RESEARCH ARTICLE

Shifts in plant composition mediate grazing effects on carbon cycling in grasslands

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Abstract

1. Carbon cycling in grasslands can be impacted by livestock grazing, partially as an indirect result of herbivory-induced compositional shifts in the plant community. However, the underlying mechanisms of how these shifts impact carbon cycling are not well-documented.

2. We conducted a long-term grazing experiment with four sheep stocking rates in the semi-arid grasslands of Inner Mongolia, China, to examine grazing effects on the ratio of C_3 to C_4 species (C_3:C_4), shoot biomass, root biomass, root:shoot, soil respiration, soil C, soil N and soil C:N between 2014 and 2018. We explored the responses of these carbon metrics to C_3:C_4 under different grazing treatments and the mechanisms driving grazing-induced carbon loss using structural equation models.

3. Livestock grazing directly shifted plant community composition (i.e. increasing C_3:C_4) and reduced vegetation carbon (i.e. shoot biomass), whereas grazing effects on below-ground carbon were mediated by the interactions of the soil profile (i.e. depth dependence) and year-to-year variation (e.g. rainfall regulation). Grazing-induced increases in C_3:C_4 suppressed soil carbon loss by inhibiting the rate of soil respiration. Furthermore, grazing intensity indirectly altered these relationships. Specifically, C_3:C_4 was positively related to shoot biomass and negatively associated with root:shoot, soil C and soil N, whereas these relationships were only significant in no-grazed plots. Meanwhile, soil respiration was negatively associated with C_3:C_4, soil C, soil N and soil C:N, but a positive relationship with shoot biomass; these relationships were significant only in grazed plots.

4. *Synthesis and applications.* Our study highlights the importance of the functional linkages between community characteristics and ecosystem processes, that is, shifts in plant community composition play a key role in regulating grassland carbon cycling. These findings provide a useful field-observed resource for model development and could improve the guidelines for livestock management and policies regarding climate mitigation.

KEYWORDS

C_3 plant, C_4 plant, depth dependence, grazing intensity, Mongolian Plateau, rangelands, sheep, soil respiration

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1 | INTRODUCTION

Anthropogenic activities are accelerating global carbon loss from terrestrial ecosystems (Delgado-Baquerizo et al., 2013; Reichstein et al., 2013). This includes overgrazing in grasslands world-wide (Asner et al., 2004; Qi et al., 2017; Sanderman et al., 2017). Covering about 40% of Earth's land surface (Parton et al., 1988), grasslands are the largest biome in arid and semi-arid regions and store ~15% of global soil carbon (Lal, 2004). The inter-annual variations of carbon sequestration in this ecosystem dominate the trends in global carbon storages and fluxes (Ahlström et al., 2015; Poulter et al., 2014). Mitigation actions (e.g. livestock management) in this biome can produce profound consequences on the global carbon budget, and therefore affect regional and global warming potentials (Crowther et al., 2016). In general, livestock grazing directly reduces above-ground carbon (i.e. leaf and shoot biomass), which affects below-ground carbon (i.e. root biomass and soil carbon storage; Derner et al., 2018). Indirectly, grazers alter carbon cycling in grasslands through changes in microclimatic conditions (e.g. soil temperature and moisture, light alteration, microbes) via trampling and altering canopy structure (Wilson et al., 2018; Zhou et al., 2019). However, experimental investigations of the complex mechanisms on the regulative effects on grassland carbon cycle dynamics remain rare.

Understanding the underlying mechanisms of grazing effects on grassland carbon cycling is especially important for seeking alternative management actions and for decision-making. Unfortunately, previous studies demonstrate the seemingly contradictory effects of grazing on carbon cycling, suggesting that further exploration based on long-term observation is still needed. For example, some studies indicate that soil respiration—a major component of the grassland carbon cycle—decreases with increasing grazing intensity (Bahn et al., 2008; Bremer et al., 1998; Cao et al., 2004; Johnson & Matchett, 2001). Yet other studies show that soil respiration is stable in the presence of grazing (Liang et al., 2016; Lin et al., 2011). Grazing can also alter soil C storage (Hewins et al., 2018; McSherry & Ritchie, 2013; Zhou et al., 2017) through effects on plant roots because plant roots substantially influence soil carbon accumulation (Rasse et al., 2005; Wilson et al., 2018). However, the responses of roots to grazing have also been shown to vary in previous studies (Milchunas & Lauenroth, 1993; Smith et al., 2014).

Livestock foraging can directly affect plants, resulting in more profound impacts on the plant community than soil carbon storage in grasslands. In brief, grazers tend to forage on perennial, tall and erect herbaceous plants; this grazing-induced compositional shift in the plant community will consequently change root dynamics, decomposition and soil physical properties (e.g. soil aggregation, soil bulk density), thereby altering carbon cycling (Figure S1a). Clearly, grazing not only *directly* changes the magnitude of carbon-related metrics, but may also affect carbon cycling indirectly by altering plant community composition, as well as shifting the relationships (hereafter, *interactive* effects) between carbon cycling metrics and plant community composition (Figure S1b). In grasslands, the ratio of C_3 to C_4 species (C_3:C_4) is an important aspect of community composition for herbivore selectivity and carbon cycling. For example, grazers generally prefer C_3 to C_4 grasses (Derner et al., 2006; Díaz et al., 2007; Epstein et al., 1997). Such foraging strategies could increase soil carbon storage due to a greater reallocation of carbon from above-ground shoots to below-ground roots (McSherry & Ritchie, 2013). Alternatively, shifting the community from C_3 to C_4 species would significantly alter forage quality and carbon sequestration in grasslands. Nonetheless, the effects of grazing-induced top-down changes in C_3:C_4 on carbon cycling have not been well-quantified, and its specific mechanism remains unclear.

