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1. Introduction

Soil respiration (RS) is a key ecosystem process that carbon dioxide (CO₂) from the soil surface to the atmosphere, because they regulate storage of large quantities of C. RS represents the second largest CO₂ efflux of the terrestrial biosphere and amounts 13 times higher than the current rate of fossil-fuel combustion. Thus, even a small change in soil respiration could significantly intensify or mitigate current atmospheric increases of CO₂, with potential feedbacks to climate change.

RS included root respiration (RR) and microbial decomposition of organic matter (RH). The RH is frequently partitioned into aboveground (leaf-litter decomposition: RL) and belowground microbial respiration (RB), because the qualities of organic matter are different. RR produced by roots and the associated rhizosphere (mycorrhizae and rhizosphere bacteria) and RH originating from soil microorganisms that decompose the organic materials from both below-ground litter and soil organic materials. The RH is frequently partitioned into aboveground (leaf-litter decomposition: RL) and belowground microbial respiration (RB), because the qualities of organic matter are different. RH was sensitive to soil temperature, the plant photosynthetic activity influenced RR. To separate RH and RR, trenching method, root biomass regression method, root excising method and isotopic method were widely used. However, compared these methods and indicated that the trench method was the best method because of more accurate data for detecting the seasonal variation in the contribution and a smaller amount of work than the other methods. However there are several problems in trench method: such as 1) the effect of residual dead root decomposition left in the trenched plots can influence soil CO₂ efflux; 2) roots still survive and respire after trenching.

In addition, fine roots (< 2 mm in diameter) are an important component of carbon cycling in forest ecosystems, because fine root dynamics (fine root production (P), mortality (M) and decomposition (D)) could provide insight into carbon uptake or return. Translocation of assimilates to belowground organs of plants in terrestrial ecosystems in
order to grow fine roots and to support mycorrhiza. Fine root production accounts for substantial part of total net primary production TNPP, especially in cool-temperate forests, therefore fine root dynamics (production, mortality and decomposition) would be closely related to RR variation, though the information about the relationship between fine-root dynamics and RS is still limited. In this study, to understand the belowground carbon cycle of forest ecosystems, a field experiment was firstly conducted in an evergreen forest in central Japan to determine the method to measure RL, and then RR, RL and RB were separately quantified through the second experiment in deciduous and evergreen forests in northern Japan. In addition, fine-root dynamics were evaluated and related to RR through the second experiment.

2. Method

2.1 To measure RL in the second field experiment, we firstly examined the reliability of the litter bag method and closed chamber method with leaf-litter addition / removal treatments (C-LART) in a temperate evergreen forest in central Japan. In June 2012, 27 aluminum chamber collars with area of 0.5 m × 0.5 m were installed 3 cm into soil to measure the soil CO2 efflux. The chamber collars were divided equally for three litter treatments: control (CT), addition (AD), and removal (RE). Each treatment has 9 replications. The chamber collars were distributed within an area of 40 m × 40 m at the study site.

We added 230 g air-dried litter to each AD collar and then fixed with cheesecloth of 50 cm × 50 cm (mesh size: 1 mm) on 2 October 2012. Simultaneously, we removed all leaf litter on litter layer from each RE collar. To find the dry weight, litter samples were oven-dried for 48 hr at 80°C. As a result, we added litter to each AD collar amounted to 199 g in oven-dried weight (796 g m⁻² / 356 gC m⁻²), and removed litter from each RE collar (mean ± 1 SD) was 44 ± 17 g in oven-dried weight (176 ± 69 g m⁻² / 82 ± 32 gC m⁻²). CO2 efflux was measured about twice a month from October 2012 to December 2013 in the site using a portable chamber system equipped with an infrared gas analyzer (LI820, Licor) and a data logger (CR1000, CSI). Simultaneously, soil temperature (Tₛ) and soil water content (SWC) were measured. RS was related to Tₛ for each chamber collar using a common exponential equation as

\[ RS = a e^{(b Tₛ)} \]  

(1)

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

\[ Q_{10} = e^{(10 b)} \]  

(2)

To assess the effect of SWC on RS, temperature-normalized soil respiration (\( R_0, \mu mol \))
m$^{-2}$ s$^{-1}$) was calculated using the following equation (Hirano et al., 2003).

$$R_b = RSe^{(b(7b-Ts))}$$  

(3)

In that equation, $T_b$ denotes base temperature, which was set for this study at 15°C.

