: CK, control / no grazing; LG, light grazing; MG, moderate grazing; HG, heavy grazing.

Figure 4: Monthly dynamics of ecosystem fluxes**.** Panels show the mean value (±SE) of net exchange of ecosystem CO_2 (a, NEE), ecosystem respiration (b, ER), gross ecosystem productivity (c, GEP) and soil respiration (d, SR) in the growing season (June-October) of 2020. The inset reflects the differences between treatments in the 2020 growing season, where positive and negative values represent net carbon release and uptake by the ecosystem and do not indicate the magnitude of the values. Different lowercase letters indicate significant differences between treatments ($p < 0.05$), Codes of different treatments are the same as in Figure 3.

Figure 5: Biplot of ecosystem carbon exchange (NEE, ER, GEP, SR) from redundancy analysis (RDA) for plant factors (a) and soil factors (b). GLM analysis was used to study the contribution of the plant and soil factors to the net exchange of ecosystem CO_2 (c, NEE), ecosystem respiration (d, ER), gross ecosystem productivity (e, GEP) and soil respiration (f, SR). a-b, Ecosystem carbon exchange is represented as red lines with arrows; plant factors (a) and soil factors (b) are represented as blue lines with arrows.

The length of the line indicates the magnitude of the correlation between the explanatory variable and ecosystem carbon exchange. The angle between the lines indicates the correlation between the variables, and the angle between the red and blue arrows is less than 90° for positive correlations. Codes of different plant factors (a) are as follows: AGB, aboveground biomass; BGB, belowground biomass; PG, perennial grass biomass; AB, annual and biennial plant biomass; PF, perennial forb biomass; SS, shrub and semi-shrub biomass; PTC, plant total carbon; PTN, plant total nitrogen; C/N, the ratio of total plant carbon content to total plant nitrogen content. Codes of different soil factors (b) are as follows: TC, total carbon; TN, total nitrogen; TP, total phosphorus; SOC, organic carbon; AN, ammonium nitrogen; NN, nitrate nitrogen; AP, available phosphorus; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen. c-f, Importance of individual environmental variables across models for ecosystem carbon exchange is shown for each indicator as variable importance weighted by % of R^2.

Figure 6: Structural equation models (SEM) examining the standard total effects of plant and soil factors on Ecosystem carbon exchange under different grazing intensities. Boxes stand for measured variables in the model. Standardized path coefficients are given. Solid black lines represent positive paths ($p < 0.05$), solid red lines represent negative paths ($p < 0.05$), and dotted black arrows represent non-significant paths ($p >$ 0.05).

Supplementary Material

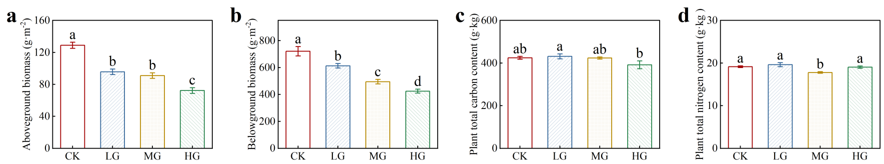
Table S1: Basic information on plant species and plant functional groups during 2020 at the study site.

