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Automated foliage chamber method for long-term measurement of CO_2 flux in the uppermost canopy

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ABSTRACT

Direct monitoring of CO_2 exchange in forest ecosystems is necessary for evaluation of the annual net CO_2 flux. We developed an automated foliage chamber system for long-term *in situ* measurement of the CO_2 flux in foliage. The chamber closes automatically for 5 min in every 30. We found a high correlation between the photosynthesis rates measured by this system and by a portable H_2O/CO_2 analyzer (LCA-3, ADC). We used the new system to continuously monitor the CO_2 flux in foliage of *Quercus serrata*. There was a positive correlation between CO_2 flux and short-wave radiation. The daily photosynthesis rate varied widely depending on the environmental conditions. It increased from May to July and decreased temporarily in August. The seasonal pattern of photosynthesis was almost the same as that measured by the eddy covariance method. These results suggest that the new system is effective for tracking long-term seasonal change of the CO_2 flux in foliage.

1. Introduction

Deciduous and evergreen mixed broadleaf forests grow widely in Japan. Deciduous and evergreen trees have individual seasonal patterns of CO_2 flux (Kozlowski and Pallardy, 1997). Yamashiro Experimental Forest is a typical mixed forest with a dramatic seasonal variation in CO_2 flux. By use of the eddy covariance method, we recently measured negative CO_2 flux even before leaf emergence, and observed a rapid increase in response to the leafing process of deciduous trees (Kominami et al., 2001).

To understand how deciduous and evergreen trees in Yamashiro Experimental Forest control CO_2 flux, we need to measure CO_2 flux directly. CO_2 flux in a tree can be studied in several ways, but long-term observation in the field is limited by the difficulty of obtaining measurements.

For taking continuous measurements, an automated chamber is effective (Kakubari and Yamamoto, 1987; Goulden and Crill, 1997). Open-top chambers are attractive but are heavy and expensive to maintain, and unsatisfactory for long-term measurement (Wong and Dunin, 1987; Yokota and Hagiwara, 1995). The branch bag technique is simple and the cost is low (Dufrêne et al., 1993; Saugier et al., 1997), and these features make it suitable for measurement of spatial variation, but its durability is not good enough for long-term measurement at our site. Moreover, dark respiration measurements using the branch bag technique were inaccurate, being disrupted by wind, which exacerbated leaks (Dufrêne et al., 1993).

One of the most important considerations in continuously measuring the long-term CO_2 flux at our site is durability, because the site has two rainy seasons and typhoons every year. Thus, we have developed a new, simplified, static foliage chamber system. The system uses an automated foliage chamber to measure seasonal patterns of CO_2 flux in foliage. This paper

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describes the new system and its use for long-term monitoring in the field.

2. Methods

The foliage chamber opens and closes automatically by means of a motor with an electromagnetic brake, under the control of a programmable controller. The bottom face of the chamber closes for 5 min in every 30, so that the CO_2 flux can be measured. Figures 1 and 2 show the automatic foliage chamber, the ventilation fan in the chamber, the cooling fan above the chamber and the infrared CO_2 gas analyzer (LI-800, Li-Cor, Lincoln, NE, USA). The LI-800 is an absolute, non-dispersive, infrared gas analyzer based on a single-path, dual-wavelength infrared detection subsystem. It uses digital signal processing to determine the temperature and pressure-corrected CO_2 concentration on the basis of optical bench signals through the use of a ratio technique. The optical bench is maintained at a constant temperature of 52 °C by a