We conducted a 5-year sheep grazing experiment in a typical steppe of Inner Mongolia, China, with four grazing intensities. We recorded the relative abundance of C_3 and C_4 plants, as well as a variety of carbon cycling metrics. Our objective was to quantify the direct and indirect effects of grazing intensity on the magnitude of plant compositional shifts (i.e. C_3:C_4) and the major components/processes of carbon cycling. We also examined the relationships between carbon cycling metrics and C_3:C_4 under different grazing intensities. Specifically, we asked: (a) How does increased grazing intensity impact plant community composition and carbon cycling dynamics?; (b) Which ecosystem processes altered by grazing are responsible for the changes in C_3:C_4?; and (c) How do these relationships change in conjunction with grazing intensity?

2 | MATERIALS AND METHODS

2.1 | Study site

This study was carried out at a typical steppe grassland in Xilin Gol Grassland Nature Reserve, Xilinhot City, Inner Mongolia, China (44°08′N, 116°19′E, 1,129 m). The site was managed for continuous low-intensity sheep grazing (~0.5 sheep day^{-1} ha^{-1}) year-round. The annual primary production (i.e. dry mass) was 980–2,200 kg/ha in grazing exclusion plots. Vegetation at the site was dominated by native perennial grasses, including big bunchgrass (*Stipa grandis* P. Smirn) and rhizome grass *Leymus chinensis* ((Trin.) Tzvel.; Liang et al., 2018). Soils are classified as Haplic Calcisol (i.e. FAO soil classification) or Calcic-Orthic Aridisol of the USDA soil classification. Mean annual air temperature was 2.6 ± 1.1°C and has been steadily increasing in the past 65 years (Figure S2a; $R^2 = 0.58$, slope = 0.045°C/year, $p < 0.0001$). Mean annual precipitation was 266.9 mm, ranging from 121.1 to 525.0 mm, with 90.5% falling in the growing season (>5°C). During 1953 – 2018, the frequency of dry years (<10% of the mean) was greater than that of wet (>10% than the mean) or normal years. The area experienced 31 dry years and 18 wet years during the last 65 years (Figure S2b).

2.2 | Grazing experiment

Because grasslands on the Mongolian Plateau are primarily used for livestock, it is impossible to find a site that has not been

disturbed by human activities. Thus, we fenced 12 equal-sized plots (120 m × 120 m) within a 650 m × 650 m area to exclude grazing from 2011 to 2012 in an area that has been lightly used for livestock grazing. Empirically, this exclusion can carry the lagged effects of previous human activities. Within these fenced plots, we implemented sheep grazing with four intensities during 2013–2018, with three replicates for each treatment. The four grazing intensities included 0, 2, 4 and 8 sheep day^{-1} ha^{-1}, respectively, for no grazing (NG), low grazing (LG), medium grazing (MG) and high grazing (HG) treatments (Figure S3a). We applied these treatments for 21 days per month in the summer months (June–September). Plant and soil sampling and measurements were conducted starting in 2014. More detailed sites and experimental descriptions can be found in Liang et al. (2019) and Wang et al. (2018).

2.3 | Shoot and root biomass of plant community

At the peak of each growing season (i.e. late July to early August), we laid out a 100-m south–north transect across each plot starting 30 m from the east boundary. We then set up five quadrats (1 m × 1 m) every 20 m (Figure S3b) to sample shoot and root biomass. The residual living above-ground tissues of all plants in each quadrat were clipped by species at ground level, oven-dried at 65°C and weighed after 48 hr to estimate above-ground biomass (g/m). All plant species were classified into C$_3$ and C$_4$ groups (Table S1). We calculated the relative abundance of each plant group as the ratio of residual living above-ground biomass to the total above-ground biomass (Liang et al., 2019).

We harvested root biomass by collecting two 7-cm diameter soil cores in each quadrat at depths of 0–5, 5–10, 10–20, 20–30, 30–40, 40–50, 50–70 and 70–100 cm (Figure S3c). We mixed the two soil samples, washed the cores free of soil through 0.15-mm mesh sieves, dried them for 48 hr at 65°C, weighed and converted the mass to volumetric weight (g/m^3).

2.4 | Soil carbon, nitrogen and respiration

We collected another soil core in each quadrat at 0–5, 5–10, 10–20, 20–30, 30–40 and 40–50 cm depth for quantifying soil carbon and nitrogen. Soil samples at each depth were air-dried, sieved over 2-mm mesh sieves to remove course roots and gravels (>2 mm) and then analysed for the concentration (g/kg) of total carbon and nitrogen with an elemental analyser (vario MACRO cube, Elementar).

