CO_2 efflux from decomposing leaf litter stacks is influenced by the vertical distribution of leaf litter water content and its temporal variation

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Abstract

We evaluated the temporal changes in the vertical distribution of leaf litter respiration （R_{LL}） due to changes of leaf litter water content （W_{LL}） within the leaf litter layer （L layer） using *in situ* direct measurements. To investigate the vertical distribution of R_{LL} and W_{LL} within the leaf litter layer over a time-series, we measured the R_{LL} and W_{LL} of 10 separate layers in vertical leaf litter stacks fixed to the forest floor using wire pins. Measurements were conducted for 30 stacks in a temperate broad-leaved secondary forest between May 2008 and January 2009. In the warm season, high R_{LL} was observed at high W_{LL}, while low R_{LL} was observed at low W_{LL}. R_{LL} was comparatively lower during the cool season than during the warm season regardless of the magnitude of W_{LL}. The temperature sensitivity of R_{LL} differed depending on W_{LL}; temperature increases under low-moisture conditions had small effects on R_{LL}, while under higher-moisture conditions, R_{LL} was more responsive to temperature increases. Small differences in position within the leaf litter stack affected the vertical variation of W_{LL} and, consequently, there was large distribution in R_{LL} during the wet period and small distribution in R_{LL} （totally small values） during the dry period. Thus, CO_2 efflux from the net L layer was strongly affected by R_{LL} distribution induced by the local wetting within the L layer. In estimating CO_2 efflux from the L layer using water content of the L layer, monitoring of the water content of the L layer, which takes into account the vertical distribution in W_{LL} within the L layer, is necessary.

Key words: Decomposition, Leaf litter respiration, Leaf litter water content, Litter layer, Warm-temperate forest.

1. Introduction

The leaf litter layer （L layer） containing a high amount of labile carbon is a reservoir of significant forest soil carbon and a habitat for the largest heterotrophic soil organisms （Anderson *et al*., 2004）. The CO_2 efflux to the atmosphere through the decomposition of the L layer affects temporal variation in heterotrophic respiration over a short-time scale because of the rapidity of decomposition （Gu *et al*., 2004）. Thus, it is an important parameter for determining the changes of belowground C stock in response to climate change （Sayer *et al*., 2007; Schaefer *et al*., 2009） and for understanding the mechanism of litter decomposition on the forest floor surface.

CO_2 efflux from the L layer, being a result of the activity of heterotrophic soil organisms, is strongly affected by environmental factors, especially the moisture （Borken *et al*., 2003; Lee *et al*., 2004）. The moisture of the L layer changes temporally because the L layer is directly exposed to solar radiation and wind. Such wetting and drying cycles of the L layer lead to transient CO_2 efflux from it. Several previous studies have attempted *in situ* continuous measurement of the moisture of the L layer to capture and model temporal variation in CO_2 efflux from

the L layer （Borken *et al*., 2003; Wilson *et al*., 2014）. In these studies, the moisture of the L layer measured using multiple sensors varied widely because the vertical distribution of moisture condition within the L layer was not considered, suggesting that CO_2 efflux from the L layer was insufficiently estimated. Additionally, such vertical distribution of moisture conditions would lead to differences in leaf litter respiration and, consequently, different decomposition processes within the L layer. However, little is known about how spatiotemporal variation of moisture in the L layer affects the vertical distribution of leaf litter respiration within the L layer and its temporal variation, and the effect on CO_2 efflux from the net L layer.

Ataka *et al*. （2014a） found the vertical distribution of moisture within the L layer, roughly separated into three sub-layers, was intensively observed over 3 days, for seven broadleaf species. The results showed similar trends in the vertical distribution of moisture in all the species; the values being highest in the lowest L sub-layer. Based on the results obtained in this short-term study, we hypothesized that the vertical distribution of moisture within the L layer would vary temporally and the moisture profile within the L layer would, in turn, affect the magnitude of CO_2 efflux from the net L layer. Thus, a detailed analysis of the vertical distribution of leaf litter water content （W_{LL}） and leaf litter respiration （R_{LL}） of a single leaf within the L layer, over time under natural conditions, was required to understand the CO_2 efflux mechanism from the L layer and the decomposition process over fine-scale environmental heterogeneity.

The main objective of this study was to evaluate the vertical distribution of W_{LL} and R_{LL} within the L layer and to determine the effect of temporal changes in these on the CO_2 efflux from the net L layer. To establish the same initial conditions among samples, we fixed leaf litter sample stacks to the forest floor using wire pins. The stacks were formed with piles of 10 leaves to duplicate the natural L layer. We measured the R_{LL} for pieces of leaves from the stack in the field over time using an infrared gas analyzer（IRGA）and a closed static chamber system. Using these data obtained from *in situ* measurements, we evaluated the effects of temporal and vertical W_{LL} variability on R_{LL} within the L layer and, consequently, on the CO_2 efflux from the net L layer.

