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

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

## 1 Introduction

Carbon (C) release from foliar litter is a primary component in C exchange among the atmosphere, vegetation, soil and water from respiration and leaching (Berg and McClaugherty, 2008), in that the majority of fixed C enters decomposition pathways (Cebrian, 1999; Ayres et al., 2009). Climatic controls on litter decomposition have gained considerable interest in recent years on account of accumulative green house gas feedback data from ecosystems (Wu et al., 2010; Aerts et al., 2012; Fraser and Hockin, 2013). Apparent positive relationships between temperature and net C release from litter and soil have widely been detected in many cold ecosystems (Trumbore et al., 1996; Moore et al., 1998; Wickland and Neff, 2008), which has led to a keen interest concerning positive feedback on global warming through increasing atmospheric concentrations of $CO_2$, $CH_4$ and other greenhouse gases (McGuire et al., 2000). However, debate has also arisen as to whether or not C release will increase with temperature (Liski et al., 1999; Giardini and Ryan, 2000) when other environmental constraints (such as freezing, thawing, drying and flooding) are taken into account. A few studies have documented that litter decomposition rates increase with increases in altitude (subsequent decreases in temperature) in cold regions due to the much stronger freezing and thawing dynamics typical of higher altitudes (Murphy et al., 1998; Withington and Sanford, 2007). Even so, results from

Bokhorst et al. (2010) showed that winter warming events had little effect on fresh litter decomposition. They suggested that their observations of extensive decomposition primarily resulted from autumn leaching that could not have occurred in the "true" winter.

Most litters fall in late autumn before soil completely freezes over (Moore et al., 1983; Yang et al., 2005). This study has designated the stage from litterfall to time when soil completely freezes as the "onset of the frozen stage" (OF). This stage is characterized by frequent freezing and thawing events as temperatures fall to the point of freezing. The subsequent stage is designated the "deep frozen stage" (DF), where temperatures remain below freezing point. Following that is the "thawing stage" (TP), when soil thawing takes place with an increase in temperature during early 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 and 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 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 has 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). Many more works must, however, be carried out 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 and thawing events. Although these forests are characterized by low temperatures, low overall primary productivity, slow decomposition and shallow and poor soil, they possess large C pools within their litter layer. C releasing from foliar litter could provide evidence of clear feedback related to climate warming through increasing/decreasing greenhouse gas flux at such sites. Distinctive temperature fluctuation stages were observed in the experimental site (Wu et al., 2010, 2011; Tan et al., 2010); the different winter stages contributed differently to fresh fir litter decomposition due to dynamical changes in freeze–thaw (Zhu et al., 2012). However, it remains uncertain as to how to discern freezing and thawing effects on C release (if it can be done at all) during litter decomposition; this is to understand feedback in relation to ongoing climate warming. Taking findings from a meta-analysis of experimental warming studies in cold biomes (a combination of 34 site species) (reviewed by Aerts, 2006), where warming resulted in slightly increased decomposition rates, it is hypothesized that changes in freezing and thawing dynamics can promote C release from foliar litter in the experimental site investigated under a scenario of climate warming.

To test the hypothesis, a 2-year field litter decomposition experiment along an altitudinal gradient ($\sim 2700$ to $\sim 3600$ m) was conducted to simulate ongoing climate warming in the eastern Tibetan Plateau, China. C release was investigated from the fresh litter of dominant species (spruce: *Picea asperata*; fir: *Abies faxoniana*; birch: *Betula albosinensis*) during five decomposition stages (OF, DF, TS, EG and LG) each year as decomposition proceeded and temperatures fluctuated. Temperature dynamics and microbial biomass were analyzed concurrently. The objectives of this study were to examine the effects of freezing and thawing dynamics on C release from foliar litter of alpine forests, and to determine the varied effects in different altitudes. Results could also be useful in explaining details of litter decomposition in cold regions, and to provide efficient knowledge and insight on the feedback of litter decomposition under climate warming scenarios.

