

# Soil Respiration Rate of Laurel-Leaved and *Cryptomeria japonica* Forests

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## II. MATERIALS AND METHODS

### A. Soil Samples

Fig. 1 illustrates the Kasugayama Hill Primeval Forest in Nara, Japan. In April 2001, soil samples were collected from laurel-leaved ( $B_{B-1}$ ), *Cryptomeria japonica* ( $B_{B-2}$ ), and Hideyoshi *C. japonica* ( $P_w$ ) forests, whose predominant vegetation is *Machilus thunbergii*, *Neolitsea aciculata*, and *C. japonica*, respectively (Fig. 2).  $B_{B-1}$  and  $B_{B-2}$  samples were composed of dry brown soil and the  $P_w$  sample of wet podzol.

Soil samples were divided into six or seven layers, consisting of organic (L, F, and H horizons) and mineral soil (A, B, and C horizons) layers (Fig. 2). Cross-sectional observation of each layer was conducted to identify the most medial point between layers, from which several sub-samples were collected. Samples were placed in polyethylene bags and preserved at 4°C or lower until the day before analysis. Samples were then cultured in an incubator (MIR-153, Sanyo) overnight at 25°C.

For measurement of soil respiration rate, the granule size of soil samples from each horizon was reduced to <2 mm using sterilized scissors. For soil chemical analyses, the granule size of soil samples <2 mm was further reduced to <0.5 mm with a grinder (WB-1, Osaka Chemical). Soil samples were analyzed for pH level and moisture, carbon (C), and nitrogen (N) content (Table I).

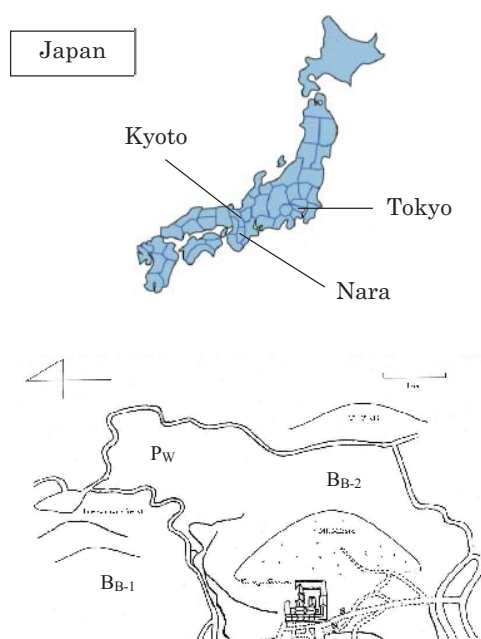


Fig. 1 Kasuga-yama Hill Primeval Forest in Nara, Japan

**Abstract**—We assessed the ecology of the organic and mineral soil layers of laurel-leaved ( $B_{B-1}$ ) and *Cryptomeria japonica* ( $B_{B-2}$  and  $P_w$ ) forests in the Kasugayama Hill Primeval Forest (Nara, Japan). The soil respiration rate was higher in the deeper horizons (F and H) of organic layers than in those of mineral soil layers, suggesting organic layers may be where active microbial metabolism occurs. Respiration rates in the soil of  $B_{B-1}$ ,  $B_{B-2}$  and  $P_w$  forests were closely similar at 5 and 10°C. However, the soil respiration rate increased in proportion to temperatures of 15°C or above. We therefore consider the activity of soil microorganisms to markedly decrease at temperatures below 10°C. At a temperature of 15°C or above, the soil respiration rate in the  $B_{B-1}$  organic layers was higher than in those of the  $B_{B-2}$  and  $P_w$  organic layers, due to differences in forest vegetation that appeared to influence several salient soil properties, particularly pH and the carbon (C) and nitrogen (N) content of the F and H horizons.