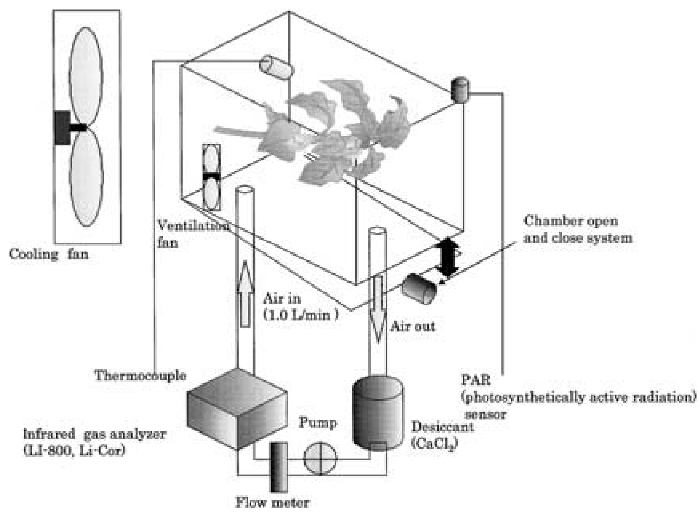

Fig. 1. Diagram of the foliage chamber.

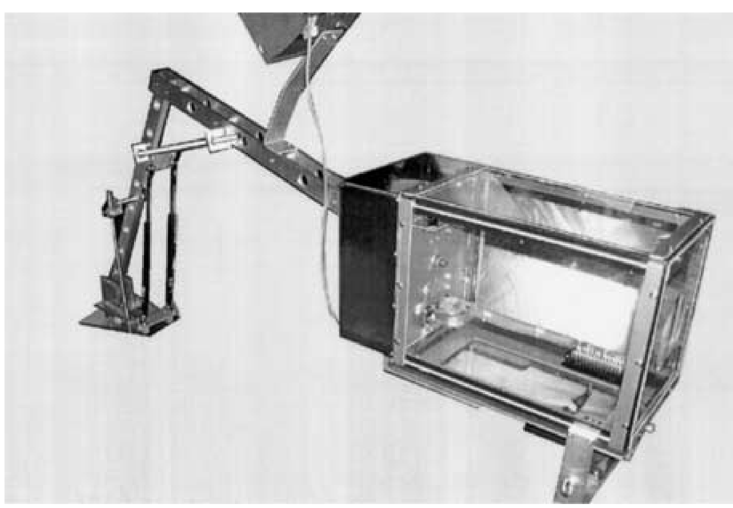

Fig. 2. The automated foliage chamber.

heater which, together with a desiccant ($CaCl_2$), minimizes the influence of moisture. The size of the foliage chamber is 36 × 50 × 30 cm and the volume is 54 000 cm^3. The chamber is made of glass and slender stainless steel frames. The daily mean light intensity inside the chamber is 96% of that outside the chamber.

We selected this system for the following reasons: (1) high durability of the weatherproof chamber and closed air circuit, (2) greatly reduced cost of power and instruments when compared to a large open-top chamber, (3) ease of adding chambers using electromagnetic valves and a programmable controller unit when we duplicate the system to measure spatial variation in the future, and (4) minimal greenhouse effect and CO_2 shortage for plants in the brief measurement time.

Foliage is inserted into the chamber through a small window and sealed with a vinyl sheet. CO_2 flux in the foliage (F_f) was calculated from the following equation:

$$F_f = \rho V / A \Delta c / \Delta t, \qquad (1)$$

where ρ is the CO_2 gas density, V is the volume of the system, A is total leaf area of the enclosed foliage, and $\Delta c / \Delta t$ is the rate of increase of CO_2 concentration (c) with time (t).

A is calculated as the product of mean leaf area (confidence interval, 10%) by the number of leaves in the chamber. $\Delta c / \Delta t$ is calculated from the difference in CO_2 concentration between 30 and 210 s (3 min), because after the chamber is closed, it takes more than 20 s for the change in air concentration to reach the infrared gas analyzer.

To verify the effectiveness of the ventilation fan, we measured the time required to ventilate 1000 ppm CO_2 from the chamber. We also compared the temperature between the sealed chamber with the cooling fan and the chamber without the fan in order to adjust the fan for effective cooling on a fine summer day, 11 August 2000.

