

1 **Grazing increases litter decomposition rate but decreases nitrogen release rate in an**  
2 **alpine meadow**

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19 **Abstract.** Litter decomposition and N release are the key processes that strongly determine the nutrient cycling at the soil-  
20 plant interface; however, how these processes are affected by grazing or grazing exclusion in the alpine grassland ecosystems  
21 on the Qinghai-Tibetan Plateau (QTP) is poorly understood. So far few studies have simultaneously investigated the influence  
22 of both litter quality and incubation site on litter decomposition and N release. Moreover, previous studies on the QTP  
23 investigating how grazing exclusion influences plant abundance and biodiversity usually lasted for many years, and the short-  
24 term effects have rarely been reported. This work studied the short-term (6 month) effects of grazing and grazing exclusion on  
25 plant community composition (i.e., plant species presented) and litter quality, and long-term (27 – 33 month) effects on soil  
26 chemical characteristics and mixed litter decomposition and N release on the QTP. Our results demonstrate that: (1) shorter  
27 term grazing exclusion had no effect on plant community composition but increased plant palatability and total litter biomass;  
28 (2) grazing resulted in higher N and C content in litter; and (3) grazing accelerated litter decomposition, while grazing exclusion  
29 promoted N release from litter and increased soil organic carbon. In addition, incubation site had significantly more impact  
30 than did litter quality on litter decomposition and N release, while litter quality affected decomposition in the early stages. This  
31 study provides insights into the mechanisms behind the nutrient cycling in alpine ecosystems. We suggest that periodic grazing  
32 and grazing exclusion is beneficial in grassland management on the QTP.

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## 36 **1 Introduction**

37 The Qinghai-Tibetan Plateau (QTP) represents an important eco-region in China (Wen et al., 2010), in which alpine grasslands  
38 cover more than 85 % of total area and are regarded as the major land unit of natural pastures in China (Dong et al., 2010).  
39 However, the grassland systems in this region have suffered from severe degradation driven by a range of factors including  
40 climate change, overgrazing, over-cultivation and poor management (Han et al., 2008; Li et al., 2009; Wu et al., 2009; Feng et  
41 al., 2010; Wu et al., 2010), and with the degraded land area increasing at 1.2-7.44 % per year (Ma et al., 2007). Since the 1990s,  
42 the restoration of degraded grasslands has attracted considerable attention (Kang et al., 2007; Han et al., 2008), and some  
43 efforts have recently been directed towards grassland restoration and maintenance by increasing aboveground plant abundance  
44 (Niu et al., 2009) and biodiversity (Wu et al., 2009; Niu et al., 2010), and improving soil organic matter content and nutrient  
45 availability (Cao et al., 2004; Wu et al., 2010; Sun et al., 2011). It is well known that grazing may change the vegetation  
46 community structure, soil structure and nutrient cycling processes, and that such changes have important consequential impacts  
47 on the structure and functioning of the ecosystem as a whole. However, litter decomposition and N release, the key factors  
48 regulating the nutrient cycle and availability at the soil-plant interface through grazing (Carrera and Bertiller, 2013), are as yet  
49 little studied in these alpine ecosystems (Luo et al., 2010; Zhu et al., 2016).

50 Herbivore grazing may induce short-term ecophysiological changes in plant tissues which in turn may translate into  
51 litter quality changes, and long-term shifts in plant community composition. At the short-term ecophysiological level, grazing  
52 may promote the plant species producing high-quality litter (Holland and Detling, 1990; Sirotiak and Huntly, 2000; Olofsson

53 and Oksanen, 2002; Semmartin et al., 2004, 2008), because the consuming of plant tissues by herbivores may favour the grazed  
54 species with a higher re-growth rate and greater nutrient contents in plant tissues due to the higher nutrient uptake (see Holland  
55 and Detling, 1990; Olofsson and Oksanen, 2002; Semmartin et al., 2008). At the long-term community level, selective foliar  
56 grazing may alter the competitive interactions and recruitment patterns of plant species, which may change their abundance  
57 and life form structure (Bardgett and Wardle, 2003; Semmartin et al., 2008; Wu et al., 2009; Niu et al., 2010). For instance,  
58 herbivores preferentially feed on the most palatable plants (e.g., species with high nutrient and low fibre contents), which may  
59 promote dominance of unpalatable species (Garibaldi et al., 2007), resulting in the high inputs of low-quality litter to soil and  
60 thus a reduction of decomposition rate, nutrient availability and nutrient cycling (Ritchie and Knops, 1998; Moretto et al.,  
61 2001; Olofsson and Oksanen, 2002). Therefore, litter in grassland subject to long-term grazing may decompose more slowly.  
62 However, some studies demonstrate that grazing *per se* may accelerate litter decomposition by modifying site conditions for  
63 litter turnover through physical changes in the soil by herbivore activities, such as trampling and urine/dung deposition (Takar  
64 et al., 1990; Fahnestock and Knapp, 1994; Semmartin et al., 2008; Luo et al., 2010; Liang et al., 2018). Empirical evidence of  
65 variance in litter quality input and decomposition caused by grazing is still subject to debate (Garibaldi et al., 2007).

66 It is often assumed that higher nutrient content in plant tissue usually results in faster litter decomposition, and in higher  
67 nutrient mineralization and availability in soil (Olofsson and Oksanen, 2002). At the ecosystem scale, the chemical  
68 characteristics of plant litter, for example the carbon:nitrogen ratio (C:N) and/or nitrogen and lignin content, are often regarded  
69 as the indicators of litter quality (Aerts, 1997; Strickland et al., 2009). Many studies have demonstrated a positive correlation  
70 between litter decomposition rate and litter N content, or a negative relationship between litter decomposition rate and initial

71 litter lignin content and C:N or lignin:N ratio (e.g., Wardle et al., 2002; Aerts et al., 2003; Semmartin et al., 2004; Garibaldi et  
72 al., 2007; Luo et al., 2010; Vaieretti et al., 2013).

