Effects of clear-cutting on decomposition and bleaching of *Camellia japonica* leaf litter in a temperate secondary forest

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Abstract

The effects of clear-cutting on the decomposition of *Camellia japonica* leaf litter were investigated in a temperate secondary forest. We focused on the pattern of occurrence of bleached area on leaf litter and the decomposition of acid-unhydrolyzable residue (AUR), which included lignin and was known as a limiting factor of decomposition. Fallen leaves of *C. japonica* were characterized by the occurrence of bleached portions attributable to colonization of leaf tissues by ligninolytic fungi and decomposition of AUR. The litterbag method was used to follow an 18-month decomposition in a clear-cut (CC) and an adjacent control (CO) plots. Mass remaining of leaf litter was significantly higher and bleached area on the leaf surface was lower in CC plot than in CO plot. AUR content was lower in bleached portion than non-bleached portion. Among explanatory variables in generalized linear model, bleach and accumulated mass loss significantly explained AUR content that was response variable, but plot did not. These results suggested that the clear-cutting affected the rate of extension of ligninolytic fungi responsible for increasing of bleached area but did not affect the activities of fungi to remove AUR from leaf tissues. The reduced mass loss of leaf litter and the suppression of expansion of bleached area in CC plot could be partly due to that the changes in microclimates of the forest floor such as desiccation that would suppress extension of hyphae of ligninolytic fungi.

**Key words:** clear-cut, leaf litter decomposition, bleaching, secondary forest

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I Introduction

Forest management, such as clear-cutting, represents human-caused disturbance of forest ecosystem (Holzner et al. 1983). Clear-cutting often alters the decomposition of leaf litter due to changes in micro-environmental conditions of the forest floor and in the activity of decomposer organisms. Effects of clear-cutting on leaf litter decomposition have been examined in a range of deciduous and evergreen broad-leaved forests and coniferous forests. Some studies showed slower decomposition in clear-cut sites than in un-cut sites (Yin et al. 1989, Prescott 1997), whereas others reported either no significant difference (Wallace and Freedman 1986, Prescott et al. 2000) or faster decomposition in clear-cut sites (Klemmedson et al. 1985, Kirmse et al. 1987, Taylor et al. 1991, Ishikawa et al. 2007). The inconsistent effects of clear-cutting on decomposition merit further studies with reference to the factors affecting the decomposition, such as chemical quality of litter and decomposer organisms.

Recalcitrant compounds in leaf litter registered as acid-unhydrolyzable residue (AUR), including condensed tannins, phenolic compounds, and lignin, accounted for 50 to 500 mg g⁻¹ leaf material (Osono 2007) and were known to limit leaf litter decomposition (Osono and Takeda 2001, 2005, Berg and McClaugherty 2003). The effects of clear-cutting on the decomposition of AUR had rarely been examined (Entry et al. 1987, Zhang and Liang 1995, Ishikawa et al. 2007). For example, Zhang and Liang (1995) reported slower decomposition of whole litter and AUR in large gaps (diameter of more than 15 m) than in small gaps (diameter of less than 5 m). In contrast, Ishikawa et al. (2007) found that the contents of recalcitrant compounds in decomposing litter were not significantly different between clear-cut and uncut sites for leaf litter of five tree species. Clearly, more studies are necessary to evaluate the effects of clear-cutting on the decomposition of AUR.

It had been shown previously that the decomposition of AUR often resulted in the occurrence of bleached portions on the surface of decomposing litter (Koide et al. 2005, Osono 2007). Thus the area of bleached portions indicated the area where ligninolytic fungi had colonized. To our knowledge, the effect of clear-cutting on the occurrence of bleached portions on the surface of decomposing leaf litter had been investigated only by Osono et al. (2008). It showed that clear-cutting reduced the bleached area on salal (Gaultheria shallon) leaf litter in clear-cut sites compared to young and old secondary regenerating sites and old growth sites in boreal forest in B.C. Canada. This reduction of bleached area in clear-cut site might be caused by two reasons that were suppression of hyphal growth and ligninolytic enzyme activity. Pietikainen et al. (2005) found that hyphae did not grow in environment where temperature exceed 40°C and Waldrop et al. (2003) showed decreasing of ligninolytic enzyme activity in leaf litter that was set on forest floor where no residual was left because of clear-cutting and following broadcast burning.

