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<td>Author(s)</td>
<td>Makita, Naoki; Kosugi, Yoshiko; Sakabe, Ayaka; Kanazawa, Akito; Ohkubo, Shinjiro; Tani, Makoto</td>
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Kyoto University
RESEARCH ARTICLE

Seasonal and diurnal patterns of soil respiration in an evergreen coniferous forest: Evidence from six years of observation with automatic chambers

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Abstract

Soil respiration (Rs) plays a key role in the carbon balance of forest ecosystems. There is growing evidence that Rs is strongly correlated with canopy photosynthesis; however, how Rs is linked to aboveground attributes at various phenological stages, on the seasonal and diurnal scale, remains unclear. Using an automated closed dynamic chamber system, we assessed the seasonal and diurnal patterns of Rs in a temperate evergreen coniferous forest from 2005 to 2010. High-frequency Rs rates followed seasonal soil temperature patterns but the relationship showed strong hysteresis. Predictions of Rs based on a temperature-response model underestimated the observed values from June to July and overestimated those from August to September and from January to April. The observed Rs was higher in early summer than in late summer and autumn despite similar soil temperatures. At a diurnal scale, the Rs pattern showed a hysteresis loop with the soil temperature trend during the seasons of high biological activity (June to October). In July and August, Rs declined after the morning peak from 0800 to 1400 h, although soil temperatures continued to increase. During that period, figure-eight-shaped diurnal Rs patterns were observed, suggesting that a midday decline in root physiological activity may have occurred in early summer. In September and October, Rs was higher in the morning than in the night despite consistently high soil temperatures. We have characterised the magnitude and pattern of seasonal and diurnal Rs in an evergreen forest. We conclude that the temporal variability of Rs at high resolution is more related to seasons across the temperature dependence.

Introduction

Knowledge of soil carbon (C) dynamics is essential for understanding the C balance in terrestrial ecosystems [1]. Gross primary production (GPP) and soil respiration (Rs) are major CO2 fluxes between the atmosphere and terrestrial ecosystems. Rs accounts for more than two-
Competing interests: The authors have declared that no competing interests exist.
The present study aimed to characterize seasonal and diurnal patterns of $R_s$ in a temperate evergreen coniferous forest consisting primarily of *Chamaecyparis obtusa* (Japanese cypress). To this end, $R_s$ was measured at 30-min intervals for 6 years by an automated closed dynamic chamber system. The present work builds on the study of Kosugi et al. [40], in which CO$_2$ gas exchange between the atmosphere and an evergreen coniferous forest was determined using eddy covariance flux data at the same study site as that of the present study. The authors reported that the temperature dependence of canopy photosynthesis decreased significantly in winter and that plant phenology must be considered to understand the seasonality of forest CO$_2$ exchange. Nevertheless, few studies have linked $R_s$ patterns in evergreen forests to seasonal differences in phenology. We tested the hypothesis that $R_s$ shows clear diurnal and seasonal changes beyond the semi-empirical model of the response of $R_s$ to soil temperature factors in an evergreen forest. Furthermore, we tested the hypothesis that the diurnal pattern of $R_s$ would be influenced by seasonality.

**Materials and methods**

**Study site**

The study was conducted in a temperate coniferous forest in Kiryu Experimental Watershed (35°N, 136°E; 190–255 m above sea level; 5.99 ha) located in Shiga Prefecture, central Japan. The region has a monsoon climate. The forest consists of 50-year-old Japanese cypress (*Chamaecyparis obtusa* Sieb. et Zucc.) planted in 1959. The mean tree height (diameter at breast height [DBH] > 5 cm) was 17.3 m based on the tree census in March 2011. The annual mean air temperature and precipitation between 2005 and 2010 at this site were 13.4°C and 1595 mm yr$^{-1}$, respectively (S1 Fig). This region has a distinct climate; it has cold winters with little snow and hot, humid summers with high rainfall owing to the significant effect of the Asian monsoon. The mean monthly air temperature was the highest in August (25.0°C) and the lowest in January (2.8°C). This area typically has snowfall on several days during a year, which melts within a few days. Rain occurs throughout the year, with two peaks in summer: the early summer *baiu* front season and the late summer typhoon season. Summer in western Japan is warm and humid with sufficient rain; however, occasional moderate drought conditions can occur (S1 Fig). The soil is classified as a Haplic Cambisol with sandy loam or loamy sand texture. The mean C/N ratio, pH, and electrical conductivity of the 0–5 cm mineral soil layer were 19.0, 5.9, and 4.9 mS/m, respectively [41].

