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

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[Title Page](#)

[Abstract](#)

[Introduction](#)

[Conclusions](#)

[References](#)

[Tables](#)

[Figures](#)

◀

▶

◀

▶

[Back](#)

[Close](#)

[Full Screen / Esc](#)

[Printer-friendly Version](#)

[Interactive Discussion](#)



Abstract

Carbon (C) release from foliar litter is a primary component in C exchange between the atmosphere and terrestrial ecosystems, but little information is currently related to the effects of freezing and thawing dynamics on C release of foliar litter in cold regions.

5 A two-year field litter decomposition experiment was conducted along an altitudinal gradient (~2700 m 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 were partitioned according to changes in freezing and thawing dynamics of each winter. High C release
10 was observed in lower altitudes during winter stages, but higher 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 degree were correlated to C release rates for the deep frozen stages in both years, and this relationship continued for the duration of the experiment,
15 indicating that changes in freezing can directly modify C release from foliar litter. The results suggested that climate warming could delay the onset of C release in fresh litter in this cold region.

1 Introduction

Carbon (C) release from foliar litter is a primary component in C exchange between the atmosphere and terrestrial ecosystems (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 has widely been detected in many cold ecosystems (Trumbore et al., 1996; Moore et al., 1998; Wick-

BGD

11, 9539–9564, 2014

Impact of changes in freezing and thawing

F. Wu et al.

Title Page	
Abstract	Introduction
Conclusions	References
Tables	Figures
◀	▶
◀	▶
Back	Close
Full Screen / Esc	
Printer-friendly Version	
Interactive Discussion	



land and Neff, 2008), which has led to a keen interest concerning positive feedbacks on global warming through increasing atmospheric concentrations of CO₂, CH₄ and other greenhouse gases (McGuire et al., 2000). However, debate has also arisen 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 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 the freezing point. Following that is the “thawing stage” (TS) when soil thawing takes place with an increase in temperature during early spring but where replicated 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

Impact of changes in freezing and thawing

F. Wu et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[◀](#)[▶](#)[◀](#)[▶](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

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 has 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 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 release from foliar litter could provide evidence of clear feedbacks 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 how to discern freezing

[Title Page](#)

[Abstract](#)

[Introduction](#)

[Conclusions](#)

[References](#)

[Tables](#)

[Figures](#)

◀

▶

◀

▶

[Back](#)

[Close](#)

[Full Screen / Esc](#)

[Printer-friendly Version](#)

[Interactive Discussion](#)



Impact of changes in freezing and thawing

F. Wu et al.

and thawing effects on C release (if it can be done at all) during litter decomposition in which to understand feedbacks 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 two-year field litter decomposition experiment along an altitudinal gradient (~2700 m to ~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*, and 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.m.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. Absolute maximum and minimum air temperatures are 23 °C in July and –18 °C

9543

in January, respectively. Annual mean precipitation ranges from 801 mm 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).

5 A 900 m vertical transitional zone was selected along an altitudinal gradient from 2700 m, 3000 m, 3300 m 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 10 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*) at 3600 m. Three sampling forest plots were established for each site at an altitude.

15 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 two weeks at room temperature.

20 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 × 20 cm nylon bags (0.50 mm on the soil side and 1 mm on the reverse side) before the bags were sealed. Chemical analysis 25 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 × two years) for each species were placed on the forest floor of the three se-

[Title Page](#)

[Abstract](#)

[Introduction](#)

[Conclusions](#)

[References](#)

[Tables](#)

[Figures](#)

◀

▶

◀

▶

[Back](#)

[Close](#)

[Full Screen / Esc](#)

[Printer-friendly Version](#)

[Interactive Discussion](#)



lected 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 5 2009 (OF2), 3 April 2010 (DF2), 28 April 2010 (TP2), 16 August 2010 (EG2) and 16 November 2010 (LG2). Selection of 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 10 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. Temperatures in litterbags were measured every two hours between 6 November 2008, 15 and 16 November 2010 (Fig. 2), using a DS1923-F5 iButton 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 20 used for nitrogen (N), phosphorus (P), cellulose and lignin analysis. N and P analyses were carried out according to Lu (1999). In brief, subsamples of 0.2500 g were acid digested using an 8 mL H₂SO₄ ($\rho = 1.84 \text{ g cm}^{-3}$) and a 3 mL H₂O₂ 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 25 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 $0.5\text{ mol L}^{-1}\text{ K}_2\text{SO}_4$ 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 two-year decomposition experiment were calculated as follows:

$$R_C(\%) = 100 \cdot (M_{i-1}C_{i-1} - M_iC_i)/M_0C_0 \quad (1)$$

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):

