

Title: Impacts of freezing and thawing dynamics on foliar litter carbon release in alpine/subalpine forests along an altitudinal gradient in the eastern Tibetan Plateau

Wu Fuzhong · Peng Changhui · Zhu Jianxiao · Zhang Jian · Tan Bo · Yang Wanqin*

Wu Fuzhong · Zhu Jianxiao · Tan Bo · Zhang Jian · Yang Wanqin (✉)

Key Laboratory of Ecological Forestry Engineering, Institute of Ecology & Forestry, Sichuan Agricultural University, Chengdu, 611130, China

e-mail: scyangwq@163.com

Tel: 86-28-86290957, Fax: 86-28-86290957

Peng Changhui

Laboratory for Ecological Forecasting and Global Change, College of Forestry, Northwest A & F University, Yangling, Shaanxi 712100, China

Wu Fuzhong · Peng Changhui

Department of Biology Sciences, Institute of Environment Sciences, University of Quebec at Montreal, C.P. 8888, Succ. Centre-Ville, Montreal H3C 3P8, Canada

1 **Abstract** Carbon (C) release from foliar litter is a primary component in C
2 exchange among the atmosphere, vegetation, soil and water from respiration and
3 leaching, but little information is currently related to the effects of freezing and
4 thawing dynamics on C release of foliar litter in cold regions. A two-year field litter
5 decomposition experiment was conducted along an altitudinal gradient (~2700 m to
6 ~3600 m) to mimic temperature increases in the eastern Tibetan Plateau. C release
7 was investigated for fresh foliar litter of spruce, fir and birch. The onset of the frozen
8 stage, deep frozen stage, and thawing stage were partitioned according to changes in
9 freezing and thawing dynamics of each winter. More rapid two-year C released from
10 fresh foliar litter at upper elevations compared to lower elevations in the
11 alpine/subalpine region. However, high C release was observed in low altitudes
12 during winter stages, but high altitudes exhibited high C release during growing
13 season stages. The deep frozen stage showed higher rates of C release than other
14 stages in the second year of decomposition. Negative degree-days showing freezing
15 degree were correlated to C release rates for the deep frozen stages in both years, and
16 this relationship continued for the duration of the experiment, indicating that changes
17 in freezing can directly modify C release from foliar litter. The results suggested that
18 the changed freezing and thawing dynamics could delay the onset of C release in fresh
19 litter in this cold region in the scenario of climate warming.

20 **Keywords:** climate warming; cold region; degree days; freezing-thawing; litter
21 decomposition

23 **1 Introduction**

24 Carbon (C) release from foliar litter is a primary component in C exchange among the
25 atmosphere, vegetation, soil and water from respiration and leaching (Berg and
26 McClaugherty 2008) in that the majority of fixed C enters decomposition pathways
27 (Cebrian 1999; Ayres et al. 2009). Climatic controls on litter decomposition have
28 gained considerable interest in recent years on account of accumulative green house
29 gas feedback data from ecosystems (Wu et al. 2010; Aerts et al. 2012; Fraser and
30 Hockin 2013). Apparent positive relationships between temperature and net C release
31 from litter and soil have widely been detected in many cold ecosystems (Trumbore et
32 al. 1996; Moore et al. 1998; Wickland and Neff 2008), which has led to a keen
33 interest concerning positive feedbacks on global warming through increasing
34 atmospheric concentrations of CO₂, CH₄ and other greenhouse gases (McGuire et al.
35 2000). However, debate has also arisen whether or not C release will increase with
36 temperature (Liski et al. 1999; Giardini and Ryan 2000) when other environmental
37 constraints (such as freezing, thawing, drying and flooding) are taken into account. A
38 few studies have documented that litter decomposition rates increase with increases in
39 altitude (subsequent decreases in temperature) in cold regions due to the much
40 stronger freezing and thawing dynamics typical of higher altitudes (Murphy et al.
41 1998; Withington and Sanford 2007). Even so, results from Bokhorst et al. (2010)
42 showed that winter warming events had little effect on fresh litter decomposition.
43 They suggested that their observations of extensive decomposition primarily resulted
44 from autumn leaching that could not have occurred in the “true” winter.

45 Most litters fall in late autumn before soil completely freezes over (Moore et al.
46 1983; Yang et al. 2005). This study has designated the stage from litterfall to time
47 when soil completely freezes as the “onset of frozen stage” (OF). This stage is
48 characterized by frequent freezing and thawing events as temperatures fall to the point
49 of freezing. The subsequent stage is designated the “deep frozen stage” (DF) where
50 temperatures remain below the freezing point. Following that is the “thawing stage”
51 (TP) when soil thawing takes place with an increase in temperature during early
52 spring but where repeated frequent freezing and thawing events also occur (Wu et al.

2011; Zhu et al. 2012). Different freezing and thawing characteristics inherent to these three stages not only physically affect litter C structure but also regulate litter C release rates due to the high sensitivity of biological processes (such as soil organism activity) (Aerts et al. 2012; García-Palacios et al. 2013). Moreover, soil surface temperature cannot parallel air temperature due to insulative effects of snow cover (Groffman et al. 2001). As a result, decreased snow cover in a warming climate will promote colder soil surface temperatures, harder freezes but less overall decomposer activity (Baptist et al. 2010; Bokhorst et al. 2013). Unfortunately, available studies on the subject have not well adequately addressed this particular decomposition stage, making the association between C release and temperature unclear.

Warm temperatures might not in and of themselves be the dominant factor that drives C release from foliar litter when temperatures have no functional effect on freeze-thaw and thereby can not limit decomposer activity. Moreover, a change in litter quality in conjunction with decomposer activation following winter will contribute a great deal to C release during the growing season. A recent publication from our experiment showed that freeze-thaw and litter chemical properties determine the winter decomposition while microbe-related factors play more important roles in decomposition in the subsequent growing season (Zhu et al. 2013). Numerous studies have documented the effects that freezing have on litter, making it more decomposable (Hobbie and Chapin 1996; Taylor and Parkinson 1988; Wu et al. 2010; Zhu et al. 2012). In doing so, C release would breakout in the “early stage of the growing season” (EG) as temperatures continually increase. After the temperatures peak in summer, C release would decrease owing to labile C components lost and decreasing temperature in the “later stage of the growing season” (LG). Nevertheless, litter or organic matter of different quality may exhibit various responses to freezing and thawing dynamics under a scenario of climate change (Pare and Bedard-Haughn 2013). Much more works must however be done to more clearly understand litter C release processes in cold biomes.

Alpine/subalpine forests in the eastern Tibetan Plateau are typical cold ecosystems subjected to low temperatures that undergo considerable seasonal freezing

83 and thawing events. Although these forests are characterized by low temperatures, low
84 overall primary productivity, slow decomposition and shallow and poor soil, they
85 possess large C pools within their litter layer. C releasing from foliar litter could
86 provide evidence of clear feedbacks related to climate warming through
87 increasing/decreasing greenhouse gas flux at such sites. Distinctive temperature
88 fluctuation stages were observed in the experimental site (Wu et al. 2010, 2011; Tan et
89 al. 2010), the different winter stages contributed differently to fresh fir litter
90 decomposition due to dynamical changes in freeze-thaw (Zhu et al. 2012). However,
91 it remains uncertain how to discern freezing and thawing effects on C release (if it can
92 be done at all) during litter decomposition in which to understand feedbacks in
93 relation to ongoing climate warming. Taking findings from a meta-analysis of
94 experimental warming studies in cold biomes (a combination of 34 site-species)
95 (reviewed by Aerts 2006) where warming resulted in slightly increased decomposition
96 rates, it is hypothesized that changes in freezing and thawing dynamics can promote C
97 release from foliar litter in the experimental site investigated under a scenario of
98 climate warming.

