In Situ CO₂ Efflux from Leaf Litter Layer Showed Large Temporal Variation Induced by Rapid Wetting and Drying Cycle

Mioko Ataka¹*, Yuji Kominami², Kenichi Yoshimura², Takafumi Miyama², Mayuko Jomura³, Makoto Tani¹

¹Laboratory of Forest Hydrology, Division of Environmental Science and Technology, Graduate School of Agriculture, Kyoto University, Kyoto, Japan, ²Kansai Research Center, Forestry and Forest Products Research Institute (FFPRI), Kyoto, Japan, ³College of Bioresource Sciences, Nihon University, Fujisawa, Kanagawa, Japan

Abstract

We performed continuous and manual in situ measurements of CO₂ efflux from the leaf litter layer (RLL) and water content of the leaf litter layer (LWC) in conjunction with measurements of soil respiration (RS) and soil water content (SWC) in a temperate forest; our objectives were to evaluate the response of RLL to rainfall events and to assess temporal variation in its contribution to RS. We measured RLL in a treatment area from which all potential sources of CO₂ except for the leaf litter layer were removed. Capacitance sensors were used to measure LWC. RLL increased immediately after wetting of the leaf litter layer; peak RLL values were observed during or one day after rainfall events and were up to 8.6-fold larger than RLL prior to rainfall. RLL declined to pre-wetting levels within 2–4 day after rainfall events and corresponded to decreasing LWC, indicating that annual RLL is strongly influenced by precipitation. Temporal variation in the observed contribution of RLL to RS varied from nearly zero to 51%. Continuous in situ measurements of LWC and CO₂ efflux from leaf litter only, combined with measurements of RS, can provide robust data to clarify the response of RLL to rainfall events and its contribution to total RS.

Introduction

Efflux of CO₂ from the soil surface (soil respiration; RS), which is the sum of respiration by autotrophs and heterotrophs, is an important component of total CO₂ efflux from forest ecosystems [1–3]. The RS of total ecosystem respiration varied from 58% to 76% in a mixed coniferous-deciduous forest [4], depending on interannual and seasonal changes in autotrophic and heterotrophic respiration; variability in RS can affect the forest carbon balance on daily and seasonal time scales. To explain the cause of variability in RS, many studies have attempted to separate differing sources of RS and to examine factors controlling CO₂ efflux rate from each source [5–7]. Especially in forest ecosystems, heterotrophic respiration consists of CO₂ efflux from various sources (e.g., leaf and root litter, woody debris, soil organic matter) and their rates are controlled by their specific environmental condition such as water content (WC) and temperature [8], physical properties of the substrate (e.g., density and structure) [9,10], and chemical properties (e.g., labile and recalcitrant carbon) [11,12]. Moreover, CO₂ efflux from the various heterotrophic sources responds differently to these controlling factors, which illustrates the complexity of RS. In recent decades, a variety of methods for separating components of heterotrophic respiration and for determining their contribution to total RS have been developed [9,13].

Among heterotrophic sources of CO₂, the leaf litter layer (L-layer) is a significant reservoir of degradable carbon and a large potential source of CO₂ efflux from forest soils [14]. In temperate forests, the contribution of CO₂ efflux from the L-layer (leaf litter respiration; RLL) to RS is reported to range from 23% to 48% [15,16]. The L-layer is in direct contact with rainfall, solar radiation, and wind, and environmental conditions (e.g., WC and temperature) can change more dynamically in the L-layer than in lower soil layers. Rapid and transient temporal variation in WC of the L-layer has been observed, especially in warm climates [16,17]. Heterotrophic respiration responds rapidly to changes in moisture status [17,18]; therefore, rapid and transient wetting and drying cycles would produce large temporal variations in RLL. This would significantly affect variation in RS [17,19], suggesting that RLL is an important controller of temporal (daily and seasonal) patterns in the carbon balance in warm regions [19,20].

Several methods for measuring RLL and for calculating its contribution to RS have been explored. Cáceres-Dozal et al. [21] used an isotope mass balance method and reported that the contribution of RLL to RS increased from 5% to 37% in response to water addition after transient drought. Deforest et al. [15]
determined that the annual contribution of $R_{LL}$ to $R_S$ was 48% ± 12% by measuring $R_S$ with and without the L-layer, and the ratio was consistent over a range of environmental conditions. However, there is little information about temporal variation in $R_{LL}$ in relation to rainfall events because of the difficulty of continuous and direct measurement of $R_{LL}$ in situ.