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 also used for the litter bag experiment. Litter bags (Photo 2.6) 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 litter samples in bags were washed gently and quickly in a laboratory to remove foreign materials and weighed after drying for 48 h at 80°C. 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 carbon loss caused by decomposition (gC gC$^{-1}$) was assessed using data of total carbon content as

$$\text{Carbon loss} = \frac{(W_0C_0 - W_tC_t)}{(W_0C_0)}$$  

(4)

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

2.2 Secondly we estimated the relationship of RR and fine root dynamics in nearby two sites (a deciduous forest dominated by *Larix kaempferi* and an evergreen coniferous plantation of *Picea glehnii*) in Tomakomai, northern Japan (42°44′N, 141°31′E, 125 m a.s.l.). The soil was volcanogenous regosol with high water permeability. An about 10-cm-thick organic surface soil layer (A horizon) undelay and a about 4-cm-thick root mat and leaf litter. Four neighboring 0.5×0.5 m$^2$ collars for control, root sampling (S), litter removal (LR) and trenching (T) treatments, respectively, were installed in five replications in each site to partition RS into RH, RR and litter decomposition (RL); litter removal and trenching was conducted in November 2014. On each collar, CO$_2$ efflux was measured about twice a month during the snow-free season from October 2014 to November 2015 in the deciduous site and to June 2016 in the evergreen site using same chamber system. Simultaneously, soil temperature ($T_s$) and soil water content (SWC) were measured. The CO$_2$ effluxes related to $T_s$ and SWC using equations (1) and (3). The three CO$_2$ effluxes of RH, RL and RR were calculated using the following simple equations:

$$\text{RH} = T - \text{RD}$$  

(5),

$$\text{RL} = RS - \text{LR}$$  

(6) and

$$\text{RR} = RS - \text{RH}$$  

(7)

where RD is the decomposition of dead roots left in the trenching collar. RD was determined using the root bag method. Meshed bags including three sizes (coarse,
medium and fine) of dead roots, respectively, were set in the soil in November 2014 and collected four times until September 2016. Using an exponential decay function derived from the root bag method, RD was calculated.

To estimate fine root dynamics, sequential core method was used to measure temporal changes in fine roots biomass ($\Delta B$, gC m$^{-2}$) with a stainless steel tube with inner diameter of 2.4 mm. Sequential core samples were collected from the top of the root mat to a depth of 14 cm at each S collar in three replications with 8 cm interval from October 2014 to November 2015 in the deciduous site and to July 2016 in the evergreen site. The collected sequential core samples were washed several times by tap water to remove soil and divided into root mat (top 4 cm) and A-horizon soil (lower 10 cm layer) to weigh after drying for 48 hours at 70°C. Moreover, fine root biomass was also separated into woody and herbaceous by their color in deciduous site because understory plants were dense in this site. Sequential core samplings were conducted at intervals of about 50 days during snow-free period. The variation of dry fine root biomass is $\Delta B$ in each interval.

The ingrowth core method was used to measure fine root production ($P$, gC m$^{-2}$). The ingrowth core (inner diameter of 2.3 mm, mesh size 2.0 mm) was filled with root-free soil which was collected in the study sites in September 2014, then separated roots from soil through a 2 mm mesh sieve. The ingrowth core samples in three replications were installed to sequential core holes in October 2014 in two sites. The ingrowth core samples were collected and newly installed as the same interval as sequential core method. Collected ingrowth core samples were washed to remove soil and divided into root mat and A-horizon soil to weigh after drying for 48 hours at 70°C. The dry mass is equivalent to $P$ in this interval.

The fine root mortality ($M$) was calculated following the equation:

$$M_{ij} = P_{ij} - \Delta B$$

where $P_{ij}$ and $M_{ij}$ denote fine root production and mortality between $t_i$ to $t_j$, respectively ($t_i > t_j$); $\Delta B$ was the changes of fine root biomass (live fine roots) between $t_i$ to $t_j$, which were measured by the sequential core sampling.

