B-6.3.2 Quantitative Analysis of the Effects of Environmental Factors on the Soil Microbial Community

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Abstract In order to study the effect of climatic condition on the soil microbial community and decomposition rate, the difference in the mass loss rate of organic matter (paper and wood chips) was examined along the altitudinal climatic gradient in the subalpine zone of Mt.Fuji. The mass loss rate tended to decrease with increasing altitude. A significant log-linear relationship was observed between the mass loss rate and the annual mean air temperature. Ergosterol, which is a component of membranes of fungal cells in the substrates tended to be smaller with increasing altitude. The difference in the ergosterol content of the moss *Hylocomium splendens* litter was also examined between forests under different climatic conditions.

1. Introduction

In many model studies, the impact of global warming on decomposition has been estimated based on the short-term responses of microbes to temperature observed in the laboratory or in the field[1]. However, long-term perturbations such as climatic change may affect on not only biological activity but also structure and/or biomass of the microbial community.

In a previous study[2], we reported that the mass loss rates of moss litter in a subalpine forest were significantly correlated with annual mean air temperature, but the temperature dependence of the mass loss rate was much larger than those expected from the temperature-soil respiration curves. This may indicate that factors other than microbial activity may also change with temperature.

In this study, we examined altitudinal differences in the mass loss rate of organic substrates (paper and wood chips) in subalpine coniferous forests.

Ergosterol, which is a component of membranes of fungal cells in the substrates, was measured to determine whether climatic conditions affect the fungal biomass. In addition, ergosterol content of the litter of the moss *Hylocomium splendens* was compared among forests under different climatic conditions.

2. Altitudinal differences in organic matter mass loss and fungal biomass in a subalpine coniferous forest

(1) Site and Methods

The study sites were set a five different altitudes – 2400, 2200, 1900, 1700 and 1500 m – on the northwestern slope of Mt. Fuji (35°23'N, 138°42–43'E). The dominant tree species were *Tsuga diversifolia* and *Abies veitchii*. Various bryophyte species were found on the forest floor in sites above 1700 m a.s.l., while the forest floor at 1500 m was dominated by ferns.

The litter bag method was employed to determine the mass loss rate of organic substrates. Cellulose filter paper (Advantec No. 526) and wood chips of beech (*Fagus crenata*) were used as the standard substrates.

The filter paper was served as an indicator of *in situ* cellulase activity, while the wood chips were used to simulate the decomposition of woody litter.

A piece of filter paper (about 5 × 10 cm, 1.6 g dry wt.) or wood chips (1.6 – 4.0 mm, about 7 g in dry wt.) were put in a nylon mesh bag (9 × 11 cm, 1 mm mesh). Twenty bags with each substrate were buried in each soil layer (L and FH) at each site in May (L layer) or in June (FH layer) 1997. Five bags of each substrate were collected from each layer in July, September and November 1997 and May 1998. They were put in polyethylene bags and brought to the laboratory in a cooled state at 5 ℃. After removal from the bags, the substrates were weighed to obtain their fresh weight and stored in a freezer at −80 ℃. They were freeze-dried to a constant mass to obtain their dry weight.

Ergosterol content in the substrates was measured according to Newell *et al.* [3] as modified by Kasai and Horikoshi [4].

Microbial respiration in the substrates was measured by a modified form of the closed-chamber method[5]. The bags (n=3–6) with filter paper were collected from L the layer of each study site in September 1997. They were brought to the laboratory and kept in a refrigerator at 5 ℃ until measurement (about one week). The samples were cut into pieces of about 5 mm_2. A portion of the sample (about. 50–250 mg dry weight) was used for the measurement of ergosterol content. The rest of the sample (about 0.5–1.1 g in dry weight) was put

in a 100-ml Erlenmeyer flask. The flask was capped with a silicon stopper. At intervals of 3-15 minutes, 3 ml of nitrogen gas was injected into the flask and the same volume of air was withdrawn with a micro-syringe to analyze CO_2 concentration. The air sample was inserted into a gas-line in which CO_2 free air streamed at 0.2 l min^{-1}, and sent to an infra-red gas analyzer (ZFUID23, Fuji Electronic, Tokyo, Japan). The respiration rates of the samples collected from 2400 and 1500 m a.s.l. were measured at 3,5,10,15,20 and 25 ℃ to obtain the Q_{10} values. The respiration of samples from 2200, 1900 and 1700 m was measured only at 15 ℃.