To continuously measure CO$_2$ efflux from the L-layer only, in parallel with measurement of $R_S$, we developed an approach for measuring $R_{LL}$ using an automated chamber method in a treatment area from which all CO$_2$ sources except for the L-layer were removed. In parallel with $R_{LL}$ and $R_S$ measurements, we continuously measured water content of the L-layer (LWC) and soil water content (SWC). LWC was measured using a method developed by Ataka et al. [22], in which intact leaf litter was attached to surrounding capacitance sensors. Sensors were also developed by Ataka et al. [22], in which intact leaf litter was discarded to avoid effects of closing the chamber. $R_{LL}$ and $R_S$ were calculated using the following equation:

$$R = \frac{\Delta C_{CO2}}{10^6} \times \frac{V}{V_{air}} \times \frac{273.2}{273.2 + T} \times M_{CO2} \times \frac{1}{A},$$

where $R$ is respiration (mg CO$_2$ m$^{-2}$ s$^{-1}$), $\Delta C_{CO2}$ is the change in CO$_2$ concentration per unit time (CO$_2$ ppm s$^{-1}$), $V$ is the volume of the system (L), $V_{air}$ is the standard gas volume (22.41 L mol$^{-1}$), $T$ is temperature inside the chamber (°C), $M_{CO2}$ is the molecular weight of CO$_2$ (44.01 g mol$^{-1}$), and $A$ is the soil surface area covered by the chamber (m$^2$).

To continuously measure CO$_2$ efflux from the L-layer only, we developed an automated chamber method in a treatment area in which all potential CO$_2$ sources (e.g., organic soil and fine roots) except for the L-layer were replaced with combusted granite soil (Fig. 1B). To prepare the treatment area (1 m$^2$), we removed surface soil (approximately 5 cm). An acrylic board was placed on the bottom side of the treatment area to prevent penetration of roots; a drain tube was placed at the bottom of the board to prevent the treatment area from flooding with rainwater. The treatment area was then filled with granite soil combusted in a muffle furnace (500°C for 1 day). For $R_{LL}$ measurement, we placed a PVC collar (320-cm$^2$ surface area) and acrylic board below the collar. The board was set at a slight incline to drain rainwater from the collar. We added 15 g of newly fallen leaf litter, which represents the average litterfall mass per unit ground surface area at this site, to the collar. We added the leaf litter to each chamber on January 2012. To acquire data on the temporal variation in $R_{LL}$ of fresh leaf litter, we replaced the litter with newly fallen leaf litter in January 2013. The collar for measurement of $R_S$ was placed near the treatment area for $R_{LL}$ measurement and the L-layer inside the collar was removed and leaf litter was supplied similarly as for measurement of $R_{LL}$. To prevent incorporation of newly fallen litter, we placed a mesh sheet (1×1 cm mesh) on the L-layer inside the chamber, and fallen litter was removed weekly. CO$_2$ efflux from combusted granite soil was measured 6 months from the start of the $R_{LL}$ measurements. The mean CO$_2$ flux rate (± standard deviation) was 0.00063 ± 0.00068 mg CO$_2$ m$^{-2}$ s$^{-1}$ ($n = 16$) when SWC ranged from 0.05 to 0.3 m$^3$ m$^{-3}$ at temperatures of 24°C. Thus, we assumed that CO$_2$ efflux from the combusted granite soil was negligible throughout the measurement period.

For continuous in situ measurement of LWC, we used capacitance sensors as described by Ataka et al. [22]. The measurements were performed on the top surface of the L-layer and at the boundary between the L-layer and mineral soil (Fig. 1B), to capture the large vertical distribution of WC within the L-layer. We estimated average LWC from the output voltage (V) of the two sensors using the conversion equation LWC = 12.73 V – 3.42 presented by Ataka et al. [22]. LWC at the forest floor shows spatial variability associated with tree canopy conditions. Thus, to reflect the LWC of the L-layer by direct measurement, two capacitance sensors were placed on the L-layer inside the chamber. To check the validity of continuous LWC monitoring, we compared the sensor values with LWC measured...
Figure 1. Schematic of the automated chamber system and the experimental design for measurement of CO$_2$ efflux from the leaf litter layer. A. Schematic of the automated dynamic-closed chamber system for measuring leaf litter respiration and soil respiration. B. The experimental design for continuous measurement of CO$_2$ efflux from the leaf litter layer only using automated chamber system.