2. Materials and Methods

2.1 Study site

Observations were conducted at the Yamashiro Experimental Forest in southern Kyoto Prefecture, Japan（34°47'N, 135°50'E, 1.7-ha）. This study site is an Asia Flux site and possesses meteorological towers to monitor CO_2 exchange between the atmosphere and the forest ecosystem, as well as environmental factors（*e.g.*, air temperature and precipitation）. Annual mean air temperature and precipitation were 15.5℃（summer hourly maximum 34.8℃; winter hourly minimum –3.9℃）and 1449 mm, respectively. The rainy season generally occurs from early June to mid-July. Daily rates of evaporation from the forest floor are 0.4–0.8 mm day^{-1} for 1–2 days after precipitation, declining thereafter to 0.2–0.3 mm day^{-1}（Tamai and Hattori, 1994）. The soils are regosols of sandy loam or loamy sand and contain fine gravel（53% by mass）composed of residual quartz crystals from the granite parent material（Kaneko *et al.*, 2007）. The soil layer is generally thin and immature. Therefore, the moisture content of the L layer rapidly changes from wet to dry within a few days following precipitation（Ataka *et al.*, 2014b）. In summer, high temperatures and frequent precipitation lead to considerable spatial variation in soil moisture content. Moreover, the terrain is complex and valley sides are steep, contributing to high proportions of rainfall being converted to runoff. Deciduous broadleaved, evergreen broadleaved, and coniferous tree species account for 66, 28 and 6% of the living tree biomass, respectively（Goto *et al.*, 2003）. The forest is dominated by *Quercus serrata* Thunb, which account for 33% of the biomass.

2.2 Arrangement of leaf litter samples for CO_2 efflux measurement

We used leaf litter of *Q. serrata*（the dominant deciduous tree species in the study area）for respiration measurements. Samples of leaf litter that had fallen at the beginning of winter（November–December 2007）were collected from the forest floor in May 2008. To avoid changing the microbial conditions of the leaf litter, we did not oven-dry the samples before use. To investigate the effect of spatial position in the L layer on R_{LL} and W_{LL}, sets of 10 dead leaves were formed into vertical stacks（Fig. 1）. A total of 30 stacks（300 leaves）were fixed to a 100 × 100 cm area of the forest floor using wire pins（2 mm diameter）to prevent wind dispersion and mixing with additional litter-fall. To maintain all sample sets under the same conditions, we manually removed additional litterfall from the top layer of all samples once a week. Each stack was approximately 3 cm thick. We numbered each leaf in the L layer from Layer 1（closest to the ground）to Layer 10（furthest from the ground）.

2.3 Measurement of leaf litter respiration（R_{LL}）in the field

We measured the R_{LL} of the 300 leaves in the 30 leaf litter stacks between June 2008 and January 2009 by destructively sampling one of the leaf litter stacks on each measurement day. Leaf litter respiration was measured using a static closed-chamber system（Fig. 1）. The system consisted of an infrared gas analyzer（IRGA, GMP343; Vaisala Group, Vanta, Finland）attached to a small cylindrical chamber（0.308 L）suitable for measuring the respiration rate of small leaf litter samples. The interior temperature of the chamber was measured with a copper-constantan thermocouple. The temperature of leaf litter（T_{LL}, ℃）was assumed to be the same as the temperature inside the chamber, because thin leaf litter rapidly adapts to the local temperature environment.
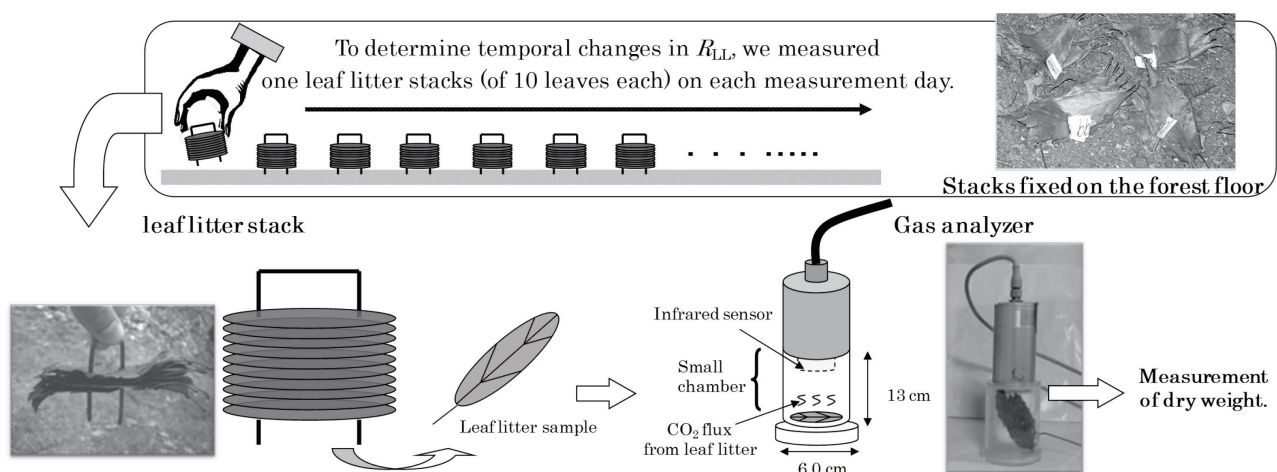

Fig. 1. Illustration of the leaf litter stacks and the device used to measure the vertical distribution of leaf litter respiration（R_{LL}）and leaf litter water content（W_{LL}）within the leaf litter layer. Sets of 10 leaves were fixed to the forest floor using wire pins. The R_{LL} of each leaf litter sample was measured using an infrared gas analyzer attached to a small cylindrical chamber. The sample was then oven-dried at 60℃ for 48 h. A total of 30 litter stacks were prepared and analyzed.

