



1 **Grazing elevates litter decomposition but slows nitrogen**
2 **release in an alpine meadow**

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11

12 **Abstract.** Nutrient cycling is a key ecosystem function whereby the processes of litter decomposition and N
13 release in the soil-plant interface are vitally important but still not clear in the alpine ecosystems. We carried out
14 a 3-year study to improve our understandings in nutrient cycle and develop strategies for restoring the degraded
15 grasslands on the Qinghai-Tibetan Plateau. We established the grazing (GP) and grazing exclusion paddocks
16 (GEP), then identified litter species composition and analyzed litter and soil chemical characteristics. Litter
17 decomposition and N release were monitored by incubating litter ‘in situ’ and across paddocks over 799 days. We
18 found that grazing did not change plant species composition, but increased litter N; while grazing exclusion
19 increased litter mass of palatable species and promoted soil organic carbon. Litter decomposed faster in GP, while
20 N release was faster in GEP. Incubation site environment had more but litter source had less impact on litter
21 decomposition and N release. Therefore, grazing and grazing exclusion had different impacts on litter



22 decomposition and N release but both elevated nutrient cycle. The implications of our findings in restoring the
23 degraded grasslands on the Qinghai-Tibetan Plateau were discussed.

24

25 **1 Introduction**

26 Qinghai-Tibetan Plateau represents an important eco-region in China (Wen et al., 2010), where the alpine
27 grasslands cover more than 85% of total area and are regarded as the major natural pastures (Dong et al., 2010).
28 However, the ecosystems in this region have continuously suffered from severe degradation mainly driven by
29 climate change, overgrazing, over-cultivation and poor management (Han et al., 2008; Li et al., 2009; Wu et al.,
30 2009; Feng et al., 2010; Wu et al., 2010), with an increasing degradation rate of 1.2-7.44% per year (Ma et al.,
31 2007). Since 1990's, the restoration of degraded grasslands has attracted highly attention (Kang et al., 2007; Han
32 et al., 2008), and some efforts have recently focused on the grassland restoration and maintenance by increasing
33 aboveground plant abundance (Niu et al., 2009) and biodiversity (Wu et al., 2009; Niu et al., 2010), and improving
34 soil property (Cao et al., 2004; Wu et al., 2010; Sun et al., 2011). However, litter decomposition and N release,
35 the key factors regulating the nutrient cycle and availability in the soil-plant interface (Carrera and Bertiller, 2013),
36 are still not clear in the alpine ecosystems (Luo et al., 2010; Zhu et al., 2016). We carried out this study to
37 investigate the effect of grazing on plant community, litter quality, litter decomposition and N release in an alpine
38 meadow, aiming to improve our understandings in the nutrient cycle and to develop management strategies for
39 restoration of ecological function in alpine pastures.

40 It is generally known that herbivore grazing may induce short-term ecophysiological changes in overall
41 litter quality as well as longer-term shifts in plant community composition. At the short-term ecophysiological
42 level, herbivore grazing may promote the plant species producing high-quality litter (Holland and Detling, 1990;
43 Sirotnak and Huntly, 2000; Olofsson and Oksanen, 2002; Semmartin et al., 2004, 2008). Because the loss of plant



44 tissues caused by grazing may favour the grazed species with a higher re-growth rate and greater nutrient contents
45 in plant tissues due to the higher nutrient uptake (see Holland and Detling, 1990; Olofsson and Oksanen, 2002;
46 Semmartin et al., 2008). At the long-term community level, selective foliar grazing of herbivores may alter the
47 competitive interaction and recruitment patterns of plant species, which will change their abundance and life form
48 structure (Bardgett and Wardle, 2003; Semmartin et al., 2008; Wu et al., 2009; Niu et al., 2010). For instance,
49 herbivores usually concentrate on the most palatable plants (e.g., species with high nutrient and low fibre contents),
50 which will favour dominance by the less unpalatable species (Garibaldi et al., 2007), resulting in the high inputs
51 of low-quality litter to soil and thus a reduction of decomposition rate, nutrient availability and nutrient cycling
52 (Ritchie and Knops, 1998; Moretto et al., 2001; Olofsson and Oksanen, 2002). However, empirical evidences of
53 variances in litter quality input and soil nutrient cycle caused by grazing are still scarce and controversial
54 (Garibaldi et al., 2007).

55 It is supposed that the higher nutrient content in plant tissue usually results in the faster litter decomposition,
56 and the high nutrient mineralization and availability in soil (Olofsson and Oksanen, 2002). At the ecosystem scale,
57 the chemical characteristics of plant litter, for example the carbon:nitrogen ratios (C:N) and/or nitrogen and lignin
58 content, are often regarded as the indicators of litter quality (Aerts, 1997; Strickland et al., 2009). Many studies
59 have demonstrated a positive correlation between litter decomposition rate and N content, or a negative
60 relationship between litter decomposition rate and initial lignin content and C:N or lignin:N ratios ratios (e.g.,
61 Wardle et al., 2002; Aerts et al., 2003; Semmartin et al., 2004; Garibaldi et al., 2007; Luo et al., 2010; Vaieretti et
62 al., 2013).

63 Except litter quality (its chemical composition), the climate (mainly temperature and humidity) and
64 decomposing organisms (their abundance and activity) are the two main factors controlling litter decomposition
65 (Coûteaux et al., 1995; Aerts, 1997; Semmartin et al., 2004; Keeler et al., 2009; Berg and McLaugherty, 2014;



66 Zhu et al., 2016). Generally, climate is the dominant factor over litter quality in areas subjected to unfavourable
67 weather conditions (Coûteaux et al., 1995), because decomposer activity regulating litter decomposition is largely
68 temperature and moisture dependent (De Santo et al., 1993). Usually the activity of decomposers increases with
69 temperature (Coûteaux et al., 1995); and as soil moisture level rises, the metabolic activity of decomposers
70 normally increases until an optimum plateau is reached (Orsborne and Macauley, 1988). Thus it is likely that the
71 optimum response of decomposers to temperature and moisture is determined largely by the local climate (De
72 Santo et al., 1993). However, under the favourable conditions, litter quality may largely prevail as the regulator
73 and remain important until the late decomposition stages (Coûteaux et al., 1995).