The study forest is one of the Asia Flux sites. Micrometeorological and CO$_2$/H$_2$O flux data were collected by the observation tower [40,42]. To compare the net ecosystem exchange estimated by the eddy covariance method, CO$_2$ and H$_2$O exchanges of leaves [40], manual soil CO$_2$ efflux [43], and soil CH$_4$ flux [41] were evaluated at this site. The average and standard deviation of annual GPP, ecosystem respiration, and net ecosystem exchange were $2044 \pm 149$, $1555 \pm 158$, and $-490 \pm 109$ g C m$^{-2}$ yr$^{-1}$, respectively [40].

**Measurement of $R_s$, soil environment, and GPP**

Three measurement plots were established in the study area, separated from each other by $\geq 25$ m. $R_s$ was measured continuously with high temporal resolution at one point per plot at 30-min intervals from 2005 to 2010. Measurements were performed with an automated closed dynamic chamber system fitted with an infrared CO$_2$/H$_2$O analyzer (Li-840; Li-corr, Lincoln, NE, USA). The system consisted of a permanently connected chamber (length 0.3 m, width 0.3 m, height 0.2 m) with an automatically controlled chamber lid. To minimize error in the CO$_2$ efflux measurements in closed dynamic chambers through pressure changes, the chambers were designed to provide sufficient volume for the steady pressure in the closed-
chamber. The soil collars were inserted tightly into the ground up to 5 cm in depth prior to the start of the sampling period and were sealed permanently to the chamber. Chamber opening and closing were controlled by an air compressor (FH-02; MEIJI, Japan). Switching between chambers was regulated by the air flow from solenoid valves (CKD USB3-6-3-E; CKD Corp., Japan) and AC/DC controller (SDM-CD16AC; Campbell Scientific, USA). To prevent shadow on the collar, all chamber material was consisted of transparent acrylic. When the chamber was closed, the air sample was dehydrated with a gas dryer to remove water vapor in the sample air and then circulated by a mass flow-controlled diaphragm pump (APN-085; Iwaki Pumps, Japan; DM-403ST-25; MFG. CO., LTD., Japan) through polyethylene tubes to the CO₂/H₂O analyzer. The flow rate using a mass flow controller (MPC0005; Yamatake, Japan) was 1.8 L min⁻¹. Because not all of the water vapor could be removed by the drying system (PD-50 T-48; Perma Pure, Toms Rivers, NJ, USA), its presence was corrected by using the H₂O concentration measured with the CO₂/H₂O analyzer. The time interval for each measurement was set to 180 s. To compensate for air disturbances caused by opening the chamber, the data for the first 90 s were discarded. Measurements were taken every 30 min. Data were recorded with a data logger (CR1000; Campbell Scientific, USA). The closed chamber flux measurement was accepted if the determination coefficient of linear regression (R²) was larger than 0.85 according to the previous reports [11,41].

ₐₚ was calculated from the rate of increase in CO₂ concentration with time using the following linear regression:

\[ R_s = \frac{dc}{dt} \times \frac{V}{A} \times \rho_{\text{airmol}} \]  

(Eq 1)

where \( dc/dt \) is the rate of increase in the gas concentration \( c \) (ppm) with time \( t \) (s) and is determined by the linear least-squares method on the slope of the change in gas concentration from 90 to 180 s at the start of measurement; \( V \) is the chamber volume (0.018 m³); \( A \) is the soil surface area in the chamber (0.09 m²); and \( \rho_{\text{airmol}} \) is the air molar density (mol m⁻³).

