J. Ecol. Field Biol. 32 (2): 67~73, 2009

Estimation of the Number of Sampling Points Required for the Determination of Soil CO_2 Efflux in Two Types of Plantation in a Temperate Region

Lee, Na-Yeon (Mi-Sun)[1]* and Hiroshi Koizumi[2]
Institute for Basin Ecosystem Studies, Gifu University, Gifu 501-1193, Japan

ABSTRACT: Soil CO_2 efflux can vary markedly in magnitude over both time and space, and understanding this variation is crucial for the correct measurement of CO_2 efflux in ecological studies. Although considerable research has quantified temporal variability in this flux, comparatively little effort has focused on its spatial variability. To account for spatial heterogeneity, we must be able to determine the number of sampling points required to adequately estimate soil CO_2 efflux in a target ecosystem. In this paper, we report the results of a study of the number of sampling points required for estimating soil CO_2 efflux using a closed-dynamic chamber in young and old Japanese cedar plantations in central Japan. The spatial heterogeneity in soil CO_2 efflux was significantly higher in the mature plantation than in the young stand. In the young plantation, 95% of samples of 9 randomly-chosen flux measurements from a population of 16 measurements made using 72-cm^2 chambers produced flux estimates within 20% of the full-population mean. In the mature plantation, 20 sampling points are required to achieve means within ± 20% of the full-population mean (15 measurements) for 95% of the sample dates. Variation in soil temperature and moisture could not explain the observed spatial variation in soil CO_2 efflux, even though both parameters are a good predictor of temporal variation in CO_2 efflux. Our results and those of previous studies suggest that, on average, approximately 46 sampling points are required to estimate the mean and variance of soil CO_2 flux in temperate and boreal forests to a precision of ± 10% at the 95% confidence level, and 12 points are required to achieve a precision of ± 20%.

Key words: Japanese cedar plantation, Number of sampling points required, Soil CO_2 efflux, Spatial heterogeneity

INTRODUCTION

CO_2 efflux from the soil surface is one of the largest and most important fluxes of carbon in terrestrial ecosystems. This efflux, which results from both root and microbial respiration, has been estimated to account for 60% to 90% of total ecosystem respiration in temperate forests (Goulden et al. 1996, Longdoz et al. 2000). Therefore, quantification of soil CO_2 efflux is important for research on topics ranging from terrestrial biosphere-atmosphere interactions to the construction of carbon budgets within ecosystems.

Soil CO_2 efflux differs among ecosystem types and varies in response to environmental conditions (Raich and Schlesinger 1992). Quantifying soil CO_2 efflux is thus essential in studies designed to evaluate biological processes that affect an ecosystem's carbon budget (Fang et al. 1998) or to examine the environmental factors that control soil CO_2 efflux and the pattern of temporal and spatial variation in soil CO_2 efflux. However, while considerable research has focused on quantifying temporal variability in soil CO_2 flux (e.g., Lee et al. 2002, Mo et al. 2005, Lee et al. 2008), less attention has been paid to spatial variability (e.g., Davidson et al. 2002, Yim et al. 2003, Ohashi and Gyokusen 2007).

Soil CO_2 efflux displays considerable spatial variability within an ecosystem, particularly in forests. Spatial heterogeneity can be addressed by choosing an appropriate number of samples. To obtain a representative value of soil CO_2 efflux within an ecosystem, a large number of sampling chambers would be required, and a considerable time and labor cost is incurred for each measurement using dynamic chamber techniques. Hence, the investigator always faces the question of how many chambers are required to adequately estimate the mean and variance of CO_2 fluxes within a site. For these reasons, it is useful to conduct analyses of the minimum sample size required in a given ecosystem. Several previous studies have provided satisfactory data to estimate the required sample size in various habitat types (e.g., Davidson et al. 2002, Yim et al. 2003, Adachi et al. 2005), but few studies have evaluated spatial hete-

---

* Corresponding author; Phone: +82-2-3290-3469, e-mail: leemsl004@gmail.com

[1] Present address: BK21 Environmental and Ecological Engineering Research Team, Korea University, Seoul 136-731, Korea.