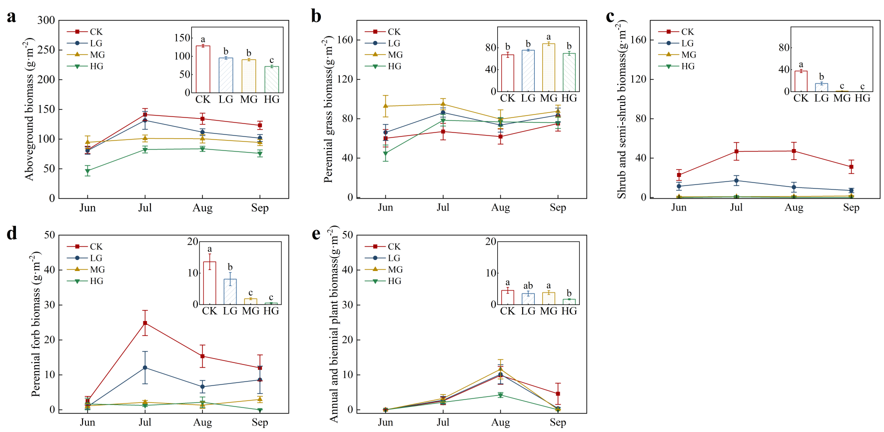
Latin name of species	**Plant functional groups**
Stipa breviflora Griseb.	perennial grass
Stipa krylovii Roshev.	perennial grass
Cleistogenes songorica (Roshev.) Ohwi.	perennial grass
Leymus chinensis (Trin.) Tzvel.	perennial grass
Cleistogenes squarrosa (Trin.) Keng.	perennial grass
Agropyron cristatum (L.) Gaertn.	perennial grass
Convolvulus ammannii Desr.	perennial forb
Allium tenuissimum L.	perennial forb
Allium mongolicum Regel.	perennial forb
Astragalus galactites Pall.	perennial forb
Lagochilus ilicifolius Bunge ex Benth.	perennial forb
Carex pediformis C. A. Mey.	perennial forb
Aster altaicus Willd.	perennial forb
Cymbaria daurica L.	perennial forb
Allium ramosum L.	perennial forb
Iris tenuifolia Pall.	perennial forb
Sibbaldianthe bifurca (L.) Kurtto & T. Erikss.	perennial forb
Artemisia frigida Willd.	subshrub
Caragana microphylla Lam.	shrub
Caragana stenophylla Pojark.	shrub
Bassia prostrata (L.) Beck.	subshrub
Kali collinum (Pall.) Akhani & Roalson.	annual and biennial plant
Neopallasia pectinata (Pall.) Poljak.	annual and biennial plant
Artemisia scoparia Waldst. et Kit.	annual and biennials plant
Chenopodium glaucum L.	annual and biennial plant
Teloxys aristata (L.) Moq.	annual and biennial plant
Euphorbia humifusa Willd.	annual and biennial plant
Lappula myosotis Moench.	annual and biennial plant

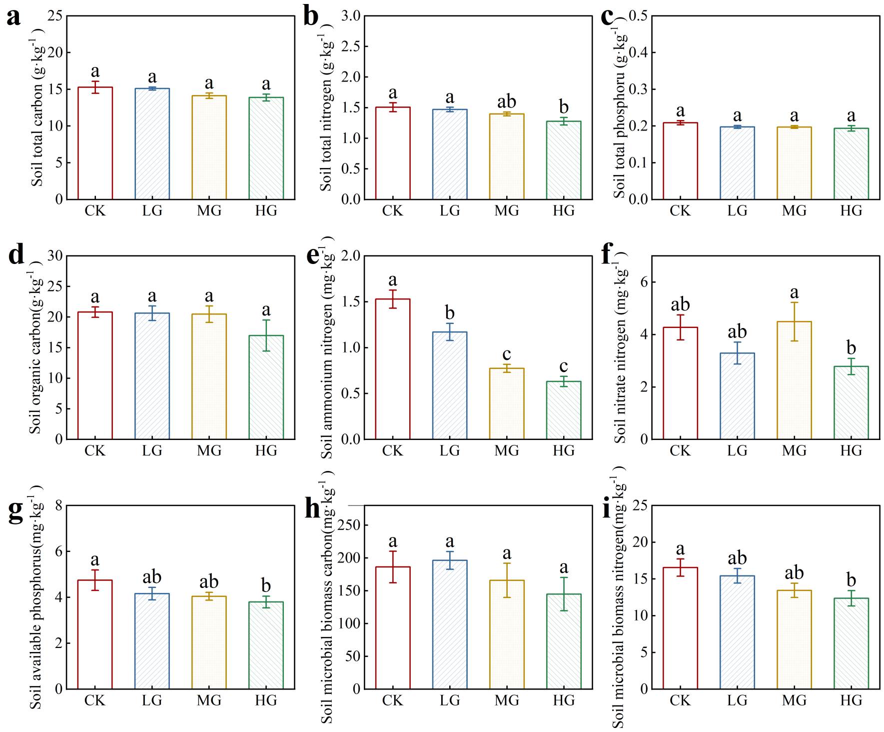
Figure S1: Daily mean air temperature (lines) and daily precipitation (bars) in the growing seasons in 2020.

Figure S2: Soil temperature (a) and soil moisture (b) at 10 cm soil depth under different grazing intensity in growing seasons (June - October 2020)

Figure S3: Correlations between air temperature (a), precipitation (b), soil temperature (c), soil moisture (d) and ecosystem CO_2 fluxes (NEE, ER, GEP, SR) in the growing season of 2020.



