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Comparison of litter-bag and chamber methods for measuring CO_2 emissions from leaf litter decomposition in a temperate forest

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Abstract

Leaf litter decomposition strongly affects the global carbon cycle through carbon dioxide (CO_2) emissions to the atmosphere. The litter bag method (LB) and chamber method with litter addition and removal treatments (C-LART) have been used to quantify the litter decomposition rate and its resultant CO_2 flux. The C-LART method measures soil CO_2 fluxes in control, litter addition, and litter removal plots, and thereby decomposition rates are calculated from differences of the fluxes. However, no report has described the applicability of C-LART in comparison with LB. This study measured the litter decomposition rate and its resultant CO_2 flux using C-LART and LB in a temperate evergreen forest in central Japan to assess the applicability of the two methods. Annual soil respiration in the control plot was 1572 gC m^{-2} yr^{-1}, which was approximately twice as high as the mean of temperate evergreen forests in the world. The litter decomposition rate was 0.42 g g^{-1} yr^{-1} in mass loss or 0.49 gC gC^{-1} yr^{-1} in carbon loss by LB, which are compatible with those reported from other temperature forests. In contrast, the decomposition rate of litter carbon ascertained using C-LART was greater than 1 (1.96-3.76 gC gC^{-1} yr^{-1}), meaning that carbon emissions increased more than applied, and that the carbon emissions were decreased more than those removed by litter treatments. The incredibly high decomposition was attributed to the enhanced or restricted microbial activities in the underlying mineral soil. Changes in microbial activity are probably caused by the alteration of material supply from the leaf litter layer to the soil by litter treatment (the priming effect). In conclusion, C-LART is not applicable to evaluate CO_2 emissions through litter decomposition. Another approach must be used to compensate the priming effect for application of the chamber method.

Key words: Litter addition, Litter removal, Priming effect, Soil CO_2 flux, Soil respiration

Introduction

Litter decomposition in terrestrial ecosystems plays an important role in the global carbon cycle through carbon dioxide (CO_2) emissions to the atmosphere (Chapin III *et al*., 2011). Ongoing global climate change can affect litter decomposition and consequently alter soil CO_2 flux both physically and microbiologically (Chapin III *et al*., 2009; Crow *et al*., 2009b). Therefore, elucidating aspects of litter decomposition is crucially important for the prediction of soil CO_2 flux under conditions of global climate change.

Litter decomposition is known to be affected by both biotic and abiotic factors (Cotrufo *et al*., 2009). As one important biotic factor, decomposer microorganisms decompose litter through catabolism. During decomposition, litter fragmentation by soil fauna and plowing effects by earthworms (Cortez and Bouche, 1998; Bradford *et al*., 2002) control the decomposition rate. Regarding abiotic factors, soil temperature and moisture control microbial activity, and in turn affect the litter decomposition rate (Cotrufo *et al*., 2009). Moreover, the litter quality influences the rate of litter decomposition (Melillo *et al*., 1982; Enriquez *et al*., 1993). Therefore, in-situ observation must be done to clarify the decomposition accurately.

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Litter bag method (LB) and closed chamber method with litter (detritus) addition and removal treatment (C-LART) have been used to ascertain litter decomposition rates. The LB determines the litter decomposition rate by evaluating the loss of mass or elements of litter in meshed bags placed on the soil surface or in the topsoil during a given time period. This simple and inexpensive method is widely applied (Kampichler and Bruckner, 2009). However, the litter bag mesh size can affect litter decomposition rates through the changes of soil fauna that can access, break down, and digest the litter in the bags (Irmler, 2000; Bradford *et al*., 2002; Yang and Chen, 2009; Bokhorst and Wardle, 2013), and thereby eventually stimulate microbial activity in the litter layer.

The C-LART is also used to evaluate the litter decomposition rate, although such studies are limited (Bowden *et al*., 1993). This method estimates the litter decomposition rate from offsets of litter-added plots and controls, and/or control and litter-removed plots, assuming that the CO_2 emissions from the mineral soil surface are equivalent. This method can resolve difficulties arising from the mesh size used for the litterbag method. Moreover, this method is anticipated for use in analyzing effects of abiotic factors (e.g. temperature and moisture) on litter decomposition because it is a non-destructive method. Nevertheless, a few shortcomings exist for this method. First, this method only assesses decomposition via CO_2 emissions. Decomposition through non-CO_2 gases (e.g. volatile organic compounds), leaching of dissolved carbon, or fragmented or digested carbon that is put into the soil to become soil organic

carbon are not considered. Moreover, the altered litter quantity can affect soil biological properties, such as biomass and activity, that affect CO_2 emissions from the underneath the mineral soil (Sulzman *et al*., 2005; Crow *et al*., 2009a; Prévost-Bouré *et al*., 2010; Wang *et al*., 2013a; Wang *et al*., 2013b).

Both LB and C-LART have been used to determine leaf litter decomposition. It is therefore important for future studies to compare the two methods and assess the reliability. Nevertheless, no report makes a direct comparison of the two methods. Therefore, we applied the LB and C-LART to measure carbon loss through litter decomposition in a temperate evergreen forest in central Japan. Based on the results, we examined the reliability of the two methods.

2. Materials and methods

2.1 Site description

The study site was an evergreen forest (36°3'N,140°7'E, 23 m a.s.l.) located within the National Institute for Environmental Studies in Tsukuba, central Japan. The forest is dominated by Japanese red pine (*Pinus densiflora*), and distributed by broadleaf species of evergreen bamboo-leaved oak (*Quercus myrsinifolia*) and deciduous konara oak (*Quercus serrata*). The average tree density, tree height, and diameter at breast height were 600 trees ha^{-1}, 25 m, and 42 cm, respectively. The forest floor was covered with dwarf bamboo (*Pleioblastus chino*). The 30-year mean annual temperature and annual precipitation were 13.8°C and 1283 mm, respectively, between 1981 and 2010 at Tateno meteorological observatory, located about 300 m distance from the study site. Field experiments were conducted in 2012–2013. The annual precipitation was greater in 2012 (1396 mm) than in 2013 (1282 mm), although the mean annual air temperatures in the two years were almost identical to the 30-year mean. The dominant soil was Andisol (Soil Survey Staff, 2006), overlain with 4-cm-thick litter. The mean of bulk density and total carbon content in the top 30-cm layer were respectively 0.537 Mg m^{-3} and 0.085 gC g^{-1}: N. Liang and M. Teramoto, unpublished data). The soil carbon–nitrogen ratio (C/N) was approximately 15 at 20 cm depth.

