

1 **Response of soil respiration and soil microbial biomass**
2 **carbon and nitrogen to grazing management in semi-arid**
3 **grassland**

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13

14 **Abstract**

15 Grazing management affects grassland carbon dynamics and soil microbial biomass, yet how
16 grazing management, including grazing intensity (GI) and grazing regime (GP), affects soil
17 respiration (Rs) and soil microbial biomass carbon (SMBC) and nitrogen (SMBN) is not fully
18 understood. To determine how GI (0, 2.7, 5.3, and 8.7 sheep ha⁻¹) and GP (warm-season grazing,
19 WG; cold-season grazing, CG) affect Rs, SMBC, and SMBN, an experiment was conducted in a
20 semi-arid grassland that had been rotationally grazed for the previous 9 years. Results suggest that
21 diurnal Rs in WG significantly decreased as stocking rate increased; however, in CG, diurnal Rs
22 was significantly higher at the GIs of 2.7 and 5.3 sheep ha⁻¹ than at 0 and 8.7 sheep ha⁻¹. Although
23 grazing (at the GIs of 2.7, 5.3, and 8.7 sheep ha⁻¹) led to increased Rs in 2010 and decreased Rs in
24 2011, when compared with Rs in the non-grazing period (0 sheep ha⁻¹), the negative indirect effect
25 of GI on Rs offset its positive indirect effect on Rs over the whole experimental period. GP
26 affected Rs both directly and indirectly through the positive effect it had on soil moisture, soil
27 temperature, and aboveground biomass. Compared with WG, CG significantly stimulated an
28 increase by 22% in annual Rs. A significant difference in the soil temperature sensitivity (Q₁₀)

29 values of R_s was observed at the four stocking rates for both WG and CG, although the Q_{10} of WG
30 was significantly higher. Interactions between GI and GP had a significant effect on SMBC and
31 SMBN, but GI alone did not affect SMBN. Regarding GP, compared with WG, CG caused a
32 significant decrease of 11% in the mean concentration of SMBN. The monthly precipitation was
33 significantly positively correlated with R_s , soil temperature, soil moisture, and SMBN, but was
34 significantly negatively correlated with SMBC. The field experimental results indicated that the
35 effects of grazing management on R_s processes in the grazing system mainly depend on GP, and
36 the effects of grazing management on SMBC and SMBN mainly depend on the interactions
37 between GI and GP. The results suggest that (1) In a long-term grazing grassland ecosystem, more
38 attention should be paid the role of GP while determining the response of R_s to grazing
39 management, and GP should be considered as an important factor in future evaluation models for
40 studying the response of soil carbon dynamics to climate change; (2) the coupling of GI and GP
41 should be taken into account in future studies on nutrient turnover in the soils of semi-arid
42 grassland ecosystems.

43

44 **1. Introduction**

45 Grasslands cover about 41% of Earth's terrestrial surface, and support domestic livestock grazing in
46 extensive agricultural grazing systems (Morgan et al. 2007). Owing to their large area and excellent
47 ability to sequester and store carbon, grasslands can provide important ecosystem services (Zhao et al.
48 2017). Grasslands in China constitute 6–8% of global grasslands and significantly contribute to global
49 carbon storage, thereby significantly affecting global carbon cycles (Ni 2002). Soil respiration (R_s) is
50 the second largest carbon flux between the atmosphere and terrestrial biomes (Cox et al. 2000; Wan et
51 al. 2007) and includes microbial and root respiration (Davidson and Janssens 2006; Jia et al. 2007; Ru
52 et al. 2017). Numerous previous studies have indicated that biological and environmental variables,
53 such as precipitation (Xu et al. 2016; Hawkes et al. 2017), soil temperature (Thomey et al. 2011), soil
54 water content (Hawkes et al. 2017), climate, and microbial community composition (Monson et al.
55 2006), are major factors determining soil and ecosystem respiration in grasslands. In addition,
56 anthropogenic factors, such as grazing, are strongly modifying biogeochemical cycles (Wang and Fang
57 2009). For example, the structure or species composition of plant communities, soil microclimates, soil
58 chemical and physical properties, and global climate, are all affected by human activities, which in turn

59 might affect Rs rates (Raich and Schlesinger 1992).

60 Although a growing number of studies have shown that the health of grassland ecosystems strongly
61 depends on grassland management strategies, such as grazing and grazing exclusion (Chen et al. 2015;
62 Deng et al. 2017), the influence of grazing management on Rs is not well understood. This limits our
63 understanding of how Rs responds to grazing management and our ability to predict carbon dynamic
64 responses under continued climate change. Grasslands are widely distributed on the Loess Plateau,
65 accounting for approximately 40% of the total area (Wang et al. 2017), and are subject to continuous
66 and widespread stress. Most of the grasslands on the Loess Plateau have degenerated owing to their
67 over-grazing and poor management (Fu et al. 2000). Efficient grassland management strategies have
68 been considered to be an important way to promote soil carbon storage and to recover degraded
69 grasslands (Conant et al. 2001; Ingram et al. 2008; Wang et al. 2011). Sustainable grazing intensity (GI)
70 and a seasonal grazing regime (GP) with periodic resting are widely used grassland management
71 practices (Cui et al. 2014; Chen et al. 2016; Wang et al. 2017, Wu et al. 2017). Grazing-induced soil
72 respiration has been well studied; however, how the effects of different GIs on soil respiration vary
73 through time remains unclear. For example, previous studies have reported that grazing had a positive
74 effect on Rs (Cao et al. 2004), had no effect on Rs (Jia et al. 2007; Wang et al. 2007; Cui et al. 2014),
75 and had a negative effect on Rs (Chen et al. 2016). Furthermore, in different types of grassland, Chen et
76 al. (2015) found that warm-season grazing (WG) decreased Rs, while Wang et al. (2017) reported that
77 cold-season grazing (CG) significantly increased Rs. These inconsistent results could be attributed to
78 the complex processes induced by GP. Therefore, limited information is available on the effects of GP,
79 especially seasonal grazing, on Rs under different levels of GI. Quantifying the effects of GI and
80 grazing season on Rs is critical to accurately estimate the carbon balance of grassland ecosystems and
81 to better understand how global changes affect grazing management practices on the Loess Plateau.

82 Soil microorganisms are an important component of terrestrial ecosystems and play important roles in
83 global nutrient cycling and organic matter decomposition (Wang et al. 2013). In this context, soil
84 microbial biomass adjusts the balance between the release of carbon during Rs and its sequestration as
85 soil microbial biomass carbon (SMBC) (Lange et al. 2015; Thakur et al. 2015). Thus, microbial
86 biomass is used to assess soil quality. Soil microbial biomass nitrogen (SMBN) is highly labile, and the
87 nitrogen pool in soils is a key regulator of C sequestration (Deng et al. 2017). Elevated soil CO₂
88 reduces available soil nitrogen, imposing nitrogen constraints on microbes and reducing microbial

89 respiration per unit biomass (Hu et al. 2001). Reports on the effects of grazing on SMBC and SMBN in
90 natural grasslands have been inconsistent (Fu et al. 2012; Liu et al. 2012; Lange et al. 2015). Ambient
91 environmental factors (precipitation, air temperature, soil moisture, and soil temperature), livestock
92 type, and grazing management have been shown to affect belowground carbon and nitrogen dynamics
93 (Zhou et al. 2017). Gallardo and Schlesinger (1992) found a succession in the control of microbial
94 biomass from nitrogen to carbon when the ratio of carbon to nitrogen decreased. Fu et al. (2012) found
95 that grazing significantly decreased SMBC and SMBN in an alpine meadow, while Wang et al. (2006)
96 and de Faccio Carvalho et al. (2010) found that cattle grazing increased SMBC. Limited evaluations of
97 soil microbial biomass in semi-arid grasslands and contradictory results regarding the effects of grazing
98 underscore the need for additional research, especially regarding GI (Mahecha et al. 2010) and GP.
99 Therefore, studies on the links between soil microbial biomass and environmental parameters provide a
100 better understanding of the factors that control nutrient cycling in grassland ecosystems.

101 Given this context, a 2-year study based on a long-term rotational grazing experiment was conducted.
102 The rotational grazing experiment began in 2001, and the Rs and soil microbial C and N were
103 measured from 2010–2011. We hypothesized that the effects of GI on Rs could be offset by
104 GP-induced direct and indirect biotic (above/below-ground biomass, SMBC, SMBN) and abiotic
105 variables (soil temperature, soil moisture) in long-term grazing grassland ecosystems. In the present
106 study, we investigated the following: 1) the effect of long-term rotational grazing on Rs under different
107 GIs and two GPs (warm-season grazing, WG; cold-season grazing, CG); 2) the effect of long-term
108 rotational grazing on SMBC and SMBN under different GIs and GPs; 3) the mechanisms driving the
109 responses of Rs, and soil microbial C and N to the different grazing management practices (GI and
110 GP).