Leaf litter of an evergreen tree, Camellia japonica, was chosen as the material in the present study, as bleached portions occur in the early stage of its decomposition due to colonization by ligninolytic endophytic fungi (Koide et al. 2005). Studying simultaneously the occurrence of bleached area and the changes in AUR content in leaf litter during the decomposition would enable us to evaluate the effects of clear-cutting on the colonization by decomposer fungi and chemical quality of leaf litter separately and provide useful insights into possible mechanisms of clear-cutting effects on bleaching of leaf litter during early stage of decomposition.

The purpose of the present study was to compare the occurrence of bleached area and the AUR contents in the leaf litter by following an 18-month decomposition of leaf litter of C. japonica in a clear-cut and an adjacent uncut control plots. We hypothesized that clear-cutting in a temperate secondary forest would reduce (1) the occurrence of the bleached portion on leaf litter during decomposition and (II) the decomposition of AUR in leaf litter, leading to the suppression of mass loss of the whole leaf litter.

II Materials and methods

1. Study site

The study site was located in Oharano Forest Park of Kyoto City (34°57′N, 135°37′E, 400 m a.s.l.), Kyoto, Japan. The mean annual temperature was 15.3°C and annual precipitation was 1,581.1 mm at the Kyoto Weather Station, about 12 km from the site (Ishikawa et al. 2007). The study site was in a mountainous area of a temperate forest that had been repeatedly harvested for fuel production until the fuel revolution in the 1950s. Two plots were used in the present study: a clear-cut (CC) plot where all trees were cut in December 2001 and a control (CO) plot where no trees were cut (Ishikawa et al. 2007). These plots were on the upper part of same slope facing west. The size of the plots was 0.03 ha (30 × 10 m) and the distance between the plots was 20 m that both plots were 10 m away from the border of clear-cutting to avoid edge effects.

The basal areas of trees were measured for trees
larger than 10 cm in diameter at breast height (Table 1). In CO plot, Quercus serrata, C. japonica, and Pinus densiflora dominated in number and basal area. The vegetation in CC plot prior to the experimental clearcutting was almost the same as that in CO plot. Canopy closure measured by taking hemispherical photographs at 60 cm above ground and analyzing the photographs with HemiView software (HemiView Canopy Analysis Software version 2.1, Delta-T Devices Ltd, England) was lower in CC than in CO plot (Table 1). The thickness of L layer measured with a scale on vertical cross-sections was thinner in CC than in CO plot (Table 1).

2. Decomposition experiment

Decomposition processes of C. japonica leaves were studied for an 18-month period from June 2006 to December 2007 by means of the litterbag method (Osono and Takeda 2005). Newly shed leaves of C. japonica were collected in May 2006, the peak period of litter fall, from the forest floor within and around the CO plot where the canopy was closed. The leaves were taken back to the laboratory and air-dried at room temperature (ca. 15°C) for one week. The leaves (3 g) were enclosed in a litter bag (15 × 15 cm) made of polypropylene shade cloth with a mesh size of approximately 2 mm. A total of 120 bags were prepared and placed on the surface of the litter layer in June 2006, 60 bags per plot. Litterbags were attached to the forest floor with metal pins to prevent possible movement or loss and to ensure good contact between the bags and litter layer. Sampling of the bags took place six times, at 3 (September 2006), 6 (December 2006), 9 (March 2007), 12 (June 2007), 15 (September 2007), and 18 months (December 2007) after placement. On each sampling occasion, a total of 20 bags were retrieved (10 bags per plot). The bags were placed in plastic bags and taken back to the laboratory. Then the leaves enclosed in the bags were taken out and extraneous matters such as soils were removed carefully. The fresh weight of the leaves was measured per litterbag. Then the leaves were sandwiched between pages of thick magazines to flatten them out and oven-dried to a constant mass at 40°C. The mass remaining of leaf litter was determined as a percentage of the original mass (3 g). Water content was determined according to the equation 1:

\[
\text{Water content (g g}^{-1}\text{)} = (\text{fresh weight (g) } - \text{ dry weight (g)}) / \text{ dry weight (g)}.
\]

Mean values of remaining mass and water content were calculated for each plot on each sampling occasion. Accumulated mass loss of litter on each sampling occasion was calculated as the mass loss relative to the initial mass, expressed in percentage. The leaves were then used for the determination of leaf area.