[2] Present address: Department of Biology, Faculty of Education and Integrated Arts and Sciences, Waseda University, Tokyo 162-8480, Japan.

rogeneity of soil CO_2 efflux in plantation ecosystems under an east Asia monsoon climate using consistent methodologies.

Japanese cedar (*Cryptomeria japonica* D. Don.) has been planted extensively throughout Japan, and now occupies approximately 45% of the country's area of plantations (Japan FAO Association 1997). The carbon cycle of these intensively managed plantations is thus important on both regional and global scales. A few previous studies have measured soil CO_2 efflux in Japanese cedar plantations (e.g. Nakane 1995, Lee et al. 2008). In particular, our previous work (Lee et al. 2008) in a Japanese cedar plantation (the "mature stand" used in the present study) revealed the magnitude of temporal variation in CO_2 efflux from soil and snow surfaces in this habitat. Therefore, it is important to consider the use of multi-point measurements to evaluate spatial heterogeneity in soil CO_2 efflux in Japanese cedar ecosystems.

Our objectives were (1) to investigate the number of sampling points required for estimation of soil CO_2 efflux in two types of Japanese cedar plantation (young and mature) ecosystems using a closed dynamic chamber technique and (2) to examine the relationship between heterogeneity in soil CO_2 efflux and soil temperature and moisture.

SITE DESCRIPTION

The study area was located 10 to 15 km east of Takayama City, in central Japan (N 36° 08' to 36° 09', E 137° 22' to 137° 25'). The site is in a cool temperate zone that is subject to the east Asian monsoon climate. We examined soil CO_2 efflux in two Japanese cedar plantations within the study area: one young and one mature.

The young Japanese cedar plantation was approximately 6 years old. A broad-leaved forest at the study site was clearcut in 1998, and then 2-year-old Japanese cedar saplings were planted in 2001. In 2005, the site contained 2,353 Japanese cedar trees ha^{-1} ranging from 1 to 2 m in height. An ecological-process research plot (20 × 20 m) was established on the middle of a north-facing slope in May 2004 at an altitude of ca. 1,400 m asl. The annual mean air temperature and precipitation during the 7 years from 1999 to 2005 were 7℃ and 2,061 mm, respectively (data from the Takayama Experimental Field Station, 1,342 m asl).

The mature Japanese cedar plantation was approximately 40 years old. At this site, Japanese cedar saplings were planted around 1965 after clearcutting of the previous rice terrace cultivations. In 2005, the site had 1,153 Japanese cedar trees ha^{-1} ranging from 20 to 25 m in height. An ecological-process research plot (30 × 50 m) was established on the middle of a slope in November 2004. The altitude of the southeast-facing plot was ca. 800 m asl, and the slope ranged from 10° to 20°. The annual mean air temperature and precipitation during the 44 years from 1961 to 2004 were 11℃ and 1,745 mm, respectively (data from the Takayama meteorological station, 560 m asl). More details on conditions in this stand are provided in Lee et al. (2008).

METHODS

To estimate spatial heterogeneity in soil CO_2 efflux, we obtained measurements at 16 lattice positions at 5-m intervals within the 20 × 20 m plot in the young plantation and at 15 lattice positions at 10-m intervals within the 30 × 50 m plot in the mature plantation, using a portable commercially available system (the Li-Cor LI-6400, Lincoln, NE, USA). The LI-6400 is a portable system for measuring leaf photosynthesis, but can also be attached to a soil chamber (the Li-Cor model 6400-09 Soil CO_2 Flux Chamber). The system was used to measure soil CO_2 efflux while fitted with a null-balance soil chamber and operated in flow-through, non-steady-state mode. The volume of the entire system was 0.99 L, and the enclosed soil surface area was 71.6 cm^2. Additional details of the system are provided by Suh et al. (2006).

Measurement error associated with disturbance of the soil and roots was minimized by permanently inserting the chamber collars 1 cm into the soil to provide an interface between the soil and the chamber. All vegetation was removed from inside the collars prior to the measurements.

In the young plantation, we measured soil CO_2 efflux in the spring (27 May), summer (15 July), early autumn (15 September), and late autumn (23 November) of 2005. In the mature plantation, we measured soil CO_2 efflux in the summer (19 July), early autumn (30 September), and late autumn (25 November) of 2005. The measurement duration was two hours, from 11:00 to 13:00, in each case.