For soil environmental monitoring, soil temperatures at 2-cm depth were measured using copper-constantan thermocouples. Soil moisture levels at 0–30 cm depth were determined with three water content reflectometers (CS615 or CS616; Campbell Scientific, USA). Data were logged continuously at each plot at 30-min intervals. Precipitation was measured with a tipping-bucket rain gauge at an open screen site near the flux tower.

For evaluating GPP, the fluxes of CO₂ (µmol m⁻² s⁻¹) were measured by open-path eddy covariance methods at a tower height of 28.5 m with a CO₂/H₂O gas analyzer (LI-7500; Li-cor, Inc., Lincoln, NE, USA). from January 2005 to December 2010. The study by Kosugi et al. [40] provides detailed information regarding the eddy covariance flux observations and calculations.

### Soil respiration models

To estimate the best fit of soil temperature control on \( R_s \) rates, two empirical models, i.e., the simple exponential function model and the Arrhenius equation model, were tested. Because of the complexity of the soil environment, many researchers depend on empirical models instead of process-based models to estimate soil respiration [7]. The simplest model is the exponential increase in respiration rate as a function of temperature. The model and its parameter space are defined as

\[ R_s = R_{s\text{ref}} \times Q_{10}^{\frac{\text{temp}_s}{\text{temp}_{\text{ref}}}} \]  

(Eq 2; Q10model)

where \( R_{s\text{ref}} > 0 \) and \( a1 > 0 \). \( R_s \) and \( R_{s\text{ref}} \) are the respiration rates (µmol m⁻² s⁻¹) at
temperatures $T_{\text{soil}}$ and $T_{\text{ref}}$, respectively. $T_{\text{soil}}$ is the observed soil temperature and $T_{\text{ref}} = 15^\circ C$. $Q_{10}$ is the temperature sensitivity and represents the relative increase in respiration as the temperature rises by $10^\circ C$. Eq 2 is often called the $Q_{10}$ model.

The second model is the Arrhenius equation. It is also used to describe temperature dependence of respiration [44]. Since respiration increases with temperature, this model and its parameter space are defined as

$$R_s = R_{s,\text{ref}}e^{\frac{E_a}{R}T_{\text{soil}}}$$

(Eq 3; Arrhenius model)

where $E_a$ is a free parameter analog to the activation energy in the standard Arrhenius model and represents the sensitivity of $R_s$ to temperature. $R$ is the gas constant ($R = 8.314 \text{ J K}^{-1} \text{ mol}^{-1}$).

Data analysis

To remove outliers, residual analyses were performed. Data points of $R_s$ were removed from the regression when the residual of an individual data point was greater than three times the standard deviation. $R_s$ was calculated as the mean of the three chambers and was used in subsequent analyses. Instrument failure and quality control procedures reduced the data by 10% during the 6 years of observation. We evaluated the empirical models of soil respiration at each soil temperature for the years from 2005 to 2010. Two commonly used models (Eqs 2 and 3), both of which fit the data well, were used to analyze the response of $R_s$ to soil temperature. The Akaike information criterion (AIC) and the root mean squared error (RMSE) were used to evaluate the goodness of fit for the $R_s$ models. The observed $R_s$ and predicted $R_s$ by the best-fit $R_s$-temperature model were calculated to determine the direction and magnitude of the seasonal dependence of $R_s$ measurements beyond temperature-response property. To better characterize seasonal $Q_{10}$ and $E_a$, monthly mean values were calculated for the years from 2005 to 2010.

The mean diurnal cycles of $R_s$ and GPP for each month were determined by calculating the average of the 30-min data at each time of day. The cycles were then used to identify the relationship between $R_s$ and soil temperature.

