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Decomposition and ergosterol content of the moss *Hylocomium splendens* litter under various climatic conditions

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Abstract: We examined the differences in the decomposition rate and fungal biomass in the litter of *Hylocomium splendens* among forests under different climatic conditions. The samples were collected from one boreal forest in Canada, three subalpine forests on Mt. Fuji and one cool temperate forest on Mt. Tsurugi, Shikoku in Japan. The decomposition rate in the cool temperate forest was much faster than those in the boreal and subalpine forests. Ergosterol, which is a component of fungal cell membranes, was used as an indicator of fungal biomass. Ergosterol was detected not only from brown moss litter but also from green shoots of the moss. In spite of the faster decomposition rate, ergosterol content of the moss litter of the cool temperate forest was about one half of those of the boreal and subalpine forests. The results suggest that the relationship between fungal biomass and decomposition rate differs significantly among forest types.

key words: decomposition, ergosterol, forest type, fungal biomass, moss, *Hylocomium splendens*

Introduction

It has been reported that mosses represent a significant proportion of the biomass production and subsequently contribute to litter formation in boreal and polar ecosystems (Longton, 1984, 1992). In addition, there is some evidence that the moss layer plays an important role in nutrient cycling in ecosystems (*e.g.* Longton, 1984). Mosses are known to efficiently absorb nutrients contained in precipitation and throughfall (Bates, 1992). Since moss litter decomposes more slowly than that of vascular plants (Rosswall *et al*., 1975; Berg, 1984), the moss layer can act as a reservoir of potentially available nutrients. Therefore, decomposition rates of moss litter may have a profound influence on the rate of nutrient cycling in the ecosystem.

The decomposition rate of litter is determined largely by climatic condition, litter quality and the nature of decomposers (species composition, biomass, activity etc.). The effects of climatic condition and litter quality on the decomposition of moss litter have been studied by several authors (*e.g.* Berg, 1984; Nakatsubo *et al*., 1997). On the

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other hand, information about the decomposer of moss litter is rather limited. A few studies so far reported indicate that fungi play a major role in litter decomposition (Parke and Linderman, 1980; Grasso and Scheirer, 1981; Redhead, 1981).

Microbial biomass levels are known to be important in determining the decomposition rate of soil organic matter (Berg and Söderström, 1979; Anderson and Domsch, 1980; Mishima *et al.*, 1999). Uchida *et al.* (2000) examined the decomposition rates and fungal biomass of organic substrates (filter paper and wood chips) in an altitudinal climatic gradient. They suggested that the altitudinal difference in decomposition rates is determined not only by climatic condition but also by difference in fungal biomass. It is expected, therefore, that fungal biomass has a significant correlation with the decomposition rate of moss litter.

In order to examine the relationship between the fungal biomass and decomposition rate of moss litter, we measured the fungal biomass of *Hylocomium splendens* (Hedw.) B.S.G. growing in forests under different climatic conditions. Ergosterol, which is a component of the fungal cell membrane, was measured as a biomaker for fungi in the litter.

Materials and methods

Study sites

The samples of *H. splendens* were collected from one boreal forest in Canada, and three subalpine forests and one cool temperate forest in Japan.

The Canadian boreal forest was situated near Candle Lake in Saskatchewan, Canada (53°50′N, 105°30′W; about 500 m a.s.l.). This study site was dominated by black spruce (*Picea mariana* (Mill.) B.S.P.) with a few aspen (*Populus tremuloides* Michx.) individuals. A thick moss layer, mainly composed of *H. splendens*, covered the ground surface. A detailed description of this study site appeared in Uchida *et al.* (1998).

The study sites in the subalpine forests were set at three different altitudes (2400, 2200 and 1700 m) on the north-western slope of Mt Fuji (35°23′N, 138°42–43′E). The smallest distance between sites was 1.5 km. The dominant tree species were *Tsuga diversifolia* (Maxim.) Masters and *Abies veitchii* Lindley. Further descriptions of the forest are given by Nakatsubo *et al.* (1997).

