Effects of Forest Gaps on Litter Lignin and Cellulose Dynamics Vary Seasonally in an Alpine Forest

Han Li 1, Fuzhong Wu 1,2, Wanqin Yang 1,2,*, Liya Xu 1, Xiangyin Ni 1, Jie He 1, Bo Tan 1,2, and Yi Hu 1

1 Long-term Research Station of Alpine Forest Ecosystems, Institute of Ecology and Forestry, Sichuan Agricultural University, Chengdu 611130, China; hannahlisc@163.com (H.L.); wufzchina@163.com (F.W.); liannah@163.com (L.X.); nixiangyin_922@163.com (X.N.); liannah@126.com (J.H.); bobotan1984@163.com (B.T.); 13803692816@163.com(Y.H.) 2 Collaborative Innovation Center of Ecological Security in the Upper Reaches of Yangtze River, Chengdu 611130, China

* Correspondence: scyangwq@163.com; Tel.: +86-288-629-1112

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Abstract: To understand how forest gaps and the associated canopy control litter lignin and cellulose dynamics by redistributing the winter snow coverage and hydrothermal conditions in the growing season, a field litterbag trial was conducted in the alpine Minjiang fir (Abies faxoniana Rehder and E.H. Wilson) forest in a transitional area located in the upper reaches of the Yangtze River and the eastern Tibetan Plateau. Over the first year of litter decomposition, the litter exhibited absolute cellulose loss and absolute lignin accumulation except for the red birch litter. The changes in litter cellulose and lignin were significantly affected by the interactions among gap position, period and species. Litter cellulose exhibited a greater loss in the winter with the highest daily loss rate observed during the snow cover period. Both cellulose and lignin exhibited greater changes under the deep snow cover at the gap center in the winter, but the opposite pattern occurred under the closed canopy in the growing season. The results suggest that decreased snowpack seasonality due to winter warming may limit litter cellulose and lignin degradation in alpine forest ecosystems, which could further inhibit litter decomposition. As a result, the ongoing winter warming and gap vanishing would slow soil carbon sequestration from foliar litter in cold biomes.

Keywords: forest gap; foliar litter; lignin; cellulose; seasonal snowpack

1. Introduction

Foliar litter decomposition plays crucial roles in bioelement cycles, energy transformation, and the maintenance of soil fertility in forest ecosystems [1–4]. As two of the most abundant and resistant components in foliar litter, cellulose and lignin play important roles in litter decomposition [5]. A number of studies have shown that throughout the litter decomposition process, lignin and cellulose changes are affected by freeze-thaw cycling, leaching and wet-dry cycling [6,7]. However, little information is available on how forest gaps and the associated hydrothermal dynamics on the forest floor influence lignin and cellulose changes in foliar litter.

Forest gap formation caused by uprooting, trunk breakage, felling, and dead standing trees is the basic mechanism of forest regeneration and succession [8,9]. The formation of forest gaps not only manipulates the regeneration of trees and plant diversity [9,10] but also determines the hydrothermal dynamics on the forest floor and the community and activity of soil organisms by redistributing light and precipitation [11,12]. In cold biomes, forest gaps and the surrounding canopy can distribute the snowpack in the winter and regulate the temperature, precipitation and solar radiation during the
growing season [13]. Consequently, forest gap formation regulates the changes in lignin and cellulose during foliar litter decomposition as well as other critical litter decomposition processes. Although previous studies have mainly been concerned with the effects of gap size on litter decomposition in various forest ecosystems [14–16], few studies have focused on the effects of gap positions on litter cellulose and lignin changes which vary seasonally in alpine forest ecosystems.

Due to spatial and temporal differences in degradability and the degradation process, a variety of mechanisms can induce significant differences in cellulose and lignin changes inside and outside gap edges at critical periods over one year of decomposition. First, in the snow cover period, the deep snow patches located in the gap center can provide stable moisture-temperature conditions [17,18], which are favorable for litter cellulose and lignin degradation by decomposers. In contrast, during snowmelt, thicker snow cover leads to acute leaching, which results in increases in cellulose and lignin contents due to the absolute losses of liable components from litter. However, thinner snow patches under a closed canopy undergo more frequent freeze-thaw cycles, and the litter at these sites is subjected to greater physical damage, which favors lignin decomposition [19]. Second, with respect to lignin photodegradation due to increased solar radiation [20], lignin loss at the gap center may be greater during the growing season, whereas a closed canopy can provide a more stable microenvironment of abundant rainfall and high temperature for decomposers, which is beneficial to cellulose and lignin loss [5] because the canopy cover excludes light and reduces the associated evaporation from the forest floor. Third, because a high lignin content has a rate-reducing influence on litter decomposition [21,22], the initial foliar litter quality may influence changes in cellulose and lignin. Finally, litter quality changes continuously over time, resulting in different chemical components that exhibit different levels of cellulose and lignin changes [23]. Previous studies have focused mainly on the mass loss, nutrient release and microbial biomass associated with winter litter decomposition in alpine forests [17,18,24–26]. However, little information is available on how cellulose and lignin changes are influenced by ongoing climate change through changing snowfall patterns [27,28]. Therefore, based on previous studies, we hypothesized that (1) both litter cellulose and lignin will exhibit more drastic changes under the stable microenvironment resulting from deep snow cover at the gap center during the winter and under suitable hydrothermal conditions under a closed canopy throughout the growing season; and (2) both litter cellulose and lignin changes will fluctuate more extensively in the winter than in the growing season.