The CO_2 concentration and temperature inside the chamber were recorded at 1-s intervals using a data logger (NR-1000; Keyence, Osaka, Japan). The measurement period for each sample was approximately 10 min; R_{LL} (mg CO_2 kg^{-1} h^{-1} with regard to the dry weight of the sample) was calculated from the increase in CO_2 concentration using a linear regression on the linear portion of the data. Linearity was checked on the logger monitor at each measurement. The IRGA response to changes in CO_2 concentration exhibits a time lag of several tens of seconds because of its in-line air filter, so data from the first 3 min of each measurement period were not used. The respiration data for the middle 5-min interval were used to calculate R_{LL}. When the measured R_{LL} was very low, the resolution of the IRGA (2–3 ppm) was insufficient to detect differences in the CO_2 concentration. Hence, when the IRGA measurements showed an increase of less than 3 ppm in CO_2 concentration over 5 min, we assumed R_{LL} to be 0 mg CO_2 kg^{-1} h^{-1}.

2.4 Measurement of leaf litter water content (W_{LL})

After measurements of R_{LL} from each of the 10 leaves in the stack in the field, each leaf litter sample was immediately enclosed in a vinyl bag and taken to the laboratory for determination of water content.

The fresh weight of the leaf litter was measured in the laboratory within 24 h after sampling. The leaf litter samples were oven-dried at 60℃ for 48 h, and the water content (W_{LL} in dry mass; g g^{-1}) was determined from the difference between the fresh and dry masses of the samples.

2.5 Analysis

To evaluate the temperature sensitivity of R_{LL} and to calculate the standard R_{LL} at 15.5℃, the annual mean air temperature at our site, the following exponential equation was applied:

$$R_{LL} = a \exp(b\, T_{LL}), \qquad (1)$$

where, *a* and *b* were constants. Nonlinear regression was conducted using a modified Levenberg-Marquardt method with Igor Pro 6.34 software (Wave Metrics, Inc., Lake Oswego, OR, USA).

3. Results

The R_{LL} from each leaf of a litter stack was measured on 30 occasions between June 2008 and January 2009 (Fig. 2d). R_{LL} ranged from 0 to 1174 mg CO_2 h^{-1}, and the mean and standard deviation were 269 and 250 mg CO_2 kg^{-1} h^{-1}, respectively ($n = 300$); W_{LL} ranged from 0.01 to 3.01 g g^{-1}, and the mean and standard deviation were 0.80 and 0.60 g g^{-1}, respectively ($n = 300$). R_{LL} showed substantial temporal variation associated with T_{LL} and W_{LL}. We defined the period during which
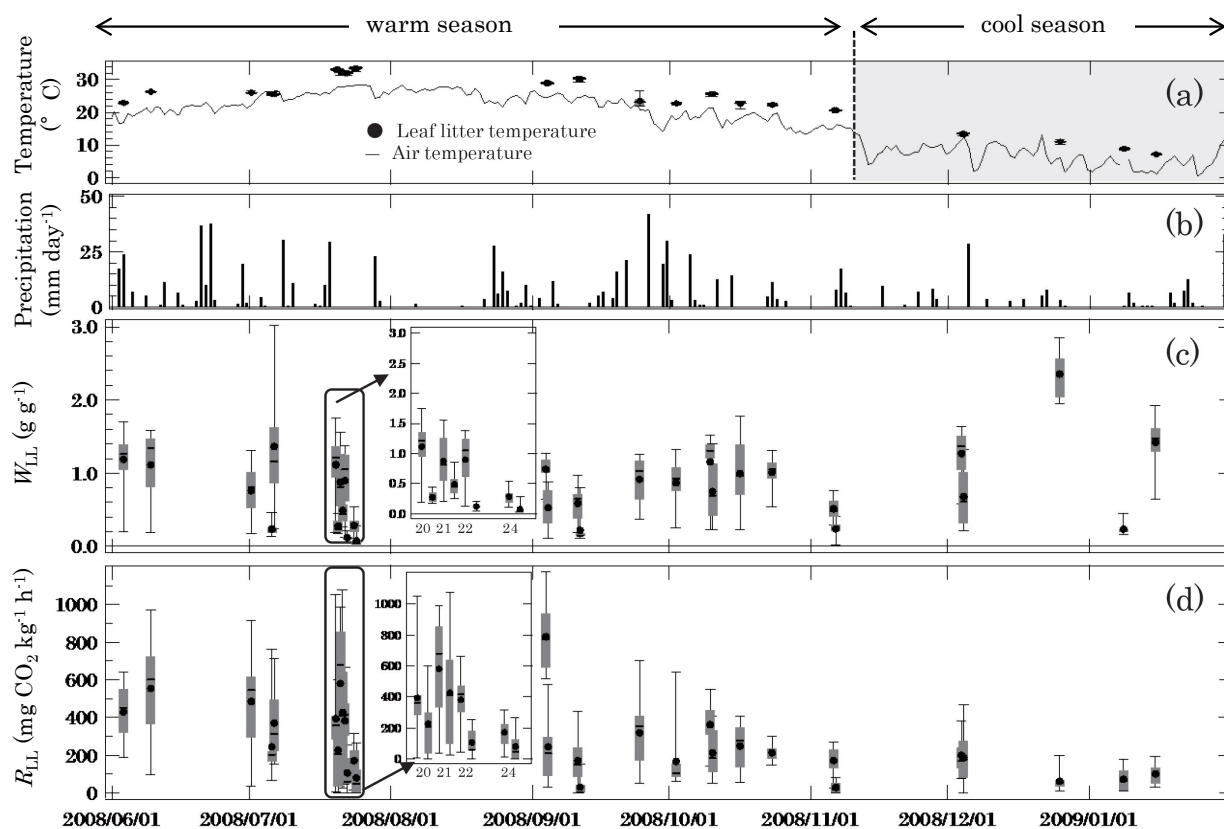