111

112 2. Materials and methods

113 The study site was conducted in the core of the Loess Plateau at Huanxian Grassland Ecosystem Trial
114 Station in eastern Gansu Province, northwest China (37.14°N, 106.84°E; 1650 m a.s.l.). The average
115 annual air temperature of the study site was 7.1 °C, with maximum temperature occurring in July and
116 minimum in January; the annual mean air temperatures were 8.7 °C and 7.9 °C in 2010 and 2011,
117 respectively (Fig. 2); the average annual rainfall was 360 mm, with >70% of rainfall occurring from
118 mid-June to September (Fig. 2), and the mean monthly precipitations were 23.2 mm and 27.8 mm in

119 2010 and 2011, respectively (Fig. 2), and the precipitation in September 2011 was 104.4 mm, which far
120 exceeded the precipitation in other months (mean monthly precipitation: 27.8 mm). The average annual
121 potential evaporation was 1993 mm. The spring and autumn seasons at the study site were typically
122 short; summer was hot and humid and occurred when most rainfall occurred; winter was long, cold,
123 and dry; soil was classified as sandy, free-draining loess, and the rangeland was a typical temperate
124 steppe (Hou et al. 2002). Dominant grassland species were *Stipa bungeana*, *Lespedeza davurica*,
125 *Pennisetum flaccidum*, *Artemisia capillaris*, and *Setaria viridis* (Hou et al. 2002).

126

127 **2.2 Experimental design**

128 2.2 Experimental design

129 Two sites with similar topography, vegetation, and cover were selected for separate warm-season
130 (summer) and cold-season (winter) grazing trial plots. Each site was divided into 12 enclosed 0.5-ha
131 replicate plots (i.e., three replicates for each of the four stocking rates); and stocked with 0, 4, 8, and 13
132 wether sheep of similar liveweight, representing stocking rates of 0, 2.7, 5.3, and 8.7 sheep ha⁻¹,
133 respectively (Fig. 1). The classification of stocking rates was based on local habitat productivity, and
134 the method of GI calculation was based on the number of wether sheep allocated to a specific GI
135 treatment divided by the combined area of 1.5 ha (i.e., 2.7 sheep ha⁻¹ = 4 sheep/1.5 ha) for the three
136 replicates in each grazing season (Chen et al. 2010). The specific methods used for rotational grazing
137 were: In each grazing season, the wethers were allocated to three replicates for each of the four
138 stocking rates, and rotationally grazed between each replicate plot allocated to that stocking rate. The
139 WG plots were rotationally grazed from June to September (90 days), with a rotation cycle length of 30
140 days (10 days grazing and 20 days rest) and three rotations. The CG plots were grazed from
141 mid-November to late December (48 days), with a rotation cycle length of 24 days (eight days grazing
142 and 16 days rest) and two rotations. The rotational grazing system field experiment began in 2001. The
143 Rs measurements were conducted in both WG and CG plots in 2010, after the trial site had been
144 rotationally grazed for the previous 9 years.

145

146 **2.3 Rs measurement**

147 For the field measurement of Rs, soil CO₂ efflux chambers (six PVC collars permanently placed in
148 each plot) were attached to each of the two gas analyzers (LI-COR 8150, Lincoln, NE, USA), which

149 were rotated in a 2-h cycle around all plots in each GP group (WG and CG). The diurnal R_s flux in
150 each plot was measured at 2h intervals between 6:00 am and 10:00 pm, on six fine days in mid-May
151 (early growth stage of herbage), September (peak aboveground biomass), and December (dormancy)
152 only in 2010 and 2011. Sampling sites were randomly selected at 30–40-m intervals along three 50-m
153 long transects per plot, with two PVC collars (11 cm diameter \times 5 cm height) per transect (i.e., six
154 collars per plot). In preparation for R_s measurement, all aboveground vegetation in each PVC collar
155 was clipped to ensure only R_s was measured. The measurement by gas analyzer took approximately 1.5
156 min to complete per chamber, connected to six soil efflux collars per plot for a total of 9 min.

157

158 **2.4 Soil temperature and soil moisture**

159 Soil temperature at 10 cm depth was measured in real time using a thermocouple probe attached to
160 every soil efflux collar during each R_s measurement. The soil moisture samples for gravimetric
161 analysis were taken from the top 10 cm, close to every soil efflux collar, once daily in mid-morning
162 (9:00–11:00 h) on the R_s sampling days, and were then oven dried at 105 °C for 48 h.

163

164 **2.5 Soil sampling and biomass measurements**

165 Soil samples were collected from random locations adjacent to each R_s measurement site at 5 cm and
166 10 cm depths during the R_s determination period. After soil samples were coarsely sieved (4.75 mm) to
167 remove rocks and large roots, they were sealed in plastic bags and immediately transported to the
168 laboratory for analysis. The **aboveground biomass** was estimated by cutting all vegetation in 1 m \times 1 m
169 quadrats (six per plot) after the plots were grazed during the second rotation in early September of 2010
170 and 2011, when the aboveground biomass peaked. The samples were oven dried at 65 °C to a constant
171 weight. Once the **aboveground biomass** and litter were harvested, soil cores (10 cm depth, 10.0 cm
172 diameter) to a depth of 1 m were collected using a soil auger to calculate the belowground biomass in
173 each quadrat, using the method described by Chen et al. (2015).

174

175 **2.6 Soil microbial carbon and nitrogen**

176 SMBC and SMBN were determined using a chloroform fumigation-extraction procedure and were
177 calculated using the difference in dissolved organic carbon and dissolved organic nitrogen between the
178 fumigated and non-fumigated soil subsamples (Brookes et al. 1985; Vance et al. 1987). Briefly, 10 g

179 soil samples were fumigated with chloroform for 24 h in a vacuum desiccator, and other 10 g samples
180 served as non-fumigated controls. Carbon and nitrogen were extracted with 50 ml of 0.5 M K₂SO₄ for
181 30 min from fumigated and non-fumigated samples, and the extracts were filtered and frozen at -20 °C
182 before analysis with a Total Dissolved Organic Carbon and Nitrogen Analyzer-multi NC 2100S
183 (Analytik Jena AG, Jena, Germany).

184

185 2.7 Statistical analysis

186 One-way analyses of variances (ANOVAs) followed by least significant difference (LSD) tests were
187 performed to examine the effects of GI (0, 2.7, 5.3, and 8.7 sheep ha⁻¹) and GP (WG and CG) on
188 diurnal fluctuations of Rs. One-way ANOVAs were also used to examine the effect of GI and GP on
189 aboveground biomass, belowground biomass, soil temperature, and soil moisture. The
190 repeated-measures ANOVAs were performed to examine the effect of GI and GP on seasonal variations
191 in Rs ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and soil microbial carbon (g kg^{-1}) and nitrogen (g kg^{-1}) in the May,
192 September, and December of 2010 and 2011 (sampling times). A Pearson correlation analysis was used
193 to test the associations between monthly precipitation and Rs, SMBC, SMBN, soil moisture, soil
194 temperature, and the responses of those variables to monthly precipitation (two-tailed test). Significant
195 differences for all statistical tests were evaluated at the level of $P \leq 0.05$. To investigate the temperature
196 sensitivity of Rs, regression analyses were conducted using $R_s = ae^{bT}$, where Rs is soil respiration, T is
197 soil temperature, coefficient a is the intercept of soil respiration at 0 °C, and coefficient b represents the
198 temperature sensitivity of Rs that was used to calculate the respiration quotient $Q_{10} = e^{10b}$ (Luo et al.
199 2001). For means, a two-sample t-test was used to determine the significance of difference between the
200 Q_{10} values under different GPs. Unless specified, the significance level was set at $P < 0.05$, and
201 uncertainty (\pm) always referred to a 95% confidence level. All statistical analyses were conducted using
202 SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

203 Structural equation modeling (SEM) was used to evaluate the pathways through which GP and GI
204 affect Rs both directly and indirectly via biotic and abiotic factors. This was carried out according to
205 the priori conceptual model to include all possible pathways (Supplementary Fig. 1), including (1)
206 direct and indirect pathways of GP and GI influence on aboveground biomass, belowground biomass,
207 soil temperature, and soil moisture; (2) direct and indirect pathways of GP and GI influence on soil
208 microbial carbon and nitrogen; and (3) direct and indirect pathways of GP or GI influence on Rs via

209 biotic or abiotic factors. Before constructing the SEM models, a correlation matrix was derived for all
210 variables using least-squares. To differentiate the effects of grazing management on Rs, grazing
211 management was divided into two sections. The first SEM was based on bivariate regressions to focus
212 on the direct and indirect effects of GI on Rs, and included aboveground biomass, belowground
213 biomass, soil moisture, soil temperature, SMBC, and SMBN. The second SEM focused on both direct
214 and indirect effects of GP on Rs. After first considering a full model that included all possible pathways,
215 non-significant pathways were sequentially eliminated, until arriving at the final model. The χ^2 test,
216 Akaike information criteria, and root mean square error of approximation were used to evaluate the fit
217 of model. The SEM analyses were conducted using AMOS 17.0 (SPSS Inc., Chicago, IL, USA).

218

219 **3. Results**

220 **3.1 Diurnal and seasonal dynamics of Rs rate under four different GIs and two GPs**

221 Daily maximum, minimum, and total Rs rates under the four GIs in the two grazing seasons are shown
222 in Supplementary Tables 1 and 2, respectively. GI significantly influenced the diurnal variation of Rs in
223 the WG (Table 1, $P < 0.001$) and CG plots (Table 1, $P < 0.001$). In a WG plot, the diurnal Rs
224 significantly decreased as the stocking rate increased (Table 1, $P < 0.001$), but in a CG plot, the diurnal
225 Rs was significantly higher at the GIs of 2.7 and 5.3 sheep ha⁻¹ than at 0 and 8.7 sheep ha⁻¹ (Table 1, P
226 < 0.001). The diurnal Rs was not significantly different between the WG and CG plots in the
227 non-grazing period (Table 1, 0 sheep ha⁻¹, $P = 0.964$); however, the Rs of a WG plot was significantly
228 lower than that for a CG plot in the grazing period (Table 1, 2.7, 5.3, and 8.7 sheep ha⁻¹, $P < 0.001$),
229 indicating that CG increases diurnal soil Rs.

