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TELLUS

Development of an automatic chamber system for long-term measurements of CO_2 flux from roots

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ABSTRACT

To separate CO_2 efflux from roots (R_r) and soil (R_s), we developed a system to measure R_r continuously. Using this system, seasonal variation in R_r was obtained in a temperate forest in Japan. We measured R_s, CO_2 efflux from mineral soil (R_m) and environmental factors simultaneously, and the characteristic and seasonality of R_r were analysed in comparison with R_s. R_r and R_s showed different responses to soil water content: R_s decreased with decreasing soil water content, whereas R_r peaked at relatively low soil water content. R_r/R_s decreased from 64.8% to 27.3% as soil water content increased from 0.075 to 0.225 cm cm^{-3}. The relationship between respiration and temperature appears to change seasonally in response to phenological and biological factors. R_r showed clear seasonal variation as a function of soil temperature. During the growing period, R_r exhibited a higher rate at the same soil temperature than during other periods, which may be due to phenological influences such as fine root dynamics. R_s decreased during the summer despite high soil temperatures. The seasonal peak for R_s occurred earlier than that for soil temperature. R_r/R_s ranged between 25% and 60% over the course of the year.

1. Introduction

Belowground processes play an important role in the carbon cycle of the biosphere. Soil respiration is the main pathway for carbon moving from the ecosystem into the atmosphere (Ryan and Law, 2005) and can strongly influence net ecosystem exchange. Therefore, soil–surface CO_2 efflux has been measured in many ecosystems (Crill, 1991; Lavigne et al., 1997). However, the efflux from a soil surface is an assemblage of multiple belowground processes such as decomposition respiration and root respiration. As a result of variations in these processes, CO_2 efflux has large spatial variability (Nakane and Lee, 1995), and it is also difficult to describe the physical processes responsible for these variations in soil respiration. According to Hanson et al. (2000), about half of soil respiration derives from metabolic activity to support and grow roots and their associated mycorrhizae. Much of the remainder is composed of heterotrophic respiration (Trumbore, 2000).

To understand the CO_2 budget of a forest ecosystem, it is important to evaluate CO_2 efflux from the soil accurately by separating autotrophic from heterotrophic respiration. In this context, autotrophic respiration means root respiration. Many reports deal with the separation of soil–surface CO_2 into autotrophic and heterotrophic respiration. In a review by Hanson et al. (2000), the authors concluded that the contribution of CO_2 efflux from roots (R_r) to total soil CO_2 efflux (R_s) might average approximately 48.5% in a forest ecosystem, but that this ratio varied widely (between 10% and 90%) depending on the measurement methods, forest type, season and location.

Various methods have been developed to separate R_r from R_s, including direct measurement of CO_2 fluxes from sample roots using a chamber (Dannoura et al., 2005). This method has the advantage that the respiration exclusively by roots can be measured, without confounding influence due to the presense of soil. However, in this sampling method, the roots are excavated from the soil and cut to fit within the chamber. As a result, continuous

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measurements are impossible and results may be biased by the impact of cutting.

The main alternative to this approach involves indirect measurement of R_r. In this approach, R_r is calculated as the difference in total R_s with and without root exclusion by means of root removal, trenching, gap creation and other methods (Nakane et al., 1996; Ohashi et al., 2000). This approach permits continuous measurements (Lee et al., 2003; Tang et al., 2005), but the influence of dead roots is included. This is a significant problem because dead roots can become major source of heterotrophic respiration.

Variation in the $^{13}C/^{12}C$ ratio is used in the isotope method for estimating R_r (Andrews et al., 1999; Rochette et al., 1999). This method permits continuous measurements (Bhupinderpal-Shingh et al., 2003) with the least amount of disturbance to the soil and roots. However, this method generally yields lower rhizosphere contributions than those obtained using other methods, and there are uncertainties about how quantitative these methodologies are when used in the field (Hanson et al., 2000).

Thus, each method of measuring R_r has specific difficulties and uncertainties. In particular, a method which permits continuous measurements with a high time resolution must be developed if the method is to be suitable for evaluating the influence of environmental factors. Dannoura et al. (2006b) reported that the smaller the root diameter, the higher the R_r value per unit root weight, independent of tree species and tree size. From the R_r values per unit area that were calculated by these authors using CO_2 flux per unit weight and root biomass in each diameter class, fine roots that accounted for only about 16% of the total root biomass accounted for more than half of total R_r. Thus, fine roots appear to be a key component of the belowground carbon cycle.

To permit continuous *in situ* measurements, we set out to develop an automated chamber system capable of providing useful measurements of R_r. In the present paper, we describe this system and present the results of more than 1 yr of continuous measurement of R_r in a temperate forest in Japan.

2. Methods

2.1. Site description

Measurements were conducted at the Yamashiro Experimental Forest (YMS), located in a hilly and mountainous region in Kyoto Pref., Japan (34°47′N, 135°51′E). The forest occupies 1.6 ha at altitude of about 220 m. Meteorological towers were established in 1999 to estimate CO_2 fluxes using the eddy covariance method (Kominami et al., 2003). The forest is temperate and contains deciduous broadleaved spiecies such as *Quercus serrata* and evergreen broadleaved spiecies such as *Ilex pedunculosa*, including some conifers. The tree density is 5953 stems ha^{-1} and tree height averages 12 m. Annual precipitation averages 1627 mm, and temperature averages 15.8 °C. We showed mean monthly air temperature and monthly precipitation in the study site during the period of this study in Table 1. The soil is classified as a Dystric Cambisol by WRB-classification and is derived from weathered granitic parent materials. Long-term monitoring of litterfall was previously performed from September 1999 to August 2003, and the annual litterfall averaged 5.16 t ha^{-1} (Goto et al., 2003). We measured DBH (diameter at breast height, 1.3 m aboveground) for all trees $\geq$ 3.0 cm in DBH in 1999 and for trees between 1.0 and 3.0 cm in DBH in a 0.17 ha subplot in 2001 (Goto et al., 2003). Aboveground and root biomass were estimated using destructive sampling (Goto et al., 2003; Dannoura et al., 2006a). As shown in Table 2, the ratio of aboveground biomass (without liana; 102.01 t ha^{-1}) to root biomass (23.41 t ha^{-1}) was 4.36. This is similar to a global average of 4.35 reported by Jackson et al. (1996). However, the root biomass at our study site was smaller than the average root biomass for a temperate forest (42 t ha^{-1}; Jackson et al., 1996). Root area index (RAI; 3.5 m m^{-2}) was also smaller than the average for temperate forest (9.8 m m^{-2}; Jackson et al., 1997). It is suggested that these characteristics resulted from the thin forest soil (A horizon; about 15 cm) as shown by Asakawa et al. (2006). Dannoura et al. (2006a) reported 80% of fine roots ($\leq$ 5 mm) were concentrated in A horizon and upper 20 cm of B horizon.