On 11 May 2002 we compared the diurnal fluctuation of CO_2 flux of *Quercus serrata* foliage measured by the foliage chamber system with that measured by a portable H_2O/CO_2 analyzer (LCA-3, Analytical Development Co. Ltd, Hoddesdon, Herts, UK). The LCA-3 is a battery-powered, portable, integrated, open system for measuring transpiration and photosynthesis. Fresh leaves were clipped into the leaf chamber for each measurement, and the mean value of three measurements was compared with the value measured by the foliage chamber system. To compare CO_2 flux exactly, the leaf area was measured with a scanner directly in this comparison.

CO_2 flux was monitored continuously with the foliage chamber system from 29 May to 7 December 2001 in Yamashiro Experimental Forest. The chamber faced south, and self-shading of the chamber was less than 10% of leaf area. The maximum number of leaves enclosed in the chamber was 128 (1572.9 cm^2). The air temperature and CO_2 concentration in the chamber were recorded every 10 s. The outside air temperature, relative humidity and short-wave radiation (R_s) were recorded every 5 min at a meteorological tower. Moreover, to interpret leaf physiology using this foliage chamber, the inside relative humidity and soil moisture will now be monitored.

Before long-term monitoring, the foliage chamber was filled with high-concentration CO_2 gas for 1 h to test for leaks. To minimize the risk of leaks, the system was lightly pressurized and tested once a month. The glass was cleaned at least once a week, and the LI-800 was calibrated to zero and 400 ppm CO_2 once a month.

3. Site description

Yamashiro Experimental Forest is located in a valley in Yamashiro-cho (34°47′N, 135°51′E), Soraku-gun, Kyoto, in a hilly, mountainous region of western Japan at an altitude of about 220 m (Table 1). The site area is 1.6 ha. The valley is underlain by weathering

Table 1. *Characteristics of the study site*

Location	Yamashiro-cho, Soraku-gun, Kyoto, Japan, 34°47′N,135°51′E
Altitude	220 m
Tree types:	
Deciduous broadleaf	*Quercus serrata*, *Lyonia neziki*
Evergreen	*Ilex pedunculosa*, *Eurya japonica*
Average tree height	12 m
Forest soil	Immature soil
Tower height:	
Eddy covariance method	26 m
Chamber method	10 m
Average temperature	15.8 °C
Average relative humidity	74.6%
Annual precipitation	1627.0 mm

granites, and the soil layer is generally thin, immature and sandy. The forest consists of deciduous broadleaved species (mainly *Q. serrata*) and evergreen broadleaved species (mainly *Ilex pedunculosa*). Biometric and optical techniques were used to measure biomass density, leaf area index (LAI), net primary productivity and litter fall. Trunk diameters were measured to show tree growth every week. The total basal areas were 6.3 m^2 ha^{-1} for evergreen species and 13.3 m^2 ha^{-1} for deciduous species. The tree density was 5953 stems ha^{-1}. LAI was measured with an LAI-2000 plant canopy analyzer (Li-Cor) and was estimated to be about 3 in the leafy season. The average canopy height was 12 m. To estimate CO_2 flux by the eddy covariance method, we built two meteorological towers (25 and 35 m high) in the forest. Eddy covariance was measured at the top of each meteorological tower (Kominami et al., 2001). Another steel tower 10 m high was built in the forest on a ridge. The automatic foliage chamber was installed at the top of the steel tower.

4. Results and discussion

4.1. Use of automatic foliage chamber system

The effect of the ventilation fan is shown in Fig. 3. The 1000-ppm CO_2 gas was exhausted from the ventilated chamber in less than 30 s. However, it took 5 min to reduce the concentration to the minimum in the unventilated chamber.

