# Soil respiration and its $Q_{10}$ response to various grazing systems of a typical steppe in Inner Mongolia, China

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## Abstract

**Background:** As one of the important management practices of grassland ecosystems, grazing has fundamental effects on soil properties, vegetation and soil microbes. Grazing can thus alter soil respiration and the soil carbon cycle, yet its impacts and mechanisms remain unclear.
**Methods:** To explore the response of soil carbon flux and temperature sensitivity to different grazing systems, soil respiration (Rs), soil temperature (ST) and soil moisture (SM) were observed from December 2014 to September 2015 in a typical steppe of Inner Mongolia under three grazing systems: year-long grazing, rest-rotation grazing and grazing exclusion. In addition, plant aboveground and root biomass, soil microbial biomass and community composition, and soil nutrients were measured during the pilot period.
**Results:** Rs was significantly different among the three grazing systems. The average Rs was highest under rest-rotation grazing (1.26 μmol·m$^{-2}$·s$^{-1}$), followed by grazing exclusion (0.98 μmol·m$^{-2}$·s$^{-1}$) and year-long grazing (0.94 μmol·m$^{-2}$·s$^{-1}$). Rs was closely associated with ST, SM, potential substrate and root and soil microbe activity. The effects of grazing among two grazing systems had generality, but were different due to grazing intensity. The root biomass was stimulated by grazing, and the rest-rotation grazing system resulted in the highest Rs. Grazing led to decreases in aboveground and microbial biomass as well as the loss of soil total nitrogen and total phosphorus from the steppe ecosystem, which explained the negative effect of grazing on Rs in the year-long grazing system compared to the grazing exclusion system. The temperature sensitivity of Rs ($Q_{10}$) was higher in the rest-rotation and year-long grazing systems, likely due to the higher temperature sensitivity of rhizosphere respiration and higher 'rhizosphere priming effect' in the promoted root biomass. The structural equation model analysis showed

38  that while grazing inhibited Rs by reducing soil aeration porosity, ground biomass and soil
39  moisture, it increased $Q_{10}$ but had a lower effect than other factors. A better understanding of the
40  effects of grazing on soil respiration has important practical implications.
41

## Introduction

43  The carbon stored in soil and vegetation plays an important role in the carbon pool of a terrestrial
44  ecosystem. The terrestrial ecosystem carbon cycle includes carbon sources and sinks in the air,
45  plants, and soil communities (Piao et al., 2009). Carbon is accumulated by plant photosynthesis
46  and released by soil respiration (Rs). Rs is fundamental to the global carbon cycle because even
47  small variations in soil respiration can influence the atmospheric $CO_2$ concentration and soil
48  organic carbon (SOC) storage (Zhang et al., 2015b). Studying Rs and its temperature sensitivity
49  can contribute to the understanding of how the terrestrial C cycle will respond to rising
50  temperatures under global climate change.
51  Temperate grassland ecosystems make up 32% of the earth's terrestrial ecosystems (Adams et
52  al., 1990). Grassland soil $CO_2$ emissions into the atmosphere contribute substantially to the
53  global carbon cycle (Craine and Wedin, 2002). Soil respiration reflects the metabolic activities of
54  roots and soil microbes. It has been acknowledged that soil respiration can be affected by soil
55  temperature, soil moisture (Raich and Schlesinger, 1992) and soil element content (Shi et al.,
56  2015). As one of the important management practices in grassland ecosystems, grazing is
57  suggested to affect the quantity of carbon released from the soil (Kioko et al., 2012; Jiao et al.,
58  2012), despite current evidence suggesting no clear relationship between grazing and the carbon
59  budget in grassland ecosystems (Liu et al., 2016; Wang et al., 2002). It is suggested that grazing
60  may decrease soil $CO_2$ flux during the growing season by reducing canopy photosynthesis and
61  carbon translocation to the rhizosphere (Bremer et al., 1998). In addition, grazing can also
62  decrease Rs by destroying the soil microenvironment and inhibiting microbial growth (Zhao et
63  al., 2017). In addition, grazing may promote nutrient cycling and thus provide more respiratory
64  substrates for microbes (Stark et al., 2002; Allard et al., 2007). Grazing exclusion, one of the
65  most common grazing systems for grassland restoration in Inner Mongolia that is usually
66  achieved by fencing, has been found to promote Rs due to its positive effect of increasing root
67  biomass and SOC (He et al., 2008). However, Chen et al. (2016) reported that Rs was decreased
68  under grazing exclusion due to reduced soil temperature and increased soil moisture. Rest-
69  rotation grazing is indicated to be a good system for balance between economic development
70  through animal husbandry and the protection of grassland ecosystems. This system involves a
71  period of rest, or short-term grazing exclusion, during the growing season. Previous studies have
72  shown that rest-rotation grazing could increase SOC, belowground biomass and soil water
73  storage (Zhang et al. 2015a). A meta-analysis of the responses of the soil microbial community
74  size and Rs to grazing showed that only heavy grazing intensity significantly reduced Rs by
75  inhibiting soil microbes (Zhao et al., 2017). Therefore, the impact of rest-rotation grazing on Rs
76  may be different from grazing exclusion or year-long grazing.

77    In East Asia, most grassland ecosystems are subjected to varying degrees of grazing pressure and
78    climate warming. The temperature sensitivity of Rs ($Q_{10}$, the increase in the $CO_2$ release for
79    every 10 ºC increase in temperature) could predict the response of Rs to climate change. $Q_{10}$ can
80    be affected by a variety of factors, including soil temperature and moisture, root activity, quality
81    and quantity of substrate, and microbial community size (Yuste et al., 2004; Zhang et al., 2015b).
82    The influence of grazing on $Q_{10}$ is still controversial (Paz-Ferreiro et al., 2012; Chen et al.,
83    2016). Grazing can debase the $Q_{10}$ of autotrophic respiration by reducing aboveground and root
84    biomass of vegetation (Xu and Qi, 2001). This decrease in aboveground biomass and litter input
85    to soil will then reduce respiratory substrates and consequently decrease the $Q_{10}$ of heterotrophic
86    respiration under grazing systems (Cao et al., 2004). However, previous studies also suggest that
87    livestock may stimulate root exudation and litter decomposition, which can increase the quality
88    of SOM and thus promote the $Q_{10}$ of heterotrophic respiration (Zhao et al., 2016). Furthermore,
89    the $Q_{10}$ of fine root respiration is higher than that of microbes (Boone, et al., 1998). Therefore,
90    grazing may alter the $Q_{10}$ of Rs by changing the composition of Rs.
91    Grazing and enclosure are the representative land management modes of the typical steppe, but
92    the effects of different management methods on the carbon cycle in grassland ecosystems have
93    not been clear. We designed and implemented an experimental study to improve the
94    understanding of the impact of different grazing systems on carbon cycling and the soil carbon
95    budget of grasslands in the Inner Mongolian plateau. This study focuses on the effects of grazing
96    on typical steppe, which may vary. Combined with soil, vegetation or microbial indicators, the
97    purpose of this study is to further explore how grazing affects Rs and its $Q_{10}$. The results will
98    help to assess the ecological effects and climate responses of typical steppe under different
99    grazing systems. The objectives of the study were to (1) reveal the annual dynamics of Rs and its
100   relationship to environmental variables (i.e., soil temperature and soil moisture) and (2) explain
101   the effects of different grazing systems on soil respiration and determine the $Q_{10}$ values of Rs in
102   different grazing systems. Our hypotheses were as follows: (1) Grazing reduces Rs by inhibiting
103   the growth and activity of the plant and soil microbial community; (2) The effect of grazing on
104   Rs may be different between rest-rotation grazing and year-long grazing systems; and (3)
105   Grazing alters $Q_{10}$ by changing the proportion of root respiration in Rs.
106
107   **Materials & Methods**
108   **Study area**
109   The study area is located in Duolun County (41°46′-42°39′N, 115°50′-116°55′E), Inner
110   Mongolia Autonomous Region, China. The annual mean air temperature is 3.3 ºC, with a
111   maximum mean temperature of 19.9 ºC in July and a minimum mean temperature of -15.9 ºC in
112   January. The mean annual precipitation is 399 mm, and 88% of the precipitation occurs during
113   the growing season extending for approximately 150 days from May to September (Miao et al.,
114   2009). The vegetation in the study area is classified as typical steppe and dominated by *Stipa*
115   *krylovii* Roshev., *Leymus chinensis* (Trin.) Tzvelev., *Artemisia frigida* Willd., *Agropyron*
116   *cristatum* (L.) Gaertn., *Allium bidentatum* Fisch., and *Cleistogenes squarrosa* (L.) Keng. (Li et