## 2 Materials and methods

### 2.1 Study area

This study was conducted in Bipenggou Valley of the Miyaluo Nature Reserve (long 102°53′ to 102°57′ E, lat 31°14′ to 31°19′ N; 2458 m to 4619 m a.s.l.), located in Li County, Sichuan Province, southwest China (Fig. 1). This is a transitional area situated between the Tibetan Plateau and the Sichuan Basin. Annual mean air temperature is 3 °C. Ab-

**Table 1.** Initial litter chemistry of each tree species expressed as potential litter quality variables ($n = 5$ means ± SE). Different letters denote significant differences ($p < 0.05$) among species.

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

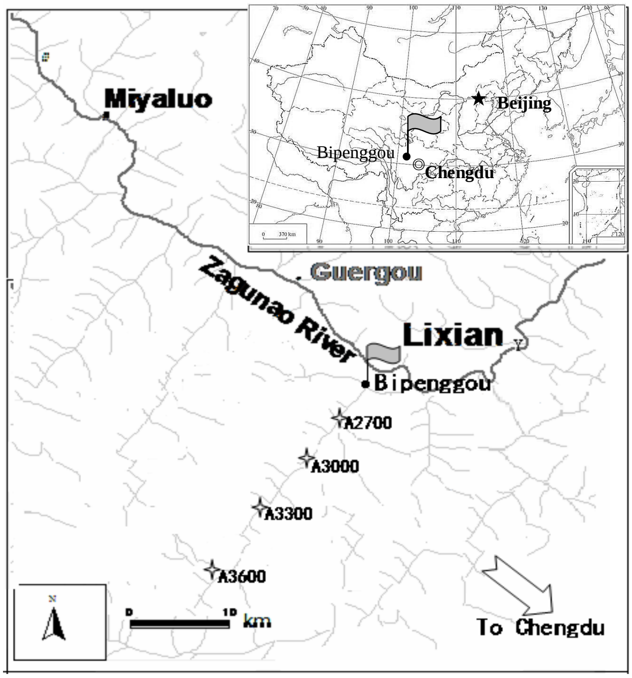

**Figure 1.** Location of sampling sites in the eastern Qinghai–Tibetan Plateau, China. A2700, A3000, A3300 and A3600 show the sampling sites along an altitudinal gratitude from 2700 to 3600 m with similar slope and direction at attributes.

solute maximum and minimum air temperatures are 23 °C in July and −18 °C in January, respectively. Annual mean precipitation ranges from 801 to 875 mm, depending on elevation. Most precipitation falls between May and August. The freeze–thaw season starts in November as soil temperatures fall below 0 °C and snow covers the ground. Soil remains frozen until the following April (Zhu et al., 2012, 2013).

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

## 2.2 Experimental design

C released during litter decomposition was determined using the widely used litterbag method. In October 2008, fresh foliar litter from spruce, fir and birch were collected from the forest floor of the sampling plots. To avoid structure damage to litter during oven-drying, the fresh litter was air-dried for more than 2 weeks at room temperature. In total, 15 g of air-dried spruce needle litter (with an approximate moisture content of 9.51 %) and fir needle litter (with an approximate moisture content of 9.15 %), and 10 g of air-dried birch broad-leaf litter (with an approximate moisture content of 9.05 %), were then separately placed in their own 20 cm × 20 cm nylon bags (0.50 mm on the soil side and 1 mm on the reverse side) before the bags were sealed. Litter of each tree species was placed in their own litter bag separately. Chemical analysis of the initial litter, as well as other calculated data, were based on the oven-dried mass (Table 1).

In total, 600 litterbags (four altitudes × five stages × five replicates × three sampling plots × 2 years) for each species were placed on the forest floor of the three selected sampling plots on 6 November 2008. Five subsamples of each litter type were oven-dried at 70 °C for 48 h to determine litter moisture content. Litterbags were randomly sampled from each forest on 8 December 2008 (OF1), 24 March 2009 (DF1), 22 April 2009 (TP1), 8 August 2009 (EG1), 12 November 2009 (LG1), 13 December 2009 (OF2), 3 April 2010 (DF2), 28 April 2010 (TP2), 16 August 2010 (EG2) and 16 November 2010 (LG2). The selection of the sampling dates was based on changes in freezing and thawing dynamics determined at previous field observations that took place between 2005 and 2007 (Tan et al., 2010; Wu et al., 2010; Zhu et al., 2012). Because of unfavorable climate and poor traffic conditions in alpine regions, sampled times were delayed in the second year of the study. Retrieved litter was then separated into two parts. One part was stored in a refrigerator at 4 °C to prepare for microbial biomass analysis. The other part was oven-dried at 70 °C for 48 h to determine dry mass and C content. Temperatures in litterbags were measured every 2 h between 6 November 2008 and 16 November

2010 (Fig. 2) in each sampling forest with different altitudes, using a DS1923-F5 iButtony logger (Maxim Integrated Products Inc., San Gabriel Drive Sunnyvale, USA).

