Research paper

Vertical and seasonal variations in temperature responses of leaf respiration in a *Chamaecyparis obtusa* canopy

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Leaf respiration (R) is a major component of carbon balance in forest ecosystems. Clarifying the variability of leaf R within a canopy is essential for predicting the impact of global warming on forest productivity and the potential future function of the forest ecosystem as a carbon sink. We examined vertical and seasonal variations in short-term temperature responses of leaf R as well as environmental factors (light and mean air temperature) and physiological factors [leaf nitrogen (N), leaf mass per area (LMA), and shoot growth] in the canopy of a 10-year-old stand of hinoki cypress [*Chamaecyparis obtusa* (Sieb. et Zucc.) Endl.] in Kyushu, Japan. Leaf respiration rate adjusted to 20 °C (R_{20}) exhibited evident vertical gradients in each season and was correlated with light, LMA and leaf N. In contrast, the temperature sensitivity of leaf R (Q_{10}) did not vary vertically throughout the seasons. Seasonally, Q_{10} was higher in winter than in summer and was strongly negatively correlated to mean air temperature. A negative correlation of R_{20} with mean air temperature was also observed for each of the three canopy layers. These results clearly indicate that leaf R was able to adjust to seasonal changes in ambient temperature under field conditions and down-regulate during warmer periods. We also found that the degree of thermal acclimation did not vary with canopy position. Overall, our results suggest that vertical and seasonal variations in temperature responses of leaf R within a hinoki cypress canopy could be predicted by relatively simple parameters (light and temperature). There was an exception of extremely high R_{20} values in April that may have been due to the onset of shoot growth in spring. Understanding thermal acclimation and variations in leaf R within forest canopies will improve global terrestrial carbon cycle models.

Keywords: climate change, foliar respiration, hinoki cypress, Q_{10}, temperature sensitivity, thermal acclimation.

Introduction

Respiration (R) is a key component of carbon balance in trees and forest ecosystems (Ryan 1991, Valentini et al. 2000) that annually releases 30–70% of the carbon fixed by photosynthesis (Ryan et al. 1996, Turnbull et al. 2005). Leaf R contributes up to two-thirds of total tree R (Hagihara and Hozumi 1991, Ryan et al. 1997) and thus plays a critical role in determining the primary productivity of forest canopies (Griffin et al. 2001, Whitehead et al. 2004). Therefore, mechanistic understanding of variability of leaf R within a canopy is essential for predicting not only changes in forest productivity but also the future carbon sequestration function of forest ecosystems in response to climatic warming.

Respiration is highly dependent on temperature (T). The short-term T response of R rates has conventionally been approximated by a simple exponential equation; Q_{10}, the proportional change in R with a 10 °C increase in T, is widely used as a measure of the T sensitivity of R (Amthor 1989, Atkin et al. 2005). This simple exponential equation assumes that Q_{10} is constant over the measurement T range. However, there is empirical evidence demonstrating that the Q_{10} of leaf R

decreases linearly as the given measurement for T increases (Tjoelker et al. 2001, Atkin and Tjoelker 2003). To incorporate the T dependence of Q_{10} into the T response curve of R, Atkin et al. (2005) proposed a modified exponential equation (see the Materials and methods). Recently, the validity of this equation was confirmed for analyzing the T response of leaf R, particularly when measurement T is varied in high-resolution intervals (e.g., $1\,°C\,min^{-1}$) and over a wide range (e.g., $10–45\,°C$) (O'Sullivan et al. 2013, Weerasinghe et al. 2014, Heskel et al. 2016). The modified exponential equation also provides Q_{10} at a given T as well as R rate at a reference T.

Over the long term, prevailing ambient air temperature can affect the T response of leaf R. For example, Q_{10} was higher in winter than in summer in evergreen species *Chamaecyparis obtusa* [(Sieb. et Zucc.) Endl.] (Paembonan et al. 1991, Yokota and Hagihara 1996a), *Picea abies* (Stockfors and Linder 1998) and *Eucalyptus pauciflora* (Atkin et al. 2000). Additionally, a negative correlation between Q_{10} and ambient air T has been observed for *C. obtusa* (Paembonan et al. 1991, Yokota and Hagihara 1996a), *Pinus banksiana* (Tjoelker et al. 2009), *Pinus radiata* and *Populus deltoides* (Ow et al. 2010). Decreases in Q_{10} with increasing air T have been attributed to the thermal acclimation of R (Atkin et al. 2000, Zaragoza-Castells et al. 2007, Ow et al. 2008, Tjoelker et al. 2008, Dillaway and Kruger 2011, Slot et al. 2014).

Recently, several meta-analyses have demonstrated global patterns in leaf R and its thermal acclimation (Atkin et al. 2015, Slot and Kitajima 2015, Vanderwel et al. 2015, Heskel et al. 2016), indicating that thermal acclimation of leaf R occurs across biomes and plant functional types. However, these studies also showed that there is notable variability in R rates and acclimation capacity within biomes and plant functional types. Therefore, it is important to accumulate evidence of species-specific acclimation capacity based on empirical studies. Yet, the majority of acclimation studies have been conducted experimentally, using growth cabinets to examine relatively small plants with fully developed leaves. This suggests that understanding of the thermal acclimation of leaf R in trees growing under field conditions, and particularly under the influence of shoot growth phenology, is still limited (Tjoelker et al. 2009, Ow et al. 2010, Heskel et al. 2014).

Canopy position affects leaf R; that is, leaf R rates increase with increasing height in the canopy. Early studies (Kira et al. 1969, Hagihara and Hozumi 1977, Yoda 1978, 1983) reported coupling of the vertical gradients of leaf R with relative light intensity. Since then, numerous studies have demonstrated that leaf R at a reference T is correlated with leaf structural and chemical traits, such as leaf mass per area (LMA) (Reich et al. 1998a, 1998b, Tissue et al. 2002), leaf nitrogen content (leaf N) (Ryan 1995, Ryan et al. 1996, Reich et al. 1998a, 1998b), and leaf carbohydrates (Griffin et al. 2001, Turnbull et al. 2001, Whitehead et al. 2004).

By contrast, it is still unclear whether Q_{10} (or activation energy estimated by a modified Arrhenius function, E_O) varies within the canopy. Turnbull et al. (2003) found that Q_{10} was higher in upper-canopy leaves than in lower-canopy leaves for three deciduous and three coniferous species. Griffin et al. (2002) also demonstrated variable Q_{10} in a canopy of 4-m-tall *P. deltoides* trees, but the relationship was inverse (i.e., Q_{10} was higher in lower leaves than in upper leaves). Conversely, Bolstad et al. (1999) concluded that Q_{10} did not vary by canopy position based on measurements of the $T–R$ relationship in 18 broad-leaved tree species. Similarly, Q_{10} (or E_O) was constant in *Quercus rubra* (Xu and Griffin 2006) and *Eucalyptus globulus* canopies (O'Grady et al. 2008, 2010). Therefore, in addition to seasonal variations in T response of leaf R, vertical variation within canopies needs to be elucidated for various forest types. Investigating simultaneous spatial and temporal variations in leaf R will be useful for scaling up from an instantaneous leaf-level R to an annual whole-canopy R, as shown for stem CO_2 efflux (Araki et al. 2010, Tarvainen et al. 2014, Araki et al. 2015a). However, there are a few studies that have examined both of these variations in leaf R in forest canopies (Xu and Griffin 2006, O'Grady et al. 2008, 2010). Furthermore, recent studies suggest that it is important to incorporate thermal acclimation of leaf R into global carbon models (Wythers et al. 2005, King et al. 2006, Atkin et al. 2008, Smith and Dukes 2013, Wythers et al. 2013, Atkin et al. 2014). However, it is still unknown whether acclimation capacity varies with canopy position.

In the present study, we examined vertical and seasonal variations in the T response of leaf dark R in the canopy of a 10-year-old stand of hinoki cypress (*C. obtusa*). In general, forest productivity is high in relatively young stands (Kira and Shidei 1967, Odum 1969, Ryan et al. 2004). Thus, we expected that leaves in a young stand would have high values for physiological activities, including R. Therefore, we chose a relatively young hinoki cypress stand for our study plot. Hinoki cypress, an evergreen conifer, is one of the most important species for timber production in Japan and is widely planted except in the northern areas (Ishii 1991, Sasse 1998).