Fig. 2. Seasonal variation in (a) temperature of leaf litter (●) and air temperature (—), (b) daily precipitation, (c) leaf litter water content (W_{LL}), and (d) leaf litter respiration (R_{LL}). The bars for leaf litter temperature show standard deviations ($n = 10$). The box plot indicates the median (thin line in the box), as well as the 25th and 75th percentiles (the boundaries of the boxes closest to, and furthest from zero, respectively; $n = 10$). The bars above and below the box show maximum and minimum values, respectively. Black dots show average values.
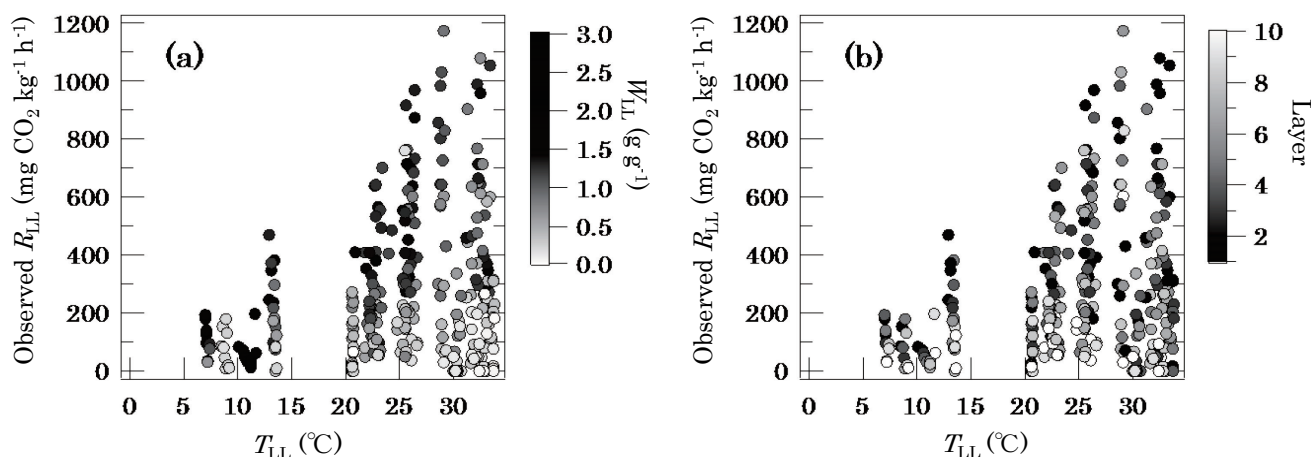
Fig. 3. (a) Relationship between leaf litter respiration (R_{LL}), leaf litter temperature (T_{LL}) and, leaf litter water content (W_{LL}). The strength of symbol shading indicates W_{LL}. (b) Relationship between R_{LL}, T_{LL}, and litter position within the leaf litter stack. The strength of symbol shading indicates litter position within the leaf litter stack as per the gradational scale (Layer 1 is closest to the ground and Layer 10 is furthest from the ground).

Table 1. Effect of leaf litter water content (W_{LL}) on temperature sensitivity (Q_{10}) of leaf litter respiration (R_{LL}). a and b indicate constants fitted with the function of T_{LL} [$y = a \exp(b \times T_{LL})$, Eq. 1]. The number represents the total number of measurements.

W_{LL} (g g^{-1})	0–0.4	0.4–0.8	0.8–1.2	1.2–	ALL
Q_{10}	1.19	2.65	1.97	1.71	1.35
a	65.0	19.5	68.6	119.4	125.4
b	0.02	0.01	0.07	0.05	0.03
Number	111	54	58	77	300

the air temperature was above the annual average (15.5℃) as the warm season and that during which temperature was below the average as the cool season (Fig. 2a). Higher R_{LL} values were frequently observed in the warm season, while R_{LL} in the cool season was relatively low. In the warm season, R_{LL} showed relatively low values, despite the high air temperature, when W_{LL} was low (Fig. 2). The relationship between R_{LL} and T_{LL} was significantly affected by W_{LL} (Fig. 3a, Table. 1). Moreover, a higher R_{LL} under each temperature condition was often detected in the lower layer within the leaf litter stack (Fig. 3b).