2.2. Development of an automatic chamber system

The concentration of CO_2 was measured using an infrared gas analyser (IRGA; LI-820, Li-Cor, Lincoln, Nebraska, USA) by closed-flow chamber method. We constructed five acrylic chambers for the measurement of CO_2 efflux from the soil surface. Each chamber is opened and closed automatically by a motor operated by a programmable controller. During measurements,

Table 1. Mean monthly air temperature and monthly precipitation in the study site

Year	2004									2005							
Month	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8
Air temperature (°C)	14.2	18.8	22.6	27.0	25.8	23.8	16.9	13.1	8.8	3.7	3.6	6.8	14.4	17.2	22.7	25.3	26.2
Precipitation (mm)	102.5	301.5	154.0	39.5	180.5	211.5	287.0	112.0	73.0	38.0	68.0	81.5	32.5	64.5	84.5	184.0	128.0

Table 2. Aboveground biomass (Goto et al., 2003) and below-ground biomass (Dannoura et al., 2006a) for the study site in the Yamashiro Experimental Forest (YMS) estimated by destructive samplingbiometric measurement

Goto et al. (2003)		3 cm ≦ DBH	1 cm ≦ DBH < 3 cm	Total
Aboveground biomass	Deciduous	63.56	5.00	68.56
(t ha^{-1})	Evergreen	27.06	0.37	27.43
	Conifer	6.02	0.00	6.02
	Liana	0.17	2.87	3.04
	Total	96.81	8.24	105.05

	Class of root diameter (mm)					
	0–2	2–5	5–20	20–50	50–	Total
Root biomass (t ha^{-1})	3.691	2.361	1.965	5.106	10.282	23.4
RAI (m^2 m^{-2})	2.631	0.481	0.128	0.137	0.127	3.5

each chamber remains closed for 5 min, and the chambers are measured sequentially. Airflow rate was 1.0 L min^{-1} controled by flowmeter and the electromagnetic valves were used for the changeover in five chambers. We passed ambient air through the tubing for 2 min before extracting air from a chamber to ensure that we removed all air from the previously measured chamber. CO_2 efflux was measured for 5 min per chamber. We use the middle 3 min data for caliculation without first and last 1 min. Thus, CO_2 efflux was measured at each chamber at interbals of about 35 min. Each chamber was left open between measurements. The inside dimensions of the chambers were 13 × 28 × 7 cm ($L \times W \times H$) and the soil surface covered by each chamber was 364 cm^2. The size was determined by pre-measurement so that we would be able to monitor R_r during the winter.

CO_2 efflux was calculated using the following equation:

$$F = \Delta CO_2 \times V/V_{air} \times 10^4/A \times T_a(T_a + T) \times M_{CO_2}/10^3, \quad (1)$$

where F is the measured CO_2 efflux from the soil surface (mg CO_2 m^{-2} s^{-1}), ΔCO_2 is the mean rate of change of the CO_2 concentration in the chamber (ppm s^{-1}), V is the volume of the chamber plus the connected tubes (m^3), V_{air} is the volume of 1 mol of an ideal gas (22.4 L mol^{-1} at STP), A is the soil surface area covered by the chamber (364 cm^2), T_a is the correction factor between Celsius and absolute temperatures (273.2), T is the temperature in the IRGA (°C) and M_{CO_2} is the molecular weight of CO_2 (44.01 g mol^{-1}).

2.3. *Measurement of temporal variations in R_r using the automatic chamber system*

To separate R_r from R_s, all soil in the A horizon was carefully removed using a portable electric vacuum cleaner, leaving only the living roots (Fig. 1-(2)). Care was taken to avoid damaging any roots during this process. An acrylic board was inserted between the A and B horizons to eliminate CO_2 efflux from the B horizon (Fig. 1-(3)). To protect the roots during subsequent measurements, we carefully replaced the removed soil with an equal depth of weathered granitic soil (obtained from a gardening supply store) that was similar to the original A horizon at the study site (Fig. 1-(4)). Before proceeding, we confirmed that this soil had a CO_2 efflux below the limits of detection. And after this study, we removed all roots inside of R_r chamber and measured root biomass, then the carbon content in the weathered granitic soil remained in R_r chamber was measured from carbon loss on combustion in a muffle furnace. Three plots for measuring R_r were established and the automatic chamber was placed above the plots (Fig. 2). We used the aluminum board for the contact between the soil and the chambers to prevent loss of gases or entry of ambient air.

We estublished one plot for measuring R_s without any treatment of the soil (i.e. no removal of soil and no installation of an acrylic board above the B horizon), and this plot served as the control. Moreover, we established one plot for measuring CO_2 efflux from the mineral soil (R_m). We constructed five chambers and used three to measure R_r, one to measure R_s and one to measure R_m. These were established randomly located within a 10 × 10 m^2 plot. Litter fall was cleared from the chambers for R_r and R_m.

Soil temperatures were monitored at depths of 1, 4 and 7 cm in each chamber using thermocouples. CO_2 efflux and soil temperatures were collected using a datalogger (NR1000, Keyence, Osaka, Japan) at 1-s intervals. We used ECH_2O probes (Decagon, Pullman, Washington, USA) to measure the soil's volumetric water content at a depth of 5 cm in each chamber at intervals of 30 min. We processed these data (CO_2 efflux, soil temperature and soil water content) to 30 min interval.