The respiratory behavior of hinoki cypress has been well studied, especially in the aboveground portion of the plant (i.e., stem, branches and leaves measured together using the enclosed whole-tree method) (Ninomiya and Hozumi 1983, Paembonan et al. 1991, 1992, Yokota et al. 1994, Yokota and Hagihara 1995, Adu-Bredu et al. 1996a, 1996b, Yokota and Hagihara 1996a, 1996b, Adu-Bredu et al. 1997a, 1997b, Yokota and Hagihara 1998) as well as in individual organs such as the stem (Oohata et al. 1971, Oohata and Shidei 1972, Hagihara and Hozumi 1981, Mori and Hagihara 1988, Ohkubo et al. 2009, Araki et al. 2010, 2015b), branches (Oohata et al. 1971, Mori and Hagihara 1995, Nagy et al. 1999, Ohkubo et al. 2009), leaves (Oohata et al. 1971, Hagihara and Hozumi

1977, Ohkubo et al. 2009) and roots (Mori and Hagihara 1991). However, to our knowledge, the *T* response of leaf *R* in hinoki cypress remains unknown.

In the current study, we also investigated the effects of environmental factors (light and mean air *T*) and physiological factors (LMA, leaf *N* and shoot growth) on *T* response of leaf *R*. The main objectives of this study were to (i) clarify vertical and seasonal patterns in the *T* response of leaf *R*, (ii) examine which factors are responsible for these variations and (iii) discuss whether the thermal acclimation capacity of leaf *R* varies according to position in the canopy. Furthermore, we verified whether variations in the *T* response of leaf *R* in a hinoki cypress canopy could be predicted by relatively simple parameters.

Materials and methods

Study site

This study was carried out in a 10-year-old stand of hinoki cypress [*C. obtusa* (Sieb. et Zucc.) Endl.] in the Tatsuda Experimental Forest of the Kyushu Research Center, Forestry and Forest Products Research Institute (FFPRI). The stand was located on a gentle and south-west facing slope of Mt Tatsuda (152 m above sea level) in Kumamoto City, Kyushu, Japan (32°49.43′ N, 130°43.97′ E). Mean annual air temperature and annual precipitation for the period 1981–2010 at Kumamoto Meteorological Observatory, near the study site, were 16.9 °C and 1986 mm, respectively (Japan Meteorological Agency 2013). The soil is brown forest soil (Forest Soil Division 1976).

The study stand was planted in 2001 after harvesting of an 85-year-old stand of hinoki cypress. At the time of study, the stand had never been thinned. The canopy was completely closed and forest floor vegetation was sparse. In March 2011, a study plot of 50 × 12 m was set up in the stand and stem diameter at breast height (DBH) and tree height were measured for all trees in the plot. Tree density was 2000 trees ha^{-1} and the mean ± SD of DBH and tree height were 10.2 ± 2.1 cm and 710 ± 89 cm, respectively. In 2011 and 2012, monthly mean air temperature ranged from 2.8 °C in January to 28.9 °C in August. Sufficient precipitation was observed during the study period, indicating that no severe drought occurred (Figure 1).

Light and LAI measurements

A scaffold tower (9.5 m in height, triangular cross section with 4-m-long sides) was built at the center of the study plot. This tower allowed us to access the canopy, which consisted of five target trees and their surrounding trees. In March 2011, the mean DBH and tree height of the target trees were 11.6 cm (range: 8.5–14.8) and 818 cm (760–930), respectively. Air temperature and relative humidity were measured every 15 min at the top of the tower with a relative humidity/air temperature sensor (1400-104; Li-cor, Lincoln, NE, USA).
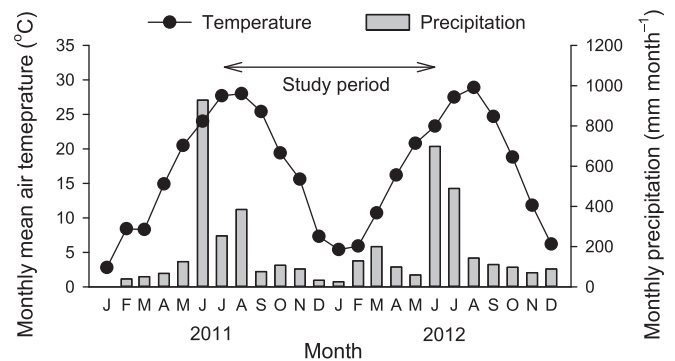

Figure 1. Monthly mean air temperature and monthly precipitation in 2011 and 2012 at Kumamoto Meteorological Observatory, near the study site, in Kumamoto City, Kyushu, Japan (Japan Meteorological Agency). The arrow indicates the period during which respiration was measured in this study (July 2011–June 2012).

We used relative photosynthetic photon flux density (RPPFD) to describe the light environment surrounding sample shoots for measuring respiration. RPPFD was defined as the ratio of integrated photosynthetic photon flux density (PPFD) at a given point within the canopy to the integrated PPFD above the canopy. Practically, we measured the leaf area index (LAI) just above the sample shoots with a plant canopy analyzer (LAI-2000, Li-cor) instead of measuring PPFD and converted the LAI values to RPPFD values using the relationship between RPPFD and LAI. To establish this relationship, PPFD sensors (LI-190SA, Li-cor) were installed at 3, 4, 5, 6 and 7 m above ground level (within the canopy) and at 9 m above ground level (above the canopy) on each of three vertical poles at the corners of the tower (i.e., 18 sensors in total). All the PPFD sensors measured instantaneous PPFD at 10-min intervals for 7 days (20–26 April 2011). This period included various weather conditions, from clear skies to rain.

Based on the integrated values of PPFD for 7 days, we calculated the relative PPFD for each 3–7 m sensor (within the canopy) against the PPFD measured at 9 m (above the canopy) on the same pole. On a uniformly cloudy day (19 April 2011), we measured LAI just above each PPFD sensor. Consequently, we determined an exponential relationship between RPPFD and LAI (i.e., the Beer–Lambert law) in this stand as follows: RPPFD = exp (−0.742 LAI) (P < 0.001, n = 18). The LAI measurements were conducted almost every month from April 2011 until April 2013. The mean LAI at 3 m (n = 3, below the lowest branch), which was re-calculated by applying the correction factor specific to hinoki cypress (1.64; Ishii et al. 1997), ranged from 5.61 (April 2011) to 8.29 (September 2012) with a mean of 6.93 (n = 20).

Shoot elongation

We selected 50 primary branches at various heights from the five target trees (10 branches per tree). Hinoki cypress is characterized by scale leaves and a lack of bud scales (Sakimoto and Takeda 1994). Thus, it is difficult to discriminate between

current-year and older shoots. Accordingly, we marked a given point near the tip of the terminal shoot of selected branches with a permanent marker before onset of shoot growth. The length between the mark and the shoot tip was measured with digital calipers and/or a steel measure every month from April 2011 to December 2012. Because 15 branches died during 2011, we selected supplementary branches in March 2012. Based on these measurements, the monthly rate of shoot elongation was calculated for three canopy layers. The means ± SD of shoot height from the ground for the upper, middle and lower canopy layers in March 2011 were 706 ± 68 cm ($n = 17$), 583 ± 28 cm ($n = 17$) and 428 ± 91 cm ($n = 16$), respectively, and those in March 2012 were 776 ± 57 cm ($n = 17$), 628 ± 34 cm ($n = 17$) and 514 ± 91 cm ($n = 16$), respectively.

Gas exchange system

Leaf gas exchange was measured in the laboratory by an open flow system with a single respiration chamber. The chamber was made from a commercially available polypropylene box (Tightbox; Jallee, Inazawa, Japan) with a volume of 1.5 L (H: 157 mm × W: 190 mm × D: 87 mm). A T-type thermocouple and a mixing fan were installed inside the chamber.

Ambient air, which was drawn by the pump (GS-5 EA; Enomoto, Tokyo, Japan) from a buffer tank situated on the roof (~10 m above the ground), was used as the reference gas. The buffer tank was 500 l in volume and had a mixing fan inside. To avoid dew condensation in the circuits, the air drawn to the laboratory was cooled with water trap (in a conical flask surrounded by ice) and dried with a dryer (MD-070-48 F; Perma Pure, Lakewood, NJ, USA). The reference gas was supplied into the chamber at a constant flow rate (0.4 or 0.5 l min^{-1}) regulated by a mass flow controller (MFC; SEC-B40; Horiba Stec, Kyoto, Japan), and fed into the reference cell of the infrared gas analyzer (IRGA; LI-6262; Li-cor) at the same flow rate, which was regulated by another MFC.