To investigate the variation in R_{LL} based on T_{LL} and W_{LL}, we classified the data into three periods using the average W_{LL} and T_{LL} of each leaf stack (mean value of ten leaves): wet period in warm season ($W_{LL} \geq 0.4$ g g^{-1}, $T_{LL} \geq 15.5$℃, 18 stacks), dry period in warm season ($W_{LL} < 0.4$ g g^{-1}, $T_{LL} \geq 15.5$℃, 7 stacks), and cool season ($T_{LL} < 15.5$℃, 5 stacks). The data in cool season were not classified because of an insufficient number of stacks. During the wet period in the warm season, the means and standard deviations of R_{LL} and W_{LL} were 348 ± 172 mg CO_2 kg^{-1} h^{-1} and 0.8 ± 0.3 g g^{-1}, respectively (Fig. 4a, b). Both R_{LL} and W_{LL} during the wet period in the warm season were generally greater in the lower layer and lesser in the upper layer. During the dry period in the warm season, the means and standard deviations of R_{LL} and W_{LL} were 98 ± 88 mg CO_2 kg^{-1} h^{-1} and 0.2 ± 0.08 g g^{-1},

respectively (Fig. 4c, d). R_{LL} during the dry period in the warm season was comparatively low in all layers, corresponding with low W_{LL}. R_{LL} during the wet period showed larger values, with larger vertical and temporal deviations, compared to those during the dry period, which corresponded to the range and variation of W_{LL}. In the cool season, the mean and standard deviation of R_{LL} and W_{LL} were 125 ± 65 mg CO_2 kg^{-1} h^{-1} and 1.2 ± 0.8 g g^{-1}, respectively (Fig. 4e, f). R_{LL} in cool season showed low values despite of large vertical and temporal variation of W_{LL}.

The CO_2 efflux from the net L layer, which was calculated using the L layer mass at the site (2.58 t C ha^{-1}; Kominami *et al.*, 2008) and the average R_{LL} (mg CO_2 kg^{-1} h^{-1}) in the leaf litter stack, ranged from 0.024 to 0.12 mg CO_2 m^{-2} s^{-1} during the wet period in the warm season, from 0.0041 to 0.035 mg CO_2 m^{-2} s^{-1} during the dry period in the warm season, and from 0.0089 to 0.029 mg CO_2 m^{-2} s^{-1} in the cool season. The average W_{LL} ranged from 0.49 to 1.37 g g^{-1} during the wet period in the warm season, from 0.077 to 0.28 g g^{-1} during the dry period in the warm season, and from 0.24 to 2.35 g g^{-1} in the cool season. The CO_2 efflux from the net L layer $_{15.5℃}$, which was standardized at 15.5℃ using the Q_{10} value (= 1.35) obtained from Table 1, ranged from 0.016 to 0.079 mg CO_2 m^{-2} s^{-1} during the wet period in warm season, from 0.0028 to 0.026 mg CO_2 m^{-2} s^{-1} during the dry period in the warm season, and from 0.010 to 0.031 mg CO_2 m^{-2} s^{-1} in the cool
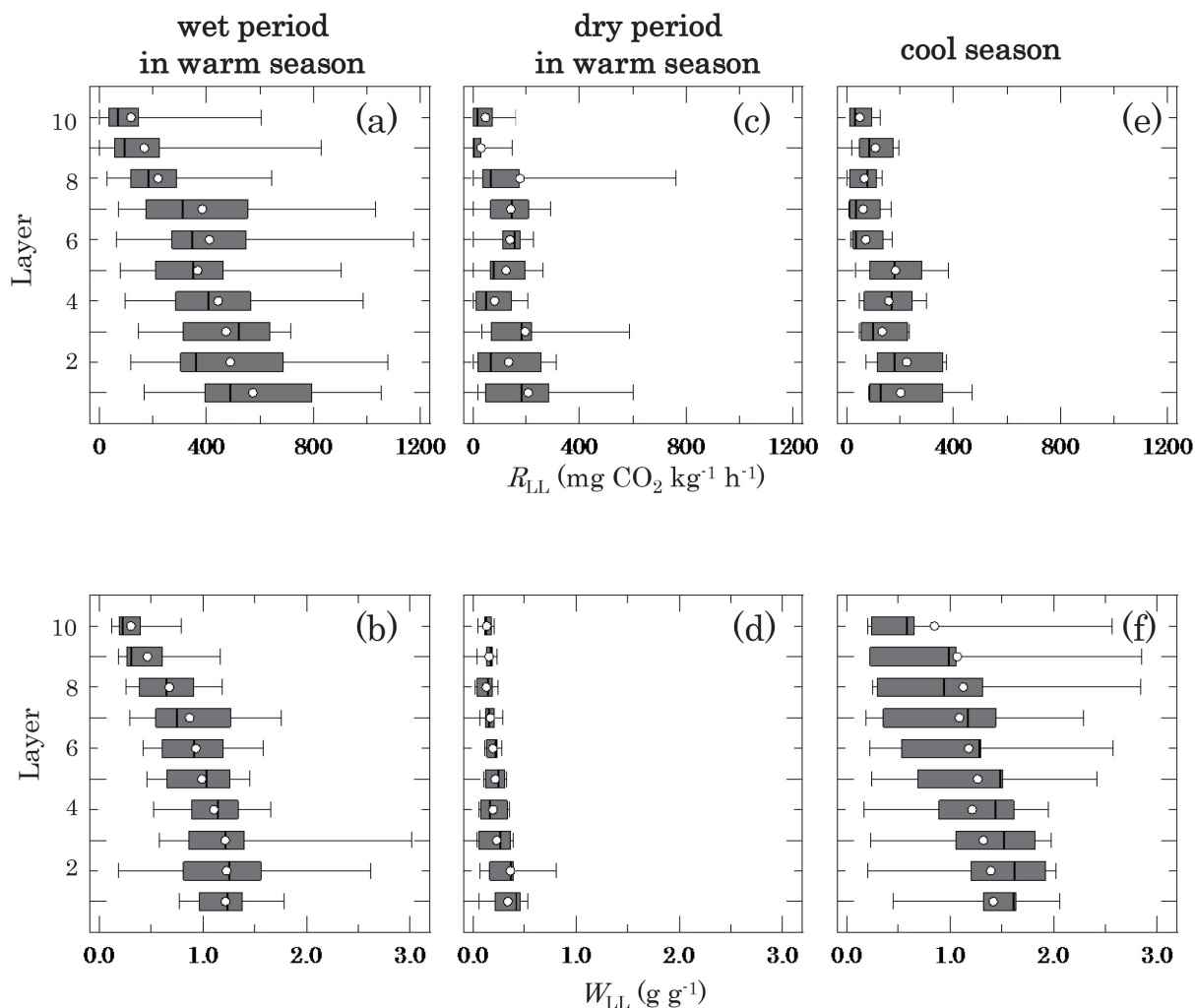
Fig. 4. Profiles of leaf litter respiration (R_{LL}; a, c, e) and leaf litter water content (W_{LL}; b, d, f) of the leaf litter stacks. The numbers on the left axis represent the ordinal numbering of layers in a stack. (Layer 1 is closest to the ground and Layer 10 is furthest from the ground.) The R_{LL} and W_{LL} profiles observed in the 30 stacks were classified into three periods; wet-warm ($W_{LL} \geq 0.4$, temperature $\geq 15.5°C$; a, b), dry-warm ($W_{LL} < 0.4$, temperature $\geq 15.5°C$; c, d), and cool (temperature $< 15.5°C$; e, f). The box plot indicates the median (thin line in the box), as well as the 25th and 75th percentiles (boundaries of the boxes closest to and furthest from zero, respectively). The right and left bars show maximum and minimum values, respectively. The white dots show average values.