### 2.3 Chemical analysis and calculation

C content in both initial and remaining litter samples was determined using the dichromate oxidation-ferrous sulfate titration method (Lu, 1999). In order to understand initial chemical characteristics, oven-dried foliar litter was ground (using a 1 mm sieve) to be used for nitrogen (N), phosphorus (P), cellulose and lignin analysis. N and P analyses were carried out according to Lu (1999). In short, subsamples of 0.2500 g were acid digested using an 8 mL $H_2SO_4$ ($\rho = 1.84\,\mathrm{g\,cm^{-3}}$) and a 3 mL $H_2O_2$ solution at 190 °C for 10 min. The digested solution was then transferred to a 100 mL volumetric flask, subsampled, and stored for N and P measurements. N and P content were determined using Kjeldahl determination for N, and the molybdenum-blue colorimetric method for P. Lignin and cellulose were measured using the acid detergent lignin method (Graca et al., 2005).

Microbial biomass C (MBC) in litter was determined according to differences between organic C extracted using $0.5\,\mathrm{mol\,L^{-1}}$ $K_2SO_4$ from fumigated and non-fumigated samples (Brookes et al., 1985; Vance et al., 1987). The efficiency factor ($K_c = 0.38$) was used to correct incomplete extractability (Vance et al., 1987). Parts of data from MBC were published by Zhou et al. (2011).

C release rates ($R_c$) throughout litter decomposition at each stage of the 2-year decomposition experiment were calculated as follows:

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

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

$$V_c = R_i/D_{Ti}(i = 1, 2, 3\ldots), \quad (2)$$

where $M_0$ and $C_0$ are the dry mass and C content ($\mathrm{g\,kg^{-1}}$) of initial litter, respectively; $M_{i-1}$ and $M_i$ are the dry mass of the remaining litter in the litterbags at the end of $T_{i-1}$ stage and $T_i$ stage after sampling, respectively; $C_{i-1}$ and $C_i$ are the C content ($\mathrm{g\,kg^{-1}}$) of the remaining litter at the end of $T_{i-1}$ stage and $T_i$ stage after sampling, respectively; $D_{T_i}$ is the length (day number) of each stage ($T_i$) as indicated earlier. The C release rate ($R_0$) during the entire 2-year decomposition experiment was the sum of C release during each stage.

It should be noted that freeze–thaw cycles should be numbered, but no efficient method currently exists. Although Konestabo et al. (2007) defined a freeze–thaw cycle as a period in which temperatures drop/rise below 0 °C for at least 3 h followed by a rise/drop above 0 °C for at least 3 h, the procedure has proven difficult to calculate in this experiment because observed temperatures in the sampling sites were often extremely close to 0 °C (Fig. 2), especially during the OF and TP stages. Therefore, since the processes of freezing and thawing can be respectively seen as thermal energy accumulating and releasing (Kayastha et al., 2003), we believe that positive-degree days and negative-degree days can be more concise and countable indicators in describing freezing and thawing. It was also determined that degree days at the experimental sites played a more important role in soil processes than other temperature indicators (Wang et al., 2012).

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

### 2.4 Statistical analysis

Prior to statistical analysis, data were tested for homogeneity of variance using Levene's test and transformed where applicable (Gaur and Gaur, 2006). To check how much variance in C release could be predicted from altitude, species and their combined interaction, $R_c$ and $V_c$ were analyzed at different stages using the univariate process of general linear model (GLM) with altitude, species and their combined interaction as factors (Gaur and Gaur, 2006). Stepwise linear regression was used to examine which factors dominated C release from foliar litter at each decomposition stage. If formerly entered indicators were removed by the stepwise process, those indicators that contributed more to higher $R$ square ($R^2$) in terminal models were chosen (Gaur and Gaur, 2006). All statistical analyses were performed using the Statistical Product and Service Solutions (SPSS) software package (standard released version 16.0 for Windows, SPSS Inc., IL., USA).