74 Most studies on litter decomposition affected by the herbivores usually focus on the forest, grassland or
75 crop ecosystems in the temperate areas, largely ignoring that in the alpine zones. Moreover, to date our knowledge
76 on the decomposition of litter particular that of mixed-litter in the grasslands in the alpine regions is still lacking,
77 though some efforts have been made (e.g., Wu et al., 2009; Luo et al., 2010; Zhu et al., 2016). In this study, we
78 firstly set up the grazing (GP) and grazing exclusion paddocks (GEP) and examined the subsequent plant
79 composition, litter quality and soil characteristics. We then subsequently investigated whether litter quality had
80 any effects on litter decomposition, N release and soil property by collecting litter mixtures from both GP and
81 GEP and incubating them 'in situ' and across GP and GEP. Based on the knowledge outlined above, we tested:
82 (1) whether grazing may improve litter quality with higher nutrient content (i.e., N) and change plant community
83 with lower biomass of palatable species detected whereas the opposite is the case in GEP, and (2) whether grazing
84 will elevate litter decomposition and N release and thus improve soil properties whereas the opposite is the case
85 in GEP. Results of the present study may provide insight into our understandings in nutrient cycle in the alpine
86 regions in general, which may also further provide a scheme to assist in the development of strategies for restoring
87 the degraded grasslands on the Qinghai-Tibetan Plateau in particular.



88

89 **2 Materials and methods**90 **2.1 Experimental site**

91 This study was conducted in a typical alpine meadow, eastern Qinghai-Tibetan Plateau, NW China (N33°59',
92 E102°00', altitude 3,500 m above sea level). The mean annual temperature is 1.2°C, ranging from -10°C in January
93 to 11.7°C in July, with approximately 270 days of frost per year. The mean annual precipitation is 620 mm over
94 the last 35 years, occurring mainly during the short and cool summer (Niu et al., 2010). The years during this
95 study (i.e., 2009 ~ 2012) were climatically typical (Sun et al., 2015), and the mean temperature and total
96 precipitation of each month during the experiments were showed in Supplemental Fig. 1.

97 The pasture selected for experiments is larger than nine hectares (including experimental and buffer areas)
98 and regularly used for Tibetan sheep and yak grazing during the grazing seasons (May ~ October). The slopes are
99 less than 5%, which is the gentle topography of the area, and the soil attributes in the experimental areas were
100 similar after a long-term grazing history with the same grazing pattern. The soil type in the experimental area is
101 alpine meadow soil, similar to the primarily Mat-Cryic Cambisols (Wu et al., 2010).

102

103 **2.2 Litter composition and quality**

104 To measure the annual litter composition and determine whether plants could recover without grazing, three
105 grazing (GP, 30 m × 20 m) and three grazing exclusion paddocks (GEP, 100 m × 200 m) were established when
106 all aboveground plants are dormant in October 2009. Grazing in GP started with an optimal moderate stocking
107 rate of 4 Tibetan sheep/ha since April 2010. The mean body weight of sheep was about 38 kg when used for the
108 experiment. In October 2010, 20 quadrats (0.5 m × 0.5 m) were randomly established within the GP or GEP, and
109 three sampling methods [i.e., half alongside, half along diagonal, and two sub-quarters (0.25 m × 0.25 m) along



110 diagonal; see Supplemental Fig. S2] were designed to minimize the sample variance caused by the uneven litter
111 distribution and to ensure the similar composition and quality of litter used for this and next experiments. The
112 aboveground portion of all dormant plants (i.e., litter) from each quadrat was sampled for two purposes: (1)
113 measurement of litter composition and quality, and (2) measurement of litter decomposition and N release.

114 To measure the litter composition, litter of different species was identified and then separated into two
115 groups according to the palatability by the Tibetan sheep (Niu et al., 2009, 2010; Wu et al., 2009; see
116 Supplementary Table 1): (1) palatable species - preferred and desirable species, and (2) unpalatable species -
117 undesirable and toxic species.

118 To measure the litter quality, two treatments were tested: (1) GP-litter – litter of all species from
119 aboveground was collected from the GP, (2) GEP-litter – litter of all species from aboveground was collected
120 from the GEP. Litter from a quadrat was mixed after the measurement of litter composition and then oven-dried
121 at 60°C for 48 h. The dry litter was ground and stored in a zip-lock bag with 10 g per bag. There were six replicates
122 for each treatment. The contents of lignin, cellulose and hemicellulose were measured by following van Soest et
123 al. (1991). Organic carbon concentration (C) was measured by the $K_2Cr_2O_7$ - H_2SO_4 oxidation method of Walkley-
124 Black (Nelson and Sommers, 1996). Briefly, a known weight of plant sample was treated with potassium
125 dichromate in the presence of concentrated sulfuric acid and digested at a low temperature ($\approx 30^\circ C$) for one hour.
126 The excess of potassium dichromate did not reduce the organic matter, as it was titrated back against a standard
127 solution of ferrous sulfate. The C was then calculated on the basis of the quantity of ferrous sulfate consumed
128 (Chen et al., 2016). The total Kjeldahl nitrogen (N) and total phosphorus (P) were analyzed using a FIAstar 5000
129 flow injection analyzer (Foss Tecator, Högnäs, Sweden) (Chen et al., 2016). We also calculated the ratios of C:N,
130 lignin:N, cellulose:N and hemicelluloses:N.

131 The soil environment modified by land use pattern is thought to be another important driver of variations



132 of litter composition and quality, and thus litter decomposition and N release. Therefore, we also examined the
133 effects of grazing and grazing exclusion on soil characteristics. We randomly selected five sampling sites from
134 each experimental paddock, and soil samples ($n = 30$ in total) were collected at depth 0-10 cm using a bucket
135 auger (10 cm in diameter) in October of 2010, 2011 and 2012. The same methods used to test litter quality (i.e.,
136 Nelson and Sommers, 1996; Chen et al., 2016) were applied to estimate the soil organic carbon (SOC), total
137 nitrogen (TN) and total phosphorus (TP).