season (Fig. 5a). The CO₂ efflux from the net L layer increased with an increase in average W_{LL}. The standard deviations for $R_{LL\_15.5°C}$ in the leaf litter stack ranged from 0.0055 to 0.031 mg CO₂ m⁻² s⁻¹ during the wet period in the warm season, from 0.0038 to 0.020 mg CO₂ m⁻² s⁻¹ during the dry period in the warm season, and from 0.0085 to 0.024 mg CO₂ m⁻² s⁻¹ in the cool season. The standard deviations for W_{LL} in the leaf litter stack ranged from 0.17 to 0.90 g g⁻¹ during the wet period in the warm season, from 0.0045 to 0.21 g g⁻¹ during the dry period in the warm season, and from 0.079 to 0.42 g g⁻¹ in the cool season (Fig. 5b).

4. Discussion

The CO₂ efflux rate from individual pieces of leaf litter measured directly in the field varied widely (0–1174 mg CO₂ kg⁻¹ h⁻¹) according to the seasonal and vertical variation of T_{LL} and W_{LL} (Fig. 2). Our results showed a wider range of R_{LL} than laboratory data in past studies in which broad-leaved litter was

incubated; Fioretto *et al.* (2007) reported CO₂ efflux from leaf litter samples ranging from 30 to 50 mg CO₂ kg⁻¹ h⁻¹ at a T_{LL} of 25°C and W_{LL} of 0.1–1.5 g g⁻¹, and Dilly and Munch (1996) indicated that CO₂ efflux from leaf litter samples ranged from roughly 100 to 700 mg CO₂ kg⁻¹ h⁻¹ at a T_{LL} of 22°C and W_{LL} of 2.5 g g⁻¹. However, these authors measured CO₂ efflux from leaf litter samples at a constant T_{LL} and/or W_{LL} in a laboratory experiment, whereas our results were produced in the field under T_{LL} values ranging from 7°C to 34°C and W_{LL} values ranging from 0.014 to 3.014 g g⁻¹. *In situ* environmental conditions in the L layer could cause large variations in R_{LL}.