117   al., 2013). SOC ranged from 1% to 3%, and soil total nitrogen (TN) ranged from 0.1% to 0.3% in
118   this study.
119   We selected three established grazing systems at Duolun Restoration Ecology Research Station
120   (part of the Institute of Botany, Chinese Academy of Sciences, 42°02′ N, 116°17′ E), which is
121   managed by the Institute of Botany, Chinese Academy of Sciences. The "year-long grazing
122   system" had been grazed by 4.75 sheep ha$^{-1}$ yr$^{-1}$ since 2002. The "rest-rotation grazing system"
123   had been grazed by 2~4.5 sheep ha$^{-1}$ yr$^{-1}$ since 2004, with an annual rest period from 10 March to
124   10 June. In the "grazing exclusion system", livestock had been completely excluded since 2002.
125   We randomly located three experimental plots, each 10 m×5 m, within the area of each grazing
126   system.
127   **Soil respiration, temperature and moisture**
128   Rs was measured on each plot in late December 2014 and during the 2015 growing season in late
129   March, May, July, August and September. To measure Rs, one PVC collar (20 cm in diameter
130   and 7 cm in height) was set up at a random location within each plot. The collars were inserted
131   into the soil where aboveground biomass had been removed at least 24 h before the Rs
132   measurement was to be taken in order to exclude $CO_2$ released by aboveground vegetation. The
133   collars were maintained in the plots for the six measurements to minimize the effect of soil
134   heterogeneity. The daily dynamics of Rs were captured by hourly measurement for 24 h. Each
135   measurement lasted 3 min, including 120 s of observation in which the first 30 s was excluded
136   due to the change in air pressure, and 30 s of pre- and post-purge time. Respiration rates were
137   measured using a LI-COR 8150 automatic soil $CO_2$ efflux measurement system with a portable
138   chamber (LI-COR Inc., Lincoln, NE, USA). The soil temperature and moisture content at a depth
139   of 5 cm adjacent to the collar were simultaneously measured using a LI-COR 8150-203
140   thermocouple probe and a LI-COR 8150-204 ECH$_2$O Model EC-5 soil volumetric moisture
141   content probe, respectively (Decagon Device, Inc., Pullman, WA, USA).
142   **Plant aboveground and root biomass**
143   Aboveground biomass was measured at the end of December 2014 and during May, July, and
144   September 2015; root biomass was measured during May, July and September 2015.
145   Aboveground biomass was removed from a randomly located 0.5 m×0.5 m quadrant in each plot
146   and oven-dried together with litter at 65 ºC to a constant weight. Root biomass was collected
147   using an 8 cm diameter soil drill sampler, and three cores were collected at 0–20 cm deep from
148   random locations in each plot to be mixed as one composite sample. The roots were washed and
149   oven-dried at 65 ºC to a constant weight.
150   **Soil microbial biomass and soil nutrients**
151   The collected soil samples were separated from the root biomass. Part of each sample was passed
152   through a 2-mm sieve to analyze soil microbial biomass carbon (MBC), dissolved organic carbon
153   (DOC) and phospholipid fatty acids (PLFAs). The composition of the microbial community
154   composition in each fraction was assessed using PLFA analysis following the method of Bossio
155   and Scow (1998). MBC was estimated using the chloroform fumigation-extraction method and
156   calculated as the difference in extracted organic carbon between the fumigated and control parts

157 divided of the soil sample divided by a conversion factor of 0.45 (Vance et al., 1987). DOC was
158 determined as the extracted organic carbon in the control soil (Vance et al., 1987). PLFAs
159 (including i14:0, 15:0, i15:0, a15:0, 16:0, 10me16:0, i16:0, 16:1w5c, 16:1w7c, 10me17:0, a17:0,
160 i17:0, cy17:0, 17:1w8c, 18:0, 10me18:0, 18:1w5c, 18:1w7, 18:1w9c, 18:2w6c cy19:0, 20:1w9c
161 and 20:4w6c) were identified by bacterial biomarkers, while fungal PLFAs, actinobacterial
162 PLFAs and protozoal PLFAs were assessed following the method of Huang et al. (2014).
163 The balance of each soil sample was passed through a 0.25-mm sieve for analysis of soil total
164 nitrogen (TN) and total phosphorus (TP). TN and TP were measured by the semi-micro-Kjeldahl
165 method (Bremner, 1960) with a PerkinElmer 2400 series II CHNS/O analyzer (PerkinElmer,
166 Inc., Norwalk CA, USA) and a SPECTRO ARCOS EOP analyzer (Spectro, Inc., GER),
167 respectively. **Data analysis**
168 $Q_{10}$ is defined as the rate of increase in soil respiration as a consequence of increasing the
169 temperature by 10 °C and is derived from the following relationships:
170 $Rs = a \cdot e^{bT}$         (1)
171 $Q_{10} = e^{10b}$         (2)
172 where $Rs$ ($\mu mol \cdot m^{-2} \cdot s^{-1}$) is soil respiration, $T$ (ºC) is the soil temperature at a depth of 5 cm, and $a$
173 and $b$ are fitted parameters.
174 The response of Rs to soil moisture was modeled using a quadratic polynomial function of the
175 following form:
176 $Rs = ex^2 + fx + g$         (3)
177 where $Rs$ ($\mu mol \cdot m^{-2} \cdot s^{-1}$) is soil respiration; $x$ (%) is the soil moisture content at a depth of 5 cm;
178 and $e$, $f$ and $g$ are fitted parameters.
179 All statistical analyses were performed using SPSS 12.0 (SPSS for Windows, Chicago, USA),
180 and the SEM analyses were conducted using SPSS AMOS 24.0.0 (SPSS Inc, Chicago, USA).
181
182 ## Results
183 **Soil temperature and moisture**
184 Soil temperature and soil moisture varied significantly among the three grazing systems in the
185 same month during the period of observation (P<0.05, Fig. 1a and b). Soil temperatures
186 exhibited consistent seasonal dynamics across all grazing systems, with the maximum soil
187 temperature occurring in July 2015 and the minimum occurring in December 2014 (Fig. 1a). The
188 low-vegetation cover plots (year-long grazing and rest-rotation grazing systems) had higher soil
189 temperatures than the high-vegetation cover plots (grazing exclusion system) due to the absence
190 of canopy shading.
191 The soil moisture content fluctuated substantially in response to irregular rainfall, peaking in
192 September 2015 (Fig. 1b). The average soil moisture content during observation was 7.89%,
193 9.87%, and 10.89% for the year-long grazing, rest-rotation grazing and grazing exclusion plots,
194 respectively.
195 **Soil respiration and $Q_{10}$**