138

139 **2.3 Litter decomposition and N release**

140 In this experiment, we carried out four treatments: (1) GP-GP, litter of all species was collected from and incubated
141 in the GP; (2) GEP-GEP, litter of all species was collected from and incubated in the GEP; (3) GP-GEP, litter of
142 all species was collected from the GP but incubated in the GEP; (4) GEP-GP, litter of all species were collected
143 from the GEP but incubated in the GP. The treatments 1 and 2 were referred as “in situ” incubation treatments.
144 The treatments 3 and 4 were across incubation treatments which were to improve our better understanding “home-
145 field advantage” effect on litter deposition (John et al., 2011).

146 For each sample soil particles attached to litter were cleaned off with a soft brush, and samples were air-dried
147 for three days. Dry litter collected from each quadrat were cut to ≈ 5 cm length and 10 g litter was packed into a
148 nylon litter-bag (15 cm \times 20 cm with mesh size of 0.35 mm) (Cornelissen, 1996), which may prevent any loss of
149 material and has no effect on litter decomposition (Cornelissen et al., 1999). On 20th Oct 2010, the packed litter
150 was incubated above the soil surface by fastening to the ground surface with four steel stakes to avoid being
151 removed by the sheep and small animals (Vaieretti et al., 2013). The small animals were the plateau pika,
152 *Ochotona curzoniae* (Hodgson) in the present study. For each treatment, 24 packed litter-bags were incubated
153 with 20 cm apart from each other to reduce the mutual interference. Three litter bags were retrieved after a



154 incubation period of 56, 141, 247, 391, 444, 582, 695 or 799 days (i.e., on 15th December 2010, 10th March, 24th
155 June and 4th September 2011, and 7st January, 24th May, 14th September and 27th December 2012, respectively).
156 There were a total of 144 packed litter-bags used in this experiment. Retrieved litter was brought back to the
157 laboratory and cleaned by removing any extraneous material attached. They were weighed after being oven-dried
158 at 60°C for 48 h. Samples were ground and stored in a zip-lock bag for further chemical analyses as mentioned
159 above. We estimated the litter decomposition and N release as the percentage of dry weight remaining at the end
160 of each incubation period (Cornelissen et al., 1999; Vaieretti et al., 2013).

161

162 **2.4 Statistical analyses**

163 A goodness-of-fit test (Shapiro-Wilk test, Univariate Procedure) was used to test the normality of data before
164 mean comparison using analysis of variance (ANOVA, GLM Procedure). All those data were normally distributed.
165 Data on litter quality (Table 1) were analyzed using ANOVA followed by Tukey's Studentized multiple range
166 test. While the difference between the palatable and unpalatable litter quantity in GP or GEP was compared by
167 paired-t test (TTEST Procedure) (Fig. 2). Data on the proportion of litter mass or N remaining (Fig. 3-5), litter
168 quality (content of organic carbon, nitrogen, phosphorous and so on) (Table 1), and soil SOC, TN and TP (Fig. 1)
169 were analyzed using ANOVA followed by least significant difference test (LSD test) for multiple comparisons.

170 The decay rate (k) of litter mass during the incubation period (Table 2) was assessed using a negative
171 exponential model according to Swift et al. (1979): $y = a \cdot e^{-(t \cdot k)}$, where y is the dry mass of litter remaining in the
172 litter bags at time t (days), a is the initial litter mass. The difference in decay rate between treatments was compared
173 according to Julious (2004), i.e. there is no significant difference in decay rate if their 83.4% CL overlap. The
174 decay rate and 83.4 % CI were estimated by fitting the negative exponential model to a nonlinear least square
175 regression model (NLIN Procedure).



176 To provide quantified information of how environmental conditions (i.e., decomposition sites, the GP and
177 GEP) and litter source (i.e., litter collected from the GP and GEP) affecting the final litter decomposition and N
178 release, a multivariate regression model (GLM Procedure) employed by Vaieretti et al. (2013) was used (Table
179 3): litter decomposition or N release = site + litter source + site × litter source + ϵ , where ϵ is the model error. The
180 proportion of site environment, litter source and their interaction contributing to variability of litter decomposition
181 or N release was calculated as: their sum of square divided by the total sum of square. The Type I sum of square
182 was used because of the balanced design of this experiment. All analyses were done using SAS 9.3 (SAS Institute
183 Inc., Cary, NC, USA). Rejection level of H_0 was set at $\alpha < 0.05$. Mean (\pm SE) was presented in Fig. 1-5.

184

185 3 Results

186 3.1 Litter composition and quality and soil property

187 Fifty-five plant species (mostly forbs and graminoid grasses with several legumes and sedges) were identified,
188 and they presented in both GP and GEP, except the *Gentiana macrophylla* Pallas, which was only found in the
189 GP (Supplemental Table 1). However, even though the annual litter mass of unpalatable species in both GP and
190 GEP was similar (ANOVA: $F_{1,38} = 3.43$, $P = 0.0717$), litter mass of palatable species was significantly greater in
191 the GEP than in the GP (ANOVA: $F_{1,38} = 75.32$, $P < 0.0001$), which contributed significantly more to the total
192 litter mass in the GEP than in the GP (ANOVA: $F_{1,38} = 114.66$, $P < 0.0001$) (Fig. 1). The litter mass was not
193 significantly different between palatable and unpalatable species in the GP (Paired-t test: $t_{19} = 0.96$, $P = 0.3510$);
194 however, in the GEP, litter mass of palatable species was significantly greater than that of unpalatable species
195 (Paired-t test: $t_{19} = 7.17$, $P < 0.0001$) (Fig. 1).

196 As shown in Table 1, litter collected from GP had significantly higher C and N but significantly lower
197 hemicelluloses and hemicelluloses:N than that collected from GEP. No significant difference was found in other



198 compounds or compound ratios between different litter sources though the cellulose, C:N, lignin:N and
199 cellulose:N were lower in litter collected from GP (Table 1).