To test these hypotheses, a field decomposition experiment was conducted in an alpine forest located along the upper reaches of the Yangtze River and the eastern Tibetan plateau to measure lignin and cellulose changes. This type of forest plays an important role in conserving the soil, maintaining headwater quality, sequestering carbon and nursing biodiversity [29]. Forest gaps and the associated crown canopy generate strong winter snowpack gradients from the gap center to the closed canopy, influence the frequency of freeze-thaw cycles in the winter, and redistribute temperature, precipitation and solar radiation during the growing season [17,18]. Remaining mass, nutrient release, and microbial biomass during the process of litter decomposition have been widely investigated in alpine forests [17–19,24,30], and the results have shown that forest gaps allow the accumulation of deep snow patches that significantly promote litter decomposition during the winter. Based on previous investigations, we measured the concentrations of litter cellulose and lignin at critical periods as well as snow coverage and temperature dynamics during a one-year decomposition period, at sites from the gap center to the closed canopy in three selected forest gaps. Our experimental test was conducted (1) to characterize the foliar litter cellulose and lignin changes in response to forest gaps in the winter and the growing season and (2) to explore the potential responses of litter cellulose and lignin changes to decreased snowpack seasonality owing to future winter warming or gap vanishing as forests are regenerated in this high-altitude forest ecosystem.
2. Materials and Methods

2.1. Site Description

The 18,000-ha research forest is in the Miyaluo Nature Reserve (102°53′–102°57′ E, 31°14′–31°19′ N, 2458–4619 m a.s.l.), which is located in Li County, Sichuan Province, southwestern China. The reserve is a transitional area located along the upper reaches of the Yangtze River and the eastern Tibetan Plateau [30]. The mean annual temperature at the study site is 2.7 °C, with mean temperatures of −8.7 °C and 9.5 °C in January and July, respectively. The mean annual precipitation is 850 mm. The freeze-thaw season typically extends from late October to late April, with a maximum snow depth of approximately 50 cm and a freeze-thaw cycle time of approximately 120 days [24]. The standing mass of the research forest is 337.31 t/ha, which is comprised mostly of Minjiang fir (Abies faxoniana Rehder and E.H. Wilson) (80% of the basal area) with smaller amounts of cypress (Sabina saltuaria (Rehder and E.H. Wilson) W.C. Cheng and W.T. Wang) (20%), red birch (Betula albosinensis Burk.) (2%–5%) and larch (Larix mastersiana Rehder and E.H. Wilson) (2%–5%). The understory shrubs are dominated by species such as Salix paraplesia C.K. Schneid., Rhododendron lapponicum (L.) Wahlenb., Berberis sargentiana C.K. Schneid., Sorbus rufopilosa C.K. Schneid. and Hippophae rhamnoides L. The herbs are dominated by Parasenecio forestii W.W. Sm. & Small., Cystopteris fragilis (L.) Bernh. and various mosses [31]. The Soil type is a cambisol; the average total C, N, and P concentrations and pH are 150.3 g/kg, 9.7 g/kg, 1.2 g/kg, and 6.1 (humus layer) and 45.2 g/kg, 1.9 g/kg, 0.7 g/kg, and 5.7 (mineral layer), respectively.

2.2. Experimental Design

Foliar litter decomposition was studied using the nylon mesh bag technique [32]. Based on previous studies, we collected freshly senesced leaves of cypress, Minjiang fir, larch and red birch from the floor of the sample site in early October 2012 [33]. Leaves that were broken into pieces and had turned dark in color were excluded. Air-dried litter samples of 200 g/m² (10 g in each bag) were placed in prepared nylon bags (20 cm × 25 cm with mesh sizes of 1.0 mm (above) and 0.5 mm (below)) to ensure that the litter was sufficiently exposed to the air, water and microorganisms.