73 In addition to litter quality (its chemical composition), two further factors controlling litter decomposition are the  
74 climate (mainly temperature and humidity) and decomposing organisms (their abundance and activity) (Coûteaux et al., 1995;  
75 Aerts, 1997; Semmartin et al., 2004; Keeler et al., 2009; Berg and McClaugherty, 2014; Zhu et al., 2016). Climate usually  
76 regulates decomposition processes at global and regional scales (Coûteaux et al., 1995; Silver and Miya, 2001), but microbial  
77 activity regulates decomposition processes through soil temperature and moisture effects (modified by grazing) at a local scale  
78 (Coûteaux et al., 1995; De Santo et al., 1993; Luo et al., 2010; Orsborne and Macauley, 1988). Generally, climatic influence  
79 dominates litter quality and decomposer activity in areas where weather conditions are unfavourable (Coûteaux et al., 1995),  
80 due to the dependence of decomposer activity on microclimate (De Santo et al., 1993). Under favourable conditions, litter  
81 quality may largely prevail as the regulator and remain important until the late decomposition stages (Coûteaux et al., 1995).  
82 However, specific temperature and moisture conditions and litter quality may interact strongly and thus the rate of litter  
83 decomposition is difficult to predict.

84 Most studies evaluating the effect of grazing on litter decomposition usually focus on forest, grassland or crop  
85 ecosystems in temperate areas (e.g., Aber and Melillo, 1980; Berg and Staaf, 1981; Luo et al., 2010; McCurdy et al., 2013),  
86 largely ignoring those in the alpine zones. On the QTP, previous studies prove that long-term grazing exclusion (> 2 years)  
87 may promote plant abundance and biodiversity (Niu et al., 2009, 2010; Wu et al., 2009, 2010); however, exclusion may limit  
88 the efficient use of grassland. By contrast, the short-term effect of grazing exclusion is seldom studied. Other previous studies

89 have focused on the dominant species only (e.g., Wu et al., 2009; Luo et al., 2010) and this approach provides less insight into  
90 the nature of nutrient cycling in the grasslands than work on mixed litter (Zhu et al., 2016; this study). Moreover, few studies  
91 have simultaneously investigated how both litter quality and incubation site affect litter decomposition (e.g., Luo et al. 2010;  
92 Zhu et al. 2016; Liang et al. 2018). In this study, we examined the short-term effect (6 month) of grazing and grazing exclusion  
93 on plant community composition and litter quality and their longer-term effect on mixed litter decomposition and N release  
94 (27 month) and soil chemical characteristics (33 month). Based on the above, this research aimed to test three hypotheses: (1)  
95 short-term grazing exclusion does not change plant community composition and litter quality (i.e., nutrient content as N and  
96 biomass of palatable plant species), (2) grazing may accelerate litter decomposition and N release and thus increase soil organic  
97 carbon and N, and (3) litter quality has less effect on litter decomposition and N release compared to incubation site. Results  
98 of the present study may improve our understanding of nutrient cycling in alpine regions in general, and this study may also  
99 further provide knowledge relevant to the development of strategies for restoring the degraded grasslands on the QTP in  
100 particular.

101

## 102 **2 Materials and methods**

### 103 **2.1 Experimental site**

104 This study site was an alpine meadow on the eastern QTP, SW China (N33°59', E102°00', altitude 3,500 m above sea level).

105 The mean annual temperature is 1.2 °C, ranging from -10 °C in January to 11.7 °C in July, with approximately 270 days with

106 frost per year. The mean annual precipitation over the last 35 years, occurring mainly during the short and cool summer is 620  
107 mm (Niu et al., 2010). The years during this study (i.e., 2009 – 2012) were climatically typical (Sun et al., 2015; Supplemental  
108 Fig. 1).

109 The grassland selected for experiments was > 9 ha in area (including 6 ha of experimental plots and 3 ha buffer areas)  
110 and regularly used for Tibetan sheep and yak grazing during the grazing season (May – October). The slopes at the site are  
111 less than 5 %, typical of the gently undulating topography of the region. The soil properties in the experimental plots were  
112 similar after a long-term grazing history with the same grazing pattern. The soil type at the experiment site is an alpine meadow  
113 soil, similar to the Mat-Cryic Cambisols described by Wu et al. (2010).

114

## 115 **2.2 Litter composition and quality**

116 To measure the annual litter composition and determine whether plants could recover without grazing, three grazing (GP, 100  
117 m × 200 m) and three grazing exclusion paddocks (GEP, 30 m × 20 m) were established when all aboveground plants were  
118 dormant in October 2009. Grazing in GP started with an optimal moderate stocking rate of 4 Tibetan sheep/ha from April 2010.  
119 The mean body weight of sheep was about 38 kg when used for the experiment. Before grazing started, 20 quadrats (0.5 m ×  
120 0.5 m) identified by GPS coordinates were randomly established within the GP or GEP, and litter was cleared soon after the  
121 establishment of quadrats. In October 2010, we collected all plant litter from each quadrat of the GP and GEP for two purposes:  
122 (1) measurement of litter composition and quality in this experiment, and (2) measurement of litter decomposition and N  
123 release in the next experiment. Three sampling methods were designed to minimize the sample variance caused by the uneven

124 litter distribution and to ensure the similar composition and quality of litter used for this and the next experiment: (1) half  
125 alongside, (2) half along diagonal, and (3) two sub-quarters (0.25 × 0.25 m) along diagonal (Supplemental Fig. 2).