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

where M_0 and C_0 are the dry mass and C content (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 (g kg^{-1}) of the remaining litter at the end of T_{i-1} stage and T_i stage after sampling, respectively; and 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 two-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 $0\text{ }^\circ\text{C}$ for at least three hours followed by a rise/drop above $0\text{ }^\circ\text{C}$ for at least three hours, the procedure has proven difficult to calculate in this experiment because observed temperatures in the sampling sites were often extremely close to $0\text{ }^\circ\text{C}$ (Fig. 2), especially during the OF and TP stages. Therefore, since the processes of freezing and thawing can be respectively looked as the thermal energy accumulating and releasing (Kayastha et al., 2003), we believe 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).

5 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 (pd) and negative degree-days (nd) were calculated (Kayastha et al., 2003) from daily average temperatures (Daily-pd and Daily-nd), daytime average temperatures (Day-pd and Day-nd) and nighttime average temperatures (Night-pd and Night-nd). 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 10 also calculated separately.

2.4 Statistical analysis

15 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 was analyzed at different stages using the univariate process of 20 general linear model (GLM) with altitude, species and their combined interaction as factors (Gaur and Gaur, 2006). Step-wise linear regression was used to examine which factors dominated C release from foliar litter at each decomposition stages. 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 chose (Gaur and Gaur, 2006). All statistical analyses were performed using the SPSS software package (standard released version 16.0 for Windows, SPSS Inc., IL., USA).

Title Page	
Abstract	Introduction
Conclusions	References
Tables	Figures
◀	▶
◀	▶
Back	Close
Full Screen / Esc	
Printer-friendly Version	
Interactive Discussion	



3 Results

3.1 R_C and V_C

At the conclusion of the two 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 two-year R_C exhibited little variance between A3300 and A3600 where values were higher than 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 observed 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 to entire two-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 two-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 relative higher V_C , regardless of species and altitudes. The litter of all three species displayed the same pattern: Higher V_C was observed in lower altitudes for winter stages, but higher V_C was observed in 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

BGD

11, 9539–9564, 2014

Impact of changes in freezing and thawing

F. Wu et al.

[Title Page](#)

[Abstract](#)

[Introduction](#)

[Conclusions](#)

[References](#)

[Tables](#)

[Figures](#)

◀

▶

◀

▶

[Back](#)

[Close](#)

[Full Screen / Esc](#)

[Printer-friendly Version](#)

[Interactive Discussion](#)



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 correlated to R_C for TP1. Daily-T and Day-T correlated to R_C for TP2 and EG2, respectively.

As it pertains to initial litter chemistry, P exhibited a strong correlation to R_C for the entire two-year experiment, the second year, the second winter, DF2 and TP2. All R_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_C for LG1, OF2 and the second winter while lignin only correlated to R_C for DF1 and the first year. C/N, C/P and lignin/N related to R_C for OF2. However, MBC showed a strong correlation to R_C for the entire two-year experiment, 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 m 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.,

BGD

11, 9539–9564, 2014

Impact of changes in freezing and thawing

F. Wu et al.

[Title Page](#)

[Abstract](#)

[Introduction](#)

[Conclusions](#)

[References](#)

[Tables](#)

[Figures](#)

◀

▶

◀

▶

[Back](#)

[Close](#)

[Full Screen / Esc](#)

[Printer-friendly Version](#)

[Interactive Discussion](#)



2012). Consequently, the highest V_C were 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 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 and litter chemical properties control winter litter decomposition but microbe-related factors control growing season (Zhu et al., 2013). It should be noted that freeze-thaw cycles could also a key factor in winter (Zhu et al., 2013), but no useful parameter 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 also

Title Page	
Abstract	Introduction
Conclusions	References
Tables	Figures
◀	▶
◀	▶
Back	Close
Full Screen / Esc	
Printer-friendly Version	
Interactive Discussion	



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_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 might together 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 two-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 stimulate litter P release. Findings from the current study that show that P is a more sensitive indicator supported these previous results, implying 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_C for LG1, OF2 and the second winter, and lignin only correlated to R_C for DF1 and the first year. On the one hand, a great deal of N 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_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.