Soil temperature was measured at depths of 1 and 5 cm using two thermometers, and the volumetric soil water content at 5 cm depth was measured simultaneously using time-domain reflectometry (TDR, TRIME-FM, IMKO, Ettlingen, Germany) in each stand. We measured these parameters three times at each sampling point where we measured soil CO_2 efflux on each sampling date, and performed our analysis using the average value of the data for each parameter.

Statistical Analysis

To estimate the number of sampling points required to obtain various degrees of precision at a specific confidence level, we used the equation provided by Petersen and Clavin (1986). According to Petersen and Clavin (1986), if more than one sampling unit is including in the sample, a random sample provides an estimate of the mean given by

$$\bar{y} = \left(\sum_{i=1}^{n} y_i\right)/n \quad (1)$$

$$V(\bar{y}) = \sum_{i=1}^{n} (y_i - \bar{y})^2/n(n-1) = s^2/n \quad (2)$$

where y_i is the value observed for the ith sampling unit and n is the number of sampling units in the sample. If more than 10% of the population is included in the sample, an adjustment should be made in the estimate of the variance of the mean (Petersen and Clavin 1986). Once the variation of the mean has been estimated, the usual confidence limits may be placed around the mean by the relationship

$$L = \bar{y} \pm t_\alpha (s^2/n)^{1/2} \quad (3)$$

in which L is the confidence limit, t_α is the Student's t with $(n-1)$ number of degrees of freedom at the α probability level, and s is the standard deviation of the full population of measurements (Petersen and Clavin 1986). If an estimate of the variance is available from previous samples from the study population or can be arrived at from knowledge of the population, then an estimate of the number of samples necessary in future sampling to estimate the mean with a given precision with a specified probability may be obtained in the following manner from Eq. (2) and (3):

$$n = \left[\frac{t_\alpha s}{D/2}\right]^2 \quad (4)$$

where "D" represents the desired confidence level for the full-population mean within which a smaller-sample mean is expected to fall (e.g., ± 20% of the full-population mean) (Petersen and Clavin 1986). We used the mean and standard deviation from our sample (e.g., the 15 to 16 measurements made in a single day in each types of plantation, as described in the previous section) to estimate the required number of measurements to estimate the population mean with specified error limits equal to 10 to 20% of the sample mean at the 95% confidence level (α).

We then performed nonlinear curve fitting using Microsoft Excel 2002 to compare CO_2 efflux with environmental parameters. The temperature functions were fitted to the following formula:

$$Flux = \beta_0 \exp(\beta_1 T) \quad (5)$$

where $Flux$ represents the measured CO_2 efflux (mg CO_2 m^{-2} h^{-1}), T is the soil temperature (℃) at a depth of 5 cm, and β_0 and β_1 are regression constants. Soil temperature was analyzed at this depth because this produced a better fit for the model than the temperature at a depth of 1 cm (data not shown).

RESULTS

Spatial Heterogeneity in Soil CO_2 Efflux in the Young Cedar Plantation

In the young plantation, the soil CO_2 efflux in May, July, September, and November averaged 338 ± 34, 693 ± 231, 519 ± 128, and 97 ± 34 (mean ± SD) mg CO_2 m^{-2} h^{-1}, respectively (Table 1). The soil CO_2 effluxes differed significantly over time (one-way ANOVA, $p < 0.05$). The spatial heterogeneity in the soil CO_2 efflux across the 16 sampling points was relatively low, with a mean coefficient of variation (CV) of 26%. However, the CV ranged from 10% to 35% among the sampling dates. The CV of soil CO_2 efflux was higher in July and November than in May and September. The numbers of measurement points required to estimate the population mean with a precision of ± 20% of the sample mean at a 95% confidence level on these dates were 1, 13, 7, and 14, while the numbers of points required for a precision of ± 10% were 5, 50, 28, and 56 (Table 1).