Measurements were performed continuously from April 2004 to September 2005. During monitoring, the LI-820 was calibrated to zero and 400 ppm CO_2 once a month.

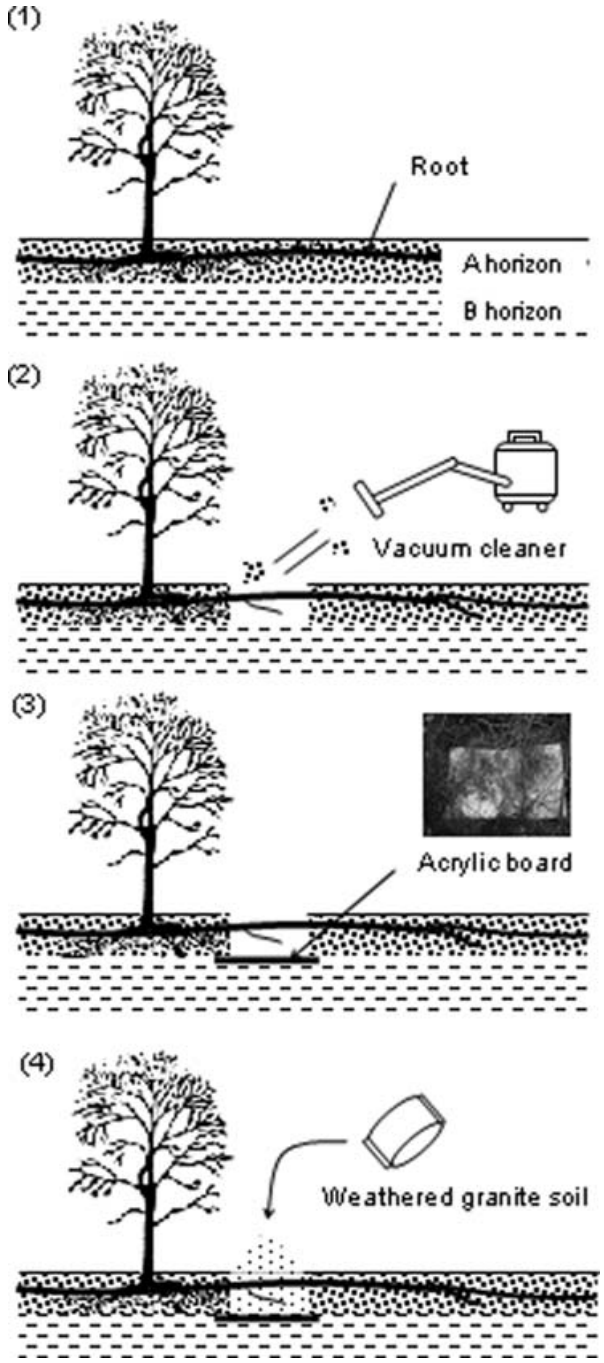

Fig. 1. Illustration of the chamber positioning and experimental design for measurements of R_r. (1) Before the experiment, the root system was concentrated in a thin A layer. (2) Soil was removed from the A horizon using a portable vacuum cleaner that produced minimal disturbance of the remaining roots. (3) An acrylic board was inserted between the A and B horizons to exclude CO_2 efflux from the B horizon in chamber R_r. (4) Weathered soil (obtained from a gardening supply store) that produced a near-zero CO_2 efflux replaced the removed forest soil.

3. Results and discussion

Figure 3 shows the seasonal courses of the daily mean soil temperature, soil water content, and CO_2 efflux during the study period. R_s, R_r and R_m decreased in winter and increased again in the following spring. Root, mycorrhizal and rhizosphere respiration, together with decomposition of recently produced litter (foliage and fine roots), contributed the majority of R_s in previous studies (Bhupinderpal-Shingh et al., 2003; Giardina et al., 2004). At our site, R_m averaged about 20% of R_s throughout the measurement period. Based on our comparison of R_s (i.e. respiration from all soil horizons combined) with R_r, about 80% of R_s originated in the thin A horizon. The carbon contents in the soil of each chamber used for the R_r measurements were small at the end of the experimental period (i.e. 21.4, 22.7 and 19.5 g kg^{-1} in the three chambers). Thus, we concluded that there were few dead roots and root exudates in this soil, and that the majority of the CO_2 efflux was generated by living roots.

Figure 4 shows an example of the daily variation in soil temperature at a depth of 4 cm, (We chose this depth in this study because it represents the mean depth (between 1 and 7 cm) for the A horizon in which we obtained our measurements and was closest to the depth at which soil water content was measured.) in soil water content at a depth of 5 cm, and in CO_2 efflux: R_r for three chambers (ch1, ch2 and ch3), R_m (ch4) and R_s (ch5). Soil water content decreased steadily during this period in each chamber, with the lowest values in the R_m plots. The reasons seemed to be the condition of the cover above the chamber and partly the capacity of forest soil to retain water. Soil temperature also varied among days, and tended to be higher in the R_r and R_m chambers than in the R_s chamber. R_s also varied among days, but decreased gradually with decreasing soil water content. On the other hand, R_r showed little change with respect to water content. Thus, the ratio of R_r to R_s increased gradually, and R_r and R_s seem to differ in their responses to environmental factors.

About R_s in YMS, Tamai et al. (2005a, b) and Nobuhiro et al. (2003) measured the spatial variation (by at 360 point measurements) and temporal fluctuation (using auromatic chamber) and reported daily R_s fluctuated at almost the same time and their relative variation ranges between 50% and 140% in one year. Their annual total value of R_s is 5.80 tC ha^{-1} yr^{-1}. R_s in this plot calculated to 8.99 tC ha^{-1} yr^{-1}, relatively higher than the average. Though R_s has large spatial variation, we used a small sample size as a 'proof of concept' demonstration. We would like to focus on demonstrating that our system solve problems posed by the use of other systems and provided acceptable measurement accuracy.