Figure 2. Schematic of the manual chamber system and the experimental design for measurement of CO$_2$ efflux from the leaf litter layer ($R_{LL}$) and soil ($R_s$).
Figure 3. Seasonal variation in environmental factors, CO2 efflux from the leaf litter layer ($R_{LL}$), and soil respiration ($R_s$). Data were measured every 30 min between September 2012 and January 2014. A. Bold and fine lines show air temperature and water content of the leaf litter layer (LWC), respectively. B. Bold and fine lines show soil temperature and soil water content (SWC), respectively. C. Black and grey lines show observed and estimated $R_{LL}$, respectively. D. Black and grey lines show observed and estimated $R_s$, respectively. E. Black and grey lines show the ratio of observed and estimated $R_{LL}$ to $R_s$, respectively. Circles and bars show mean values and standard deviation of manual measurements. Estimated $R_{LL}$ and $R_s$ were calculated from regression equations using temperature (T) and water content (WC): $R_{LL} = 0.29 e^{0.059T/WC/(95.04+WC)}$ and $R_s = 0.031 e^{0.037T/WC/(0.032+WC)}$. doi:10.1371/journal.pone.0108404.g003

Table 1. Q_{10} of leaf litter respiration ($R_{LL}$) and soil respiration ($R_s$) for different water contents of the leaf litter layer (LWC) and soil (SWC).

|       | $R_{LL}$ |       |       | $R_s$ |       |       |
|-------|----------|-------|-------|-------|-------|-------|
|       | LWC≤1    | 1<LWC≤2 | 2<LWC | SWC≤0.1 | 0.1<SWC≤0.15 | 0.15<SWC |
| $Q_{10}$ | 1.54 | 1.88 | 2.07 | 1.97 | 2.12 | 2.73 |
| a     | 0.0019 | 0.0044 | 0.0064 | 0.027 | 0.032 | 0.025 |
| b     | 0.043 | 0.063 | 0.073 | 0.068 | 0.075 | 0.10 |

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manually as described in the following section. In parallel with LWC measurement, soil temperature (copper-constantan thermocouple) and soil volumetric water content (ECH2O EC-5 sensors; Decagon Devices, Pullman, WA, USA) were measured at 5-cm depth near each chamber. The output voltage of all environmental data was recorded every 1 min with a data logger (Datamark LS3000 PtV; Hakusan, Japan) and average values were computed every 30 min. The environmental data, $R_{LL}$, and $R_S$ were measured continuously between September 2012 and January 2014. Malfunction of IRGA resulted in a lack of data for $R_{LL}$ and $R_S$ for 31% of the measurements.

**Manual chamber method for measuring leaf litter respiration and soil respiration**

To determine the validity of $R_{LL}$ and $R_S$ measured using the automated chamber method, respiration was measured using the manual chamber method. We assumed that manual chamber method allow to measure under conditions that were closer to natural than the automated chamber method. We measured $R_{LL}$ and $R_S$ manually using a static chamber system at midday on 18 days between April 2013 and January 2014. Twelve PVC collars (320 cm$^2$ surface area) were placed in a 2×4 m area in January 2013. The edges of the collars were inserted approximately 1.5 cm into the soil. To measure $R_{LL}$, mesh baskets (1×1 mm mesh, the
same diameter as the PVC collars; 20 cm) were set into each collar and 15 g (dry weight) of newly fallen leaf litter was placed on the L-layer inside each basket (Fig. 2). To prevent supply of newly fallen litter, we placed a mesh sheet (1 x 1 mm mesh) on the L-layer inside the chamber, and fallen litter was removed weekly.

For measurement of $R_S$, the collars were completely covered with lids to which an IRGA and copper-constantan thermocouple were attached. Soil temperature and SWC (5 cm depth) were measured close to the collars when $R_S$ was measured. After completing the measurements of $R_S$, the mesh baskets were carefully removed from the collars and placed in PVC chambers (20 cm diameter, 7 cm high; Fig. 2). We measured $R_{LL}$ using the same methods as used for $R_S$ measurement. The temperature and CO$_2$ concentrations in the chamber were recorded at 1-s intervals using a data logger (GL220). Linearity of the CO$_2$ flux was checked on the data logger monitor at each measurement. The measurement period for each chamber was 10 min and CO$_2$ data for the middle 5-min intervals were used to determine $R_{LL}$ according to Eq. (1), excluding data from the first 3 min.