Results

Soil environmental factors and carbon exchange over six years

The mean soil water content at 0–30 cm depth ranged from 0.05 to 0.24 $\text{m}^3 \text{m}^{-3}$ of soil (Fig 1A). Seasonal soil temperature patterns were observed (Fig 1B). The mean soil temperature at 2 cm depth varied seasonally, ranging from 0°C in February to 25°C in August during the years from 2005 to 2010. The half-hourly $R_s$ rates measured with the automated chamber ranged from 0.1 to 10.9 $\mu\text{mol m}^{-2} \text{s}^{-1}$ during the years from 2005 to 2010 (Fig 1C). $R_s$ showed strong seasonality; it was the lowest in February and the highest in mid-August. Seasonal variations in daily GPP over the course of this study are illustrated in Fig 1D.

Seasonal variation of soil respiration in relation to temperature and gross primary production

Two models of the correlation between $R_s$ and soil temperature were tested to obtain the best-fit curves. RMSE and AIC based on the $R_s$-soil temperature relationship were smaller in the Arrhenius model than in the $Q_{10}$ model (Table 1). When pooling data of all seasons, the $Q_{10}$ and $E_a$ value was 2.42 and 61.69 $\text{kJ mol}^{-1}$, respectively. A better fit for the Arrhenius model
was found for the relationship of $R_s$ with soil temperature for the years from 2005 to 2010 and was used in further analyses.

In all seasons, $R_s$ exponentially increased with soil temperature (Fig 2). The Arrhenius model explained a significant portion of the variation in $R_s$ in response to soil temperature (Table 1). Monthly mean values of observed $R_s$ were the highest in July and the lowest in February. In contrast, the monthly predicted $R_s$ were the highest in August and the lowest in February. The underestimations of the predicted- to observed $R_s$ were found for June-July. In contrast, the overestimations were observed for January–May and August-September.

There was a seasonal relationship between GPP and $R_s$ of an evergreen conifer (Fig 3). We observed greater $R_s$ relative to GPP in autumn for September to November when compared with spring for March to May.

Seasonal patterns in $Q_{10}$ and $E_a$ values

The $Q_{10}$ and $E_a$ values of the monthly $R_s$ were 1.09–2.43 and 5.61–56.89 kJ mol$^{-1}$, respectively (Table 2). Changes in $Q_{10}$ and $E_a$ values were related to seasonal patterns; the values were higher in winter than in summer. For all collected samples, the $Q_{10}$ and $E_a$ values of $R_s$ declined markedly with increasing soil temperature, according to the seasons, which explained a significant proportion of the variation in the temperature sensitivity of $R_s$ ($r = 0.88$, $p < 0.001$; Fig 4A, $r = 0.83$, $p < 0.001$; Fig 4B).

![Fig 1. Time courses of (a) mean soil water content at 0–30 cm depth (n = 3) and precipitation levels, (b) mean soil temperature at 2 cm depth (n = 3), (c) half-hourly mean soil respiration rates (n = 3), (d) gross primary production (GPP) according to eddy covariance tower observations during the years from 2005 to 2010.](https://doi.org/10.1371/journal.pone.0192622.g001)

Table 1. Empirical equations and parameter estimates describing the relationship between soil respiration and temperature from 2005 to 2010 (n = 94904). The Akaike information criterion (AIC) and the root mean squared error (RMSE) are used to evaluate the best fit for the models.

<table>
<thead>
<tr>
<th>Model</th>
<th>Equation and parameter estimates</th>
<th>RMSE</th>
<th>AIC</th>
</tr>
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<td>Q10 model</td>
<td>$R_s = R_{s_{ref}} \times Q_{10}^{\frac{T_{soil}}{T_{ref}}} = 0.57 \times 2.42^{\frac{T_{soil}}{15}}$</td>
<td>0.68</td>
<td>196173</td>
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<tr>
<td>Arrhenius model</td>
<td>$R_s = R_{s_{ref}}e^{\frac{E_a}{T_{ref}}} = 2.18e^{\frac{61692}{T_{soil}}}$</td>
<td>0.67</td>
<td>194786</td>
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https://doi.org/10.1371/journal.pone.0192622.t001
Diurnal variation in soil respiration with seasons