99 To test the hypothesis, a two-year field litter decomposition experiment along an
100 altitudinal gradient (~ 2700 m to ~3600 m) was conducted to simulate ongoing
101 climate warming in the eastern Tibetan Plateau, China. C release was investigated
102 from the fresh litter of dominant species (spruce: *Picea asperata*, fir: *Abies faxoniana*,
103 and birch: *Betula albosinensis*) during five decomposition stages (OF, DF, TS, EG and
104 LG) each year as decomposition proceeded and temperatures fluctuated. Temperature
105 dynamics and microbial biomass were analyzed concurrently. The objectives of this
106 study were to examine the effects of freezing and thawing dynamics on C release
107 from foliar litter of alpine forests, and to determine the varied effects in different
108 altitudes. Results could also be useful in explaining details of litter decomposition in
109 cold regions, and to provide efficient knowledge and insight on the feedback of litter
110 decomposition under climate warming scenarios.

111 **Materials and methods**

113 Study area

114
115 This study was conducted in Bipenggou Valley of the Miyaluo Nature Reserve (long
116 102°53' to 102°57'E, lat 31°14' to 31°19'N, 2458 m to 4619 m AMSL), located in Li
117 County, Sichuan Province, southwest China (Fig. 1). This is a transitional area
118 situated between the Tibetan Plateau and the Sichuan Basin. Annual mean air
119 temperature is 3 °C. Absolute maximum and minimum air temperatures are 23 °C in
120 July and -18 °C in January, respectively. Annual mean precipitation ranges from 801
121 mm to 875 mm, depending on elevation. Most precipitation falls between May and
122 August. The freeze-thaw season starts in November as soil temperatures fall below
123 0 °C and snow covers the ground. Soil remains frozen until the following April (Zhu et
124 al. 2012, 2013).

125 A 900 m vertical transitional zone was selected along an altitudinal gradient
126 from 2700 m, 3000 m, 3300 m to 3600 m, each site exhibiting similar topographical
127 and environmental attributes such as slope, aspect and canopy density. The dominant
128 tree species in the forests at four sites are as follows; spruce and birch interspersed
129 with dense shrubs, including dwarf bamboo (*Fargesia nitida*) at 2700 m; spruce, fir
130 and birch, including dwarf bamboo, *Lonicera* spp. and *Rubus corchorifolius* at 3000
131 m; fir and birch, including dwarf bamboo at 3300 m; and fir, larch (*Larix mastersiana*)
132 and cypress (*Sabina saltuaria*) interspersed with shrubs of a few azaleas
133 (*Rhododendron* spp.) and willow (*Salix paraplesia*) at 3600 m. Three sampling forest
134 plots were established for each site at an altitude.

135
136 Experimental design

137
138 C released during litter decomposition was determined using the widely-used litterbag
139 method. In October 2008, fresh foliar litter from spruce, fir and birch were collected
140 from the forest floor of the sampling plots. To avoid structure damage to litter during
141 oven-drying, the fresh litter was air-dried for more than two weeks at room
142 temperature. In total, 15 g of air-dried spruce needle litter (with an approximate

143 moisture content of 9.51%) and fir needle litter (with an approximate moisture content
144 of 9.15%), and 10 g of air-dried birch broad-leaf litter (with an approximate moisture
145 content of 9.05%) were then separately placed in their own 20×20 cm nylon bags
146 (0.50 mm on the soil side and 1 mm on the reverse side) before the bags were sealed.
147 Litter of each tree species was placed in their own litter bag separately. Chemical
148 analysis of the initial litter as well as other calculated data were based on the
149 oven-dried mass (Table 1).

150 In total, 600 litterbags (four altitudes × five stages × five replicates × three
151 sampling plots × two years) for each species were placed on the forest floor of the
152 three selected sampling plots on 6 November 2008. Five subsamples of each litter
153 type were oven-dried at 70 °C for 48 h to determine litter moisture content. Litterbags
154 were randomly sampled from each forest on 8 December 2008 (OF1), 24 March 2009
155 (DF1), 22 April 2009 (TP1), 8 August 2009 (EG1), 12 November 2009 (LG1), 13
156 December 2009 (OF2), 3 April 2010 (DF2), 28 April 2010 (TP2), 16 August 2010
157 (EG2) and 16 November 2010 (LG2). Selection of sampling dates was based on
158 changes in freezing and thawing dynamics determined at previous field observations
159 that took place between 2005 and 2007 (Tan et al. 2010; Wu et al. 2010; Zhu et al.
160 2012). Because of unfavorable climate and poor traffic conditions in alpine regions,
161 sampled times were delayed in the second year of the study. Retrieved litter was then
162 separated into two parts. One part was stored in a refrigerator at 4 °C to prepare for
163 microbial biomass analysis. The other part was oven-dried at 70 °C for 48 h to
164 determine dry mass and C content. Temperatures in litterbags were measured every
165 two hours between 6 November, 2008, and 16 November, 2010 (Fig. 2) in each
166 sampling forest with different altitudes, using a DS1923-F5 iButtonny logger (Maxim
167 Integrated Products, Inc., San Gabriel Drive Sunnyvale, USA).

168

169 Chemical analysis and calculation

170

171 C content in both initial and remaining litter samples was determined using the
172 dichromate oxidation-ferrous sulfate titration method (Lu 1999). In order to

173 understand initial chemical characteristics, oven-dried foliar litter was ground (using a
174 1 mm sieve) to be used for nitrogen (N), phosphorus (P), cellulose and lignin analysis.
175 N and P analyses were carried out according to Lu (1999). In brief, subsamples of
176 0.2500 g were acid digested using an 8 mL H₂SO₄ ($\rho = 1.84 \text{ g cm}^{-3}$) and a 3 mL H₂O₂
177 solution at 190 °C for 10 min. The digested solution was then transferred to a 100 mL
178 volumetric flask, subsampled, and stored for N and P measurements. N and P content
179 were determined using Kjeldahl determination for N, and the molybdenum-blue
180 colorimetric method for P. Lignin and cellulose were measured using the acid
181 detergent lignin method (Graca et al. 2005).

182 Microbial biomass C (MBC) in litter was determined according to differences
183 between organic C extracted using 0.5 mol L⁻¹ K₂SO₄ from fumigated and
184 non-fumigated samples (Brookes et al. 1985; Vance et al. 1987). The efficiency factor
185 ($K_c = 0.38$) was used to correct incomplete extractability (Vance et al. 1987). Parts of
186 data from MBC were published by Zhou et al. (2011).

187 C release rates (R_c) throughout litter decomposition at each stage of the two-year
188 decomposition experiment were calculated as follows:

$$189 \quad R_c (\%) = 100 \times (M_{i-1}C_{i-1} - M_iC_i) / M_0C_0 \quad (1)$$

190 To exclude the effects of time length (day number) on the C release rate of each
191 stage, C release rates per day (V_c) were calculated as follows (Zhu et al. 2012):

$$192 \quad V_c = R_i / D_{T_i} \quad (i = 1, 2, 3, \dots) \quad (2)$$

193 where M_0 and C_0 are the dry mass and C content (g kg^{-1}) of initial litter,
194 respectively; M_{i-1} and M_i are the dry mass of the remaining litter in the litterbags at the
195 end of T_{i-1} stage and T_i stage after sampling, respectively; C_{i-1} and C_i are the C content
196 (g kg^{-1}) of the remaining litter at the end of T_{i-1} stage and T_i stage after sampling,
197 respectively; and D_{T_i} is the length (day number) of each stage (T_i) as indicated earlier.
198 The C release rate (R_0) during the entire two-year decomposition experiment was the
199 sum of C release during each stage.