3.1. The response to soil temperature

Soil temperature was correlated with R_s, R_r and R_m. Using the data collected at 30 min intervals from the chambers, the relationship between CO_2 efflux from each chamber and soil

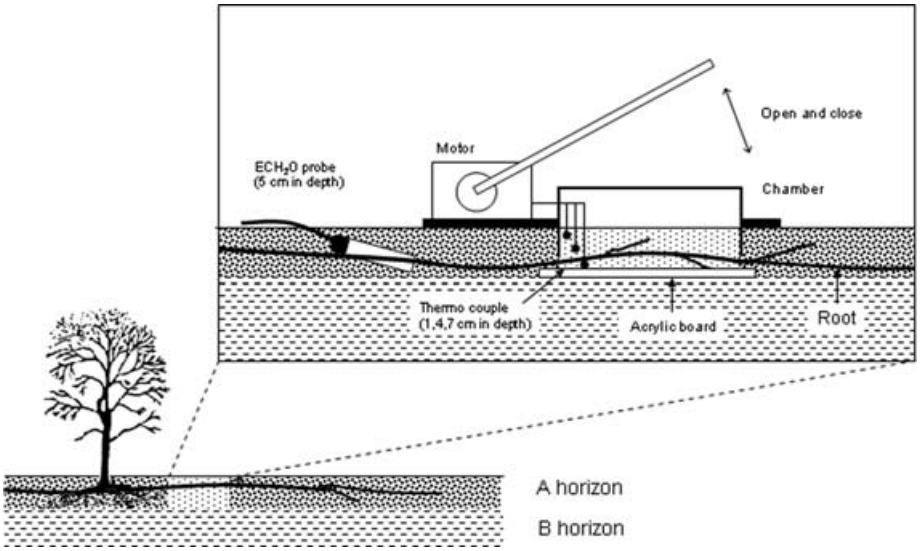

Fig. 2. Illustration of the devices and system setup used in the R_r measurements. A chamber with automatic opening and closing was installed above the root system. Chambers had no fan for not raise a cloud of dust because the chamber made with low height for the abailability of measuring CO_2 flux even if it is low temperature and few root. We use the meth at the entrance of air tube prevention of transportion of sand. The rain was drained out from the inserted acrylic board.

Three plots were established to measure R_r, and one plot each was established to measure R_s (total soil respiration) and R_m (CO_2 efflux from mineral soil with no roots) near the R_r plots. Soil temperature and soil water content were measured in all five chambers.

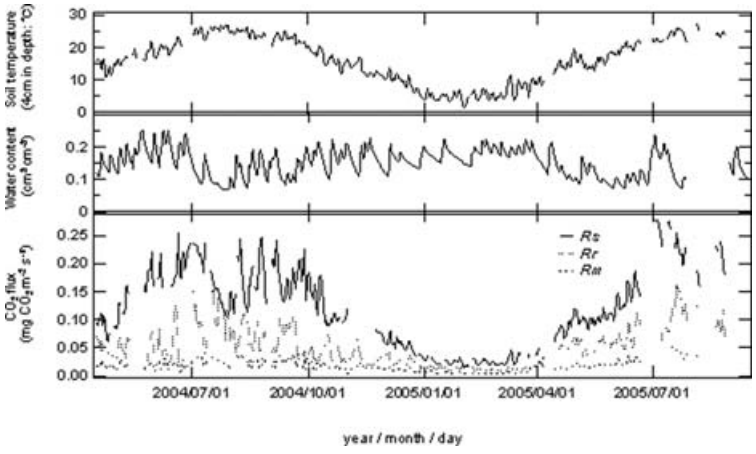

Fig. 3. Seasonal changes in soil temperature (top), soil water content (middle) and CO_2 efflux (bottom) indicated using data of daily averages. We used the mean value of three R_r chambers for soil temperature and soil moisture.

temperature at a depth of 4 cm was calculated using the following exponential function:

$$R = a\mathrm{e}^{bT_s}, \tag{2}$$

where R is the CO_2 efflux from each chamber, T_s is the soil temperature at a depth of 4 cm, and a and b are regression coefficients. The Q_{10} value, which represents the proportional increase in respiration rate for a 10 °C increase in temperature, can be calculated as follows:

$$Q_{10} = \mathrm{e}^{10b}, \tag{3}$$

where b is the regression coefficient from equation (2). The Q_{10} values were 2.64, 2.38 and 2.12 for the three R_r chambers, 1.93 for the R_m chamber, and 2.97 for the R_s chamber. Fig. 5 shows the resulting temperature–respiration curves. The differences among the three R_r chambers may have been caused by differences in root biomass. The biomass of roots <2 mm in diameter for the three chambers (ch1, ch2 and ch3) was 84.41, 134.69 and 32.70 (g m^{-2}), respectively, versus 165.06, 157.38 and 69.59 (g m^{-2}), respectively, for roots from 2 to 5 mm in diameter. The highest R_r value was observed in the chamber with the largest amount of root biomass. In addition, the average value of root biomass in A horizon is 125.64 g m^{-2} (SD = 89.0) <2 mm in diameter and 80.79 g m^{-2} (SD = 102.9) from 2 to 5 mm in diameter.

Simulation models of regional and global carbon cycling generally use a single, fixed Q_{10} coefficient for the exponential relationship between CO_2 efflux and soil temperature.