During the data collection periods, we picked a day when it was not raining and used a soil respiration chamber and an LI-6400 CO$_2$/H$_2$O analyser (Li-Cor) to measure soil respiration (μmol CO$_2$ m^{-2} s^{-1}) between 08:00 hr and 12:00 hr. The chamber was connected to a PVC collar buried at 6-cm depth (Figure S3d). Prior to each measurement, we removed all live plants inside the chamber. Two pairs of PVC rings inside the plots were used to take two measurements

(Figure S3b). LI-6400 CO$_2$/H$_2$O analyser records the data three times in approximately 270 s (90 s × 3 readings).

2.5 | Statistical analysis

To test how grazing directly altered the plant community composition and carbon cycling in our system, we performed multiple two-way ANOVAs with grazing intensity (**GI**), year (**Y**: 2014, 2015, 2016, 2017 and 2018) and their interaction (**GI × Y**) as categorical fixed effects. Dependent variables used in the models were the relative abundance of C$_3$ and C$_4$ plants (i.e. C$_3$:C$_4$), shoot biomass, root biomass, root: shoot, soil respiration, soil total carbon, soil total nitrogen and soil C:N, as well as these below-ground carbon metrics at different soil depths. Separate models were fit for each dependent variable. To explore the importance of soil depth (**D**) on below-ground carbon cycling variables (i.e. root biomass, root: shoot, soil total carbon, soil total nitrogen and soil C:N), we also performed a three-way ANOVA to test the effects of **GI**, **Y** and **D**, and their interactions.

To examine the indirect and interactive effects of grazing on carbon cycling via changes in plant community (C$_3$:C$_4$), we explored how the responses of carbon cycling variables to the compositional changes in plant community varied by grazing intensities. To do this, we conducted ordinary least squares linear regression models (OLS-LM) between the carbon cycling variables (i.e. independent variable) and C$_3$:C$_4$ (i.e. dependent variable). The models were applied for different carbon cycling variables (i.e. shoot biomass, root biomass, root: shoot, soil total carbon, soil total nitrogen, soil C:N and soil respiration) under different grazing treatments (i.e. NG: no grazing, LG: low grazing intensity, MG: medium grazing intensity, and HG: high grazing intensity). Because grazing may alter the functional linkage between soil respiration and shoot biomass, root biomass, soil total carbon and soil total nitrogen, we also ran the OLS-LM between soil respiration (i.e. independent variable) and other carbon cycling variables (i.e. dependent variable). A best-fit model based on Akaike information criteria (AIC) was selected for each model.

We also performed structural equation modelling (SEM) to explore how compositional shifts of plant community regulate grazing-induced changes in carbon cycling. Our null hypothesis was that an increase in sheep grazing intensity (from 0, 2, 4 to 8 sheep day^{-1} ha^{-1}) regulated soil carbon processes via foraging, trampling and excretion directly. This was expected to indirectly shape plant community composition (i.e. C$_3$:C$_4$), which in turn would stimulate root turnover, and therefore alter carbon cycling. To evaluate and confirm the final SEM, we used Shipley's test of d-separation (e.g. Fisher's *C*) and chose the model with the lowest AIC in the R package PIECEWISESEM (Lefcheck & Freckleton, 2016). The model presented the standardized correlation coefficients between predictors to dependent variables for comparing the effect sizes (Fan et al., 2016; Grace, 2006; Grace et al., 2018). The data we used in all analyses were paddock based in each treatment as four treatments × three replicates × five years (*n* = 60). In both the OLS-LM and SEM, we used the accumulated values of root biomass and the averaged values for root:shoot,

soil total carbon, nitrogen and C:N through the entire soil depth. All analyses were conducted in R v3.5.2 (R Development Core Team, 2019).

3 | RESULTS

3.1 | Grazing-induced compositional shifts

Grazing significantly changed the plant community composition of grasslands. The C_3:C_4 showed a decreasing trend in both grazed and no-grazed plots from 2014 to 2018; between 2017 and 2018, the C_3:C_4 was 15-fold higher (i.e. $\frac{\bar{v}_{HG} - \bar{v}_{NG}}{\bar{v}_{NG}} \times 100\% = 15$) on average in high grazing plots (HG) compared to the no-grazed plots (Figure 1A). Overall, grazing maintained a high portion of C_3 species (Figure 1D; Table S2, $F_{3,40} = 4.80$, $p = 0.006$), while a large shift from C_3 to C_4 occurred in no-grazed plots over time (Table S1).

3.2 | Grazing effects on carbon cycling

Grazing significantly decreased grassland above-ground carbon storage in shoot biomass (Figure 1B; Table S2, $F_{3,40} = 37.56$, $p < 0.0001$). Overall, high sheep grazing decreased shoot biomass by 39% compared to no-grazed plots, with 12% inter-annual variation. Interestingly, grazing did not significantly change grassland carbon flux (Figure 1F; Table S2, $F_{3,40} = 1.67$, $p = 0.188$). However, high and medium grazing decreased soil respiration rate by ~24%

($p = 0.038$) and ~39% ($p = 0.040$) compared to no-grazed plots in 2017 (Figure 1C); soil respiration demonstrated significant inter-annual variabilities in all plots (Figure 1C; Table S2, $F_{3,40} = 31.37$, $p < 0.0001$).