2.2 Chamber experiment

In June 2012, 27 aluminum chamber bases with area of 0.5 m × 0.5 m were installed 3 cm into soil to measure the soil CO_2 flux. The chamber bases were divided equally for three litter treatments: control (CT), addition (AD), and removal (RE). Each treatment has 9 replications. The chamber bases were distributed within an area of 40 m × 40 m at the study site.

To simplify the experiment, we used leaf litter of only bamboo-leaved oak. The fresh leaf litter of bamboo-leaved oak which fell in summer of 2012 was collected from surface litter layer in September 2012. We determined whether the litters were fresh by color (close to green) and intactness. The leaf litter was then air-dried in a laboratory for two weeks. To dose the same amount of litter for the litter bag and chamber experiments on an areal basis, we added 230 g air-dried litter to each AD base on 2 October 2012. Simultaneously, we removed all leaf litter on litter layer from each RE base. To find the dry weight, litter samples were oven-dried for 48 h at 80°C. As a result, we added litter to each AD base amounted to 199 g in oven-dried weight (796 g m^{-2} / 356 gC m^{-2}), and removed litter from each RE base (mean ± 1 SD) was 44 ± 17 g in oven-dried weight (176 ± 69 g m^{-2} / 82 ± 32 gC m^{-2}). The amount of litter addition was ascertained according to total litterfall from August through early February, which accounts for approximately 60% of the annual litter input (585 gC m^{-2}; N. Liang, *unpublished data*). During the experiment, litterfall input was not prevented. Leaf litter was naturally supplied to chamber bases for all three treatments.

Soil CO_2 flux was measured on each chamber base monthly for July 2012 – December 2012 and twice a month for January 2013 – December 2013 with a closed chamber system, which was produced according to the guidelines proposed by Liang *et al*. (2004; 2010). The system used two 50-cm-tall cubic chambers made of transparent acrylic plastic. The CO_2 concentration and air temperature inside the chamber were measured every 5 s with an IRGA (LI820; Li-Cor Inc., Lincoln, NB, USA) and a thermocouple probe (MHP; Omega Engineering, Stamford, CA, USA). Their outputs were recorded using a data logger (CR1000; Campbell Scientific Inc., Logan, UT, USA). Each chamber was closed for 180 s. All measurements were made around noon between 10:00 and 12:00 local time under the no-rain condition. Soil CO_2 flux (R_s, μmol m^{-2} s^{-1}) was calculated from an increasing rate of CO_2 concentration during the last 140 s of chamber closure using the following equation:

$$R_s = \frac{VP}{RST}\frac{dC}{dt} \quad (1)$$

where V is the chamber volume (m^3), S is the soil surface area inside a chamber (m^2), P is the air pressure (Pa), T is the air temperature (K), dC/dt is the changing rate of CO_2 concentration (μmol mol^{-1} s^{-1}) determined using least-squares method, and the R is the gas constant (8.314 Pa m^3 K^{-1} mol^{-1}). A test of linearity was applied to control the quality of dC/dt following Aguilos *et al*. (2013).

Simultaneously, soil temperature (T_s) at a depth of 5 cm and volumetric soil water content (SWC) of the top layer of 5 cm were measured respectively at each plot with a thermocouple probe (MHP; Omega Engineering, Stamford, CT, USA) and a soil moisture sensor (SM150; Delta-T Devices Ltd., Cambridge, UK). In addition, the soil temperature at 5 cm depth and soil water content at a depth of 10 cm were monitored hourly at one point in the study site using the same-type sensors throughout the study period.

2.3 Data analysis of chamber measurement

R_s was related to T_s for each chamber base using a common exponential equation as

$$R_s = a\, e^{(b\,Ts)} \quad (2)$$

where a is R_s at 0°C, and b is the temperature coefficient of R_s. The b was used to calculate Q_{10}, which is the relative increase in R_s with a 10°C increase.

$$Q_{10} = e^{(10\,b)} \quad (3)$$

To assess the effect of SWC on R_s, temperature-normalized soil respiration (R_b, μmol m^{-2} s^{-1}) was calculated using the

following equation (Hirano *et al*., 2003).

$$R_b = R_s e^{(b(T_b - T_s))} \quad (4)$$

In that equation, T_b denotes the base temperature, which was set for this study at 15℃.

Annual R_s (gC m^{-2} yr^{-1}) was assessed as the sum of hourly R_s calculated from hourly T_s using equation (2) for each chamber base for an annual period from October 2012, when litter was added or removed. The fitted coefficients (a and b) were determined from data in the same period. In the AD plot, however, the measured R_s exceeded the estimated R_s from T_s considerably during October–December 2012. Consequently, R_s of October –December 2012 was estimated by linear interpolation. The R_s of December 2012–October 2013 was estimated using equation (2), of which the fitted coefficients (a and b) were determined from data in the same period. Then, annual R_s at the AD plot was calculated as the sum of R_s before and after December 2012.

For the C-LART experiment, to minimize undesirable errors caused by localized high CO_2 emissions (hot spots), which were expected to result from decomposition of below-ground detritus, R_s data on any chamber base were not used for further analyses if the annual R_s exceeded mean + 1 SD (n = 9) for each treatment (CT, AD, and RE). Therefore, we used data of 8, 7, and 7 chamber bases, respectively, at CT, AD, and RE plots. Annual changes in CO_2 emissions through litter decomposition were calculated as

$$R_{LA} = R_{AD} - R_{CT} \quad (5)$$

$$R_{LR} = R_{CT} - R_{RE} \quad (6)$$

where R_{LA} and R_{LR} respectively represent CO_2 emissions (gC m^{-2} yr^{-1}) through litter addition and litter removal treatments. Also, R_{CT}, R_{AD} and R_{RE} respectively denote the annual R_s in CT, AD, and RE plots.

2.4 Litter bag experiment

Litter decomposition was measured using the litter bag method (LB) for one year: December 2012–December 2013. The air-dried fresh leaf litter of bamboo-leaved oak, which was collected for the chamber experiment, was used for the litter bag experiment. Litter bags of 20 cm × 30 cm (mesh size: 1 mm) were sewn with cheesecloth. Air-dried 50 g litter was put into each bag, which was equivalent to 44 g (743 g m^{-2}/345 gC m^{-2}) dry weight. The amount of litter corresponded to the litter addition (796 g m^{-2} / 356 gC m^{-2}) of the chamber experiment on an areal basis. The 15 bags were placed on the soil surface around chamber bases on 16 December 2012. We collected five bags respectively on 13 June, 13 September, and 15 December 2013. The litter samples in bags were washed gently and quickly in a laboratory to remove foreign materials and weighed after drying for 48 h at 80C. For the dry samples, concentrations of total nitrogen and carbon were measured using an NC elemental analyzer (Flash EA 1112 Series; Thermo Electron Corp, Waltham, MA, USA).