200 It should be noted that freeze-thaw cycles should be numbered, but no efficient
201 method currently exists. Although Konestabo et al. (2007) defined a freeze-thaw cycle
202 as a period in which temperatures drop/rise below 0 °C for at least three hours

203 followed by a rise/drop above 0 °C for at least three hours, the procedure has proven
204 difficult to calculate in this experiment because observed temperatures in the sampling
205 sites were often extremely close to 0 °C (Fig.2), especially during the OF and TP
206 stages. Therefore, since the processes of freezing and thawing can be respectively
207 looked as the thermal energy accumulating and releasing (Kayastha et al. 2003), we
208 believe positive degree-days and negative degree-days can be more concise and
209 countable indicators in describing freezing and thawing. It was also determined that
210 degree-days at the experimental sites played a more important role in soil processes
211 than other temperature indicators (Wang et al. 2012).

212 After ascertaining temperature data from 2005 to 2007, it was determined that
213 daytime exhibited stronger temperature fluctuations than nighttime. To better express
214 temperature characteristics (especially freezing and thawing throughout the different
215 stages), positive degree-days (pd) and negative degree-days (nd) were calculated
216 (Kayastha et al. 2003). Since there are significant freezing-thawing differences
217 between daytime and nighttime from our field observations, daily-pd and daily-nd
218 were calculated from daily average temperatures, day-pd and day-nd were calculated
219 from daytime average temperatures, and night-pd and night-nd were calculated from
220 nighttime average temperatures. 0 °C was considered to be the normal threshold. Daily
221 (Daily-T), daytime (Day-T) and nighttime (Night-T) average temperatures for each
222 stage were also calculated separately.

223

224 Statistical analysis

225

226 Prior to statistical analysis, data were tested for homogeneity of variance using
227 Levene's test and transformed where applicable (Gaur and Gaur 2006). To check how
228 much variance in C release could be predicted from altitude, species and their
229 combined interaction, R_c and V_c was analyzed at different stages using the univariate
230 process of general linear model (GLM) with altitude, species and their combined
231 interaction as factors (Gaur and Gaur 2006). Step-wise linear regression was used to
232 examine which factors dominated C release from foliar litter at each decomposition

233 stage. If formerly entered indicators were removed by the stepwise process, those
234 indicators that contributed more to higher R square (R^2) in terminal models were
235 chose (Gaur and Gaur 2006). All statistical analyses were performed using the SPSS
236 software package (standard released version 16.0 for Windows, SPSS Inc., IL., USA).

237

238 **Results**

239 R_c and V_c

240

241 At the conclusion of the two year decomposition experiment, foliar litter C release
242 reached from 49.6% to 64.9%, depending on species and altitude (Fig. 3). Regardless
243 of species, the entire two-year R_c exhibited little variance between A3300 and A3600
244 where values were higher than lower altitudes. The majority (42.5%–58.5%) of C
245 released from foliar litter occurred in the first year of the decomposition. When
246 compared to the other decomposition stages, higher R_c was observed for DF2 during
247 the second year (Fig. 3). The contribution of foliar litter C release at EG1 was great
248 (accounting for 29.9%–44.8% of the C release rates to entire two-year experiment),
249 regardless of altitude and species, followed by DF1 and OF1. With the exception of
250 DF1 for which species had only insignificant ($p > 0.05$) effects on R_c , both altitude
251 and species had significant ($p < 0.05$) effects on R_c for all other stages (Table 2).

252 Altitude and species had statically significant ($p < 0.05$) effects on V_c for all
253 stages of the two-year decomposition experiment (Table 2). Regardless of altitude, the
254 highest V_c for fir and birch were observed for OF1, followed by EG1 (Fig 4).
255 Although V_c was also highest for OF1 in lower altitudes (A2700 and A3000) for
256 spruce, it was higher for EG1 in higher altitudes (A3000 and A3600). Compared to
257 other stages in the second year of the decomposition experiment, DF2 showed relative
258 higher V_c , regardless of species and altitudes. The litter of all three species displayed
259 the same pattern: Higher V_c was observed in lower altitudes for winter stages, but
260 higher V_c was observed in higher altitudes for growing season stages.

261

262 Multiple correlations

263
264 According to stepwise regression multiple correlations (Table 3), R_c was strongly
265 correlated to Day-pd for the entire experiment, but in the first and the second year R_c
266 correlated more to Night-nd and Day-nd, respectively, compared to other temperature
267 indicators. Night-nd and Day-nd also correlated to R_c for DF1 and DF2, respectively,
268 while Night-pd strongly correlated to R_c for both OF1 and EG1. Day-pd correlated to
269 R_c for TP1. Daily-T and Day-T correlated to R_c for TP2 and EG2, respectively.

270 As it pertains to initial litter chemistry, P exhibited a strong correlation to R_c for
271 the entire two-year experiment, the second year, the second winter, DF2 and TP2. All
272 R_c during the first year, the second growing season, OF1, DF1, EG1, TP2 and EG2
273 correlated to initial C content. N only correlated to R_c for LG1, OF2 and the second
274 winter while lignin only correlated to R_c for DF1 and the first year. C/N, C/P and
275 lignin/N related to R_c for OF2. However, MBC showed a strong correlation to R_c for
276 the entire two-year experiment, the second year, the second winter, the first growing
277 season and TP2.

278

279 **Discussion**

280

281 Contrary to the hypothesis that changes in freezing and thawing can promote C
282 release from foliar litter under a scenario of climate warming, results from this study
283 indicate that C release from foliar litter was more rapid at higher altitudes (> 3300m)
284 than lower altitudes (2700 m to 3000m) in the alpine/subalpine forest region under
285 investigation, regardless of species. Previous observations reported that temperature
286 stimulated C release might be attributable to permafrost thaw and the microbial
287 decomposition of previously frozen organic C (Schuur et al. 2009). This agrees with
288 results from Aerts (2006) and Murphy et al. (1998) who found that the higher
289 decomposition rates in the higher and colder sites were primarily due to freezing and
290 thawing characteristics. As a result, C release from fresh foliar litter would be delayed
291 under a scenario of global warming in these cold regions.

292 Most C was released from foliar litter during the first winter (OF1 and DF1)

293 and the subsequent early growing season (EG1), which can be explained by at least
294 three distinct processes. Firstly, the presence of fresh litter with relatively more labile
295 C components may undergo a relatively rapid C release rate (Rouifed et al. 2010; Zhu
296 et al. 2012). Consequently, the highest V_c were observed for OF1 for fir and birch,
297 regardless of altitude (Fig. 4). Secondly, the physically destructive effects that occur
298 during freezing processes with temperatures decreasing in winter can directly increase
299 litter decomposability (Hobbie and Chapin 1996; Taylor and Parkinson 1988; Zhu et
300 al. 2012). Stepwise regression multiple correlations also provided evidence in that
301 Night-nd entered R_c model regression for DF1 (Table 3), implying negative degree
302 days could be a good indicator of freezing intensity. Thirdly, rapid increase in
303 temperature during the early growing season can stimulate and promote an increase in
304 activity of decomposing organisms (Moorhead and Sinsabaugh 2006; Schadt et al.
305 2003; Weintraub et al. 2007). When this interacts with an increase in litter
306 decomposability after winter concludes, it could contribute to C release peaking
307 events. Higher V_c was also observed for all three species for EG1 (Table 2).
308 Furthermore, Night-pd was determined to be one of the dominate factors of R_c for
309 both OF1 and EG1 (Table 3), indicating that accumulated heat could play an
310 important role in C release in this temperature-limited region. This could also explain
311 why lower altitudes exhibited higher V_c during winter stages but higher V_c in higher
312 altitudes during the growing season, and the results agree with the opinion that
313 freeze-thaw control winter litter decomposition but microbe-related factors control
314 growing season (Zhu et al. 2013). It should be noted that freeze-thaw cycles could
315 also a key factor in winter (Zhu et al. 2013), but no useful parameter can effectively
316 specify them in the field because observed temperatures were extremely close to 0°C
317 for both OF and TP (Wu et al. 2010; Zhu et al. 2012; Fig 2). Clearly, more work on
318 freezing and thawing in cold regions is required.