One study site was set in the *Fagus crenata* Blume forest of Mt. Tsurugi (33°52′N, 134°05′E; about 1350 m a.s.l.) in Ehime Prefecture. The forest floor was dominated by bamboo grass (*Sasa nipponica* Makino et Shibata). *H. splendens* was found mainly on large rocks and fallen trees on the forest floor.

Monthly mean air temperatures at the study sites were estimated from data recorded at nearby weather stations (*cf.* Nakatsubo *et al.*, 1997). Temperatures at the Mt. Tsurugi study site were estimated from data recorded at the summit of Mt. Tsurugi (33°51′N, 134°06′E; 1945 m a.s.l.) for 30 years (1961–1990) using the local environmental lapse rate of 0.60°C 100 m^{-1}. Locations and climatic conditions of these study sites are shown in Table 1.

Table 1. Location, temperature, dominant tree species and annual mass loss rates of *Hylocomium splendes* litter at each study site.

Site	Altitude (m)	Mean annual air temperature (°C)[a]	Dominant tree species	Annual mass loss rate (%)
Candle Lake	500	0.6	*Picea mariana*	18.6[b]
Mt. Fuji	2400	1.2	*Tsuga diversifolia*	10.1[b]
Mt. Fuji	2200	2.3	*Tsuga diversifolia*	14.1[b]
Mt. Fuji	1700	5.2	*Abies veitchii*	22.9[b]
Mt. Tsurugi	1350	7.8	*Fagus crenata*	24.5

[a] Values are estimated from data recorded at nearby weather stations.
[b] Data from Nakatsubo *et al.* (1997).

Mass loss rate

Six or seven almost pure stands of *H. splendens* were selected at each study site. Part of each stand, 15×15 cm in surface area, was cut vertically to the FH layer, and a moss block composed of green shoots and the L layer was collected. The shoots were divided into segments of each age class (see below). Then, these segments and the moss litter in the L layer were freeze-dried to obtain their dry weight.

The annual mass loss rate of the moss litter was calculated by the simple model proposed by Nakatsubo *et al.* (1997) assuming constant litter production (L) and a constant litter mass loss rate (k') on an annual basis. *H. splendens* produces a new, readily identifiable segment each year (*e.g.* Tamm, 1953). Each segment increases its weight for 1 or 2 years to attain its full size. These fully grown segments constitute the largest age class. If the annual net production rate and annual mass loss rate are constant, the biomass of the largest age class corresponds to the annual litter production L (Skre and Oechel, 1979). The amount of litter, including the largest age class, in the steady state (M_m) is given by the following simple equation (Jenny *et al.*, 1949).

$$M_m = L/k' \qquad k' = L/M_m. \tag{1}$$

Mass loss rates at the study sites of Candle Lake, and Mt. Fuji (2400, 2200 and 1700 m a.s.l.) were given by Nakatsubo *et al.* (1997). Moss blocks of Mt. Tsurugi were collected in November 1998 and the mass loss rate was estimated as described above.

Ergosterol content

Ergosterol content in each segment of the *H. splendens* litter was measured according to Newell *et al.* (1988) as modified by Kasai and Horikoshi (1997). Samples of boreal, subalpine and cool temperate forests were collected in July 1995, June 1998 and November 1998, respectively. For comparison, samples of needle litter (composed mainly of *Tsuga diversifolia* or *Abies veitchii*) were also collected from the forest floor at subalpine sites in July 1997.

The samples were cut fine by after dividing them into segments of each age class. For samples collected from the boreal site, a portion of the sample (*ca.* 40–180 mg dry matter) and 20 m*l* methanol were put in a 30-m*l* bottle and capped. The sample was brought to Japan and stored in a refrigerator at 5°C until the measurement (within 1

month). The rest of the sample (*ca.* 80–150 mg) was dried to constant weight at 80°C to obtain the dry weight. For samples collected from other sites (subalpine and cool temperate forests), freeze-dried moss litter (10–500 mg dry matter) was placed in 20 m*l* methanol and stored in a refrigerator at 5°C until the measurement (within 1 week).

Quantitative determination of ergosterol was performed by reversed-phase HPLC analysis (Shimadzu Co., Kyoto, Japan) by using a main column 25 cm by 4.6 mm Shim-pack HRC-ODS with a 5 μm gasket with a mobile phase of methanol, a 1.0 m*l* min^{-1} flow rate and UV detection at 282 nm. Retention of ergosterol with this system is about 15 min.