Litter decomposition or litter loss (g g^{-1}) at time t was calculated as

$$\text{Litter decomposition} = (W_0 - W_t) / W_0 \quad (7)$$

where W_0 and W_t respectively denote the mean dry weight of litter (n = 5) at the beginning and time t. Litter carbon loss caused by decomposition (gC gC^{-1}) was assessed using data of total carbon content as

$$\text{Carbon loss} = (W_0 C_0 - W_t C_t) / (W_0 C_0) \quad (8)$$

where C_0 and C_t respectively represent the mean carbon contents of litter (n = 5) at the beginning and time t.

2.5 Statistical analysis

The coefficients a and b in equation (2) were determined using a least squares fit. To check whether R_b is influenced by SWC, the significance of linear or quadratic regressions was analyzed. In C-LART measurements, a Tukey-Kramer test was applied to compare R_s among litter treatments. In LB experiment, a Tukey test was applied to check whether the amount of remained litter differs among sampling time. All statistical analyses were performed using a statistical software package (R 3.2.1; The R Foundation for Statistical Computing).

3. Results

3.1 Seasonal variation in soil CO_2 flux

The start dates of C-LART and LB experiments differed by about two months. Although the period for the C-LART experiment had lower precipitation than that for the LB experiment, the annual mean T_s and SWC were almost identical (Table 1). Therefore, the litter decomposition rates between C-LART and LB experiments are comparable.

The R_s in three litter treatments showed seasonal fluctuations (high in summer and low in winter) in accordance with the change of T_s (Fig. 1). SWC was stable at around 0.20 m^3 m^{-3}, except during summer, with lower SWC (Fig. 1a). Before the litter addition/removal treatments, R_s showed no significant difference among CT, AD, and RE treatments (Fig. 1b). After litter addition, R_s in AD increased rapidly, although R_s in CT and RE did not increase (Fig. 1b). The highest and lowest R_s were recorded respectively in August 2013 and January 2013, corresponding to the highest and lowest T_s: those were 9.8 ± 1.3 (CT), 11.9 ± 2.1 (AD) and 8.0 ± 1.0 (RE) μmol m^{-2} s^{-1} (mean ± 1 SD) for the highest, and 1.1 ± 0.5 (CT), 1.4 ± 0.1 (AD) and 0.9 ± 0.2 (RE) μmol m^{-2} s^{-1} for the lowest (Fig. 1b). Mean annual R_s were 4.5, 6.9, and 3.7 μmol m^{-2} s^{-1} in CT, AD, and RE, respectively, during October 2012 – October 2013 (Fig. 1b).

Table 1. Precipitation, air temperature, soil temperature, and soil water contents in two periods of October 2012 − September 2013 (chamber method) and mid-December 2012 − mid-December 2013 (litter bag method). Precipitation and air temperature were measured at Tateno Meteorological Observatory.

Period	Precipitation (mm yr^{-1})	Mean air emperature (°C)	Mean soil temperature (°C)	Mean soil water content (m^3 m^{-3})
Oct. 2012 − Oct. 2013	1138	14.4	14.7	0.20
Dec. 2012 − Dec. 2013	1282	14.6	14.8	0.21

3.2 Effects of soil temperature and soil water content on soil CO_2 flux

For all chamber bases, equation (2) was significantly fitted to R_s ($p < 0.05$) (Fig. 2a). The combined exponential curves were the following.

CT: $R_s = 1.15 \times \exp(0.0772 \times T_s)$

AD: $R_s = 1.98 \times \exp(0.0640 \times T_s)$

RE: $R_s = 0.865 \times \exp(0.0806 \times T_s)$

The values of Q_{10} were 2.2 ± 0.1 in CT, 1.9 ± 0.2 in AD, and 2.4 ± 0.1 in RE, which were significantly lower in AD than in RE ($p < 0.05$, Tukey–Kramer test). The temperature-normalized CO_2 flux at 15℃ (R_b) shows neither a linear nor quadratic relation with SWC ($p > 0.05$, Fig. 2b).

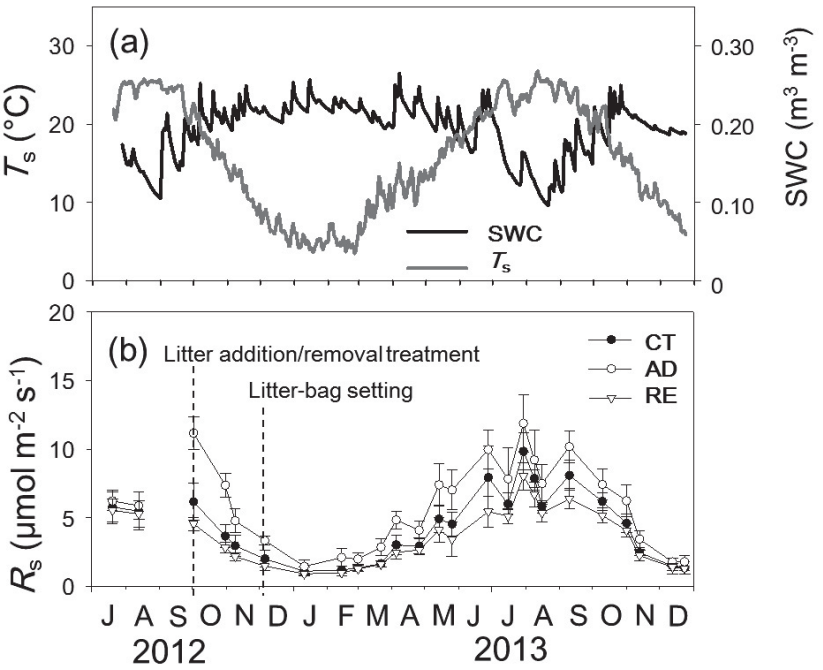

Fig. 1. Seasonal variations in (a) daily means of soil temperature (5 cm) and volumetric soil water content (10 cm) and (b) measured soil CO_2 fluxes (R_s) in three treatments (CT, control; AD, litter addition; RE, litter removal) during July 2012 − December 2013. In (b), each symbol denotes the mean of 7 or 8 chamber bases after excluding outliers (R_s data on any chamber base if the annual R_s exceeded mean + 1 SD ($n = 9$) for each treatment). Vertical bars denote 1 standard deviation.