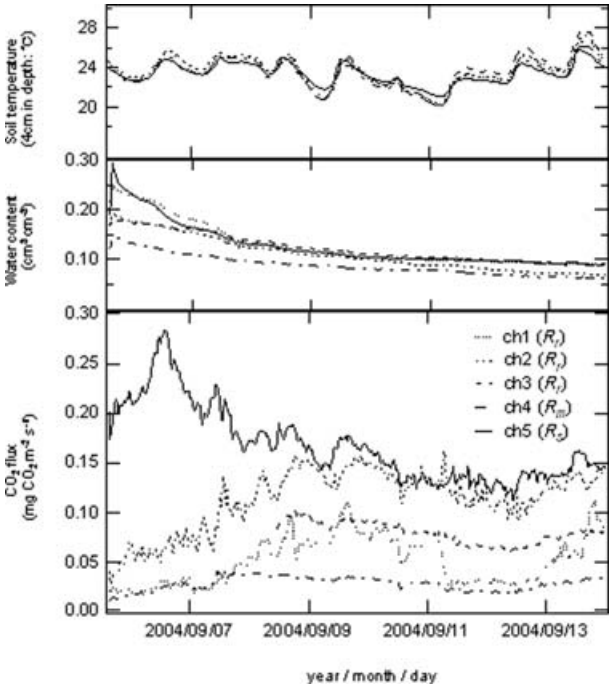

Fig. 4. An example of a typical pattern of daily variation in soil temperature, soil water content, CO_2 flux and the ratios of R_r and R_m to R_s. These data was collected every 30 min for 9 days after a rainfall occurred on 5 September 2004.

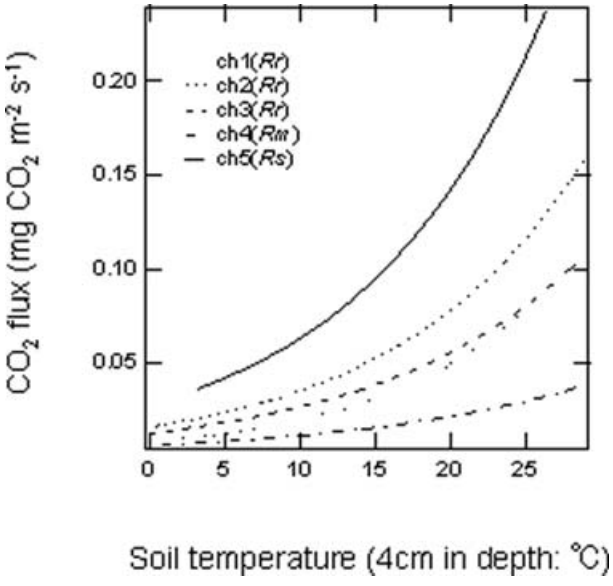

Fig. 5. Curves for the relationship between CO_2 efflux and soil temperature at a depth of 4 cm. Each curve represents an exponential function (eq. 3 in the text) based on data recorded every 30 min in the five chambers.

However, variations in the relationship between CO_2 efflux and soil temperature were large, especially at high soil temperature. For example, at a soil temperature of 25 °C, R_s ranged from 0.10 to 0.28 mg CO_2 m^{-2} s^{-1} and R_r ranged from 0.01 to 0.18 mg CO_2 m^{-2} s^{-1}. R_r and R_s changed diurnally, and different values were observed at the same temperature. The variation suggested that it was necessary to consider other factors such as soil water content and seasonality. Several previous studies suggested that factors such as plant photosynthetic activity and carbon supply may be as important or more important than soil temperature in governing the rates of root respiration (Fitter et al., 1998; Giardina and Ryan, 2002; Bhupinderpal-Shingh et al., 2003).

Figure 6 shows the relationship of the residuals (measured value – fitted value) from each fitting curve for R_s, R_r and R_m to soil temperature and soil water content. Here, we show data of chamber 1 for R_r. The residuals for R_s tended to be negative at higher soil temperature (i.e. the fitted value was greater than the measured value) and at low soil water content. This suggests that low soil water content tended to suppress CO_2 efflux from the soil. In contrast, the influence of low soil water content on R_r appeared smaller than its impact on R_s. The residuals for R_m did not so much appear to change with respect to either soil temperature or soil moisture, and they remained near zero for all values of both parameters; this suggests that the CO_2 efflux from the mineral soil was stable and unaffected by either parameter. The variations in the residuals for R_s and R_r, however, showed considerable responsiveness to changes in soil temperature and moisture, suggesting that biological factors in the soil dominated abiotic factors.

Figure 7 shows the relationship between CO_2 efflux and soil temperature using the mean daily data. R_r is the average value of three chambers. R_s, R_r and R_m increased exponentially with increasing soil temperature. Each component of the CO_2 efflux was approximated as follows:

$$R_s = 0.015e^{0.1099T_s} \quad (4)$$

$$R_r = 0.011e^{0.0861T_s} \quad (5)$$

and

$$R_m = 0.0057e^{0.0675T_s}, \quad (6)$$

where T_s is the daily average soil temperature at a depth of 4 cm. The Q_{10} values for R_s, R_r and R_m were 3.00, 2.37 and 1.96. For all three respiration rates, the variation in the measured CO_2 efflux increased with increasing temperature; the increase was greatest for R_s and smallest for R_m.

Boone et al. (1998) reported that the Q_{10} value for calculated 'root' respiration as the difference between the control and plots without roots was 4.6. This is higher than the values in other reports (Burton et al., 1996, 1998; Ryan et al., 1996; Zogg et al., 1996).

Moreover, this value was higher than the value in the present study for the control (R_s). Boone et al. (1998) concluded that their Q_{10} values reflected not only root respiration but also respiration by mycorrhizae and the decomposition of labile root-derived organic material (e.g. detritus and exudates) by microbiota in the rhizosphere. They emphasized that roots exert a strong influence