To check air tightness in the system, the flow rate of a sample gas from the chamber was monitored with a flowmeter (Floline SEF-21 A; Horiba Stec). Differences in CO_2 concentration between reference and sample gases were measured using the IRGA in differential mode. Simultaneously, the absolute values of CO_2 concentrations in the reference gas were monitored with another IRGA (LI-840; Li-cor). The CO_2 concentration was found to be highly stable, probably owing to the large buffer tank containing the mixing fan. Voltage outputs from the IRGAs, the MFCs, the flowmeter and the thermocouple installed in the chamber were recorded by a data logger (GL220; Graphtec, Yokohama, Japan) at 30-s intervals.

Leaf respiration measurement

Several studies have demonstrated that there are no differences in R rates between attached and detached leaves (Mitchell et al. 1999, Xu and Griffin 2006, Cavaleri et al. 2008). Accordingly,

we measured dark R rates of hinoki cypress leaves with detached shoots six times between July 2011 and June 2012. The measurement periods were 28 June–22 July (hereafter, denoted as July), 10 September–2 October (September), 20–27 December in 2001, and 27 February–6 March (March), 16–24 April, and 15–28 June in 2012. During each measurement month, 15 terminal shoots of a primary or secondary branch located at the crown surface were randomly selected from various heights (3.2–8.9 m; approximately three branches from each 1-m stratum) from trees surrounding the target trees (i.e., excluding the five target trees).

At the tip of the selected shoots, the shoot height and LAI were measured with the plant canopy analyzer under a uniformly cloudy sky. The LAI readings were converted to RPPFD values using the exponential regression mentioned above. Subsequently, a single shoot was harvested and immediately cut under water in the field. The detached shoot, whose base was placed in a container of water, was brought to the laboratory (10-min transport time). The sample shoot was recut under water and placed into the chamber. The chamber had a 5-mm-diameter hole in one side to allow the bottom section of the sample shoot to be inserted into a container of water outside the chamber. The gap between the shoot and the hole was filled with putty to ensure airtightness. The chamber was placed in darkness in a cooling incubator (MIR-154; Sanyo, Osaka, Japan).

We measured short-term responses of leaf R rates to changes in the measurement T with a 20 °C range using the incubator's programming function. The measurement T ranged from 15 to 35 °C for July, September and June; 10–30 °C for April; 5–25 °C for December and March. In the case of the 15–35 °C range, for example, the T inside the incubator was controlled to decrease from 25 °C to 15 °C, then increase to 35 °C, and decrease again to 25 °C. The incubator T was varied by 1 °C at 10-min intervals, and maintained for 30 min at the middle, upper and lower ends of the measurement T range, while R rates and T inside the chamber were recorded at 30-s intervals with the gas exchange system described above. For a single shoot, the process took 8–9 h, which meant we obtained 1042–1082 data points for measuring the R–T relationship.

It is important to consider substrate limitations when taking leaf R measurements of detached shoots because a decrease in the substrate may result in a subsequent decrease in R rates with elapsed time. To confirm that this limitation did not affect our measurements, R rates at the middle of the measurement T range (e.g., 25 °C in the 15–35 °C range) were measured three times: at the beginning, middle and end of the measurement period, as described above. In most cases, the three R rates were almost identical (Figure 2), indicating that R rates did not decrease over the measurement period. Thus, we concluded that substrate limitation was not a relevant issue for our R measurement.

In total, 90 shoots ($n = 15$ per month) were sampled from the crown surfaces of 11 trees. The mean ± SD of shoot length
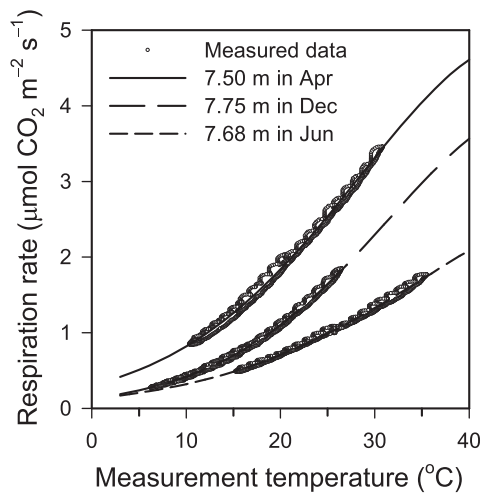
Figure 2. Short-term temperature-response curves of leaf respiration rates in hinoki cypress (*Chamaecyparis obtusa*) calculated by a modified exponential function that includes temperature-dependent Q_{10} (Atkin et al. 2005) (see the main text for details). Examples of respiration curves for shoots taken from the upper layer in three different months are shown: solid, long- and short-dashed lines indicate curves for shoots at 7.50 m in April, 7.75 m in December and 7.68 m in June, respectively. Small open circles indicate respiration rates measured every 30 s over 8 h (n = 1042). The measurement temperature varied 20 °C in range (see Table 1). For example, in April, the temperature was controlled to start at 20 °C, decreased to 10 °C, then increased to 30 °C, and decreased again to 20 °C. Almost identical respiration rates at the beginning and end of the measurements indicate that respiration rates did not decrease due to substrate limitations over the measurement period.

and leaf area enclosed by the chamber were 251 ± 28 mm and 196 ± 56 cm^2, respectively. Sample shoots included not only newly formed current-year leaves but also older leaves. The proportions of new to old leaves differed according to the sampling height and month. We observed that sample shoots from the upper canopy consisted almost entirely of current-year leaves except for those collected in April. In contrast, older leaves were dominant in lower shoots, as shown previously for *Cryptomeria japonica* (Kiyono and Akama 2016). We did not measure R rates separately according to leaf age owing to the technical difficulty of correctly distinguishing leaf ages in hinoki cypress.

Leaf traits

Following the R measurements, the leaves of the sample shoots were detached from their axes, then projected leaf area (m^2) was measured using a flatbed scanner (MP970; Canon, Tokyo, Japan) and image analysis software (LIA32, Yamamoto 2005). The leaves were dried at 65 °C for 72 h, weighed to calculate leaf dry mass per unit area (LMA, g m^{-2}), and then ground to a fine powder. The total nitrogen concentration in leaves was determined using the dry combustion method with an NC analyzer (Sumigraph NC-22 F; SCAS, Tokyo, Japan). Nitrogen contents per unit leaf dry mass (N_{mass}, mg g^{-1}) and per projected leaf area (N_{area}, mg m^{-2}) were calculated.

Data analysis

We obtained high-resolution measurement T over a relatively wide range (20 °C). To quantify the short-term T response of R rates in hinoki cypress leaves, we employed the following modified exponential equation proposed by Atkin et al. (2005):

$$R_T = R_{T_{ref}} \left[x - y \left(\frac{T + T_{ref}}{2} \right) \right]^{\frac{T - T_{ref}}{10}}$$

where R_T is the rate of R at a given T, $R_{T_{ref}}$ is R at a reference T_{ref}, x and y are constants that describe a linear decline in Q_{10} with increasing T (i.e., Q_{10} value at a given T can be calculated as: $Q_{10}[T] = x - y \, [(T + T_{ref})/2]$). We used 20 °C as the T_{ref} in this study and the equation was fitted by nonlinear least-square regression using R version 3.0.3 (R Development Core Team 2014) (Figure 2). Consequently, area- and mass-based leaf R rates adjusted to 20 °C (R_{20}) and Q_{10} at 20 °C ($Q_{10}[20]$) as well as R rates and Q_{10} at ambient air temperature (T_{amb}, described below) were obtained for each sample shoot.

To determine how many days in the field prior to shoot sampling affected the T sensitivity of leaf R, we conducted linear regressions between $Q_{10}[20]$ and the 1–14 day averages of mean air T (n = 88), according to the methods of Ow et al. (2010). Then, we compared the coefficients of determination (r^2) of the regressions as a function of the number of days before shoot sampling (including the day of sampling). In accordance with the results of the comparison, we defined ambient air temperature (T_{amb}) as the mean air temperature for 7 days prior to shoot sampling in this study.

Fifteen shoots sampled in each month were divided into three canopy layers, according to the shoot height (n = 5 for each layer, with the exception of n = 4 for the upper and lower layers in July owing to a measurement failure). The mean shoot height in each layer gradually increased with measurement month because of tree height growth. For example, the values of mean ± SD of shoot height for the upper, middle and lower canopy layers in July 2011 were 760 ± 91 cm, 550 ± 50 cm and 415 ± 66 cm, respectively, and, those in June 2012 were 812 ± 53 cm, 655 ± 63 cm and 498 ± 58 cm, respectively.

We examined the effects of canopy position (upper, middle or lower layer) and measurement month on the parameters of respiratory characteristics by two-way analysis of variance. Subsequently, we compared the differences in mean values between each layer with multiple comparisons using Holm's method at P = 0.05. The same analyses were conducted for environmental (RPPFD and ambient air T) and physiological (shoot elongation, LMA, N_{area} and N_{mass}) factors.