Based on our previous field investigation and previously collected local data, three forest gaps, measuring at least 25 × 25 m each and with similar canopy densities were randomly selected within a representative Minjiang fir forest (31°14′ N, 102°53′ E, 3579–3582 m) in the nature reserve. In each gap, 4 positions measuring 4 × 4 m each were established from the gap center to the closed canopy (gap center south, gap edge, expanded gap and closed canopy) along a downwind direction at 3–4 m intervals to ensure adequate sampling of the heterogeneous microenvironmental conditions (Figure 1). There were 144 litterbags of each species to allow four collections of three bags of each species from each of the twelve sites (four positions in each forest gap). These litterbags were placed on the forest floor from the gap center to the closed canopy at approximately the same time on 15 November 2012. The temperature in the litterbags was measured every 2 h using iButton recorders (iButton DS1923-F5, Maxim/Dallas Semiconductor, Sunnyvale, CA, USA). The recorders were placed in two litterbags at each gap position. The air temperature and the temperature in the litterbags were measured from 15 November 2012 to 30 October 2013, and the following measures were calculated to clearly describe the temperature characteristics: daily mean temperature, positive accumulated temperature (sum of temperatures above 0 °C), negative accumulated temperature (sum of temperatures below 0 °C) [17,18], and the number of freeze-thaw cycles per day (one freeze-thaw cycle was completed when the threshold of 0 °C was crossed twice within at least 3 h) [34] of each critical period (Table 1).
To quantify the cellulose and lignin concentrations in the litter at different critical periods, which were identified based on Olsson’s period division of the cold season [35] and the field investigation of our previous studies [17,18,36,37], we sampled the litterbags at the end of each critical period over the first year of litter decomposition on 26 December 2012 (snow formation period, SFP), 8 March 2013 (snow cover period, SCP), 24 April 2013 (snow melt period, SMP), and 30 October 2013 (growing season, GS). The litterbags were transported back to the laboratory for analysis. Additionally, as the depth of the snow cover could not be monitored in real-time due to low temperature and the lack of electrical power in the mountains, it was measured at multiple points (3 points at each gap position in each forest gap) using a ruler in situ when we uploaded the temperature data recorded by the iButton recorders, which had limited memory (Figure 2). Four of the eight dates were our litter sampling dates, which means that all the snow depths measured were representative of the snow coverage conditions at each critical period of the first year of litter decomposition.

**Table 1.** Characteristics of the daily mean temperature (DMT, °C), positive accumulated temperature (PAT, °C), negative accumulated temperature (NAT, °C) and the number of freeze-thaw-cycles per day (FTC, times) across each period of air and the four gap positions (gap center, gap edge, expanded gap, closed gap) over the first year of litter decomposition in an alpine Minjiang fir forest.

<table>
<thead>
<tr>
<th>Position</th>
<th>Temperature Characteristics</th>
<th>Snow Formation Period</th>
<th>Snow Cover Period</th>
<th>Snow Melt Period</th>
<th>Winter</th>
<th>Growing Season</th>
<th>1st Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>DMT</td>
<td>−4.11</td>
<td>−4.46</td>
<td>1.43</td>
<td>−2.38</td>
<td>7.46</td>
<td>2.54</td>
</tr>
<tr>
<td></td>
<td>PAT</td>
<td>1</td>
<td>30</td>
<td>82</td>
<td>113</td>
<td>1417</td>
<td>1530</td>
</tr>
<tr>
<td></td>
<td>NAT</td>
<td>−166</td>
<td>−351</td>
<td>−15</td>
<td>−532</td>
<td>−7</td>
<td>−539</td>
</tr>
<tr>
<td></td>
<td>FTC</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Gap center</td>
<td>DMT</td>
<td>−3.55</td>
<td>−3.44</td>
<td>1.88</td>
<td>−1.70</td>
<td>8.90</td>
<td>3.60</td>
</tr>
<tr>
<td></td>
<td>PAT</td>
<td>0</td>
<td>14</td>
<td>91</td>
<td>106</td>
<td>1683</td>
<td>1789</td>
</tr>
<tr>
<td></td>
<td>NAT</td>
<td>−142</td>
<td>−262</td>
<td>−3</td>
<td>−407</td>
<td>0</td>
<td>−408</td>
</tr>
<tr>
<td></td>
<td>FTC</td>
<td>0.45</td>
<td>0.41</td>
<td>0.12</td>
<td>0.44</td>
<td>0.08</td>
<td>0.26</td>
</tr>
<tr>
<td>Gap edge</td>
<td>DMT</td>
<td>−2.88</td>
<td>−3.14</td>
<td>0.93</td>
<td>−1.70</td>
<td>7.66</td>
<td>2.98</td>
</tr>
<tr>
<td></td>
<td>PAT</td>
<td>1</td>
<td>24</td>
<td>53</td>
<td>79</td>
<td>1451</td>
<td>1529</td>
</tr>
<tr>
<td></td>
<td>NAT</td>
<td>−116</td>
<td>−250</td>
<td>−9</td>
<td>−375</td>
<td>−2</td>
<td>−378</td>
</tr>
<tr>
<td></td>
<td>FTC</td>
<td>0.59</td>
<td>0.61</td>
<td>0.09</td>
<td>0.49</td>
<td>0.10</td>
<td>0.29</td>
</tr>
<tr>
<td>Expanded gap</td>
<td>DMT</td>
<td>−2.91</td>
<td>−4.20</td>
<td>1.94</td>
<td>−1.73</td>
<td>7.87</td>
<td>3.03</td>
</tr>
<tr>
<td></td>
<td>PAT</td>
<td>0</td>
<td>17</td>
<td>93</td>
<td>111</td>
<td>1493</td>
<td>1604</td>
</tr>
<tr>
<td></td>
<td>NAT</td>
<td>−117</td>
<td>−320</td>
<td>−2</td>
<td>−439</td>
<td>−5</td>
<td>−444</td>
</tr>
<tr>
<td></td>
<td>FTC</td>
<td>0.52</td>
<td>0.53</td>
<td>0.21</td>
<td>0.53</td>
<td>0.10</td>
<td>0.31</td>
</tr>
<tr>
<td>Closed canopy</td>
<td>DMT</td>
<td>−3.87</td>
<td>−4.05</td>
<td>1.51</td>
<td>−2.14</td>
<td>7.70</td>
<td>2.78</td>
</tr>
<tr>
<td></td>
<td>PAT</td>
<td>0</td>
<td>24</td>
<td>79</td>
<td>104</td>
<td>1458</td>
<td>1561</td>
</tr>
<tr>
<td></td>
<td>NAT</td>
<td>−155</td>
<td>−316</td>
<td>−8</td>
<td>−479</td>
<td>−3</td>
<td>−482</td>
</tr>
<tr>
<td></td>
<td>FTC</td>
<td>0.63</td>
<td>0.59</td>
<td>0.28</td>
<td>0.56</td>
<td>0.04</td>
<td>0.30</td>
</tr>
</tbody>
</table>
2.3. Analyses and Calculations