Impact of changes in freezing and thawing

F. Wu et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[◀](#)[▶](#)[◀](#)[▶](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

[Title Page](#)

[Abstract](#)

[Introduction](#)

[Conclusions](#)

[References](#)

[Tables](#)

[Figures](#)

[◀](#)

[▶](#)

[◀](#)

[▶](#)

[Back](#)

[Close](#)

[Full Screen / Esc](#)

[Printer-friendly Version](#)

[Interactive Discussion](#)



crease in this cold region under a scenario of climate warming. This could be a 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 a question,” and, if this proves definitively to be the case, increases in temperature might delay C cycling processes.

Author Contribution

F. W. and W. Y. designed the experiments, J. Zhu, J. Zhang and B. T. carried them out.
10 F. W. and C. P. prepared the manuscript with contributions from all co-authors.

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15 References

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

20 Aerts, R., Callaghan, T. V., Dorrepaal, E., van Logtestijn, R. S. P., and Cornelissen, J. H. C.: Seasonal climate manipulations have only minor effects on litter decomposition rates and N dynamics but strong effects on litter P dynamics of sub-arctic bog species, *Oecologia*, 170, 809–819, 2012.

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

25 Baptist, F., Yoccoz, N. G., and Choler, P.: Direct and indirect control by snow cover over decomposition in alpine tundra along a snowmelt gradient, *Plant Soil*, 328, 397–410, 2010.

[Title Page](#)

[Abstract](#)

[Introduction](#)

[Conclusions](#)

[References](#)

[Tables](#)

[Figures](#)

◀

▶

◀

▶

[Back](#)

[Close](#)

[Full Screen / Esc](#)

[Printer-friendly Version](#)

[Interactive Discussion](#)



Berg, B. and McClaugherty, C.: Plant Litter: Decomposition, Humus Formation, Carbon Sequestration, 2nd edn., Springer, New York, 2008.

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

Bokhorst, S., Bjerke, J. W., Melillo, J., Callaghan, T. V., and Phoenix, G. K.: Impacts of extreme winter warming events on litter decomposition in a sub-Arctic heathland, *Soil Biol. Biochem.*, 42, 611–617, 2010.

Bokhorst, S., Metcalfe, D. B., and Wardle, D. A.: Reduction in snow depth negatively affects decomposers but impact on decomposition rates is substrate dependent, *Soil Biol. Biochem.*, 62, 157–164, 2013.

Brookes, P. C., Landman, A., Pruden, G., and Jinkenson, D. S.: Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil, *Soil Biol. Biochem.*, 17, 837–842, 1985.

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

Fraser, L. H. and Hockin, A. D.: Litter decomposition rates of two grass species along a semi-arid grassland–forest ecocline, *J. Arid Environ.*, 88, 125–129, 2013.

Freschet, G. T., Aerts, R., and Cornelissen, J. H. C.: Multiple mechanisms for trait effects on litter decomposition: moving beyond home-field advantage with a new hypothesis, *J. Ecol.*, 100, 619–630, 2012.

García-Palacios, P., Maestre, F. T., Kattge, J., and Wall, D. H.: Climate and litter quality differently modulate the effects of soil fauna on litter decomposition across biomes, *Ecol. Lett.*, 16, 1045–1053, 2013.

Gaur, A. S. and Gaur, S. S.: Statistical Methods for Practice and Research: a Guide to Data Analysis using SPSS, Sage Publications, Thousand Oaks, CA, 2006.

Giardina, C. P. and Ryan, M. G.: Evidence that decomposition rates of organic carbon in mineral soil do not vary with temperature, *Nature*, 404, 858–861, 2000.

Graca, M. A. S., Bärlocher, F., and Gessner, M. O.: Methods to Study Litter Decomposition: a Practical Guide, Springer, New York, 2005.