3. Results and discussions

3.1 In the first experiment, seasonal variations in $T_s$ and SWC were shown during the experiment period. SWC was stable at around 0.20 m$^3$ m$^{-3}$, except during summer with lower SWC. The RS in three litter treatments showed seasonal fluctuations (high in summer and low in winter) in accordance with the change of $T_s$. Before the litter
addition/removal treatments, RS showed no significant difference among CT, AD, and RE treatments. After litter addition, RS in AD increased rapidly, although RS in CT and RE did not increase. The highest and lowest RS were recorded respectively in August 2013 and January 2013, corresponding to the highest and lowest T_s.

For all chamber collars, equation (1) was significantly fitted to RS ($p < 0.05$). The values of $Q_{10}$ were $2.2 \pm 0.2$ in CT, $1.9 \pm 0.2$ in AD, and $2.4 \pm 0.1$ in RE, which were significantly lower in AD than in RE ($p < 0.05$, Tukey–Kramer test). The temperature-normalized CO2 efflux at 15°C shows neither a linear nor quadratic relation with SWC ($p > 0.05$). In AD, however, the measured RS was considerably higher than the estimated RS for about two months after litter addition. The annual soil RS were calculated at $1572 \pm 170$ ($R_{CT}$), $2247 \pm 204$ ($R_{AD}$), and $1263 \pm 144$ ($R_{RE}$) gC m$^{-2}$ yr$^{-1}$ (mean ± 1 SD). Annual RS were significantly different among litter treatments: $R_{AD} > R_{CT} > R_{RE}$ ($p < 0.05$, Tukey–Kramer test). The different annual CO2 efflux of each treatment was due to 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. 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$^{-2}$).

Litter mass, carbon content, and C/N in litter bags decreased significantly from December 2012 to December 2013. 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$^{-1}$. 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. Carbon contents decreased through decomposition. Therefore, the relative carbon loss was greater than the relative mass loss. 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. 2.12).

The results imply that C-LART is inapplicable to evaluation of CO2 emissions through leaf litter decomposition. To apply the chamber method for direct measurement of CO2 efflux, a new approach that compensates the priming effect is necessary. One possible practical approach is the placement of a litter bag in a chamber collar. For C-LART, the litter bag is removed temporarily from the chamber collar. By measuring CO2 efflux twice on the same chamber collar before and after the litter bag removal, decomposition CO2 efflux can be determined as the difference of the two effluxes.
3.2 In the second experiment. Among treatments \((n = 5)\), \(T_s\) was not significantly different in both the two sites, whereas \(T_s\) was higher in the deciduous site than in the evergreen site \((p < 0.05)\) probably because of different leaf area indices. In the evergreen site, SWC in trenching collars was significantly higher than that in control collars because of no water uptake by living roots.

The decomposition constant \((k)\) of the decay function was highest for fine roots, followed by medium roots and coarse roots, and higher in the deciduous site than in the evergreen site, partly because fine roots have a higher ratio of surface area / volume and the deciduous site was higher in \(T_s\), which both would have enhanced microbial decomposition. Using the \(k\) and initial amount of dead roots, the annual RD from November 2014 to November 2015 in the trenching collars was calculated to be 112 and 81 gC m\(^{-2}\) yr\(^{-1}\), respectively, in the deciduous and evergreen sites.

CO\(_2\) efflux was not significantly different among the four collars until November 2014, before the treatments of litter removal and trenching. In all collars, CO\(_2\) efflux showed seasonal variation with the peak in late July 2015. CO\(_2\) efflux increased exponentially with \(T_s\) \((p < 0.05)\). The temperature sensitivity of CO\(_2\) efflux \((Q_{10})\) was significant higher \((p < 0.05)\) in the deciduous site, whereas no significant difference was found among treatments. In contrast, CO\(_2\) efflux showed no correlation with SWC, even after it was normalized by \(T_s\) using \(Q_{10}\). Thus, the annual CO\(_2\) effluxes of RS, RR, RH and RL were estimated from half-hourly \(T_s\) using simple exponential equations between November 2014 and November 2015. Annual RS, RR, RH and RL were 1255 ± 401, 592 ± 447, 452 ± 273 and 211 ± 239 gC m\(^{-2}\) yr\(^{-1}\) \((\text{mean ± 1 standard deviation, } n = 5)\), respectively, in the deciduous site, and 1139 ± 386, 386 ± 356, 642 ± 248 and 111 ± 118 gC m\(^{-2}\) yr\(^{-1}\), respectively, in the evergreen site. The annual values of two sites were not significantly different in each component \((p > 0.05)\). The contribution of RR to RS was 47 and 34%, in the deciduous and evergreen sites.