Results and discussion

Figure 1 shows shoots of *Hylocomium splendens* collected from the study sites in the Canadian boreal forest, subalpine forests of Mt. Fuji and cool temperate deciduous forest of Mt. Tsurugi. In all samples, the current year (0-year-old) and 1-year-old segments were green. The 2-year-old segments in the boreal and subalpine forest samples were also green, while those from Mt. Tsurugi were yellow and appeared to be senescing. In the boreal and subalpine forests, decomposition of old segments (older than 3-year-old segment) appeared to be slow (Fig. 1a–c). For example, at the highest study site on Mt. Fuji (2400 m a.s.l.), the dry weight of the 5-year-old segment was nearly 80% of the weight of the largest segment. On the other hand, old segments from Mt. Tsurugi rapidly decomposed so that the 5-year-old segments almost totally lost their leaves and branches (Fig. 1d). The dry weight of the 5-year-old segment was less than one half of the weight of the largest segment at Mt. Tsurugi (data not shown).

The high decomposition rate at the Mt. Tsurugi study site was confirmed by the litter mass loss rate estimated by the model. The annual mass loss rate at Mt. Tsurugi

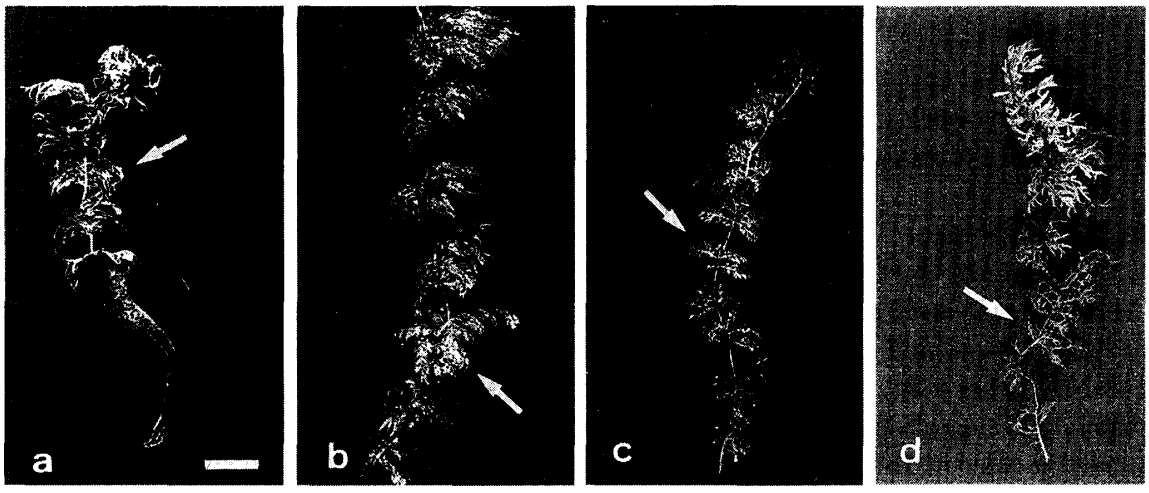

Fig. 1. Shoots of *Hylocomium splendens*.
(a) Canadian boreal forest. (b) Subalpine forest on Mt. Fuji (2200 m a.s.l.). (c) Subalpine forest on Mt. Fuji (1700 m a.s.l.). (d) Cool temperate forest on Mt. Tsurugi. Arrows indicate the 5-year-old segments. Scale bar = 10 mm.