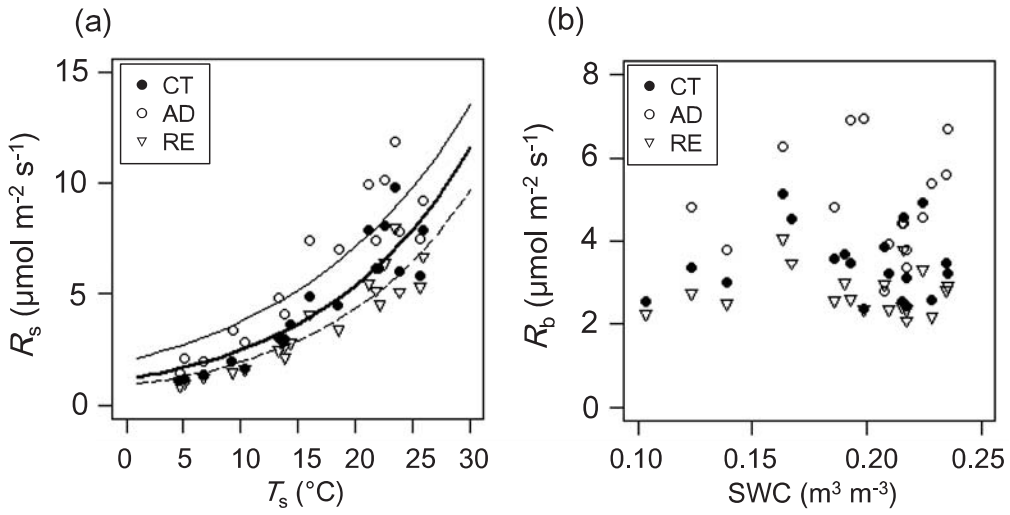

Fig. 2. Responses of soil CO_2 fluxes to abiotic factors: relations (a) between soil CO_2 fluxes (R_s) and soil temperature, and (b) between temperature-normalized R_s at soil temperature of 15℃ (R_b) and soil water content in control (CT), litter addition (AD), and litter removal (RE) treatments. Significant exponential curves are drawn for the three treatments in (a). In (b), neither a linear nor quadratic relation was significant.

3.3 Comparison of annual soil CO_2 flux among litter treatments

Measured R_s fell within the range of mean ± 1SD of estimated R_s during most of the study period (Fig. 3). In AD, however, the measured R_s was considerably higher than the estimated R_s for about two months after litter addition (Fig. 3b), as described above. The annual soil R_s were calculated at 1572 ± 170 (R_{CT}), 2247 ± 204 (R_{AD}), and 1263 ± 144 (R_{RE}) gC m^{-2} yr^{-1} (mean ± 1 SD, n = 7–8, Fig. 4). Annual R_s were significantly different among litter treatments: $R_{AD} > R_{CT} > R_{RE}$ ($p < 0.05$, Tukey–Kramer test). Results show that R_{LA} and R_{LR} were, respectively, 675 and 308 gC m^{-2} yr^{-1}. The R_{LA} and R_{LR} accounted respectively for 190% and 376% of litter addition (356 gC m^{-2}) and removal (82 gC m^{-2}).

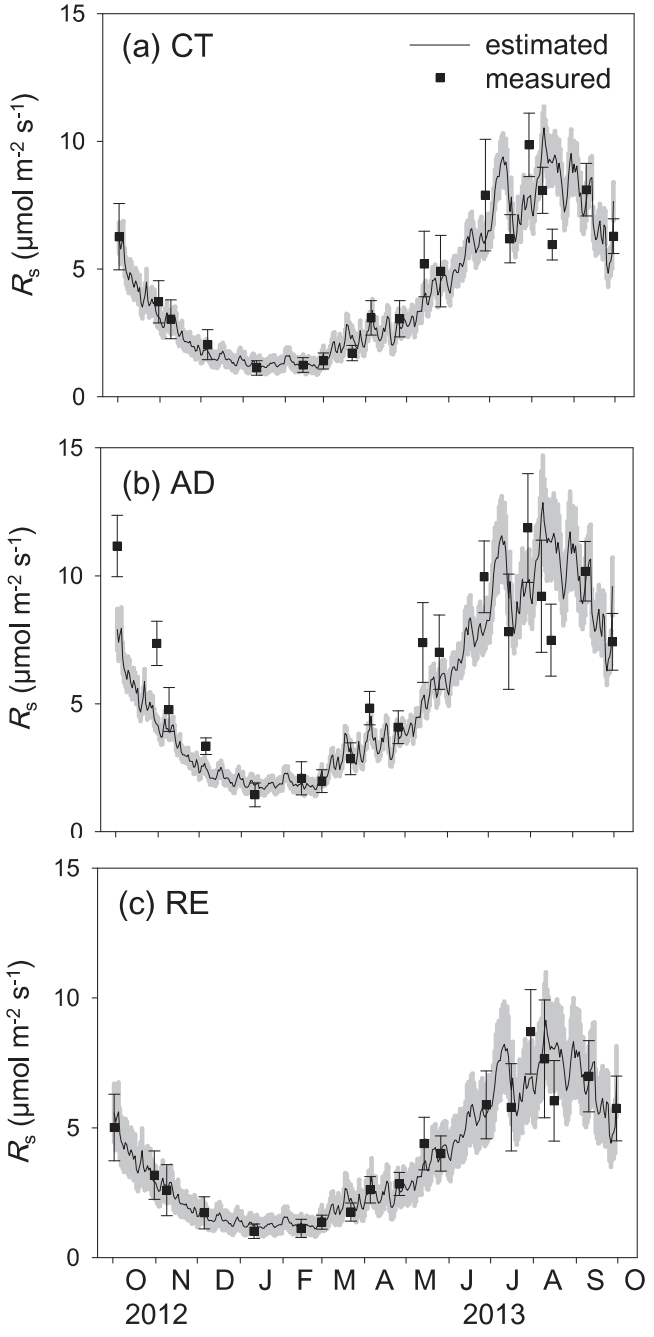

Fig. 3. Seasonal variations in calculated daily mean soil CO_2 flux (R_s) from soil temperature with measurements in (a) control (CT), (b) litter addition (AD), and (c) litter removal (RE) plots during October 2012 − September 2013. Lines and gray bands respectively show the mean and 1 standard deviation of calculated fluxes (n = 8 in CT and n = 7 in AD and RE). Closed squares and vertical bars denote the mean and 1 standard deviation of measured fluxes (n = 8 in CT and n = 7 in AD and RE).

3.4 Litter bag experiment

Litter mass, carbon content, and C/N in litter bags decreased significantly from December 2012 to December 2013 (Table 2). The litter decomposition rate was highest during fall, from September through December 2013. The annual mass loss and its corresponding carbon loss amounted respectively to 313 g m^{-2} yr^{-1} and 171 gC m^{-2} yr^{-1} (Table 2). The values were convertible into 0.42 g g^{-1} yr^{-1} (mass) and 0.49 gC gC^{-1} yr^{-1} (carbon) relative to their initial conditions (Tables 2 and 3). Carbon contents decreased through decomposition (Table 2). Therefore, the relative carbon loss was greater than the relative mass loss.