319 In contrast, obvious higher C release rates were detected for the deep frozen
320 stage (DF2) than other stages in the second year of decomposition. This is consistent
321 with the results from Hobbie and Chapin (1996) who reported that litter mass was
322 mainly lost during winter in Alaskan tussock tundra after the first year of

323 decomposition. This may also be attributable to freezing since Day-nd was deemed
324 the affecting factor in C release for DF2 (Table 3). Freezing does not only directly
325 promote the loss of recalcitrant C components by physical destruction (Taylor and
326 Parkinson 1988), but also indirectly contributes to C release in subsequent thawing
327 processes, making litter more decomposable (Hobbie and Chapin 1996; Baptis et al.
328 2010). As a result, R_c in the first and second year showed strong correlations to
329 Night-nd and Day-nd, respectively. Results from both this and other studies suggest
330 that changing winter temperatures and their related freezing and thawing
331 characteristics in the long run will play essential roles in C release from foliar litter
332 under a scenario of climate change. In the future, more attention should be paid to
333 ecological processes that take place in winter.

334 Berg et al. (1993) and Freschet et al. (2012) have documented that climate and
335 substrate quality might together explain at least 57% of global scale variation in leaf
336 decomposition. Results from the current study stand in agreement with them, showing
337 that initial litter chemistry was also the main factor in explaining C release from foliar
338 litter. To take one example, P was strongly related to C release for the entire two-year
339 experiment, the second year, the second winter, DF2 and TP2. Moore et al. (2011)
340 found that P mineralization in decomposing litter is mainly affected by environmental
341 controls, and Aerts et al. (2012) reported increased temperatures stimulate litter P
342 release. Findings from the current study that show that P is a more sensitive indicator
343 supported these previous results, implying initial P concentration might determine
344 litter decomposition as an earlier plant nutrient study in this region reported (Wu et al.
345 2009). Initial C content in litter showed strong correlations to C release in the first
346 year, the second growing season, OF1, DF1, EG1, TP2 and EG2, suggesting that the
347 C pool mainly determines release processes. However, lignin and N are well known to
348 be sensitive indicators in litter decomposition (Zhu et al. 2012), but N here only
349 correlated to R_c for LG1, OF2 and the second winter, and lignin only correlated to R_c
350 for DF1 and the first year. On the one hand, a great deal of N lost before LG1, such as
351 the rapid loss that occurred with labile fresh C components for OF1, thawing
352 processes for TP1 (Zhu et al. 2012) and the breakout of C release for EG1. At the

353 same time, C/N was determined to be one of the factors that affected R_c during the
354 first winter and throughout the first year (Table 3). As a result, N can be an important
355 factor in controlling C release in this ecosystem as many other studies have reported.
356 On the other hand, lignin has been documented as a recalcitrant C component that
357 limits litter decomposition (Taylor et al. 1989). In the current study, lignin exhibited
358 good correlations to R_c for DF1 and the first year, and lignin/N strongly correlated to
359 R_c for OF1 and the second winter. These results also provide evidence for freezing
360 effects on foliar litter C release.

361 Additionally, both previous studies from the authors of this study as well as other
362 studies have detected relatively high microbial activity and rich microbial biodiversity
363 during the deep frozen and thawing stages (Schadt et al. 2003; Wang et al. 2012).
364 However, microbial activity (expressed as MBC in the current study) was only
365 examined as one of the dominant factors in C release from foliar litter for TP2 alone.
366 Results testify to the fact that climate change together with litter chemistry had a
367 greater effect on C release from foliar litter than microbial activity, which is in
368 agreement with previous studies (Freschet et al. 2012; García-Palacios et al. 2013). At
369 the same time, results support that microbe contribution of microbial activity in foliar
370 litter C release since MBC was correlated to R_c in the first growing season, the second
371 winter, the second year and the entire two-year experiment. It may be that the strong
372 correlation found in this study between MBC and C release in the first growing season
373 is direct evidence that winter decomposition increases litter decomposability by
374 physical effects such as freezing and thawing (Aerts 2006; Baptis et al. 2010), and
375 temperature by itself actually limits decomposer activity (Rouifed et al. 2010; Tan et
376 al. 2010) in winter.

377 In summary, results from two-year observation give evidence of more rapid C
378 release from fresh foliar litter at upper elevations compared to lower elevations in the
379 alpine/subalpine region investigated. After the majority of C was lost during the first
380 year, clear signs of C release could only be detected during the deep frozen stage.
381 Including other factors, negative-degree days correlated to R_c for the deep frozen
382 stages in the first and second year, and subsequently maintained this relationship with

383 C release during the entire first and second year. This indicates that freezing plays a
384 dominant role in C release from foliar litter decomposition. In the short term, foliar
385 litter C release could decelerate due to changes in freezing when annual temperatures
386 increase in this cold region under a scenario of climate warming. This could be a
387 positive climate change feedback since numerous studies reported that increases in
388 climate change will likely occur at higher altitudinal/latitudinal locales (Groffman et
389 al. 2001; IPCC 2007; Schuur et al. 2009), although C loss can also be attributed to
390 other processes, such as leaching (Bokhorst et al. 2010). However, “to release or not
391 to release, that is a question,” and, if this proves definitively to be the case, increases
392 in temperature might delay C cycling processes during the first two-year foliar litter
393 decomposition.

394

395 **Acknowledgements**

396

397 This research was financially supported by the National Natural Science Foundation
398 of China (31170423 & 31270498), the National Key Technologies R&D of China
399 (2011BAC09B05), and the Sichuan Youth Sci-tech Foundation (2012JQ0008 &
400 2012JQ0059).

401

402 **References**

403

404 Aerts R (2006) The freezer defrosting: global warming and litter decomposition rates
405 in cold biomes. *J Ecol* 94: 713–724.

406 Aerts R, Callaghan TV, Dorrepaal E, van Logtestijn RSP, Cornelissen JHC (2012)
407 Seasonal climate manipulations have only minor effects on litter decomposition
408 rates and N dynamics but strong effects on litter P dynamics of sub-arctic bog
409 species. *Oecologia* 170: 809–819.

410 Ayres E, Steltzer H, Berg S, Wall DH (2009) Soil biota accelerate decomposition in
411 high-elevation forests by specializing in the breakdown of litter produced by the
412 plant species above them. *J Ecol* 97: 901–912.

413 Baptist F, Yoccoz NG, Choler P (2010) Direct and indirect control by snow cover
414 over decomposition in alpine tundra along a snowmelt gradient. *Plant Soil* 328:
415 397–410.

416 Berg B, Berg MP, Bottner P, Box E, Breymeyer A, Ca de Anta R, Couteaux MM,
417 Escudero A, Gallardo A, Kratz W, Madeira M, Mäkkönen ME, McClaugherty C,
418 Meentemeyer V, Muñoz F, Piussi P, Remacle J, Vide Santo A (1993) Litter mass
419 loss rates in pine forests of Europe and Eastern United States: some relationships
420 with climate and litter quality. *Biogeochemistry* 20: 127–159.