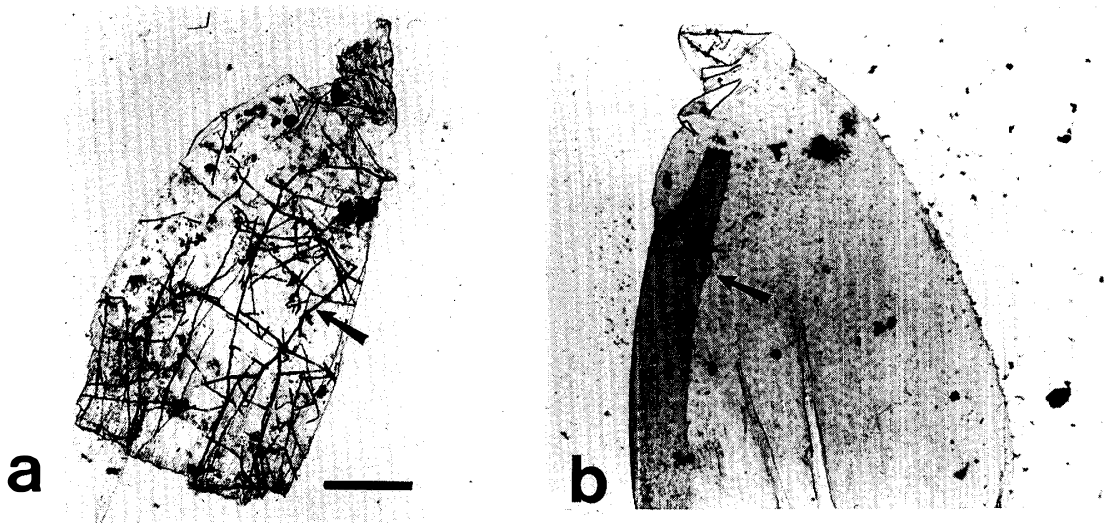

Fig. 2. Light microscopy of *Hylocomium splendens* at the site on Mt. Fuji (2400 m a.s.l.).
(a) The 4-year-old segment. (b) The 1-year-old segment. Arrows indicate filamentous fungi.
Scale bar = 200 μm.

(25%) was larger than the rates at the boreal and subalpine sites (10–23%; Table 1).

Figure 2 shows the microscopic observation of *H. splendens* of a different age class. The brown shoots were densely colonized by filamentous fungi (Fig. 2a). A few filamentous fungi were also observed in green shoots (Fig. 2b).

Fungal colonization in green shoots was also confirmed by ergosterol analysis. A significant amount of ergosterol was detected from green shoots for all sites (Table 2). The samples collected from the five study sites showed similar patterns of change in ergosterol content with age. Ergosterol content tended to increase with segment age for 3 years. Then, no significant difference in the ergosterol content was detected between ages 4 and 5 (Tukey-Kramer $P > 0.05$). Ergosterol content of the moss litter (older than 3-year-old segment) was within the range from 54 to 201 μg g^{-1} dry matter which is similar to or less than that of litter of salt marsh plants (202–392 μg g^{-1} dry matter) (Newell, 1988).

The ergosterol content of the 3-year-old segments collected from the study sites on Mt. Fuji was within the range from 99 to 138 μg g^{-1} dry matter (Table 2, Fig. 3). The relationship between the ergosterol content and mean annual air temperature was not necessarily clear, but the ergosterol content of the 3-year-old segments at the highest altitude (2400 m a.s.l., mean annual air temperature; 1.2°C) was significantly lower than those at lower altitudes on Mt. Fuji (Tukey-Kramer $P < 0.05$). The ergosterol content of the 2-year-old segments showed a similar tendency of altitudinal change as those for 3-year-old segments (Table 2). The ergosterol content of the sample collected from the boreal site was similar to those collected from the subalpine sites (Table 2).

On the other hand, in spite of higher temperature condition, the ergosterol content of the Mt. Tsurugi samples was about one half of those from the boreal and subalpine sites (Table 2, Fig. 3). This result is in contrast with the result of Uchida *et al.* (2000), who reported that fungal biomass in decomposing organic matter tended to decrease with increasing altitude (decreasing temperature) in the subalpine zone. They also

Table 2. Ergosterol content of *Hylocomium splendens* at the study sites.

Segment age	Ergosterol content ($\mu g\ g^{-1}$ dry matter)				
	Candle Lake	Mt. Fuji			Mt. Tsurugi
		2400 m	2200 m	1700 m	
0,1	31 (5)	35 (5)	55 (8)	55 (6)	6 (2)
2	74 (19)	59 (6)	92 (14)	83 (7)	26 (4)
3	166 (11)	99 (7)	138 (11)	137 (14)	54 (6)
4	186 (17)	150 (10)	158 (7)	201 (25)	79 (6)
5	193 (12)	142 (15)	155 (12)	160 (10)	94 (9)

Values are means with SE in parentheses; $n=6$ (Candle Lake) or 7 (other sites).