**Keywords**—Forest soil, mineralization rate, soil respiration rate.

## I. INTRODUCTION

THE Kasugayama Hill Primeval Forest in Nara, Japan, is a World Heritage Site consisting of lowland laurel-leaved forest where natural conditions have been preserved for more than 1,000 years [1]. This primeval forest has attracted considerable attention for the number of unique species of plants, animals, and insects found nowhere else on earth [1]. However, the microbial ecology of the soil supporting this forest has not been studied in detail.

Soil respiration is the absorption of oxygen and release of carbon dioxide generated through decomposition of organic soil matter by soil microorganisms to obtain energy [2], [3]. The soil respiration rate is an important index for determining the rate of metabolism and bioactivity of whole soil organic matter.

Here, to estimate the decomposition rate of soil organic matter mediated exclusively by soil microorganisms, the respiration rate of soil containing heterotrophs was measured *in vitro* [3], [4]. In addition, the annual soil respiration and mineralization rate of the soil organic matter decomposed by soil microorganisms were estimated.

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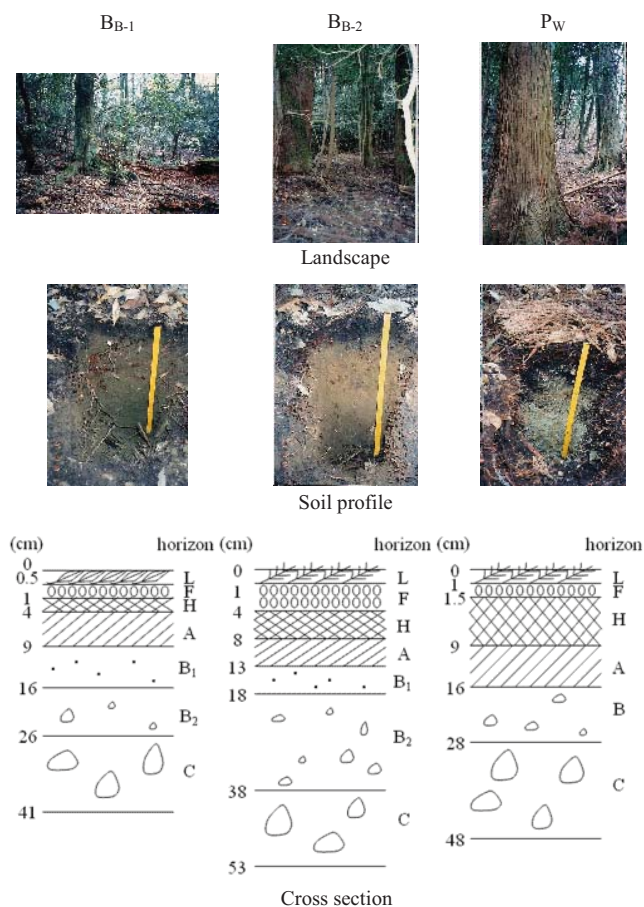


Fig. 2 Landscape, soil profile, and cross section of laurel-leaved, *C. japonica*, and Hideyoshi *C. japonica* forests

TABLE I  
PROPERTIES OF LAUREL-LEAVED, *C. JAPONICA*, AND HIDEYOSHI *C. JAPONICA* FOREST SOILS

Vegetation	Horizon	Moisture Content (%)	pH (H <sub>2</sub> O)	Total C (g kg <sup>-1</sup> )	Total N (g kg <sup>-1</sup> )	C/N ratio	Volume weight (g cm <sup>-3</sup> )
BB-1	L	13.7	4.52	467.3	12.6	37.1	0.048
	F	43.8	4.34	402.2	16.9	23.8	0.180
	H	56.5	4.51	318.9	16.9	18.9	0.297
	A	42.5	3.88	189.1	10.5	18.0	0.685
	B <sub>1</sub>	25.0	3.64	38.2	2.0	19.1	0.823
	B <sub>2</sub>	20.0	4.37	21.8	1.2	18.2	1.048
	C	17.6	4.20	12.2	0.6	20.3	1.301
BB-2	L	13.7	5.04	498.7	5.8	89.1	0.032
	F	62.0	4.37	361.2	11.9	30.4	0.066
	H	53.4	3.87	201.4	9.5	21.2	0.155
	A	42.0	3.81	106.0	5.8	18.3	0.493
	B <sub>1</sub>	28.4	3.95	30.7	2.0	15.4	0.628
P <sub>w</sub>	B <sub>2</sub>	25.6	4.14	19.1	1.3	14.7	0.700
	C	22.5	4.31	8.8	0.6	14.7	0.890
	L	19.3	4.95	505.0	7.4	68.2	0.033
	F	65.6	4.04	464.0	13.4	34.6	0.108
	H	68.0	3.95	269.0	12.3	21.9	0.176
P <sub>w</sub>	A	68.4	3.58	142.0	6.4	22.2	0.699
	B	19.1	4.23	13.2	0.7	18.9	0.815
	C	15.7	4.05	5.8	0.4	14.5	1.211