421 Berg B, McClaugherty C (2008) *Plant litter: decomposition, humus formation, carbon*
422 *sequestration*, 2nd ed. New York, Springer.

423 Bokhorst S, Bjerke JW, Melillo J, Callaghan TV, Phoenix GK (2010) Impacts of
424 extreme winter warming events on litter decomposition in a sub-Arctic heathland.
425 *Soil Biol Biochem* 42: 611–617.

426 Bokhorst S, Metcalfe DB, Wardle DA (2013) Reduction in snow depth negatively
427 affects decomposers but impact on decomposition rates is substrate dependent.
428 *Soil Biol Biochem* 62: 157–164.

429 Brookes PC, Landman A, Pruden G, Jinkenson DS (1985) Chloroform fumigation and
430 the release of soil nitrogen: a rapid direct extraction method to measure microbial
431 biomass nitrogen in soil. *Soil Biol Biochem* 17: 837–842.

432 Cebrian J (1999) Patterns in the fate of production in plant communities. *Am Nat* 154:
433 449–468.

434 Fraser LH, Hockin AD (2013) Litter decomposition rates of two grass species along a
435 semi-arid grassland–forest ecocline. *J Arid Environ* 88: 125–129.

436 Freschet GT, Aerts R, Cornelissen JHC (2012) Multiple mechanisms for trait effects
437 on litter decomposition: moving beyond home-field advantage with a new
438 hypothesis. *J Ecol* 100: 619–630.

439 Garc á-Palacios P, Maestre FT, Kattge J, Wall DH (2013) Climate and litter quality
440 differently modulate the effects of soil fauna on litter decomposition across
441 biomes. *Ecol Lett* 16: 1045–1053.

442 Gaur AS, Gaur SS (2006) *Statistical methods for practice and research: A guide to*

443 data analysis using *SPSS*. Thousand Oaks, CA, Sage publications.

444 Giardina CP, Ryan MG (2000) Evidence that decomposition rates of organic carbon in
445 mineral soil do not vary with temperature. *Nature* 404: 858–861.

446 Graca MAS, B ärlöcher F, Gessner MO (2005) Methods to study litter decomposition:
447 A practical guide. New York, Springer.

448 Groffman PM, Driscoll CT, Fahey TJ, Hardy JP, Fitzhugh RD, Tierney GL (2001)
449 Colder soils in a warmer world: A snow manipulation study in a northern
450 hardwood forest ecosystem. *Biogeochemistry* 56: 135–150.

451 Hobbie SE, Chapin FS (1996) Winter regulation of tundra litter carbon and nitrogen
452 dynamics. *Biogeochemistry* 35: 327–338.

453 IPCC (Intergovernmental Panel on Climate Change) (2007) Climate change
454 2007—the physical science basis. Cambridge University Press, Cambridge

455 Kayastha RB, Ageta Y, Nakawo M, Fujita K, Sakai A, Matsuda Y (2003) Positive
456 degree-day factors for ice ablation on four glaciers in the Nepalese Himalayas and
457 Qinghai-Tibetan Plateau. *B Glaciol Res* 20: 7–14.

458 Konestabo HS, Michelsen A, Holmstrup M (2007) Responses of springtail and mite
459 populations to prolonged periods of soil freeze-thaw cycles in a sub-Arctic
460 ecosystem. *Appl Soil Ecol* 36: 136–146.

461 Liski J, Ilvesniemi H, Makela A, Westman CJ (1999) CO₂ emissions from soil in
462 response to climatic warming are overestimated—the decomposition of old soil
463 organic matter is tolerant of temperature. *Ambio* 28: 171–174

464 Lu RK (1999) Soil and agro-chemical analytical methods (In Chinese). Beijing: China
465 Agricultural Science and Technology Press.

466 McGuire AD, Clein JS, Melillo JM (2000) Modeling carbon responses of tundra
467 ecosystems to historical and projected climate: Sensitivity of Pan-Arctic carbon
468 storage to temporal and spatial variation in climate. *Global Change Biol* 6:
469 141–159

470 Moore TR (1983) Winter-time litter decomposition in a subarctic woodland. *Arct.*
471 *Antact Alp Res* 15: 413–418.

472 Moore TR, Roulet NT, Waddington JM (1998) Uncertainty in predicting the effect of

473 climatic change on the carbon cycling of Canadian peatlands. *Climatic Change* 40:
474 229–245.

475 Moore TR, Trofymow AJ, Prescott CE, Titus B, CIDET Working Group (2011)
476 Nature and nurture in the continuum of C, N and P from litter to soil organic
477 matter in Canadian forests. *Plant Soil* 339: 163–175.

478 Moorhead DL, Sinsabaugh RL (2006) A theoretical model of litter decay and
479 microbial interaction. *Ecol Monogr* 76: 151–174.

480 Murphy KL, Klopatek JM, Klopatek CC (1998) The effects of litter quality and
481 climate on decomposition along an elevational gradient. *Ecol Appl* 8: 1061–1071.

482 Pare MC, Bedard-Haughn A (2013) Soil organic matter quality influences
483 mineralization and GHG emissions in cryosols: a field-based study of sub- to high
484 Arctic. *Global Change Biol* 19: 1126–1140.

485 Rouifed S, Handa IT, David JF, Hätenschwiler S (2010) The importance of biotic
486 factors in predicting global change effects on decomposition of temperate forest
487 leaf litter. *Oecologia* 163: 247–256.

488 Schadt CW, Martin AP, Lipson DA, Schmidt SK (2003) Seasonal dynamics of
489 previously unknown fungal lineages in tundra soils. *Science* 301: 1359–1361.

490 Schuur EAG, Vogel JG, Crummer KG, Lee H, Sickman JO, Osterkamp TE (2009)
491 The effect of permafrost thaw on old carbon release and net carbon exchange
492 from tundra. *Nature* 459: 556–559.

493 Tan B, Wu FZ, Yang WQ, Liu L, Yu S (2010) Characteristics of soil animal
494 community in the subalpine/alpine forests of western Sichuan at the early stage of
495 freeze-thaw season. *Acta Ecol Sin* 30: 93–99.

496 Taylor BR, Parkinson D (1988) Does repeated freezing and thawing accelerate decay
497 of leaf litter? *Soil Biol Biochem* 20: 657–665.

498 Taylor BR, Parkinson D, Parsons WFJ (1989) Nitrogen and lignin content as
499 predictors of litter decay rates: A microcosm test. *Ecology* 70: 97–104.

500 Trumbore SE, Chadwick OA, Amundson R (1996) Rapid exchange between soil
501 carbon and atmospheric carbon dioxide driven by temperature change. *Science*
502 272: 393–396.