Grazing did not change total carbon storage in grassland soils, while it shifted its vertical distributions. Grazing produced no effect on total root biomass (a cumulative value from 0 to 100 cm depths), soil total carbon and soil C:N (the average values from 0 to 50 cm depths), resulting in root:shoot (0–100 cm) being higher (98 ± 9.6%) in high grazing than that in the non-grazed plots (Table S2, $F_{3,40} = 20.41$, $p < 0.0001$). However, significant grazing effects on soil total carbon and nitrogen, as well as root biomass and root:shoot were apparent at some depths, suggesting a depth-dependent effect of grazing (Table 1; Figures S4–S8). Finally, below-ground carbon variables at some depths showed apparent inter-annual variations (Table 1).

3.3 | Consequences of compositional shifts on carbon cycling

Grazing weakened the responses of carbon storage variables to compositional shifts in grassland communities. This was evidenced that the regressions between shoot biomass, root:shoot, soil total carbon, and soil total nitrogen and C_3:C_4 were significant in no grazing plots ($p < 0.05$), whereas these relationships were not significant in the grazed plots (Figure 2; Table S3). Note that an increase in C_3 plants increased shoot biomass but decreased
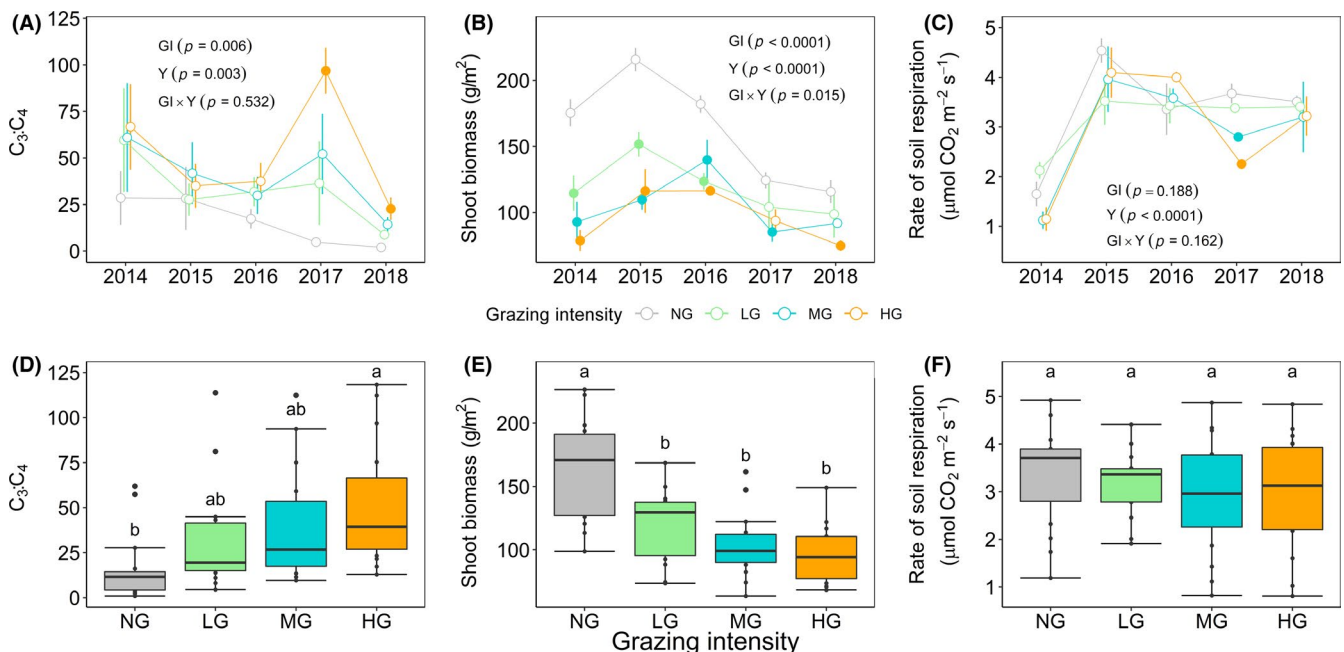
FIGURE 1 Plant community composition, above-ground carbon storage and carbon fluxes under different grazing intensities (Mean ± *SE*, $n = 3$). Shown are the dynamics of (A, D) C_3:C_4, (B, E) shoot biomass, (C, F) soil respiration with statistics (i.e. Tukey's range test) indicating the results from the ANOVA models of grazing intensity, year and their interactions (see Table S2). Key: NG = no grazing (grey), LG = low grazing intensity (light green), MG = medium grazing intensity (blue), HG = high grazing intensity (yellow), GI = grazing intensity, Y = year. Filled dots indicate the significant ($p < 0.05$) grazing effects in comparison to no grazing treatments [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Grazing effects on below-ground carbon storage. Shown are the results of three-way ANOVA (F-value and contribution percent [i.e. SS_i/SST]) for the effects of grazing intensity (GI), year (Y), soil depth (D) and their interactive forcing on root biomass of plant community (g/m²) and root: shoot ($df_{GI} = 3$, $df_Y = 4$, $df_D = 7$, $df_{residual} = 320$), soil total carbon (g/kg, STC), soil total nitrogen (g/kg, STN) and soil C:N ($df_{GI} = 3$, $df_Y = 4$, $df_D = 5$, $df_{residual} = 240$) respectively. SS_i denotes the sum of square by factors, where i represent GI, Y, D and their interactions; SST is the total sum of squares by factors and residuals; the bold values represent significant effects ($p < 0.05$)