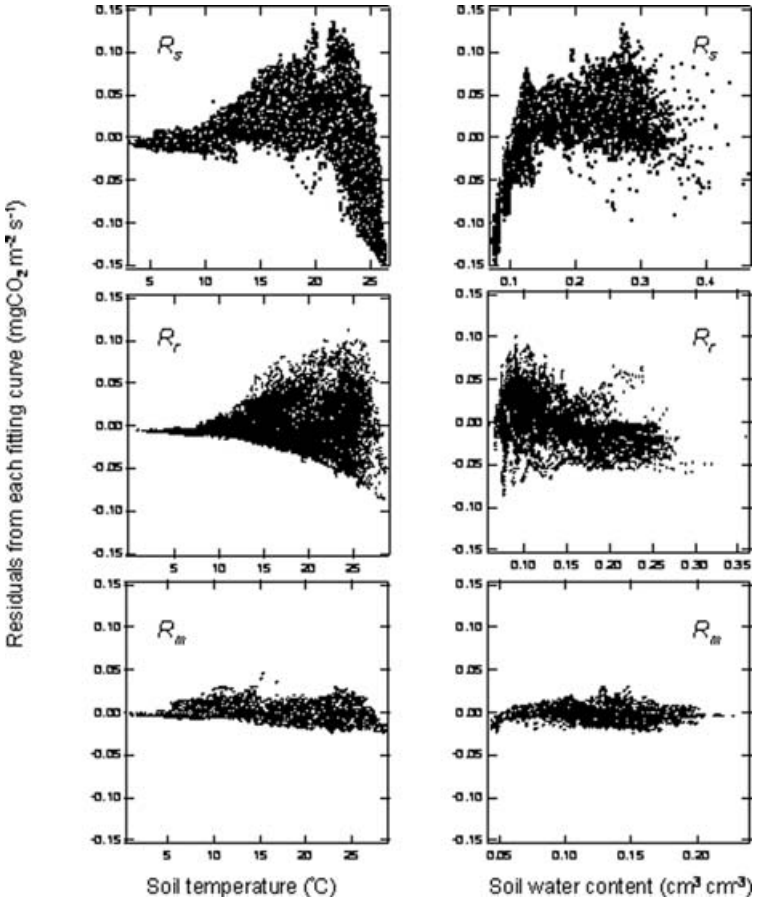

Fig. 6. The relationship between the residuals (measured value – fitted value) for the regression of R_s, R_r and R_m versus soil temperature and soil water content. Data on CO_2 efflux were recorded every 30 min, and the corresponding soil temperature and soil water content measured in that chamber were used. Data represent the values from three chambers for R_r and one chamber each for R_s and R_m. The differences between R_s and R_r were most apparent at high soil temperature and low soil water content.

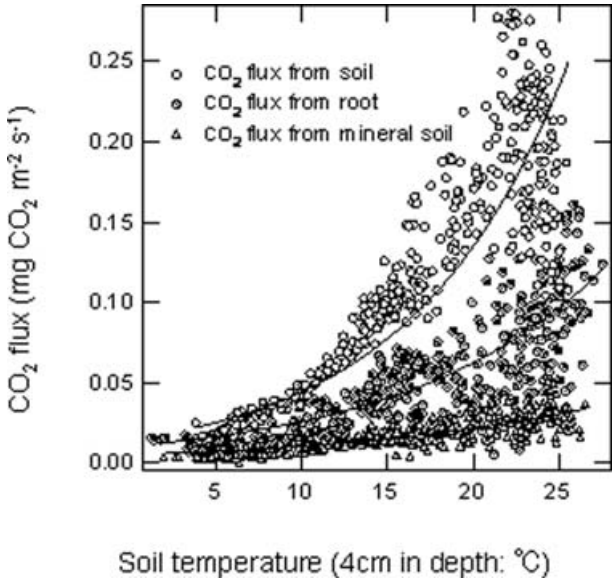

Fig. 7. The relationship between daily averages of R_s, R_r and R_m and daily mean soil temperature at a depth of 4 cm. R_r represents the average value from three chambers; R_s and R_m represent the values from a single chamber.

on the overall temperature sensitivity of soil respiration. However, the Q_{10} value of R_s in our study (3.0) was higher than that of R_r (2.37). The difference between our study and that of Boone et al. (1998) appears to be whether or not the roots contacted the soil. In both studies, R_s includes respiration by mycorrhizae and the decomposition of organic matter by rhizosphere microorganisms. Respiration from the rhizosphere was included in the R_r values reported by Boone et al. (1998) but not in the present study. This suggests that rhizosphere respiration may respond strongly to soil temperature.

3.2. The response to soil water content

R_s decreased rapidly with decreasing soil water content, as shown in Fig. 4. The influence of soil water content on R_s resembles that observed in measurements of decomposition respiration. For example, Jomura et al. (2005) reported that the maximum value of decomposition respiration occurred at relatively high water content. The similarity in response between R_s and decomposition respiration may result from the fact that R_s is composed primarily of R_r and decomposition respiration. Also, R_r did not decrease as much as R_s in response to the decrease of soil water content during short period (Fig. 4).