The initial organic carbon (C), total nitrogen (N) and total phosphorus (P) concentrations were determined using the methods of dichromate oxidation, Kjeldahl determination (KDN, Top Ltd., Zhejiang, China) and phosphomolybdenum yellow spectrophotometry (TU-1901, Puxi Ltd., Beijing, China), respectively [38] (Table 2). After the roots and foreign materials were separated, the remaining litter was removed from the litterbags. The samples were oven-dried at 65 °C for at least 48 h to constant weight and then very finely ground, using a mill to allow passage through a 0.25-mm mesh. The cellulose and lignin concentrations in the foliar litter were measured using the Van Soest method [39]. This method yields cellulose and acid-detergent lignin, which has recently been referred to as AUR (Acid Unhydrolyzable Residue) [40,41], by calculating the weight remaining after washing the litter with different reagents. All of the analyses were carried out in triplicate. The litter mass loss (%) [42], and cellulose and lignin absolute change percentage (%) and daily rate (mg/day) were calculated as follows:

\[
\text{Mass loss (\%)} = \frac{(M_0 - M_t)}{M_0} \times 100
\]

\[
\text{Absolute change percentage (\%)} = \frac{[(C_{t-1} \times M_{t-1}) - (C_t \times M_t)]/(C_0 \times M_0) \times 100}{\text{Absolute change daily rate (mg/day)}} = \frac{[(C_{t-1} \times M_{t-1}) - (C_t \times M_t)]}{D_t}
\]

where \(M_t\) and \(M_{t-1}\) represent the remaining dry mass of litter on the current and previous sampling date, respectively; \(C_t\) and \(C_{t-1}\) represent the concentration (%) of cellulose or lignin on the current and previous sampling date, respectively; \(M_0\) is the initial litter dry mass; \(C_0\) is the initial concentration of cellulose or lignin; and \(D_t\) is the number of days between the current and previous sampling dates.

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**Figure 2.** Snow depth (cm) on the four gap positions (gap center, gap edge, expanded gap, closed canopy) during the first year of litter decomposition in an alpine Minjiang fir forest. * indicate there are significant differences between different gap positions, \(p < 0.05\) (mean ± SE, n = 3).
Table 2. Initial quality of the four types of foliar litter (cypress, Minjiang fir, larch, red birch) in an alpine Minjiang fir forest (mean ± SD, n = 3).

<table>
<thead>
<tr>
<th>Species Name</th>
<th>C (%) ± SD</th>
<th>N (%) ± SD</th>
<th>P (%) ± SD</th>
<th>Lignin (%) ± SD</th>
<th>Cellulose (%) ± SD</th>
<th>C/N ± SD</th>
<th>C/P ± SD</th>
<th>N/P ± SD</th>
<th>Lignin/N ± SD</th>
<th>Lignin/Cellulose ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cypress</td>
<td>51.64 ± 1.77&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.88 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.12 ± 0.01&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>14.07 ± 0.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.22 ± 0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.86 ± 2.21&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>416.02 ± 14.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.08 ± 0.41&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>16.04 ± 1.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.15 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Minjiang Fir</td>
<td>50.56 ± 2.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.88 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.11 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.85 ± 0.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.19 ± 0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>57.77 ± 3.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>443.51 ± 36.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.68 ± 0.72&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.11 ± 0.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.30 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Larch</td>
<td>54.35 ± 0.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.86 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.13 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>27.21 ± 2.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.45 ± 0.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>63.32 ± 3.49&lt;sup&gt;c&lt;/sup&gt;</td>
<td>407.08 ± 2.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.44 ± 0.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.95 ± 1.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.36 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Red birch</td>
<td>49.69 ± 1.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.33 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.09 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.68 ± 0.62&lt;sup&gt;d&lt;/sup&gt;</td>
<td>12.47 ± 0.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37.24 ± 1.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>544.94 ± 31.72&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.63 ± 0.36&lt;sup&gt;c&lt;/sup&gt;</td>
<td>25.99 ± 0.37&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.78 ± 0.13&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Different lowercase letters indicate a significant difference between species for the same variable (one-way ANOVA, p < 0.05).

Table 3. Multivariate analysis of variance (MANOVA) for the effects of period, gap position and species on litter cellulose and lignin absolute change daily rates over the first year of litter decomposition in an alpine Minjiang fir forest. df, degree of freedom.