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

205

206 3.2 Litter decomposition

207 The proportion of litter mass remaining significantly decreased with incubation duration (LSD test: LSD = 3.23
208 ~ 4.96, $P < 0.0001$) (Fig. 3). It is found that the lower environmental temperature ($< 0^{\circ}\text{C}$) between November and
209 March (Supplemental Fig. 1) might have significantly slowed the litter decomposition (Fig. 3); but increasing
210 temperature since April (Supplemental Fig. 1) significantly accelerated litter decomposition (Fig. 3). The time
211 period required to achieve 50 % decomposition of litter mass was about 19 months in GP-litter, which was faster
212 than that in GP-GEP-litter (i.e., about 23 months) (Fig. 3).

213 As shown in Table 2, the decomposition rate (k) of litter incubated in the GP was significantly higher than
214 that in the GEP (non-overlap of 83.4% CL), i.e., for the 'in situ' treatments k in GP-GP $> k$ in GEP-GEP) and for
215 the across treatments k in GEP-GP $> k$ GP-GEP. The final proportion of litter mass remaining was significantly
216 lower in GP-GP and GEP-GP than in GP-GEP (LSD test: LSD = 2.51, $P < 0.0001$) (Fig. 5a).

217

218 3.3 N release

219 Compared to litter decomposition, the dynamics of N release were more complicate. Generally, the percentage of



220 total N release did not significantly change during the first winter when temperature < 0°C, except that it
221 significantly increased during the incubation period of December 2010 and March 2011 (first winter) in treatments
222 of GEP-GP (Fig. 4). Since January in the second winter (2012), the percentage total N remaining significantly
223 decreased until the end of experiments (Fig. 4). The final proportion of total N remaining was significantly higher
224 in the GEP-GP and significantly lower in the GEP-GEP and GP-GEP (LSD test: LSD = 5.36, P = 0.0002) (Fig.
225 5b).

226

227 **3.4 Contribution of site environment and litter source to litter decomposition and N release**

228 The multivariate regression model indicates that both site environment and litter source significantly affected litter
229 decomposition and N release with various contributions (Table 3). Site environment contributed respectively near
230 25% and 50% more to litter decomposition and N release than did litter source (Table 3). Furthermore, the model
231 predicts that GP resulted in significantly greater litter decomposition (8.13%) but significantly fewer N release
232 (9.73%) than did GEP ($F_{1,8} = 62.48$ and 57.49 for litter decomposition and N release, respectively; $P < 0.0001$).
233 Results show that litter collected from GEP decomposed significantly faster (2.5%) but released N significantly
234 slower (9.27%) than that collected from GP ($F_{1,8} = 34.99$ and 15.80 for litter decomposition and N release,
235 respectively; $P < 0.01$).

236 Significant interaction of site environment and litter source was found on litter decomposition ($F_{2,8} = 7.10$,
237 $P = 0.0286$), i.e., litter collected from GEP but incubated in GP decomposed significantly faster (also see Fig. 5a).
238 However, interaction of site environment and litter source was not significant on N release ($F_{2,8} = 2.76$, $P = 0.1350$).

239

240 **4 Discussion**

241 **4.1 Litter Composition and Quality**



242 Grazing of herbivores may indirectly alter the species composition and functioning of grasslands by inducing
243 shifts in plant competitive interactions and recruitment patterns, and thus changes in species abundances and life
244 form structure (Bardgett and Wardle, 2003; Garibaldi et al., 2007; Semmartin et al., 2008; Wu et al., 2009; Niu et
245 al., 2010; Chaneton, 2011). However, our results indicate that herbivore grazing did not alter plant community
246 composition in terms of species inventory, as species found in the GEP mostly presented in the GP. According to
247 Sun et al. (2011), herbivores caused changes in species composition is grazing-intensity dependent. Thus our
248 results imply that a moderate stocking rate of 4 Tibetan sheep/ha with a short annual grazing period (i.e. from
249 April to October 2010) in the GP did not change the species composition. To confirm this conclusion, a long-term
250 grazing exclusion experiment will provide more confident proofs.

251 However, our results show that herbivore grazing significantly altered species composition in terms of
252 species abundance or palatability with significantly less palatable litter produced in the GP than in GEP (Fig. 1).
253 The low mass of palatable species may attribute to two causations. First, on the Qinghai-Tibetan Plateau herbivore
254 grazing will can in the short plant height and small leaf area of palatable species (Sun et al., 2011). This may be
255 an effective ecological strategy of palatable species to avoid being grazed and the continuous disturbance, through
256 increasing resistance to grazing with lower competition for light (van der Wal et al., 2000; Falster and Westoby,
257 2003; Niu et al., 2009, 2010). Second, most palatable species on the Qinghai-Tibetan Plateau (mostly the grasses
258 and sedges, see Supplemental Table 1) are tall and more likely grazed by preferential grazing (Sun et al., 2011),
259 subsequently resulting in the lower mass of palatable species in the GP. Furthermore, our results show that there
260 was no significant difference in the litter mass of unpalatable species between the GP and GEP (Fig. 1),
261 disagreeing with the assumption that removing the canopy of palatable species will allow the intra- and inter-
262 specific competition for light which ultimately favours the establishment of short, less-palatable species
263 (Sternberg et al., 2000; Pavlů et al., 2008; Wu et al., 2009; Sun et al., 2011).

264 It is well known that litter quality is usually determined by the content of different chemical compounds
265 such as soluble C, N and P, as well as lignin or lignin:N ratio, and litter of high quality usually has higher N
266 content but lower lignin and lignin:N ratio (e.g., Aerts, 1997; Olofsson and Oksanen, 2002; Wardle et al., 2002;
267 Garibaldi et al., 2007; Strickland et al., 2009). In this study, grazing might have improved litter quality at least to
268 some degrees by significantly increasing the N content and potentially lowering the hemicellulose content and
269 C:N, lignin:N, cellulose:N and hemicellulose:N ratios (Table 1). Therefore, our results agree with previous studies
270 that grazing may promote litter quality (e.g., Sirotnak and Huntly, 2000; Olofsson and Oksanen, 2002; Semmartin
271 et al., 2004).