- 503 Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring
504 soil microbial biomass C. *Soil Biol Biochem* 19: 703–707.
- 505 Wang A, Wu FZ, Yang WQ, Wu ZC, Wang XX, Tan B (2012) Abundance and
506 composition dynamics of soil ammonia-oxidizing archaea in an alpine fir forest
507 on the eastern Tibetan Plateau of China. *Can J Microbiol* 58: 572–580.
- 508 Weintraub MN, Scott-Denton LE, Schmidt SK, Monson RK (2007) The effects of tree
509 rhizodecomposition on soil exoenzyme activity, dissolved organic carbon, and
510 nutrient availability in a subalpine forest ecosystem. *Oecologia* 154: 327–338.
- 511 Wickland KP, Neff JC (2008) Decomposition of soil organic matter from boreal black
512 spruce forest: environmental and chemical controls. *Biogeochemistry* 87: 29–47.
- 513 Withington CL, Sanford RL (2007) Decomposition rates of buried substrates increase
514 with altitude in the forest-alpine tundra ecotone. *Soil Biol Biochem* 39: 68–75.
- 515 Wu FZ, Yang WQ, Zhang J, Deng RJ (2010) Fine root decomposition in two
516 subalpine forests during the freeze-thaw season. *Can J Forest Res* 40: 298–307.
- 517 Wu F, Yang W, Wang K, Wu N, Lu Y (2009) Effects of dwarf bamboo (*Fargesia*
518 *denudata* Yi) density on leaf nutrient dynamics and nutrient-use efficiency.
519 *Pedosphere* 19: 496–504.
- 520 Wu FZ, Yang WQ, Zhang J, Liu L, Wang A (2011) Changes in soil microbial biomass
521 and bacterial diversity during the transition from winter to growing season in the
522 subalpine/alpine forests. *Afr J Microbiol Res* 5: 5575–5583.
- 523 Yang WQ, Wang KY, Kellomäki S, Gong HD (2005) Litter dynamics of three
524 subalpine forests in western Sichuan. *Pedosphere* 15: 653–659.
- 525 Zhou X, Wu F, Yang W, Zhu J (2011) Dynamics of Microbial Biomass during Litter
526 Decomposition in the Alpine Forest. *Acta Ecol Sin* 31: 4144–4152.
- 527 Zhu J, He X, Wu F, Yang W, Tan B (2012) Decomposition of *Abies faxoniana* litter
528 varies with freeze–thaw stages and altitudes in subalpine/alpine forests of
529 southwest China. *Scand J Forest Res* 27: 586–596.
- 530 Zhu J, Yang W, He X (2013) Temporal Dynamics of Abiotic and Biotic Factors on
531 Leaf Litter of Three Plant Species in Relation to Decomposition Rate along a
532 Subalpine Elevation Gradient. *Plos One* 8(4): e62073.

533

534

535 Fig. 1 Location of sampling sites in the eastern Qinghai-Tibetan Plateau, China. A2700, A3000,
536 A3300 and A3600 show the sampling sites along an altitudinal gradient from 2700m to 3600m
537 with similar slope and direction attributes.

538
539 Fig. 2 Graph of temperature taken at two-hour intervals in litterbags at four sampling plots
540 positioned at different altitudes from 06 November, 2008, to 16 November, 2010. Sampling stages
541 were partitioned from differences ascertained in freezing and thawing characteristics as
542 temperatures changed.

543 OF: onset of frozen stage exhibiting frequent soil temperature fluctuation around 0 °C from
544 November to December; DF: deep frozen stage where soil temperature remains constant below
545 0 °C from December to the following March; TP: thawing stage where soil temperature remains
546 close to around 0 °C as temperature increases from March to April; EG: early stage of growing
547 season where soil temperature continuously increases from April to August; and LG: later stage of
548 growing season where soil temperature decreases continuously from August to November.

549
550 Fig. 3 Carbon release rates (R_c) for foliar litter of the three species investigated (spruce, fir and
551 birch) at four different altitudes and ten decomposition stages. Bars indicate SE, $n=3$.

552
553 Fig. 4 Carbon release rate per day (V_c) for foliar litter of the three species investigated at
554 different decomposition stages along an altitudinal gradient from 2700 m to 3600 m. Bars indicate
555 SE, $n=3$.

Table 1 Initial litter chemistry of each tree species expressed as potential litter quality variables

(n=5, means \pm SE). Different letters denote significant differences ($p<0.05$) among species.

Species	C (g kg ⁻¹)	N (g kg ⁻¹)	P (g kg ⁻¹)	Lignin (%)	Cellulose (%)	C/N	Lignin/N
Spruce	527.34 \pm 6.86a	12.06 \pm 0.26a	1.41 \pm 0.03ab	28.31 \pm 0.35a	25.58 \pm 0.60a	43.73 \pm 6.51a	23.48 \pm 2.56a
Fir	545.82 \pm 6.94b	13.81 \pm 0.31ab	1.32 \pm 0.05a	32.82 \pm 0.49b	24.85 \pm 0.61a	39.52 \pm 6.20ab	23.77 \pm 3.21a
Birch	526.02 \pm 6.65a	14.61 \pm 0.43b	1.51 \pm 0.02b	28.44 \pm 0.54a	25.77 \pm 0.36a	36.00 \pm 3.25b	19.47 \pm 1.59b

Table 2 *F* values derived from statistical analyses expressed as the effects of altitude, species and their combined interaction of altitude and species on *R_c* (carbon release rate) and *V_c* (carbon release rate per day) for each decomposition stage.

	<i>F</i> value	OF1	DF1	TP1	EG1	LG1	OF2	DF2	TP2	EG2	LG2
<i>R_c</i>	<i>F</i> _{altitude}	5.55**	21.52**	56.76**	4.85**	13.61**	8.46**	10.44**	122.46**	116.42**	246.35**
	<i>F</i> _{species}	4.95*	0.28	18.71**	7.98**	100.89**	28.26**	17.33**	178.70**	158.38**	6.37**
	<i>F</i> _{altitude×species}	5.92**	1.47	28.94**	0.45	4.48**	6.15**	41.22**	74.82**	128.26**	38.97**
<i>V_c</i>	<i>F</i> _{altitude}	19.33**	14.11**	181.69**	7.18**	25.76**	72.81**	17.28**	244.29**	144.67**	306.26**
	<i>F</i> _{species}	17.27**	106.71**	59.89**	11.80**	190.94**	243.17**	28.67**	167.40**	196.17**	7.92**
	<i>F</i> _{altitude×species}	20.61**	7.28**	92.62**	0.68	8.48**	52.94**	68.19**	102.28**	159.37**	48.445**

* $p < 0.05$, ** $p < 0.01$, $n=15$ for species, $n=20$ for altitude.

Table 3 Summary tables (R^2 and step number in brackets, n=60) of stepwise regression multiple correlations expressed as carbon release rate affected by factors during different foliar litter decomposition stages.

	OF1	DF1	TP1	EG1	LG1	OF2	DF2	TP2	EG2	LG2	1 st winter	1 st GP	2 nd winter	2 nd GP	1 st year	2 nd year	2 years
MBC								0.67(4)				0.42(1)	0.39(2)			0.29(1)	0.70(3)
C	0.23(2)	0.73(3)		0.26(1)				0.48(2)	0.21(1)					0.13(1)	0.63(4)		
N					0.75(1)	0.64(4)							0.61(5)				
P							0.18(1)	0.61(3)					0.26(1)			0.45(2)	0.41(1)
Cellulose											0.20(1)				0.29(1)		
Lignin		0.68(1)													0.69(5)		
C/N						0.60(3)					0.44(2)		0.47(3)		0.55(3)	0.65(4)	
C/P						0.56(2)											0.57(2)
Lignin/N						0.43(1)							0.55(4)				
Daily-T									0.31(2)								
Day-T								0.25(1)									
Night-T												0.66(2)					
Daily-pd																	
Day-pd			0.15(1)														0.75(4)
Night-pd	0.12(1)			0.45(2)													
Daily-nd																	
Day-nd							0.23(2)									0.55(3)	
Night-nd		0.72(2)													0.42(2)		

MBC denotes microbial biomass C at corresponding decomposition stage; Daily-T, Day-T and Night-T denote the mean temperature during the entire day, daytime and nighttime at the corresponding stage, respectively; Daily-pd, Day-pd and Night-pd denote the positive degree-days during the entire day, daytime and nighttime at the corresponding stage, respectively; Daily-nd, Day-nd and Night-nd denote the negative degree-days during the entire day, daytime and nighttime at the corresponding stage, respectively; GP denotes growing season. winter = OF+DF+TP, GP = EG+LG, 1st year = winter + GP; 1st and 2nd denote the first and second decomposition year