	Root biomass (g/m²)			Root: shoot			STC (g/kg)			STN (g•kg)			Soil C:N		
	F	SS/SST	p	F	SS/SST	p	F	SS/SST	p	F	SS/SST	p	F	SS/SST	p
GI	6.80	0.89	**0.0002**	100.28	18.37	**<0.0001**	2.70	2.23	**0.046**	9.46	1.10	**<0.0001**	1.73	0.37	0.162
Y	126.03	21.90	**<0.0001**	61.12	14.93	**<0.0001**	4.86	5.32	**0.0009**	8.12	1.34	**<0.0001**	5.95	1.75	**0.0001**
D	178.32	54.22	**<0.0001**	82.77	35.39	**<0.0001**	8.99	12.28	**<0.0001**	417.06	84.77	**<0.0001**	211.79	77.31	**<0.0001**
GI × Y	2.14	1.11	**0.015**	5.88	4.31	**0.015**	0.70	2.28	0.753	0.91	0.49	0.530	0.79	0.70	0.656
GI × D	1.43	1.31	0.100	2.12	2.72	**0.003**	1.97	8.05	**0.018**	1.36	0.85	0.170	0.72	0.78	0.764
Y × D	3.39	4.12	**<0.0001**	1.43	2.44	0.079	0.21	1.16	1.000	0.77	0.61	0.750	0.52	0.76	0.959
GI × Y × D	0.70	2.56	0.973	0.45	2.29	1.000	0.19	3.09	1.000	0.43	1.10	1.000	0.19	0.82	1.000

root:shoot, soil total carbon and soil total nitrogen in the no-grazed plots.

Grazing enhanced the response of carbon fluxes to compositional shifts of grassland communities. The regressions indicated that soil respiration was negatively associated with $C_3:C_4$ ($R^2 = 0.30$, $p = 0.034$) in the high grazing plots (HG), but was not significant in the no-grazing plots (Figure 3A; Table S4). Grazing also amplified the functional linkages between soil respiration and other carbon cycling variables. For example, the regressions demonstrated that soil respiration was positively correlated with shoot biomass (Figure 3B, HG: $R^2 = 0.27$, $p = 0.046$), but negatively correlated with soil total carbon (Figure 3D, HG: $R^2 = 0.35$, $p = 0.021$), soil total nitrogen (Figure 3E, medium grazing plots (MG): $R^2 = 0.27$, $p = 0.048$) and soil C:N ratio (Figure 3F, HG: $R^2 = 0.40$, $p = 0.011$). Interestingly, soil respiration was also positively related to shoot and root biomass but negatively associated with soil carbon and nitrogen in grazed plots (Figure 3; Table S4).

Our final SEM disentangled the pathways through which grazing influenced grassland carbon cycling, demonstrating significant indirect pathways of grazing effects on soil respiration and soil nitrogen through $C_3:C_4$. An increase in grazing intensity elevated $C_3:C_4$, which suppressed soil respiration and decreased soil nitrogen storage (Figure 4). Combining all pathways, an increase in grazing intensity indirectly led to decreased soil respiration (Table S5, total effect size or TES = −0.136) and soil total nitrogen (TES = −0.204).

4 | DISCUSSION

By quantifying grazing effects on plant community composition ($C_3:C_4$) and carbon cycling in a grassland ecosystem, we found that grazing influences carbon cycling via direct, indirect and interactive pathways. Importantly, we found that the indirect and interactive effects of grazing were more profound than the direct effects of grazing on carbon cycling, which could be potentially explained by herbivory selectivity and the identity dependence of carbon cycling.