Fig 5 shows the monthly time course of $R_s$ and GPP. On a diurnal scale, $R_s$ rates were frequently higher from 1200 to 1800 h, decreasing overnight and reaching their minimum values in the early morning. GPP was highest at 1100–1300 h and decreased slightly during the afternoon. There was a lag between the time when maximum GPP and maximum $R_s$ were reached.
A relationship between diurnal \( R_s \) and soil temperature was observed for each month, and a strong seasonal fluctuation in the relationship was also observed (Fig 6). For example, the diurnal pattern of \( R_s \) rates during July and August differed from that in other seasons. In August after the morning peaks, the \( R_s \) rates decreased around noon but soil temperatures remained high. \( R_s \) recovered in the afternoon, lagging behind the peak in soil temperature and resulting in a figure-eight curve (Fig 6H). In September and October, \( R_s \) relative to the temperature was higher in the morning than in the night, despite nearly constant soil temperatures (Fig 6I and 6J). Therefore, diurnal \( R_s \) rates showed a hysteresis pattern in seasons with high biological activity (Fig 6). In contrast, the \( R_s \) rates in seasons where biological activity ceases changed exponentially and showed negligible hysteresis.

Discussion

From six years of observation by automated chambers, we characterised the magnitude and pattern of seasonal and diurnal \( R_s \) in an evergreen coniferous forest. This information may enable more accurate prediction of soil C dynamics and their associated ecosystem processes.

Our results support the hypothesis that high-frequency observations of \( R_s \) rates clearly indicate the seasonal changes in the response of \( R_s \) to soil temperature in field conditions, so that soil temperature alone is clearly insufficient to predict \( R_s \). In this study, \( R_s \) increased exponentially with increasing soil temperature. This correlation explained 80% of the variation in \( R_s \) across seasons when the best-fit Arrhenius model was used. In addition, the temperature sensitivity in this study was consistent with the findings of previous studies [45]. Our \( Q_{10} \) values were well within the global median of 2.4 [46] and the range (2.0–6.3) reported for European and North American forest ecosystems [47,48]. The Arrhenius function reveals the reactions with \( E_a \) around 50 kJ mol\(^{-1}\) [7], in agreement with our field observations. Nevertheless, there was a strong seasonal fluctuation in the relationship between \( R_s \) and soil temperature. The predicted \( R_s \) underestimated the actual \( R_s \) for June and July and overestimated \( R_s \) for August and September (Fig 2). Our results corroborate those of previous studies that reported increases in the contributions of \( R_s \) to ecosystem respiration during early summer [14,49]. This is probably due to the compensation of the model bias in late summer and autumn (overestimation) and early summer (underestimation), without explicit dependence of \( R_s \) on phenological attributes.

<table>
<thead>
<tr>
<th>Month</th>
<th>Soil temperature</th>
<th>( Q_{10} )</th>
<th>( E_a )</th>
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<tr>
<td>1</td>
<td>3.90</td>
<td>3.90</td>
<td>56885</td>
</tr>
<tr>
<td>2</td>
<td>4.16</td>
<td>2.08</td>
<td>50937</td>
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<tr>
<td>3</td>
<td>5.73</td>
<td>1.91</td>
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<tr>
<td>4</td>
<td>10.21</td>
<td>2.55</td>
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<tr>
<td>5</td>
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<td>2.96</td>
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</tr>
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<td>6</td>
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<td>9</td>
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<td>2.30</td>
<td>28634</td>
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<tr>
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<td>1.56</td>
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<td>0.95</td>
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<td>12</td>
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<td>0.48</td>
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https://doi.org/10.1371/journal.pone.0192622.t002
We found that there was a hysteresis in the seasonal relationship between GPP and $R_s$ of an evergreen conifer (Fig 3). Seasonal patterns in $R_s$ rates may be due to root production and respiration levels. Endogenous and phenological C assimilation rates are strongly correlated with belowground C allocation to roots, mycorrhizae, and rhizosphere microorganisms [28,29,50,51]. Root growth is assumed to peak early in the growing season and is therefore correlated with aboveground growth [52]. When a pulse of root growth occurs to support leaf production, the amount of respiring tissue and root CO$_2$ emission simultaneously increase. In this study site, GPP relative to the solar radiation and temperature was higher during the spring and summer [40]. Kosugi et al. [40] noted that red leaf pigmentation in the winter prevented light inhibition at low temperatures and affected stomatal conductance and photosynthetic rates in an evergreen coniferous forest. Substrate limitation in the rhizosphere during the winter may reduce root growth and autotrophic respiration rates. Therefore, seasonal