## 3 Results

### 3.1 $R_c$ and $V_c$

At the conclusion of the 2-year decomposition experiment, foliar litter C release reached from 49.6 to 64.9 %, depending on species and altitude (Fig. 3). Regardless of species, the entire 2-year $R_c$ exhibited little variance between A3300 and A3600, where values were higher than at lower altitudes. The majority (42.5–58.5 %) of C released from foliar litter occurred in the first year of the decomposition. When compared to the other decomposition stages, higher $R_c$ was ob-

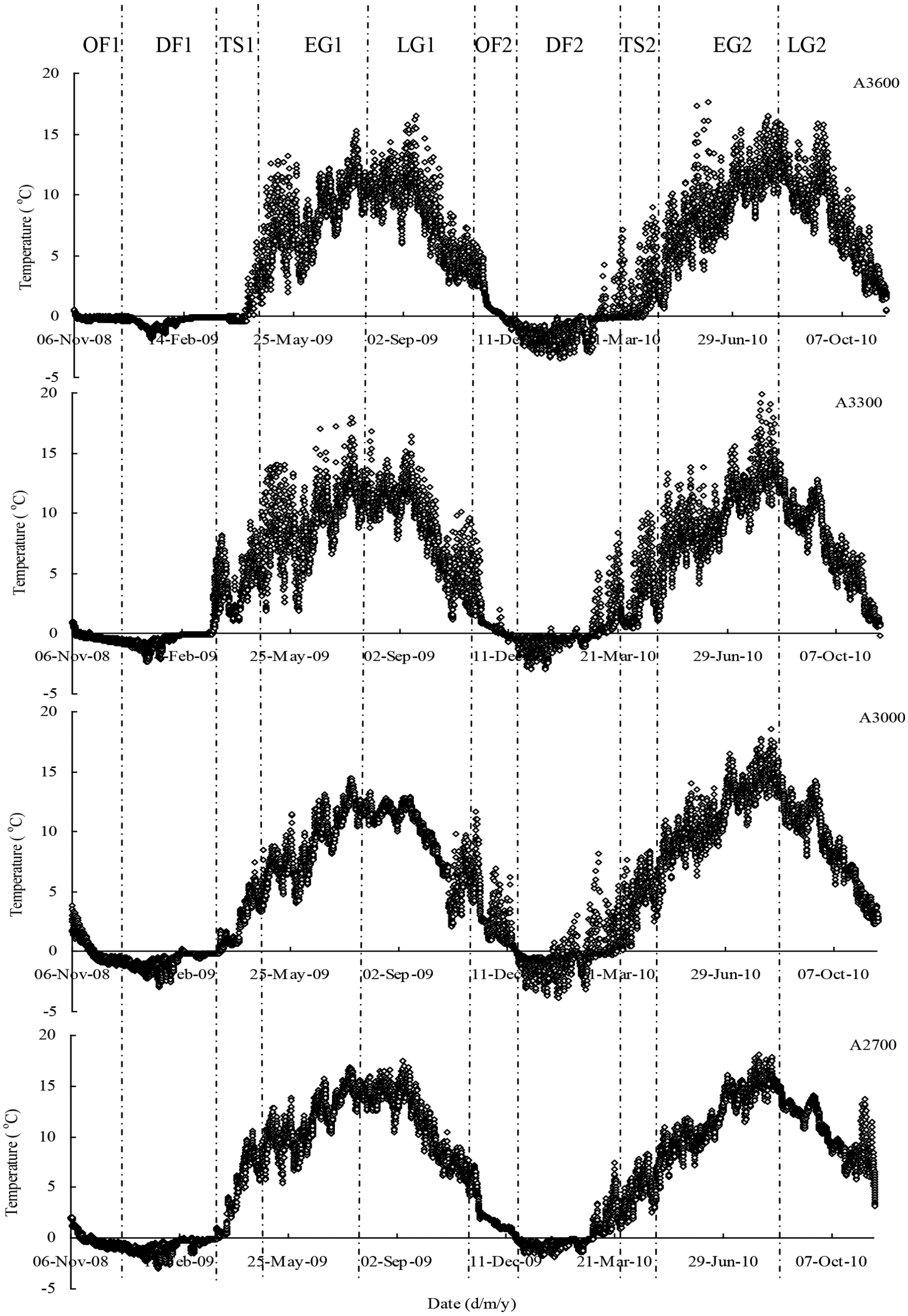

**Figure 2.** Graph of temperature taken at 2-hour intervals in litterbags at four sampling plots positioned at different altitudes from 6 November 2008 to 16 November 2010. Sampling stages were partitioned from differences ascertained in freezing and thawing characteristics