### B. Measurements of Soil Respiration Rate

Fig. 3 shows the quantification of respiration rate in soil samples containing heterotrophs. Size-adjusted soil samples (L,

F, and H horizons: 20 g; A, B, and C horizons: 30 g) were placed in an Erlenmeyer flask. Sodium hydroxide solution (10 mL, 0.2N) was pipetted into a small beaker, which was then suspended inside the flask. Soil samples were cultured for 24 h in an incubator (MIR-153, Sanyo) at 5, 10, 15, 20, and 25 °C. Carbon dioxide generated from soil samples was absorbed by the sodium hydroxide solution. After cultivation, barium hydroxide was added, and barium carbonate was precipitated. After the addition of several drops of phenolphthalein, the remaining sodium hydroxide was back-titrated with 0.1N hydrochloric acid until pink. Soil respiration rate (mg CO<sub>2</sub> 100 g<sup>-1</sup> day<sup>-1</sup>) was measured three times, and differences from experimental values were calculated.

The fixed numbers (a, b) and correlation coefficient (r) between soil respiration rate of each horizon and soil temperature were determined using

$$\text{Log } Y = a T + b \quad (1)$$

where Y denotes soil respiration rate (g CO<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup>), T denotes soil temperature (°C), and a/b denotes fixed number.

Soil respiration per unit area (t C ha<sup>-1</sup> y<sup>-1</sup>) was then substituted by the average monthly soil temperature of the Kasuga-yama Hill Primeval Forest and calculated using (1) and the thickness and volume weight of each horizon (Table I). The mineralization rate of the soil organic matter decomposed by soil microorganisms was then calculated using

$$\text{MR} = \frac{\text{SRC}}{\text{TOC}} \times 100 \quad (2)$$

where MR denotes mineralization rate (%), SRC denotes annual soil respiration carbon per unit area (t C ha<sup>-1</sup> y<sup>-1</sup>) and TOC denotes total soil organic carbon per unit area (t C ha<sup>-1</sup>).

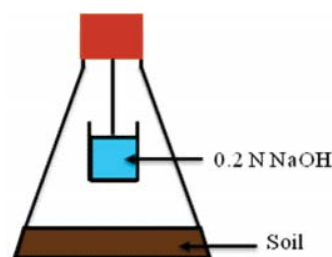


Fig. 3 Apparatus for measuring soil respiration rate

## III. RESULTS

### A. Soil Respiration Rate

In the soil of BB-1, BB-2 and P<sub>w</sub> forests, the respiration rate (Fig. 4) was higher in the organic layer (F and H horizons) than in the mineral soil layer. Comparison of the three organic layer horizons revealed that the soil respiration rate was lowest in the L horizon and highest in the F horizon.

Respiration rates were markedly similar at 5 and 10°C but increased in proportion to temperature at 15°C or above.