4. Discussion

4.1 Annual soil respiration and Q_{10} value

Annual soil respiration in temperate evergreen forests is reported to be 779 ± 377 gC m^{-2} yr^{-1} (mean ± 1SD) based on the world forest database (Bond-Lamberty and Thomson 2014). In this study, the annual soil respiration in control (CT) (1572 ± 170 gC m^{-2} yr^{-1}) is approximately twice as high as the mean value (779 gC m^{-2} yr^{-1}). Such high soil respiration is often measured in evergreen forests in Appalachia (Bolstad and Vose, 2005; Vose and Bolstad, 2007), Oregon (Campbell and Law, 2005), and southwestern Japan (Kirita, 1971; Nakane *et al.*, 1983). These regions have warm and humid climate. For that reason, soil respiration is rarely inhibited by desiccation.

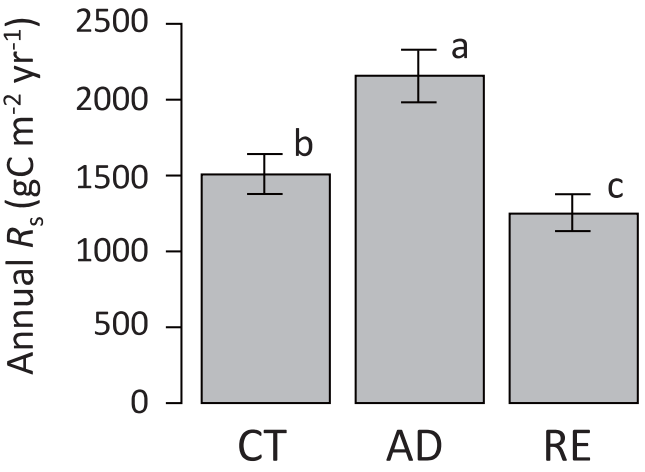

Fig. 4. Comparison of annual soil CO_2 flux (R_s) from October 2012 to September 2013 among three litter treatments: CT, control; AD, litter addition; and RE, litter removal. Vertical bars denote 1 standard deviation (n = 7−8). Different alphabet letters denote significant difference at $p < 0.05$ according to Tukey–Kramer tests.

Table 2. Litter mass remains, mass loss, carbon (C) content, carbon to nitrogen ratio (C/N), carbon remains, carbon loss, and daily carbon release rate during the litter bag experiment. Values of mass remains, C content, C/N and C remains denote mean ± 1 ($n = 5$). Different alphabet letters in each column denote significant difference at $p < 0.05$ according to Tukey's HSD.

Date	Period (days)	Mass remains (g m^{-2})	Mass loss (g m^{-2})	C content (%)	C/N ratio	C remains (gC m^{-2})	C loss (gC m^{-2})	C release rate (gC m^{-2} d^{-1})
Dec. 16, 2012	–	743 ± 0a	–	46.5 ± 0.4a	33.4 ± 2.6a	346 ± 0a	–	–
June 13, 2013	180	641 ± 5b	103	44.2 ± 0.7b	21.3 ± 1.0b	283 ± 6b	62.8	0.349
Sep. 13, 2013	92	566 ± 29c	74.8	44.2 ± 1.6b	21.0 ± 0.4b	250 ± 13c	32.7	0.355
Dec. 13, 2013	91	430 ± 81d	135	40.5 ± 0.6c	20.7 ± 1.0b	174 ± 33d	75.8	0.833
Total	363		313				171	

Table 3. Comparison of annual decomposition rates in temperate forests using the litter-bag (LB) method and the chamber method with litter addition and removal treatments (C-LART).

Decomposition rate (g g^{-1} yr^{-1})	Decomposition constant k	Treatment [1]	Species of leaf litter	Country/region	Forest type[2]	Reference
Litter bag method (LB)						
0.42 ± 0.11	–	–	***Quercus myrsinifolia***	**central Japan**	**E**	**This study**
0.65–0.71	0.29–0.31 yr^{-1}	–	*Quercus serrata, Pinus densiflora*	eastern Japan	ED	Salamanca et al. 1998
0.34	0.0081 $week^{-1}$	–	*Eucalyptus nitens*	New Zealand	E	Wedderburn and Carter 1999
0.29	0.0066 $week^{-1}$	–	*Acacia melanoxylon*	New Zealand	E	Wedderburn and Carter 1999
0.84	1.81 yr^{-1}	–	*Eucalyptus globulus*	New Zealand	E	Guo and Sims 2002
0.55	0.80 yr^{-1}	–	*Eucalyptus botryoides*	New Zealand	E	Guo and Sims 2002
0.41	0.52 yr^{-1}	–	*Eucalyptus ovata*	New Zealand	E	Guo and Sims 2002
0.20	0.0186 $month^{-1}$	–	*Picea abies*	Germany	E	Albers et al. 2004
0.32–0.39	0.38–0.50 yr^{-1}	–	*Nothofagus pumilio*	Chile	D	Caldentey et al. 2001
0.21–0.25	0.75–0.79 yr^{-1}	–	*Larix leptolepis*	South Korea	D	Son et al. 2004
0.85	0.0368 $week^{-1}$	–	*Alnus glutinosa*	New Zealand	D	Wedderburn and Carter 1999
0.31	0.0070 $week^{-1}$	–	*Quercus rubra*	New Zealand	D	Wedderburn and Carter 1999
0.22	0.0210 $month^{-1}$	–	*Fagus sylvatica*	Germany	D	Albers et al. 2004
0.34	0.42 yr^{-1}	–	*Fagus sylvatica*	Germany	D	Jakob et al. 2010
0.59	0.90 yr^{-1}	–	*Fagus sylvatica, Fraxinus excelsior, Tilia spp*	Germany	D	Jakob et al. 2010
0.82	1.71 yr^{-1}	–	*Tilia spp., Fagus sylvatica*	Germany	D	Jakob et al. 2010
Chamber method with litter addition and removal treatments (C-LART)						
1.90	–	**AD**	***Quercus myrsinifolia***	**central Japan**	**E**	**This study**
3.76	–	**RE**				
0.225	–	AD	*Quercus borealis, Acer rubrum, Betula*	Massachusetts, USA	D	Bowden et al. 1993
0.399	–	RE	*papyrifera*			

(1) AD, litter addition; RE, litter removal. (2) E, evergreen forest; ED, evergreen-deciduous mixed forest; D, deciduous forest

The Q_{10} values for soil respiration found in this study (1.9 – 2.4) are similar to those reported for temperate evergreen broadleaf forests (2.2 ± 0.8 (mean ± 1 SD), $n = 18$) (Wang *et al*., 2010). Lower Q_{10} in AD suggests a higher contribution of leaf litter decomposition to soil respiration than in CT and RE. Higher carbon quality (more easily decomposable substrates) achieved by the litter addition would decrease the temperature sensitivity (Q_{10}) of microbial decomposition (Bosatta and Ågren, 1999; Fierer *et al*., 2005).