To determine the relationships between respiratory characteristics and each of the environmental and physiological factors (X), we conducted regression analysis. We divided the six measurement months into dormant (December, March and April) and growing (June, July and September) seasons to equate the

number of sample for each of two seasons for this analysis. The effects of X and season on respiratory characteristics were examined using linear models. If an interaction ($X \times$ season) was not significant ($P > 0.05$, Type II analysis of variance), we tested the difference between the seasons (analysis of covariance). All statistical analyses were performed with R version 3.0.3 (R Development Core Team 2014).

Results

Vertical and seasonal variations in leaf respiratory characteristics

According to the average values for each measurement month, the area-based respiration rates at ambient air temperature (R_{amb}) and R_{20} of hinoki cypress leaves ranged from 0.20 to 0.76 μmol CO_2 m^{-2} s^{-1} and 0.37 to 1.05 μmol CO_2 m^{-2} s^{-1}, respectively (Table 1). Mass-based R_{20} ranged from 2.30 to 4.81 nmol CO_2 g^{-1} s^{-1}. The effects of canopy position and measurement month were significant for these three R rates, but the interaction between canopy and measurement month was significant only for R_{amb} (Table 2). The R rates exhibited apparent vertical gradients during all seasons, except for R_{amb} in December (Figure 3a and b). The R rates were highest in the upper canopy layer, intermediate in the middle layer and lowest in the lower layer. However, seasonal trends in R_{amb} and R_{20} were apparently different. R_{amb} was lower during winter (December and March) and was not significantly different among other months (Table 1), whereas R_{20} was highest in April, drastically decreased in summer, and then gradually increased through the fall and winter. The vertical and seasonal patterns observed in mass-based R_{20} were almost identical to those of area-based R_{20} (data not presented). Thus, only area-based R_{20} was used for further analysis (hereafter, R_{20} indicates area-based values).

Based on monthly averages, Q_{10} at ambient air temperature ($Q_{10}[T_{amb}]$) and $Q_{10}[20]$ ranged from 1.94 to 2.63 and 1.98 to 2.30, respectively (Table 1). Canopy position did not affect either Q_{10} significantly (Table 2); that is, Q_{10} showed no vertical difference throughout the seasons (Figure 3c and d). In contrast, seasonal patterns in these Q_{10} were obvious and similar (Table 1, Figure 3c and d). In particular, $Q_{10}[T_{amb}]$ was much higher during winter than during the growing season (June, July and September).

Vertical and seasonal variations in environmental and physiological factors

RPPFD displayed a distinct vertical gradient within the canopy but had no seasonal variation (Table 2, Figure 4a). Conversely, ambient air T (i.e., mean air T for 7 days before shoot sampling), which ranged from 4.5 °C in December to 26.5 °C in July, showed an obvious seasonal change but no vertical variation (Table 2, Figure 4b).

The effects of canopy position and measurement month were significant for all four of the physiological factors (Table 2). The values of these factors all tended to be greater in the upper than the middle and lower layers (Figure 4c–f). In particular, the vertical gradients of LMA and N_{area} were consistent throughout the seasons. In contrast, vertical trends in monthly shoot elongation rate and N_{mass} differed by measurement month. Seasonally, LMA was greatest in March, decreased after March until the summer, and then increased gradually through the fall and winter. The seasonal pattern of N_{area} was similar to that of LMA, although the peak occurred in spring. An opposite seasonal trend was observed for shoot elongation rate and N_{mass}. Shoot elongation began at the end of April, peaked in July and then decreased until winter. An increase in N_{mass} was observed in spring and early summer, especially in the upper layer. This increase might have been associated with shoot growth because cumulative shoot elongation resulted

Table 1. Number of sample shoots (n), measurement temperature ranges used for determining the short-term temperature response of leaf respiration in hinoki cypress (*Chamaecyparis obtusa*) as well as means $\pm$ standard errors of ambient air temperature (T_{amb}) and leaf respiratory characteristics for each measurement month.

Month	n	Measurement temperature range (°C)	Ambient air temperature (°C)	$Q_{10}[T_{amb}]$	$Q_{10}[20]$	Area-based R_{amb} (μmol CO_2 m^{-2} s^{-1})	Area-based R_{20} (μmol CO_2 m^{-2} s^{-1})	Mass-based R_{20} (nmol CO_2 g^{-1} s^{-1})
July	13	15–35	26.5 $\pm$ 0.2 a	1.94 $\pm$ 0.02 a	1.98 $\pm$ 0.03 a	0.55 $\pm$ 0.10 a,b,c,d	0.37 $\pm$ 0.07 a	2.30 $\pm$ 0.41 a
September	15	15–35	22.3 $\pm$ 0.6 b	1.98 $\pm$ 0.03 a	1.98 $\pm$ 0.03 a	0.58 $\pm$ 0.10 b,d	0.52 $\pm$ 0.10 a	3.01 $\pm$ 0.53 a,b
December	15	5–25	4.5 $\pm$ 0.1 c	2.63 $\pm$ 0.03 b	2.30 $\pm$ 0.02 b	0.20 $\pm$ 0.03 c	0.85 $\pm$ 0.13 a,b	4.28 $\pm$ 0.54 a,b
March	15	5–25	8.2 $\pm$ 0.1 d	2.55 $\pm$ 0.03 b	2.30 $\pm$ 0.02 b	0.26 $\pm$ 0.03 c,d	0.80 $\pm$ 0.09 a,b	3.49 $\pm$ 0.29 a,b
April	15	10–30	16.0 $\pm$ 0.3 e	2.25 $\pm$ 0.03 c	2.19 $\pm$ 0.03 c	0.76 $\pm$ 0.12 a,b	1.05 $\pm$ 0.16 b	4.81 $\pm$ 0.69 b
June	15	15–35	22.2 $\pm$ 0.1 b	2.03 $\pm$ 0.03 a	2.04 $\pm$ 0.03 a	0.66 $\pm$ 0.10 a,b	0.56 $\pm$ 0.09 a	3.26 $\pm$ 0.46 a,b

The relationship between respiration rate and measurement temperature was approximated by a modified exponential equation proposed by Atkin et al. (2005) (see the main text for details).

Ambient air temperature (T_{amb}) was defined as the mean air temperature for 7 days prior to shoot sampling.

Means with the same letter within the same column were not significantly different ($P > 0.05$, paired multiple comparisons using Holm's method).

Abbreviations: $Q_{10}[T_{amb}]$, Q_{10} at ambient air temperature; $Q_{10}[20]$, Q_{10} at 20 °C; R_{amb}, respiration rate at ambient air temperature; R_{20}, respiration rate at 20 °C.

Table 2. Effects of canopy position (upper, middle or lower layer), measurement month, and their interaction on leaf respiratory characteristics and environmental and physiological factors in a hinoki cypress canopy ($n = 88$, two-way analysis of variance).

Category	Dependent variables	Independent variables					
		Layer		Month		Layer × Month	
		df	F	df	F	df	F
Respiration	$Q_{10}[T_{amb}]$	2	0.9 ns	5	150.6***	10	1.37 ns
	$Q_{10}[20]$	2	2.8 ns	5	29.3***	10	0.90 ns
	Area-based R_{amb}	2	51.8***	5	16.4***	10	3.2**
	Area-based R_{20}	2	47.1***	5	10.2***	10	1.0 ns
	Mass-based R_{20}	2	44.2***	5	6.4***	10	1.1 ns
Environment	RPPFD	2	67.6***	5	1.7 ns	10	1.0 ns
	T_{amb}	2	2.9 ns	5	804.8***	10	1.0 ns
Physiology	Shoot elongation	2	68.5***	5	19.5***	10	4.9***
	LMA	2	33.2***	5	27.4***	10	1.9 ns
	N_{area}	2	38.7***	5	13.4***	10	1.6 ns
	N_{mass}	2	16.1***	5	9.6***	10	3.2**