Further, at 15°C or above, the soil respiration rates were higher in the organic layers and A horizon of the mineral soil layer of B<sub>B-1</sub> samples than in those of B<sub>B-2</sub> and P<sub>w</sub> samples. In contrast, respiration rates of the B and C horizons of the mineral soil layer were relatively low, with no marked differences between forests. The soil respiration rate for P<sub>w</sub> soil samples from the F and H horizons of the organic layer and A horizon of the mineral soil layer were higher than those of B<sub>B-2</sub>. However, no markedly differences were observed in rates in the L horizon of the organic layer or deeper (B and C) horizons of the mineral soil layer.

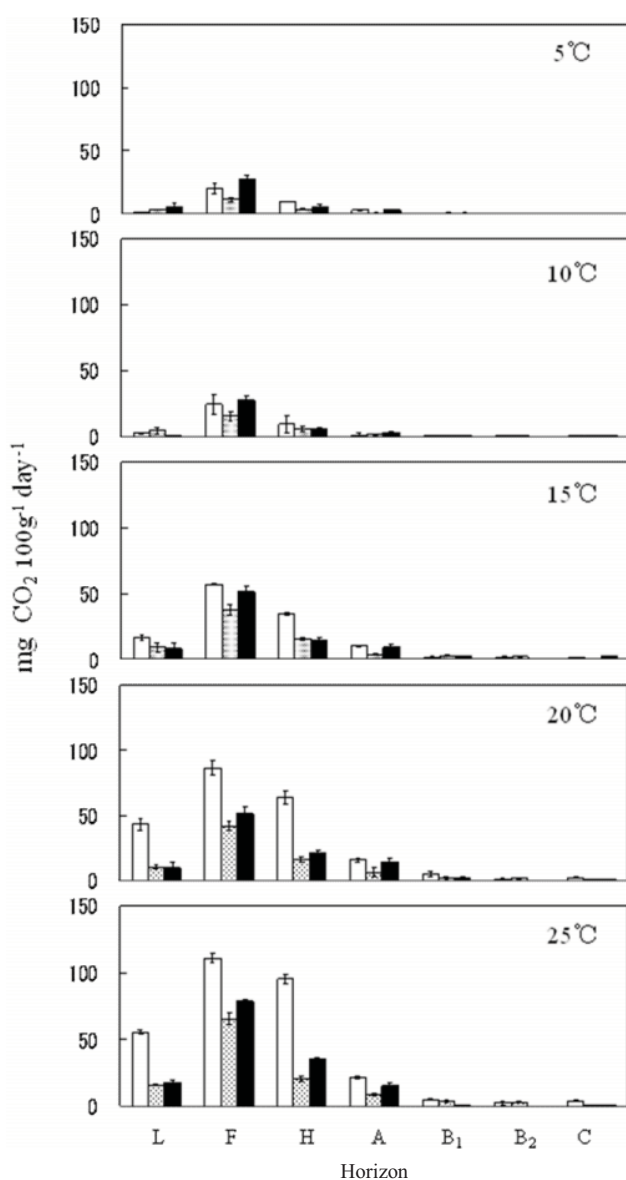


Fig. 4 Soil respiration rate at various temperature for laurel-leaved, *C. japonica*, and Hideyoshi *C. japonica* forest soils

The vertical distribution of soil respiration rate in the Kasugayama Hill Primeval Forest was similar to that in the *Betula ermanii* and *Tsuga diversifolia/Abies mariesii* forests [5]. The soil respiration rate peaked in the F horizon and was

higher in broad-leaved forest than in needle-leaved forest.

The soil respiration rate of the *B. ermanii* forests is reported to range from 27-40 (F horizon) and 9-15 (H horizon) mg CO<sub>2</sub> 100 g<sup>-1</sup> day<sup>-1</sup> (20°C) and that of *T. diversifolia/A. mariesii* forests from 9-22 (F horizon) and 3-6 (H horizon) mg CO<sub>2</sub> 100 g<sup>-1</sup> day<sup>-1</sup> (20°C) [5]. The soil respiration rate of laurel-leaved (B<sub>B-1</sub>) forests was 87 (F horizon) and 64 (H horizon) mg CO<sub>2</sub> 100 g<sup>-1</sup> day<sup>-1</sup> (20°C), and that of *C. japonica* (B<sub>B-2</sub> and P<sub>w</sub>) ranged from 42-52 (F horizon) and 16-22 (H horizon) mg CO<sub>2</sub> 100 g<sup>-1</sup> day<sup>-1</sup> (20°C). These rates were significantly higher than those of *B. ermanii* and *T. diversifolia/A. mariesii* forests due to the warmer climate of the Kasugayama Hill Primeval Forest, which contributes to an increased rate of respiration.