126 To measure the litter composition, litter of different species collected from each quarter was identified. After litter  
127 species identification, litter was separated into two groups of contrasting palatability to the Tibetan sheep (Niu et al., 2009,  
128 2010; Wu et al., 2009; see Supplementary Table 1): (1) palatable species - preferred and desirable species, and (2) unpalatable  
129 species - undesirable and toxic species. To measure the dry biomass, the palatable and unpalatable litter was separately oven-  
130 dried at 60 °C for 48 h and then weighed.

131 To measure the quality of litter collected from GP (GP-litter treatment) or from GEP (GEP-litter treatment), the  
132 palatable and unpalatable litter from a quarter was mixed again, then ground and stored in a zip-lock bag with 10 g per bag for  
133 a quality test. There were six replicates for each treatment. The contents of lignin, cellulose and hemicellulose were measured  
134 as described by van Soest et al. (1991). Organic carbon concentration (C) was measured by the  $K_2Cr_2O_7$ - $H_2SO_4$  oxidation  
135 method of Walkley-Black (Nelson and Sommers, 1996). The total Kjeldahl nitrogen (N) and total phosphorus (P) were  
136 analyzed using a FIAstar 5000 flow injection analyzer (Foss Tecator, Högnäs, Sweden) (Chen et al., 2016). We also calculated  
137 the ratios of C:N, lignin:N, cellulose:N and hemicellulose:N.

138 We also examined the effects of grazing and grazing exclusion on soil characteristics. We randomly collected five soil  
139 samples in each experimental paddock (n=30 in total) from the 0 – 10 cm depth using a bucket auger (10 cm in diameter) in  
140 October of 2010, 2011 and 2012. The same methods used to test litter quality (i.e., Nelson and Sommers, 1996; Chen et al.,  
141 2016) were applied to estimate the soil organic carbon (SOC), total nitrogen (TN) and total phosphorus (TP).



142

### 143 **2.3 Litter decomposition and N release**

144 In this experiment, we included four treatments: (1) GP-GP, litter of all species was collected from and incubated in the GP;  
145 (2) GEP-GEP, litter of all species was collected from and incubated in the GEP; (3) GP-GEP, litter of all species was collected  
146 from the GP but incubated in the GEP; (4) GEP-GP, litter of all species was collected from the GEP but incubated in the GP.  
147 Treatments 1 and 2 were designated “in situ” incubation treatments, while treatments 3 and 4 were designated “across” grazing  
148 category incubation treatments, and these were included to improve understanding of the “home-field advantage” effect on  
149 litter deposition (John et al., 2011).

150 For each sample soil particles attached to the litter were cleaned off with a soft brush, and samples were air-dried for three  
151 days. Dry litter collected from each quadrat was cut to  $\approx 5$  cm length and 10 g litter was packed into a nylon litter-bag (15 cm  
152  $\times$  20 cm with mesh size of 0.35 mm) (Cornelissen, 1996), which should have prevented any loss of material and had no effect  
153 on litter decomposition (Cornelissen et al., 1999). On 20<sup>th</sup> Oct 2010, the packed litter was incubated above the soil surface by  
154 fastening to the ground surface with four steel stakes to prevent removal by sheep and small animals (Vaieretti et al., 2013),  
155 such as the plateau pika *Ochotona curzoniae* (Hodgson). For each treatment, 24 litter-bags were incubated with 20 cm apart  
156 from each other to reduce the mutual interference. Three litter bags from each treatment were retrieved after incubation periods  
157 of 56, 141, 247, 391, 444, 582, 695 or 799 days (i.e., on 15<sup>th</sup> December 2010, 10<sup>th</sup> March, 24<sup>th</sup> June and 4<sup>th</sup> September 2011,  
158 and 7<sup>st</sup> January, 24<sup>th</sup> May, 14<sup>th</sup> September and 27<sup>th</sup> December 2012, respectively). There were a total of 96 litter-bags used in  
159 this experiment. Retrieved litter was brought back to the laboratory, cleaned by removing any extraneous material attached,

160 and then weighed after being oven-dried at 60 °C for 48 h. Samples were ground and stored in a zip-lock bag for further  
161 chemical analyses as mentioned above. We estimated the litter decomposition and N release as the percentage of dry weight  
162 lost during each incubation period (Cornelissen et al., 1999; Vaieretti et al., 2013).

163

#### 164 **2.4 Statistical analyses**

165 A goodness-of-fit test (Shapiro-Wilk test, Univariate Procedure) was used to test the normality of data before mean comparison  
166 using analysis of variance (ANOVA, GLM Procedure). All data were normally distributed. Data on the initial chemical  
167 characteristics of litter (Table 1) were analyzed using ANOVA followed by Tukey's Studentized multiple range test. Data on  
168 the biomass of palatable or unpalatable species and that on the total biomass between GP and GEP were also analyzed using  
169 ANOVA, while for GP or GEP the difference in litter biomass between the palatable and unpalatable species was compared  
170 by paired-t test (TTEST Procedure) (Fig. 1). Data on the final proportion of litter biomass or N remaining (Fig. 4), litter quality  
171 (content of organic carbon, nitrogen, phosphorous and other chemical characteristics of litter) (Table 1), and soil SOC, TN and  
172 TP (Fig. 2) were analyzed using ANOVA followed by least significant difference test (LSD test) for multiple comparisons.