Abbreviations: $Q_{10}[T_{amb}]$, Q_{10} at ambient air temperature; $Q_{10}[20]$, Q_{10} at 20 °C; R_{amb}, respiration rate at ambient air temperature; R_{20}, respiration rate at 20 °C; T_{amb}, mean air temperature for 7 days prior to shoot sampling; RPPFD, relative photosynthetic photon flux density; shoot elongation, monthly elongation rate of terminal shoots measured for primary branches; LMA, leaf dry mass per unit leaf area; N_{area} and N_{mass}, area- and mass-based leaf nitrogen content, respectively.
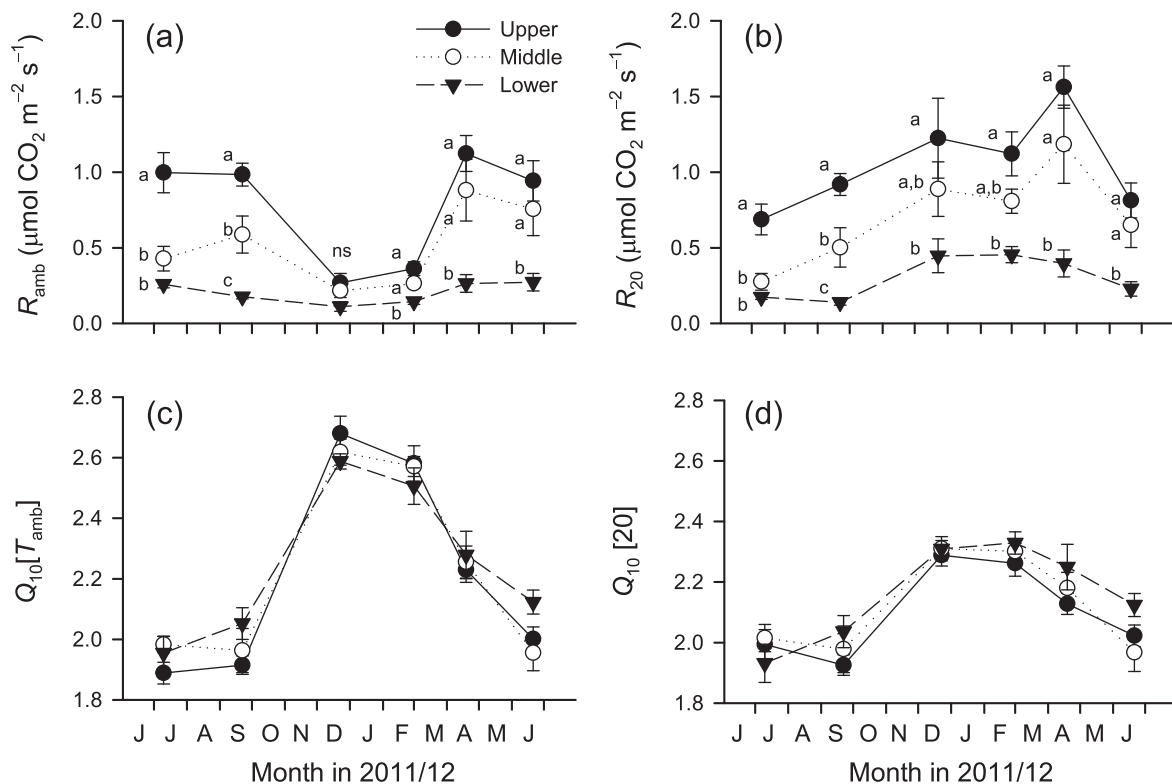Significance level: ns, not significant; **$P < 0.01$; ***$P < 0.001$.

Figure 3. Vertical and seasonal variations in respiratory characteristics of hinoki cypress leaves: the area-based respiration rates at ambient air temperature (R_{amb}) and at 20 °C (R_{20}) decreased with depth in the canopy but their seasonal patterns were different (a, b); Q_{10} at ambient air temperature ($Q_{10}[T_{amb}]$) and at 20 °C ($Q_{10}[20]$) did not vary vertically and were higher in winter than in summer (c, d). Closed and open circles and triangles indicate mean values in the upper, middle and lower canopy layers, respectively ($n = 5$, except for upper and lower layers in July when $n = 4$). The vertical bars denote standard errors of the means (SEM). The same letter within the same month indicates that mean values were not significantly different between canopy layers ($P > 0.05$, multiple comparisons using Holm's method). The effects of canopy layers on both Q_{10} were not significant (see Table 2).
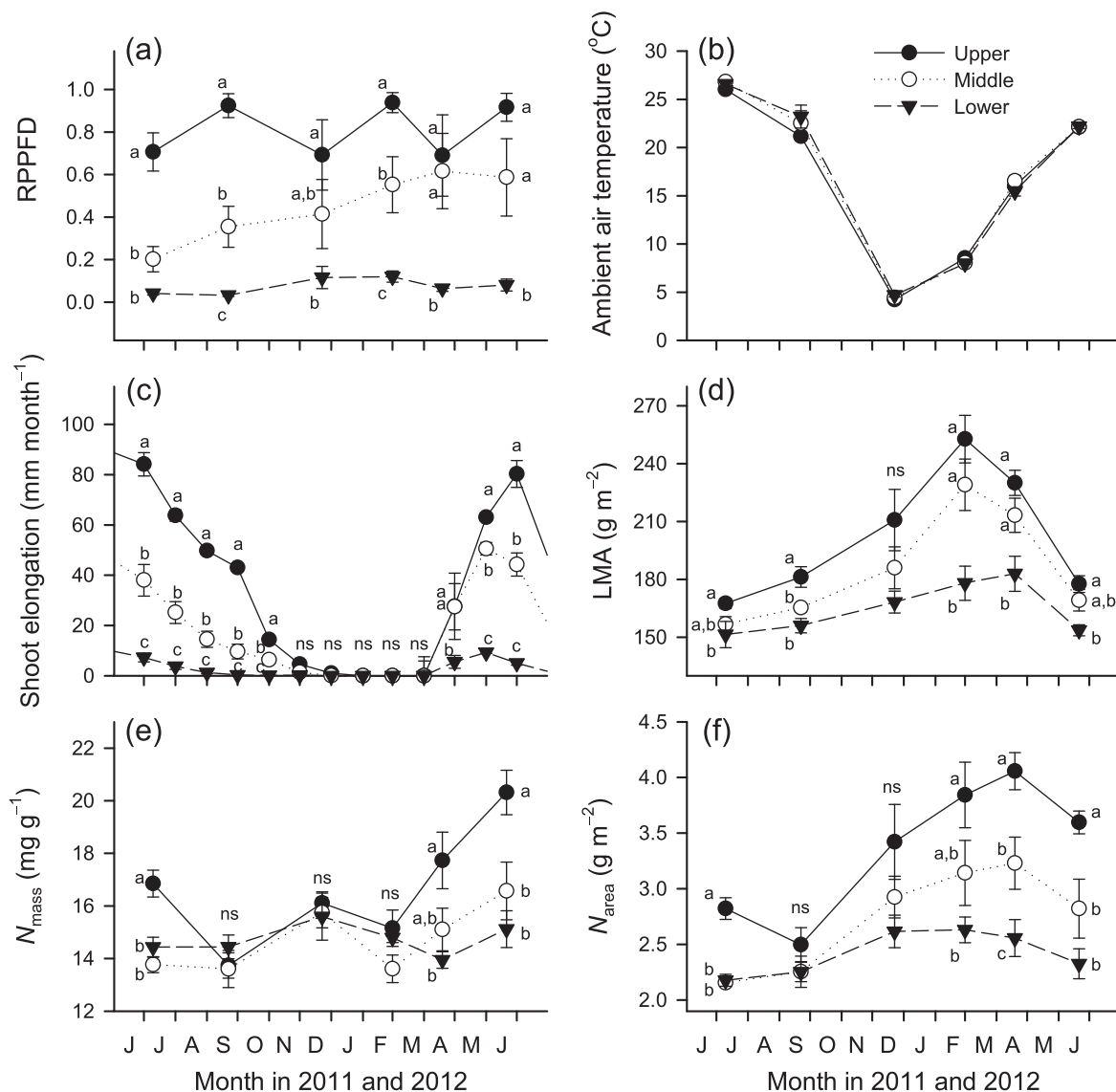
Figure 4. Vertical and seasonal variations in environmental and physiological factors within a hinoki cypress canopy: RPPFD at the tip of the sample shoot for respiration measurement varied vertically but had no seasonal variation (a); ambient air temperature, defined as the mean air temperature for 7 days before shoot sampling, varied seasonally but had no vertical variation (b); monthly elongation rate of the terminal shoot of primary branches of five target trees adjacent to the trees used for respiration measurement ($n = 17$ for upper and middle and $n = 16$ for lower canopy layers) (c); leaf dry mass per area (LMA) (d); mass- and area-based leaf nitrogen contents (e, f). Symbols, bars and letters are same as in Figure 3 (ns: not significant). The effect of canopy layers on ambient air temperature was not significant (see Table 2). (d–f) Modified from Araki et al. (2015b).

in a higher proportion of newly formed current leaves with high N_{mass} in the sample shoots.