Figure 4 indicates that the cooling fan was effective in reducing air temperature inside the chamber on 11 August 2000. The maximum R_s on the day was 1092 W m^{-2}. However, temperature differences between inside and outside of several degrees were common at higher temperatures. For example, a ΔT of >3 °C was recorded between 14:00 and 15:20 h. This result prompted us to improve the wind direction and the cooling fan to cool the side panels. On the basis of a temperature–photosynthesis curve (300 ppm CO_2; adequate light; young potted plant), Kai (1984) reported that the optimum temperature for photosynthesis in *Q. serrata* was approximately 30 °C. The photosynthesis rate decreased between 35 and 40 °C. Thus, overheating may cause large underestimates in rate, which must be compensated for. However, temperatures of >35 °C are uncommon at our site, and field measurement includes many kinds of environment effect, so no compensation was performed.

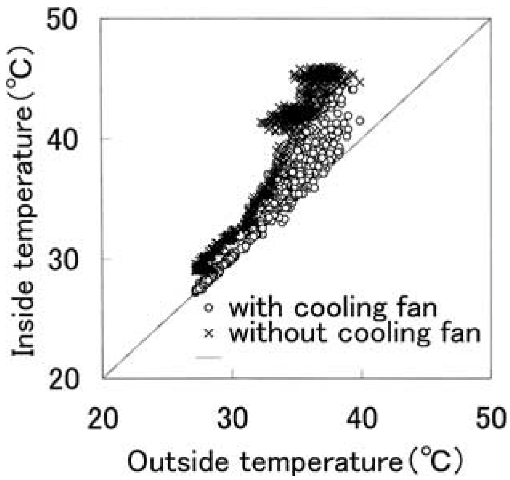

Fig. 4. Effect of the cooling fan.

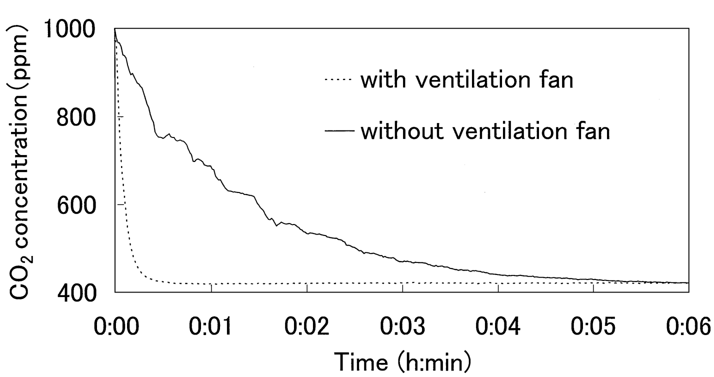

Fig. 3. Effect of the ventilation fan.

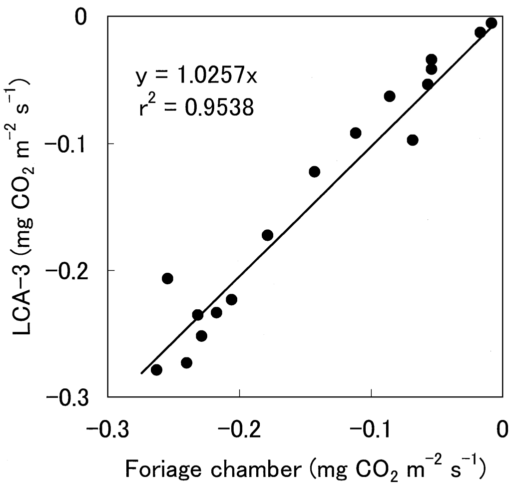

Fig. 5. Relationship between values derived from the LCA-3 and the foliage chamber.

Figure 5 compares CO_2 flux rates measured by the foliage chamber system and the LCA-3. The values were in close agreement ($r^2 = 0.9538$). This result indicates that the foliage chamber system can predict the mean value of the LCA-3.