230 From 2010 to 2011, Rs showed obvious seasonal and interannual changes in both WG and CG plots
231 (Fig. 3, Table 2). Compared with the non-grazed plots (0 sheep ha⁻¹), GI significantly increased the Rs
232 between sampling seasons by approximately 22% in 2010 (Table 2, $P = 0.007$), but decreased it by
233 approximately 16% in 2011 (Table 2, $P = 0.011$). The interactions between the GIs and sampling
234 seasons showed no effect on Rs in 2010 and 2011. The GIs (Table 2, $P = 0.826$) and the interactions
235 between the GIs and years (Table 2, $P = 0.070$) had no significant effect on Rs over the whole
236 experiment period. A significant difference in Rs was observed between the WG and CG plots in 2010
237 (Fig. 3, Table 2, $P < 0.001$), but not in 2011 (Table 2, $P < 0.964$). In both years, GP and year, as well as
238 their interactions, had a significant effect on Rs ($P < 0.001$), and Rs was approximately 22% higher in

239 the CG plots than in the WG plots ($P < 0.001$) and was significantly and positively correlated with
240 monthly precipitation (Table 3, $P < 0.001$).

241

242 3.2 SMBC and SMBN

243 SMBC and SMBN at 5 and 10 cm soil depths were characterized by pronounced temporal dynamics
244 between and within sampling seasons over the investigated time across 2010 and 2011 (Figs 4 and 5).
245 Compared with WG, CG significantly increased SMBC by 6% in 2010 (Table 2, $P < 0.001$), but
246 decreased it by 19% in 2011 (Table 2, $P < 0.001$). GI affected SMBC neither in 2010 (Table 2, $P =$
247 0.129), nor in 2011 (Table 2, $P = 0.208$). SMBC was significantly affected by the interactions between
248 GPs and GIs in 2011 (Table 2, $P < 0.001$) and by the GPs and sampling seasons and their interactions in
249 both 2010 (Table 2, $P < 0.05$) and 2011 (Table 2, $P < 0.05$). In both years, GP and GI alone did not
250 significantly affect SMBC (Table 2, $P > 0.05$), but their interactions had a significant effect on SMBC
251 (Table 2, $P < 0.001$). Moreover, SMBC was significantly affected by the interaction between the year
252 and GP (Table 2, $P < 0.001$), but not by the interaction between the year and GI (Table 2, $P = 0.224$).
253 When compared with 2010, the mean concentrations of SMBC decreased by 58% and 69% in the WG
254 and CG plots, respectively, in 2011 (Fig. 4). SMBC was significantly negatively correlated with
255 monthly precipitation (Table 3, $P < 0.001$). SMBN was significantly affected by GP (Table 2, $P < 0.05$),
256 sampling season (Table 2, $P < 0.001$), and the interactions between GI and GP (Table 2, $P < 0.05$) in
257 2010 and 2011. GI alone significantly affected SMBN (Table 2, $P = 0.042$) in 2011, but not in 2010
258 (Table 2, $P = 0.601$). In both years, a significant difference in SMBN was observed between the WG
259 and CG plots ($P < 0.001$); the interaction between GP and GI had a significant effect on SMBN (Table 2,
260 $P = 0.007$). Relative to WG, CG significantly decreased the mean concentration of SMBN by 11% over
261 the entire study period (Table 3, $P < 0.001$). SMBN was significantly positively correlated with monthly
262 precipitation (Table 3, $P < 0.001$).

263

264 3.3 Effects of grazing management on soil temperature, soil moisture, aboveground biomass, and 265 belowground biomass.

266 The diurnal variation in soil temperature, measured at 2-h intervals at depths of 0–10 cm, changed
267 significantly over time (Supplementary Fig. 2, $P < 0.05$). No significant differences in soil temperature

268 were observed in both CG and WG plots under different stocking rates (2.7, 5.3, and 8.7 sheep ha⁻¹) (P
269 = 0.63). Soil temperature in the CG plots was 3.1 °C higher (P = 0.02) than that in the WG plots
270 (Supplementary Fig. 2). The soil temperature varied significantly (P <0.001) by year, with the highest
271 values observed in 2011 and the lowest in 2010 (Supplementary Fig. 2). The annual mean soil moisture
272 in 2011 was 7.9% higher (P <0.001) than that in 2010 (Supplementary Fig. 3). GI did not affect soil
273 moisture (P = 0.87); however, the CG plots increased soil moisture by 13.2% compared with that in the
274 WG plots (P = 0.005). A significant positive correlation was observed between monthly precipitation
275 and soil temperature and soil moisture (Table 3, P <0.001). The GI significantly decreased the
276 aboveground (Supplementary Fig. 4, P <0.001) and belowground biomass (Supplementary Fig. 4, P =
277 0.001). GP significantly affected the aboveground biomass (P <0.001), but not the belowground
278 biomass (P = 0.071). The aboveground biomass was higher in the CG plots than in the WG plots (by
279 16%).

280

281 **3.4 Effect of grazing management on temperature sensitivity of soil respiration (Q_{10})**

282 During the whole experimental period, a significant exponential positive correlation between the rates
283 of R_s change and soil temperature was observed in both WG plots and CG plots, indicating that R_s is
284 strongly influenced by soil temperature (P <0.001, Table 3). A significant difference in the Q_{10} values
285 of R_s was observed among the GIs in both WG and CG plots (P <0.001, Table 3). With regard to the
286 GPs, the Q_{10} values of the WG plots were significantly higher than those of the CG plots (Table 3).

287

288 **3.5 Structural equation models**

289 The structural equation models showed that R_s was indirectly explained by GI, which explained 57%
290 of the total variation in R_s in the grazing ecosystem (Figs 6 and 7). GI decreased R_s via its negative
291 effect on aboveground biomass, whereas it increased R_s via its positive effect on soil microbial
292 biomass (Fig. 6). These negative and positive indirect effects of GI on R_s offset the direct effect of GI
293 on R_s (Fig. 6). GP explained 57% of the total variation in R_s in the grazing ecosystem (Fig. 7). We not
294 only observed a direct positive effect of GP on R_s , but also an indirect effect of GP on R_s via its
295 positive effects on soil moisture, soil temperature, and aboveground biomass. In addition, the negative
296 effect of GP on R_s via its negative effect on SMBC and SMBN weakened the positive influence of GP
297 on R_s .

298

299 4. Discussion

300 4.1 Effect of grazing management on Rs

301 The diurnal changes in Rs under different GPs (warm and cold season) with four GIs (0, 2.7, 5.3, and
302 8.7 sheep ha⁻¹) observed in our study were within the range of the daily Rs reported by several
303 previous studies (Zhang et al. 2014; Wang et al. 2015; Rong et al. 2017). We found that GI significantly
304 affected the diurnal changes of Rs in both WG ($P < 0.001$) and CG plots ($P < 0.001$), but the response
305 was different. Since the diurnal changes in Rs followed a unimodal pattern through time, consistent
306 with soil temperature, this could be due to the differences in sensitivity to temperature in grazing
307 seasons or could be due to the spatial heterogeneity of Rs (Wang et al. 2013; Wang et al. 2017). Our
308 study revealed that CG enhances the diurnal Rs rate. These results are consistent with those of a
309 previous study that performed a meta-analysis of Tibetan grasslands (Wang et al. 2017). The GI had a
310 significant effect on seasonal changes in Rs in both 2010 ($P = 0.007$) and 2011 ($P = 0.011$); however,
311 the interaction between season and GI had no significant effect on Rs in 2010 ($P = 0.253$) and 2011 (P
312 $= 0.153$). It was also found that both GI ($P = 0.826$) and the interaction between GI and year ($P = 0.070$)
313 had no effect on Rs throughout the whole experimental period; this could be explained by both biotic
314 and abiotic pathways (Fig. 6) in the following two ways: 1) The negative impact of GI on Rs was
315 achieved indirectly by directly negatively affecting the aboveground biomass or by indirectly
316 negatively affecting the microbial biomass; 2) At the same time, GI had a positive and indirect effect on
317 Rs, which was achieved by directly negatively affecting the belowground biomass or by directly
318 affecting soil moisture. Consequently, these two different types of pathways offset each other within a
319 certain range.