![Fig 4. Relationship between (a) $Q_{10}$ and (b) activation energy ($E_a$) of soil respiration and temperature for each month. Numbers in the figure indicate months.](https://doi.org/10.1371/journal.pone.0192622.g004)

![Fig 5. Diurnal variations in soil respiration and gross primary production (GPP) for each month. Error bars represent the standard errors of the mean for each month from 2005 to 2010. Each figure shows the fixed-width from bottom to top in Y-axis in all months.](https://doi.org/10.1371/journal.pone.0192622.g005)
plant phenology patterns may lead to variation in the substrate supply and belowground C allocation, and partly affect variation in \( R_s \) [39].

The level of heterotrophic respiration is also indicative of the seasonal patterns of \( R_s \), particularly for the decline in observed \( R_s \) rates during August and September. In Asian monsoon areas, microbial decomposition is often enhanced during the early summer rainy season and
suppressed by the late summer drought conditions [53]. Heterotrophic respiration is sensitive to seasonal rainfall patterns because soil water content strongly affects microbial physiology [12]. The biodiversity and metabolic activity of most soil microbial communities decrease with soil water content [54,55]. In fact, we found a significantly negative relationship between the temperature sensitivity of Rs and temperature; monthly Q10 and Ea were highest in winter and lowest in summer (Fig 4). These seasonal patterns in temperature sensitivity may be related to degradation of soil C, microbial physiological acclimation and community adjustment [55,56] by changing their lipid composition, synthesizing new proteins, and changing resource allocation from growth to survival mechanisms [57,58]. Previous studies reported that heterotrophic respiration and nutrient mineralization under drought also declined [58–60]. Consequently, the decline in Rs during the late summer is mostly related to a changed temperature response due to changed sensitivity of microbial degradation to water stress.

However, the seasonal Rs pattern in the present study contrasts with those reported previously [61]. Lee et al. [62] showed that Rs in a cool-temperate Japanese deciduous broad-leaved forest was lower in spring and early summer than in late summer and autumn. This difference may be explained by seasonal changes in soil heat transport and CO2 fluxes [34,63]. In spring, when soils are covered with snow, the contributions of root and microbial activity are reduced by the low temperatures in deeper soil layers, but the opposite occurs in late summer and autumn. In late summer, the Rs components increase in response to the warming of the deeper soil layers. Soils usually warm from the top downward in spring and cool from the top downward in autumn. The presence of snow and the timing of early spring thaw and late autumn frost affect the vertical distribution of soil temperature. In addition, high Rs in a deciduous forest in autumn could also be related to the high input of litter during autumn. Therefore, variation in CO2 production with soil depth during the growing season may affect heat transport-based hysteresis.

The coordination of aboveground and belowground phenological patterns would contribute to the seasonality of the Rs diurnal scale hysteresis. In September and October, Rs relative to the soil temperature was higher in the morning than at night. Diurnal hysteresis in the relationship between Rs and soil temperature is an example of multiple processes interacting to produce highly variable photosynthetic attributes [30,31]. Liu et al. [17] showed that the diurnal cycle of Rs in a mixed deciduous forest was related more to differences in photosynthetically active radiation than to variations in soil environmental conditions, suggesting that diurnal Rs patterns were associated with photosynthesis. In the present study, diurnal Rs was higher in the morning than in the nighttime, especially in September and October. The diurnal Rs pattern of the relationship between Rs and soil temperature showed a hysteresis loop. The Rs morning peaks in September and October suggest faster transfer of recent photosynthates to belowground in warm-temperate ecosystems. In fact, the Rs peaks occurred later than GPP peaks (Fig 5I and 5J). Our results suggest that soil temperature does not fully explain variations in diurnal Rs dynamics.