For measurement of LWC in the mesh baskets, four or five leaves were removed from each basket and immediately placed in sealed plastic bags. Fresh weight of the leaf litter was measured in the laboratory within 24 h of sampling. Leaf litter samples were oven dried at 65°C for 48 h, and water content (WC; g g$^{-1}$) was calculated using Eq. 2 as follows:

$$WC = \frac{FW - DW}{DW},$$  \hspace{1cm} (2)

where $FW$ is the fresh mass of the sample (g), and $DW$ is the dry mass of the sample (g). Samples were returned to each mesh basket within 1 week after sampling.

Leaf litter respiration and soil respiration rates as a function of environmental factors

Respiration models are fundamentally described by nonlinear functions. We used the following function to investigate the response of respiration to temperature:

$$R = a \exp(bT),$$  \hspace{1cm} (3)

where $T$ is temperature (leaf litter temperature for $R_{LL}$ measurement or soil temperature for $R_S$ measurement) and $a$ and $b$ are constants. Leaf litter temperature was assumed to be same as air temperature. $b$ is related to the Q10 parameter ($Q_{10} = e^{10b}$). To determine the effects of temperature and water content on $R_{LL}$ and $R_S$, we used a function that was previously applied to estimate soil respiration by Subke and Schlesinger [26]:

$$R = a \exp(bT) \left( \frac{WC}{\epsilon + WC} \right),$$  \hspace{1cm} (4)

where $a$, $b$, and $\epsilon$ are constants. LWC or SWC was used as WC in this equation. These nonlinear regressions were performed using a modified Levenberg–Marquardt method with Igor Pro 6.0 software (WaveMetrics, Lake Oswego, OR, USA). The estimated respiration values presented in this manuscript were calculated using Eq. 4.

Short-term changes in $R_{LL}$ and LWC on wetting and drying cycle

To evaluate short-term changes in $R_{LL}$ and LWC after rainfall events, we chose eight typical periods that included one wetting and drying cycle and had consecutive no rainfall days for at least 3 days. We used daily mean $R_{LL}$ and LWC before the day on which precipitation occurred as the pre-wetting condition, and these values after precipitation as the post-wetting condition. Daily mean $R_{LL}$ was calculated from $R_{LL}$ values observed using the automated chamber method.

Effect of wetting and drying cycle of the L-layer on $R_{LL}$ and $R_S$ on the annual time scale

To investigate the effects of wetting and drying of the L-layer on $R_{LL}$ on the annual time scale, we separated the estimated daily mean $R_{LL}$ in 2013 into ‘Dry’ and ‘Wet’ periods based on daily mean LWC as a threshold value. The threshold LWC value that separated ‘Dry’ and ‘Wet’ periods for $R_{LL}$ was estimated by the abovementioned short-term analyses. Daily mean $R_{LL}$ was calculated from the estimated $R_{LL}$ values because there were gaps in the continuous $R_{LL}$ data observed using the automated chamber data. The estimated $R_{LL}$ was calculated using a function based on temperature and water content (Eq. 8, 9).
chambers. We estimated the contribution of \( R_{LL} \) accumulated during the wet and dry period to total \( R_S \).

**Results**

Seasonal variation in \( R_{LL} \) and \( R_S \)

The magnitude of the peak in the observed \( R_{LL} \) pulse was higher in summer than in winter (Fig. 3C). \( R_{LL} \) values were low when LWC was low (Fig. 3A, C). \( R_S \) changed substantially according to temperature (Fig. 3B, D), with higher values in summer than in winter. The relationships between respiration and temperature were described by the following functions:

\[
R_{LL} \left( \text{mg CO}_2 \text{ m}^{-2} \text{s}^{-1} \right) = 0.0038 \exp \left( 0.065 \times T_{LL} \right),
\]

(5)

\[
R_S \left( \text{mg CO}_2 \text{ m}^{-2} \text{s}^{-1} \right) = 0.0031 \exp \left( 0.19 \times T_S \right),
\]

(6)

where \( T_{LL} \) is leaf litter temperature and \( T_S \) is soil temperature (°C).