173 The decomposition rate ( $k$ ,  $\text{g} \cdot 10 \text{ g}^{-1} \cdot \text{day}^{-1}$ ) of litter biomass during the incubation period (Table 2) was assessed using a  
174 negative exponential model according to Swift et al. (1979):  $y = a \cdot e^{-(t/k)}$ , where  $y$  is the dry biomass of litter remaining in the  
175 litter bags at time  $t$  (days),  $a$  is the initial litter biomass (i.e., 10 g in this study). The difference in decomposition rate between  
176 treatments was compared according to Julious (2004), i.e. there is deemed to be no significant difference in decomposition  
177 rate if their 83.4 % CL overlap. The decomposition rate and 83.4 % CL were estimated by fitting the negative exponential

178 model to a nonlinear least square regression model (NLIN Procedure).

179 A multivariate regression model (GLM Procedure) employed by Vaieretti et al. (2013) was applied to quantify the  
180 effect of incubation site and litter quality (the two independent factors) on the final litter decomposition or N release (the  
181 dependent factor) (Table 3): litter decomposition or N release = Site + Quality + Site×Quality +  $\epsilon$ , where ‘Site’ is the paddock  
182 category where the litter was incubated (i.e., incubation site: GP and GEP), ‘Quality’ is the litter quality reflecting the sources  
183 where the litter was collected from (i.e., GP and GEP), and  $\epsilon$  is the model error. The proportional contribution of incubation  
184 site, litter quality and their interaction to variability of litter decomposition or N release was calculated as the sum of squares  
185 for each of the terms, divided by the total sum of squares. The Type I sum of squares was used because of the balanced design  
186 of this experiment. All analyses were done using SAS 9.3 (SAS Institute Inc., Cary, NC, USA). The rejection level for  $H_0$  was  
187 set at  $\alpha < 0.05$ . Values of the means ( $\pm$  SE) are presented in Figs. 1-4.

188

### 189 **3 Results**

#### 190 **3.1 Litter composition and quality and soil property**

191 55 plant species (mostly forbs and grasses along with several legumes and sedges) were identified, and all were found in both  
192 GP and GEP, except *Gentiana macrophylla* Pallas, which was only found in the GP (Supplemental Table 1). However, even  
193 though the annual litter biomass of unpalatable species in both GP and GEP was similar (ANOVA:  $F_{1,38} = 3.43$ ,  $P = 0.0717$ ),  
194 litter biomass of palatable species was significantly greater in the GEP than in the GP (ANOVA:  $F_{1,38} = 75.32$ ,  $P < 0.0001$ ),  
195 and this difference, which contributed significantly more to the total litter biomass in the GEP than in the GP (ANOVA:  $F_{1,38}$

196 = 114.66,  $P < 0.0001$ ) (Fig. 1). The litter biomass was not significantly different between palatable and unpalatable species in  
197 the GP (Paired-t test:  $t_{19} = 0.96$ ,  $P = 0.3510$ ); however, in the GEP, litter biomass of palatable species was significantly greater  
198 than that of unpalatable species (Paired-t test:  $t_{19} = 7.17$ ,  $P < 0.0001$ ) (Fig. 1).

199 GP-litter had significantly higher C and N but significantly lower hemicellulose and hemicellulose:N than GEP-litter  
200 (Table 1). Although other quality characteristics were lower in GP-litter than in GEP-litter, the differences were not significant  
201 (Table 1).

202 The concentrations of soil TN and TP were not significantly different between the GP and GE for any year (LSD =  
203 0.0002 - 0.0015 and 0.0003 - 0.0004 for TN and TP, respectively;  $P > 0.05$ ) (Fig. 2a-b). Similarly, there was no significant  
204 difference in SOC concentration between the GP and GE in 2010 and 2011 (LSD = 0.0169 and 0.0111 for 2010 and 2011,  
205 respectively;  $P > 0.05$ ), while in 2012 SOC was significantly higher in GEP than in GP (LSD = 0.0138,  $P = 0.0279$ ) (Fig. 2c).

206

### 207 **3.2 Litter decomposition**

208 The proportion of litter biomass remaining continuously decreased with incubation duration and litter decomposed faster in  
209 the first year (i.e., 44.6, 38.5, 41.46 and 31.6 % decomposition in GP-GP, GEP-GEP, GEP-GP and GP-GEP, respectively)  
210 than in the second year (i.e., 18.8, 24.1, 27.6 and 23.4 % decomposition in GP-GP, GEP-GEP, GEP-GP and GP-GEP,  
211 respectively) (Fig. 3a). As shown in Table 2, the decomposition rate ( $k$ ) of litter incubated in GP was significantly higher than  
212 that in GEP (non-overlap of 83.4 % CL), i.e., for the “in situ” treatments  $k$  in GP-GP  $>$   $k$  in GEP-GEP and for the “across”  
213 treatments  $k$  in GEP-GP  $>$   $k$  GP-GEP. The final proportion of litter biomass remaining was significantly lower in GP-GP and

214 GEP-GP than in GP-GEP (LSD = 2.51,  $P < 0.0001$ ) (Fig. 4a).

215

### 216 **3.3 N release**

217 Generally, the percentage of total N release did not change during the first winter when temperature was  $< 0$  °C, except that it

218 increased during the incubation period of December 2010 and March 2011 (first winter) in the treatment GEP-GP (Fig. 3b).