as temperatures changed. OF: onset of frozen stage exhibiting frequent soil temperature fluctuation around 0 °C from November to December; DF: deep frozen stage at which soil temperature remains constant below 0 °C from December to the following March; TP: thawing stage at which soil temperature remains close to around 0 °C as temperature increases from March to April; EG: early stage of growing season at which soil temperature continuously increases from April to August; LG: later stage of growing season at which soil temperature decreases continuously from August to November.

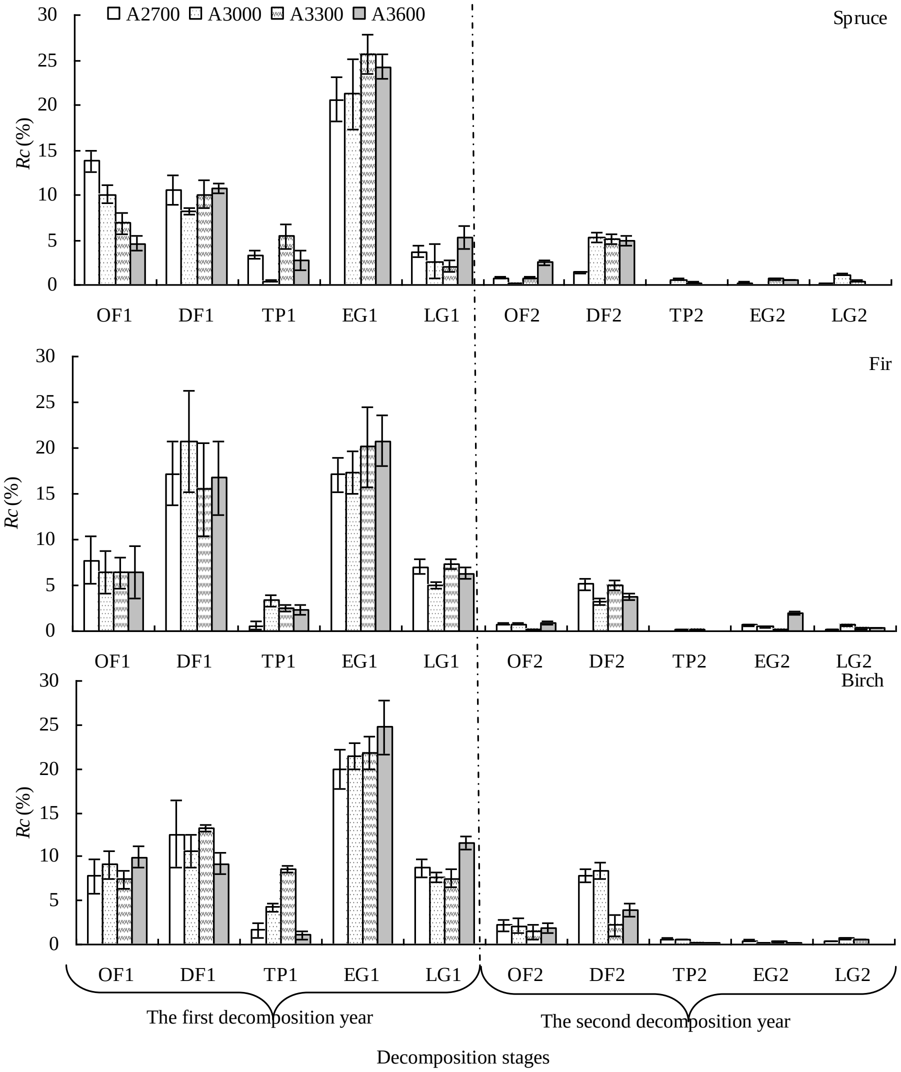

**Figure 3.** Carbon release rates ($R_c$) for foliar litter of the three species investigated (spruce, fir and birch) at four different altitudes and 10 decomposition stages. Bars indicate SE; $n = 3$.