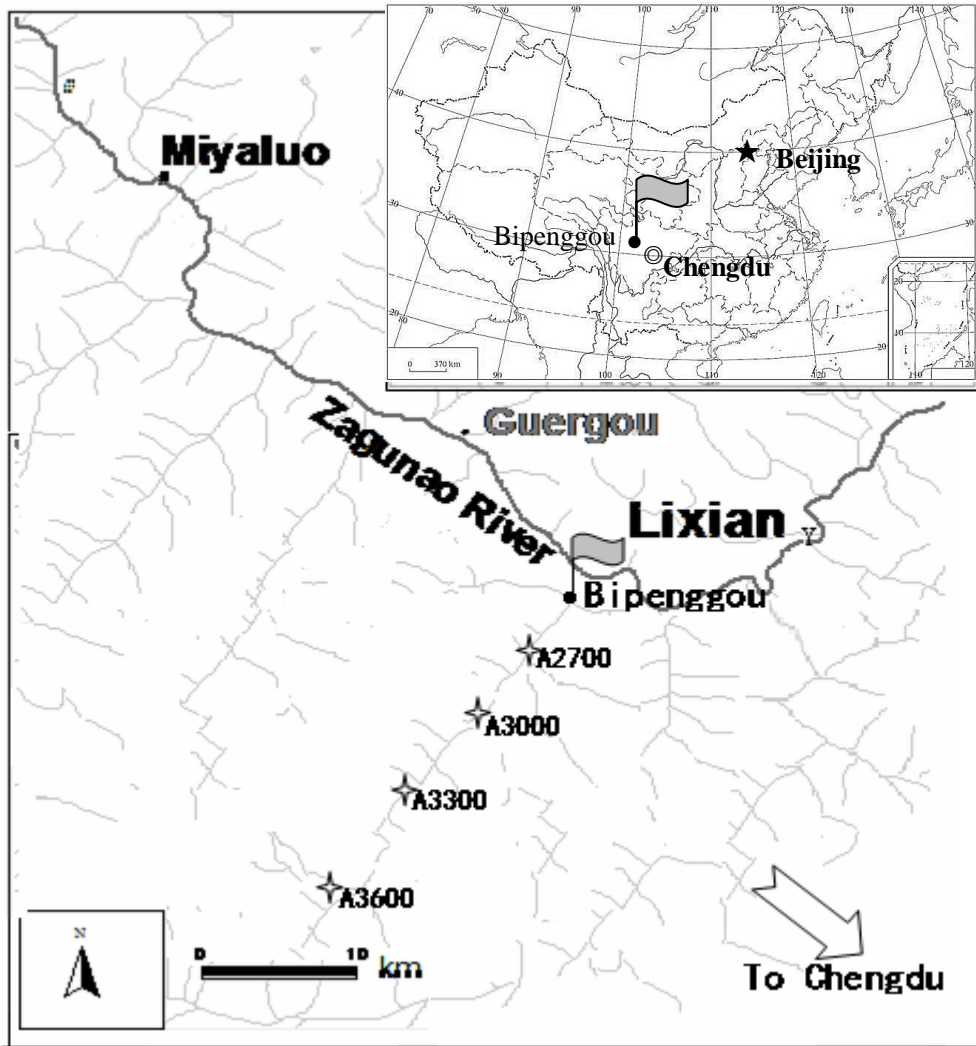


Fig.1

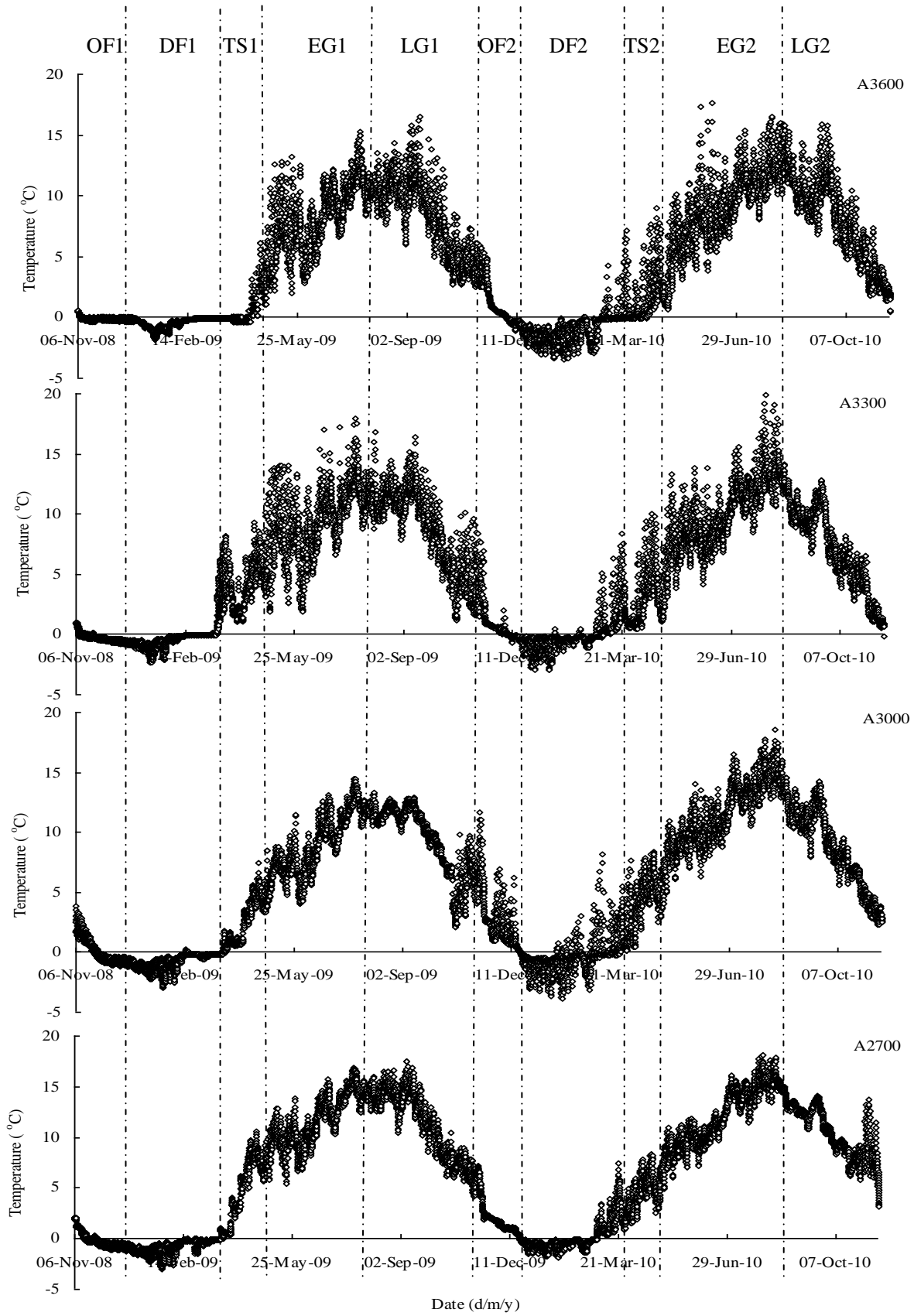


Fig.2