320

321 The results of this study indicate that GP ($P < 0.001$), year ($P < 0.001$), and their interaction ($P < 0.001$)
322 have strong effects on Rs. Compared to WG, CG promoted soil respiration by 22%. These results were
323 supported by the SEM analyses performed in this and previous studies (Jiang et al. 2010; Wang et al.
324 2013; Chen et al. 2015; Xu et al. 2016). The response of Rs to GP can be explained by the following
325 three mechanisms (Fig. 7): (1) The CG increased soil temperature by 3.1 °C and increased soil moisture
326 by 13.2%, and both soil temperature and soil moisture had direct positive effects on Rs. Soil
327 temperature and water availability effect Rs by altering the activity of plant roots and soil microbes,

328 and they also indirectly affect soil respiration by altering plant growth and substrate supply (Wan et al.
329 2007). In addition, the effect of soil temperature on Rs can be explained by the distribution of seasonal
330 precipitation and interannual precipitation (Ru et al. 2017). This study found a significant correlation
331 between monthly precipitation and Rs ($P < 0.001$); the precipitation of the semi-arid grassland peaked in
332 September 2011, strongly influencing soil respiration. The significant interannual variations in Rs
333 might be mainly caused by seasonal precipitation fluctuations; this could be proved by setting up a
334 controlled field experiment. (2) Compared with WG, CG significantly increased the aboveground
335 biomass ($P < 0.001$), which directly affected Rs. This might be due to the increased sensitivity of the
336 aboveground biomass to low temperatures (Abdalla et al. 2010). On one hand, CG reduces litter and
337 increases sunshine exposure, which is beneficial for plant growth in the subsequent year (Coughenour
338 1991; Altesor et al. 2005; Wang et al. 2017). On the other hand, grazing leads to a warm and dry
339 microclimate by removing aboveground plants and compacting the soil (Rong et al. 2017). (3) GP has a
340 negative effect on Rs through its negative effects on soil microbial biomass and microbial nitrogen.
341 Since Rs is a process of converting organic carbon to inorganic carbon, the rate of Rs is ultimately
342 controlled by the supply of carbon substrates (Xu et al. 2016; Bagchi et al. 2017). Soil microbial
343 communities exhibit high substrate utilization rates at low temperatures (Monson et al. 2006); thus, a
344 decrease in microbial biomass will reduce soil carbon emissions (Allison et al. 2010). Previous studies
345 have reported that SMBC primarily determines microbial respiration (Zhang et al. 2014). Significant
346 changes in SMBC and SMBN have significant effects on CO_2 emissions under different land-use
347 patterns (Iqbal et al. 2010).

348 Previous studies have been conducted on the temperature sensitivity of Rs (Q_{10}), both globally and in
349 different ecosystems of China (Luo et al. 2001; Curiel et al. 2004; Davidson et al. 2006; Chen et al.
350 2015; Chen et al. 2016). In our study, under different grazing rates, the Q_{10} values ranged between 1.31
351 and 1.57 for the WG plots and between 1.21 and 1.36 for the CG plots, agreeing with the results of
352 Chen et al. (2015). The Q_{10} values of the WG plots were higher than those of the CG plots. This might
353 be due to soil water freezing at low temperatures, which inhibits soil microbial activity, and thus
354 reduces Q_{10} in the CG seasons (Mahecha et al. 2010; Chen et al. 2016). The Q_{10} values for different
355 ecosystems on a global scale are different, but the median is 1.4 (Mahecha et al. 2010). Our results are
356 similar to this median, indicating that it is necessary to consider the impact of Rs on climate warming
357 under different GIs and different grazing systems in order to accurately assess the carbon cycle of

358 semi-arid grassland ecosystems. Overall, our results indicate that the mechanisms underlying the
359 effects of grazing management on Rs mainly depend on GP, but not on GI. This indicates that the
360 effects of GP, especially seasonal and long-term grazing, should be considered in future manipulation
361 experiments and carbon models to accurately simulate soil carbon dynamics in semi-arid grassland
362 ecosystems.

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364 **4.2 Effects of grazing management on SMBC and SMBN**

365 The results of our study revealed that SMBC was higher at the beginning (May) and in the middle
366 (September) of the growing season than in the dormant period (December) (Fig. 4). These results are
367 consistent with the findings of previous studies conducted in the grassland ecosystems of the
368 trans-Himalaya (Bagchi et al. 2017) and the Tibetan Plateau (Fu et al. 2012). We found that SMBN in
369 the WG plots was significantly higher than that in the CG plots ($P < 0.001$). The reason for this might
370 be that the response of SMBN is more sensitive to grazing than that of SMBC (Fu et al. 2012). CG
371 increased the soil temperature, which increased microbial biomass (Lu et al. 2013; Wang et al. 2017).
372 Our results show that GI did not have an effect on SMBC ($P > 0.05$) and SMBN ($P > 0.05$), but the
373 interactions between GI and GP significantly affected SMBC ($P < 0.001$) and SMBN ($P < 0.007$). These
374 results indicate that the response of SMBC is coupled with GI and GP. On one hand, high GI increases
375 bulk density and urine input and decreases soil porosity and aggregation, affecting microorganism
376 metabolism (Prieto et al. 2011; Liu et al. 2012,). On the other hand, GI leads to a variation in SMBC as
377 a direct result of the soil water content from higher precipitation and temperature.

378 According to our SEM analysis, there are two pathways to explain the effects of GI (Fig. 6) and GP
379 (Fig. 7) on SMBC and SMBN. (1) The effect of GI on SMBC and SMBN occurs mainly via its adverse
380 effects on aboveground biomass, which directly stimulates SMBC. Recent studies have shown that the
381 effects of grazing on soil microbial community size are largely dependent on GI via biotic factors
382 (Zhao et al. 2017); grazing decreases the aboveground and belowground biomass (Koerner and Collins
383 2014). Our results support the theory that grazing management could change soil microbial activities
384 by regulating the aboveground and belowground biomass, which in turn changes the microbial biomass
385 in the soil (Stark et al. 2015; Xu et al. 2017). (2) GP positively affects soil temperature and soil
386 moisture, both of which stimulate SMBC and SMBN. In our study, compared with WG, CG
387 significantly increased soil moisture ($P = 0.005$) and soil temperature ($P = 0.020$), which might

388 stimulate more efficient enzymes to catalyze the reactions of soil organic matter decomposition (Stark
389 et al. 2015). Moreover, the dissolved organic carbon was metabolized only after rewetting, and the
390 chemical signals released by the roots regulate the microbial communities, some of which have
391 powerful feedbacks in carbon cycling (Schimel et al. 2013). Monthly precipitation events were
392 significantly negatively related to SMBC ($P < 0.001$), but significantly positively related to SMBN in
393 our study ($P < 0.001$). This agrees with the previous studies that found that the precipitation events
394 stimulating microbial activity might shift the C-balance of grassland ecosystems (Curiel et al. 2007)
395 and that grazing interacts with precipitation to affect the belowground biomass (Koerner and Collins
396 2014). Overall, our results indicate that the effects of grazing management on SMBC and SMBN
397 mainly depend on the interactions between GI and GP. This suggests that integrated grazing
398 management strategies should be taken into account in future studies on nutrient turnover in the soils of
399 semi-arid grassland ecosystems.

400

401 *Author contributions.* Fujiang Hou designed and directed the study, Zhen Wang carried out the
402 data analysis, and wrote the manuscript. Xiuli Wan, Junbo Chen, Mei Tian, Xiayan Wang,
403 Xianjiang Chen, Shenghua Chang collected samples, analyzed the data, and contributed to the
404 final writing of the manuscript.

405

406 *Data availability.* from the corresponding author, Fujiang Hou (cyhoufj@lzu.edu.cn), upon request.

407

408 *Competing interest.* The authors declare that they have no conflicts of interest.

409

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629 **Table 1.** Effect of warm-season grazing (WG) and cold-season grazing (CG) on daily soil
630 respiration (Rs) under different grazing intensities (GI). F- and P-values were obtained by the
631 one-way ANOVA analyses. Different letters in the same column indicate statistical significant
632 differences between grazing intensity under certain grazing season ($P < 0.05$). Data are shown as
633 the mean \pm SE (n=800).

GI (sheep ha ⁻¹)	Rs (μ mol CO ₂ m ⁻² s ⁻¹)				
	WG	CG	df	F-Value	P-Value
0	0.91 \pm 0.02a	0.91 \pm 0.02c	1.00	0.002	0.964
2.7	0.81 \pm 0.02b	1.30 \pm 0.02a	1.00	307.444	0.001
5.3	0.76 \pm 0.02c	1.28 \pm 0.02a	1.00	380.222	0.001
8.7	0.76 \pm 0.02c	1.18 \pm 0.02b	1.00	288.455	0.001

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654 **Table 2.** Effects of grazing regime (GP), grazing intensities (GI), year, sampling season , and their

655 interactions on the rates of Rs, SMBC, and SMBN. F-and P-values are obtained by the

656 **repeated-measures ANOVA analyses.** Values in bold indicate significance levels of $P < 0.05$.