To evaluate effect of WC on the temperature sensitivity of respiration, the measured respiration data was separated into three groups based on WC (Table 1). More than 14% of total respiration data was included in each WC group. \( R_{LL} \) showed low values when WC values were low in spite of high temperature. Consequently, calculated \( Q_{10} \) values for not only \( R_{LL} \) but also \( R_S \) decreased with decreasing WC. The relationships between respiration and temperature and WC were described by the following functions:

\[
R_{LL} \left( \text{mg CO}_2 \text{ m}^{-2} \text{s}^{-1} \right) = 0.29 \exp \left( 0.059 \times T_{LL} \right) \left( \frac{LWC}{95.04 + LWC} \right),
\]

(7)

\[
R_S \left( \text{mg CO}_2 \text{ m}^{-2} \text{s}^{-1} \right) = 0.031 \exp \left( 0.10 \times T_S \right) \left( \frac{SWC}{0.032 + SWC} \right),
\]

(8)

Figure 6. Temporal variation in environmental factors, CO2 efflux from the leaf litter layer (\( R_{LL} \)), soil respiration (\( R_S \)), and the ratio of \( R_{LL} \) to \( R_S \). Data was measured at one collar every 30 min between May 17 and June 6, 2013. A. Soil and air temperature. Spikes on the x-axis indicate precipitation events (mm h\(^{-1}\)). B. \( R_{LL} \) and water content of the leaf litter layer (LWC). C. \( R_S \) and soil water content (SWC). D. The ratio of \( R_{LL} \) to \( R_S \) (%). doi:10.1371/journal.pone.0108404.g006
where LWC (g g$^{-1}$) and SWC (m$^3$ m$^{-3}$) are water content of leaf litter and soil, respectively. The RMSE between observed and estimated daily mean respiration based on temperature ($R_{LL}$, 0.0080 mg CO$_2$ m$^{-2}$ s$^{-1}$; $R_S$, 0.060 mg CO$_2$ m$^{-2}$ s$^{-1}$) was larger than that based on temperature and WC ($R_{LL}$, 0.0046 mg CO$_2$ m$^{-2}$ s$^{-1}$; $R_S$, 0.012 mg CO$_2$ m$^{-2}$ s$^{-1}$) (Fig. 4). Estimated respiration was calculated using the equation based on temperature and WC because of the lower RMSE. Throughout the measurement period, the contribution of observed $R_{LL}$ to variation in $R_S$ changed from nearly zero to 51% following a rainfall event (Fig. 3E).

To consider the validity of $R_{LL}$ and $R_S$ estimated from continuous measurement, we compared these values with respiration rates measured using the manual chamber method (Fig. 5). Estimated respiration was very similar to that observed using manual measurements. The RMSE between estimated and observed respiration were 0.0041 and 0.061 mg CO$_2$ m$^{-2}$ s$^{-1}$ for $R_{LL}$ and $R_S$, respectively.

Temporal changes in $R_{LL}$ and $R_S$ on the short-term scale
To show clear temporal variation in $R_{LL}$ and $R_S$, the period between May 17 and June 6, 2013 (Fig. 6) was chosen because this

Figure 7. Temporal variation in water content of the leaf litter layer (LWC) and CO$_2$ efflux from the leaf litter layer ($R_{LL}$) after rainfall events. LWC (A) and $R_{LL}$ (B) show the daily mean values. The rainfall intensity of each precipitation event was 23.5 mm in 2 days (2012/12/20–12/27, mean air temperature: 3.6°C); 30.0 mm in 3 days (2012/12/27–1/9, 3.9°C); 49.8 mm in 2 days (2013/2/17–2/24, 2.4°C); 52.4 mm in 3 days (2013/3/17–3/26, 11.2°C); 3.8 mm in 2 days (2013/3/26–3/31, 10.5°C); 11.6 mm in 2 days (2013/5/18–5/27, 21.0°C); 5.4 mm in 3 days (2013/5/27–6/9, 21.5°C); and 3.8 mm in 4 days (2013/10/1–10/8, 22.0°C).