196   Rs showed significant variation among the grazing systems in all months (P<0.05). The Rs
197   ranges were 0.07-1.89, 0.06-2.57 and 0.03-3.47 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for the year-long grazing, rest-
198   rotation grazing and grazing exclusion systems, respectively (Fig. 1c). Rs was lowest in
199   December 2014 for all three grazing systems. It peaked in July for the grazing exclusion and
200   rest-rotation grazing plots and in August for the year-long grazing plots (Fig. 1c). Averaged
201   across the period of observation, Rs was highest in the rest-rotation plots (1.26 $\mu mol \cdot m^{-2} \cdot s^{-1}$),
202   followed by the grazing exclusion (0.98 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and year-long grazing (0.94 $\mu mol \cdot m^{-2} \cdot s^{-1}$)
203   plots. During the 2015 growing season (from May to September), the average Rs values were
204   1.32, 1.81 and 1.42 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for the year-long grazing, rest-rotation grazing and grazing
205   exclusion systems, respectively.
206   Significant exponential relationships were found between Rs and soil temperature for all grazing
207   systems and sample times, with $R^2$ values ranging from 0.506 to 0.920 (Table 1). The $Q_{10}$ values
208   for the grazing exclusion plots were lower than those for the year-long grazing plots, except in
209   July and September 2015, and were lower for the rest-rotation plots than for the year-long
210   grazing plots, except in December 2014, July 2015 and September 2015 (Table 1). Very high $Q_{10}$
211   values were recorded for all grazing systems in December 2014, and a relatively high value was
212   recorded for the grazing exclusion system in September 2015, indicating the heightened
213   sensitivity of Rs to temperature at these times (Table 1).
214   A quadratic relationship between Rs and soil moisture was derived from the monthly soil
215   moisture measurements in each grazing system (Fig. 2). All relationships were significant
216   (P<0.001), with $R^2$ values ranging from 0.69 to 0.77. The effect of increasing soil moisture on Rs
217   was nonlinear at this study site.
218   **Plant and soil properties**
219   Aboveground biomass under the three grazing system increased over time within a growing
220   season, and it was higher in the grazing exclusion system than in the other two systems for the
221   same month (Fig. 3a). Root biomass showed remarkable variation between months, with the
222   maximum biomass occurring in July 2015 (Fig. 3b). Root biomass was significantly higher in the
223   rest-rotation grazing system than in the year-long grazing system in July and September and
224   higher than in the grazing exclusion system in May, July and September (P<0.05). Rs increased
225   linearly with root biomass in all grazing systems as the growing season progressed (Fig. 4).
226   TP and TN remained steady during the experiment in all grazing systems (Fig. 3c, d). The
227   average TP content was 0.37, 0.32 and 0.42 g kg$^{-1}$, while the average TN content was 1.57, 1.63
228   and 2.36 kg$^{-1}$ for the year-long grazing, rest-rotation grazing and grazing exclusion systems,
229   respectively. Both the TN and TP contents of the grazing exclusion system were significantly
230   higher than the other two systems (P<0.05, Fig. 3c, d). MBC increased during the 2015 growing
231   season in all systems (Fig. 3e). MBC of the grazing exclusion system was 49% higher than that
232   of the year-long grazing system in July and 42% higher in September. DOC decreased as the
233   growing season progressed and was higher in the year-long grazing system than in the other two
234   systems (Fig. 3f).
235   **Phospholipid fatty acids**

236    The total PLFAs declined at first and then increased from December 2014 to September 2015,
237    and each kind of PLFA increased during the course of the growing season (Fig. 5a-e). The mean
238    total PLFA values for the year-long grazing, rest-rotation grazing, and grazing exclusion systems
239    in the growing season were 29.77, 31.9, 35.88 nmol g$^{-1}$, respectively. The highest levels for all
240    types of PLFAs were observed in the grazing exclusion system during September (Fig. 5a-e).
241    The average ratios of fungi to bacteria during the growing season were 0.17, 0.17 and 0.14 in the
242    year-long grazing, rest-rotation grazing, and grazing exclusion systems, respectively.
243    **Relationships among $Q_{10}$, Rs and other factors under different grazing systems**
244    Rs and $Q_{10}$ were affected by grazing, climate, DOC, plant and soil microbes; these factors were
245    related to each other. Therefore, the structural equation model (SEM) was used to explore the
246    influence mechanism of Rs and $Q_{10}$. The SEM explained 70% and 63% of the variance of Rs and
247    $Q_{10}$, respectively, under different grazing systems (Fig. 6). Among the variables influencing Rs
248    and $Q_{10}$, the increase in PLFAs and soil temperature resulted in a highly significant enhancement
249    of Rs (P<0.001). The standardized total effect coefficients of PLFAs and soil temperature on Rs
250    were 0.634 and 0.621, respectively. The increase in the soil temperature by seasonal variation
251    resulted in a highly significant decrease in $Q_{10}$ (P<0.001), and the standardized total effect
252    coefficient was -0.775. Grazing had an insignificant negative direct effect (-0.051) on Rs, and an
253    indirect negative effect (-0.078) by decreasing aboveground biomass, PLFAs and soil moisture.
254    Grazing had a small indirect positive effect (0.026) by decreasing the aboveground biomass and
255    increasing soil moisture on $Q_{10}$. The result of the SEM indicated that increased grazing inhibited
256    Rs and enhanced $Q_{10}$. When the standardized total effects were compared (Table 2), the effect of
257    grazing on Rs and $Q_{10}$ was lower than the effects of soil temperature or moisture.
258
259    ## Discussion
260    **Response of plant biomass, soil properties and microbial community to grazing**
261    Grazing is well-acknowledged to have impacts on the growth of vegetation (Porensky et al.,
262    2016), basic soil properties and soil microbial biomass (Raich and Nadelhoffer, 1989) in
263    grassland ecosystems. In the present study, we found that root biomass in year-long grazing and
264    rest-rotation grazing systems were higher than that in grazing exclusion systems (Fig. 3). These
265    results can be explained by the grazing optimization hypothesis proposed by McNaughton
266    (1979), which suggested that moderate grazing stimulates vegetation regeneration. Moreover,
267    plants would supply more nutrients to roots instead of shoots as a change in growth strategy due
268    to grazing (Bai et al., 2015; Frank et al., 2002). But the root biomass increased significantly only
269    in rest-rotation grazing systems, it indicating that rest-rotation grazing could result in economic
270    benefits and grassland restoration.
271    We found that soil total carbon and total phosphorus were more abundant in the grazing
272    exclusion system than in the year-long grazing and rest-rotation grazing systems (Fig. 3).
273    Similarly, previous studies also found that grazing had a negative effect on the storage of soil
274    nutrients (Han et al., 2008), whereas fencing improved the physical and chemical properties of
275    soil (Dormaar et al., 1989). Loss of soil nutrient elements may limit the growth of vegetation and