### 4.1 | Herbivory selectivity versus rainfall regulations in shaping plant dominance

Our findings suggested that sheep grazing increased $C_3:C_4$, particularly in a dry year. For example, grazing-increased $C_3:C_4$ was remarkably apparent in 2017—a low precipitation year with mean annual precipitation 37% lower than the 65-year average value. This is mainly due to herbivory selectivity and plant anti-herbivory strategies. Grazers, in general, prefer perennial to annual plants, tall to short plants and erect over prostrate plants (Díaz et al., 2007; Milchunas & Lauenroth, 1993). On the Mongolia Plateau, *Stipa* species are the most dominant plants in grasslands; they are tall, erect, perennial C_3 grasses (Bai et al., 2012; Liu et al., 2015)
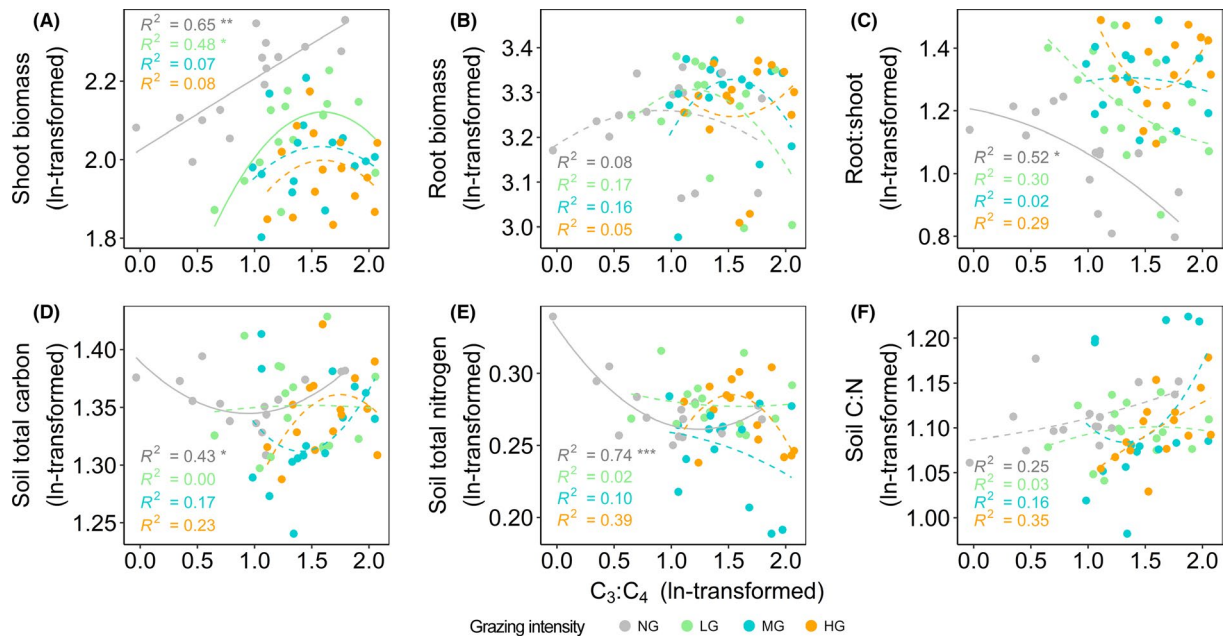
FIGURE 2 Carbon storage response to plant community composition of under different grazing intensities. Lines indicate the best-fit regressions of (A) shoot biomass, (B) root biomass, (C) root:shoot, (D) soil C, (E) soil N and (F) soil C:N to $C_3:C_4$, respectively, according to Akaike information criterion. Solid and dashed lines denote significant and insignificant, respectively. Significant level: *$p < 0.05$; **$p < 0.001$; ***$p < 0.0001$. Key: NG = no grazing (grey), LG = low grazing intensity (light green), MG = medium grazing intensity (blue), HG = high grazing intensity (yellow) [Colour figure can be viewed at wileyonlinelibrary.com]