Year	Factors	Rs			SMBC			SMBN		
		df	F Value	P	df	F Value	P	df	F Value	P
2010	GP	1	83.672	<0.001	1	21.270	<0.001	1	5.008	0.027
	GI	3	4.234	0.007	3	1.922	0.129	3	0.623	0.601
	GI×GP	3	9.427	<0.001	3	44.321	<0.001	3	5.976	<0.001
	Season	2	218.389	<0.001	2	68.065	<0.001	2	25.685	<0.001
	GP×Season	2	6.186	0.003	2	15.389	<0.001	2	5.976	<0.001
	GI×Season	6	1.317	0.253	6	3.828	<0.001	6	4.515	0.013
	GI×GP×Season	6	0.867	0.521	6	5.417	<0.001	6	2.192	0.047
2011	GP	1	0.002	0.964	1	28.930	<0.001	1	12.725	<0.001
	GI	3	3.853	0.011	3	1.534	0.208	3	2.812	0.042
	GI×GP	3	0.205	0.893	3	2.186	0.092	3	2.966	0.034
	Season	2	333.087	<0.001	2	29.162	<0.001	2	81.204	<0.001
	GP×Season	2	6.640	0.002	2	3.353	0.038	2	20.360	<0.001
	GI×Season	6	1.593	0.153	6	3.682	0.002	6	9.269	<0.001
	GI×GP×Season	6	1.498	0.183	6	2.955	0.009	1	0.000	0.526
Overall	GP	1	12.391	<0.001	1	0.197	0.658	1	18.129	<0.001
	GI	3	0.299	0.826	3	2.470	0.062	3	1.873	0.134
	GI×GP	3	1.395	0.244	3	22.313	<0.001	3	4.154	0.007
	Year	1	36.942	<0.001	1	1280.544	<0.001	1	0.995	0.319
	GP×Year	1	16.867	<0.001	1	16.170	<0.001	1	3.473	0.063
	GI×Year	3	2.375	0.070	3	1.396	0.244	3	2.051	0.107
	GI×GP×Year	3	1.867	0.135	3	20.096	<0.001	3	3.195	0.024

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666 **Table 3.** Pearson correlation between monthly precipitation (P) and soil temperature (ST),soil
667 moisture (SM) soil respiration (Rs), soil microbial biomass carbon (SMBC) and soil microbial
668 biomass nitrogen (SMBN). *** indicate significance level at $P < 0.001$.

Variables	ST	SM	SR	SMBC	SMBN
P	0.298***	0.737***	0.475***	-0.162***	0.543***

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694 **Table 4.** Temperature sensitivity of soil respiration (Q_{10}) for the grazing regimes (warm-season
695 grazing, WG; cold-season grazing, CG) with different grazing intensities (GI, 0, 2.7, 5.3, 8.7 sheep
696 ha^{-1}). a and b are two coefficients in the regression line $R_s = ae^{bT}$, where R_s is soil respiration and T is
697 soil temperature. r^2_{adj} is the adjustive determinant coefficient, Q_{10} is the temperature quotient ($= e^{10b}$).
698 Significance levels of $P < 0.05$ indicated in bold.

GP	GI (sheep ha^{-1})	a	b	r^2_{adj}	P	Q_{10}
WG	0	0.510	0.027	0.212	<0.001	1.310
	2.7	0.318	0.045	0.478	<0.001	1.568
	5.3	0.313	0.043	0.458	<0.001	1.537
	8.7	0.343	0.039	0.373	<0.001	1.477
CG	0	0.473	0.031	0.271	<0.001	1.363
	2.7	0.689	0.019	0.155	<0.001	1.209
	5.3	0.643	0.022	0.200	<0.001	1.246
	8.7	0.598	0.023	0.196	<0.001	1.259

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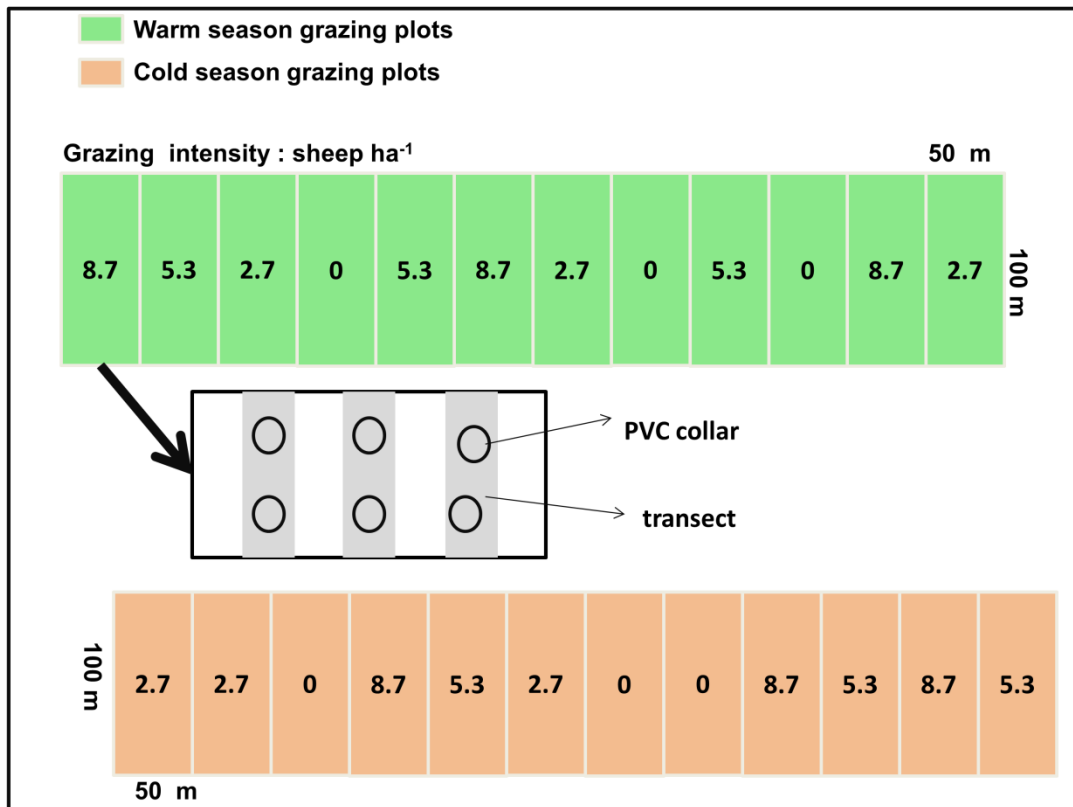
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708 **Figure 1.** Long-term rotational grazing experimental design at the study site. Green box represent warm
 709 season grazing plots; brown boxes represent cold season grazing plots..

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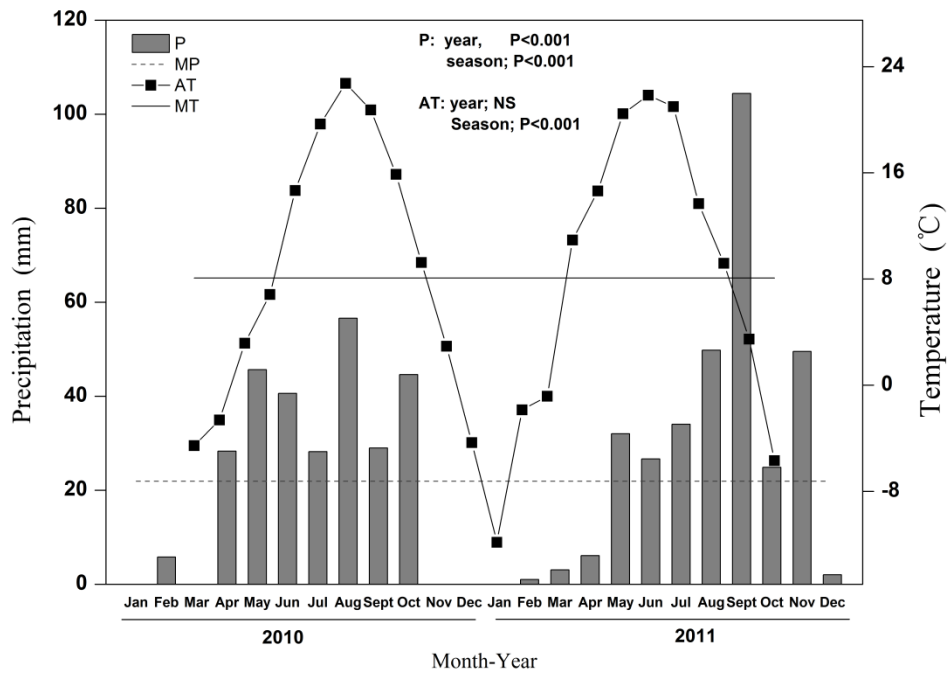
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723 **Figure. 2.** Temporal variation in measured values of air temperature, precipitation, at the study site
 724 from January 2010 to December 2011. Straight line and line of dashes represents mean annual
 725 precipitation represents and mean annual air temperature from 2001 to 2009. The vegetation of the
 726 grassland starts to regreen in late April to early May, and starts to wither in late October. Air
 727 temperature and precipitation did not change significantly during 2010 and 2011.