4.2. *Measurement of daily variation*

Figure 6 shows the typical daily variations in CO_2 flux in *Q. serrata* foliage (F_f), CO_2 flux above the canopy (F_c) measured by the eddy covariance method, downward R_s and air temperature (T) from 28 June to 2 July 2001. F_f on fine days was negative because of photosynthesis. F_f responded to R_s even for brief small fluctuations. The relationship between R_s and F_f in the daytime for 4 days (Fig. 7) shows a strong correlation.

The patterns of diurnal variation in F_f and F_c are similar (Fig. 6). The times of increase, maximum increase and duration of decrease of CO_2 flux all agree. However, nocturnal variation is different. F_f was approximately constant, and the absolute value of nocturnal respiration rate was approximately one-seventh or less that of the diurnal net photosynthesis. On the other hand, nocturnal F_c was variable and indicated sharp fluctuation. F_c might be affected by local advection (Kominami et al. 2002) at night, when stability is high. This suggests that verification using the chamber method is effective for presuming an accurate CO_2 flux in the forest under complex geographical features

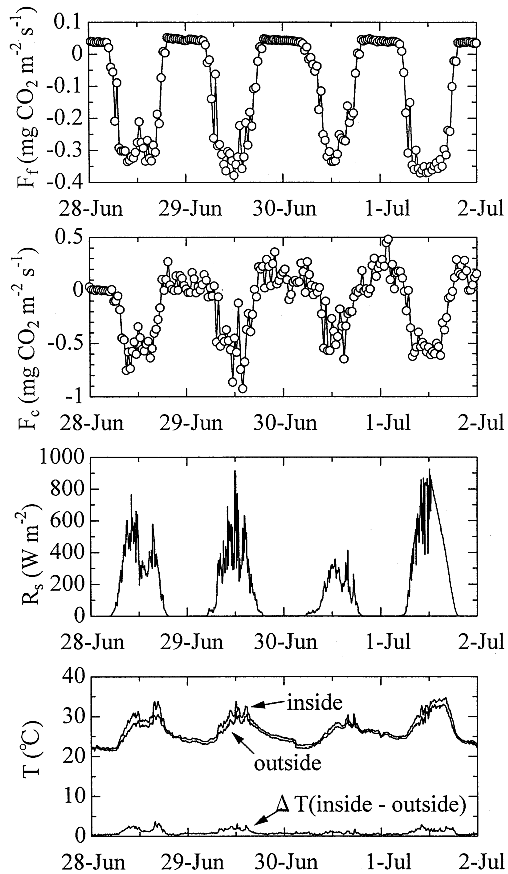

Fig. 6. Typical daily variations CO_2 flux in foliage (F_f), CO_2 flux above canopy (F_c), downward short-wave radiation (R_s), and air temperature (T).

such as those present in the Yamashiro Experimental Forest.

The air temperature difference between inside and outside the chamber (ΔT) is shown at the bottom of

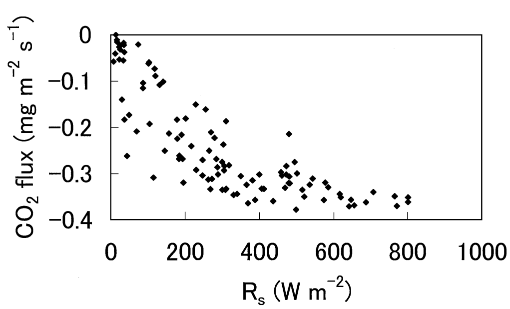

Fig. 7. Relationship between short-wave radiation (R_s) and foliage CO_2 flux (F_f).