Interestingly, figure-eight-shaped diurnal Rs patterns were observed in July and August (Fig 6). This finding suggests that midday declines in root physiological activity may have occurred in early summer. Under natural field conditions, plants adapt to changes in the prevailing irradiance to protect and optimize photosynthesis. As a result, continuous daily variations occur. Photooxidative damage to leaf thylakoid membranes causes photoinhibition and stomatal closure. The leaf protects the photosynthetic apparatus by down-regulating it at higher temperatures under high photon flux [64]. Photoinhibitory damage and stomatal closure contribute significantly to midday photosynthetic depression and, indirectly, to the decline in C supply to the root system. Makita et al. [31] showed that weather conditions under high temperature stress cause a midday depression of CO2 assimilation in deciduous trees and then a sharp
reduction in autotrophic respiration rate. The flux of new photosynthate to the rhizosphere significantly accelerates microbial activity there. This process affects the relative amount of heterotrophic respiration from decomposition of soil organic matter [33,65]. The results of the present study indicate how canopy processes affect the phase lags between the diurnal signals of soil temperature and forest floor $R_s$. Some studies have suggested that the autotrophic component of $R_s$ is controlled by carbohydrate production and internal transport in trees more than by diurnal variations in environmental variables [13,30]. Therefore, diurnal variation in $R_s$ may explain the hysteresis loop observed in this study. Nevertheless, there remains some debate over the relative importance of temperature- and substrate-dependent processes as drivers of midday photosynthesis depression in actual $R_s$ rates. There is little evidence that root growth and other C sinks are determined by substrate availability [66]. The associations between photosynthesis and $R_s$ may be controlled by multiple factors, including photosynthate transport distance, root depth, plant physiology, growth stage, and environmental conditions [15,67]. Recent advances in isotopic labeling techniques have enabled the quantification of C partitioning in forests and the assessment of its role in tree growth, resource acquisition, and C sequestration at temporal scales [37,38]. Further investigation is needed to establish the mechanisms of aboveground–belowground interactions and the factors that control them.

In conclusion, continuous monitoring of $R_s$ rates in a warm-temperate evergreen coniferous forest with an automated chamber system demonstrated diverse biological phases of the $R_s$ rate at different time scales independently of soil temperature. We found that the magnitude and pattern of temporal $R_s$ was depend on seasons across the temperature dependence. Additionally, more research is needed to elucidate whether the impact of linkage between aboveground and belowground C allocation depends on vegetation types and features of the soil environment, such as moisture. Soil CO$_2$ efflux data with a high temporal resolution would help to quantify the contributions of abiotic and biotic effects on C flux and sequestration in forest soils.

Supporting information
S1 Fig. Mean monthly air temperature (°C) precipitation (mm) for the period 2005–2010. Error bars represent standard deviations. Data were from Y. Kosugi et al. [40]. (TIF)

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Author Contributions
Conceptualization: Naoki Makita, Yoshiko Kosugi, Ayaka Sakabe.
Data curation: Naoki Makita, Yoshiko Kosugi, Akito Kanazawa, Shinjiro Ohkubo.
Formal analysis: Naoki Makita.
Funding acquisition: Yoshiko Kosugi, Makoto Tani.
Investigation: Naoki Makita.
Methodology: Naoki Makita, Ayaka Sakabe, Shinjiro Ohkubo.
Project administration: Naoki Makita.
Resources: Naoki Makita.
Software: Naoki Makita.
Supervision: Naoki Makita.
Validation: Naoki Makita.
Visualization: Naoki Makita.
Writing – original draft: Naoki Makita, Yoshiko Kosugi, Ayaka Sakabe.
Writing – review & editing: Naoki Makita, Akito Kanazawa, Makoto Tani.

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Seasonal and diurnal patterns of soil respiration by automatic chamber systems