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Figure 8. Histograms of the relative frequency of “Dry” and “Wet” periods in relation to water content of the leaf litter layer (LWC), and the relative contribution of estimated leaf litter respiration ($R_{LL}$) in 2013. The daily mean LWC (A) and $R_{LL}$ (B) were used to present histograms. Estimated respiration rates were calculated using a function based on temperature (T) and water content (WC). $R_{LL} = 0.29e^{0.059T}[WC/(95.04+WC)]$. The daily mean LWC and $R_{LL}$ were defined as Dry or Wet based on LWC. Days in which daily mean LWC $\geq$0.75 g g$^{-1}$ were defined as Dry periods, while days in which daily mean LWC $<$0.75 g g$^{-1}$ were defined as Wet periods.

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Rapid Temporal Variation in CO$_2$ Efflux from Leaf Litter Layer
period included two characteristic rainfall events. The rainfall intensity was 11.6 mm over 13 h during the first event and 5.4 mm over 46 h during the second event. LWC and SWC increased from 0.11 to 2.64 g g\(^{-1}\) and from 0.14 to 0.16 m\(^3\) m\(^{-3}\), respectively, following the first rainfall event (Fig. 6B, C). LWC increased from 0.16 to 1.58 g g\(^{-1}\) but SWC did not increase after the second rainfall event.

Temporal variation in \(R_{LL}\) measured using the automated chamber system changed according to wetting and drying of the L-layer (Fig. 6B), reaching a maximum of 0.060 and 0.047 mg CO\(_2\) m\(^{-2}\) s\(^{-1}\) during first and second rainfall events, respectively. \(R_s\) increased following the increase in SWC and subsequently decreased gradually with diurnal variation according to temperature (Fig. 6C). Between May 17 and June 6, 2013, the contribution of \(R_{LL}\) to \(R_s\) increased from 6.5% to 51%, with a peak value of 51% during the first rainfall event and 37% during the second rainfall event (Fig. 6D).

Both \(R_{LL}\) and LWC reached a peak during or one day after rainfall events (Fig. 7). The peak of \(R_{LL}\) and LWC varied from 0.0020 to 0.026 mg CO\(_2\) m\(^{-2}\) s\(^{-1}\) and from 0.50 to 2.66 g g\(^{-1}\), respectively. Peak value of each rainfall event highly depended on air temperature. High peaks of \(R_{LL}\) were observed in the warm season (0.017 mg CO\(_2\) m\(^{-2}\) s\(^{-1}\); 2013/5/18–5/27, 0.026 mg CO\(_2\) m\(^{-2}\) s\(^{-1}\); 2013/5/27–6/9 in Fig. 7). Also, the peak value was related to LWC: low peak of \(R_{LL}\) was observed when LWC was low (0.004 mg CO\(_2\) m\(^{-2}\) s\(^{-1}\); 2013/10/1–10/8 in Fig. 7). The relationship between LWC and amount of precipitation was not clear. In the cold season, peak values of \(R_{LL}\) were relatively low (e.g., 0.005 mg CO\(_2\) m\(^{-2}\) s\(^{-1}\); 2013/2/17–2/24, 0.006 mg CO\(_2\) m\(^{-2}\) s\(^{-1}\); 2012/12/20–12/27 in Fig. 7) even when the L-layer was wet enough (LWC more than 1.5 g g\(^{-1}\)). The peak values of \(R_{LL}\) were 1.2- to 8.6-fold higher than the \(R_{LL}\) values before rainfall events, and \(R_{LL}\) fell to pre-wetting levels within 2–4 days after rainfall events and peak LWC values were 1.3- to five-fold higher than LWC before rainfall, and LWC also dropped to pre-wetting levels within 2–4 days after rainfall events. We defined \(R_{LL}\) from the period just after rainfall events through 2–4 days later as the “\(R_{LL}\) pulse”.

Effects of wetting and drying of the L-layer on \(R_{LL}\) and \(R_s\) on the annual time scale

Estimated daily mean \(R_{LL}\) in 2013 was separated into ‘Dry’ and ‘Wet’ periods based on daily mean LWC. Days for which mean LWC was <0.75 g g\(^{-1}\) were categorized as Dry, while days for which mean LWC ≥0.75 g g\(^{-1}\) were categorized as Wet. The threshold value (0.75 g g\(^{-1}\)) was obtained from mean LWC 3 days after a rainfall event (Fig. 7A). The relative frequency of Dry and Wet periods in 2013 were 47.6% and 52.8%, respectively, while the relative contributions of daily mean \(R_{LL}\) during the Dry and Wet periods in 2013 were 26.9% and 73.2%, respectively (Fig. 8). Annual \(R_{LL}\) and \(R_s\) in 2013 were estimated to be 0.69 and 7.94 t C ha\(^{-1}\) y\(^{-1}\), respectively. The RMSE between continuous respiration measured and estimated based on temperature and WC was 0.011 and 0.029 t C ha\(^{-1}\) y\(^{-1}\), respectively.