219 From January in the second winter (2012), the percentage of total N remaining decreased steadily until the end of the

220 experiment (Fig. 3b). The final proportion of total N remaining was significantly higher in GEP-GP and significantly lower in

221 GEP-GEP and GP-GEP (LSD test: LSD = 5.36,  $P = 0.0002$ ) (Fig. 4b).

222

### 223 **3.4 Contribution of incubation site and litter quality to litter decomposition and N release**

224 The multivariate regression model indicates that both incubation site and litter quality significantly affected litter

225 decomposition and N release (Table 3). Incubation site contributed respectively near 25 % and 50 % more to litter

226 decomposition and N release than did litter quality (Table 3). Furthermore, the model predicts that GP resulted in significantly

227 greater litter decomposition (8.13 %) but significantly lower N release (9.73 %) than did GEP ( $F_{1,8} = 62.48$  and  $57.49$  for litter

228 decomposition and N release, respectively;  $P < 0.0001$ ). Results show that GEP-litter decomposed significantly faster (2.5 %)

229 but released N significantly more slowly (9.27 %) than GP-litter ( $F_{1,8} = 34.99$  and  $15.80$  for litter decomposition and N release,

230 respectively;  $P < 0.01$ ).

231 A significant interaction between incubation site and litter quality for litter decomposition was found ( $F_{2,8} = 7.10$ ,  $P =$

232 0.0286), i.e., litter collected from GEP but incubated in GP decomposed significantly faster (also see Fig. 4a). However, the  
233 interaction between incubation site and litter quality on N release was not significant ( $F_{2,8} = 2.76$ ,  $P = 0.1350$ ).

234

## 235 **4 Discussion**

### 236 **4.1 Litter Composition and Quality**

237 Grazing or grazing exclusion of herbivores may indirectly alter the species composition and functioning of grasslands by  
238 inducing shifts in plant competitive interactions and recruitment patterns, and thus changes in species abundance and life form  
239 structure (Bardgett and Wardle, 2003; Garibaldi et al., 2007; Semmartin et al., 2008; Wu et al., 2009; Niu et al., 2010; Chaneton,  
240 2011). However, our results indicate that herbivore grazing or grazing exclusion did not alter plant community composition in  
241 terms of species inventory, as species found in the GEP mostly also occurred in the GP. On the QTP, species composition is  
242 grazing-intensity-dependent (Niu et al. 2010; Sun et al., 2011) and/or grazing exclusion-period-dependant (Wu et al., 2009).  
243 Thus our results imply that a stocking rate of 4 Tibetan sheep/ha in the GP or a short period of grazing exclusion (i.e., from  
244 April to October) in the GEP did not change the species composition.

245 However, our results show that herbivore grazing significantly altered species composition in terms of species  
246 abundance or palatability with significantly less palatable as well as total litter produced in the GP compared to the GEP (Fig.  
247 1). The low biomass of palatable species in GP may be attributed to the more palatable species (mostly the grasses and sedges,  
248 see Supplemental Table 1) on the QTP being taller (Sun et al., 2011), and therefore more accessible to herbivore grazing, in  
249 addition to being more likely to be grazed by preferential grazing. Through these two mechanisms, the biomass of palatable

250 species in the GP would subsequently be reduced. Results of this study indicate that grazing exclusion for a short period may  
251 allow the recovery of palatable species in the alpine meadows. However, there was no significant difference in the litter  
252 biomass of unpalatable species between the GP and GEP (Fig. 1), which provides evidence against the assumption that  
253 removing the canopy of palatable species may allow the intra- and inter-specific competition for light which ultimately favours  
254 the establishment of short, less-palatable species (Sternberg et al., 2000; Pavlů et al., 2008; Wu et al., 2009; Sun et al., 2011).

255 It is generally accepted that litter quality is usually determined by the levels of various chemical compounds such as  
256 soluble C, N and P, as well as lignin or lignin:N ratio, and litter of high quality usually has higher N content but lower lignin  
257 and lignin:N ratio (e.g., Aerts, 1997; Olofsson and Oksanen, 2002; Wardle et al., 2002; Garibaldi et al., 2007; Strickland et al.,  
258 2009). In this study, grazing might have improved litter quality at least to some extent by significantly increasing the N content  
259 and potentially reducing the hemicellulose content and C:N, lignin:N, cellulose:N and hemicellulose:N ratios (Table 1).  
260 Therefore, our results align with previous studies that have indicated grazing may promote litter quality due to the high nutrient  
261 uptake (e.g., Sirotnak and Huntly, 2000; Olofsson and Oksanen, 2002; Semmartin et al., 2004).

262

#### 263 **4.2 Litter decomposition**

264 For a given climatic region, the ecological processes of litter decomposition are regulated by incubation  
265 microenvironment (i.e., grazing/grazing exclusion and soil property in this study) and litter quality. Our results suggest that  
266 herbivore grazing played a major role in litter decomposition on the QTP. Many studies have demonstrated that litter quality  
267 is one of the most important factors affecting the litter decomposition, and litter with higher N content but lower lignin and

268 lignin:N ratio will decompose faster (Aerts, 1997; Olofsson and Oksanen, 2002; Wardle et al., 2002; Garibaldi et al., 2007;  
269 Strickland et al., 2009). Therefore, it may be expected that regardless of incubation site, GP-litter that had significantly higher  
270 N content should decompose faster than GEP-litter. However, evidence for this expectation was only detected for the “in situ”  
271 incubation treatments (i.e., greater decomposition rate in GP-GP than in GEP-GEP), while for the “across” incubation  
272 treatments opposite results were found (i.e., greater decomposition rate in GEP-GP than in GP-GEP) (Table 2 and Fig. 4a).  
273 Our experimental site is located at high altitude with a low mean annual temperature of 1.2 °C; hence the activity of  
274 decomposers may be inhibited during the cold seasons (Coûteaux et al., 1995). Therefore, litter quality may not be a good  
275 predictor of litter decomposability in cold temperate regions (Aerts, 1997), where climate is more important than litter quality  
276 in the regulation of litter decomposition (Coûteaux et al., 1995). On the QTP, decomposition rate ( $k = 1.04 \times 10^{-3} - 1.34 \times 10^{-3}$   
277  $\text{g} \cdot 10 \text{ g}^{-1} \cdot \text{day}^{-1}$  in Table 2, i.e.  $k = 0.38 - 0.48 \text{ g} \cdot \text{g}^{-1} \cdot \text{year}^{-1}$ ) was much lower than that of the global mean ( $k \approx 0.75 \text{ g} \cdot \text{g}^{-1} \cdot \text{year}^{-1}$ )  
278 with similar latitudes (N30° - 40°) (Zhang et al., 2008).