[Title Page](#)

[Abstract](#)

[Introduction](#)

[Conclusions](#)

[References](#)

[Tables](#)

[Figures](#)

◀

▶

◀

▶

[Back](#)

[Close](#)

[Full Screen / Esc](#)

[Printer-friendly Version](#)

[Interactive Discussion](#)



Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

1

B

Close

Full Screen / Esc

[Printer-friendly Version](#)

Interactive Discussion



Impact of changes in freezing and thawing

F. Wu et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[◀](#)[▶](#)[◀](#)[▶](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

Wu, F., Yang, W., Wang, K., Wu, N., and Lu, Y.: Effects of dwarf bamboo (*Fargesia denudata* Yi) density on leaf nutrient dynamics and nutrient-use efficiency, *Pedosphere*, 19, 496–504, 2009.

5 Wu, F. Z., Yang, W. Q., Zhang, J., and Deng, R. J.: Fine root decomposition in two subalpine forests during the freeze-thaw season, *Can. J. Forest Res.*, 40, 298–307, 2010.

Wu, F. Z., Yang, W. Q., Zhang, J., Liu, L., and Wang, A.: Changes in soil microbial biomass and bacterial diversity during the transition from winter to growing season in the subalpine/alpine forests, *Afr. J. Microbiol. Res.*, 5, 5575–5583, 2011.

10 Yang, W. Q., Wang, K. Y., Kellomäki, S., and Gong, H. D.: Litter dynamics of three subalpine forests in western Sichuan, *Pedosphere*, 15, 653–659, 2005.

Zhou, X., Wu, F., Yang, W., and Zhu, J.: Dynamics of microbial biomass during litter decomposition in the Alpine forest, *Acta Ecol. Sin.*, 31, 4144–4152, 2011.

15 Zhu, J., He, X., Wu, F., Yang, W., and Tan, B.: Decomposition of *Abies faxoniana* litter varies with freeze-thaw stages and altitudes in subalpine/alpine forests of southwest China, *Scand. J. Forest Res.*, 27, 586–596, 2012.

Zhu, J., Yang, W., and He, X.: Temporal dynamics of abiotic and biotic factors on leaf litter of three plant species in relation to decomposition rate along a subalpine elevation gradient, *Plos One*, 8, e62073, doi:10.1371/journal.pone.0062073, 2013.

[Title Page](#) [Abstract](#) [Introduction](#) [Conclusions](#) [References](#) [Tables](#) [Figures](#) [◀](#) [▶](#) [◀](#) [▶](#) [Back](#) [Close](#) [Full Screen / Esc](#)

[Printer-friendly Version](#) [Interactive Discussion](#)



Table 1. Initial litter chemistry of each tree species expressed as potential litter quality variables ($n = 5$, \pm SE).

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

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[◀](#)[▶](#)[◀](#)[▶](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

Impact of changes in freezing and thawing

F. Wu et al.

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	
R_C	$F_{altitude}$	5.55**	21.52**	56.76**	4.85**	13.61**	8.46**	10.44**	122.46**	116.42**	246.35**
	$F_{species}$	4.95*	0.28	18.71**	7.98**	100.89**	28.26**	17.33**	178.70**	158.38**	6.37**
	$F_{altitude \times species}$	5.92**	1.47	28.94**	0.45	4.48**	6.15**	41.22**	74.82**	128.26**	38.97**
V_C	$F_{altitude}$	19.33**	14.11**	181.69**	7.18**	25.76**	72.81**	17.28**	244.29**	144.67**	306.26**
	$F_{species}$	17.27**	106.71**	59.89**	11.80**	190.94**	243.17**	28.67**	167.40**	196.17**	7.92**
	$F_{altitude \times species}$	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.

- [Title Page](#)
- [Abstract](#) [Introduction](#)
- [Conclusions](#) [References](#)
- [Tables](#) [Figures](#)
- [◀](#) [▶](#)
- [◀](#) [▶](#)
- [Back](#) [Close](#)
- [Full Screen / Esc](#)
- [Printer-friendly Version](#)
- [Interactive Discussion](#)



	OF1	DF1	TP1	EG1	LG1	OF2	DF2	TP2	EG2	LG2	1st winter	1st GP	2nd winter	2nd GP	1st year	2nd 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(2)		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(8)	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)				0.66(2)					
Night-T																	
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		0.62(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; winter = $OF + DF + TP$; $GP = EG + LG$. 1st year = winter + GP; 1st and 2nd denote the first and second decomposition year.