In the warm season, the highest R_{LL} was observed during the wet period, whereas comparatively low R_{LL} values, similar to those observed in the cool season, were observed during the dry period (Fig. 2). This result indicates that annual CO₂ efflux from the L layer is significantly influenced by W_{LL} status in the warm season. Frequent summer rainfall events occur under the Asian
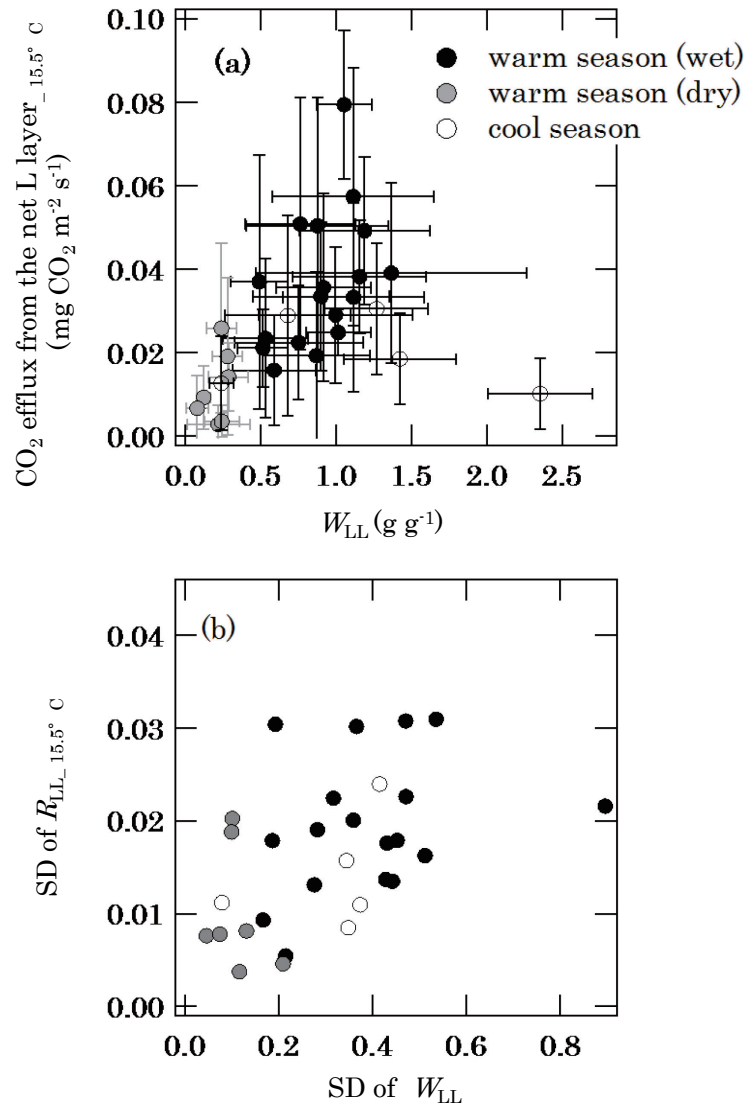
Fig. 5. (a) Relationship between leaf litter water content (W_{LL}) and CO_2 efflux from the net L layer$_{\_15.5℃}$. The CO_2 efflux from the net L layer $_{\_15.5℃}$ was standardized at a temperature of 15.5℃. Black, gray, and white circles show CO_2 efflux from the net L layer$_{\_15.5℃}$ at three periods; wet-warm ($W_{LL} \geq 0.4$, temperature $\geq 15.5℃$), dry-warm ($W_{LL} < 0.4$, temperature $\geq 15.5℃$), and cool (temperature $< 15.5℃$), respectively. The bars for CO_2 efflux from the net L layer $_{\_15.5℃}$ and W_{LL} show standard deviations ($n = 10$). (b) Relationship between standard deviations of W_{LL} and leaf litter respiration (R_{LL}) in the leaf litter stack. R_{LL} was standardized at a temperature of 15.5℃ ($R_{LL\ 15.5℃}$).

monsoon climate; at the present site, the evaporation rate from the L layer was higher for the 1 to 2 days after precipitation and rapidly declined after that. Hanson *et al.* (2003) also reported that the water status of the L layer was highly dynamic, with wetting and drying cycles occurring within 1–2 days in association with rain events in temperate forest, which lead to transient CO_2 efflux from the L layer. The temporal variation in the CO_2 efflux from the L layer due to moisture dynamics of the L layer has been observed in both temperate and tropical forests. Such variations in the CO_2 efflux from the L layer induce sudden and transient increases in soil respiration and forest CO_2 exchange (Goulden *et al.*, 2004; Borken and Matzner 2006; Cisneros-Dozal *et al.*, 2007; Savage *et al.*, 2009), and the magnitude of annual CO_2 efflux from the L layer is therefore determined by the frequency of precipitation because of the rapid evaporation in the warm season.

The interaction of temperature and moisture on R_{LL} differed depending on W_{LL} (Table. 1). Under low-moisture conditions ($W_{LL} < 0.4$ g g^{-1}), temperature increases had a small effect on R_{LL}, while under higher-moisture conditions ($W_{LL} \geq 0.4$ g g^{-1}), R_{LL} was more responsive to temperature increases. The comparatively small Q_{10} under dry conditions was caused by the small R_{LL} despite the magnitude of temperature. These results were similar to those of Xu and Qi (2001) and Reichstein *et al.* (2003), who also examined the effects of water content on Q_{10} of soil respiration. Moreover, the L layer has more rapid drying and wetting cycles compared to other organic materials (*e.g.*, soil organic matter, root litter, and woody debris). Consequently, the CO_2 efflux from the L layer varied dynamically from about zero to the peak value associated with the drying and wetting cycles under natural condition (Schimel *et al.*, 1999; Ataka *et al.*,

2014c). The lower potential of leaf litter to retain water due to its properties and position (Jomura *et al*., 2012) result in the relatively low temperature sensitivity of R_{LL} over the observed total range of W_{LL} (Q_{10} = 1.35, Table. 1). Such intrinsic characteristic of organic material affect moisture conditions and the temperature sensitivity of CO_2 efflux.