276   the soil microbial community, and it also could be an important reference index for the
277   establishment of grassland management mode. In contrast to inorganic elements, DOC increased
278   with intensified grazing during the growing season, except in July of 2015 when the rest-rotation
279   grazing system was being implemented (Fig. 3f). DOC has been recognized to play an important
280   role in regulating soil carbon mineralization and soil respiration in grassland ecosystems (Zhou
281   et al., 2016). The increase in DOC caused by grazing provided more respiration substrates, which
282   may consequently enhance Rs.
283   Soil microbes are crucial in regulating Rs by transforming soil carbon into $CO_2$ flux during
284   decomposition (Chapin et al., 2011). We found that grazing generally suppressed the MBC in the
285   2015 growing season (Fig. 3e), which is consistent with results from Bardgett and Leemans
286   (1995). The result of the PLFA assays also showed that grazing significantly decreased the total
287   PLFAs, bacterial PLFAs and actinomycelial PLFAs in the middle and late growing season (Fig.
288   5). The decrease in microbial biomass is likely attributed to livestock trampling disturbing the
289   soil microenvironment and thus inhibiting soil microbe reproduction, although the increase in the
290   root biomass caused by grazing may provide more exudates, promoting microbial growth. We
291   also found that the F:B ratio in year-long grazing and rest-rotation grazing systems was higher
292   than that in grazing exclusion systems. This increased F:B ratio by grazing might be attributed to
293   the grazing-induced increase in soil C:N ratio by increasing SOC and decreasing TN since
294   substrates with high C:N ratios are conducive to fungal growth. Fungi play an important role in a
295   large number of soil ecosystem processes, particularly in nutrient cycling and maintaining soil
296   structure (Wal et al., 2006). As a higher F:B ratio in soil could reflect a more stable ecosystem
297   status (Vries et al., 2006), moderate grazing might to some extent improve the stability of
298   grassland ecosystems.
299   **Relationships between soil respiration and other factors under grazing**
300   In accordance with the results of previous studies (Bao et al., 2016; Li and Sun, 2011), tight
301   linkages among Rs and plant and soil properties were found in this study (Fig. 6). Compared to
302   other factors, soil temperature had a significantly direct effect on Rs ($R^2 =$, $p =$) in the
303   constructed SEM. Rs in all grazing systems increased exponentially with rising soil temperature,
304   which is consistent with other findings (Yuste et al., 2004; Zhang et al., 2015b). Nonetheless, we
305   also found that in August 2015, Rs decreased by 71.8% compared to that in July in the grazing
306   exclusion system, while soil temperature was similar between these two months. This result
307   indicates that in addition to soil temperature, other factors, e.g., soil moisture, might also
308   contribute to monthly variation in Rs.
309   Soil moisture is one of the factors limiting ecological recovery in semiarid regions (Zhang et al.,
310   2015b). Soil moisture was considered a dominant driver of Rs when temperature was above 20
311   ºC in a semiarid steppe ecosystem in Spain (Rey et al., 2011). According to the results of the
312   SEM, soil moisture directly inhibited Rs (Fig. 6). It is suggested that high soil moisture may
313   reduce Rs because the water in the soil interstices affects gaseous diffusion (Liu et al., 2014).
314   However, high soil moisture can also enhance the transport of organic matter among soil and
315   metabolic activity of soil microbes (Michalzik et al., 2003). The positive and negative effects of

316  moisture on Rs exist simultaneously, and their relationship fit the quadratic function well,
317  echoing the findings of Suseela et al., (2012). It may also explain why the Rs of the three grazing
318  systems peaked in different months. In July and August, higher soil temperature caused moisture
319  to become a major limiting factor of Rs; therefore, the difference in Rs between year-long
320  grazing and grazing exclusion aligned with changes in soil moisture. This resulted in the Rs of
321  grazing exclusion systems peaking in July, while the Rs of year-long grazing systems peaked in
322  August. In the rest-rotation grazing system, Rs peaked in July but did not show a significant
323  difference ($P<0.05$) between July and August. This finding was probably due to root biomass
324  being the highest in rest-rotation grazing systems (Fig. 3), so rhizosphere respiration became the
325  main component of Rs, and it was thus less sensitive to surface soil moisture. Due to the
326  nonlinear effect of soil moisture on Rs, identifying the nonlinearities and thresholds could reduce
327  some uncertainties associated with climate change decision-making.
328  In the present study, the average Rs from May to September was highest in the rest-rotation
329  grazing system (1.81 $\mu mol \cdot m^{-2} \cdot s^{-1}$), followed by the grazing exclusion system (1.41 $\mu mol \cdot m^{-2} \cdot s^{-1}$)
330  and the year-long grazing system (1.32 $\mu mol \cdot m^{-2} \cdot s^{-1}$). This result indicated that grazing had a
331  significant impact on Rs in a temperate typical steppe that was not only inhibitory, thus
332  contradicting our first hypothesis that grazing inhibited Rs. Similarly, a study conducted under
333  different land uses in Inner Mongolia reported that the average Rs during the growing season
334  was $1.43\pm0.04$ $\mu mol \cdot m^{-2} \cdot s^{-1}$ for free grazing (only during the growing season with 9 sheep $ha^{-1}$)
335  and $1.37\pm0.03$ $\mu mol \cdot m^{-2} \cdot s^{-1}$ for enclosed grassland grazing (Xue and Tang, 2018). In contrast to
336  the findings of the study, the higher Rs observed under rest-rotation grazing system in our study
337  was likely due to the lower grazing intensity (2~4.5 vs 9 sheep $ha^{-1}$). Rest-rotation grazing
338  accelerated the soil carbon mineralization rate, and also reduced soil carbon stocks and increased
339  the greenhouse effect.
340  In the SEM, grazing showed a minor negative direct effect on Rs, which could be explained as
341  an increase in soil bulk density and compactness by grazing that might block gas exchange. The
342  indirect effects of grazing on Rs were mainly mediated by plant and microbial factors. Our study
343  found that the mean Rs and root biomass were highest under rest-rotation grazing, probably
344  because grazing accelerated the nutrient cycle (Allard et al., 2007) and increased the transfer of
345  plant biomass to roots (Milchunas and Lauenroth, 1993). Previous research (Tu et al., 2013; Sun
346  et al., 2013) has shown that environmental factors affect root respiration through changes in root
347  biomass. The root biomass was higher in grazing systems, and Rs increased linearly with root
348  biomass in all grazing systems (Fig. 4). This finding suggests that grazing enhanced soil
349  respiration by increasing root biomass.
350  Soil microbial respiration was one of the main contributors to Rs. A previous study showed that
351  the response of Rs to grazing was positively correlated with soil microbial biomass under
352  moderate and heavy grazing intensities (Zhao et al., 2017). In addition, the substrate supply from
353  photosynthesis is a main factor affecting Rs, and the aboveground biomass is proportional to Rs
354  (Kuzyakov and Gavrichkova, 2010). In this study, Rs was positively correlated positively to
355  aboveground biomass and microbial biomass (Fig. 6). Previous studies have shown that DOC