The contribution of annual \(R_{LL}\) to \(R_s\) was 8.6%. The relative frequency of LWC was similar during Dry and Wet periods, while the contribution of \(R_{LL}\) during the Wet period was approximately three-fold higher than that during the Dry period (Fig. 8).

Discussion

As seen in Fig. 6, \(R_{LL}\) immediately increased with wetting of the L-layer and decreased to pre-wetting levels within 2–4 days after rainfall events, which was consistent with observations made in previous studies [17,19]. \(R_{LL}\) showed no diurnal variation despite a diurnal temperate range >10°C. Consequently, the \(Q_{10}\) of \(R_{LL}\) increased with increasing LWC (Table. 1). The variation in \(Q_{10}\) would be directly related to water stress experienced by microorganism. This indicated that LWC can reach to adequate low value, suspected as water stress for microorganism, within several days after rainfall. On the one hand, \(R_s\) increased during rainfall and subsequently decreased, showing chamber variation. The \(Q_{10}\) of \(R_s\) also increased with increasing SWC. Daminou et al. [27] reported that root respiration showed little change with variation in SWC compared with changes in \(R_s\). Therefore, the increased \(Q_{10}\) of \(R_s\) with increasing SWC might be highly affected by not only \(R_{LL}\) but also by respiration from other heterotrophic sources.

Although the relative frequency of LWC was similar during Dry and Wet periods, the contribution of annual \(R_{LL}\) during the Wet period was approximately three-fold higher than that during the Dry period (Fig. 8), indicating strong effect of rainfall on \(R_{LL}\). Although the \(R_{LL}\) pulse can last for only 3–4 days after a rainfall event, this pulse would determine a large part of annual \(R_{LL}\). This suggests that the magnitude of total \(R_{LL}\) may be influenced by the frequency of rainfall events, especially in summertime, rather than the intensity of rainfall. Still, the cumulative \(R_{LL}\) in the Dry period contributed 26.9% of annual \(R_{LL}\) in 2013, even though instantaneous \(R_{LL}\) was very low. There may be large vertical variability in WC and \(R_{LL}\) within the L-layer, indicating that higher WC and \(R_{LL}\) occur in lower parts of the L-layer during the drying process because the upper L-layer dries more rapidly [28]. In that case, although the mean WC of the L-layer was very low, local wetting in lower sections would produce small CO\(_2\) fluxes. Despite low instantaneous \(R_{LL}\), the accumulation of \(R_{LL}\) over a long time period (approximately 6 mo) resulted in a substantial contribution (27%) of Dry-period respiration to annual \(R_s\).

Raindrops first reach the L-layer and then percolate to the soil layers below. Small amounts of precipitation caused no change in SWC or \(R_s\), but \(R_{LL}\) increased rapidly with increasing LWC (Fig. 6). In semi-arid and arid ecosystems, wetting of the L-layer and surface soil by small fog-drop pulses during the dry season can contribute up to 35% of \(R_s\) [29]. Although such small water inputs (e.g., brief rain showers and fog), which mainly affect the surface of the forest floor, can be significant drivers of temporal variation in \(R_s\), the soil water content sensors (generally inserted at depths > 5 cm) could not capture these inputs. Continuous measurement of LWC allowed for realistic modeling of the effects of rapid changes in LWC on \(R_{LL}\).