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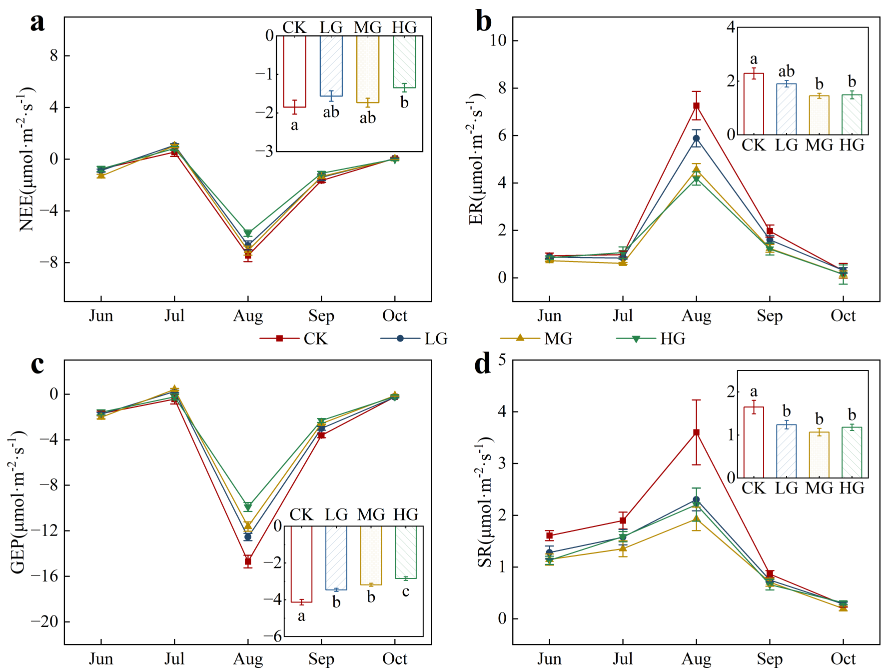
Soil total carbon (g·kg-1)
b
Soil total nitrogen (g·kg-1)
c
Soil total phosphoru (g·kg-1)
d
Soil organic carbon(g·kg-1)
e
Soil ammonium nitrogen (mg·kg-1)
f
Soil nitrate nitrogen (mg·kg-1)
g
Soil available phosphorus(mg·kg-1)
h
Soil microbial biomass carbon(mg·kg-1)
i
Soil microbial biomass nitrogen(mg·kg-1)
CK
LG
MG
HG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NEE(μmol·m-2·s-1)
b
ER(μmol·m-2·s-1)
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GEP(μmol·m-2·s-1)
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SR(μmol·m-2·s-1)