(2) Results

Figure 1 shows the time course of mass loss (% remaining) of the substrates (filter paper and wood chips) placed in L and FH layers. The mass loss (100 - % remaining weight) of each substrate tended to be smaller with increasing altitude though there were some exceptions. For example, in July, the mass loss of filter paper in the FH layer at 1500m was more than six times faster than that of 2200 m or 2400 m (Fig. 1b). The substrates lost their weight rapidly in summer (from July to September) in every study sites. From September to November, the substrates showed no significant change in their dry weight (Scheffe's F, $P>0.05$). Low but significant mass loss was detected from November 1997 to May 1998 for the most study sites (Scheffe's F, $P<0.05$).

The filter paper placed in the L layer showed the similar patterns of mass loss as those in the FH layer, though the mass loss rate at 2200 m was exceptionally rapid.

The mass loss rates of wood chips were much slower than those of filter paper. However, as with the filter paper, the mass loss rates of wood chips tended to be smaller with increasing altitude.

The annual mass loss rates were significantly correlated with the annual mean air temperature in a log-linear fashion. We calculated the apparent Q_{10} values for the mass loss rate (the proportional increase in mass loss rate for a 10 ℃ increase in temperature) from the mean annual temperatures at the study sites. The Q_{10} values calculated for filter paper in the L and FH layers and wood chips in the L and FH layers were 1.7, 7.9, 3.7 and 19.9, respectively.

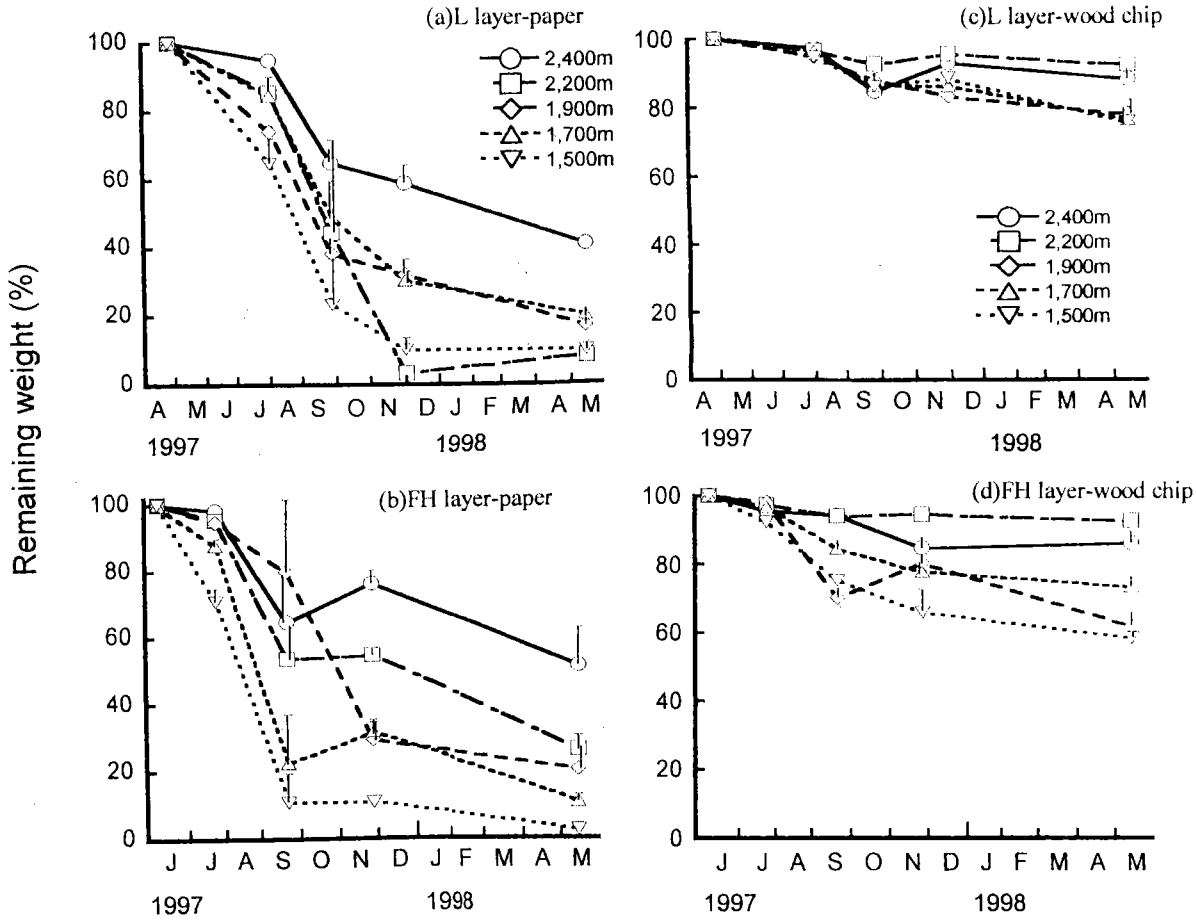

Fig. 1. Mass loss (% remaining dry weight) of organic substrates in L and FH layers at different altitudes.