served for DF2 during the second year (Fig. 3). The contribution of foliar litter C release at EG1 was great (accounting for 29.9–44.8 % of the C release rates of the entire 2-year experiment), regardless of altitude and species, followed by DF1 and OF1. With the exception of DF1, for which species had only insignificant ($p > 0.05$) effects on $R_c$, both altitude and species had significant ($p < 0.05$) effects on $R_c$ for all other stages (Table 2).

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

### 3.2 Multiple correlations

According to stepwise regression multiple correlations (Table 3), $R_c$ was strongly correlated to Day-pd for the entire experiment, but, in the first and the second year, $R_c$ correlated more to Night-nd and Day-nd, respectively, compared to other temperature indicators. Night-nd and Day-nd also correlated to $R_c$ for DF1 and DF2, respectively, while Night-pd strongly correlated to $R_c$ for both OF1 and EG1. Day-pd

**Table 2.** $F$ values derived from statistical analyses expressed as the effects of altitude, species and their combined interaction of altitude and species on $R_\mathrm{c}$ (carbon release rate) and $V_\mathrm{c}$ (carbon release rate per day) for each decomposition stage.

| | $F$ value | OF1 | DF1 | TP1 | EG1 | LG1 | OF2 | DF2 | TP2 | EG2 | LG2 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $R_\mathrm{c}$ | $F_\mathrm{altitude}$ | $5.55^2$ | $21.52^2$ | $56.76^2$ | $4.85^2$ | $13.61^2$ | $8.46^2$ | $10.44^2$ | $122.46^2$ | $116.42^2$ | $246.35^2$ |
| | $F_\mathrm{species}$ | $4.95^1$ | 0.28 | $18.71^2$ | $7.98^2$ | $100.89^2$ | $28.26^2$ | $17.33^2$ | $178.70^2$ | $158.38^2$ | $6.37^2$ |
| | $F_{\mathrm{altitude}\times\mathrm{species}}$ | $5.92^2$ | 1.47 | $28.94^2$ | 0.45 | $4.48^2$ | $6.15^2$ | $41.22^2$ | $74.82^2$ | $128.26^2$ | $38.97^2$ |
| $V_\mathrm{c}$ | $F_\mathrm{altitude}$ | $19.33^2$ | $14.11^2$ | $181.69^2$ | $7.18^2$ | $25.76^2$ | $72.81^2$ | $17.28^2$ | $244.29^2$ | $144.67^2$ | $306.26^2$ |
| | $F_\mathrm{species}$ | $17.27^2$ | $106.71^2$ | $59.89^2$ | $11.80^2$ | $190.94^2$ | $243.17^2$ | $28.67^2$ | $167.40^2$ | $196.17^2$ | $7.92^2$ |
| | $F_{\mathrm{altitude}\times\mathrm{species}}$ | $20.61^2$ | $7.28^2$ | $92.62^2$ | 0.68 | $8.48^2$ | $52.94^2$ | $68.19^2$ | $102.28^2$ | $159.37^2$ | $48.445^2$ |

[1] $p < 0.05$, [2] $p < 0.01$, $n = 15$ for species, $n = 20$ for altitude.