356  was the substrate of soil microbial respiration, and its content and degree of decomposition
357  positively correlated with Rs (Moinet et al., 2016). However, DOC had a negative effect on Rs in
358  this study mainly because the decrease in soil microbes by grazing weakened the restrictive
359  effect of DOC on Rs. In conclusion, grazing inhibited respiration by suppressing aboveground
360  biomass, soil moisture and soil microbial biomass. These results were consistent with the second
361  hypothesis that grazing enhanced rhizosphere respiration but inhibited microbial respiration.
362  **The relationships between the temperature sensitivity of soil respiration and other factors**
363  **under grazing**
364  Previous studies have reported that the $Q_{10}$ of Rs is negatively correlated with soil temperature in
365  northern temperate grasslands (Flanagan and Johnson, 2005). The higher values of $Q_{10}$ in
366  December of 2014 and March of 2015 in our study were consistent with that result, indicating
367  that Rs could be extremely sensitive to temperature changes at very low soil temperatures. The
368  decrease in the $Q_{10}$ with increasing temperature could be related to a decline in $Q_{10}$ of microbial
369  respiration, which contributed a great proportion to Rs. A previous study conducted in forest
370  soils found that the $Q_{10}$ of SOM decomposition rates decreases with the increase of soil
371  temperature and moisture (Schindlbacher et al., 2010), which was similar to the $Q_{10}$ of Rs in our
372  study (Fig. 6). This finding can be explained by the differential response of enzyme activity and
373  substrate affinity to increased temperature. Enzyme activity was inhibited at low temperatures
374  and could be enhanced by soil temperature, but the decline in enzyme substrate affinity with
375  increased temperature could offset the promotion of soil enzyme activity (Li et al., 2017),
376  resulting in higher $Q_{10}$ and decomposition potential of SOM at low temperatures. Rs increased
377  rapidly after removing the temperature limit; the limiting effect of temperature and the rate of
378  increase in Rs decreased gradually in this process, meaning that $Q_{10}$ decreased with increasing
379  soil temperature. $Q_{10}$ decreased more slowly as limitations were removed.
380  The main components of Rs are autotrophic respiration (rhizosphere respiration) and
381  heterotrophic respiration (microbial respiration). Sensitivity of different components to
382  temperature determine the total $Q_{10}$ of Rs. Evidence regarding the response of $Q_{10}$ to grazing has
383  been inconsistent. $Q_{10}$ increased under grazing exclusion in a meadow grassland (Chen et al.,
384  2016) but was stimulated by grazing in another temperate grassland (Paz-Ferreiro et al., 2012).
385  In this study, the temperature sensitivity of Rs in the two grazing systems was higher than that in
386  the grazing exclusion system. Consistent with the third hypothesis, the increased $Q_{10}$ from
387  grazing in this study could be explained by the higher fine root biomass in grazing systems and
388  higher $Q_{10}$ value for rhizosphere respiration (Rr) (Boone, et al., 1998). In addition, the
389  'rhizosphere priming effect' increased the availability of labile carbon by root exudation or fresh
390  litter, which enhanced the $Q_{10}$ of heterotrophic respiration (Hopkins et al., 2013; Zhu and Cheng,
391  2011). These results indicate that Rs in the grassland is more susceptible to rising temperature
392  under grazing.
393  Compared with year-long grazing, rest-rotation grazing could be a reasonable avenue to
394  grassland sustainable development, but the increased of soil $CO_2$ emissions and loss of soil
395  nutrient elements caused by rest-rotation grazing are inescapable. Given grassland degradation

396   caused by long-term grazing in typical steppe, grazing exclusion could be an effective way to
397   promote grassland restoration. Due to lack of long-term observational data and multiple
398   replicates in each plot, and because respiration of different components cannot be characterized
399   accurately by microbial biomass and root biomass, the results of this study have some
400   limitations. Future studies can set multiple gradients of grazing time or intensity to explore the
401   varying effects of grazing, and focus on changes of different components of Rs under grazing by
402   more effective means. Long-term measurements of Rs and soil organic matter should be
403   performed.
404