279         Additionally, incubation site had significantly greater effect on litter decomposition ( $\approx 25\%$ ) than did litter quality  
280 (Table 3), and regardless of litter quality, litter decomposed faster in GP (19.9 % for GP-litter and 11.8 % for GEP-litter) than  
281 in GEP (Fig. 4a; Table 2). This may be attributed to the effect of herbivore grazing activity, which modifies the incubation site  
282 conditions for litter turnover (Takar et al., 1990; Fahnestock and Knapp, 1994; Semmartin et al., 2008; Luo et al., 2010; Liang  
283 et al., 2018). Our results further demonstrate that regardless of incubation site and litter quality, litter decomposed faster in the  
284 first year (31.6 – 44.6 % decomposition) than in the second year (18.8 – 27.6 % decomposition) (Fig. 3a). Berg (2014) and  
285 Berg and McClaugherty (2014) have stated that litter decomposition rate varies at different stages. In the early stages of



286 decomposition litter mass is lost rapidly via the leaching of soluble compounds, while in the later stages decomposition can  
287 even cease after only recalcitrant litter compounds remain (Berg 2014; Berg and McClaugherty 2014). Therefore, litter quality  
288 regulates decomposition processes mainly in the early stages of decomposition (Berg and McClaugherty, 2014), which is  
289 supported by our results, i.e., when incubated in GP in the first year, the percentage decomposition of GP-litter (44.6 %) was  
290 higher than that of GEP-litter (41.5 %). However, we could not reject the above conclusion that incubation site is the dominant  
291 factor affecting litter decomposition over litter quality, as in the first year litter always decomposed faster in GP (i.e., 44.6 %  
292 in GP-GP and 41.5 % in GEP-GP) than in GEP (i.e., 38.5 % in GEP-GEP and 31.6 % in GP-GEP) (Fig. 3a). These data also  
293 imply that a home-field advantage is detected only for GP-litter during the first year of incubation (i.e., percentage  
294 decomposition 44.6 % in GP-GP > 31.6 % in GP-GEP) with no evidence for GEP-litter (i.e., percentage decomposition 38.5 %  
295 in GEP-GEP < 41.5 % in GEP-GP).

296 In a long-term (9 year) study on the QTP, Wu et al. (2009) reported that grazing exclusion favors the increase of soil  
297 total nitrogen, soil organic matter, soil organic carbon, soil microbial biomass carbon and soil carbon storage. It is interesting  
298 that in the present study, only soil organic carbon (SOC) significantly increased after three-years grazing exclusion (Fig. 2).  
299 The increase in SOC in GEP may be because grazing exclusion prevents the reduction of removal of palatable litter by the  
300 herbivores (Fig. 1), and the organic C locked within plant tissues may be returned to the soil during litter decomposition instead  
301 (Bardgett and Wardle, 2003; Wu et al., 2009). Holland and Detling (1990) and Ågren et al. (1999) stated that increasing carbon  
302 availability in soil may promote decomposer growth and activity even at low nitrogen concentrations. However, the expected  
303 results, i.e. significantly higher litter decomposition rate caused by the possible increasing decomposer mass and/or activity in

304 the grazing exclusion grasslands (Wu et al., 2009), were not observed in the GEP in this study (Table 2, Fig. 4a). Thus, soil  
305 properties are unlikely to be significantly changed through grazing or grazing exclusion over relatively short periods, indicating  
306 that limited grazing events have a smaller effect on litter decomposition under cool environments on the QTP than in  
307 experiments conducted in warmer climates.

308

### 309 4.3 N release

310 N release is a more complex process, compared to litter decomposition. N release may involve any one or both processes of N  
311 immobilization and N mineralization, where the former results in the accumulation of N in the litter and the latter causes the  
312 release of N from the litter (Manzoni et al., 2008). Swift et al. (1979) and Berg and McClaugherty (2014) reported that the  
313 biological decomposition of litter is mainly carried out by microbial decomposers, which *per se* have a higher N:C ratio  
314 compared with most litter types. This property of decomposers creates a high N demand for decomposer growth (Manzoni et  
315 al., 2008). Results show that regardless of litter quality or source, N remaining was significantly higher when litter was  
316 incubated in GP than in GEP (Fig. 4b). Bosatta and Balesdent, (1996) and Manzoni et al. (2008), a promising candidate  
317 mechanism may be that the faster decomposition rate of litter in GP increases the utilization of C by the decomposers which  
318 in turn increases the N:C ratio in litter; when N:C ratio is high, large amounts of mineral N are immobilized, increasing the N  
319 concentration in litter. This mechanism may also account for the dynamics of N release over the incubation period (Fig. 3b).  
320 For instance, decomposer activity resulted in continuous C consumption and litter decomposition (March – June 2011), while  
321 high N:C ratio due to decomposition progress induced the release of accumulated N (June – August 2011). The inverse pattern

322 of N release and litter decomposition found in this study (Fig. 4) is frequently reported (e.g., Aber and Melillo, 1980; Fahey et  
323 al., 1991; Gallardo and Merino, 1992).