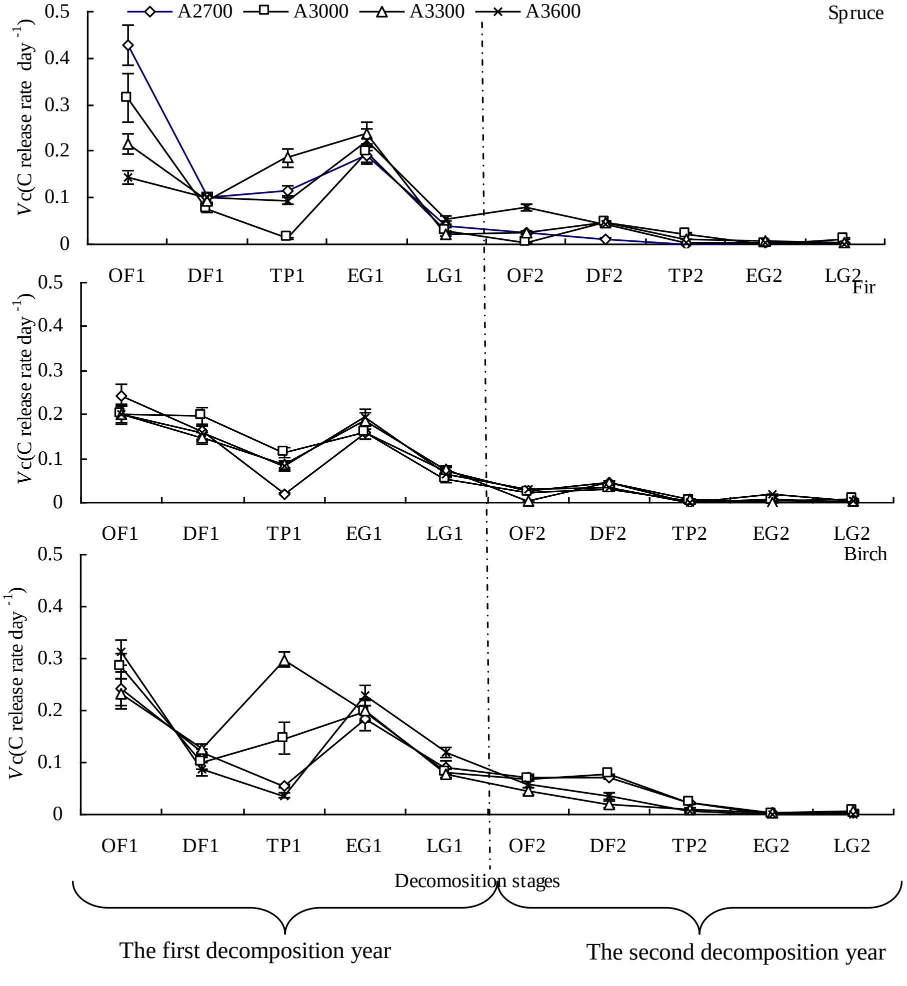

**Figure 4.** Carbon release rate per day ($V_\mathrm{c}$) for foliar litter of the three species investigated at different decomposition stages along an altitudinal gratitude from 2700 to 3600 m. Bars indicate SE; $n = 3$.

correlated to $R_\mathrm{c}$ for TP1. Daily-T and Day-T correlated to $R_\mathrm{c}$ for TP2 and EG2, respectively.

As it pertains to initial litter chemistry, P exhibited a strong correlation to $R_\mathrm{c}$ for the entire 2-year experiment, the second year, the second winter, DF2 and TP2. All $R_\mathrm{c}$ during the first year, the second growing season, OF1, DF1, EG1, TP2 and EG2 correlated to initial C content. N only correlated to $R_\mathrm{c}$ for LG1, OF2 and the second winter, while lignin only correlated to $R_\mathrm{c}$ for DF1 and the first year. C / N, C / P and lignin / N related to $R_\mathrm{c}$ for OF2. However, MBC showed a strong correlation to $R_\mathrm{c}$ for the entire 2-year experiment,

**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 | First winter | First GP | Second winter | Second GP | First year | Second 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, first year = winter + GP; first and second denote the first and second decomposition year.

the second year, the second winter, the first growing season and TP2.

## 4 Discussion

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

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

In contrast, obvious higher C release rates were detected for the deep frozen stage (DF2) than other stages in the second year of decomposition. This is consistent with the results from Hobbie and Chapin (1996) who reported that litter mass was mainly lost during winter in Alaskan tussock tundra after the first year of decomposition. This may also be attributable to freezing, since Day-nd was deemed the affecting factor in C release for DF2 (Table 3). Freezing does not only directly promote the loss of recalcitrant C components by physical destruction (Taylor and Parkinson, 1988), but it also indirectly contributes to C release in subsequent thawing processes, making litter more decomposable (Hobbie and Chapin, 1996; Baptis et al., 2010). As a result, $R_\mathrm{c}$ in the first and second year showed strong correlations to Night-nd and Day-nd, respectively. Results from both this and other studies suggest that changing winter temperatures and their related freezing and thawing characteristics in the long run will play essential roles in C release from foliar litter under a scenario of climate change. In the future, more attention should be paid to ecological processes that take place in winter.