(a) Cellulose filter paper in L layer. (b) Cellulose filter paper in FH layer.
(c) *Fagus crenata* wood chips in L layer. (d) *F. crenata* wood chips in FH layer.
Each value is the mean of five samples with SE (n = 5).

Figure 2 shows the changes in ergosterol content per bag in L and FH layers at different altitudes. The initial increase in ergosterol content of filter paper was more rapid at low altitudes than at higher altitudes. At low altitudes, the ergosterol content stopped increasing and began to decrease in autumn, possibly because of the rapid decline in substrate weight (Fig. 2a). On the other hand, ergosterol content of filter paper continued to increase until next season at 2400 m (Fig. 2a).

The ergosterol content of wood chips showed the similar patterns of seasonal change as those of filter paper (Fig. 2c,d). However, the ergosterol of wood chips continued to increase until the late growing season in most of the sites. The initial increase of ergosterol content was very rapid at 1500 m especially in the FH layer. The ergosterol content of wood chips in the FH layer at 1500 m was more than four times as large as those at higher altitudes in November.

The microbial respiration in cellulose filter paper increased with rising temperature from 3 to 25 ℃ with Q_{10} values of about 2 (2.1 – 2.4).

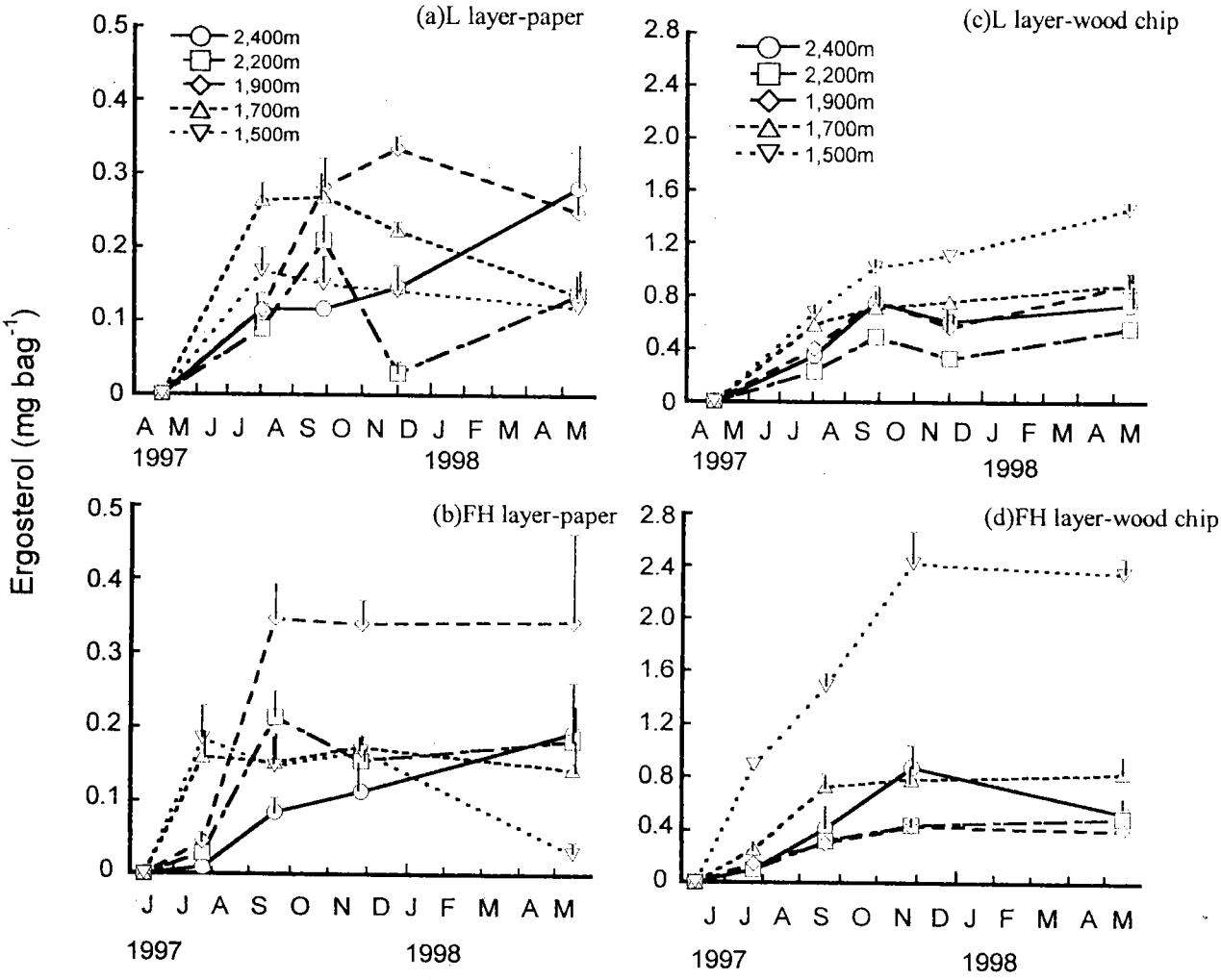

Fig. 2. Changes in ergosterol content of organic substrates in L and FH layers at difference altitudes.

(a) Cellulose filter paper in L layer.(b) Cellulose filter paper in FH layer. (c) *Fagus crenata* wood chips in L layer. (d) *F. crenata* wood chips in FH layer.

Each value is the mean of five samples with SE (n = 5).

(3) Discussion

In this study, the apparent Q_{10} values for the mass loss rate were much larger than the Q_{10} obtained for microbial respiration (about 2) except for cellulose filter paper in the L layer. This indicates that the temperature dependence of the mass loss rate in the field can not be explained solely by the temperature dependence of microbial activity.

If the chemical quality of the substrates is almost the same, the mass loss rate of the substrates is determined mainly by climatic factors (temperature and moisture) as well as the quality and quantity of decomposers. In this study, soil moisture condition did not differ significantly among study sites.

One possible explanation is that altitudinal difference, thus the length of the snow-free season, affected mass loss rate. Another possibility is that the quantity (biomass) and/or quality (composition) of decomposers changed with altitude. The measurement of ergosterol content indicated that fungal biomass increased more rapidly at low altitudes than at high altitudes.