#### B. Correlation between Soil Respiration Rate and Soil Temperature

While soil respiration rate per unit area is known to have an exponential relationship with soil temperature, most respiration rate measurements thus far have been taken outdoors [6]-[9], with the relationship between temperature and respiration rate indoors remaining relatively unstudied.

We determined the fixed number (a, b) and correlation coefficient (r) between the soil respiration rate of each horizon by heterotroph and soil temperature of B<sub>B-1</sub>, B<sub>B-2</sub> and P<sub>w</sub> forests (Table II).

A significant equilateral correlation was observed between soil respiration rate by heterotroph and soil temperature of each type of vegetation. This result shows that the annual soil respiration rate can be estimated from the soil respiration rate by heterotroph, outdoor soil respiration rate, and thickness and volume weight of each horizon.

The monthly soil respiration rate (Fig. 5) was higher when ground temperature was higher in summer and lower in winter throughout the year. Monthly respiration rates in B<sub>B-1</sub> forests were higher than in B<sub>B-2</sub> and P<sub>w</sub> forests, and differences in rates between these two forests increased in summer and decreased in winter. The monthly soil respiration rate of the P<sub>w</sub> forest was higher than that of the B<sub>B-2</sub> forest. Differences in respiration rates between B<sub>B-2</sub> and P<sub>w</sub> remained fairly constant value regardless of ground temperature.

The annual soil respiration rate of each type of vegetation was 44.3 t CO<sub>2</sub> ha<sup>-1</sup> y<sup>-1</sup> (B<sub>B-1</sub>), 20.8 t CO<sub>2</sub> ha<sup>-1</sup> y<sup>-1</sup> (B<sub>B-2</sub>), and 32.1 t CO<sub>2</sub> ha<sup>-1</sup> y<sup>-1</sup> (P<sub>w</sub>).

#### C. Mineralization Rate of Soil Organic Matter

Table III shows the mineralization rate of soil organic matter decomposed by soil microorganisms. The mineralization rate of the B<sub>B-1</sub> forest was higher than that of B<sub>B-2</sub>, and that of the P<sub>w</sub> forest was higher than that of the B<sub>B-2</sub> forest.

TABLE II

THE FIXED NUMBER (A, B) AND CORRELATION COEFFICIENT (R) BETWEEN SOIL RESPIRATION RATE AND SOIL TEMPERATURE OF LAUREL-LEAVED, *C. JAPONICA*, AND HIDEYOSHI *C. JAPONICA* FOREST

Horizon	B <sub>B-1</sub>		
	a	b	r
L	0.083	-2.819	0.959**
F	0.040	-0.964	0.978**
H	0.055	-0.419	0.967**
A	0.043	-0.151	0.992**
B <sub>1</sub>	0.059	-0.908	0.944*
B <sub>2</sub>	0.036	-0.487	0.984**
C	0.036	-0.040	0.942*
Total	0.044	0.407	0.994**

Horizon	B <sub>B-2</sub>		
	a	b	r
L	0.029	-2.053	0.977**
F	0.040	-0.848	0.977**
H	0.042	-0.792	0.973**
A	0.048	-0.845	0.974**
B <sub>1</sub>	0.043	-0.857	0.927*
B <sub>2</sub>	0.053	-0.784	0.933*
C	0.037	-0.619	0.988**
Total	0.040	0.135	0.929*