Spatial Heterogeneity in Soil CO_2 Efflux in the Mature Cedar Plantation

In the mature plantation, the CO_2 efflux in July, September, and November averaged 595 ± 239, 433 ± 140, and 184 ± 90 (mean ±

Table 1. Number of sampling points required to obtain mean measurements of soil CO_2 efflux with different degrees of precision (within ± 10% and within ± 20% of the full sample mean) with a 95% confidence interval using the LI-6400 chamber in the young Japanese cedar plantation

Measurement date	No. of sampling points actually measured	Soil CO_2 efflux (mean ± S.D.) (mg CO_2 m^{-2} h^{-1})	Coefficient of variation (%)	No. of sampling points required for measurements	
				Within ± 10%	Within ± 20%
27 May	16	338 ± 34	10	5	1
15 July	16	693 ± 231	33	50	13
15 Sept.	16	519 ± 128	25	28	7
23 Nov.	16	97 ± 34	35	56	14

SD) mg CO_2 m^{-2} h^{-1}, respectively (Table 2). Again, the soil CO_2 effluxes differed significantly over time (one-way ANOVA, $p < 0.05$). The spatial heterogeneity of soil CO_2 efflux across the 15 sampling points in the mature stand was significantly higher than that in the young stand, with a mean CV of 40% ($p < 0.05$). The CV ranged from 32% to 49% among the sampling dates. The CV of soil CO_2 efflux was higher in July and November than in September. The numbers of measurement points required to produce a sample mean within ± 10% of the full-population mean at a 95% confidence level on the sampling dates were 75, 48, and 110 points; the corresponding numbers were 19, 12, and 28 for a precision of ± 20%. In the mature plantation, the number of samples required to obtain a precision of ± 10% was prohibitively high, so we have also presented the required sample sizes for precisions of ± 30% (Table 2).

The Effects of Soil Temperature and Moisture

Table 3 shows the soil temperature and soil moisture in the young and mature Japanese cedar plantations on each sampling date. Soil temperature in the young plantation increased from May to July and then decreased during the autumn (Table 3). In the mature plantation, soil temperature increased from July to September at a depth of 1 cm, but decreased during this period at a depth of 5 cm. Temperatures at both depths then decreased from September to November. In the young plantation, the maximum soil temperatures (mean ± SD) were recorded in July (22.1 ± 1.4℃ at 1 cm and 20.8 ± 1.5℃ at 5 cm). The maximum soil temperatures in the mature plantation were 22.4 ± 5.7℃ at 1 cm in September and 19.9 ± 0.3℃ at 5 cm in July. In the young plantation, the minimum soil temperatures at both depths were recorded in November (−0.1 ± 1.0℃ at 1 cm and 0.3 ± 0.5℃ at 5 cm). In the mature plantation, the minimum soil temperatures at both depths also occurred in November, but were warmer than in the young plantation (4.3 ± 0.6℃ at 1 cm and 4.5 ± 0.6℃ at 5 cm). While the soil temperatures in September were lower in the young plantation than in the mature plantation, the soil CO_2 efflux rates were slightly higher in the young plantation (Tables 1, 2 and 3).

Table 3. Soil temperatures and volumetric water contents in the young and mature Japanese cedar plantations in 2005

Measurement date	No. of sampling points	Soil temperature (1 cm, ℃)	Soil temperature (5 cm, ℃)	Soil volumetric water content (5 cm, %)
Young plantation				
27 May	16	20.0 ± 2.9	14.9 ± 1.8	23.0 ± 4.2
15 July	16	22.1 ± 1.4	20.8 ± 1.5	27.3 ± 4.4
15 Sept.	16	19.7 ± 1.3	18.7 ± 0.9	30.0 ± 3.1
23 Nov.	16	−0.1 ± 1.0	0.3 ± 0.5	20.3 ± 7.1
Mature plantation				
19 July	15	16.2 ± 6.0	19.9 ± 0.3	19.7 ± 0.4
30 Sept.	15	22.4 ± 5.7	15.3 ± 0.3	15.3 ± 0.2
25 Nov.	15	4.3 ± 0.6	4.5 ± 0.6	28.0 ± 8.6

Volumetric soil moisture contents (mean ± SD) adjacent to the soil chambers in July averaged 27.3 ± 4.4% in the young plantation and 19.7 ± 0.4% in the mature plantation (Table 3). In September, the corresponding values increased to 30.0 ± 3.1% and decreased to 15.3 ± 0.2%, respectively. In November, the value in the young stand decreased to 20.3 ± 7.1% whereas the value in the mature stand increased to 28.0 ± 8.6%.