272

273 **4.2 Litter decomposition**

274 For a given climate region, the ecological processes of litter decomposition are regulated by incubation
275 environment (i.e., grazing/grazing exclusion and soil property in this study) and litter quality. Our results indicate
276 that herbivore grazing played a major role in litter decomposition on the Qinghai-Tibetan Plateau. Firstly many
277 studies have demonstrated that litter quality is one of the most important factors affecting the litter decomposition,
278 and litter with higher N content but lower lignin and lignin:N ratio will decompose faster (Aerts, 1997; Olofsson
279 and Oksanen, 2002; Wardle et al., 2002; Garibaldi et al., 2007; Strickland et al., 2009). By following this line, it
280 may be assumed that regardless of incubation site, litter collected from the GP will decompose faster than that
281 collected from GEP. It is true for the “in situ” incubation treatments, i.e., decomposition rate was significantly
282 greater in GP-GP than in GEP-GEP; however, for the across incubation treatments opposite results were found,
283 i.e., it was significantly greater in GEP-GP than in GP-GEP (Table 2 and Fig. 5a). In fact the multivariate
284 regression model shows that litter collected from the GEP had less mass remaining (i.e., 2.5%) compared to that
285 collected from the GP. In the study of tissue and fertilizer N affecting decomposition of conifer litter, Perakis et



286 al. (2012) revealed that high initial litter N slows decomposition rate in both early and late stages in unfertilized
287 plots. Fertilizer N is usually not applied in the pastures on Qinghai-Tibetan Plateau (F.H personal comment).
288 Moreover, Aerts (1997) suggests that litter chemistry is not a good predictor on litter decomposability in the cold
289 temperate region. Our experimental area locates 3,500 m above sea level with a typical alpine climate having a
290 mean annual temperature of 1.2°C and ranging from -10°C in January to 11.7°C in July.

291 Alternatively, for a given litter source (i.e., collected from GP or GEP) a greater decomposition rate might
292 be caused by the grazing herbivores when litter was incubated in GP (Table 2 and Fig. 5a), because grazing
293 herbivores may modify site conditions for litter turnover through physical changes in the soil through herbivore
294 activities, such as trampling and urine/dung deposition (Takar et al., 1990; Fahnestock and Knapp, 1994; Luo et
295 al., 2010). Such argument is demonstrated by the results of multivariate regression model: (1) the significantly
296 greater effect of incubation site environment ($\approx 25\%$) than that of litter source or quality, and (2) the the
297 significantly greater effect of GP (8.13%) than that of GEP (Table 3). Grazing elevates litter decomposition is
298 widely reported (e.g., Takar et al., 1990; Fahnestock and Knapp, 1994; Garibaldi et al., 2007; Semmartin et al.,
299 2008; Luo et al., 2010).

300 On the Qinghai-Tibetan Plateau, Wu et al. (2009) report that a long-term (9 years) grazing exclusion favors
301 the increase of soil total nitrogen, soil organic matter, soil organic carbon, soil microbial biomass carbon and soil
302 carbon storage. It is interesting that in the present study, only the SOC significantly increased after three-years
303 grazing exclusion (Fig. 2c); however, grazing exclusion did not significantly modify soil property in terms of TN
304 and TP. The increase of SOC in GEP may be because grazing exclusion prevents the reduction of outflow of
305 palatable litter by the herbivores (Fig. 1), and the organic C locked within plant tissues will be returned to the soil
306 during litter decomposition (Bardgett and Wardle, 2003; Wu et al., 2009). Holland and Detling (1990) and Ågren
307 et al. (1999) state that increasing carbon availability in soil will promote decomposer growth and activity even at



308 the low nitrogen concentrations. However, the expected results, i.e. significant higher litter decomposition rate
309 caused by the possible increasing decomposer mass and/or activity in the GEP (Wu et al., 2009), were not observed
310 (Table 2 and Fig. 5a). Therefore, during a relatively short period of time the soil property is unlikely significantly
311 changed through herbivore urine/dung deposition and may have less effect on litter decomposition under cool
312 environments on the Qinghai-Tibetan Plateau.

313 Our study site is a typical alpine meadow with a long and cold winter and spring (Supplemental Fig. 1).
314 Climate is the dominant factor regulating litter decomposition and nutrient cycling in such area, and the activity
315 of decomposers may be inhibited during the cold seasons (Coûteaux et al., 1995). These may have interpreted the
316 slow progresses of litter decomposition (Fig. 3) during the first winter and spring seasons when the low
317 temperature inhibited decomposer growth and activity. While the optimum response of decomposers to
318 temperature and moisture occurs during summer and early autumn, elevating the litter decomposition rate (Fig. 3)
319 when both temperature and precipitation simultaneously increase (Supplemental Fig. 1), as reported by Orsborne
320 and Macauley (1988) and De Santo et al. (1993). Because of the slow biological processes, the decomposition
321 rates estimated in this study ($k = 0.38 \sim 0.49$, Table 2) is lower than global ones ($k \approx 0.75$) with similar latitudes
322 ($N30 \sim 40^\circ$) (Zhang et al., 2008).

323

324 **4.3 N release**

325 N release is more complex compared to litter decomposition. N release from litter may involve any one or both
326 procedures of N immobilization and N mineralization, where the former results in the accumulation of N in the
327 litter and the latter causes the release of N from the litter (Manzoni et al., 2008). Swift et al. (1979) and Berg and
328 McClaugherty (2014) report that the biological decomposition of litter is mainly carried out by microbial
329 decomposers, including bacteria and fungi, which have higher N/C value compared with most litter types. This
330 property of decomposers creates a high N demand for growth. Therefore, Manzoni et al. (2008) state that even



331 though a considerable fraction of assimilated C is respired, the decomposers often still require some inorganic N
332 uptake during at least the early phases of decomposition. The increase of N remaining in the GP-GP, GEP-GEP
333 and GEP-GP at beginning of incubation season (i.e., from October to December 2010) in the present study (Fig.
334 4a-c) provided direct evidence supporting the above assumption of Manzoni et al. (2008).