Berg et al. (1993) and Freschet et al. (2012) have documented that climate and substrate quality together might explain at least 57 % of global scale variation in leaf decomposition. Results from the current study stand in agreement with them, showing that initial litter chemistry was also the main factor in explaining C release from foliar litter. To take one example, P was strongly related to C release for the entire 2-year experiment, the second year, the second winter, DF2 and TP2. Moore et al. (2011) found that P mineralization in decomposing litter is mainly affected by environmental controls, and Aerts et al. (2012) reported increased temperatures to stimulate litter P release. Findings from the current study that show that P is a more sensitive indicator supported these previous results, implying that initial P concentration might determine litter decomposition as an earlier plant nutrient study in this region reported (Wu et al., 2009). Initial C content in litter showed strong correlations to C release in the first year, the second growing season, OF1, DF1, EG1, TP2 and EG2, suggesting that the C pool mainly determines release processes. However, lignin and N are well known to be sensitive indicators in litter decomposition (Zhu et al., 2012), but N here only correlated to $R_\mathrm{c}$ for LG1, OF2 and the second winter, and lignin only correlated to $R_\mathrm{c}$ for DF1 and the first year. On the one hand, a great deal of N was lost before LG1, such as the rapid loss that occurred with labile fresh C components for OF1, thawing processes for TP1 (Zhu et al., 2012) and the breakout of C release for EG1. At the same time, C / N was determined to be one of the factors that affected $R_\mathrm{c}$ during the first winter and throughout the first year (Table 3). As a result, N can be an important factor in controlling C release in this ecosystem as many other studies have reported. On the other hand, lignin has been documented as a recalcitrant C component that limits litter decomposition (Taylor et al., 1989). In the current study, lignin exhibited good correlations to $R_\mathrm{c}$ for DF1 and the first year, and lignin / N strongly correlated to $R_\mathrm{c}$ for OF1 and the second winter. These results also provide evidence for freezing effects on foliar litter C release.

Additionally, both previous studies from the authors of this study, as well as other studies, have detected relatively high microbial activity and rich microbial biodiversity during the deep frozen and thawing stages (Schadt et al., 2003; Wang et al., 2012). However, microbial activity (expressed as MBC in the current study) was only examined as one of the dominant factors in C release from foliar litter for TP2 alone. Results testify to the fact that climate change, together with litter chemistry, had a greater effect on C release from foliar litter than microbial activity, which is in agreement with previous studies (Freschet et al., 2012; García-Palacios et al., 2013). At the same time, results support that microbe contribution of microbial activity in foliar litter C release since MBC was correlated to $R_\mathrm{c}$ in the first growing season, the second winter, the second year and the entire 2-year experiment. It may be that the strong correlation found in this study between MBC and C release in the first growing season is direct evidence that winter decomposition increases litter decomposability by physical effects, such as freezing and thawing (Aerts, 2006; Baptis et al., 2010), and temperature by itself actually limits decomposer activity (Rouifed et al., 2010; Tan et al., 2010) in winter.

In summary, results from 2-year observations give evidence of more rapid C release from fresh foliar litter at upper elevations compared to lower elevations in the alpine/subalpine region investigated. After the majority of C was lost during the first year, clear signs of C release could only be detected during the deep frozen stage. Including other factors, negative-degree days correlated to $R_\mathrm{c}$ for the deep frozen stages in the first and second year, and subsequently maintained this relationship with C release during the entire first and second year. This indicates that freezing plays a dominant role in C release from foliar litter decomposition. In the short term, foliar litter C release could decelerate due to changes in freezing when annual temperatures increase in this cold region under a scenario of climate warming. This could be positive climate change feedback since numerous studies reported that increases in climate change will likely occur at higher altitudinal/latitudinal locales (Groffman et al., 2001; IPCC, 2007; Schuur et al., 2009), although C loss can also be attributed to other processes, such as leaching (Bokhorst et al., 2010). However, “to release or not to release, that is the question”, and, if this proves definitively to be the case, increases in temperature might delay C cycling processes during the first 2-year foliar litter decomposition.

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