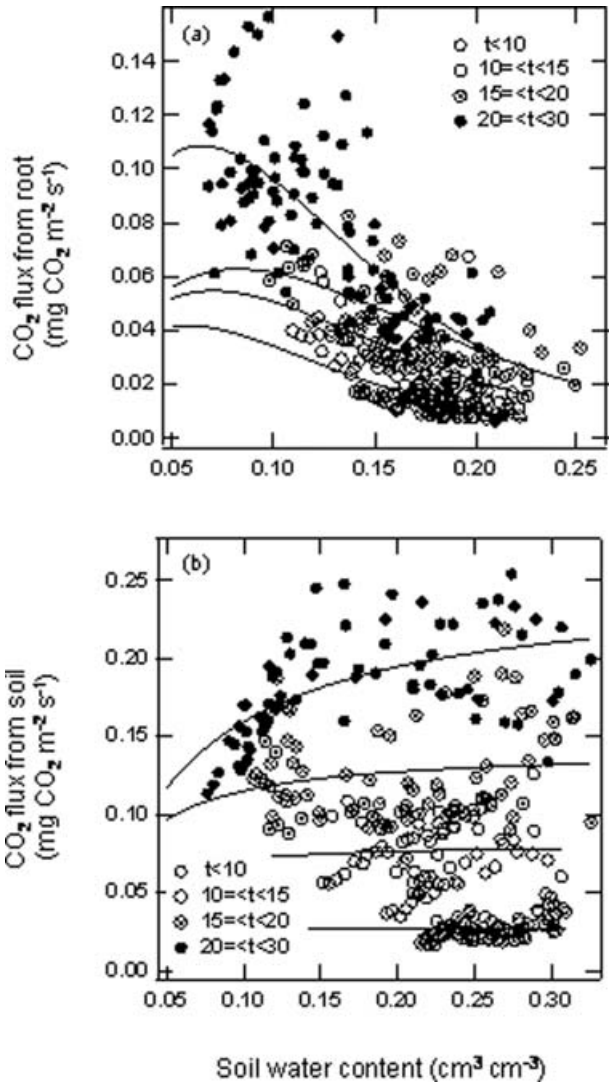

Fig. 8. The relationship between volumetric soil water content (θ) and (a) R_r and (b) R_s in four soil temperature classes using daily averages. R_r represents the average value from three chambers. The curve for R_r is described by $R_r = A(\theta - b)\,(c - \theta)^d$ based on the analysis of Mielnick and Dugas (2000). In this equation, A is a constant related to soil temperature, and b, c and d are invariables. The curve for R_s is described by $R_s = E(\theta)/(f + \theta)$ where E is a constant value related to soil temperature and f is invariables. This approach is suitable for the study site because the site was not constrained by excessive soil water content (Tamai et al., 2005a,b).

Figure 8 shows the relationship between soil water content and R_s or R_r as a function of mean daily soil temperature. Separate regression curves were generated for four different soil temperature classes. R_s did not increase at high soil water content at a given temperature. R_s increased at high temperature and high soil water content, as was previously shown by Tamai et al. (2005a, b) for the study site. The trend in R_s thus seems to be a characteristic of this site and may apply to other sites covered

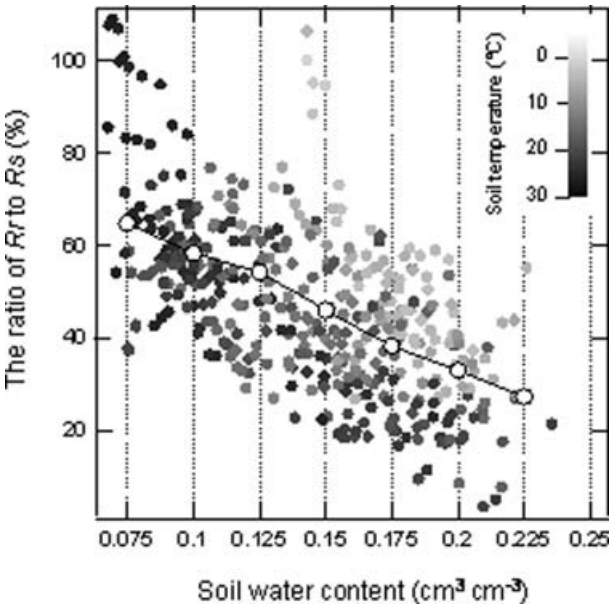

Fig. 9. Changes in the ratio of daily averages of R_r to R_s as a function of soil water content. Colour means soil temperature. White circles and the line drawn between them represent the average value for each 0.05 $cm^3\ cm^{-3}$ range of soil water content.

by thin sandy soils. Moreover, the decrease in R_s was observed in low water content, as shown in Fig. 6.

On the other hand, the peak for R_r occurred at a lower soil water content (about 0.05–0.10 $cm^3\ cm^{-3}$) though R_r decreased under extremely high water content. This tendency could be shown in total data of long-term measurement (Fig. 8) though it was not so clear in short-term measurement (Fig. 4). The differences between R_r and R_s were preserved at each soil temperature.

It is possible that R_r remained relatively constant due to a low soil water content. Irvine et al. (2005) found that R_s increased on the dry side of trees watered on only one side, where hydraulic redistribution provided water to roots on the dry side. They estimated that root and rhizosphere respiration doubled in response to the watering because photosynthesis increased for the whole tree. This suggests a strong influence of recently fixed carbon on R_r during the growing season.

In our results, the contribution of R_r to R_s decreased with increasing soil water content (Fig. 9). As you can see in Fig. 4, R_r can reach higher value than R_s in highly dry condition. Thus, the R_r /R_s ratios are in excess of 1 occasionally. The ratio decreased from an average of 0.65 at soil water content of 0.075 cm cm^{-3} to 0.27 at a soil water content of 0.225 cm cm^{-3}. The rate of decrease with increasing water content was greatest at low temperature, and it slowed as temperature increased.

3.3. *Seasonal changes in of R_r and R_s*

The relationship between respiration and temperature appears to change seasonally as a result of changing phenological and

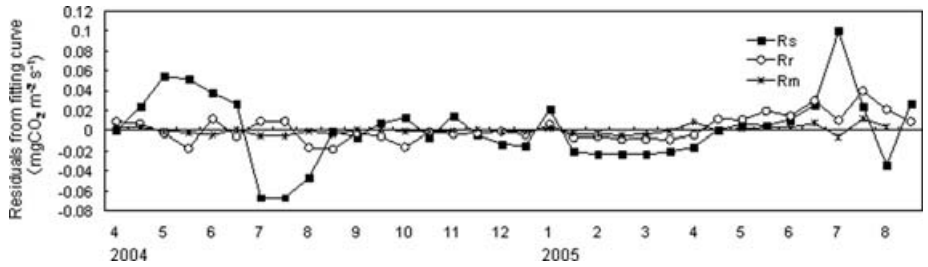

Fig. 10. Seasonal changes in the residuals from fitting the curve for CO_2 efflux as a function of soil temperature. The value of residuals was two weeks averages.