<table>
<thead>
<tr>
<th>Source Variance</th>
<th>df</th>
<th>Cellulose F-Value</th>
<th>Cellulose P-Value</th>
<th>Lignin F-Value</th>
<th>Lignin P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period (P)</td>
<td>3</td>
<td>70.520</td>
<td>&lt;0.001 **</td>
<td>53.771</td>
<td>&lt;0.001 **</td>
</tr>
<tr>
<td>Gap position (G)</td>
<td>3</td>
<td>3.410</td>
<td>0.020 *</td>
<td>4.905</td>
<td>0.003 **</td>
</tr>
<tr>
<td>Species (S)</td>
<td>3</td>
<td>8.838</td>
<td>&lt;0.001 **</td>
<td>41.719</td>
<td>&lt;0.001 **</td>
</tr>
<tr>
<td>P x G</td>
<td>9</td>
<td>10.741</td>
<td>&lt;0.001 **</td>
<td>7.727</td>
<td>&lt;0.001 **</td>
</tr>
<tr>
<td>P x S</td>
<td>9</td>
<td>9.551</td>
<td>&lt;0.001 **</td>
<td>18.847</td>
<td>&lt;0.001 **</td>
</tr>
<tr>
<td>G x S</td>
<td>9</td>
<td>0.762</td>
<td>0.651</td>
<td>3.651</td>
<td>0.001 **</td>
</tr>
<tr>
<td>P x G x S</td>
<td>27</td>
<td>3.918</td>
<td>&lt;0.001 **</td>
<td>23.413</td>
<td>&lt;0.001 **</td>
</tr>
</tbody>
</table>

Significant effects: * p < 0.05; ** p < 0.01, n = 48.

Table 4. Dominant factors and coefficients of determination (R²) for step-wise regression analysis of litter cellulose and lignin absolute change percentages affected by environmental factors (DMT, daily mean temperature; PAT, positive accumulated temperature; NAT, negative accumulated temperature; FTC, frequency of freeze-thaw cycles per day) and initial litter quality (initial C, N, P, cellulose, lignin, C/N, C/P, N/P, lignin/N, lignin/cellulose) for each decomposition period over the first year of litter decomposition in an alpine Minjiang fir forest.

<table>
<thead>
<tr>
<th>Dominant Factors</th>
<th>Snow Formation Period</th>
<th>Snow Cover Period</th>
<th>Snow Melt Period</th>
<th>All of Winter</th>
<th>Growing Season</th>
<th>1st Year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cellulose</td>
<td>Lignin</td>
<td>Cellulose</td>
<td>Lignin</td>
<td>Cellulose</td>
<td>Lignin</td>
</tr>
<tr>
<td>1</td>
<td>DMT (0.139)</td>
<td>Initial Lignin/N (0.265)</td>
<td>Initial Lignin/N (0.270)</td>
<td>Initial Cellulose (0.289)</td>
<td>Initial Lignin/Celulose (0.227)</td>
<td>Initial Cellulose (0.773)</td>
</tr>
<tr>
<td>2</td>
<td>DMT (0.410)</td>
<td>NAT (0.411)</td>
<td>DMT (0.425)</td>
<td>Initial N (0.824)</td>
<td>Initial Lignin/N (0.674)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>FTC (0.544)</td>
<td>Initial N (0.487)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Initial P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significant effects: * p < 0.05; ** p < 0.01, n = 48.
2.4. Statistical Analyses

One-way ANOVA (analysis of variance) and LSD (least significant difference) tests at the 0.05 level were used to evaluated differences between the gap positions with respect to the initial substrate quality, litter mass losses, cellulose and lignin concentrations, and the absolute change percentage of cellulose and lignin during the decomposition of the four types of foliar litter, as well as the absolute change daily rates of cellulose and lignin between different periods. The effects of gap position, species and period on the absolute change daily rates of cellulose and lignin were analyzed using multivariate analysis of variance (MANOVA) (Table 3). The influence of environmental factors (daily mean temperature, DMT; positive accumulated temperature, PAT; negative accumulated temperature, NAT; the number of freeze-thaw cycles per day, FTC) and initial quality (initial C, initial N, initial P, initial Cellulose, initial Lignin, initial C/N, initial C/P, initial N/P, initial Lignin/N, initial lignin/cellulose) on the cellulose and lignin absolute change percentages at each critical period were screened through step-wise (backward) regression analysis [36] (Table 4). All of the statistical analyses were performed using the SPSS (Standard release version 19.0 for Windows, SPSS Inc., Chicago, IL, USA) software package, and the data for all of the tests were normally distributed.

3. Results

3.1. Snow Depth and Temperature

Snow thickness decreased in the following order throughout the freeze-thaw season: gap center > gap edge > expanded gap > closed canopy (Figure 2). In the winter, DMT was higher at the gap center and gap edge than at the expanded gap and under the closed canopy. FTC was highest under the closed canopy and lowest at the gap center. In the growing season, compared with the other gap positions, DMT was significantly higher at the gap center compared with the other gap positions. Among the four gap positions, FTC was lowest at the gap center during the snow cover and formation periods, and it reached its maximum value during the snow melt period (Table 1).