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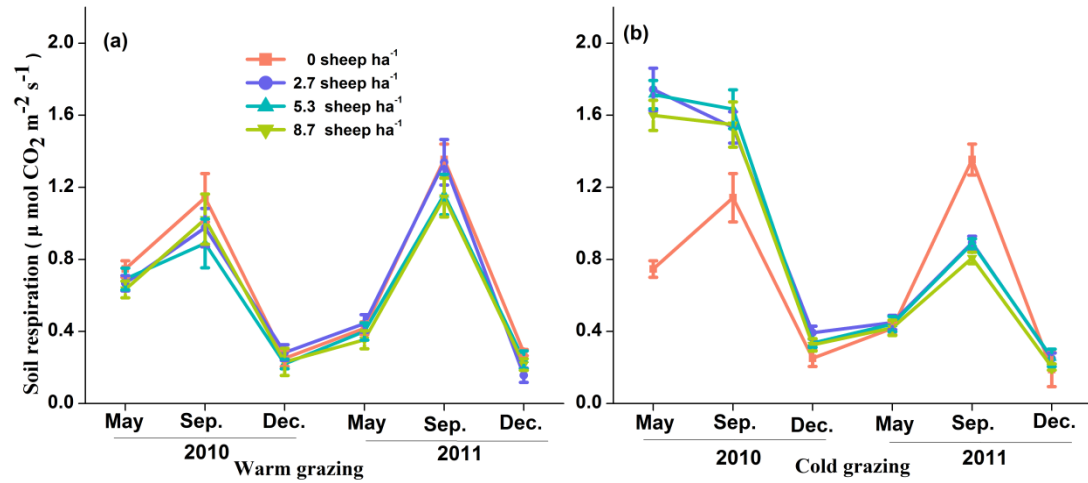
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741 **Figure 3.** Seasonal dynamics of Rs with different grazing intensities (a) in warm season grazing area,

742 (b) in cold season grazing area. Vertical bars indicate mean bars standard errors (n=9).

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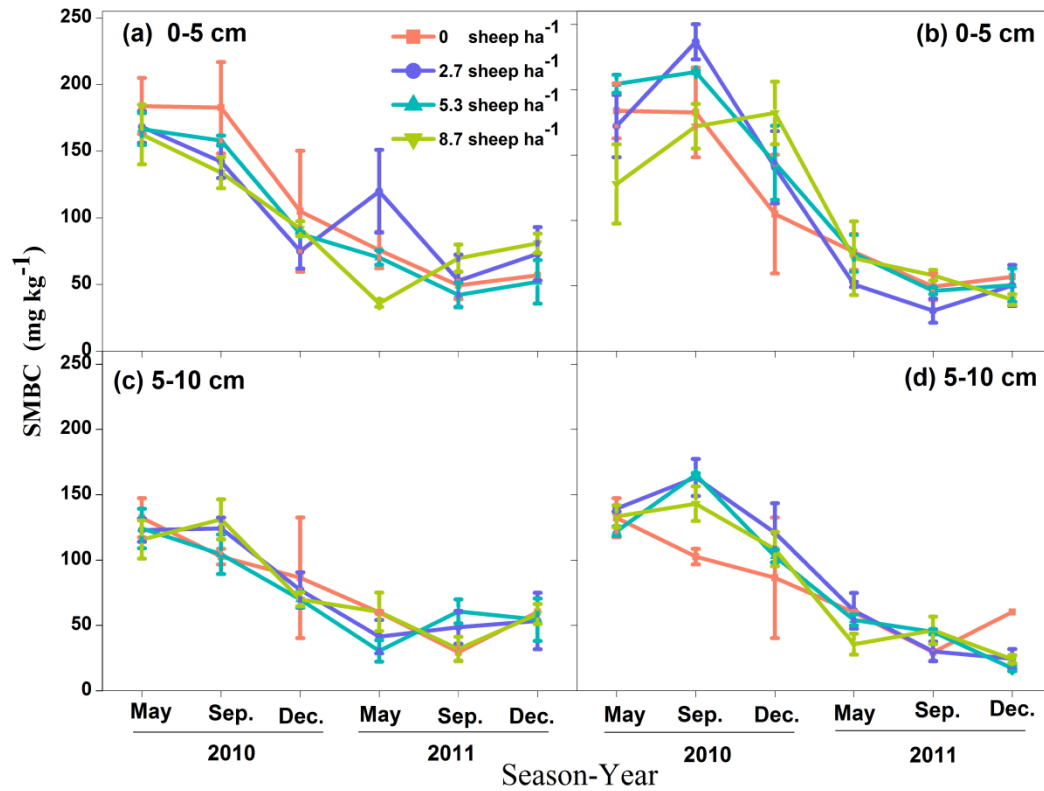
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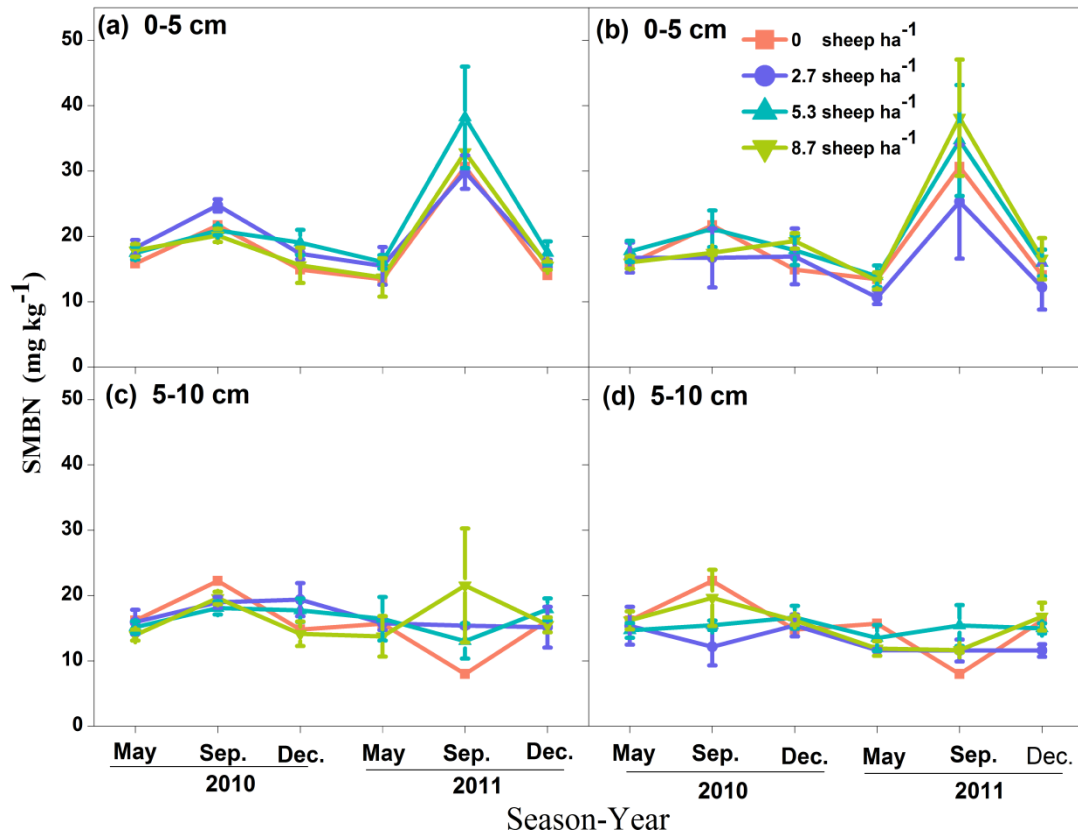
753 **Figure 4.** Seasonal dynamics of Soil microbial biomass carbon (SMBC) (a) 0-5 cm soil layers in
 754 warm-season grazing plots; (b) 0-5 cm in cold-season grazing plots; (c) 5-10 cm in warm-season
 755 grazing plots; (d) 5-10 cm in cold-season grazing plots. Vertical bars represent the standard error of the
 756 measurement mean (n=3) for each observation date.

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762 **Figure 5.** Seasonal dynamics of soil microbial biomass nitrogen (SMBN) (a) 0-5 cm in warm-season
 763 grazing plots; (b) 0-5 cm in cold-season grazing plots; (c) 5-10 cm in warm-season grazing plots; (d)
 764 5-10 cm in cold-season grazing plots. Vertical bars represent the standard error of the measurement
 765 mean (n =3) for each observation date.

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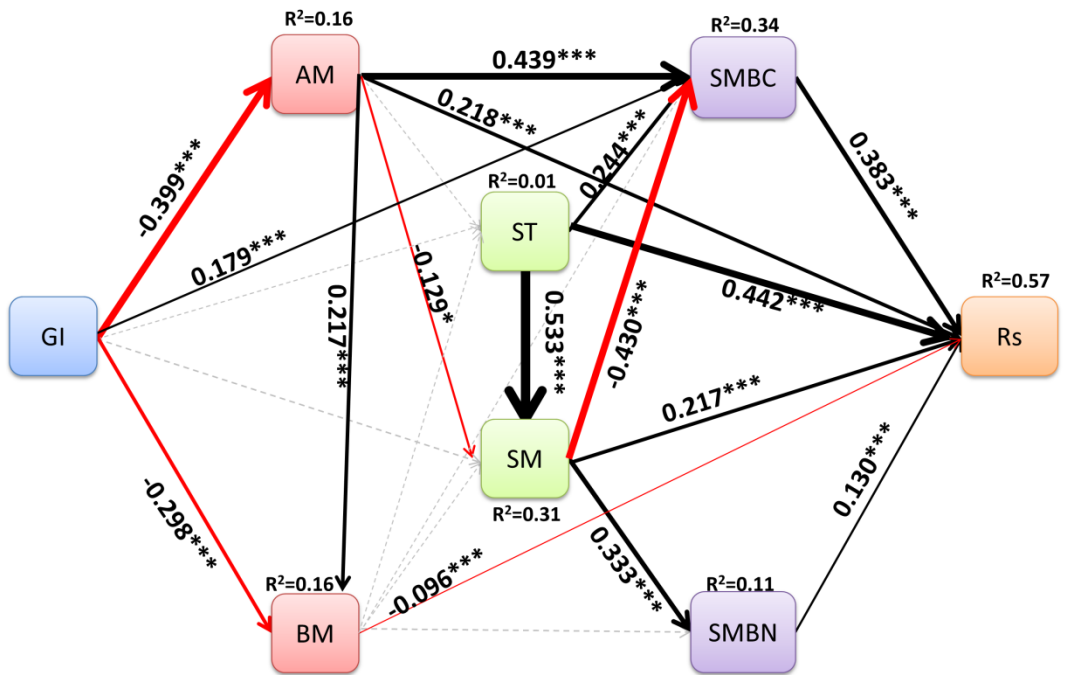
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781 **Figure. 6.** Structural equation model of grazing intensities (Mahecha et al.) effects on soil respiration

782 (Rs) via direct or indirect effect on aboveground biomass (AM), belowground biomass(BM), soil

783 temperature (ST), SM (soil moisture), SMBC (soil microbial carbon), soil microbial nitrogen (SMBN).