\(B\) increased significantly in June 2015 \((p < 0.05)\) in the deciduous site, whereas no significant difference was found among sampling dates in the evergreen site. Annual mean \(B\) was 209±75 and 264±59 gC m\(^{-2}\), respectively, in the deciduous and evergreen sites. Both in the two sites, fine root production \((P)\) showed a seasonal variation with the peak occurring between June and August and the minimum during the snowy season. Annual \(P\) from November 2014 was calculated to be 184 ± 21 and 176 ± 31 gC m\(^{-2}\) yr\(^{-1}\), respectively, in the deciduous and evergreen sites. \(P\) measured by the ingrowth core method showed a significant positive relationship with soil temperature \((T_s)\). Correlation coefficients \((R^2)\) were 0.92 ± 0.10 and 0.74 ± 0.14 \((\text{mean ± 1 standard deviation, } n = 5)\), respectively, in the deciduous and evergreen sites. In contrast, the \(P\) showed a
significant negative correlation with SWC in the deciduous site \((p < 0.05)\), whereas relationships with SWC were not significant in the evergreen site. In addition, the \(P\) measured by the ingrowth core method showed a significant positive correlation with root respiration (RR) at a majority of plots both in the two sites. The \(P\) measured by the sequential core method showed no significant relationship with RR, \(T_s\), and SWC.

4. Conclusions

Leaf litter decomposition has been measured commonly by the litter bag and chamber methods. Comparison of the two methods suggests that the litter bag method provided more reliable results, judging from results of leaf litter decomposition reported by previous studies, although the litter bag method also has an unavoidable fault attributable to its use of meshes. However, the chamber method with litter addition and removal treatments greatly overestimated \(CO_2\) emissions derived from litter decomposition probably because of the alteration of the material flows from the litter layer into underlying soil (the priming effect on soil respiration). Therefore, an improved chamber method was used to estimate leaf litter decomposition in deciduous and evergreen forests in Hokkaido, northern Japan. In the improved method, soil \(CO_2\) efflux was measured on collars, immediately after leaf-litter removal, and the leaf litter was set in the collars again after the chamber measurement to keep the soil condition natural in collars.

In the field experiment conducted in deciduous and evergreen forest sites in Hokkaido, soil respiration (total soil \(CO_2\) efflux) was separated into root respiration and heterotrophic respiration using the trenching method, which killed roots in trenching collars. \(CO_2\) emissions through the decomposition of dead roots left in trenching collars were estimated using the root-litter bag method. The decomposition contact \((k)\) of fine root was highest among three classes of roots with different diameter, because the ratio of surface area / volume increases as diameter decreases. As a result, the dead root decomposition accounted for 11% and 8% of \(CO_2\) emissions from trenching collars, respectively, in the deciduous and evergreen forests on an annual basis. This fact indicates that the \(CO_2\) emissions potentially overestimate heterotrophic respiration and, consequently, underestimate root respiration.

Fine root biomass mainly accumulated into the surface root mat and underling A-horizon, which only occupied top 14 cm of soil profile, both in the two sites. The result of the fine root biomass showed a corresponding seasonal variation with a peak in summer, whereas the variation was slightly different between the deciduous and evergreen forests, because these have different phenology. Necromass of fine roots was
less than those in previous studies, because it would have been underestimated in this study owing to some technical difficulties. Fine root productions were determined by the two methods: the sequential core sampling method and the ingrowth method. Although, fine root productions from the two methods were not significantly different, the ingrowth core method was probably more reliable, because the sequential core sampling method frequently resulted in considerably lower fine root mortality and undesirable production.

Fine root production determined by the ingrowth core method showed a significant positive relationship with soil temperature both in the deciduous and evergreen forests, which indicates that soil temperature was an important environmental factor controlling fine root production. In addition, fine root production showed a significant positive relationship with root respiration both in the two sites. This fact confirmed the close relationship between fine root production and root respiration.

Through this study, Root respiration and fine-root production was measured simultaneously in the same plots in nearby deciduous and evergreen forests. The result of the field experiment showed a corresponding seasonal variation of root respiration and fine-root production with a peak in summer, whereas the variation was slightly different between the deciduous and evergreen forests, because these have different phenology. These results contribute to understanding the below-ground carbon cycle of forest ecosystems.