3.2. Cellulose and Lignin Concentrations

In the four species studied, the litter cellulose concentration tended to decrease over the winter and then significantly increase by the end of the growing season with the exception of red birch, for which the cellulose concentration continued to decrease (Figure 3). After the snow formation period, there were no significant differences in the cellulose concentration among the different gap positions for cypress litter, whereas higher concentrations were found in larch, Minjiang fir and red birch litter at the gap center and the expanded gap. All four types of foliar litter exhibited the highest cellulose concentration at the gap center and the lowest concentration at the gap edge at the end of the snow cover period. In all four types of foliar litter, the cellulose concentrations were higher at the expanded gap and under the closed canopy than at the gap center and the gap edge after snow melt. Cypress and larch litter had higher cellulose concentrations at the gap center at the end of the growing season, whereas Minjiang fir and red birch litter had lower concentrations at the gap center at this time.

In contrast, the lignin concentrations in the four types of foliar litter increased after the first year of litter decomposition regardless of gap position. After the entire year of decomposition, the lignin concentrations of the cypress, Minjiang fir and larch litter were approximately twice the concentrations in fresh litter, while a relatively lower increase in red birch litter lignin concentration was observed at the end of each critical period (Figure 3). The gap edge exhibited a higher lignin concentration than the gap center and closed canopy after snow formation, and the gap edge and expanded gap exhibited higher lignin concentrations than did the other positions at the end of the snow cover period. Sharp increases in the lignin concentrations of the foliar litter were observed at the end of the growing season, and all four types of foliar litter had the lowest concentrations at the gap center except for Minjiang fir, which showed no significant differences in lignin concentration among gap positions.
3.3. Cellulose and Lignin Absolute Changes

As shown in Table 3, the absolute change daily rates of both cellulose and lignin in litter were significantly affected by period, gap position and species, as the interaction effects were pronounced. Over the first year of litter decomposition, the litter exhibited mass losses ranging from 18.73% to 33.62%, depending on species (Figure 4). The absolute loss percentage of litter cellulose ranged from 10.83% to 58.78% depending on species. In addition, lignin accumulated at an absolute percentage of 19.94% to 63.20% in cypress, Minjiang fir and larch foliar litter, whereas a small absolute loss of lignin, 6.94%, was detected in red birch litter after the first year of decomposition (Figure 5). Overall, litter cellulose exhibited greater absolute losses during the winter, especially at the snow cover period, when the daily loss rate was high relative to that of other periods (Figure 6). Cypress, Minjiang fir and larch litter all had obvious levels of absolute lignin accumulation over the year except for cypress in the growing season, in which a decrease in absolute lignin loss from the gap center to the closed canopy was observed. The lignin in red birch litter exhibited slight absolute losses throughout the entire winter, with higher loss rates at the snow formation period, but exhibited a marked absolute accumulation in the growing season. As shown in Table 4, the litter quality appeared to exert stronger effect than the temperature factors on the absolute changes in both cellulose and lignin.

![Figure 3](image-url)

**Figure 3.** Cellulose and lignin concentrations (%) in the four types of foliar litter (cypress, Minjiang fir, larch, red birch) in the four gap positions (gap center, gap edge, expanded gap, closed canopy) at each sampling time (the end of each critical period) over the first year of litter decomposition in an alpine Minjiang fir forest. Different lowercase letters indicate significant differences among gap positions for the same sampling date, *p* < 0.05 (mean ± SE, *n* = 3).
At the snow formation period, there were no significant differences among gap positions in the absolute change percentages of cellulose or lignin for cypress and red birch, and lignin for Minjiang fir. In this latter species, cellulose accumulation was observed at the gap center in contrast to the other gap positions. Over the winter, the litter exhibited both higher absolute cellulose losses and absolute lignin accumulation at the gap center and the gap edge compared with the expanded gap and closed canopy. An exception was found for the absolute loss percentage of lignin in red birch litter, which was highest under the closed canopy. In contrast, litter cellulose and lignin exhibited higher absolute change percentages and daily rates under the closed canopy compared with the other gap positions; however, litter cellulose in Minjiang fir and red birch litter accumulated to a greater extent at the gap edge, and litter lignin had a higher absolute loss percentage at the gap center.