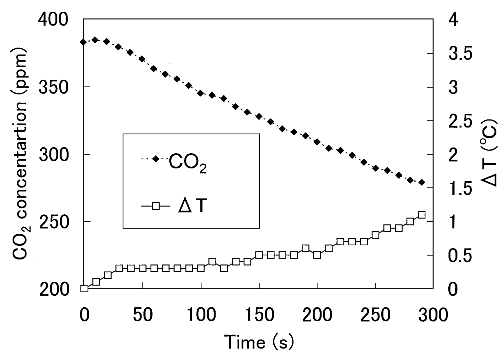

Fig. 8. Decrease in CO_2 and increase in ΔT with time after chamber closure at a high irradiance (817 W m^{-2}, 27 °C).

Fig. 6. The average ΔT for four summer days was 1.0 °C. The actual ΔT in field measurements might be reduced by the effects of the cooling and ventilation fans.

Figure 8 gives an example of the decrease in CO_2 and the increase in ΔT with time during an assimilation measurement under high irradiance (817 W m^{-2}, 27 °C). The decrease in CO_2 was almost linear over the measurement period (approximately 21 ppm min^{-1}). The increase in air temperature was 0.22 °C min^{-1}. The relationship at noon was checked once a week and was always almost linear. In normal use, the CO_2 level and the temperature inside the chamber may be acceptable. Although the relationship was almost linear, the initial rate of decrease was a little faster than the rate near the end of the 5-min period. This phenomenon might be caused by the decrease in CO_2 concentration in the chamber, because the rate of carbon assimilation depends on the concentration of CO_2. To more correctly estimate CO_2 flux, a correction for CO_2 concentration decrease might be needed.

Figure 9 shows the relationship between net CO_2 assimilation rate and CO_2 concentration over 3 min. At steady high irradiance, the relationship was linear. On the basis of an ambient CO_2 concentration–photosynthesis curve (25 °C; 40 klx), Shirahata and Hashimoto (1992) reported that the net photosynthesis rate of *Q. serrata* cuttings increased linearly with ambient CO_2 concentration up to 1000 ppm, and then reached a plateau at about 1500 ppm. Maruyama and Liang (1995) reported the same linearity in young potted plants of several C_3 species between 0 and 900 ppm (25 °C; PAR of 1200 μmol m^{-2} s^{-1}). These results indicate that this linear relationship can be used to correct decreases in CO_2 concentration.

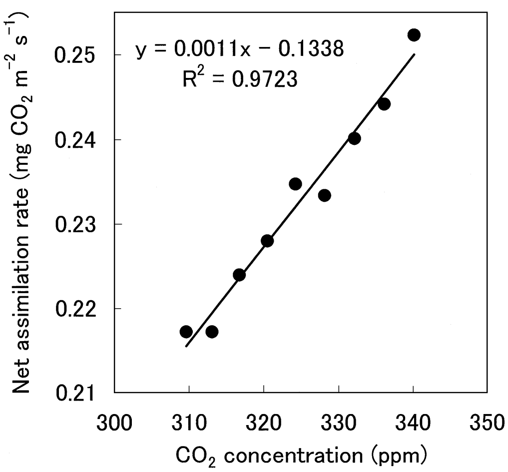

Fig. 9. Relationship between net CO_2 assimilation rate and CO_2 concentration after chamber closure at high irradiance (817 W m^{-2}, 27 °C).

4.3. Measurement of seasonal variation

The automatic foliage chamber system operated for 92.1% of the time. Lightning interrupted measurement by cutting off the electricity, but there was no damage at any time. The chamber has survived weather hazards for a growing season and appears reliable with respect to seasonal variations.

Figure 10 shows the seasonal variations in diurnal net photosynthesis (P_n, estimated from the negative value of F_f) and daily average of downward R_s. P_n was closely related to R_s, and varied widely from day to day. In particular, both decreased with the advancing season. This result suggests that P_n as measured by the foliage chamber is controlled mainly by R_s in autumn. Linder and Troeng (1980) reported that shortening days and low irradiance in autumn reduced the rate of photosynthesis. P_n increased rapidly from late May to July and decreased temporarily in the rainy season (June to early July and late September) and August. Maximum P_n was recorded on 3 July 2001, 11.79 mg CO_2 m^{-2} d^{-1}. Kakubari and Yamamoto (1987) recorded the maximum P_n of sun-leaves in July and that of shade-leaves in August, and found that P_n increased more rapidly in sun-leaves than in shade-leaves. To estimate correctly the seasonal variation of CO_2 flux in the whole tree, both sun-leaves and shade-leaves should be monitored. The low-cost foliage chamber system may be appropriate for measuring such spatial variations.