335 However, our results also show an increase of N remaining from a lower content in June 2011 to a higher
336 content in September 2011 (Fig. 4). It may be because that the fast decomposition rate of litter during summer
337 (Fig. 3) results in utilization of C by decomposers which increases N:C ratio. Similarly, Bosatta and Balesdent
338 (1996) and Manzoni et al. (2008) have demonstrated a positive correlation between the decomposer N:C and
339 respiration rate (the efficient carbon use) defining the actual nutrient requirement of decomposers; thus the higher
340 respiration rate of decomposers during summer will elevate N:C ratio. Manzoni et al. (2008) also indicate that
341 when N:C is high, large amounts of mineral N are immobilized, which will in turn increase the litter N
342 concentration. However, N release starts after the accumulative N level reaches a critical level (Berg and Staaf,
343 1981), causing a significant decrease of N remaining during April ~ June 2011 and during the end incubation
344 period (May ~ December 2012) (Fig. 4).

345 Based on the above knowledge, inverse patterns of litter decomposition and N release were found, i.e., the
346 greater litter decomposition rate was (Fig. 5a), the lower N release rate became (Fig. 5b). Many studies of litter
347 decomposition have found an increase in relative nitrogen concentration in litter as the litter decomposition
348 proceeds (e.g., Aber and Melillo, 1980; Fahey et al., 1991; Gallardo and Merino, 1992). Aber and Melillo (1980)
349 state that an inverse-linear relationship between the remaining biomass and nitrogen concentration in litter is
350 validated for a large number of litter decomposition studies.

351 The inverse patterns of N release compared to litter decomposition is also demonstrated by the results of
352 multivariate regression model: (1) although the incubation site environment had significantly greater effect (\approx



353 50%) on N release than did litter source or quality (Table 3), N release was significantly lower in GP (9.27%)
354 than in GEP; (2) litter collected from GP had significantly lower N remaining than did GEP. The latter result
355 contradicts the assumption of Berg and Staaf (1981) that the relatively higher initial nitrogen concentration (see
356 Table 1 for GP litter) may contribute to the higher nitrogen immobilization in litter. The cause is unknown but
357 this phenomenon indicates that litter chemistry may not be a good predictor on litter N release as Aerts (1997)
358 suggests for litter decomposability in the cold temperate region.

359

360 **5 Conclusion**

361 Our findings provide insight into our understandings in the litter decomposition and N release affected by
362 herbivore grazing in a typical alpine meadow. Compared to grazing exclusion, grazing with a moderate stocking
363 rate could improve litter quality with higher N but lower hemicellulose and hemicellulose:N ratio, and herbivore
364 activity significantly elevated litter decomposition rate regardless of litter source. While grazing exclusion was
365 more likely to maintain significantly more palatable litter, promoted N release and increased SOC. The different
366 effects of livestock grazing and grazing exclusion on litter decomposition and N release may have implications in
367 the management of alpine meadows on the Qinghai-Tibetan Plateau. For example, grazing exclusion for one
368 season following by livestock grazing will be a good option which allows plant recovery, promotes N release,
369 elevates litter decomposition, and thus contributes to the restoration of degraded grasslands.

370

371 *Author contributions.* YS and FH designed the experiments, YS, ZW and SH Chang performed research and
372 collected data. XZH and YS analysed data and prepared the manuscript, and all authors contributed to the writing.

373

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

375



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548 **Table titles**

549 **Table 1.** Mean (\pm SE) initial contents of carbon (C, mg g^{-1}), nitrogen (N, mg g^{-1}), phosphorus (P, mg g^{-1}), lignin (mg g^{-1}),

550 cellulose (mg g^{-1}) and hemicellulose, and C:N, lignin:N, cellulose:N and hemicellulose:N ratios in litter*.

551

552 **Table 2.** Estimated litter decay rate (k) in different incubation environments.

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554 **Table 3.** Estimated contribution (%) of incubation site environment (Site, GP and GEP) and litter source (Source, GP and

555 GEP) to litter decomposition and N release by multivariable regression model.*

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571 **Figure captions**

572 **Figure 1.** Estimated mean (\pm SE) annual litter mass in GP and GEP. For each category, columns with different letters are
573 significantly different (ANOVA: $P < 0.05$).

574

575 **Figure 2.** Comparison of soil property between GP and GEP: (a) TN, (b) TP and (c) SOC. *Significant difference was only
576 found between GP and GEP for SOC in 2012 ($P < 0.05$).

577

578 **Figure 3.** Decrease of litter mass remaining with incubation time. For each treatment columns with the different letters are
579 significantly different ($P < 0.05$). Vertical bar is the LSD value. Grey lines under months indicates the air temperature < 0 °C.

580

581 **Figure 4.** Dynamics of total N remaining with incubation time. For each treatment columns with the different letters are
582 significantly different ($P < 0.05$). Vertical bar is the LSD value. Grey lines under months indicates the air temperature < 0 °C.

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584 **Figure 5.** Percentage of litter mass (a) and total N remaining (b) at the end of experiments. Columns with the different letters
585 are significantly different ($P < 0.05$). Vertical bars are the LSD values.

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593 **Table 1.** Mean (\pm SE) initial contents of carbon (C, mg g⁻¹), nitrogen (N, mg g⁻¹), phosphorus (P, mg g⁻¹), lignin
594 (mg g⁻¹), cellulose (mg g⁻¹) and hemicellulose, and C:N, lignin:N, cellulose:N and hemicellulose:N ratios in litter*.

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

595 *Litter collected from different paddocks in GP or GEP was mixed well before test. Means with the different

596 letters in each row are significantly different (ANOVA: P < 0.05).

597 **Table 2.** Estimated litter decay rate (k) in different incubation environments.

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

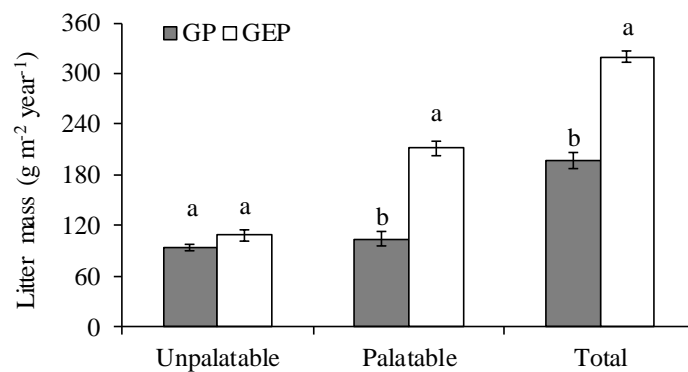
598 Mean (\pm SE) followed by the different letters are significantly (non-overlap of 83.4% CL). The values of R^2 , F
599 and P are estimated from the negative exponential model of Swift et al. (1979).