**Figure 4.** Litter mass loss (%) for the four types of foliar litter (cypress, Minjiang fir, larch, red birch) in the four gap positions (gap center, gap edge, expanded gap, closed canopy) for each decomposition period (SFP, snow formation period; SCP, snow cover period; SMP, snow melt period; Winter, all of winter; GS, growing season; 1st Year, the first year of litter decomposition) over the first year of litter decomposition in an alpine Minjiang fir forest. Different lowercase letters indicate significant differences among gap positions for the same decomposition period, $p < 0.05$ (mean $\pm$ SE, $n = 3$).
Figure 5. Cellulose and lignin absolute change percentages (%) for the four types of foliar litter (cypress, Minjiang fir, larch, red birch) in the four gap positions (gap center, gap edge, expanded gap, closed canopy) for each decomposition period (SFP, snow formation period; SCP, snow cover period; SMP, snow melt period; Winter, all of winter; GS, growing season; 1st Year, the first year of litter decomposition) over the first year of litter decomposition in an alpine Minjiang fir forest. Different lowercase letters indicate significant differences among gap positions for the same degradation period, $p < 0.05$ (mean ± SE, n = 3).
Figure 6. Cellulose and lignin absolute change daily rates (mg/day) for the four types of foliar litter (cypress, Minjiang fir, larch, red birch) in the four gap positions (gap center, gap edge, expanded gap, closed canopy) for each decomposition period (SFP, snow formation period; SCP, snow cover period; SMP, snow melt period; GS, growing season) over the first year of litter decomposition in an alpine Minjiang fir forest. Different lowercase letters indicate significant differences among periods, \( p < 0.05 \) (mean ± SE, \( n = 3 \)).
3.4. Ratio between Lignin and Cellulose

After the first year of litter decomposition, the ratio of the lignin to the cellulose concentration (lignin/cellulose) increased significantly in all of the types of foliar litter except for the larch litter (which increased by 2.67) (Figure 7). The lignin/cellulose ratio increased throughout the entire year for all four litter types except cypress litter, in which lignin/cellulose ratio decreased slightly at the snow melt period and in the growing season. At the snow formation period, a higher lignin/cellulose ratio was observed in the gap edge than in the other gap positions for all types of foliar litter, which is consistent with the observation from the snow cover period. However, the lowest lignin/cellulose ratios in litter were observed under the closed canopy at the snow melt period.

![Figure 7](image_url)  
*Figure 7. The lignin/cellulose concentration ratio in the four types of foliar litter (cypress, fir, larch, red birch) in the four gap positions (gap center, gap edge, expanded gap, closed canopy) at each sampling date over the first year of litter decomposition in an alpine Minjiang fir forest. Different lowercase letters indicate significant differences among gap positions within the same sampling time, p < 0.05 (mean ± SE, n = 3).*

4. Discussion

Our first hypothesis was well supported in the present study. We observed that both the cellulose and lignin exhibited more drastic changes under the stable microenvironment provided by deep snow cover at the gap center during the winter and under the suitable environmental conditions formed under the closed canopy throughout the growing season. Our results revealed that changes in both lignin and cellulose were significantly affected by gap position, species and period as well as their interactions over the first year of litter decomposition.
As recalcitrant materials, cellulose and lignin degrade slowly compared with the release of nutrients that occurs shortly after foliar litter is shed \[14,29,43\]. This slow degradation of cellulose and lignin plays a major role in regulating the rate of litter decomposition \[44\]. The mechanisms of cellulose and lignin degradation differ due to differences in the degradability of these components \[5\]. One well-known difference is that lignin exhibits accumulates following leaf fall, whereas cellulose may either immediately decrease or accumulate before subsequently decreasing \[5\]; the results of the present study are consistent with this observation. However, the acid detergent lignin we obtained using the Van Soest method has recently been referred as AUR and is composed of both lignin and other acid resistant compounds that are created by microbes during litter decomposition \[37,38\]. Therefore, the absolute increase in lignin observed in the present study might be partially or wholly due to the accumulation of both lignin and lignin-like compounds originating from microbial products. Fresh litter in the autumn \[45,46\] is characterized by abundant available nutrients, resulting in the rapid loss of soluble and low-molecular-weight compounds \[47,48\]. This process accounts for the observed increases in cellulose and lignin concentrations at the snow formation period in our study. Our finding that litter cellulose showed a slight absolute accumulation in Minjiang fir and larch litter at the snow formation period is consistent with the observation that a slight decrease in the cellulose level is not observed until after approximately one year \[49\]. In general, lignin loss begins later due to the accumulation of lignin-like compounds accumulation and the low degradability of lignin \[50\]; however, both red birch (36.68%) and larch (27.21%) litter, which exhibited higher initial lignin concentrations than the other species, exhibited absolute losses of lignin at the snow formation period. These results support the hypothesis that lignin loss in the early decomposition period occurs only in litter that has a high initial lignin content (>30%) \[51\]. As the temperature decreases and the a thin layer of snow cover forms, the differences among snow cover microenvironments generated by the forest gaps become insignificant \[17,18\]. As a result, neither cellulose nor lignin degradation was influenced by position relative to the forest gap.

All four litter types showed higher absolute daily loss rates of cellulose at the snow cover period compared with the other periods due to the favorable microenvironment underneath the snow cover generated by the accumulated snowfall. This result supports previous findings, demonstrating that snow cover can promote the loss of litter cellulose \[19,26\]. Cellulose is degraded by extracellular enzymes secreted by numerous species of bacteria and fungi \[5\]. Therefore, the warmer conditions resulting from the deep snow coverage, which are capable of maintaining high microbial diversity and activity \[52\], were favorable for litter biodegradation. Another potential explanation for the higher absolute daily loss rate of cellulose observed at the snow cover period involves the domination of litter decomposition by cellulose degradation after the loss of soluble and low-molecular-weight compounds during the first period of litter decay \[53\]. Previous studies have shown that deep snow coverage can protect organisms from cold temperatures, promoting litter decomposition \[19,54,55\]. Therefore, the gap center and gap edge likely exhibited higher absolute losses of cellulose at the snow cover period due to decreases in the abundance and activity of decomposers as snow cover decreased from the gap center to the closed canopy \[56\]. Red birch litter, which exhibited the highest initial lignin concentration, was the first litter type to show an absolute lignin loss. Lignin physically protects most of the surrounding cellulose and hemicellulose from enzymatic hydrolysis \[5\], and lignified cellulose and hemicellulose as well as lignin are sufficiently interwoven in the fiber structure such that they are incapable of undergoing degradation separately \[5\]; therefore, the lignin in red birch litter may degrade earlier than in the other litter types.