Relationships between respiratory characteristics and environmental and physiological factors

Area-based R_{20} was significantly correlated with all factors except for ambient air T, although the effect of season (dormant or growing) was different among the factors (Table 3). By contrast, $Q_{10}[T_{amb}]$ and $Q_{10}[20]$ were found to be correlated with only ambient air T, and none of the physiological factors had a significant effect on both Q_{10} (statics for $Q_{10}[T_{amb}]$ not shown).

R_{20} was strongly correlated with RPPFD for each season separately; that is, the slope was not significantly different but the intercept was ($r^2 = 0.75$, $P = 0.002$, $n = 9$ for the dormant season; $r^2 = 0.98$, $P < 0.001$, $n = 9$ for the growing season) (Table 3, Figure 5a). R_{20} was correlated with shoot elongation rate only during the growing season ($r^2 = 0.70$, $P = 0.005$, $n = 9$) (Figure 5b). The effect of LMA on R_{20} differed by season; that is, the slope was significantly different ($r^2 = 0.55$, $P = 0.022$, $n = 9$ for the dormant season; $r^2 = 0.95$, $P < 0.001$, $n = 9$ for the growing season) (Table 3, Figure 5c). R_{20} was correlated with N_{area} without seasonal differences (i.e., the slope and intercept were not different)

Table 3. Results of linear models to examine the effects of each environmental and physiological factors (X) and season (dormant or growing) on leaf respiratory characteristics (area-based R_{20} and $Q_{10}[20]$) in hinoki cypress.

Dependent variables	Independent variables (X)	P-value			
		X	Season	$X \times$ Season	ANCOVA
R_{20}	RPPFD	<0.001***	<0.001***	0.150 ns	<0.001***
	T_{amb}	0.725 ns	0.168 ns	0.201 ns	0.177 ns
	Shoot elongation	0.005**	<0.001***	0.176 ns	0.001***
	LMA	<0.001***	0.452 ns	0.049*	—
	N_{area}	<0.001***	0.655 ns	0.202 ns	0.662 ns
	N_{mass}	0.031*	0.001**	0.119 ns	0.013*
$Q_{10}[20]$	RPPFD	0.131 ns	<0.001***	0.740 ns	<0.001***
	T_{amb}	0.011*	0.037*	0.441 ns	0.034*
	Shoot elongation	0.067 ns	<0.001***	0.007**	—
	LMA	0.105 ns	<0.001***	0.614 ns	<0.001***
	N_{area}	0.161 ns	<0.001***	0.195 ns	<0.001***
	N_{mass}	0.765 ns	<0.001***	0.129 ns	<0.001***

The analysis was conducted based on mean values presented in Figures 3 and 4. Measurement months were divided into dormant (December, February and April) and growing (July, September and June) seasons. If an interaction ($X \times$ season) was not significant (i.e., the slope did not differ between seasons; $n = 18$, Type II analysis of variance), then the differences in the intercepts between seasons were tested by analysis of covariance (ANCOVA). Abbreviations are same as in Table 2.
Significance level: ns, not significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
Dash indicates the test was not conducted.

($r^2 = 0.78$, $P < 0.001$, $n = 18$ for the two seasons combined) (Table 3, Figure 5d).

A strong negative relationship was found between each Q_{10} and ambient air T ($Q_{10}[T_{amb}] = 2.798–0.034 \ T_{amb}$, $r^2 = 0.98$, $P < 0.001$, $n = 6$; $Q_{10}[20] = 2.409–0.017 \ T_{amb}$, $r^2 = 0.94$, $P < 0.001$, $n = 6$) (Figure 6a). As described above, ambient air T had no significant effect on R_{20} when all data were pooled (Table 3). However, when the relationship was separately examined by canopy layer, excluding the high R_{20} values observed for the upper and middle layers in April, a negative relationship was found between R_{20} and ambient air T for the upper ($r^2 = 0.96$, $P = 0.003$, $n = 5$), middle ($r^2 = 0.85$, $P = 0.026$, $n = 5$) and lower layers ($r^2 = 0.88$, $P = 0.005$, $n = 6$) (Figure 6b). The regression slopes were not significantly different, but the intercepts were significantly different between each canopy layer according to analysis of covariance.

Predicting variations in temperature responses of leaf respiration

Our results indicated that Q_{10} did not vary across the hinoki cypress canopy positions (Table 2, Figure 3c and d), and its temporal variation could be predicted from seasonal changes in ambient air T (Figure 6a). Furthermore, the vertical variation in R_{20} correlated well to within-canopy light gradients for each season (Figure 5a). The seasonal variation in R_{20} in each canopy layer could be also explained by the ambient air T (Figure 6b). Moreover, RPPFD and ambient air T were not correlated. Accordingly, we developed a generalized linear model for predicting R_{20} in which RPPFD and ambient air T are independent variables (Table 4). For the model development, we excluded the extremely high values observed for the upper and middle layers in April. As a result, predictions of R_{20} using the model showed fairly good agreement with the measured values ($r = 0.98$, $P < 0.001$, $n = 16$), except for the two high values (Figure 7). These results suggest that vertical and seasonal variations in T responses of leaf R in the hinoki cypress canopy could be predicted by relatively simple parameters, light and temperature.

Discussion

Seasonal temperature acclimation of leaf respiration

Previous studies that measured the nighttime respiration of all the aboveground parts of field-grown hinoki cypress trees together have demonstrated that Q_{10} decreased with increasing nighttime air temperature in 12-year-old trees (Paembonan et al. 1991) and 17-year-old trees (Yokota and Hagihara 1996b). Our current results on leaf R in 10-year-old hinoki cypress trees confirm these findings. Moreover, not only Q_{10} but also R_{20} for each canopy layer was negatively correlated to ambient air T (Figure 6). Other environmental and physiological factors did not affect the seasonal pattern of Q_{10} (Table 3). These results indicate that the short-term T response of leaf R in hinoki cypress varied primarily by adjusting to long-term (seasonal) changes in air T. Similar declines in Q_{10} and R at a reference T with increases in air T were found in 33-year-old *P. banksiana* trees grown in a common garden (Tjoelker et al. 2009) as well as 8-year-old *P. radiata* and 20-year-old *P. deltoides* trees grown in a field (Ow et al. 2010). Taken together, these findings suggest
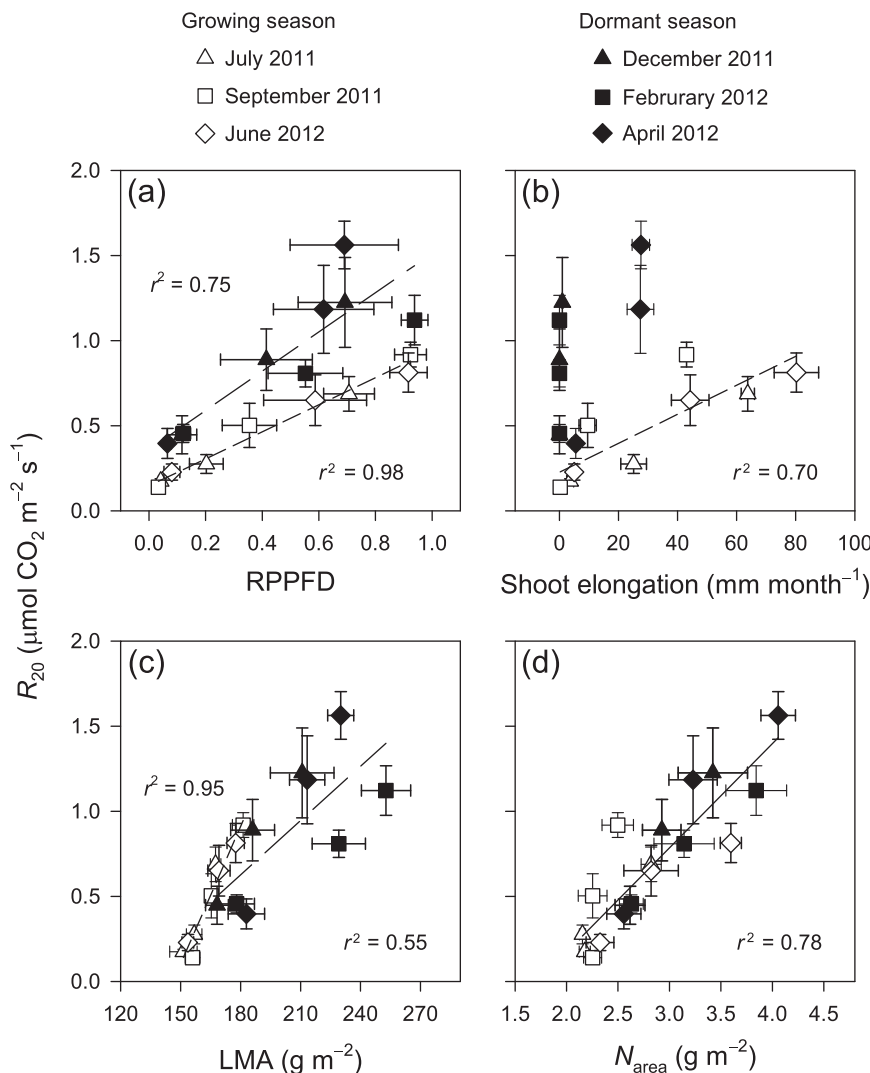
Figure 5. Relationships between area-based R_{20} and environmental and physiological factors of hinoki cypress leaves: R_{20} was strongly correlated with RPPFD for each season separately (a); monthly shoot elongation rate affected R_{20} during the growing season (b); effect of LMA on R_{20} differed by seasons (c); R_{20} was correlated with area-based leaf N (N_{area}) without seasonal differences. Different symbols indicate different measurement months. Closed and open symbols indicate data from the dormant and the growing seasons, respectively. Differences in slope and intercept between the seasons were tested by Type II analysis of variance and analysis of covariance, respectively (Table 3). Based on these results, regression lines for the dormant (long-dashed) and/or the growing (short-dashed) seasons are shown separately (a–c). The solid line denotes the linear regression for all months combined (d). Vertical and horizontal bars denote SEM ($n = 5$).