4.2 Comparison of C-LART and LB experiments

Strictly, the extent of "decomposition" differs between C-LART and LB. In LB, decomposition includes leaching, emissions of volatile organic compound (VOC) and other trace gases (e.g. methane and nitrous oxide), and litter fragmentation by soil fauna and slip through the litter-bag mesh as well as the loss by aerobic decomposition (CO_2 emission). In C-LART, by contrast, decomposition is confined to aerobic decomposition. Therefore, the CO_2 emission estimated from the litter mass loss by LB is potentially overestimated. In this study, however, CO_2 emissions by C-LART greatly exceeded those by LB. The reason is discussed later.

The annual decomposition rate of fresh litter measured by LB (0.42 g g^{-1} yr^{-1}) was within the range reported for world

temperate forests (0.20 − 0.84 g g^{-1}) (Salamanca *et al*., 1998; Wedderburn and Carter, 1999; Caldentey *et al*., 2001; Guo and Sims, 2002; Albers *et al*., 2004; Son *et al*., 2004; Jacob *et al*., 2010), which were estimated from the exponential decomposition function. Reportedly, the decomposition rate by LB tends to increase with mesh size because the accessibility of soil fauna into litter bags depends on the mesh size (Irmler, 2000; Bradford *et al*., 2002; Yang and Chen, 2009; Bokhorst and Wardle, 2013). Soil fauna break down and digest the litter, and consequently enhance the microbial decomposition (Petersen and Luxton, 1982; Anderson *et al*., 1983; Maraun and Scheu, 1996). In this study, the decomposition rate might be underestimated because we used 1-mm mesh. Indeed, the litter decomposition rate of 1-mm mesh bags is 20–30% lower than that of 5-mm mesh bags in a temperate broadleaved forest (Cortez and Bouche, 1998).

Mesh of litter bags can prevent leaf litter from contacting with the soil surface, and can be expected to eventually affect microbial activities through the alteration of the moisture conditions. Reportedly, moisture conditions influence CO_2 emissions through leaf litter decomposition (Kim *et al*., 2005b). Particularly, the vertical moisture distribution in the litter layer affects the decomposition (Ataka *et al*., 2015). Although the effect of mesh on moisture was not measured in this study, the presence of the mesh can underestimate the decomposition rate.

Although C-LART is expected to solve unavoidable difficulties arising from the use of LB, the differences of CO_2 emissions between the control and treatment plots ($R_{AD} - R_{CT}$ and $R_{CT} - R_{RE}$) were, respectively, more than 1.9 times the amount of litter addition and removal. Such overestimation was not found for a deciduous forest (Bowden *et al.,* 1993). We observed that the leaf litter remained in the AD plot even at the end of the experiment. The enhanced soil respiration of more than additional carbon inputs as leaf litter has been reported as a "priming effect" (Sulzman *et al*., 2005; Crow *et al*., 2009a; Prévost-Bouré *et al*., 2010; Wang *et al*., 2013a; Wang *et al*., 2013b). The mechanism of the priming effect is explainable by the enhancement of soil microbial activities caused by the supply of available substrates and nutrients from the litter layer into underlying soil through leaching and fragmentation (Kuzyakov, 2010). In RE, the supply of substrate and nutrient was probably decreased by the litter removal in comparison with the CT plot. For that reason, soil CO_2 flux from litter would have been overestimated in RE. Although the initial stage of litter decomposition is important because of the high litter quality (Kim *et al*., 2005b), the mass loss rate of LB in this study is highest after nine months of litter bag installation after an experience of summer (Table 2). The leaves of dominant *Q. myrsinifolia* at this study site are characterized as physically tough, with a waxy surface. Such structural defense of leaves might cause a delay in decomposition. Indeed, the litter loss rate is negatively correlated with the toughness of the litter constituents (Gallardo and Merino, 1993). Additionally, the activity of soil fauna is low at the start of the LB experiment in winter. The delay of litter fragmentation or digestion by soil fauna is partly attributable to the delay of peak decomposition rates in LB.

The remarkably high R_s in the AD plot in comparison with in CT plot after the initial two months is probably attributable, to a great degree, to the priming effect after adding litter rather than to litter decomposition. The mass loss of litter is well known to occur by leaching at the initial stage of decomposition (Gallardo and Merino, 1993; Chapin III *et al*., 2009). Indeed, the initial decrease in C/N of the litter bag sample (Table 2) probably occurred because of leaching (Kim *et al*., 2005b) (Table 3). The supply of material from litter enhances microbial activity in the underlying soil.

The results imply that C-LART is inapplicable to evaluation of CO_2 emissions through leaf litter decomposition. To apply the chamber method for direct measurement of CO_2 flux, a new approach that compensates the priming effect is necessary. One possible practical approach is the placement of a litter bag in a chamber base. For C-LART, the litter bag is removed temporarily from the chamber base. By measuring CO_2 flux twice on the same chamber base before and after the litter bag removal, decomposition CO_2 flux can be determined as the difference of the two fluxes.

4.3 The amount of priming effect

A high priming effect was observed in this study. We discuss the amount of priming effect (PE) by litter-addition and -removal treatments. The amount of priming effect is defined as the amount of CO_2 emission from the mineral soil which was enhanced by the plant-litter addition. The amount of priming effect is calculated as follows.

$$\text{Litter addition PE} = (R_{AD} - D_{LA}) - R_{CT} = R_{LA} - D_{LA} \quad (9)$$

$$\text{Litter removal PE} = (R_{CT} - D_{LC}) - R_{RE} = R_{LR} - D_{LC} \quad (10)$$

where D_{LA} is the amount of litter decomposition from added litter, which is estimated at 174 gC m^{-2} yr^{-1} (= 356 gC m^{-2} yr^{-1} × 0.49 gC gC^{-1}). D_{LC} is the amount of litter decomposition from the litter at the beginning of the experiment in control, which is estimated at 40 gC m^{-2} yr^{-1} (= 82 gC m^{-2} yr^{-1} × 0.49 gC gC^{-1}).