At the snow melt period, rising temperature as well as intense leaching and frequent freeze-thaw cycling due to the melting snow play important roles in litter decomposition \[19,31\]. Increased moisture results from the snow melting, which is beneficial for microbial activity \[57\], together with the mechanical disruption and intense leaching caused by frequent freeze-thaw cycling. These processes lead to absolute losses of cellulose and lignin through biodegradation and physical destruction \[58\]. Nevertheless, neither cellulose nor lignin absolute changes exhibited a consistent trend in the four types
of foliar litter. Both the significant effect of species and the relationship between the cellulose and lignin absolute changes and their initial quality may explain this result. It has been reported that lignin can be degraded into short polymers, such as cellulose and condensed tannins, during the process of litter decomposition [5]. The close relationship between the cellulose absolute changes and the initial lignin concentration observed in the step-wise regression also provides a possible explanation for the absolute increase in cellulose observed in cypress, Minjiang fir and larch litter. Additionally, a higher cellulose absolute loss was observed in the gap center than in the other gap positions for all four litter types, and the gap center experienced the greatest number of freeze-thaw cycles, which supports previous findings that cellulose degradation is improved by frequent freeze-thaw cycles through the repeated damage caused to cellulose structures [17]. Conversely, lignin showed an absolute accumulation in cypress, Minjiang fir and larch litter, which can be attributed to the increase in lignin-like compounds demonstrated in previous studies [59]. This increase in lignin-like compounds may be the result of the absolute accumulation of newly formed substances combined with microbial products and imported nutrients [5].

In the growing season, which is characterized by warmer temperature, litter cellulose and lignin should degrade more rapidly due to the more stable conditions, which are beneficial for the recovery of decomposer abundance. The forest gap center receives greater sunlight exposure and precipitation but also experiences more rapid evaporation compared with than do the other gap positions, which can adversely affect cellulose degradation [60]. The more suitable temperature and more favorable balance between precipitation and evaporation under the closed canopy and in the expanded gap could contribute to cellulose absolute loss [17]. In addition, due to the lack of deep snow coverage under the closed canopy and in the expanded gap during the winter, litter decomposition is likely promoted by strong freezing and thawing, suggested by our finding that all four types of foliar litter exhibited greater cellulose absolute loss under the closed canopy and in the expanded gap. However, lignin absolute loss in cypress litter was highest at the gap center, which might have been caused by greater lignin photodegradation due to increased solar radiation [20].

Our second hypothesis, i.e., that cellulose and lignin changes will be greater in the winter than in the growing season was well supported. First, due to the uniqueness of the alpine ecosystem, microbial activity remained high in the winter [24,25]. Furthermore, the step-wise regression showed that the cellulose and lignin absolute changes were closely related to the initial N, which is associated with microbial activity. As a result, litter cellulose and lignin exhibited more dramatic changes in the winter. Second, over the first year of litter decomposition, the winter period was assumed to be the earlier stage of decomposition, whereas the growing season was assumed to be the later stage. These assumptions were based on a previous finding that litter decomposition occurred at a much higher rate during the early stage of decomposition [5], leading to more dynamic processes of cellulose and lignin degradation in the winter compared with later, in the growing season. In addition, cellulose and lignin absolute changes were strongly influenced by the initial litter quality, which indicated that the processes of cellulose and lignin degradation were not consistent among species due to differences in the initial substrate.

5. Conclusions

The changes in litter cellulose and lignin were significantly affected by the interactions between and among gap position, period and species. During the first year of decomposition of foliar litter from four species in an alpine forest, cellulose loss and lignin accumulation was observed for all species except red birch. The changes in both litter cellulose and litter lignin were greater in the winter. Litter cellulose exhibited a greater loss in the winter, with the highest daily loss rate observed at the snow cover period. Both cellulose and lignin exhibited more extensive changes under the deep snow cover at the gap center in the winter, but the opposite pattern occurred under the closed canopy in the growing season. Overall, our results provide insight into the influence of forest gaps on the degradation processes of litter cellulose and lignin in an alpine forest ecosystem. Forest gaps in
the alpine forest appear to accelerate cellulose and lignin degradation during the winter. Decreased snowpack seasonality due to winter warming might limit litter cellulose and lignin degradation in alpine forest ecosystems, which could further inhibit litter decomposition. Consequently, the ongoing winter warming and gap vanishing would slow soil carbon sequestration from foliar litter in cold biomes.

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