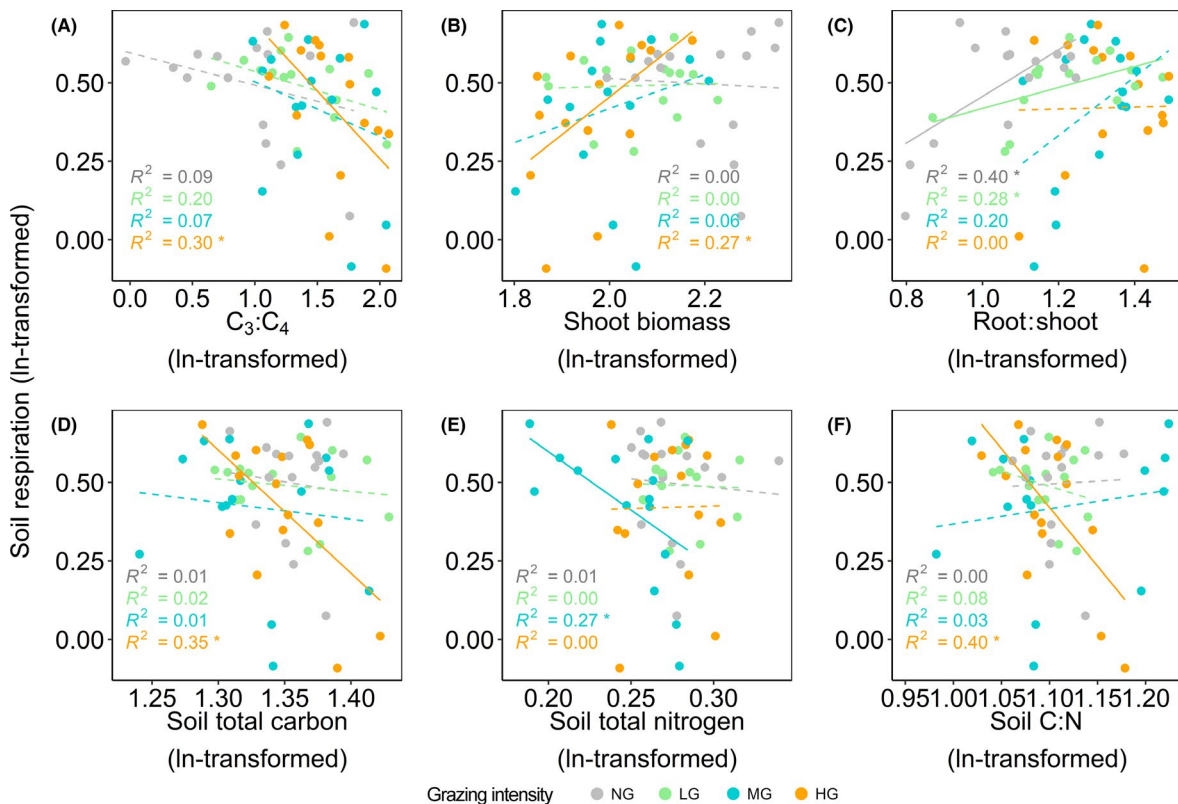
FIGURE 3 Carbon fluxes response to plant community composition and carbon storage variables under different grazing intensities. Lines indicate the best linear regressions of soil respiration to (A) $C_3:C_4$, (B) shoot biomass, (C) root:shoot, (D) soil C, (E) soil N and (F) soil C:N, respectively, according to Akaike information criterion. Solid and dashed lines denote significant and insignificant, respectively. Significant level: *$p < 0.05$. Key: NG = no grazing (grey), LG = low grazing intensity (light green), MG = medium grazing intensity (blue), HG = high grazing intensity (yellow) [Colour figure can be viewed at wileyonlinelibrary.com]
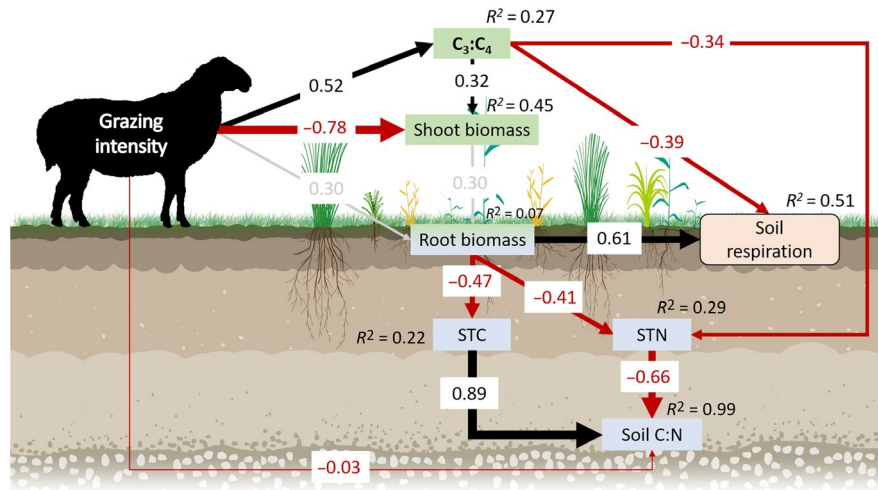
FIGURE 4 Final structural equation modelling (SEM) of grazing intensity to predict the dynamics of carbon cycling. Shown are that the numbers (red/negative vs. black/positive) denote the standardized path correlation coefficients (Pearson's r) with 95% confidence level (significance: $p < 0.05$); the direction of pathways with red (i.e. negative) and black arrows (i.e. positive); grey numbers and pathways are not statistically significant ($p > 0.05$); and the proportion of variance (R^2) explained by fixed factors in the model. Fisher's $C = 23.101$, $df = 30$, probability level $= 0.811$, AIC $= 77.101$, the sample size $n = 60$ [Colour figure can be viewed at wileyonlinelibrary.com]