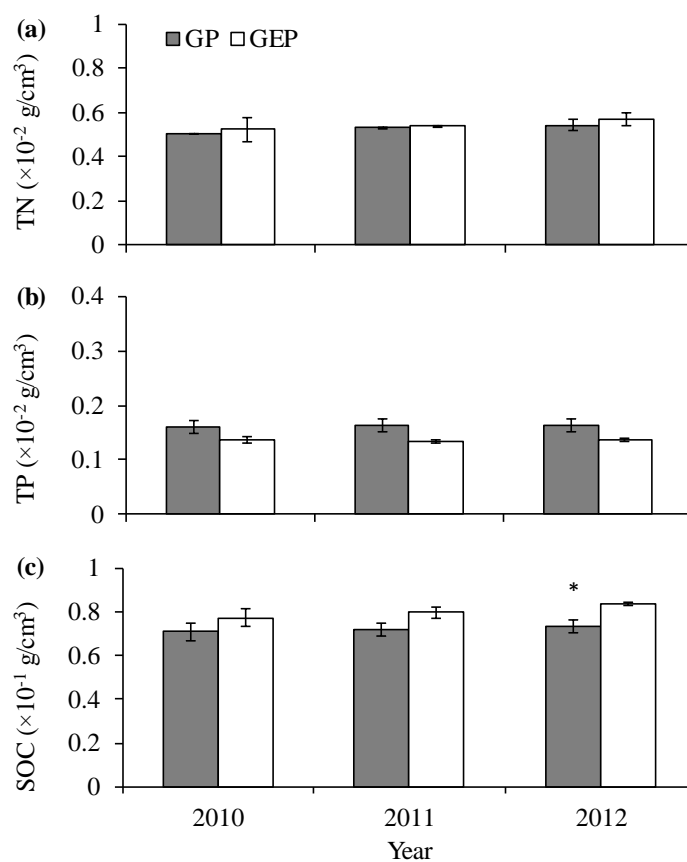
600 **Table 3.** Estimated contribution (%) of incubation site environment (Site, GP and GEP) and litter source (Source,
601 GP and GEP) to litter decomposition and N release by multivariable regression model.*

Parameter	df	Type I SS	Contribution	F	P
<i>Litter decomposition</i>					
Site	1	110.84	55.51	62.48	< 0.0001
Source	1	62.06	31.08	34.99	0.0004
Site×Source	1	12.59	6.30	7.1	0.0286
Error	8	14.19	7.11		
<i>N release</i>					
Site	1	466.25	68.39	57.49	< 0.0001
Source	1	128.18	18.80	15.81	0.0041
Site×Source	1	22.41	3.29	2.76	0.1350
Error	8	64.88	9.52		

602 * For litter decomposition: $F_{3,8} = 34.86$, $P < 0.0001$, $R^2 = 0.9289$; for N release: $F_{3,8} = 25.35$, $P = 0.0002$, $R^2 =$
603 0.9048.



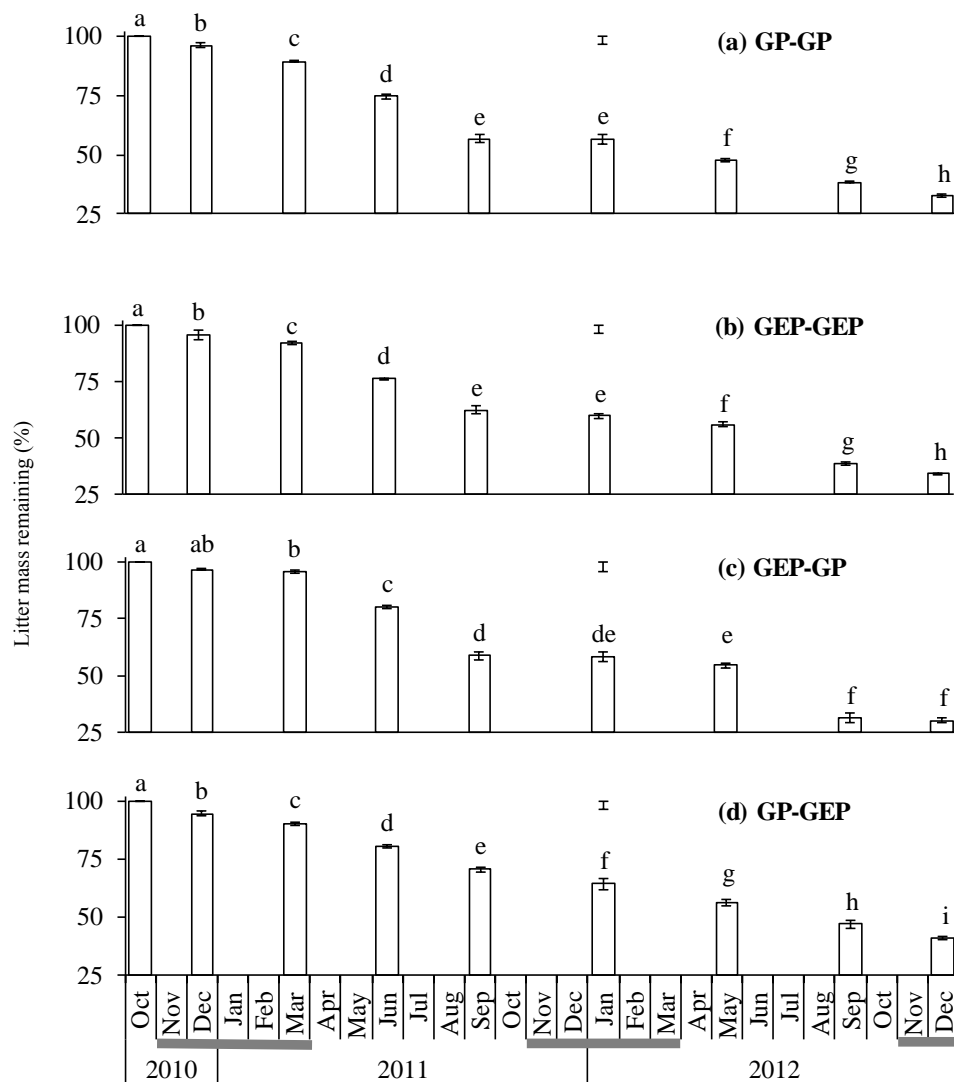
604 **Figure 1.** Estimated mean (\pm SE) annual litter mass in GP and GEP. For each category, columns with different
605 letters are significantly different (ANOVA: $P < 0.05$).