Jun
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CK
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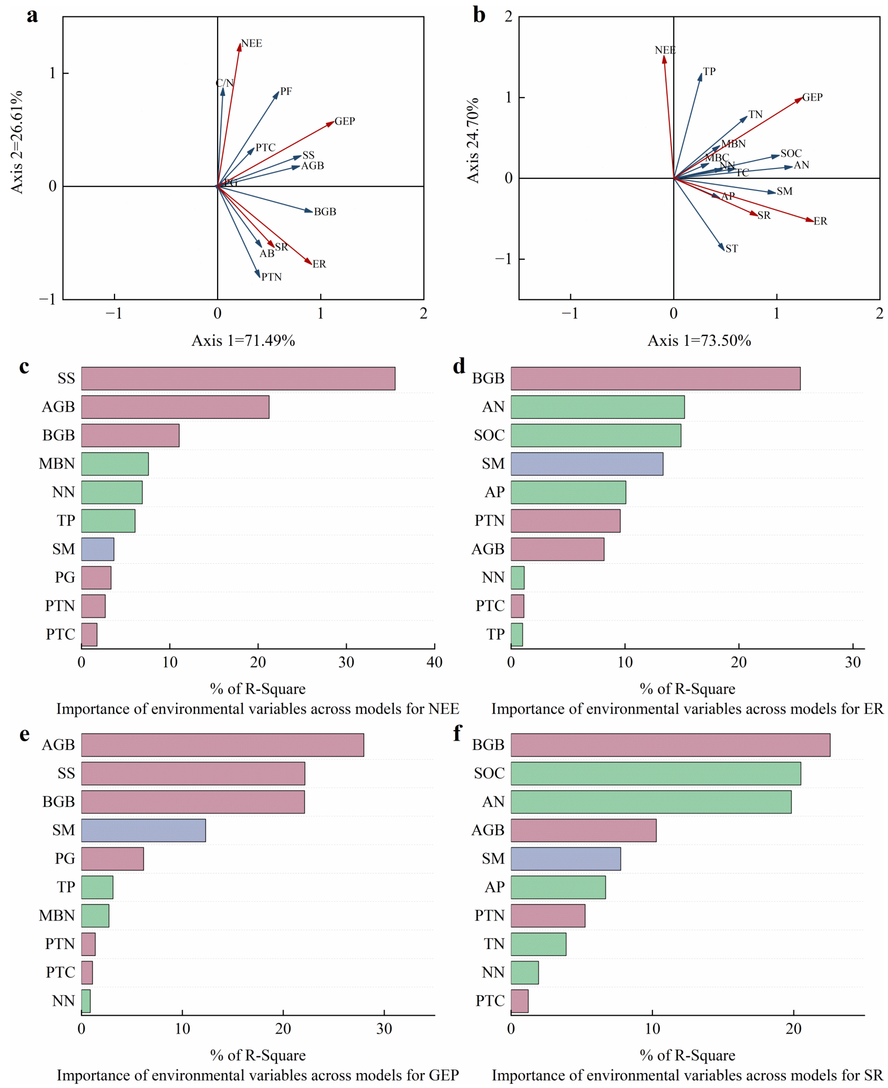
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C/N
PF
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b
NEE
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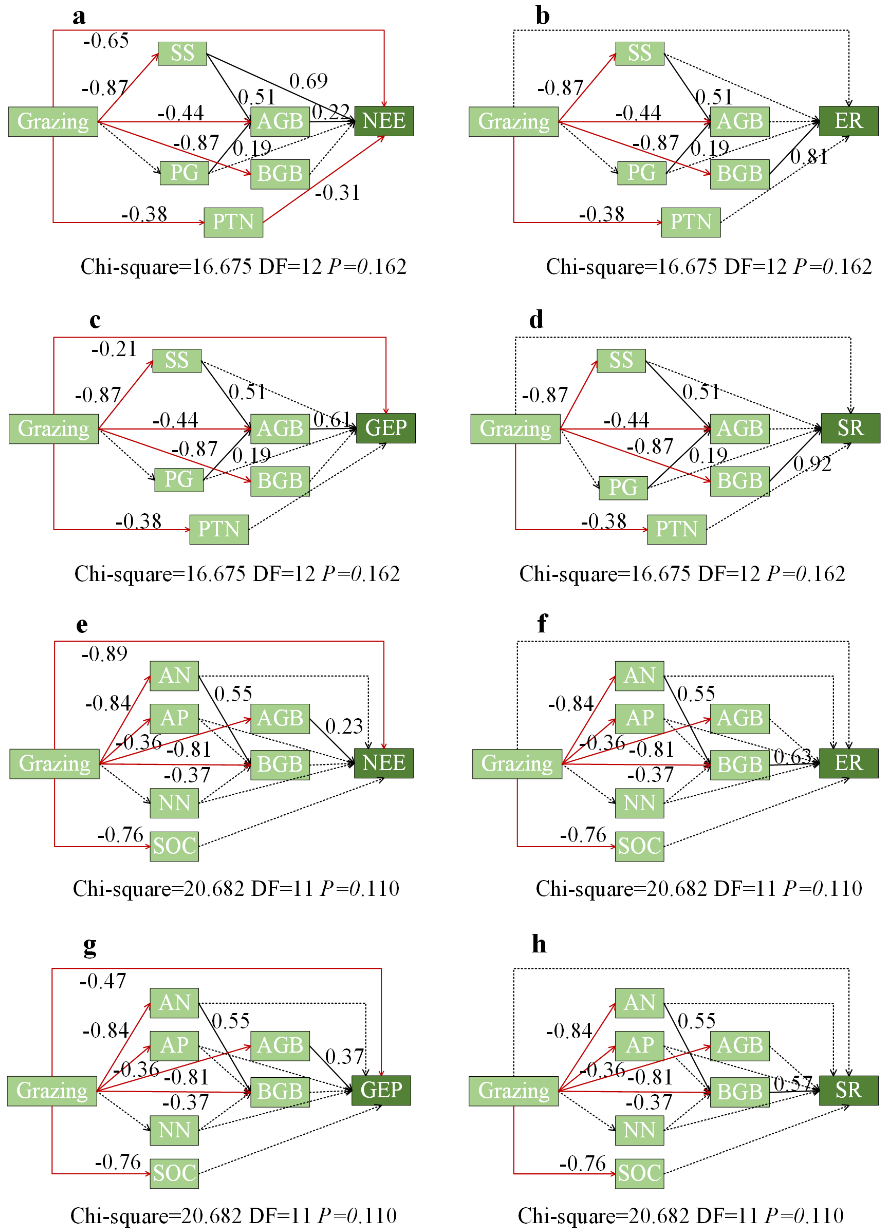
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0.69
0.22
0.19
-0.38
-0.31
Chi-square=16.675 DF=12 P=0.162
b
Grazing
SS
AGB
PG
BGB
PTN
ER
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-0.38