## Conclusions

406   In this study, the mean Rs varied significantly among different grazing systems. Diverse effects
407   of grazing management on soil $CO_2$ flux occurred in this temperate grassland, as the mean Rs
408   was lowest in the year-long grazing system but highest in the rest-rotation grazing system. The
409   difference in Rs among the grazing systems was closely associated with changes in soil
410   temperature, soil moisture, potential substrate availability and soil microbial activity. Since
411   rhizospheric respiration increased with increasing root biomass stimulated by grazing and was
412   more sensitive to increased temperature, the temperature sensitivity ($Q_{10}$) of Rs was enhanced in
413   grazing systems. Therefore, grazing has the potential to make Rs more susceptible to global
414   warming. In addition, the occurrence of the highest contents of TN, TP and MBC in the grazing
415   exclusion system demonstrated the value of grazing exclusion for conserving soil nutrients and
416   microbial biomass. Higher F:B ratios in the grazing systems were likely due to more suitable soil
417   management. By altering plant roots and their control over the soil microbial community,
418   grazing profoundly influences soil decomposition and thus can regulate the carbon budget in
419   grassland ecosystems. A better understanding of the effects of grazing on Rs has practical
420   implications for ecological restoration in a typical steppe.
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422
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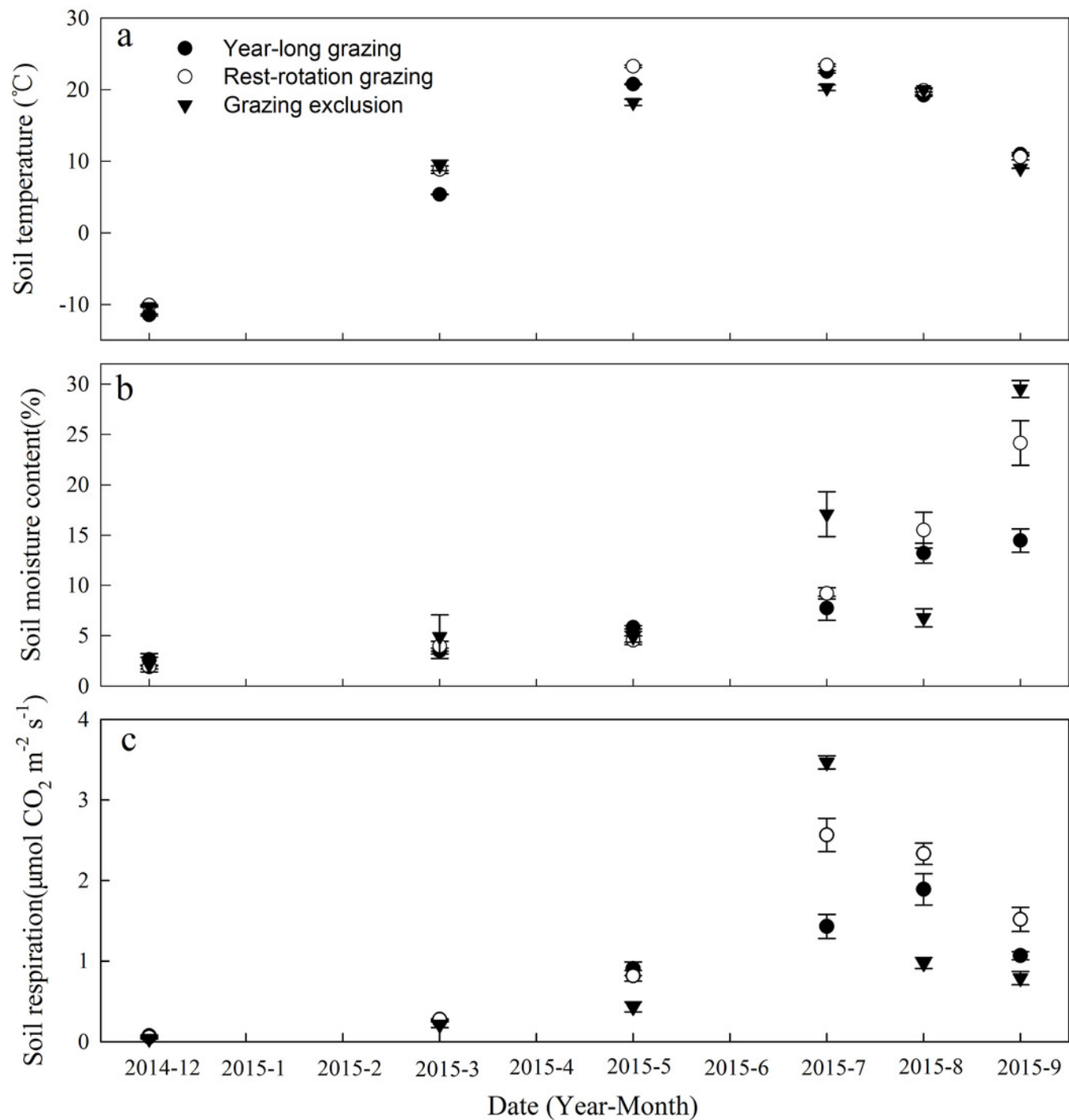
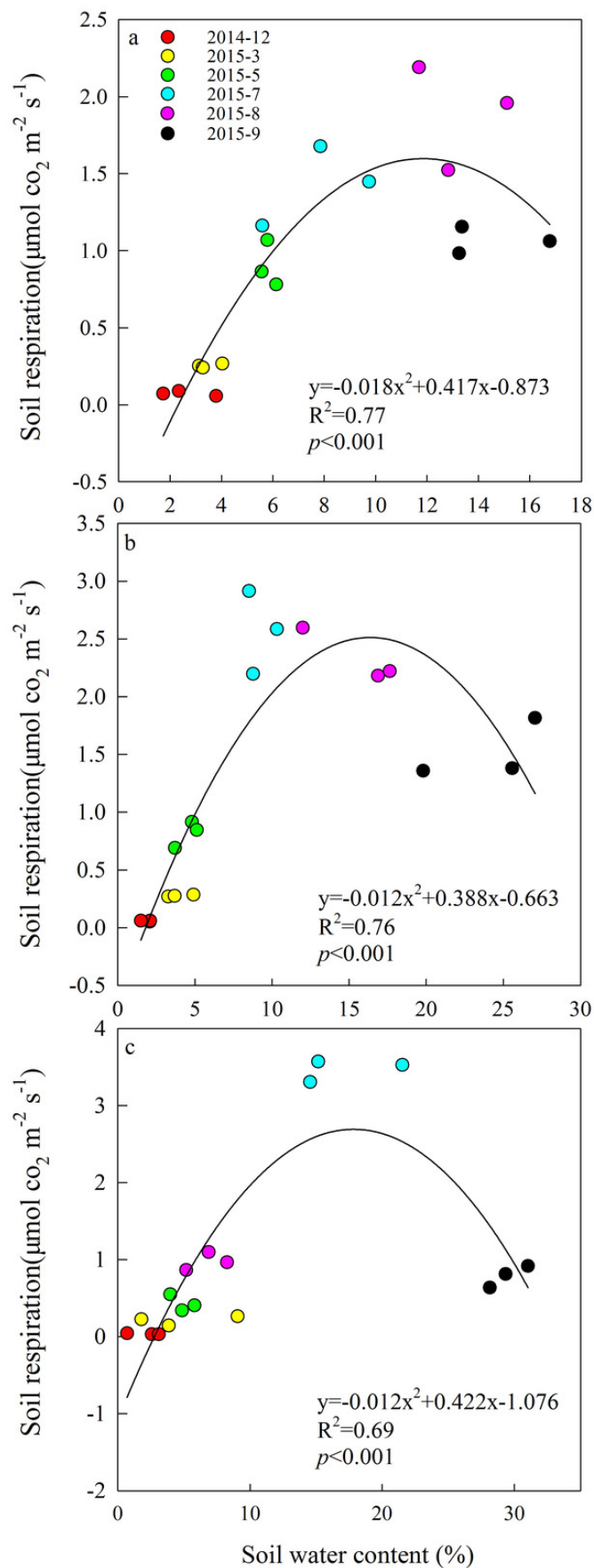
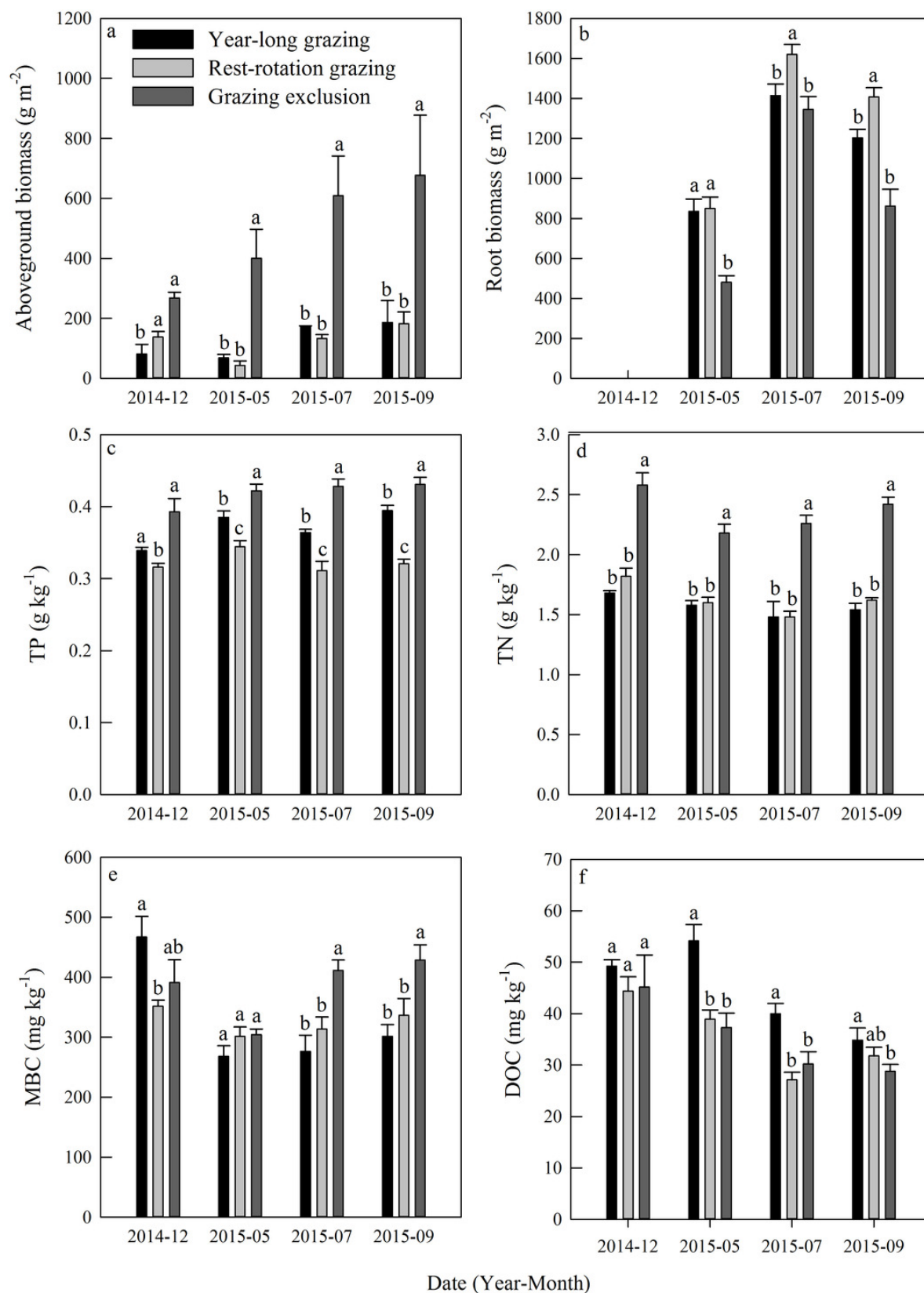
# Figure 1