Horizon	P <sub>W</sub>		
	a	b	r
L	0.025	-1.922	0.966**
F	0.024	-0.980	0.961**
H	0.034	-0.261	0.950*
A	0.039	-0.013	0.939*
B	0.019	-0.224	0.520
C	0.026	-0.142	0.472
Total	0.031	0.498	0.881*

(Significant difference: \* 5 % \*\* 1 %, n = 5)

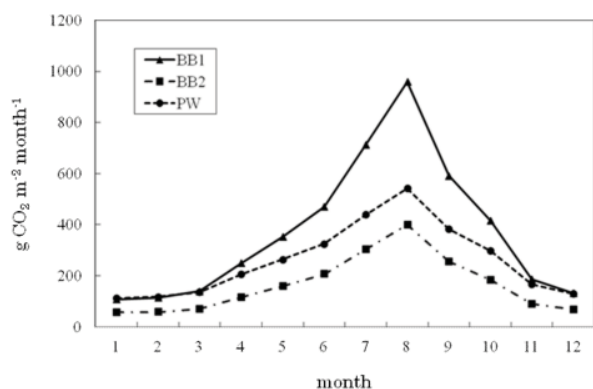


Fig. 5 Average monthly soil respiration rate of laurel-leaved, *C. japonica*, and Hideyoshi *C. japonica* forest

TABLE III

MINERALIZATION RATE (MR) OF THE SOIL ORGANIC MATTER (TOC) OF LAUREL-LEAVED, *C. JAPONICA*, AND HIDEYOSHI *C. JAPONICA* FOREST

Vegetation	SRC (t C ha <sup>-1</sup> yr <sup>-1</sup> )	TOC (t C ha <sup>-1</sup> )	MR (%)
B <sub>B-1</sub>	12.1	287.2	4.21
B <sub>B-2</sub>	5.7	157.0	3.62
P <sub>W</sub>	8.8	228.2	3.73

#### IV. DISCUSSION

The soil respiration rate was higher in the deeper horizons (F and H horizons) of organic layers than in mineral soil layers for all forest types, suggesting that active microbial metabolism takes place in the organic layers. The uppermost L horizon consists of high levels of fresh plant residue and high C/N ratio, but low levels of moisture content (Table I) and microorganisms [10]. In the F horizon, the proportion of microorganisms that absorbed plant nutrients increased as decomposition progressed. In the H horizon, which contains decayed organic matter, the proportion of these microorganisms in the soil decreased. This finding is consistent with the number of bacteria and fungi obtained by the dilution-plate count method [10].

In the soil of both laurel-leaved and *C. japonica* forests, the soil respiration rate at 5 and 10°C was relatively low but increased in proportion to temperature at 15°C or above. We believe the activity of soil microorganisms decreases at temperatures less than 10°C. At 15°C or above, laurel-leaved organic layers exhibited more soil microorganism activity than *C. japonica* organic layers, possibly due to differences in forest vegetation, which appear to influence several salient soil properties—particularly the pH and C and N content in the F and H horizons (Table I).

*In vitro* comparison of mineralization rates of soil organic matter showed that the soil in laurel-leaved forest had faster metabolic activity than that in the *C. japonica* forest, which was also due to differences in forest vegetation. This result is consistent with the fact that laurel-leaved forest soil generally contains more easily decomposable organic matter, such as water-soluble polysaccharides, hemicellulose, cellulose, and protein, and less decomposable organic matter, such as lipid and resin. Similarly, the soil of the Hideyoshi *C. japonica* forest also had faster metabolic activity than that of the *C. japonica* forest, as while lipid, resin, and cellulose content was similar between the two, the Hideyoshi *C. japonica* forest's soil contained more water-soluble polysaccharides, hemicellulose, and protein.

#### V. CONCLUSIONS

The soil respiration rate of the organic and mineral soil layers of the laurel-leaved and *C. japonica* forests in the Kasuga-yama Hill Primeval Forest (Nara, Japan) are influenced by forest vegetation. Mineralization rates of the soil organic matter revealed that the soil of the laurel-leaved forest had faster metabolic activity than that of the *C. japonica* forest.

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