784 Red and black arrows represent significant negative and positive pathways, respectively. Bold numbers

785 indicate the standard path coefficients. Arrow width is proportional to the strength of the relationship.

786 R^2 represent the proportion of variance explained for each dependent variable in the model.

787 $***P<0.001$, $**P<0.01$, $*<0.05$; $\chi=10.746$; $P=0.057$; root mean square error of approximation

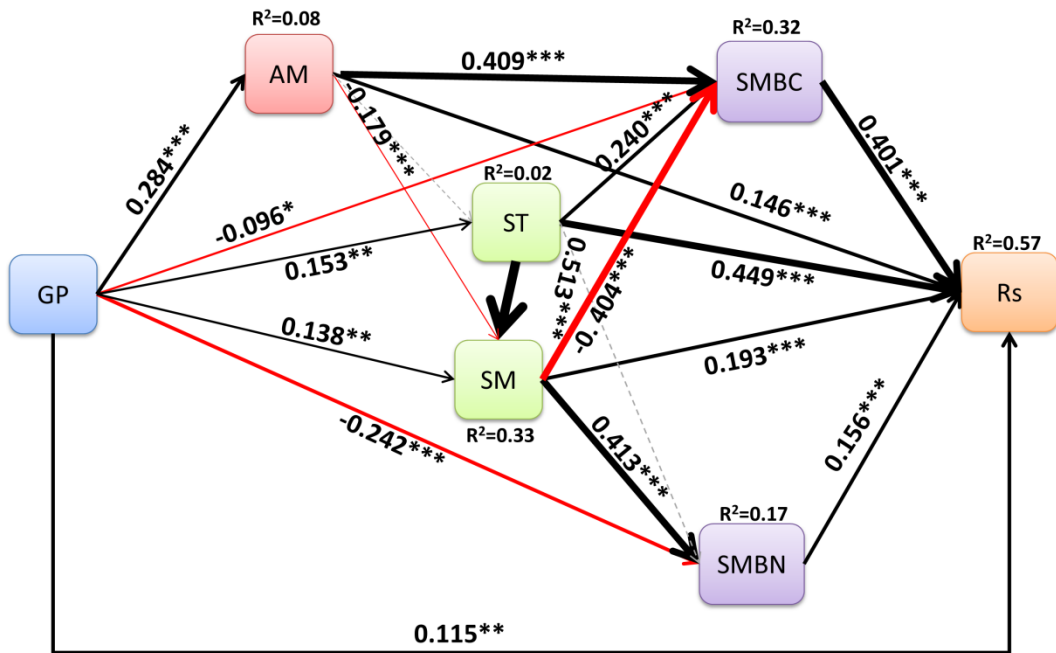
788 (RMSEA) =0.059; $P=0.057$; Akaike information criteria (AIC) =88.746.

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794 **Figure 7.** Structural equation model of grazing regime (GP) effects on soil respiration (Rs) via direct
 795 or indirect effect on aboveground biomass (AM), soil temperature (ST), SM (soil moisture), SMBC
 796 (soil microbial carbon), soil microbial nitrogen (SMBN). The structural equation model considered all
 797 plausible pathways through which experimental treatments influence Rs. Red and black arrows
 798 represent significant negative and positive pathways, respectively. Bold numbers indicate the standard
 799 path coefficients. Arrow width is proportional to the strength of the relationship. R^2 represent the
 800 proportion of variance explained for each dependent variable in the model. $***P<0.001$, $**P<0.01$,
 801 $*<0.05$; $\chi=2.418$; $P=0.299$; root mean square error of approximation (RMSEA) =0.025; $P=0.299$;
 802 Akaike information criteria (AIC) =68.418.

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810 **Supplementary materials**

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812 **Supplementary Table 1.** Daily variation in measured values of soil respiration (Rs) at the warm
 813 grazing site from May 2010 to December 2011 with different grazing intensities (GI).
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Year	Month	GI (sheep ha ⁻¹)	Rs ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$)				Daily Total C efflux ($\text{g CO}_2 \text{m}^{-2} \text{d}^{-1}$)	
			Maximum	time	Minimum	time		Mean \pm SE
2010	May	0	0.90	10:00	0.48	6:00	0.75 \pm 0.06	0.77
		2.7	0.86	10:00	0.49	6:00	0.67 \pm 0.04	0.69
		5.3	1.02	10:00	0.41	6:00	0.69 \pm 0.06	0.72
		8.7	0.82	10:00	0.39	6:00	0.63 \pm 0.03	0.65
	Sep	0	1.69	14:00	0.60	22:00	1.16 \pm 0.05	1.20
		2.7	1.38	14:00	0.54	22:00	0.99 \pm 0.08	1.03
		5.3	1.41	14:00	0.49	22:00	0.91 \pm 0.09	0.94
		8.7	1.62	12:00	0.45	22:00	1.04 \pm 0.09	1.08
	Dec	0	0.32	14:00	0.16	6:00	0.25 \pm 0.04	0.26
		2.7	0.37	14:00	0.23	6:00	0.28 \pm 0.04	0.29
		5.3	0.25	14:00	0.17	6:00	0.22 \pm 0.03	0.23
		8.7	0.360	14:00	0.099	6:00	0.23 \pm 0.08	0.24
2011	May	0	0.55	10:00	0.31	22:00	0.42 \pm 0.05	0.44
		2.7	0.68	10:00	0.30	6:00	0.44 \pm 0.04	0.46
		5.3	0.58	10:00	0.21	6:00	0.39 \pm 0.04	0.40
		8.7	0.58	10:00	0.15	6:00	0.35 \pm 0.03	0.36
	Sep	0	1.69	16:00	1.02	22:00	1.35 \pm 0.08	1.40
		2.7	1.84	16:00	0.81	6:00	1.34 \pm 0.09	1.39
		5.3	1.58	14:00	0.69	6:00	1.16 \pm 0.09	1.20
		8.7	1.62	16:00	0.78	6:00	1.14 \pm 0.06	1.18
Dec	0	0.30	14:00	0.16	6:00	0.24 \pm 0.04	0.25	
	2.7	0.22	14:00	0.084	6:00	0.16 \pm 0.04	0.16	
	5.3	0.34	14:00	0.185	6:00	0.24 \pm 0.05	0.25	
	8.7	0.27	14:00	0.16	6:00	0.22 \pm 0.03	0.22	

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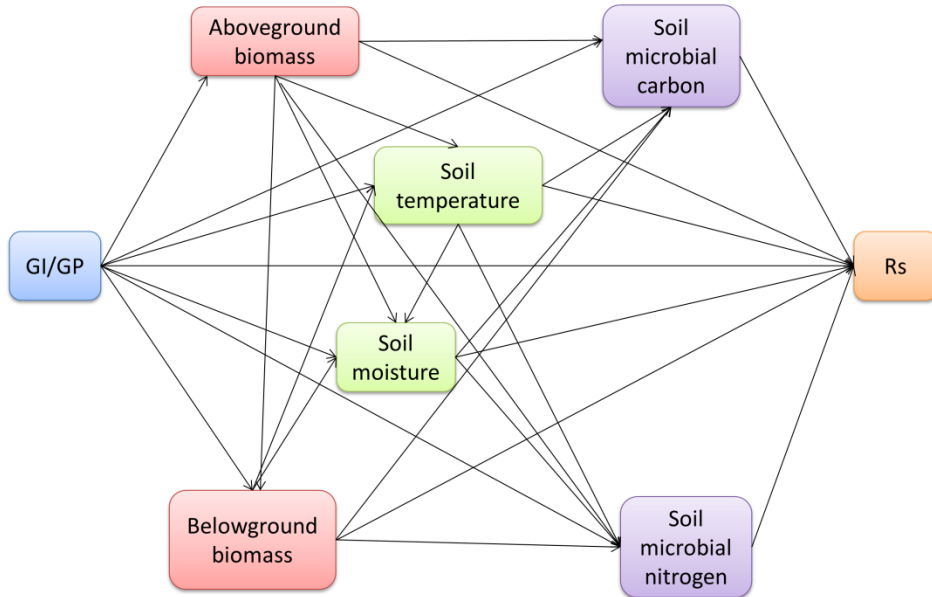
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Supplementary Table 2. Daily variation in measured values of soil respiration (Rs) at the cold grazing site from May 2010 to December 2011 with different grazing intensities (GI).