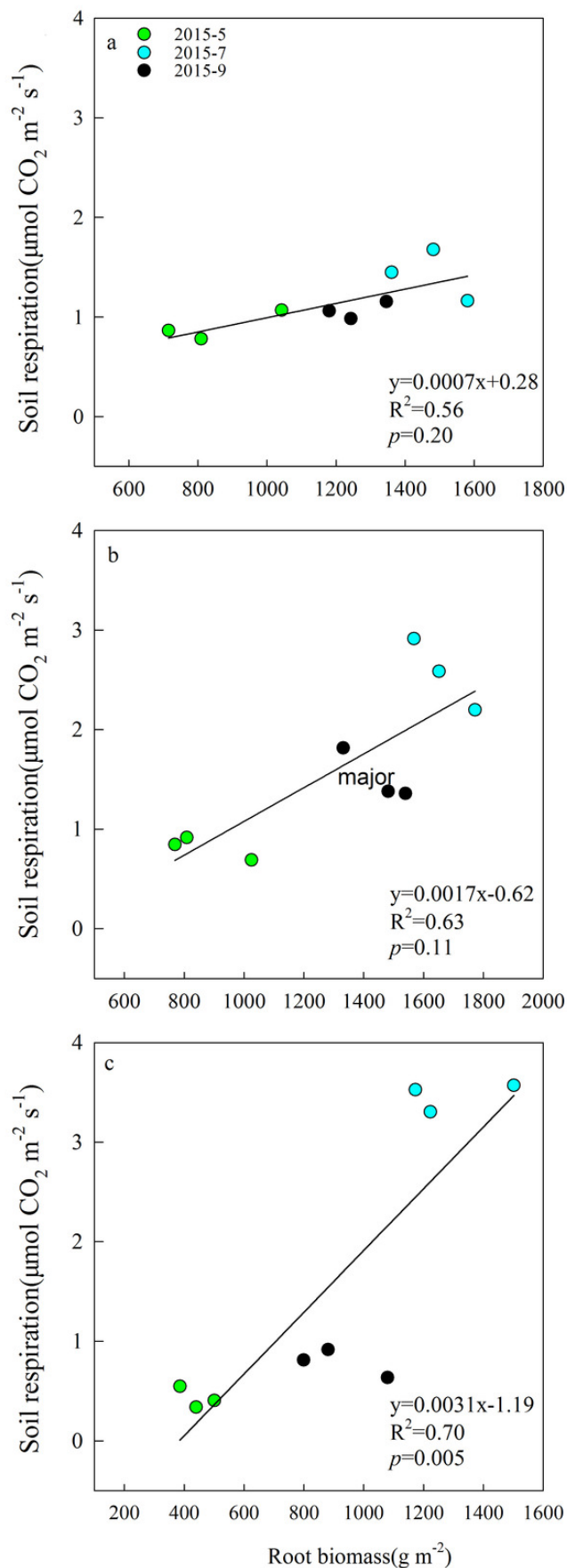
Dynamics of (a) soil temperature, (b) soil moisture content and (c) soil respiration under three different grazing systems from December 2014 to September 2015.

# Figure 2

Quadratic relationships between soil respiration and soil moisture from December 2014 to September 2015 in (a) year-long grazing system,(b) rest-rotation grazing system, (c) grazing exclusion system.

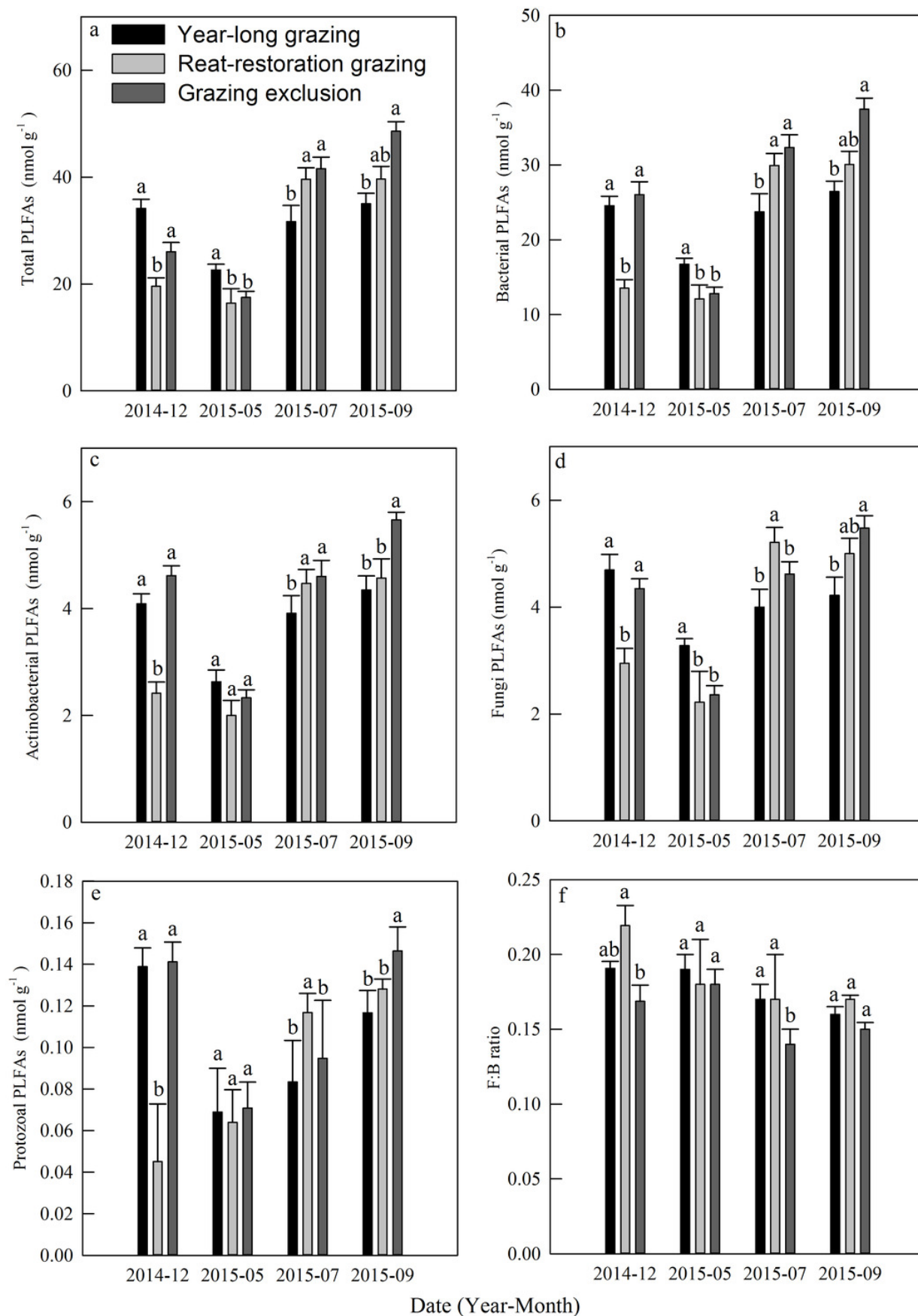
$R^2$ is the goodness of fit for the function, and the P value is the significance of the overall function.

# Figure 3

Dynamics of (a) aboveground biomass, (b) root biomass, (c) total phosphorus (TP), (d) total nitrogen (TN), (e) microbial biomass carbon (MBC)and (f) dissolved organic carbon (DOC) under three different grazing systems from December 2014 to September 2015.