324 It is not surprising that because both litter decomposition and N release are regulated by decomposers synchronously,  
325 incubation site also had a significantly greater effect ( $\approx 50\%$ ) on N release as on litter decomposition (Table 3) than did litter  
326 quality, indicating the latter is not a good predictor on litter N release in the cold temperate region (Aerts, 1997).

327

## 328 **5 Conclusion**

329 Results of our study are not completely consistent with previously proposed hypotheses. On the cold QTP, short-term grazing  
330 exclusion did not promote species abundance but increases plant palatability and total litter biomass. Grazing improves litter  
331 quality through higher N content but lower hemicellulose and hemicellulose:N ratio. Grazing significantly accelerated litter  
332 decomposition, while grazing exclusion promoted N release and increased SOC. Although litter quality may affect  
333 decomposition at the early stages, incubation site had significantly more impact on both litter decomposition and N release.  
334 The different effects of grazing and grazing exclusion functioning on the grassland ecosystems may have implications in the  
335 management of alpine meadows on the QTP. For example, periodic grazing and grazing exclusion may be a good option which  
336 allows plant recovery and promotes nutrient cycling, and thus contributes to the restoration of degraded grasslands.

337

338 *Author contributions.* YS and FH designed, and FH supervised the experiments, YS, FH, ZFW and SHC performed research  
339 and collected data. XZH, FH and YS analysed data and prepared the manuscript, and all authors contributed to the writing.

340

341 *Competing interests.* The authors declare that they have no conflict of interest.

342

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349

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497 **Tables**

498 **Table 1.** Initial chemical characteristics (mean  $\pm$  SE) of litter collected grazing paddocks (GP-litter) and grazing exclusion  
 499 paddocks (GEP-litter). Unit of chemical characteristics is mg/g litter for C, N, P, lignin, cellulose and hemicellulose. Different  
 500 letters in each row indicate significant difference (ANOVA:  $P < 0.05$ ).

Compound	GP-litter		GEP-litter		LSD	P
C	576.44 $\pm$ 4.20	a	553.03 $\pm$ 3.35	b	14.92	0.0121
N	7.41 $\pm$ 0.32	a	5.35 $\pm$ 0.67	b	2.05	0.0494
P	1.39 $\pm$ 0.27	a	1.05 $\pm$ 0.27	a	1.05	0.4197
Lignin	22.94 $\pm$ 4.57	a	18.83 $\pm$ 1.67	a	13.51	0.4456
Cellulose	328.61 $\pm$ 11.55	a	385.18 $\pm$ 19.27	a	62.38	0.0655
Hemicellulose	296.76 $\pm$ 6.82	b	324.56 $\pm$ 5.52	a	24.37	0.0340
C:N	78.08 $\pm$ 3.62	a	106.82 $\pm$ 13.96	a	40.05	0.1171
Lignin:N	3.15 $\pm$ 0.72	a	3.70 $\pm$ 0.74	a	2.87	0.6210
Cellulose:N	44.40 $\pm$ 1.35	a	75.23 $\pm$ 13.15	a	36.70	0.0800
Hemicellulose:N	40.12 $\pm$ 1.21	b	62.58 $\pm$ 7.69	a	21.61	0.0447

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504 **Table 2.** Litter decay rate ( $k$ ,  $\text{g} \cdot 10 \text{ g}^{-1} \cdot \text{day}^{-1}$ ) in different incubation environments. GP-GP, mixed litter was collected from  
 505 grazing paddocks (GP) and incubated in GP; GEP-GEP, mixed litter was collected from grazing exclusion paddocks (GEP)  
 506 and incubated in GEP; GEP-GP, mixed litter was collected from GEP and incubated in GP; GP-GEP, mixed litter was collected  
 507 from GP and incubated in GEP.  $k$  values followed by different letters are significantly different (non-overlap of 83.4 % CL).  
 508 The  $R^2$ , F and P are estimated from the negative exponential model of Swift et al. (1979).

Treatment	$k \pm \text{SE} (\times 10^{-3})$	83.4 % CL ( $\times 10^{-3}$ )	$R^2$	$F_{1,26}$	P
GP-GP	$1.34 \pm 0.04$ a	1.28 - 1.40	0.9666	6716.09	< 0.0001
GEP-GEP	$1.20 \pm 0.04$ b	1.14 - 1.27	0.9545	5646.09	< 0.0001
GEP-GP	$1.30 \pm 0.07$ ab	1.12 - 1.44	0.9149	2382.19	< 0.0001
GP-GEP	$1.04 \pm 0.02$ c	0.10 - 1.07	0.9809	8524.80	< 0.0001

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510 **Table 3.** Contribution (%) of incubation site (Site: GP, grazing paddocks; GEP, grazing exclusion paddocks) and litter quality  
 511 (Quality: GP-litter, mixed litter collected from grazing paddocks; GEP-litter, mixed litter collected form grazing exclusion  
 512 paddocks) to litter decomposition and N release.

Parameter	df	Type I SS	Contribution	F	P
<b>Litter decomposition</b>					
Site	1	110.84	55.51	62.48	< 0.0001
Quality	1	62.06	31.08	34.99	0.0004
Site×Quality	1	12.59	6.30	7.10	0.0286
Error	8	14.19	7.11		
<b>N release</b>					
Site	1	466.25	68.39	57.49	< 0.0001
Quality	1	128.18	18.80	15.81	0.0041
Site×Quality	1	22.41	3.29	2.76	0.1350
Error	8	64.88	9.52		

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517 **Supplemental table**518 **Table S1.** The list of all species in grazing and grazing exclusion paddocks (Niu et al., 2009, 2010; Wu et al., 2009).