Fig. 1 shows the relationship between soil CO_2 efflux and soil temperature in both stands. There was a significant exponential relationship between soil CO_2 efflux and soil temperature at a depth of 5 cm in both the young plantation ($\beta_0 = 87.75$, $\beta_1 = 0.094$, $R^2 = 0.85$, $p < 0.05$, $n = 64$) and the mature plantation ($\beta_0 = 116.78$, $\beta_1 = 0.080$, $R^2 = 0.64$, $p < 0.05$, $n = 45$) (Fig. 1). However, the graph shows that temperature could not accurately predict spatial variation in efflux at typical temperatures observed during the growing sea-

Table 2. Number of sampling points required to obtain mean measurements of soil CO_2 efflux with different degrees of precision (within ± 20% and within ± 30% of the full sample mean) with a 95% confidence interval using the LI-6400 chamber in the mature Japanese cedar plantation

Measurement date	No. of sampling points actually measured	Soil CO_2 efflux (mean ± S.D.) (mg CO_2 m^{-2} h^{-1})	Coefficient of variation (%)	No. of sampling points required for measurements		
				Within ± 10%	Within ± 20%	Within ± 30%
19 July	15	595 ± 239	40	75	19	8
30 Sept.	15	433 ± 140	32	48	12	5
25 Nov.	15	184 ± 90	49	110	28	12

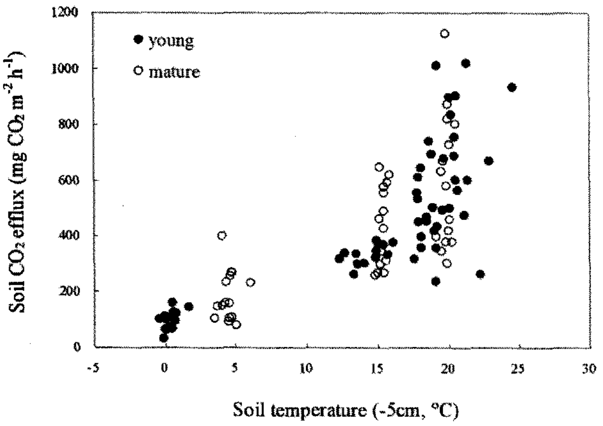

Fig. 1. The relationship between soil temperature at a depth of 5 cm and soil CO_2 efflux measured using the Li-6400 in the young and mature Japanese cedar plantations in the 2005 growing season.

son; despite the overall success of the regression, efflux still varied by nearly 400% at higher temperatures (e.g., around 19℃). The soil CO_2 efflux rates measured throughout the growing season were not significantly related to moisture content at a depth of 5 cm in both stands ($n = 64$ and 45, $p > 0.05$) (Fig. 2).

DISCUSSION

Coefficients of Variation (CV) in Soil CO2 Efflux and Soil Temperature and Moisture in Two Plantations

Spatial heterogeneity in soil CO_2 efflux within an ecosystem can be described by the coefficient of variation (CV). In the present study, the high CV in the mature plantation (40%) might have been caused by the history of disturbance. The mature plantation originated as a cultivated rice terrace, and some positions had sandy soil whereas others had a gravely soil. The soil CO_2 effluxes under litter-covered surfaces were higher than those under sand and gravel surfaces (data not shown). In contrast, the young plantation was established on a site formerly occupied by natural forest, and was likely to have been more homogeneous in its soils. Therefore, the spatial heterogeneity in soil CO_2 efflux in the mature plantation stand should be expected to be higher than that in the young plantation, and indeed, the CV for soil CO_2 efflux in the young plantation (26%) was lower than that in the mature plantation.