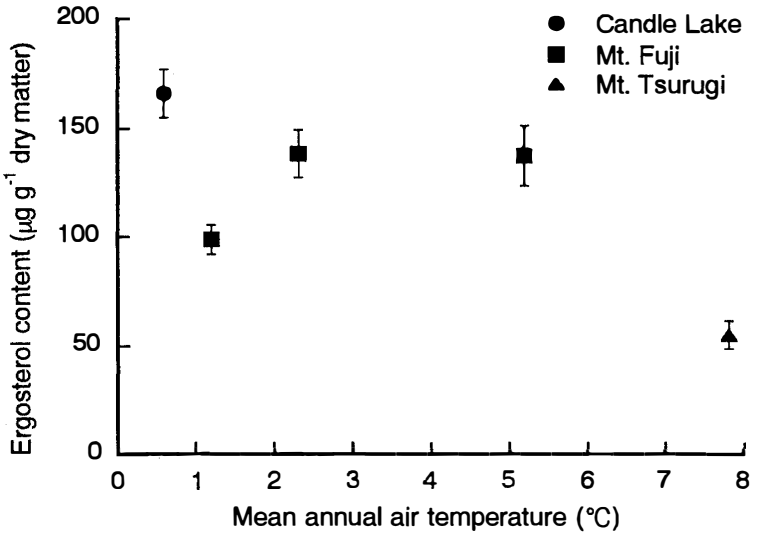

Fig. 3. The relationship between mean annual air temperature and ergosterol content of the 3-year-old segments of *Hylocomium splendens*. Each value is the mean of 6 (Candle Lake) or 7 (other sites) samples with SE.

reported that significant increase of the ergosterol content occurred mainly from spring to summer. Therefore, it is expected that the ergosterol content of litter determined in autumn is larger than that in early summer. Nevertheless, the ergosterol content on Mt. Tsurugi in November was much smaller than that at other sites which were determined in June or July.

One possible explanation of this inconsistency is that the quality (composition) of decomposers differs among forest types. Mishima *et al.* (1999) reported that there are significant differences in metabolic quotient (respiration rate per unit biomass) among forests dominated by different tree species. Although the species of litter examined in our study is common to all study sites (*H. splendens*), the forest type on Mt. Tsurugi is quite different from those at the other sites (deciduous broad-leaved forest vs. evergreen

coniferous forests).

It is also possible that the higher ergosterol contents at boreal and subalpine study sites are partly due to the external hypha of ectomycorrhizal fungi. External hypha of mycorrhizal fungi constitutes a considerable proportion of the total fungal biomass in forests dominated by ectomycorrhizal trees (*e.g.* Fogel and Hunt, 1983). Although all genuses of dominant tree species at the study sites, *i.e. Picea, Tsuga, Abies* and *Fagus,* are known to be ectomycorrhizal (Maeda, 1954; Harley and Harley, 1987; Allen, 1992; Li, 1996; Smith and Read, 1997), the ratio of saprophytic to mycorrhizal fungi may differ significantly among forest types. At present, however, there is no reliable method to distinguish the biomass of mycorrhizal fungi from that of saprophytic fungi (*e.g.* Colpaert *et al.*, 1992).

The results of this study indicate that fungal biomass in moss litter can vary widely among forest types even if the moss species are the same. It is also suggested that the relationship between fungal biomass and decomposition rates differ significantly among forest types.

Ergosterol contents of the needle litter collected at the study sites on Mt. Fuji were within the range from 193 to 293 μg g^{-1} dry matter. These values were similar to the ergosterol content of the 4-year-old segments of *H. splendens* determined in this study (150–201 μg g^{-1} dry matter). It has been reported that decomposition of moss litter was much lower than that of needle litter (Berg, 1984). However, the data of the present study suggest that the moss layer is important as a growth substrate of saprophytic and/or mycorrhizal fungi.

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