that seasonal T acclimation of leaf R is evident in forest canopies under field conditions; furthermore, down-regulation of leaf R can occur during warmer periods (Table 1, Figure 3a).

According to the two-component functional model, plant R can be divided into maintenance and growth components (Thornley 1970, Hesketh et al. 1971, Amthor 1989). Practically and theoretically, maintenance R is highly dependent on T and is directly proportional to plant mass with a constant maintenance respiration coefficient (m). Growth R is proportional to growth rate with a constant growth respiration coefficient (g). Based on seasonal observations of R of the entire aboveground portion of young hinoki cypress trees, Paembonan et al. (1992) and Yokota and Hagihara (1995) demonstrated seasonal changes in

the proportions of maintenance and growth R to total R. Moreover, Adu-Bredu et al. (1997b) found that the value of m increases exponentially with monthly mean air T, whereas g is independent of air T (i.e., g is constant throughout seasons). These findings suggest that maintenance cost per unit biomass is higher in warmer periods and that growth R is highly dependent on growth rate. Although this idea is based on measurements of R from the whole aboveground portion of trees, it might be helpful for understanding how the Q_{10} of leaf R decreases with increasing air T. In the current study, the monthly shoot growth rate (Figure 4c) was zero during winter and closely followed seasonal changes in monthly mean air T (Figure 1) during the growing season. This seasonal trend may indicate a pattern
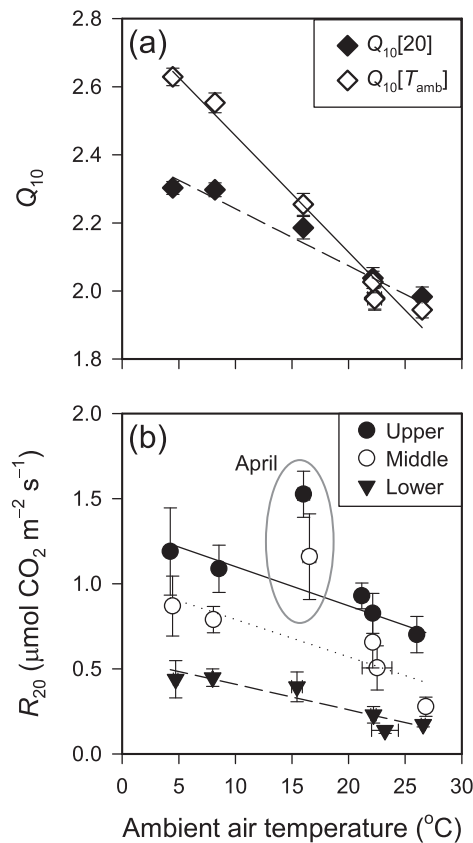
Figure 6. Q_{10} of leaf respiration in hinoki cypress decreased with increasing ambient air temperature, which was defined as the mean air temperature for 7 days prior to shoot sampling (a) and area-based R_{20} of each canopy layer was correlated negatively with ambient air temperature separately, except for the high R_{20} values observed for the upper and middle layers in April (surrounded by a gray ellipse) (b). Vertical and horizontal bars denote SEM. The solid and broken lines in (a) indicate the linear regression for $Q_{10}[T_{amb}]$ ($Q_{10}[T_{amb}] = 2.798-0.034\ T_{amb}$, $r^2 = 0.98$, $P < 0.001$, $n = 6$) and $Q_{10}[20]$ ($Q_{10}[20] = 2.409-0.017\ T_{amb}$, $r^2 = 0.94$, $P < 0.001$, $n = 6$), respectively. The solid, dotted and broken lines in (b) indicate regressions for upper ($R_{20} = 1.327-0.023\ T_{amb}$, $r^2 = 0.96$, $P = 0.003$, $n = 5$), middle ($R_{20} = 1.009-0.022\ T_{amb}$, $r^2 = 0.85$, $P = 0.026$, $n = 5$) and lower ($R_{20} = 0.559-0.015\ T_{amb}$, $r^2 = 0.88$, $P = 0.005$, $n = 6$) canopy layers, respectively. Note that linear regressions for the upper and middle layers in (b) exclude the high R_{20} values observed in April.

Table 4. Results of a generalized linear model to predict area-based R_{20} of hinoki cypress leaves using light and ambient air temperature.

Variables	Estimate	Standard error	*t*-value	*P*-value
Intercept	0.563	0.053	10.65	<0.001
RPPFD	0.803	0.061	13.25	<0.001
T_{amb}	−0.018	0.002	−7.44	<0.001

The interaction between RPPFD and T_{amb} was not significant.
Notably high R_{20} values observed for the upper and middle layers in April were excluded from analysis ($n = 16$).
Abbreviations: R_{20}, respiration rate at 20 °C; RPPFD, relative photosynthetic photon flux density; T_{amb}, mean air temperatures for 7 days prior to shoot sampling.
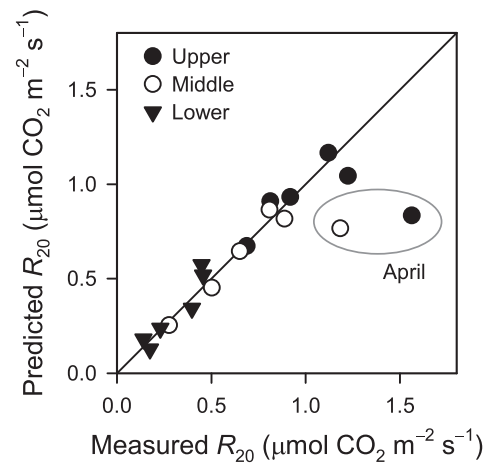

Figure 7. Good agreement of predicted and measured values for the R_{20} of hinoki cypress leaves. Predictions were calculated by a generalized linear model with RPPFD and ambient air temperature as the independent variables (Table 4). The high R_{20} values observed in April (surrounded by a gray ellipse) were excluded from model development. The solid line indicates 1:1.

in the growth component of leaf R, assuming that g of leaf R is constant throughout seasons. Certainly, a seasonal pattern in the maintenance component of leaf R would also be dependent on air T. Therefore, we speculate that an increase in the relative contribution of T-independent R (i.e., the growth component) in relation to T-dependent R (i.e., the maintenance component) with increasing air T may cause the responses of leaf R to short-term temperatures changes to be less sensitive.

Two types of thermal acclimation have been recognized in R (Atkin and Tjoelker 2003, Atkin et al. 2005, Slot and Kitajima 2015). Type I acclimation is characterized predominantly by a change in Q_{10} (i.e., the slope of the T response curve of R), probably under the influence of regulatory changes in existing respiratory enzymes. Type II acclimation is characterized by a change in the elevation [base R, such as R at 5 °C (R_5)] of the T response curve of R (without a change in Q_{10}) and typically involves a change in overall respiratory capacity. Tjoelker et al. (2009) compared the extent of seasonal adjustments of Q_{10} and R_5 of *P. banksiana* and concluded that mainly Type II acclimation contributed to seasonal variations in leaf R.