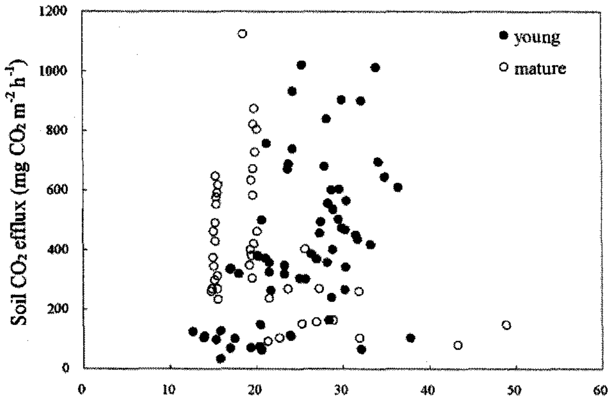

Fig. 2. The relationship between soil water content and soil CO_2 efflux measured using the Li-6400 in the young and mature Japanese cedar plantations in the 2005 growing season.

We found when the CV of soil CO_2 flux in our ecosystems exceeded 30%, it was necessary to increase the number of sampling positions to obtain a given level of precision with 95% confidence (see Table 1 and 2). The CV for soil CO_2 efflux reported by Xu and Qi (2001) for a young ponderosa pine (*Pinus ponderosa* P.& C. Lawson) plantation in northern California was about 30%, which is lower than the value of 55% reported for a slash pine (*Pinus elliotii* Engelm.) plantation in Florida by Fang et al. (1998). Russell and Voroney (1998) found that the CV for soil CO_2 efflux ranged from 16% to 45% along a 40-m transect (with a 2- to 4-m sampling interval) in a mature boreal aspen (*Populus tremuloides* Michx.) forest. The CV was also usually on the order of 30% for soil CO_2 fluxes in the Harvard forest (Davidson et al. 1998, 2002) and in the eastern Amazon (Davidson et al. 2000).

Temporal variability in soil CO_2 efflux is known to depend strongly on soil temperature and moisture. In our results, however, variation in the soil temperature could not account for the observed spatial heterogeneity (400%) in soil CO_2 efflux. The spatial heterogeneity in soil moisture was not as large as the observed variation in soil CO_2 efflux rates: the CV values for moisture contents were generally relatively small (1.3% to 18.3%) in both plantations, but were higher in November (35% and 31% in the young and mature plantations, respectively). These results suggest that the major environmental factors that control the temporal variability in soil CO_2 efflux did not strongly influence its spatial variability, at least in the Japanese cedar ecosystems in this study. Our study did not provide adequate data for a thorough analysis of the relationships between soil CO_2 efflux and soil temperature and moisture because our research was conducted only on a limited set of sampling dates in each ecosystem type. Our results, however, were comparable to the results obtained for a slash pine plantation in which the influence of these two factors was assumed to be negligible (Fang et al. 1998). Epron et al. (2004) also reported that soil water content was the main determinant of temporal variation of soil CO_2 efflux, but accounted poorly for its spatial variability, in a young *Eucalyptus* plantation. Similarly, Xu and Qi (2001) found that soil temperature and moisture explained less than 34% of the spatial variation in soil CO_2 efflux, though the two variables together explained most (76%

to 95%) of the temporal variation. Further study of the factors controlling spatial variation in soil CO_2 efflux is needed.

The Number of Sampling Points Required for Measurement

Table 4 summarizes the results of several studies in temperate and boreal forests of the number of sampling points required for measurements within ± 10% and ± 20% of the full-population mean with a 95% confidence level. In these studies, the number of sampling points that would be required to produce mean values within ± 10% of the full-population mean ranged from 11 to 87, versus 3 to 22 points for a precision of ± 20%; these values compare well with the values of 5 to 110 points and 1 to 28 points, respectively, in our study. In previous studies, Liang et al. (2004) used three chamber sizes (8,100-cm^2, 706-cm^2, and 72-cm^2) to measure soil CO_2 efflux in a larch plantation using automatic, open-top and Li-6400 chamber systems, respectively. However, Liang et al. (2004) made in error in calculation. If we recalculate using the authors' published data, 11, 47, 87 and 3, 12, 22 sampling points are required to achieve means within ± 10% and ± 20% of the full-population mean, respectively, at a 95% confidence level. Interestingly, the number of sampling points required to adequately estimate the mean and variance of the CO_2 fluxes fell within a narrow range (around 46 and 12 for ± 10% and ± 20% of the full-population mean, respectively). Davidson et al. (2002) suggested that relatively homogeneous sites can be characterized with about 6～8 flux measurements performed within an hour, and that 4～8 such sites can be measured within a day. In contrast, Adachi et al. (2005) reported that the spatial heterogeneity in four tropical forests and plantations necessitates the use of more measurement points than are required in temperate forests. Clearly, the routine collection of large numbers of flux measurements would be ideal, but logistical constraints (labor and time) often limit the number of measurements that are feasible