Year	Season	GI (sheep ha ⁻¹)	Rs ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)				Average \pm SE	Daily Total C efflux (g CO ₂ m ⁻² d ⁻¹)
			Maximum	time	Minimum	time		
2010	May	0	0.90	10:00	0.48	6:00	0.75 \pm 0.06	0.77
		2.7	2.17	16:00	1.35	22:00	1.74 \pm 0.08	1.81
		5.3	1.90	16:00	1.27	6:00	1.71 \pm 0.05	1.78
		8.7	1.87	16:00	1.13	6:00	1.60 \pm 0.05	1.66
	Sep	0	1.69	14:00	0.60	22:00	1.16 \pm 0.11	1.20
		2.7	1.86	14:00	1.23	6:00	1.53 \pm 0.05	1.59
		5.3	2.11	16:00	1.18	22:00	1.63 \pm 0.06	1.69
		8.7	2.02	14:00	1.07	6:00	1.55 \pm 0.09	1.61
	Dec	0	0.32	14:00	0.16	6:00	0.25 \pm 0.04	0.26
		2.7	0.46	14:00	0.34	6:00	0.39 \pm 0.04	0.41
		5.3	0.38	14:00	0.30	6:00	0.34 \pm 0.02	0.35
		8.7	0.37	14:00	0.26	6:00	0.33 \pm 0.03	0.34
2011	May	0	0.55	10:00	0.31	22:00	0.42 \pm 0.05	0.43
		2.7	0.69	16:00	0.31	6:00	0.45 \pm 0.03	0.47
		5.3	0.61	10:00	0.23	22:00	0.44 \pm 0.03	0.46
		8.7	0.61	10:00	0.25	6:00	0.42 \pm 0.03	0.44
	Sep	0	1.69	16:00	1.02	22:00	1.35 \pm 0.08	1.40
		2.7	1.02	16:00	0.72	6:00	0.89 \pm 0.03	0.93
		5.3	1.01	10:00	0.77	6:00	0.89 \pm 0.03	0.92
		8.7	0.95	10:00	0.68	22:00	0.81 \pm 0.03	0.84
Dec	0	0.30	14:00	0.16	6:00	0.24 \pm 0.04	0.25	
	2.7	0.28	14:00	0.19	6:00	0.25 \pm 0.03	0.26	
	5.3	0.34	14:00	0.17	6:00	0.25 \pm 0.05	0.26	
	8.7	0.23	14:00	0.18	6:00	0.21 \pm 0.02	0.21	

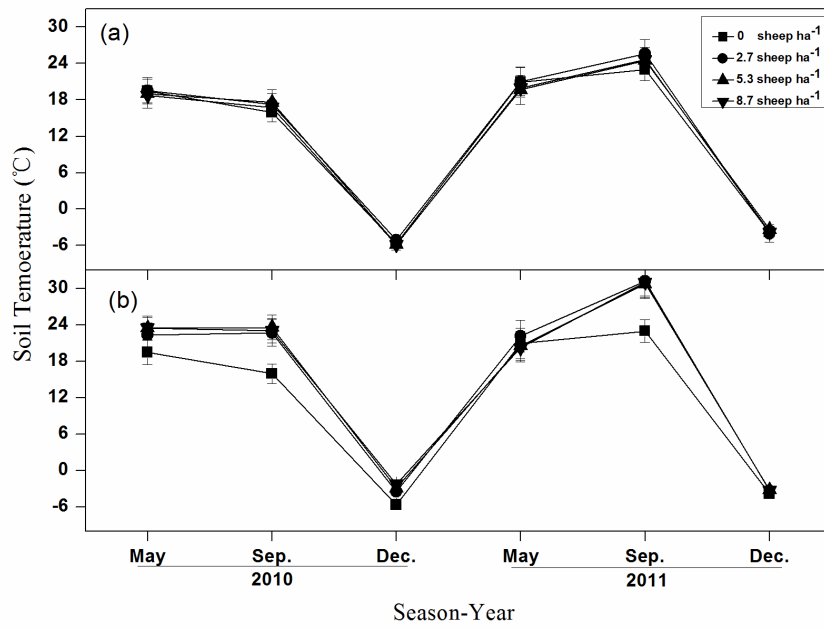
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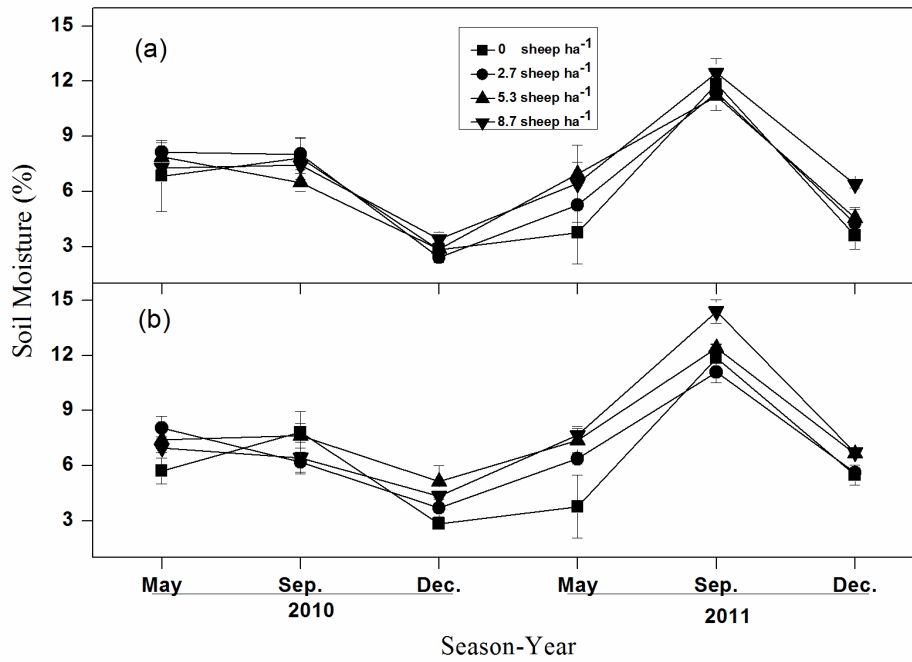
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Supplementary Figure 1 An a priori conceptual model of how grazing intensity (GI), grzing regime (GP) altered soil respiration (Rs), soil microbial carbon and nitrogen. The model contains all possible pathways that cause changes in the abiotic and biotic variables and soil respiration. Box represents variables Arrow direction indicates the hypothesized direction of causation.



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 855 **Supplementary Figure 2** Seasonal dynamics of soil temperature (a) in warm season grazing plots; (b)
 856 in cold season grazing plots; Vertical bars represent the standard error of the measurement mean (n=9)
 857 for each observation date.

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870 **Supplementary Figure 3** Seasonal dynamics of soil moisture (a) in warm season grazing plots; (b)

871 in cold season grazing plots; Vertical bars represent the standard error of the measurement mean (n =3)

872 for each observation date.

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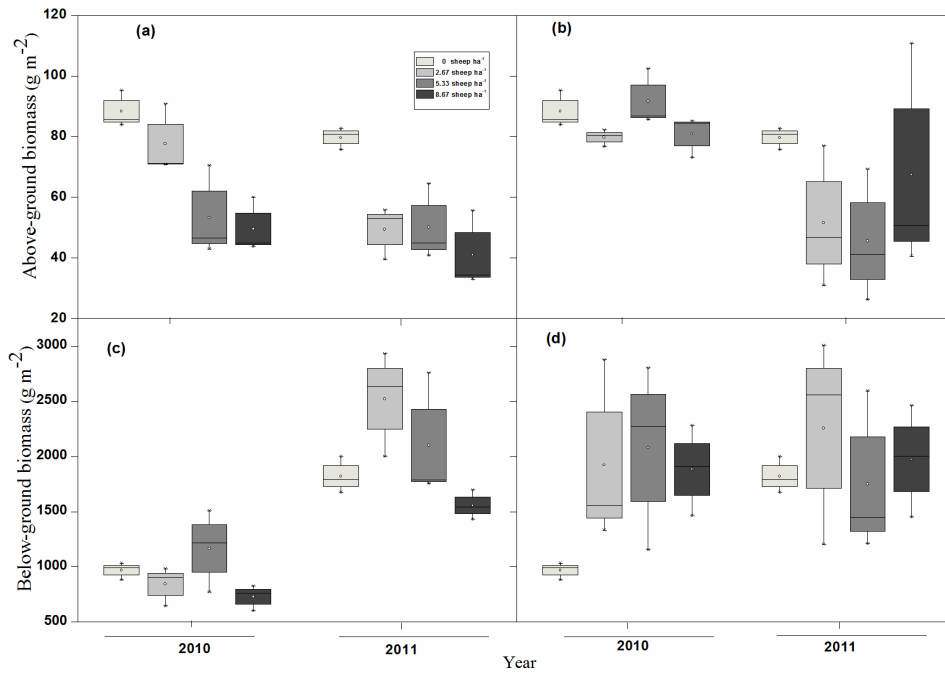
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896 **Supplementary Figure 4.** Aboveground biomass within (a) warm season grazing plots; (b) cold season

897 grazing plots; belowground biomass within (c) warm season grazing plots; (d) cold season grazing

898 plots from 2010 to 2011. Horizontal lines in boxes show medians and dashed whiskers show data

899 extremes. Open circles and solid whiskers show means \pm propagated standard errors.

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