Since microbial biomass and the length of snow-free season change with altitude, these two factors might have a significant influence on the apparent temperature dependence of the mass loss rate. The relative importance of these two factors in determining the apparent Q_{10} value of the mass loss rate was examined using a simple simulation model. The basic assumption was that the annual decomposition rate (R) at each altitude was determined by four factors, i.e. microbial activity per biomass (metabolic quatient), temperature (T), microbial biomass (B) and the length of the snow-free season.

$$R = B R_0 Q_{10}^{(T-T_0)/10}$$

where R_0 is the microbial activity per biomass at T_0 and Q_{10} is the Q_{10} value obtained for organic substrate (filter paper) respiration. In this calculation, R_0 is assumed to be constant since no clear altitudinal change of microbial activity per fungal biomass was detected in this study.

The following four possible cases were considered.

1) Microbial activity responds to temperature with a Q_{10} of 2 throughout the year. Microbial biomass is not affected by temperature (control).

2) Microbial activity responds to temperature with a Q_{10} of 2, but it is completely suppressed at freezing temperatures (< 0 ℃).

3) The temperature dependence of microbial activity is the same as in 1) and microbial biomass change with altitude.

4) The temperature dependence of microbial activity is the same as in 2) and microbial biomass changes with altitude.

In 3) and 4), the ergosterol content in wood chips of the FH layer in July was used as the relative value of microbial biomass. We used the data of soil temperature recorded at 15 cm beneath the ground surface. The annual decomposition rate was calculated for each altitude and the proportional increase in mass loss rate for a 10 ℃ increase in temperature (Q_{10}) was re-calculated from mean annual temperatures at the study sites.

The result of the simulation is shown in Fig. 3. In the control, the Q_{10} value was equal to the Q_{10} of respiration (about 2). If microbial activity was negligible at freezing temperatures, Q_{10} was calculated to be 3. The effect of microbial biomass is much larger than that of freezing temperatures. When both factors (freezing temperatures and microbial biomass) were considered, Q_{10} was estimated to be 28, a value higher than observed in the field.