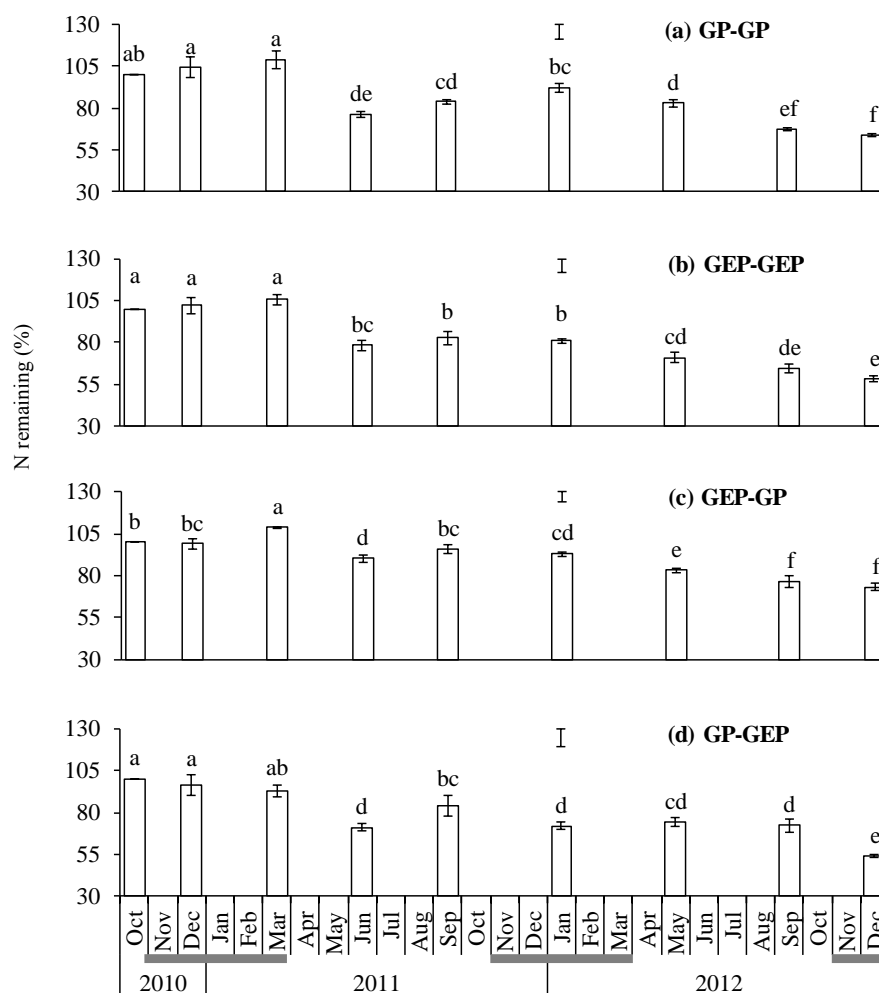
606

607 **Figure 2.** Comparison of soil property between GP and GEP: (a) TN, (b) TP and (c) SOC. *Significant difference

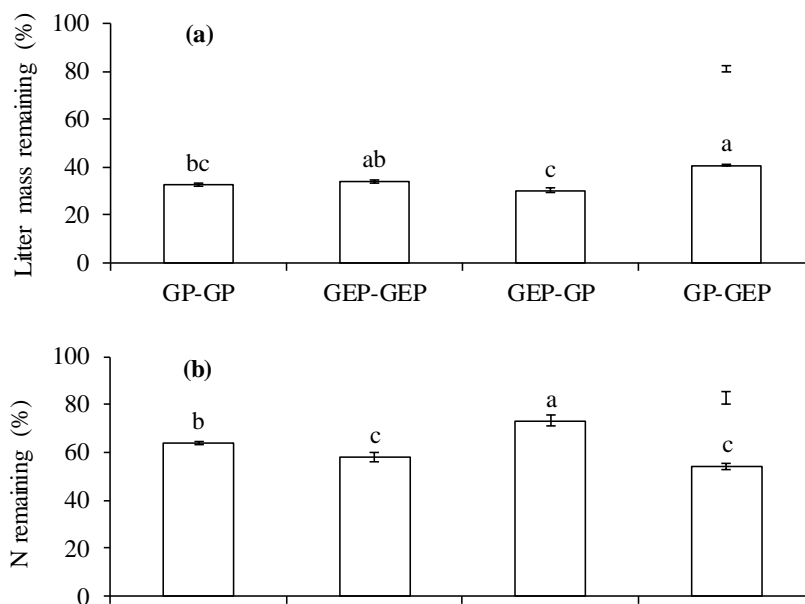
608 was only found between GP and GEP for SOC in 2012 ($P < 0.05$).



609 **Figure 3.** Decrease of litter mass remaining with incubation time. For each treatment columns with the different
 610 letters are significantly different ($P < 0.05$). Vertical bar is the LSD value. Grey lines under months indicates the
 611 air temperature < 0 °C.



612 **Figure 4.** Dynamics of total N remaining with incubation time. For each treatment columns with the different
 613 letters are significantly different ($P < 0.05$). Vertical bar is the LSD value. Grey lines under months indicates the
 614 air temperature < 0 °C.



615 **Figure 5.** Percentage of litter mass (a) and total N remaining (b) at the end of experiments. Columns with the
616 different letters are significantly different ($P < 0.05$). Vertical bars are the LSD values.