Table 4. Number of sampling points required to obtain mean measurements within ± 10% and ± 20% of the full population mean with a 95% confidence level in temperate and boreal forests

Site description	No. of sampling points required for measurements		No. of sampling points measured	Chamber size (cm^2)	Reference
	Within ± 10%	Within ± 20%			
Red pine (*Pinus resinosa*) plantation at Harvard Forest in Massachusetts, USA (～80-year-old) (a single day)	71 (Within ± 5%)	/	41	594	Raich et al. (1990)
Boreal aspen (*Populus tremuloides*) forest in Saskatchewan, Canada (70-year-old)	40	10	10 to 24	79	Russell and Voroney (1998)
Ponderosa pine (*Pinus ponderosa*) plantation in Berkeley, CA, USA (7 to 8-year-old)					Xu and Qi (2001)
Growing season	41	10	18	72	
Non-growing season	54	14			
All seasons	42	10			
Mixed hardwood (red oak, red maple) forest at Harvard forest in Massachusetts, USA (60-year-old)	41	10	36	490	Davidson et al (2002)
Larch (*Larix kaempferi*) plantation at the Tomakomai Flux Site, Hokkaido, Japan (40-year-old) (Sampling date:28 and 30 August)	30	8	50 and 49	125	Yim et al. (2003)
Larch (*L. kaempferi*) plantation at the Tomakomai Flux Site, Hokkaido, Japan (45-year-old)					Liang et al. (2004)
Automated chamber system	11	3	16	8,100	
Open-top chamber system	47	12	9	706	
Li-6400 system	87	22	20	72	
Japanese cedar (*Cryptomeria japonica*) plantation (young stand) at Takayama, Gifu, Japan (6-year-old)	35 (5 to 56)	9 (1 to 14)	16	72	Present study
Japanese cedar (*C. japonica*) plantation (mature stand) at Takayama, Gifu, Japan (40-year-old)	78 (48 to 110)	20 (12 to 28)	15	72	Present study
Mean	46	12			

(Davidson et al. 2002). Stratified sampling techniques can be used to further improve estimation accuracy and reduce the necessary sample sizes, especially in highly heterogeneous ecosystems (Fang et al. 1998).

ACKNOWLEDGMENTS

We thank Dr. M. Inatomi of the Frontier Research Center for Global Change and Mr. Nobuhiro Sode, Ms. A. Nishiwaki, Mr. H. Kawai, Mr. K-S Kim and Mr. K. Kurumado of the Institute for Basin Ecosystem Studies, Gifu University, for their assistance in the field. This study was supported financially by a Japan Society for the Promotion of Science (JSPS) postdoctoral fellowship for foreign researchers and the 21st-Century Centers of Excellence (COE) program "Satellite Ecology" at Gifu University.

LITERATURE CITED

Adachi M, Bekku YS, Konuma A, Kadir AR, Okuda T, Koizumi H. 2005. Required sample size for estimating soil respiration rates in large areas of two tropical forests and of two types of plantation in Malaysia. For Ecol Manage 210: 455-459.

Davidson EA, Belk E, Boone RD. 1998. Soil water content and temperature as independent or confounded factors controlling soil respiration in a temperate mixed hardwood forest. Global Change Biol 4: 217-227.

Davidson EA, Savage K, Verchot LV, Navarro R. 2002. Minimizing artifacts and biases in chamber-based measurements of soil respiration. Agr For Meteorol 113: 21-37.