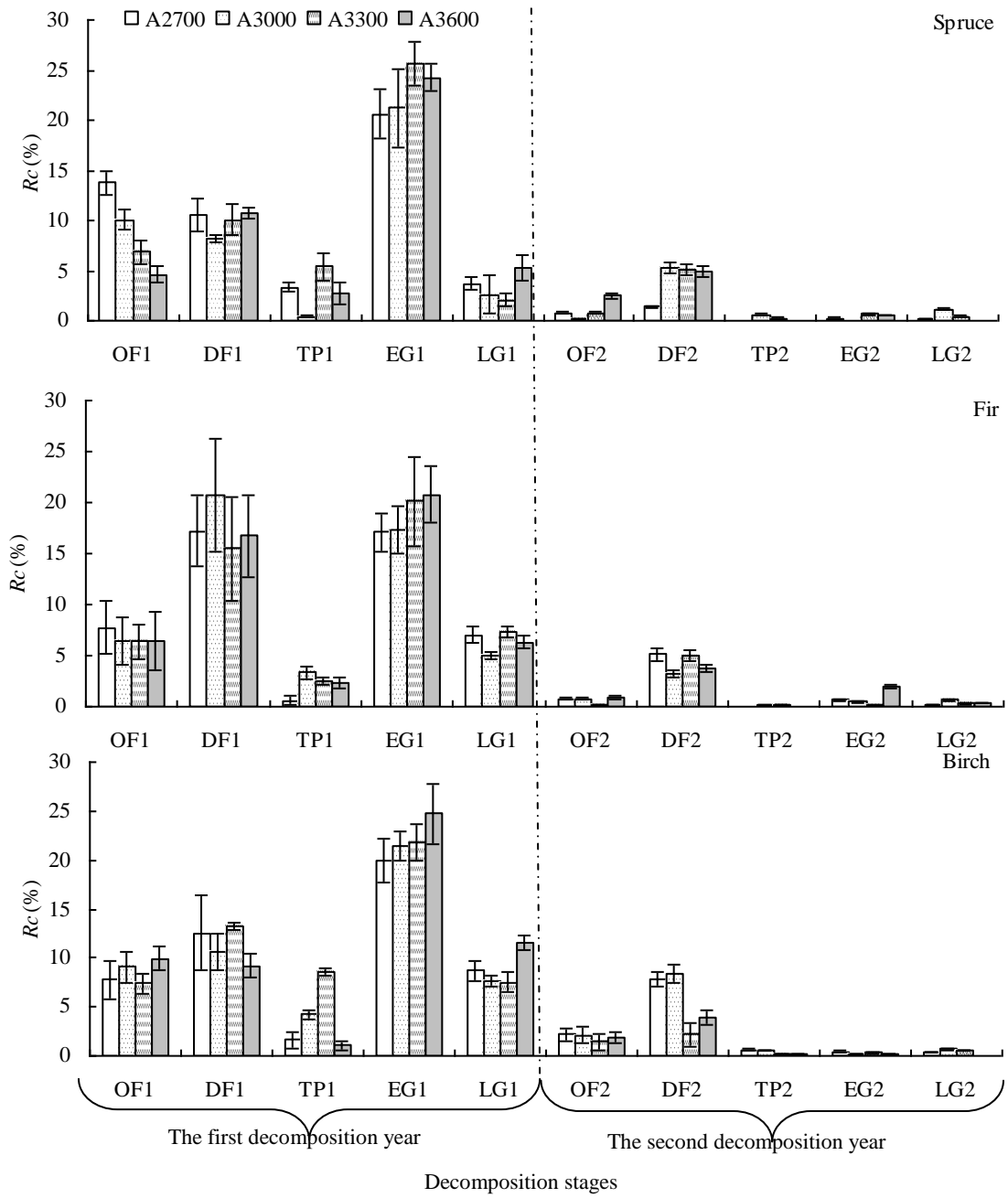


Fig. 3

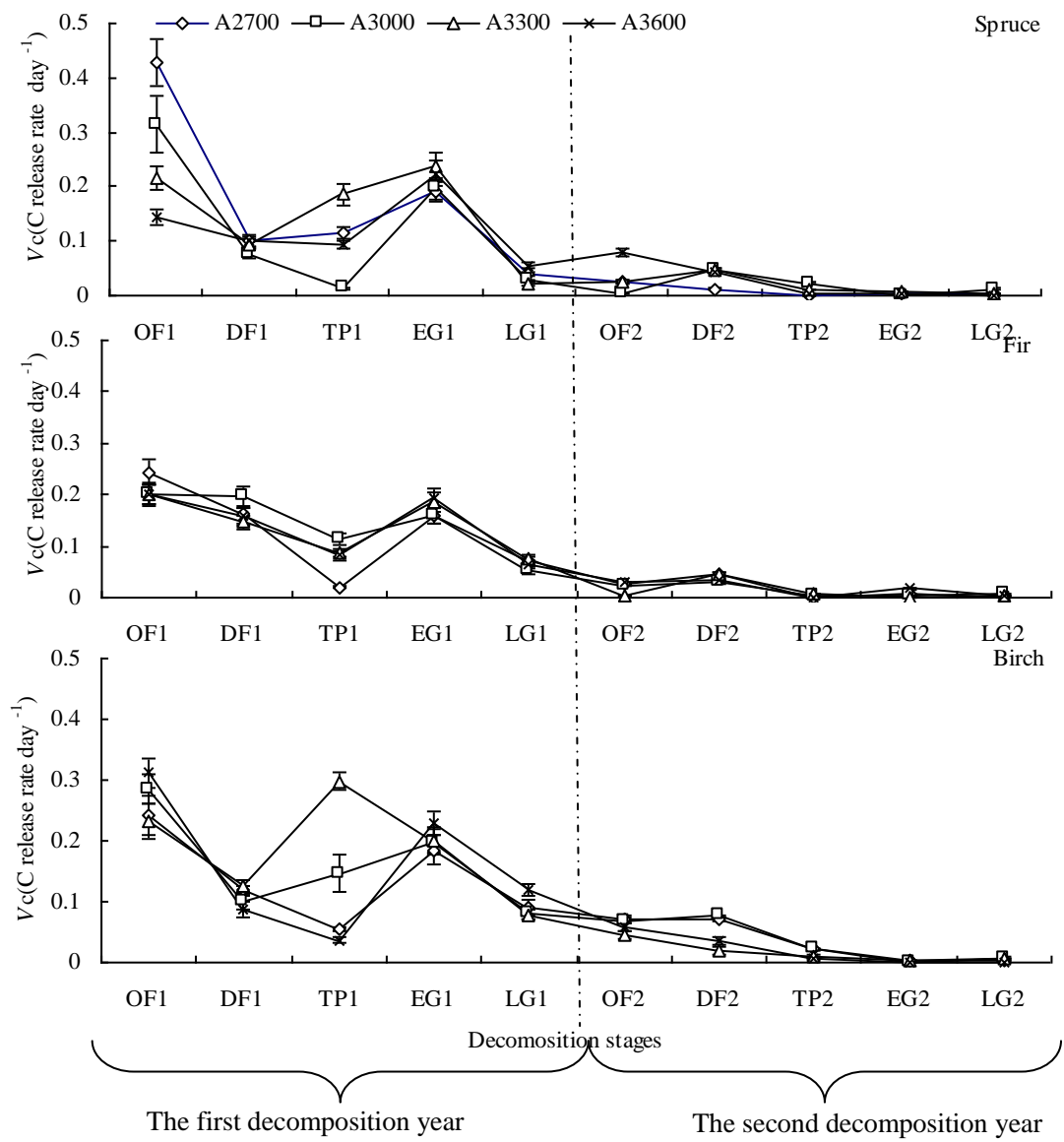


Fig. 4