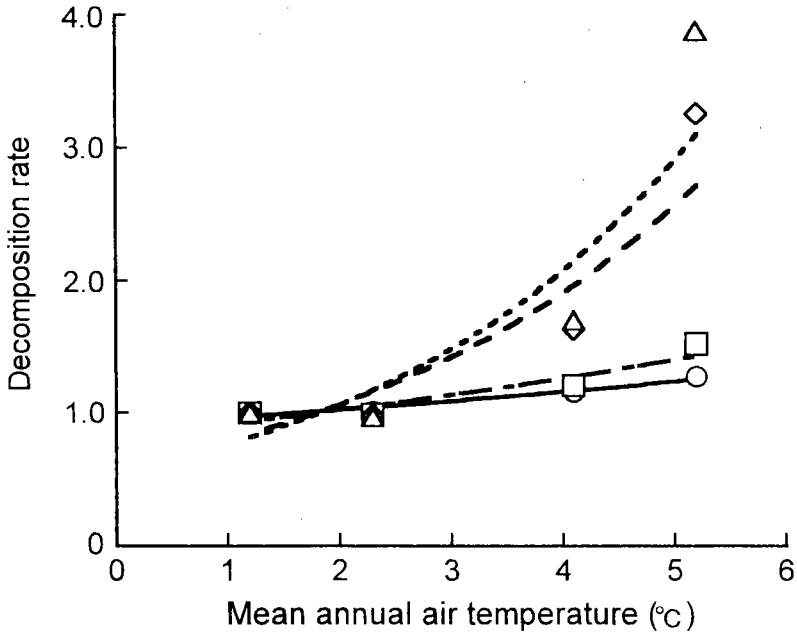

Fig. 3. Effects of freezing temperature and microbial biomass on the decomposition rate and its apparent Q_{10} value.
○ Microbial activity responds to temperature with a Q_{10} of 2 throughout the year (control). □ Microbial activity is completely suppressed at freezing temperatures (< 0°C). △ The temperature dependence of microbial activity is the same as in the control and microbial biomass change with altitude. ◇ Microbial activity is completely suppressed at freezing temperatures and microbial biomass change with altitude.

In this study, altitudinal changes of bacteria and soil animals were not examined. However, the results clearly demonstrated that altitudinal difference in microbial biomass and freezing time have a significant influence on the *in situ* decomposition rate. If similar changes of microbial biomass and/or freezing temperatures occur, the impact of future climatic change could be larger than those expected from short-term experimental studies.

3. Mass loss rate and fungal biomass in moss litter

(1) Materials and Methods

The moss *Hylocomium splendens* shows a very wide distribution in the Northern Hemisphere making a significant contribution to the net primary production of boreal forest ecosystems. Because this species produces readily identifiable segment each year, annual growth rate and annual mass loss rate of the litter can be easily estimated. Thus, this species can be used as a useful indicator of litter decomposition in boreal and subalpine regions.

The sample were collected from a boreal forest near Candle Lake in Saskatchewan, four subalpine coniferous forests of Mt. Fuji and a *Fagus crenata* forest of Mt. Tsurugi (Table 1).

Six or seven almost pure stands of *H. splendens* were selected in each study site. Part of each stand, 15 cm × 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 number of shoots in each block was counted to obtain the shoot density. Then, the shoots were divided into segments of each age class. Mass loss rate of the moss litter was estimated according to Nakatsubo *et al.*[2] Ergosterol content in the segments was measured Newell *et al.*[3] according to Kasai and Horikoshi[4].

(2) Results and Discussion

The annual mass loss rates of H. splendens litter in Candle Lake and in Mt. Fuji were 18 and 8.5 - 19.3 %, respectively, corresponding well with the previous study[2]. The mass loss rate in the study site of Mt. Tsurugi (about 25 %) was significantly larger than those of other study sites.

There was a significant correlation between the annual mass loss rates and mean annual temperature in the study site ($r=0.7$, $P<0.05$).

Figure 4 shows ergosterol content of segments of different age class of *H. splendens* shoots collected from Candle Lake. Ergosterol was detected not only from brown moss litter but also from green shoots (age 1). Ergosterol content tended to increase with segment age for 3 years. Then, no significant change in the ergosterol content was detected from age 3 to age 4. Almost the same pattern of change in the ergosterol content was observed for samples collected from other study sites.

Table 1. Location, mean annual air temperature and dominant tree species.