Subtle differences (on the order of centimeters) in position within the leaf litter stack affected vertical variations in W_{LL} and R_{LL}, and these values changed with the moisture conditions and the seasonal temperature changes (Fig. 4). During the cool season, R_{LL} was comparatively small throughout the entire layer despite the large variation in W_{LL} within the leaf litter stack. Conversely, during the warm season, the vertical distribution in R_{LL} largely varied in association with W_{LL}. The temporal and vertical variation in W_{LL} depends on the strength and frequency of rainfall events, the upper layer generally experienced repeated drying and wetting cycles, while the lower layer experienced relatively stable moisture conditions (Hatton *et al*., 1988). Such spatial variation in moisture conditions caused by the position of litter affects fungal growth (Osono *et al*., 2006) and decomposition progress (Jomura *et al*., 2007), which suggests that there is different decomposition process within the L layer.

The CO_2 efflux from the net L layer was strongly affected by R_{LL} variation associated with the vertical distribution of W_{LL} within the L layer and its temporal variation (Fig. 4 and Fig. 5). As seen in Fig. 4, there were large distributions in W_{LL} and R_{LL} within the L layer during the wet period and small distributions in W_{LL} and R_{LL} (totally small values) within the L layer during the dry period. In the warm season, the averages and standard deviations of W_{LL} and R_{LL} during the wet period (348 ± 172 mg CO_2 kg^{-1} h^{-1} and 0.8 ± 0.3 g g^{-1}; Fig. 4a, b) within the L layer were larger than those during the dry period (98 ± 88 mg CO_2 kg^{-1} h^{-1} and 0.2 ± 0.08 g g^{-1}; Fig. 4c, d). As a result, the standard deviations of $R_{LL\_15.5℃}$ increased with an increase in W_{LL} in the leaf litter stack (Fig. 5b), and the ranges of W_{LL} and R_{LL} in the wet-warm season (0.0055 to 0.031 mg CO_2 m^{-2} s^{-1} and 0.17 to 0.90 g g^{-1}, respectively) were larger than those in the dry-warm season (0.0038 to 0.020 and 0.0045 to 0.21 g g^{-1}, respectively). Furthermore, the CO_2 efflux from the net L layer$_{15.5℃}$ decreased with the average W_{LL}, which was determined by the vertical distribution in W_{LL} within the L layer (Fig. 5a). These data indicated that the CO_2 efflux from the net L layer was strongly affected by R_{LL} distribution, which was induced by the local wetting within the L layer that often occurs in the lower part of the net L layer. Thus, our results suggest that monitoring of the water content of the L layer that take into account the vertical distribution in W_{LL} within the L layer is necessary in estimating CO_2 efflux from the L layer using water content of the L layer.

Additionally, the average CO_2 efflux from the net L layer$_{15.5℃}$ (0.028 mg CO_2 m^{-2} s^{-1}) contributed 29% of the average soil respiration measured at around 15.5℃ at the same site (0.098 mg CO_2 m^{-2} s^{-1}; Tamai *et al*., 2005). This was similar to the 48% and 23% contribution to soil respiration by CO_2 efflux from the net L layer observed in temperate forest, respectively, by DeForest *et al*. (2009) and Hanson *et al*. (2003). On the one hand, the range of CO_2 efflux from the net L layer (0.0041 to 0.12 mg CO_2 m^{-2} s^{-1}) was relatively larger than that directly measured with an automated chamber system at the same site (0–0.077 mg CO_2 m^{-2} s^{-1}; Ataka *et al*., 2014c). It might have been possible because of the excess CO_2 efflux stimulated by human-induced disturbances (*e.g*., cutoff of fungal hyphae and CO_2 diffusion within the leaf litter stack) when a leaf was separated from the leaf litter stack, which suggests that we should pay attention to the accumulated R_{LL} as the CO_2 efflux from the L layer.

In this study, seasonal variation in R_{LL} was relatively strongly affected by temperature, while temporal variation in R_{LL} was strongly affected by W_{LL}. The vertical distribution in W_{LL} could cause large heterogeneity in R_{LL} within the L layer, indicating that local wetting could strongly depend on the CO_2 efflux from the net L layer. Such temporal and spatial variation in the water content of the substrate could affect heterotrophic respiration from the substrate. In the estimation of CO_2 efflux from the L layer using water content of the L layer, particular attention should be paid to large vertical variation of W_{LL} within the L layer.

Moreover, we occasionally observed hotspots of R_{LL} that were exceptionally larger than those expected from T_{LL} and W_{LL} (for example, Layer 8 during the dry period in warm season as depicted in Fig. 4c). This may possibly be due to the distribution of microbial biomass within the fine-scale as the leaf litter layer. Detailed measurement of W_{LL} and R_{LL} within the L layer would help us to understand the mechanism of litter decomposition on the forest floor surface, which is driven by small size heterotrophic organisms.

Acknowledgments

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