In the current study, seasonal variation in Q_{10} was obvious. Moreover, when we considered monthly averages for R_5 ($n = 13$ for July and $n = 15$ for other months, data not shown), seasonal variation in R_5 became smaller than that in R_{20}. Furthermore, significant differences in R_5 were only observed between July and April ($P = 0.013$, multiple comparisons using Holm's method). These results suggest that Type I acclimation contributed largely to the seasonal adjustment of leaf R in our study. Evidence of Type I acclimation is also shown in Figure 2. The slope of the T–R response curve (Q_{10}) decreased from December to June, but the elevation (R_5) was almost identical between these two examples. Therefore, regulatory changes in existing respiratory enzymes

might be largely responsible for the seasonal T acclimation of leaf R in hinoki cypress.

However, extremely high R_{2O} values, which deviated from the regression lines in Figure 6b, were observed for the upper and middle layers in April. The T–R response curve for April was located considerably higher on the y-axis (R rate) than the curve for December over the whole T range (Figure 2), although Q_{10} was lower in April than in December (Figure 3). Thus, the high R rates in April were caused by a remarkable increase in base R (i.e., R_5). Considering the mechanism of Type II acclimation, this period-specific phenomena may reflect an increase in overall respiratory capacity. The most likely explanation is that April R rates were strongly affected by shoot growth, which would entail high metabolic demands for biosynthesis associated with localized cell division/expansions processes (Amthor 2000).

However, the shoot elongation rate alone could not explain the extremely high R rates in April (Figure 5b) because the monthly elongation rates were much higher in June and July (Figure 4c). Shoot growth began at the end of April, suggesting that the onset of shoot growth must demand more energy, probably for processes such as nutrient and/or carbohydrate reallocation (Egger et al. 1996, Schaberg et al. 2000, Kitao et al. 2004, Wyka et al. 2016). However, the effects of the onset of shoot growth and/or leaf development on leaf R remain unclear. This issue will require further study.

Thermal acclimation capacity in tree canopies

There are several methods of evaluating the degree of thermal acclimation of R (Atkin et al. 2005, Slot and Kitajima 2015). For example, the set temperature method quantifies the degree of acclimation as $Acclim_{SetTemp} = R_{Cold}$ at T_{Set}/R_{Warm} at T_{Set}, where R_{Cold} and R_{Warm} indicate the rates of R in cold-grown and warm-grown plants, respectively (Loveys et al. 2003). If we apply this method to our monthly averaged R_{2O} shown in Table 1 (in this case, $T_{Set} = 20\,°C$) by setting R_{2O} in the coldest month (December; $T_{amb} = 4.5\,°C$) as R_{Cold} and R_{2O} in the warmest month (July; $T_{amb} = 26.5\,°C$) as R_{Warm}, then $Acclim_{SetTemp}$ would be 2.30. This value is higher than other $Acclim_{SetTemp}$ values reported for evergreen trees in temperate climates according to a recent meta-analysis (1.54 ± 0.41 SD and 1.03 ± 0.14 SD from laboratory and field measurements, respectively) (Slot and Kitajima 2015). However, in our case, the estimated value of $Acclim_{SetTemp}$ differed according to the chosen combination of measurement months. For example, the combination of December and September (the second warmest month; $T_{amb} = 22.3\,°C$) results in 1.63 as the $Acclim_{SetTemp}$. Other methods for quantifying the degree of acclimation [e.g., LTR_{10}, $Acclim_{LTR10}$ and $Acclim_{Homeo}$, see Atkin et al. (2005)] are useful for experimental data, but involve potentially similar problems when applied to seasonal data like ours.

For this reason, we propose that the slope of the linear regression between R at a reference T and ambient air T can represent overall seasonal acclimation capacity in addition to the linear decrease in Q_{10} with increasing ambient air T. In this study, we found that the regression slopes did not differ among the three canopy layers (Figure 6b), suggesting that the degree of thermal acclimation of leaf R did not vary vertically within the hinoki cypress canopy. To our knowledge, this is the first report exploring the possibility of vertical variation in acclimation capacity within a forest canopy. Our slope values (−0.0227, −0.0228 and −0.0151 for the upper, middle and lower layers, respectively) are comparable to values reported for *P. radiata* (−0.0175) and *P. deltoides* (−0.0205) (Ow et al. 2010). Moreover, the slope of the regression between $Q_{10}[20]$ and ambient air T (−0.0167) in our study is identical to that of *P. deltoides* (−0.0167) and similar to that of *P. radiata* (−0.0115) (Ow et al. 2010). These similarities suggest the usefulness of regression slopes for comparing acclimation capacity. Nevertheless, the validity of this method should be further verified.

Vertical variation in temperature response of leaf respiration

In the present study, we found that there were no vertical differences in Q_{10} in a hinoki cypress canopy throughout the year (Figure 3). This agrees with previous studies demonstrating that Q_{10} (or E_0) was constant in the tree canopies of *Q. rubra* in the northeastern United States (Xu and Griffin 2006), *E. globulus* in Australia (O'Grady et al. 2008, 2010) and 18 deciduous tree species in the southern Appalachian mountains (Bolstad et al. 1999). If Q_{10} can be assumed to be constant within a tree canopy, this is helpful for simplifying the representation of leaf R in carbon cycle models. However, there is evidence showing that Q_{10} varied according to canopy height (Griffin et al. 2002, Turnbull et al. 2003). Further studies are needed to further our understanding of vertical trends in Q_{10} within tree canopies for various forest types.

While Q_{10} remains poorly understood, the fact that leaf R rate decreases with decreasing height in the canopy has been well studied, including in hinoki cypress (Oohata et al. 1971, Hagihara and Hozumi 1977, Ohkubo et al. 2009). Moreover, numerous studies have demonstrated that within-canopy variation in leaf R during a given season is associated with LMA, leaf N and leaf carbohydrates (e.g., Ryan 1995, Reich et al. 1998a, 1998b, Griffin et al. 2001, Turnbull et al. 2001, 2003, Tissue et al. 2002). Our results agree well with these studies, though we did not measure leaf carbohydrates. In our case, N_{area} was a better predictor of R_{2O} than LMA in terms of not only vertical but also seasonal variations, because there was no seasonal difference in the regression between R_{2O} and N_{area} (Table 3, Figure 5d). However, it is generally difficult to separate the effects of these variables because N_{area} is product of LMA and N_{mass}. Furthermore, these leaf structural and chemical traits often co-vary within canopies and their vertical patterns are determined primarily by within-canopy light gradients (see Araki et al. 2015b in our case, also see review by Niinemets et al. 2015).

These results further indicate that light gradients, which can be measured relatively easily, could be a good predictor of vertical patterns of leaf R rates, as shown by early studies (Kira et al. 1969, Hagihara and Hozumi 1977, Yoda 1978). Indeed, in the present study, R_{20} correlated well with RPPFD (Figure 5a). Furthermore, the combination of RPPFD and ambient air T was able to predict vertical and seasonal variations in R_{20} well (Table 4, Figure 7).

Leaf age may also affect vertical variations in R_{20}. Katsuno-Miyaura et al. (1996) showed for Japanese cedar (*C. japonica*) that leaf R was higher in current-year needles than in older ones. Similar effects of needle age on leaf R have been reported for *Pinus sylvestris* (Zha et al. 2002) and *P. banksiana* (Tjoelker et al. 2009). Miyamoto et al. (2013) reported that leaf longevity in hinoki cypress ranges from ~4 to 6 years, based on stand-level estimates. We were not able to consider leaf age in this study. However, we observed that current-year leaves were dominant in sample shoots from the upper canopy and older leaves were dominant in lower shoots, as indicated by cumulative shoot elongation rates (Figure 4c). Thus, differences in leaf age structure in the sample shoots might be partly responsible for the vertical gradient of R_{20} we observed. In contrast, the lack of vertical difference in Q_{10} in this study suggests that leaf age might not influence the Q_{10} of leaf R.

Concluding remarks

The present study clearly demonstrates vertical and seasonal variations in leaf R within a hinoki cypress canopy. Our results indicate that spatial and temporal variations in the short-term T responses of leaf R could be predicted by relatively simple parameters (i.e., light and temperature). This simple model will be useful not only for scaling up to the annual rate of whole-canopy respiration (i.e., stand-level leaf R) but also for predicting the impacts of global warming on forest productivity and the potential future function of hinoki cypress stands as carbon sinks. However, the applicability of the model should be further verified. Nevertheless, a better understanding of thermal acclimation and spatial and temporal variations in leaf R within forest canopies can help to improve the representation of leaf R in global terrestrial carbon cycle models (Smith and Dukes 2013, Wythers et al. 2013, Atkin et al. 2014).

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