Davidson EA, Verchot LV, Cattanio JH, Ackerman IL, Carvalho JEM. 2000. Effects of soil water content on soil respiration in forest and cattle pastures of eastern Amazonia. Biogeochemistry 48: 53-69.

Epron D, Nouvellon Y, Roupsard O, Mouvondy W, Mabiala A, Saint-Andre L, Joffre R, Jourdan C, Bonnefond J, Berbigier P, Hamel O. 2004. Spatial and temporal variations of soil respiration in a *Eucalyptus* plantation in Congo. For Ecol Manage 202: 149-160.

Fang C, Moncrieff JB, Gholz HL, Clark KL. 1998. Soil CO_2 efflux and its spatial variation in a Florida slash pine plantation. Plant Soil 205: 135-146.

Goulden ML, Munger JW, Fan SM, Daube BC, Wofsy SC. 1996. Exchange of carbon dioxide by a deciduous Forest: Response to interannual climate variability. Science 271: 1576-1578.

Japan FAO Association. 1997. Forests and forestry in Japan, 2nd Ed. Japan FAO Association, Tokyo.

Lee M, Lee J, Koizumi H. 2008. Temporal variation in CO_2 efflux from soil and snow surfaces in a Japanese cedar (*Cryptomeria japonica*) plantation, central Japan. Ecol Res 23: 777-785.

Lee M, Nakane K, Nakatsubo T, Mo W, Koizumi H. 2002. Effects of rainfall events on soil CO_2 flux in a cool temperate deciduous broad-leaved forest. Ecol Res 17: 401-409.

Liang NS, Nakadai T, Hirano T, Qu LY, Koike T, Fujinuma Y, Inoue G. 2004. In situ comparison of four approaches to estimating soil CO_2 efflux in a northern larch (*Larix kaempferi* Sarg.) forest. Agr For Meteorol 123: 97-117.

Longdoz B, Yernauz M, Aubinet M. 2000. Soil CO_2 efflux measurements in a mixed forest: impact of chamber disturbances, spatial variability and seasonal evolution. Global Change Biol 6: 907-917.

Mo W, Lee M, Uchida M, Inatomi M, Saigusa N, Mariko S, Koizumi H. 2005. Seasonal and annual variations in soil respiration in a cool-temperate deciduous broad-leaved forest, Japan. Agr For Meteorol 134: 81-94.

Nakane K 1995. Soil carbon cycling in a Japanese cedar (*Cryptomeria japonica*) plantation. For Ecol Manage 72: 185-197.

Ohashi M, Gyokusen K. 2007 Temporal change in spatial variability of soil respiration on a slope of Japanese cedar (*Cryptomeria japonica* D. Don) forest. Soil Biol Biochem 39: 1130-1138.

Petersen RG, Clavin LD. 1986. Sampling. In Methods of Soil Analysis. Part 1. Physical and Mineralogical Methods (Klute A, ed). Agronomy Monograph No. 9 (2nd edition), pp 33-51.

Raich JW, Bowden RD, Steudler PA. 1990. Comparison of two static chamber techniques for determining carbon dioxide efflux from forest soils. Soil Sci Soc Am J 54: 1754-1757.

Raich JW, Schlesinger WH. 1992. The global carbon dioxide flux in soil respiration and its relationship to climate. Tellus 44B: 81-99.

Russell CA, Voroney RP. 1998. Carbon dioxide efflux from the floor of a boreal aspen forest. I. Relationship to environmental variables and estimates of C respired. Can J Soil Sci 78: 301-310.

Suh S, Chun Y, Chae N, Kim J, Lim J, Yokozawa M, Lee M, Lee J. 2006. A chamber system with automatic opening and closing for continuously measuring soil respiration based on an open-flow dynamic method. Ecol Res 21: 405-414.

Xu M, Qi Y. 2001. Soil-surface CO_2 efflux and its spatial and temporal variations in a young ponderosa pine plantation in northern California. Global Change Biol 7: 667-677.

Yim M, Joo S, Shutou K, Nakane K. 2003. Spatial variability of soil respiration in a larch plantation: estimation of the number of sampling points required. For Ecol Manage 175: 585-588.

(Received December 23, 2008; Accepted April 28, 2009)