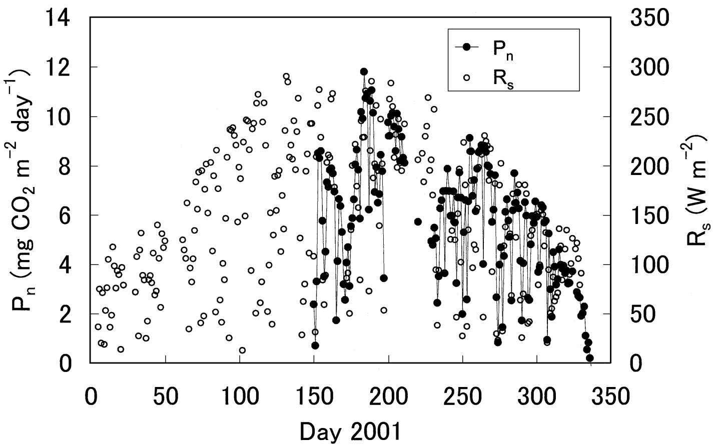

Fig. 10. Seasonal variations in diurnal net photosynthesis (P_n) and daily average short-wave radiation (R_s).

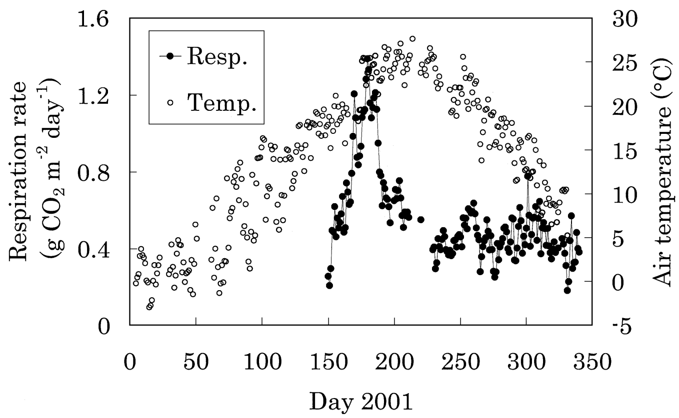

Fig. 11. Seasonal variations in respiration rate and average air temperature at night.

Figure 11 shows seasonal variations in respiration rate (estimated from the positive value of F_f) and average air temperature at night. The respiration rate at night varied rapidly at the beginning of the growing season. A rapid increase in respiration at the same time has been observed in other species (Kakubari and Yamamoto, 1987; Kozlowski and Pallardy, 1997). The highest night temperatures were recorded from July to August, even though the highest dark respiration rate was recorded at the end of June. As a result, there was a negative correlation between temperature and respiration for a short period after the peak of respiration. The relationship between temperature and respiration rate at night is shown in Fig. 12. This figure indicates that the relationship differs according to season.

Figure 13 shows seasonal variations in the number of leaves in the chamber and LAI. Variations in the

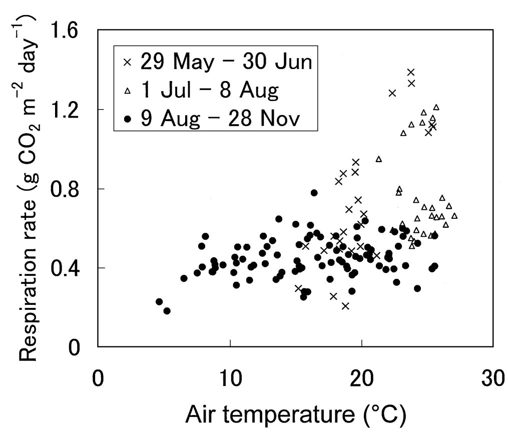

Fig. 12. Relationship between temperature and respiration rate at night.