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Grazing
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Grazing
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SOC
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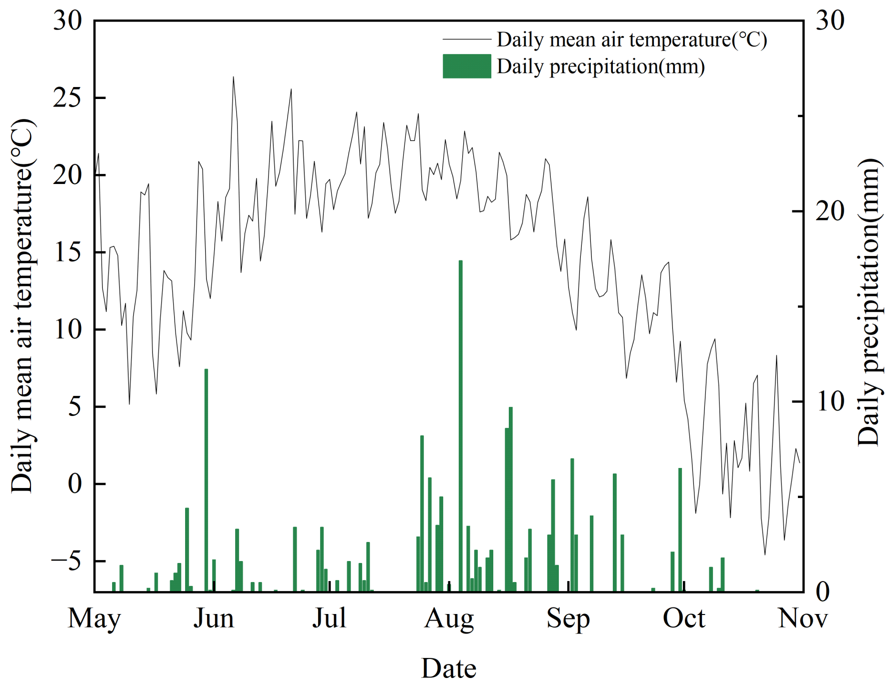
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Daily precipitation(mm)
Daily mean air temperature(°C)
Daily precipitation(mm)
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30
20
10
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May
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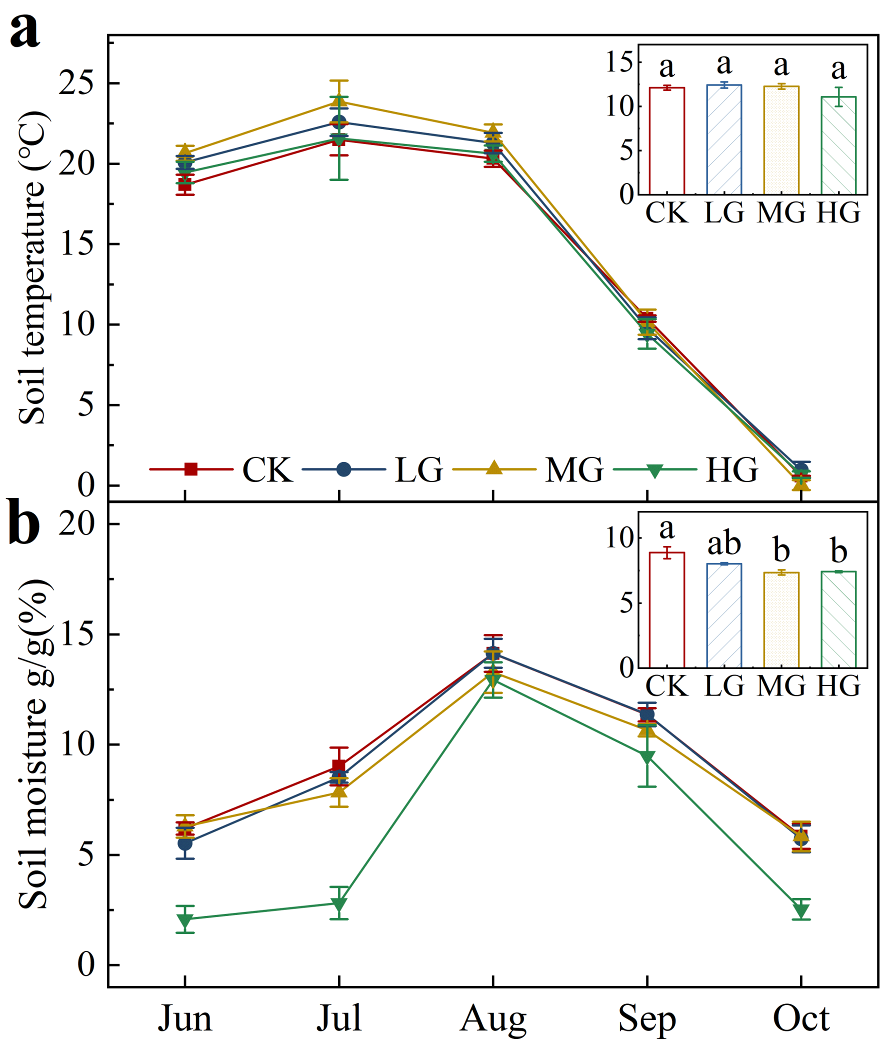
Date