Site	Latitude	Altitude (m)	Mean Annual air[a] temperature (℃)	Dominant tree species
Candle Lake	53°50′ N	500	0.6	*Picea mariana*
Mt. Fuji	35°23′ N	2,400	1.2	*Tusga diversifolia*
Mt. Fuji	35°23′ N	2,200	2.3	*Tusga diversifolia*
Mt. Fuji	35°23′ N	1,900	4.1	*Abies veitchii*
Mt. Fuji	35°23′ N	1,700	5.2	*Abies veitchii*
Mt. Tsurugi	33°52′ N	1,400	7.8	*Fagus crenata*

[a] Values were estimated from data recorded at nearby weather stations.

Figure 5 shows ergosterol content of 2-year-old segments in the six study sites. The ergosterol content of the samples collected from the study sites of Mt. Fuji was within the range from 43 to 170 μg g^{-1} dry weight. The relationship between the ergosterol content and altitude was not necessarily clear, but the ergosterol content at the highest altitude (2400 m) was significantly lower than those at 2200 m and 1900 m.

The ergosterol content of the sample collected from Candle Lake was similar to those collected from Mt. Fuji. On the other hand, in spite of higher mass loss rate, the samples of Mt. Tsurugi showed much smaller ergosterol content than those of the boreal and subalpine sites.

This result disagrees with the previous data that fungal biomass in organic substrates tended to increase with increasing temperature (cf. Fig. 2).

One possible explanation of these apparently contradictory results is that the quality (composition) of decomposers differ among forest types. Mishima *et al.*[6] reported that there were significant differences in microbial biomass and metabolic quatient (respiration rate per unit biomass) between 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 of Mt. Tsurugi is quite different from those of the other sites (deciduous broad-leaved forest vs. evergreen coniferous forests). Therefore, it is likely that the composition of decomposer, and thus the decomposing activity per biomass, at the study site of Mt. Tsurugi were different from those at the other study sites.

The data of this study support the earlier assumption that long-term perturbations such as climatic change affect on not only biological activity but also structure and/or biomass of the microbial community. In order to assess the long-term effect of the climatic change on decomposition, improvement of our understanding about these effects seems to be necessary.

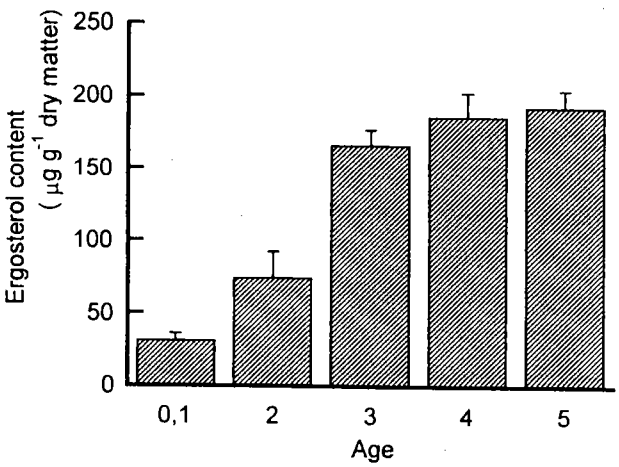

Fig. 4. Ergosterol content of *H. splendens* litter at Candle Lake.
Each value is the mean of 3 samples with SE.

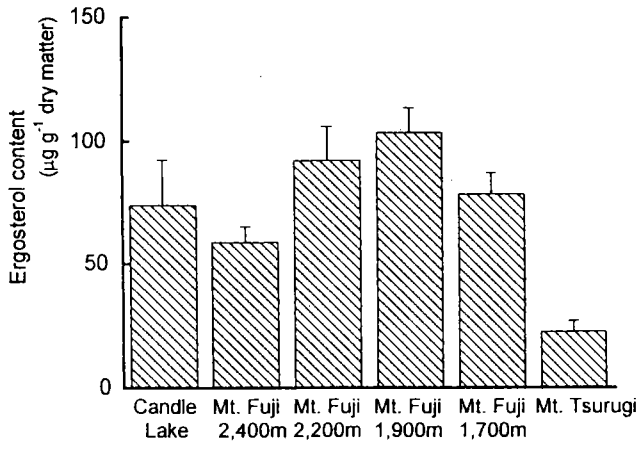

Fig. 5. Ergosterol content of *H. splendens* two-year-old segment among the study sites.
Each value is the mean of 3-7 samples with SE.

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