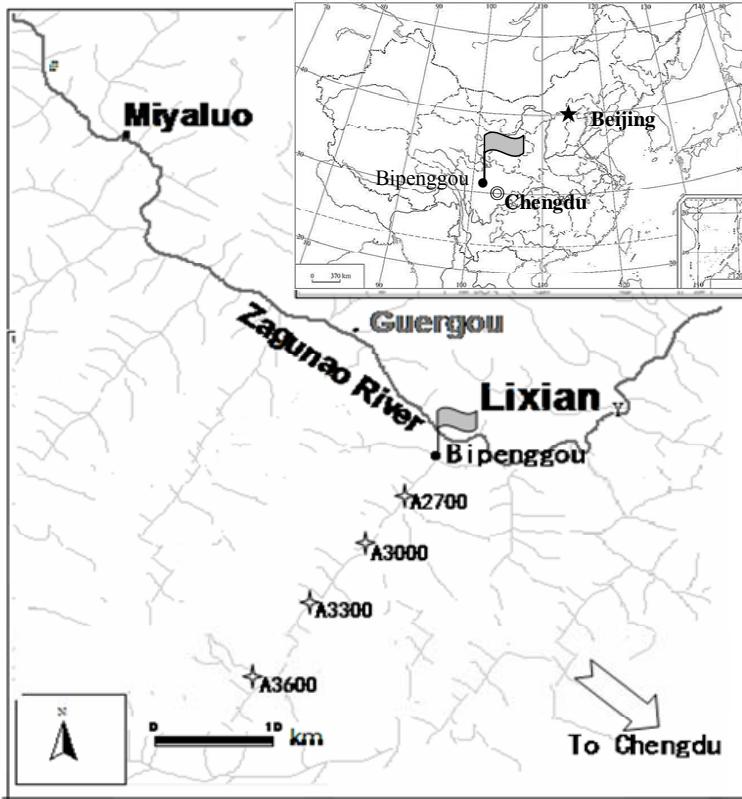


Figure 1. Location of sampling sites in eastern Qinghai-Tibet Plateau, China. A2700, A3000, A3300 and A3600 show the sampling sites along an altitudinal gradient from 2700 m to 3600 m with similar slope and direction attributes.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[◀](#)[▶](#)[◀](#)[▶](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

Impact of changes in freezing and thawing

F. Wu et al.

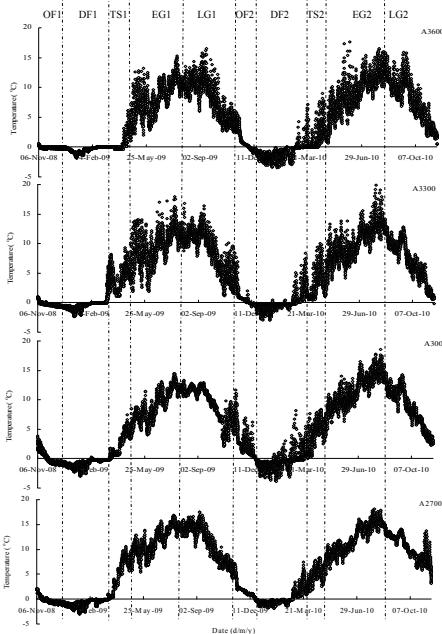


Figure 2. Graph of temperature taken at two-hour intervals in litterbags at four sampling plots positioned at different altitudes from 6 November 2008 to 16 November 2010. Sampling stages were partitioned from differences ascertained in freezing and thawing characteristics as temperatures changed. OF: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

- [Title Page](#)
- [Abstract](#) [Introduction](#)
- [Conclusions](#) [References](#)
- [Tables](#) [Figures](#)
- [◀](#) [▶](#)
- [◀](#) [▶](#)
- [Back](#) [Close](#)
- [Full Screen / Esc](#)
- [Printer-friendly Version](#)
- [Interactive Discussion](#)