At our site, *S. grandis* was the most abundant species and it is less palatable and lower in nutrition than other plants in the focal community (Liang et al., 2019). During the heading period (August–September), it produces long needle seeds with a barb, which could suppress sheep foraging via hooking on their fur or damaging their digestive systems. Meanwhile, C_4 grasses are small, annual and biennial plants, which would be benefited from livestock grazing while highly reliant on water availability (i.e. developed in a wet season or year). These grasses preferentially allocate more resources to reproduction instead of growth when resources and environmental conditions are sufficient (Liang et al., 2018). These findings are seemly contradictory to previous studies in North American grasslands where grazing favoured C_4 plants over C_3 plants (Derner et al., 2006). Nevertheless, this further confirms the substantially climatic-driven productions of C_3 and C_4 plants (Epstein et al., 1997) as grazing substantially increased C_3:C_4 of rainfall-dependent grasslands in our study area.

4.2 | Identity-dependent responses of carbon cycling to grazing

Grazing effects on carbon cycling were primarily associated with the identity of carbon cycling variables and their response to abiotic factors. This appears the result of indirect grazing disturbances. First, sheep directly forage plant shoots, but not roots; thus, the grazing effects on below-ground carbon is a slow process that possibly manifests via trampling-induced stimulation of carbon turnover and mineralization rates via the alteration in soil physical properties (Wang et al., 2018). Second, grazing effects on below-ground carbon are complicated (Milchunas & Lauenroth, 1993; Smith et al., 2014) by, for example, the depth-dependent response of root biomass, soil C and

soil N (Bai et al., 2012; Derner et al., 2006). The correlations of soil C and N to root biomass were significant (Figure S9). In this study, we found that both soil C and soil N were resistant to year-to-year variations. This is probably true in grasslands because soil C and N are more stable in grasslands than other ecosystems (Lal, 2004), which is evidenced by the fact that both a 25-year and 74-year disturbance (grazing or fire) did not change soil C and N (Derner et al., 2018; Wilcox et al., 2016). Interestingly, we found a strong interactive year effect with grazing on soil respiration. Soil respiration decreased linearly with grazing intensity in the water-limited year (2017) when the mean annual precipitation was 37% lower than a 65-year average. Finally, grazing effects were essentially complicated in the water-sufficient year (e.g. 2015 and 2016), which is likely because soil respiration is highly sensitive to environmental conditions (Luo et al., 2001).

4.3 | Grazing effects through regulating plant compositional shifts

Grazing appeared to decouple the functional linkages between carbon storage and plant community composition, as evidenced by the fact that the correlations of root:shoot, soil C and soil N to C_3:C_4 in no-grazed plots were not apparent in grazed plots. This suggests a profound forcing of carbon sequestration in grasslands, that is, grazing could alter the dynamics of carbon storage via indirect shaping of plant community composition. Specifically, this was the result of grazing-induced increases in C_3:C_4, which altered carbon allocations between above- and below-ground components. A low C_3:C_4 denotes a high relative abundance of C_4 plants in the community (e.g. in no grazing plots). The fact that high C inputs to the biomass and soil due to C_4 plants is characterized as light-use efficient, with a

high capacity of photosynthetic C inputs to their leaves, stems and roots (McSherry & Ritchie, 2013). Additionally, the relationships of root:shoot, soil C and soil N to C_3:C_4 were generally negative. In our system, the C_3 plants, especially perennial grasses, are more resource conservative and nutrient acquisitive than C_4 plants (Bai et al., 2012; Liang et al., 2019). Compared to the C_4 plants, more abundant C_3 plants would regulate biomass carbon allocations differently, resulting in different carbon storage regimes. Thus, it is possible that, in grasslands on the Mongolian Plateau, grazers may change soil carbon indirectly via re-allocations of shoot and root biomass, which is accompanied by grazing-induced shifts on plant community composition.

Finally, our results suggested that grazing could amplify soil respiration via biotic pathways. Specifically, C_3:C_4, shoot and root biomass, root:shoot, soil C and N, and soil C:N became important biotic factors (particular in high grazing intensity plots) because of the importance of top-down grazing effects on carbon cycling in grasslands. Soil respiration is a principal component of below-ground carbon cycling in grasslands (Raich & Schlesinger, 1992; Schlesinger & Andrews, 2000), which includes considerable root autotrophic respiration (Bahn et al., 2006; Boone et al., 1998). Our previous study confirmed that grazing-induced compositional shifts in the plant community drive the rate of soil respiration via shaping the vertical distributions of root biomass (Liang et al., 2016). An increase in the proportion of C_4 plants would increase the magnitude and proportion of fine root mass within the top-soil profile (Derner et al., 2006), which would support more microbial biomass (Wilson et al., 2018), and thus increase microbial respiration rates in the soils (Dacal et al., 2019). In this system, most focal C_4 plants are annual and biennials that are shallow-rooted species. Thus, grazing-driven decreases in the relative abundance of C_4 plants could alter root architecture and dynamics resulting in a change in soil respiration and, thereby, carbon cycling.

4.4 | Implications for livestock management in grasslands

Grasslands can potentially mitigate climate warming because of the considerable carbon stored within the system (Lal, 2004; Parton et al., 1988). However, the dynamics of carbon cycling in grasslands are highly sensitive to inter-annual rainfall variability (Ahlström et al., 2015; Poulter et al., 2014). Additionally, livestock grazing, as the most prevalent land-use activity in grasslands, could alter the magnitudes and dynamics of carbon cycling processes (Derner et al., 2018; Zhou et al., 2017). Importantly, climate change appears to be a critical driver regulating ecosystem processes of grazed lands (Koerner et al., 2018; Liang & Gornish, 2019; Sloat et al., 2018). Therefore, understanding the mechanisms underlying grazing effects on carbon cycling in grasslands is crucial for decision-making agencies to future climate change. Through our on-going sheep grazing experiment, we demonstrated that grazing caused carbon loss by foraging but it could suppress soil carbon

loss by indirectly shifting the plant community composition. Our results provide a useful field-observed resource for studying the influences of anthropogenic perturbations on carbon cycling, which has direct implications in improving livestock management that facilitates the carbon budgets of global rangelands under the uncertainty of climate change (Qi et al., 2017).

AUTHORS' CONTRIBUTIONS
M.L. and C.L. conceived the ideas and designed the study; M.L., Y.W. and Z.G. collected the data; Y.W. and Z.G. measured soil C and N contents; M.L. analysed the data; M.L., N.G.S., J.C. and E.S.G. wrote the first draft; all the authors contributed to the development of the manuscript.

DATA AVAILABILITY STATEMENT
Data available via the Dryad Digital Repository https://doi.org/10.5061/dryad.prr4xgxkg (Liang et al., 2020).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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