The PEs from litter addition and removal experiments are respectively 501 gC m^{-2} yr^{-1} and 269 gC m^{-2} yr^{-1}, which correspond respectively to 22% and 17% of CO_2 emissions from AD and CT plots. These fractions are similar to the results of incubation experiments using the soil of a subtropical evergreen plantation (25.9%; Wang *et al*., 2013b) and of in-situ observations in temperate evergreen forests in Germany and Oregon (up to 21.6%; Subke *et al*., 2004; Crow *et al*., 2009a).

The remarkable enhancement of CO_2 emission from the soil (269–501 gC m^{-2} yr^{-1}) was observed in this study. The source of CO_2 is probably derived from the abundant soil carbon and belowground biomass of understory vegetation. The total carbon content in the top 0.3-m layer in this research site was 13700 gC m^{-2} (= 0.0851 gC g^{-1} × 0.537 Mg m^{-3} × 0.3 m from total carbon content and bulk density of soil in the Materials and Methods section). The amount of priming effect is estimated to be less than 4% of the total carbon in the soil. Even though we did not determine the quality of soil carbon (labile or recalcitrant), it is not impossible that the CO_2 evolves from the mineral soil by the priming effect. Moreover, the litter of belowground biomass of undergrowth (mainly dwarf bamboo) in chamber bases, of which aboveground biomass was removed before the experiment, also could become the substrate of the primed CO_2.

4.4 Contribution of fresh-litter decomposition on soil respiration

The annual fresh-litter decomposition by LB (annual decomposition rate × annual litterfall = 286 gC m^{-2} yr^{-1}) accounted for 18% of total soil respiration (1572 gC m^{-2} yr^{-1} in CT; Fig. 4). That contribution is slightly higher than those reported for other temperate forests (9–12%) in northern Japan (Sakuma *et al*., 1994; Kim *et al*., 2005a), Tennessee (Edwards and Harris, 1977), Massachusetts (Bowden *et al*., 1993), and Florida (Moncrieff and Fang, 1999). The result of this study, as well as earlier reports, indicates that the litter decomposition is an important pathway for carbon emissions to the atmosphere from temperate evergreen forest ecosystems.

5. Conclusions

The litter bag method provided comparable results of leaf litter decomposition to those reported from previous studies, although the method has an unavoidable fault attributable to its use of meshes. However, the chamber method with litter addition and removal treatments greatly overestimated the CO_2 emissions derived from litter decomposition, probably because of the alteration of the material flows from the litter layer into underlying soil.

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References

Aguilos M, Takagi K, Liang N, Watanabe Y, Teramoto M, Goto S, Takahashi Y, Mukai H, Sasa K, 2013: Sustained large stimulation of soil heterotrophic respiration rate and its temperature sensitivity by soil warming in a cool-temperate forested peatland. *Tellus* **65B**, 20792.

Albers D, Migge S, Schaefer M, Scheu S, 2004: Decomposition of beech leaves (*Fagus sylvatica*) and spruce needles (*Picea abies*) in pure and mixed stands of beech and spruce. *Soil Biology and Biochemistry* **36**, 155−164.

Anderson JM, Ineson P, Huish SA, 1983: Nitrogen and cation mobilization by soil fauna feeding on leaf litter and soil organic matter from deciduous woodlands. *Soil Biology and Biochemistry* **15**, 463−467.

Ataka M, Kominami Y, Jomura M, Yoshimura K, Miyama T, Kosugi Y, Tani M, 2015: CO_2 efflux from decomposing leaf litter stacks is influenced by the vertical distribution of leaf litter water content and its temporal variation. *Journal of Agricultural Meteorology* **71**, 263−270.

Bokhorst S, Wardle DA, 2013: Microclimate within litter bags of different mesh size: Implications for the 'arthropod effect' on litter decomposition. *Soil Biology and Biochemistry* **58**, 147−152.

Bolstad PV, Vose JM, 2005: Forest and pasture carbon pools and soil respiration in the southern Appalachian Mountains. *Forest Science* **51**, 372−383.

Bond-Lamberty B, Thomson A, 2014: A global database of soil respiration data, Version 3.0. Oak Ridge National Laboratory Distributed Active Archive Center available at http://daac.ornl.gov. (accessed on 2 Jun. 2016)

Bosatta E, Ågren GI, 1999: Soil organic matter quality interpreted thermodynamically. *Soil Biology and Biochemistry* **31**, 1889−1891.

Bowden RD, Nadelhoffer KJ, Boone RD, Melillo JM, Garrison JB, 1993: Contributions of aboveground litter, belowground litter, and root respiration to total soil respiration in a temperate mixed hardwood forest. *Canadian Journal of Forest Research* **23**, 1402−1407.

Bradford MA, Tordoff GM, Eggers T, Jones TH, Newington JE, 2002: Microbiota, fauna, and mesh size interactions in litter decomposition. *Oikos* **99**, 317−323.

Caldentey J, Ibarra M, Hernández J, 2001: Litter fluxes and decomposition in *Nothofagus pumilio* stands in the region of Magallanes, Chile. *Forest Ecology and Management* **148**, 145−157.

Campbell JL, Law BE, 2005: Forest soil respiration across three climatically distinct chronosequences in Oregon. *Biogeochemistry* **73**, 109−125.

Chapin III FS, Matson PA, Vitousek P, 2011: *Principles of terrestrial ecosystem ecology. Second edition*. Springer, New York, USA. pp.529.

Chapin III FS, McFarland J, McGuire DA, Euskirchen ES, Ruess RW, Kielland K, 2009: The changing global carbon cycle: linking plant-soil carbon dynamics to global consequences. *Journal of Ecology* **97**, 840−850.

Cortez J, Bouche MB, 1998: Field decomposition of leaf litters: Earthworm-microorganism interactions – the ploughing-in effect. *Soil Biology and Biochemistry* **30**, 795−804.

Cotrufo MF, Del Galdo I, Piermatteo D, 2009: Litter decomposition: concepts, methods and future perspectives. In: *Soil carbon dynamics: an integrated methodology* (ed. by Kutsch, W. L. *et al*.). Cambridge University Press, Cambridge, pp 76−90.

Crow SE, Lajtha K, Bowden RD, Yano Y, Brant JB, Caldwell BA, Sulzman EW, 2009a: Increased coniferous needle inputs accelerate decomposition of soil carbon in an old-growth forest. *Forest Ecology and Management* **258**, 2224−2232.

Crow SE, Lajtha K, Filley TR, Swanston CW, Bowden RD, Caldwell BA, 2009b: Sources of plant-derived carbon and stability of organic matter in soil: implications for global change. *Global Change Biology* **15**, 2003−2019.