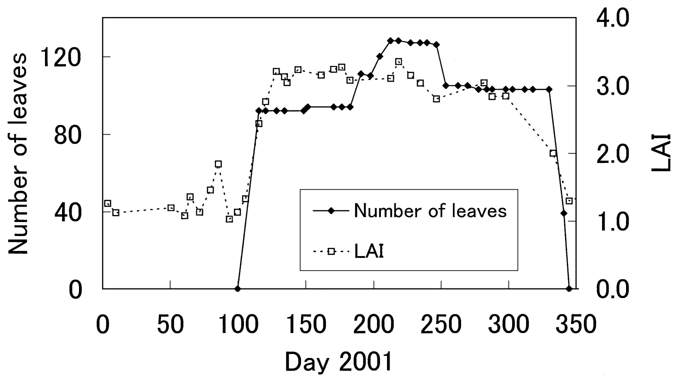

Fig. 13. Seasonal variations in leaf count and LAI.

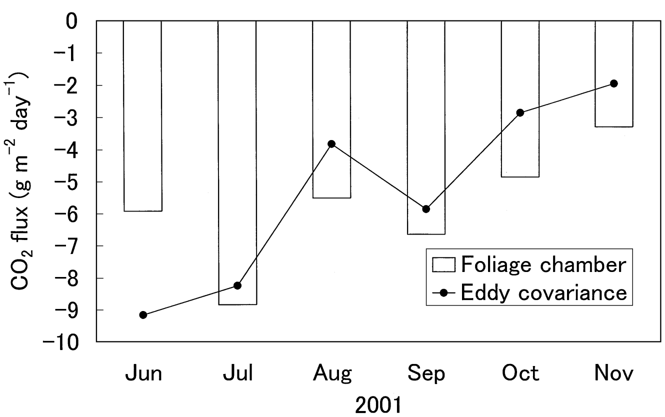

Fig. 14. Comparison between foliage chamber method and eddy covariance method in measuring CO_2 flux.

two parameters agree closely. Both began to increase rapidly to a maximum in late April. A second increase in the number of leaves was recorded in July. The seasonal variation in respiration had two peaks (29 June and 9 September, Fig. 10), which came about 2 months after the leaf increases. The rapid increase in respiration might be caused by the high metabolic demand of producing leaves. The absolute value of nocturnal respiration rate is lower than that of the diurnal photosynthesis rate significantly. However, these results indicate that the seal of the chamber was effective in order to measure the seasonal variation of nocturnal respiration.

Figure 14 compares the monthly average values of CO_2 flux measured by the foliage chamber method and the eddy covariance method. The seasonal variations are similar. Both methods showed a decrease in August. However, the foliage chamber value in June is much lower than that of the eddy covariance method. There is possibility of that net photosynthesis rate of mature evergreens is high in June. Unfortunately, the foliage chamber system was not operated on another species. This result suggests that it is necessary to compare the seasonal patterns of F_c and that of F_f on several species.

Monitoring CO_2 flux by the eddy correlation method CO_2 flux over a forest on complex topography is difficult, especially during rainfall and at night (Kominami et al., 2001). In contrast, we found the newly developed foliage chamber system to be useful for monitoring it even during the rainy season and at night at a reasonable cost. Longer continuous observation is necessary to estimate the characteristics of CO_2 flux in the Yamashiro Experimental Forest. It is also necessary to evaluate the differences in CO_2 flux characteristics by season between sun-leaves and shade-leaves, and between evergreen and deciduous species. The automated foliage chamber may be useful for the evaluation.

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