# Figure 4

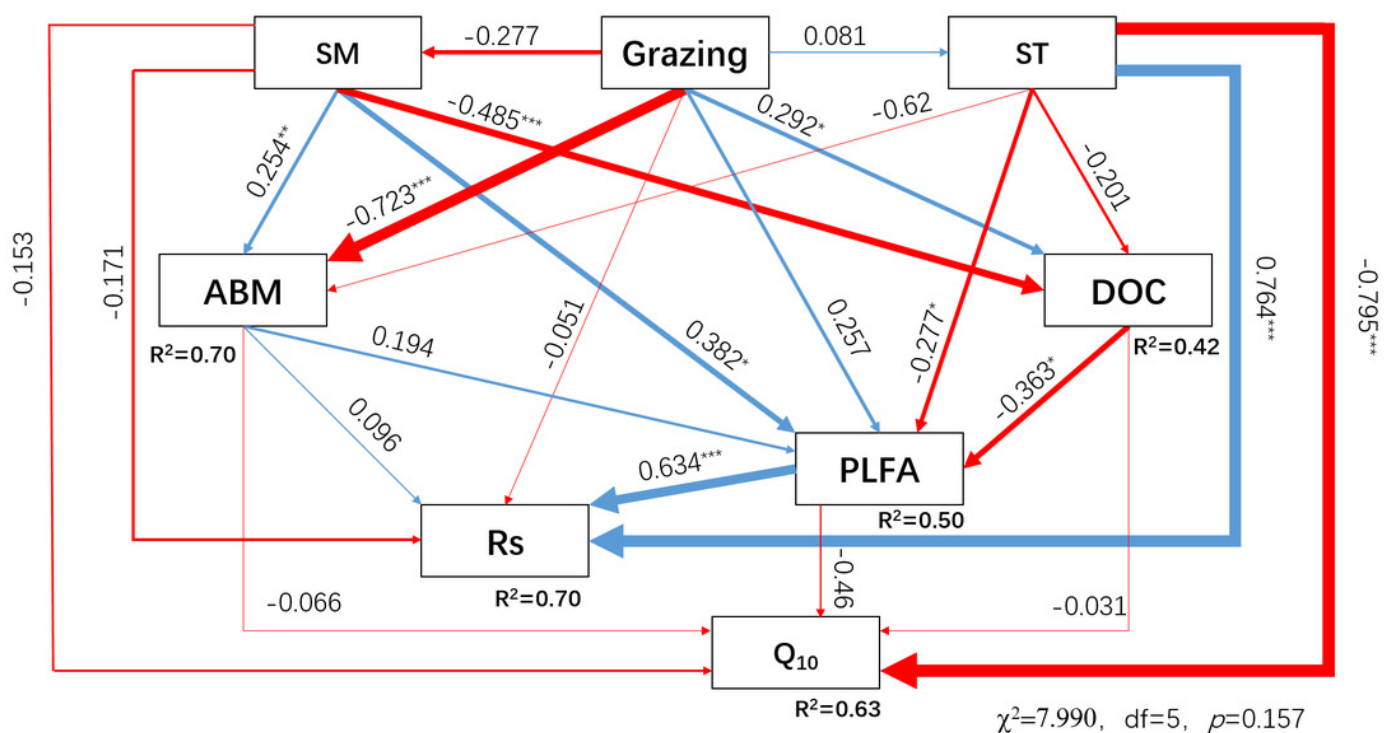
Linear relationships between soil respiration and root biomass in May, July and September 2015 under (a) year-long grazing system, (b) rest-rotation grazing system and (c) grazing exclusion system.

$R^2$ is the goodness of fit for the function, and the P value is the significance of the overall function.

# Figure 5

Dynamics of phospholipid fatty acids (PLFAs, nmol g$^{-1}$) under different grazing systems from December 2014 to September 2015.

(a) total PLFAs, (b) bacterial PLFAs, (c) actinobacterial PLFAs, (d) fungalPLFAs, (e) protozoal PLFAs and (f) the ratio of fungal: bacterial PLFAs (F:B ratio). The different letters represent significant differences (P < 0.05) among the different grazing systems in the same month. The error bars represent the standard errors.

# Figure 6

Structural equation model (SEM) describing the effects of grazing,environmental factors, and biotic factors on soil respiration and $Q_{10}$.

The SEM was performed with data from December 2014 and from May, July and September 2015. ST, SM, ABM, DOC, PLFA, Rs and $Q_{10}$ represent the soil temperature, soil moisture, aboveground biomass, dissolved organic carbon, phospholipid fatty acids, soil respiration and temperature sensitivity for Rs, respectively. The blue or red lines, the direction of the arrows and the thickness of the lines indicate positive or negative effects, the direction of the effects and the severity of the effects, respectively. $R^2$ denotes the proportion of variables explained by these drivers. The numbers close to the lines are the standardized direct influence coefficients. The *, ** and *** at the top right of the numbers indicate that the effects were significant at $P<0.05$, $P<0.01$ and $P<0.001$, respectively. $\chi^2$, chi-square; df, degrees of freedom; $p$, probability level.

Temporal variation in the soil respiration–temperature relationship (equation 1) and $Q_{10}$ among three grazing systems. The $R^2$ and P values refer to equation 1.

| Date Year/month | Grazing systems | Equation 1 $R_s = a \cdot e^{bT}$ | $Q_{10}$ | $R^2$ | P |
|---|---|---|---|---|---|
| 2014-12 | Year-long grazing | $2.298e^{0.308T}$ | 21.76 | 0.804 | <0.001 |
| | Rest-rotation grazing | $1.191e^{0.331T}$ | 27.39 | 0.740 | <0.001 |
| | Grazing exclusion | $0.541e^{0.298T}$ | 19.69 | 0.644 | <0.001 |
| 2015-03 | Year-long grazing | $0.14e^{0.100T}$ | 2.72 | 0.506 | <0.001 |
| | Rest-rotation grazing | $0.113e^{0.092T}$ | 2.51 | 0.655 | <0.001 |
| | Grazing exclusion | $0.111e^{0.06T}$ | 1.82 | 0.677 | <0.001 |
| 2015-05 | Year-long grazing | $0.171e^{0.067T}$ | 1.95 | 0.736 | <0.001 |
| | Rest-rotation grazing | $0.288e^{0.053T}$ | 1.70 | 0.883 | <0.001 |
| | Grazing exclusion | $0.166e^{0.049T}$ | 1.63 | 0.889 | <0.001 |
| 2015-07 | Year-long grazing | $0.810e^{0.024T}$ | 1.28 | 0.743 | <0.001 |
| | Rest-rotation grazing | $0.975e^{0.041T}$ | 1.51 | 0.883 | <0.001 |
| | Grazing exclusion | $1.496e^{0.04T}$ | 1.49 | 0.750 | <0.001 |
| 2015-08 | Year-long grazing | $0.614e^{0.056T}$ | 1.75 | 0.920 | <0.001 |
| | Rest-rotation grazing | $0.950e^{0.044T}$ | 1.55 | 0.901 | <0.001 |
| | Grazing exclusion | $0.545e^{0.028T}$ | 1.32 | 0.789 | <0.001 |
| 2015-09 | Year-long grazing | $0.655e^{0.043T}$ | 1.54 | 0.746 | <0.001 |
| | Rest-rotation grazing | $0.948e^{0.043T}$ | 1.54 | 0.915 | <0.001 |
| | Grazing exclusion | $0.125e^{0.193T}$ | 6.89 | 0.855 | <0.001 |

Standardized total effects of ST, SM, grazing, ABM, DOC and PLFAs on ABM, DOC, Rs and $Q_{10}$.

ST, SM, ABM, DOC, PLFAs, Rs and $Q_{10}$ represent soil temperature, soil moisture, aboveground biomass, dissolved organic carbon, phospholipid fatty acids, soil respiration and temperature sensitivity of soil respiration respectively.

|  | Grazing | SM | ST | ABM | DOC | PLFA |
|---|---|---|---|---|---|---|
| SM | -0.277 |  |  |  |  |  |
| ST | 0.081 |  |  |  |  |  |
| ABM | -0.799 | 0.254 | -0.062 |  |  |  |
| DOC | 0.409 | -0.485 | -0.201 |  |  |  |
| PLFA | -0.174 | 0.607 | -0.216 | 0.194 | -0.363 |  |
| $Q_{10}$ | 0.026 | -0.183 | -0.775 | -0.075 | -0.014 | -0.046 |
| Rs | -0.129 | 0.238 | 0.621 | 0.219 | -0.230 | 0.634 |