Edwards NT, Harris WF, 1977: Carbon cycling in a mixed deciduous forest floor. *Ecology* **58**, 431−437.

Enríquez S, Duarte CM, Sandjensen KAJ, 1993: Patterns in decomposition rates among photosynthetic organisms – the importance of detritus C-N-P content. *Oecologia* **94**, 457−471.

Fierer N, Craine JM, McLauchlan K, Schimel JP, 2005: Litter quality and the temperature sensitivity of decomposition. *Ecology* **86**, 320−326.

Gallardo A, Merino J, 1993: Leaf decomposition in two Mediterranean ecosystems of southwest Spain – influence of substrate quality. *Ecology* **74**, 152−161.

Guo LB, Sims REH, 2002: Eucalypt litter decomposition and nutrient release under a short rotation forest regime and effluent irrigation treatments in New Zealand: II. Internal effects. *Soil Biology and Biochemistry* **34**, 913−922.

Hirano T, Kim H, Tanaka Y, 2003: Long-term half-hourly measurement of soil CO_2 concentration and soil respiration in a

temperate deciduous forest. *Journal of Geophysical Research* **108**(D20), 4631, doi:10.1029/2003JD003766.

Irmler U, 2000: Changes in the fauna and its contribution to mass loss and N release during leaf litter decomposition in two deciduous forests. *Pedobiologia* **44**, 105−118.

Jacob M, Viedenz K, Polle A, Thomas FM, 2010: Leaf litter decomposition in temperate deciduous forest stands with a decreasing fraction of beech (*Fagus sylvatica*). *Oecologia* **164**, 1083−1094.

Kampichler C, Bruckner A, 2009: The role of microarthropods in terrestrial decomposition: a meta-analysis of 40 years of litterbag studies. *Biological Reviews* **84**, 375−389.

Kim H, Hirano T, Koike T, Urano S, 2005a: Contribution of litter CO_2 production to total soil respiration in two different deciduous forests. *Phyton* **45**, 385−388.

Kim H, Hirano T, Urano S, 2005b: Seasonal variation in CO_2 production of leaf litter from different deciduous forests at the early decomposition stage. *Journal of Agricultural Meteorology* **61**, 95−104.

Kirita H, 1971: Studies of soil respiration in warm-temperate evergreen broadleaf forests of southwestern Japan. *Japanese Journal of Ecology* **21**, 230−244.

Kuzyakov Y, 2010: Priming effects: Interactions between living and dead organic matter. *Soil Biology and Biochemistry* **42**, 1363−1371.

Liang N, Hirano T, Zheng Z, Tang J, Fujinuma Y, 2010: Soil CO_2 efflux of a larch forest in northern Japan. *Biogeosciences* **7**, 3447−3457.

Liang N, Nakadai T, Hirano T, Qu L, 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. *Agricultural and Forest Meteorology* **123**, 97−117.

Maraun M, Scheu S, 1996: Changes in microbial biomass, respiration and nutrient status of beech (*Fagus sylvatica*) leaf litter processed by millipedes (*Glomeris marginata*). *Oecologia* **107**, 131−140.

Melillo JM, Aber JD, Muratore JF, 1982: Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. *Ecology* **63**, 621−626.

Moncrieff JB, Fang C, 1999: A model for soil CO_2 production and transport 2: Application to a Florida *Pinus elliotte* plantation. *Agricultural and Forest Meteorology* **95**, 237−256.

Nakane K, Yamamoto M, Tsubota H, 1983: Estimation of root respiration rate in a mature forest ecosystem. *Japanese Journal of Ecology* **33**, 397−408.

Petersen H, Luxton M, 1982: A comparative analysis of soil fauna populations and their role in decomposition processes. *Oikos* **39**, 287−388.

Prévost-Bouré NC, Soudani K, Damesin C, Berveiller D, Lata J, Dufrêne E, 2010: Increase in aboveground fresh litter quantity over-stimulates soil respiration in a temperate deciduous forest. *Applied Soil Ecology* **46**, 26−34.

Sakuma T, Tomita A, Shibata H, Tanaka Y, 1994: Distribution and cycling of sulfur in deciduous and coniferous forest ecosystems as influenced by acidic deposition II: Input, cycling, and output of sulfur. *Japanese Journal of Soil Science and Plant Nutrition* **65**, 684−691 (in Japanese).

Salamanca E, Kaneko N, Katagiri S, 1998: Effects of leaf litter mixtures on the decomposition of *Quercus serrata* and *Pinus densiflora* using field and laboratory microcosm methods. *Ecological Engineering* **10**, 53−73.

Soil Survey Staff, 2006: Keys to Soil Taxonomy Tenth edition. USDA Natural Resources Conservation Service, Washington DC, USA.

Son Y, Jun YC, Lee YY, Kim RH, Yang SY, 2004: Soil carbon dioxide evolution, litter decomposition, and nitrogen availability four years after thinning in a Japanese larch plantation. *Communications in Soil Science and Plant Analysis* **35**, 1111−1122.

Subke JA, Hahn V, Battipaglia G, Linder S, Buchmann N, Cotrufo MF, 2004: Feedback interactions between needle litter decomposition and rhizosphere activity. *Oecologia* **139**, 551−559.

Sulzman EW, Brant JB, Bowden RD, Lajtha K, 2005: Contribution of aboveground litter, belowground litter, and rhizosphere respiration to total soil CO_2 efflux in an old growth coniferous forest. *Biogeochemistry* **73**, 231−256.

Vose JM, Bolstad PV, 2007: Biotic and abiotic factors regulating forest floor CO_2 flux across a range of forest age classes in the southern Appalachians. *Pedobiologia* **50**, 577−587.

Wang Q, He T, Wang S, Liu L, 2013a: Carbon input manipulation affects soil respiration and microbial community composition in a subtropical coniferous forest. *Agricultural and Forest Meteorology* **178**, 152−160.

Wang Q, Liu S, Wang S, 2013b: Debris manipulation alters soil CO_2 efflux in a subtropical plantation forest. *Geoderma* **192**, 316−322.

Wang X, Piao S, Ciais P, Janssens IA, Reichstein M, Peng S, Wang T, 2010: Are ecological gradients in seasonal Q_{10} of soil respiration explained by climate or by vegetation seasonality?. *Soil Biology and Biochemistry* **42**, 1728−1734.

Wedderburn ME, Carter J, 1999: Litter decomposition by four functional tree types for use in silvopastoral systems. *Soil Biology and Biochemistry* **31**, 455−461.

Yang X, Chen J, 2009: Plant litter quality influences the contribution of soil fauna to litter decomposition in humid tropical forests, southwestern China. *Soil Biology and Biochemistry* **41**, 910−918.