Although the annual contribution of \(R_{LL}\) to \(R_s\) was relatively small (8.6%), this contribution showed large temporal variation according to rainfall, ranging from nearly zero to 51%. Several other studies have described similar results [17,21]. For example, Borken et al. [17] reported that peaks in \(R_{LL}\) during addition of water ranged from 0.031 to 0.071 mg CO\(_2\) m\(^{-2}\) s\(^{-1}\) in vitro, which represented 11–26% of maximum in situ \(R_s\) in the Harvard forest, although \(R_{LL}\) before addition of water was nearly zero. These findings indicate that \(R_{LL}\) is a significant component of rapid and transient temporal variation in \(R_s\) in relation to rainfall events. Although numerous studies have examined CO\(_2\) efflux from mineral soils in relation to the intensity, duration, and frequency of rainfall [30,31], few studies have focused on \(R_{LL}\) because of the difficulty in measuring this dynamic. Here, \(R_{LL}\) pulses were observed only during and several days after rainfall events. Thus, periodic sampling (e.g., twice per week) might be insufficient to capture the contribution of the \(R_{LL}\) pulse to \(R_s\). Moreover, manual flux measurements are usually not performed during precipitation events because of difficulties that can occur
with electronic instruments and sampling methods. In our view, conducting in situ measurements of CO$_2$ efflux from the L-layer only over short time intervals (e.g., up to 1 h) produces robust data for understanding the response of $R_{LL}$ to rainfall events and its contribution to $R_S$.

The contribution of $R_{LL}$ to annual $R_S$ was 8.6% in our site. In an oak forest, the contribution of $R_{LL}$ to $R_S$ was 23%, according to model simulation based on temperature and LWC by Hanson et al. [13]. Ngao et al. [32] reported a lower contribution (8%) in a beech forest, estimated using an isotope mass balance approach, which was close to the value observed at our site (8.6%). However, simple quantitative comparisons between studies are difficult because of the use of different methods. In addition, some technical problems remain at our site. First, we performed $R_{LL}$ measurements in the treatment area in which the mineral soil below the L-layer was replaced with combusted granite soil. This treatment may have affected the microbial community and environmental conditions in the L-layer. Secondly, each continuous measurement of $R_{LL}$ and $R_S$ was performed with single chambers, so spatial heterogeneity in $R_{LL}$ and $R_S$ were not considered. Automated chamber methods allowed high-interval measurements of temporal variation in respiration but had poorer spatial distribution compared with the manual chamber method. The balance of trade-offs between automated and manual chamber method is subject to the relative importance of characterizing temporal and spatial variability of individual CO$_2$ sources. The number of chambers used can enhance the accuracy of measured mean values. Loescher et al. [33] reported that the number of chambers needs to be $>100$ to adequately represent spatial variability. However, this is not a feasible experimental design because of practical limitations to sampling efforts. To improve estimation of $R_{LL}$ and $R_S$ at the forest stand level, and to better understand the soil carbon budget, a comprehensive comparison of the diverse C pools and fluxes in forest soils is required.

Conclusions

In our study, the rapid and transient variation in $R_{LL}$ induced by rainfall; the peak $R_{LL}$ was observed during or one day after rainfall, and $R_{LL}$ subsequently decreased to pre-wetting levels within 2–4 days after rainfall events, following the decrease in LWC. On the one hand, CO$_2$ efflux from coarse woody debris found in our site decreased during rainfall events, and subsequently, a gradual increase in CO$_2$ efflux continued for at least 14 days until next rainfall [34]. Therefore, coarse woody debris was a CO$_2$ efflux source over longer time scales, while $R_{LL}$ approached nearly zero within few days after rainfall events, even at high temperatures. Such specific temporal CO$_2$ efflux patterns for each heterotrophic source when subjected to wetting and drying cycles would be a result of substrate properties (e.g., specific surface area).

In our view, continuous and direct measurements of CO$_2$ efflux and environmental conditions characterized by substrate properties of individual CO$_2$ sources could improve understanding of the processes that regulate variation in heterotrophic respiration and $R_S$ and enable progress beyond empirical models that are primarily based on simple temperature and SWC relationships.

Moreover, the magnitude of heterotrophic respiration under wetting and drying cycles is strongly related to microbial physiology and community composition. For example, Schnurer et al. [35] showed that longer-duration wetting could promote microbial biomass, causing an increase in basal respiration. Fierer et al. [36] showed the influence of drying and rewetting frequency on microbial (fungi and bacteria) community composition. To improve understanding of heterotrophic respiration associated with response and adaptation of microorganisms under climatic changes, collected continuous in situ data for CO$_2$ efflux and environmental conditions (e.g., temperature and WC) of individual CO$_2$ sources should be combined with analyses of microbial physiology and community composition.

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Author Contributions

Conceived and designed the experiments: MA YK TM. Performed the experiments: MA YK MT. Analyzed the data: MA YK MT. Contributed reagents/materials/analysis tools: MA MJ. Contributed to the writing of the manuscript: MA.

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