Species	Palatability	Functional group	Species	Palatability	Functional group
<i>Agrostis hugoniana</i>	P	GG	<i>Euphorbia esula</i>	UP	FG
<i>Agrostis micrantha</i>	P	GG	<i>Euphrasia regelii</i>	UP	FG
<i>Allium sikkimense</i>	P	FG	<i>Gentiana macrophylla*</i>	UP	FG
<i>Astragalus polycladus</i>	P	LG	<i>Gentiana sino-ornata</i>	UP	FG
<i>Blysmus sinocompressus</i>	P	SG	<i>Gentianopsis barbata</i>	UP	FG
<i>Carex brunnescens</i>	P	SG	<i>Geranium wilfordii</i>	UP	FG
<b><i>Elymus nutans</i></b>	P	GG	<i>Halenia elliptica</i>	UP	FG
<i>Festuca sinensis</i>	P	GG	<i>Lamiophlomis rotata</i>	UP	FG
<i>Gueldenstaedtia diversifolia</i>	P	LG	<i>Lancea tibetica</i>	UP	FG
<b><i>Kobresia graminifolia</i></b>	P	SG	<i>Leontopodium souliei</i>	UP	FG
<i>Kobresia humilis</i>	P	SG	<i>Ligularia virgaurea</i>	UP	FG
<i>Kobresia kansuensis</i>	P	SG	<i>Oxytropis kansuensis</i>	UP	LG
<i>Kobresia tibetica</i>	P	SG	<i>Parnassia palustris</i>	UP	FG
<i>Plantago depressa</i>	P	FG	<i>Pleurospermum pulszkyi</i>	UP	FG
<b><i>Poa chalarantha</i></b>	P	GG	<i>Polygonum viviparum</i>	UP	FG
<i>Poa poophagorum</i>	P	GG	<i>Potentilla anserine</i>	UP	FG
<i>Potentilla fragarioides</i>	P	FG	<i>Ranunculus tanguticus</i>	UP	FG
<b><i>Roegneria nutans</i></b>	P	SG	<i>Rumex patientia</i>	UP	FG
<i>Scirpus pumilus</i>	P	SG	<i>Saussurea hieracoides</i>	UP	FG
<b><i>Stipa aliena</i></b>	P	GG	<i>Saussurea pachyneura</i>	UP	FG
<i>Anaphalis lactea</i>	UP	FG	<i>Saussurea stella</i>	UP	FG
<i>Anemone obtusiloba</i>	UP	FG	<i>Swertia bimaculata</i>	UP	FG
<b><i>Anemone rioularis</i></b>	UP	FG	<i>Thalictum alpinum</i>	UP	FG
<i>Anemone trullifolia</i>	UP	FG	<i>Thermopsis lanceolata</i>	UP	LG
<i>Arenaria serpyllifolia</i>	UP	FG	<i>Thlaspi arvense</i>	UP	FG
<i>Aster alpinus</i>	UP	FG	<b><i>Trollius farreri</i></b>	UP	FG
<i>Cerastium arvense</i>	UP	FG	<i>Veronica eriogyne</i>	UP	FG
<i>Cremanthodium lineare</i>	UP	FG			



519 Bold font indicates the dominant species. P, palatable species; UP, unpalatable species; GG, group of grass species; SG, group  
520 of sedge species; LG, group of leguminous species; FG, group of forbs species; \**G. macrophylla* presented in GP only.

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537 **Figure captions:**

538 **Figure 1.** Mean ( $\pm$  SE) annual biomass of litter collected from grazing paddocks (GP) and grazing exclusion paddocks (GEP).

539 For each category, columns with different letters are significantly different (ANOVA:  $P < 0.05$ ).

540 **Figure 2.** Mean ( $\pm$  SE) soil total nitrogen (TN) (**a**), soil total phosphorous (TP) (**b**), and soil organic carbon (SOC) (**c**) between

541 the grazing paddocks (GP) and grazing exclusion paddocks (GEP). Significant difference was only found between GP and

542 GEP for SOC in 2012 (LSD test:  $P < 0.05$ ).

543 **Figure 3.** Dynamics (mean  $\pm$  SE) of litter decomposition (**a**) and N lease (**b**) on the QTP. GP-GP, mixed litter was collected

544 from grazing paddocks (GP) and incubated in GP; GEP-GEP, mixed litter was collected from grazing exclusion paddocks

545 (GEP) and incubated in GEP; GEP-GP, mixed litter was collected from GEP and incubated in GP; GP-GEP, mixed litter was

546 collected from GP and incubated in GEP. Grey lines under months indicate the mean air temperatures  $< 0$  °C.

547 **Figure 4.** Percentage of litter mass (**a**) and total N remaining (**b**) at the end of experiments. GP-GP, mixed litter was collected

548 from grazing paddocks (GP) and incubated in GP; GEP-GEP, mixed litter was collected from grazing exclusion paddocks

549 (GEP) and incubated in GEP; GEP-GP, mixed litter was collected from GEP and incubated in GP; GP-GEP, mixed litter was

550 collected from GP and incubated in GEP. Vertical bars are the least significant different (LSD) values. Columns with the

551 different letters are significantly different (LSD test:  $P < 0.05$ ).

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553 **Supplemental figures**

554 **Figure S1.** Mean temperature (°C) and total precipitation (mm) during experiments.

555 **Figure S2.** Field sampling methods: **(a)** half alongside, **(b)** half along diagonal, **(c)** two sub-quarters along diagonal, and **(d)**  
556 additional quarter for supplementary litter collection to make up 20 g in quarter **a**, **b** or **c**.