a
b
Soil temperature (°C)
Soil moisture g/g(%)
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LG
MG
HG
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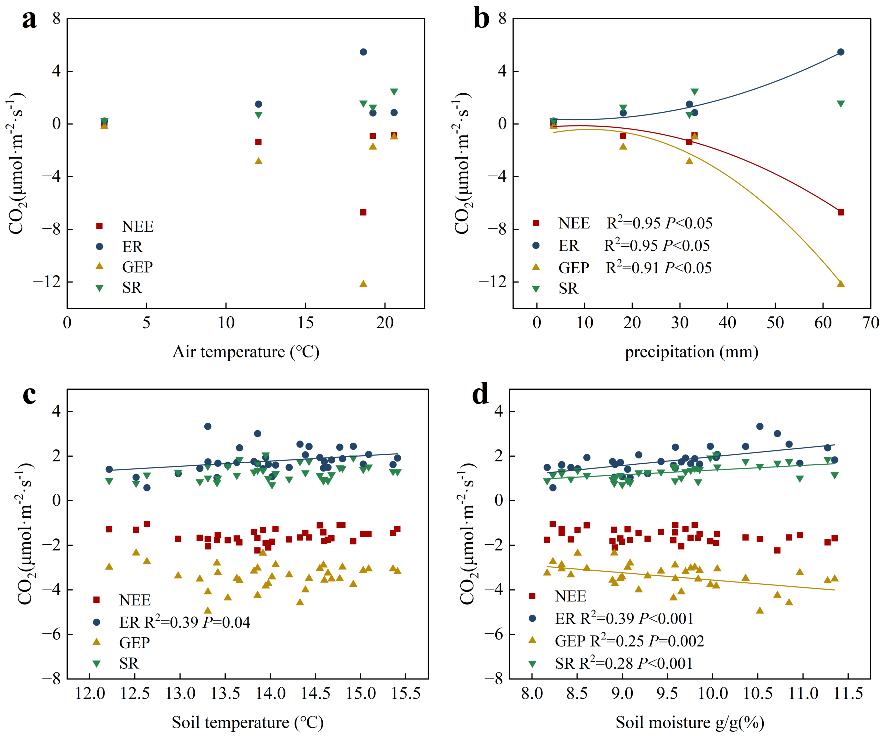
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CO2(μmol·m-2·s-1)
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ER
GEP
SR
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GEP R2=0.91 P<0.05
ER R2=0.39 P=0.04
ER R2=0.39 P<0.001
GEP R2=0.25 P=0.002
SR R2=0.28 P<0.001
Air temperature (℃)
precipitation (mm)
Soil temperature (℃)
Soil moisture g/g(%)