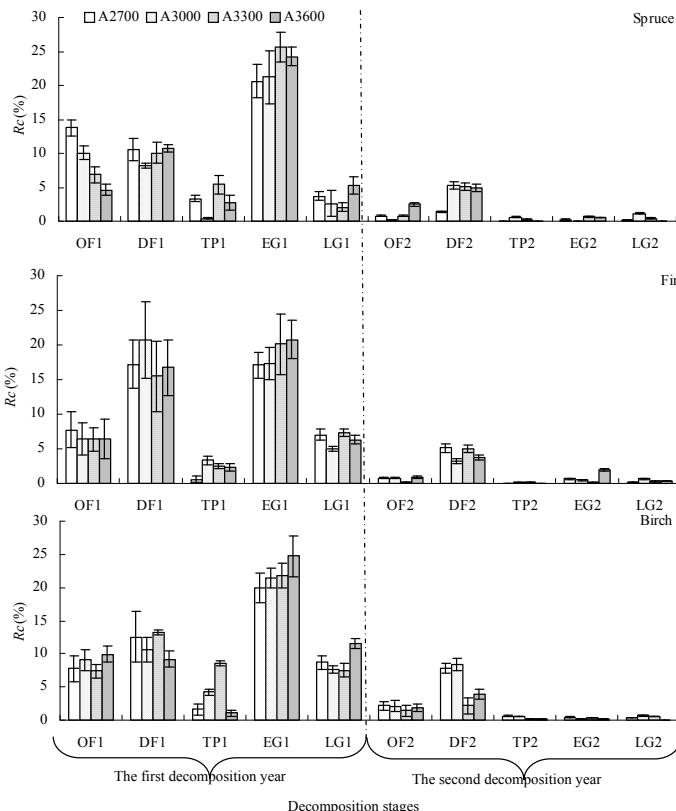


Figure 3. Carbon release rates for foliar litter of the three species investigated (spruce, fir and birch) at four different altitudes and ten decomposition stages. Bars indicate SE, $n = 3$.

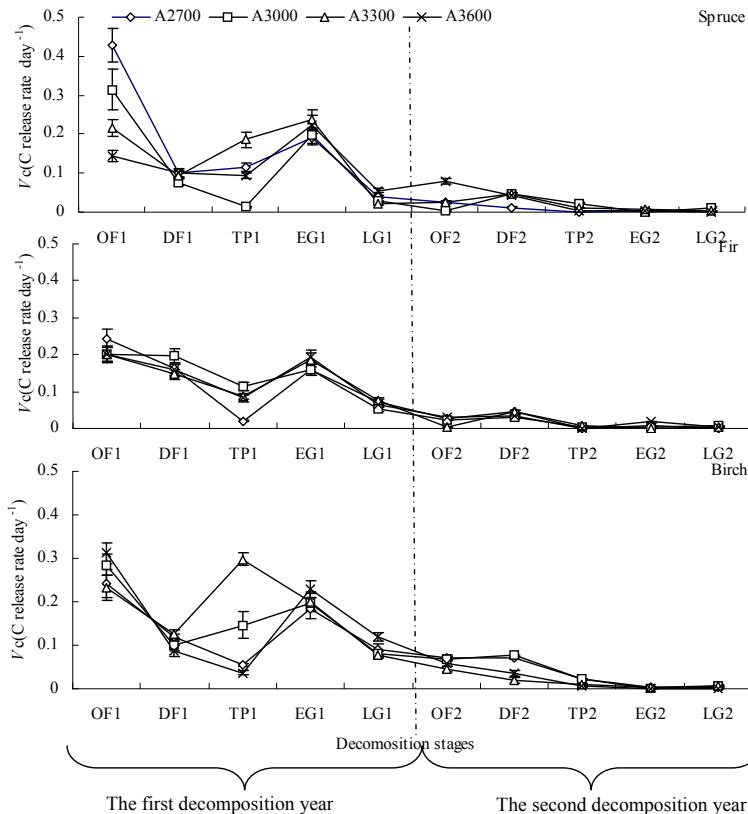


Figure 4. Carbon release rate per day (V_c) for foliar litter of the three species investigated at different decomposition stages along an altitudinal gradient from 2700 m to 3600 m. Bars indicate SE, $n = 3$.