3. Leaf area measurement

Flattened leaves were photocopied and scanned by a scanner (EPSON GT-9800F) with gray scale and 150 dpi in resolution. While photocopying, the bleached portions where the color was clearly paler than surrounding non-bleached area were recorded on copied image. The area of bleached portions and total leaf area were measured by image analysis software performed on a Windows computer using Scion image software (version 4.0.3, windows version of NIH image, Scion Corporation). The proportion of bleached area to the total leaf area was expressed as a percentage, and the mean values of the proportions were calculated for each plot.

4. Chemical analysis

Each of bleached and non-bleached portions in samples and initial leaf litter were punched out with a paper punch (6 mm in diameter) and combined to make one sample per plot on each sampling occasion. Then the combined samples were ground in a laboratory mill to pass through a 0.5-mm screen. Total nitrogen (N) and carbon (C) contents were measured by automatic gas chromatography (Sumigraph NC-900 NC analyzer; Sumitomo Chemical, Osaka, Japan).

Table 1 Basal area, canopy closure, and thickness of L and FH layers in clear-cut (CC) and control (CO) plots. Values indicate means ± standard errors (n=6 for canopy closure, n=10 for thickness of L and FH layers). Values that do not share a common letter are significantly different at the 5% level by t-test.

<table>
<thead>
<tr>
<th>Plot</th>
<th>BA (m² ha⁻¹)</th>
<th>Canopy closure (%)</th>
<th>Thickness (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>L layer</td>
</tr>
<tr>
<td>CC</td>
<td>0</td>
<td>78.7 ± 0.02 a</td>
<td>0.57 ± 0.45 a</td>
</tr>
<tr>
<td>CO</td>
<td>64.2</td>
<td>91.3 ± 0.01 b</td>
<td>2.48 ± 0.45 b</td>
</tr>
</tbody>
</table>

表 1 昔伐区と対照区における、胸高断面積合計、林冠閉鎖率、L 層および FH 層の厚さ。値は平均±標準誤差（繰り返し数は、林冠閉鎖率では 6、L 層および FH 層の厚さでは 10）。異なるアルファベットを有する値は t-検定で 5% 水準で有意な差がある。
The AUR content in the samples was estimated by gravimetry, according to a standardized method using hot sulfuric acid digestion (King and Heath 1967). The methods of chemical analysis were described in detail elsewhere in Osono and Takeda (2005). In the present study, we referred to the final residual fraction remaining after proximate analysis as AUR. Acid-unhydrolyzable residue fraction contains a mixture of organic compounds in various proportions, including condensed tannins, phenolic compounds, carboxylic compounds, alkyl compounds such as cutins, and true lignin (Preston et al. 1997). Samples collected at 18th month in CO plot were not used for chemical analysis because of the low amount of bleached leaf tissues.

5. Data analysis

Decay constant of litter were calculated as Olson's $k$ (Olson 1963) according to the equation 2:

$$W_t = W_0 \times \exp (-kt)$$ (2)

where $W_t$ is the litter mass at time $t$, $W_0$ is the original litter mass, $t$ is time in year, and $k$ is the decay constant. The $t$-test was used to evaluate the differences between CC and CO plots in canopy closure, thickness of L and FH layers, and remaining mass loss, water content, and bleached area of leaf litter.

Generalized linear model (GLM) was formulated to show the relationship between response variable that was AUR content (AUR) and explanatory variables that were bleached or non-bleached area (BL), clearcut or control plot (PLOT), and accumulated mass loss (AML) where BL and PLOT were categorical variables with two modalities. AUR content was assumed to follow gaussian distribution for sub-samples in each retrieving occasion hence identity was chosen as the link function. We assessed significance of explanatory variables that were BL, PLOT, and AML in relation to AUR content. The data of initial samples (i.e. AML = 0 %) were not included in this analysis because the changes in remaining mass during the first three month was not only due to the decomposition of AUR or other insolubles such as hemicellulose but also the eluviation of soluble components. The statistical analyses for this GLM were performed using the statistical environment R 2.11 (R Development Core Team, 2010).