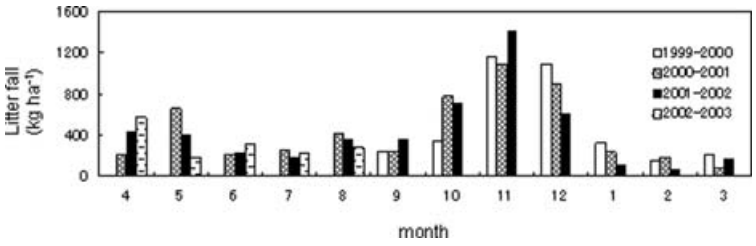

Fig. 11. Seasonal changes in litter-fall at the YMS study site.

biological factors (Lavigne et al., 1997). Figure 10 shows the seasonal change in the residuals from the fitted curve based on the half-month average data. Positive values mean that the measured CO_2 efflux was higher than the value estimated using only soil temperature; this difference reflects the influence of factors other than temperature (i.e. seasonality). The residuals from the curves for R_s and R_r changed seasonally. R_s in the spring and fall were higher than the estimated values. These seasons were the periods of peak litterfall at our study site. The YMS study site is a mixed-wood forest with deciduous and evergreen broadleaved trees, which have different defoliation seasons (during the spring and fall, respectively). This explains the two peaks in litterfall at the YMS study site (Fig. 11). Moreover, R_s was high during the rainy season (June and July), but the soils were dry enough to suppress transpiration during the summer (Kominami et al., 2003), and measured R_s was lower than the estimated value.

The residuals for R_r were near zero in winter but high in spring and after the rainy season. This suggests that during the growing period, R_r was higher at a given soil temperature than during other periods. Bhupinderpal-Shingh et al. (2003) suspected that the high Q_{10} values previously reported for R_r were due to the fact that R_r startes later but stop earlier during a growing season than does heterotrophic respiration in their data. And they suggested R_r shows a large seasonal variation during a period of a small variation in soil temperature. For R_r one should consider not only environmental factor but also above-ground plant photosynthetic activity and C supply and so on. In our result, the high responsivity of R_r was also shown in the growing season.

Figure 12 shows the temporal changes in monthly average values of R_s, R_r and R_m. The seasonal peak in R_s was reached earlier than the seasonal peak in soil temperature.

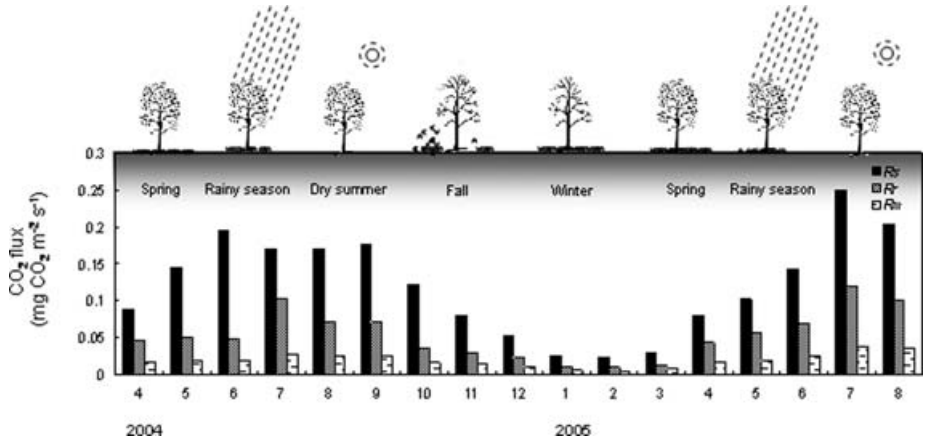

Fig. 12. Temporal changes in the monthly mean values of R_s, R_r and R_m.

The ratio of R_r to R_s was relatively greater in spring than in fall and changed from about 25% in fall to about 60% in spring. Similarly, Tang et al. (2005) found that the ratio of R_r to R_s averaged 56% during the growing season and 16% during the dormant season in a ponderosa pine plantation. The decomposition rate might be accelerated by the higher temperatures and high soil water content during the rainy season (June and July), and most litter may have been decomposed by summer at the YMS study site. This lack of litter may have caused a decrease in R_s during the summer even at high soil temperatures. We suspect many factors affect variation of R_s, not only change of environmental factors, but also change of biological factors and these affect each other in short and long-term because R_s consist of R_r and heterotrophic respiration basically. These results suggest the importance of long-term measurements of each component separately for understanding forest carbon cycle.

4. Conclusions

The eddy covariance method has commonly been used to study net ecosystem exchange of CO_2 in forest ecosystems (Baldocchi et al., 2001). However, uncertainties in the long-term carbon uptake by such ecosystems arise from systematic underestimation of flux measurements at night by this method (Goulden et al., 1996). Kominami et al. (2003) concluded that most dark respiration could not be measured during calm night conditions in a forest growing on complex terrain using the eddy covariance method based on a comparison of respiration measured using this method with the results from automated chambers. Because measurements of each component of respiration are required to provide a complete picture of carbon exchange for a complex forest, the chamber method may offer a significant advantage over the eddy covariance method because it provides accurate, continuous, direct measurements of respiration that are largely unaffected by atmospheric conditions such as wind. In various measurements of respiration by aboveground parts of trees, the responses to temperature and other seasonal changes were also caused by phenological effects (Lavigne et al., 1997; Miyama et al., 2005). However, considerable uncertainties remain surrounding measurement methods for belowground respiration. Bond-Lamberty et al. (2004) analysed published data for 54 forest sites and showed that autotrophic respiration and heterotrophic respiration were strongly correlated with annual R_s across a wide range of forest ecosystems. They discussed why the two types of respiration might be related on large scales, concluding that the reason was that both ultimately depend on a forest's carbon balance and photosynthate supply. Nonetheless, there is a strong need for technological advances that will allow researchers to separate the autotrophic and heterotrophic components of soil respiration under a wide range of site conditions (Ryan and Law, 2005).

The automatic chamber approach described in this paper represents a promising method for measuring R_r over long time periods. Our results demonstrated high R_r in the spring in the relation to soil temperature. The response of R_r to environmental factors differed from that of R_s, and both showed different seasonal patterns (Fig. 12). Thus, the ratio of R_r to R_s fluctuates throughout year though same measurement plots. This means that R_r cannot be predicted from R_s and should be evaluated separately from R_s. The results of the present study show that, for understanding the carbon budget of forest ecosystems, it is indispensable to evaluate R_r accurately and to clarify its properties.

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