III Results

The initial leaf litter had 14.4 mg cm$^{-2}$ in leaf mass per area, 246.9 mg g$^{-1}$ of AUR, 17.1 mg g$^{-1}$ of N, and 522.7 mg g$^{-1}$ of C. The decay constant $k$ during the experimental period was 0.57 year$^{-1}$ in CC plot and 0.69 year$^{-1}$ in CO plot. The remaining mass (% original mass) was significantly ($t$-test, $P<0.05$, n=10) greater in CC plot than in CO plot for the 3rd, 9th, 12th, and 18th months of decomposition (Fig. 1a). The water content of leaf litter was significantly ($t$-test, $P<0.05$, n=10) lower in CC plot than in CO plot for the 3rd month of decomposition (Fig. 1b) but was not significantly ($P>0.05$) different between the plots for the rest of the study period. The proportion of bleached area was significantly ($t$-test $P<0.05$, n=10) lower in CC plot than in CO plot for the 6th and 9th months of decomposition (Fig. 1c).

In GLM analysis of the AUR content, $P$ values for each coefficients of terms were significant ($P<0.05$) with that of 0.03 for BL and 4.9 $\times$ 10$^{-6}$ for AML, whereas $P$ value was not significant for PLOT ($P=0.07$). The coefficient of BL indicated that AUR content in non-bleached area was generally 95.8 mg g$^{-1}$ higher than bleached area at the same sampling occasion and that of AML indicated that AUR content increased 3.8 mg g$^{-1}$ as AML increased 1 % in both bleached and non-bleached part of leaf litter.

$$AUR = 102.8 + 95.8 \times BL - 55.2 \times PLOT + 3.8 \times AML$$

In the model, the estimated content of AUR was increased when the leaf litter was not bleached and as accumulated mass loss of leaf litter increased (Fig. 2).

IV Discussion

In the present study, decomposition of C. japonica leaf litter was suppressed in CC plot compare to CO plot (Fig 1a), which was consistent with previous studies (Yin et al. 1989, Prescott 1997). Bleached area that occurred on decomposing leaf litter was also reduced in CC plot compare to CO plot (Fig. 1c), which was consistent with Osono et al. (2008). Koide et al. (2005) reported that Rhytismataceae which was endophytic fungi of C. japonica was responsible for bleaching of C. japonica leaf litter. The reduction of bleached area in CC plot indicated that clear-cutting suppressed the growth of the endophytic fungi.

Clear-cutting can alter the micro-environmental conditions such as temperature and moisture, which account for the changes in the decomposition of leaf litter (Kirshbaum 1995, Van Meeteren et al. 2007). Pietikainen et al. (2005) demonstrated that fungi could not grow over 40°C in forest humus, thus increasing in temperature could be thought as the factor of suppressing the bleached area in CC plot in the present study, although no data of soil temperature was available. In contrast to the suppression of growth of ligninolytic fungi, the ability of ligninolytic fungi to decompose AUR was not affected by clear-cutting because coefficient of PLOT was not significant
Fig. 1 Change in remaining mass (a), water content of leaf litter (b), and proportion of bleached portions to total leaf area (c) during decomposition. Squares and open bars indicate CC plot, circles and filled bars indicate CO plot. Error bars indicate standard errors. * indicates that there is a significant difference between CC and CO plots in t-test (n=10, P<0.05).

Fig. 2 Relationship between accumulated mass loss and AUR content (mg g⁻¹). Open squares indicate bleached portions in CC, filled squares indicate non-bleached portions in CC, open circles indicate bleached portions in CO, filled circles indicate non-bleached portions in CO, and cross sign indicates initial leaf litter.

In GLM. This result did not agree with the study of Waldrop et al. (2003) that showed suppression of ligninolytic enzyme activity of leaf litter set on the broadcast burning site, where residues of forest harvesting were burned prior to sampling, compare to forest site. Nutrient left in ash may be reason of this inconsistency, and the underlying mechanism is needed to be revealed.

Increasing in soil temperature could cause occasional desiccation of the forest floor, which could also suppress the growth of fungi at CC plot, but we did not find significant differences in water content of leaf litter between CC and CO plots on the most of sampling occasions (Fig. 1b).

In conclusion, in the present study, the slower decomposition of leaf litter in the clear-cut site was presumable to the suppressed growth of ligninolytic fungi which was determined by the shift in the area of bleached portion. However, the abilities of fungi to decompose AUR were not suppressed since there were no significant differences in AUR content between CC and CO plots. Further studies are necessary to determine growth and enzyme activity of ligninolytic fungi in relation to temperature gradient in leaf litter.

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Literature cited


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