New Phytologist

Research

Temperature responses of leaf respiration in light and darkness are similar and modulated by leaf development

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Received: *25 August 2023*
Accepted: *6 November 2023*

New Phytologist (2024) **241:** 1435–1446
doi: 10.1111/nph.19428

Key words: carbon balance, chlorophyll fluorescence, global warming, Kok method, leaf age, leaf respiration in the light, mesophyll conductance, Q_{10}.

Summary

- Our ability to predict temperature responses of leaf respiration in light and darkness (R_L and R_{Dk}) is essential to models of global carbon dynamics. While many models rely on constant thermal sensitivity (characterized by Q_{10}), uncertainty remains as to whether Q_{10} of R_L and R_{Dk} are actually similar.
- We measured short-term temperature responses of R_L and R_{Dk} in immature and mature leaves of two evergreen tree species, *Castanopsis carlesii* and *Ormosia henry* in an open field. R_L was estimated by the Kok method, the Yin method and a newly developed Kok-iterC_c method.
- When estimated by the Yin and Kok-iterC_c methods, R_L and R_{Dk} had similar Q_{10} (*c.* 2.5). The Kok method overestimated both Q_{10} and the light inhibition of respiration. R_L/R_{Dk} was not affected by leaf temperature. Acclimation of respiration in summer was associated with a decline in basal respiration but not in Q_{10} in both species, which was related to changes in leaf nitrogen content between seasons. Q_{10} of R_L and R_{Dk} in mature leaves were 40% higher than in immature leaves.
- Our results suggest similar Q_{10} values can be used to model R_L and R_{Dk} while leaf development-associated changes in Q_{10} require special consideration in future respiration models.

Introduction

Leaf respiration, which is crucial to provide energy and C-skeletons for biosyntheses, is highly temperature-sensitive (Atkin & Tjoelker, 2003; Heskel *et al.*, 2016). Responses of respiration to climate warming may have a considerable impact on carbon uptake and the size of terrestrial carbon sinks (Huntingford *et al.*, 2013; Dusenge *et al.*, 2019) as well as on global carbon exchange (Smith & Dukes, 2013; Dusenge *et al.*, 2019; O'Leary *et al.*, 2019). More detailed knowledge of respiratory responses to warming is thus essential to obtain reliable estimates of future carbon budgets at different scales.

Many terrestrial biosphere models use Q_{10} to model leaf respiration (Running & Coughlan, 1988; Melillo *et al.*, 1993; Cox *et al.*, 2000), and most models assume leaf respiration roughly doubles with a 10°C rise in temperature (i.e. $Q_{10} = 2.0$; Amthor, 1984; Atkin *et al.*, 2017; Crous *et al.*, 2017). However, in contrast to respiration in the darkness (R_{Dk}), respiration in the light (R_L, also termed day respiration) interacts with light-related processes and products thereof (Brooks & Farquhar, 1985; Atkin *et al.*, 2000; Tcherkez *et al.*, 2017a). For example, photorespiration produces CO_2 within plant leaves and is temperature-sensitive, potentially complicating the temperature response of R_L. It is also broadly accepted that R_L is inhibited by light due to reduced enzymatic activity in glycolytic and tricarboxylic acid pathways and fundamental differences in pathways of respiration between light and dark (Tcherkez *et al.*, 2012, 2017b). There is no *a priori* reason for the temperature response of leaf respiration in light and darkness to be the same (Gulías *et al.*, 2002; Dusenge *et al.*, 2019). Also, experimental studies of the temperature sensitivity of R_L are inconsistent. Some studies suggest R_L has greater or lower temperature sensitivity than R_{Dk} (McLaughlin *et al.*, 2014; Way *et al.*, 2019; Fang *et al.*, 2022), while others report little difference or even no temperature sensitivity of R_L (Shapiro *et al.*, 2004; Atkin *et al.*, 2005a; Zaragoza-Castells *et al.*, 2007; Crous *et al.*, 2017). Clearly, there is a need for further research.

R_{Dk} can also acclimate to changes in thermal regimes across temporal scales. Atkin & Tjoelker (2003) summarized that acclimation can be classified into two broad types: reduction in thermal sensitivity and reduced basal respiration. Both acclimation types lead to reductions in respiration at any given greater temperature. While acclimation has been extensively studied, underlying mechanism(s) remain unclear (Araki *et al.*, 2017; Crous *et al.*, 2017, 2022) adding difficulty to incorporating acclimation in estimates of respiratory CO_2 release under global warming (e.g. Smith & Dukes, 2013; McLaughlin *et al.*, 2014; Dusenge *et al.*, 2019). Furthermore, studies on the thermal acclimation of R_L are limited (Crous *et al.*, 2017).

The classical growth-maintenance paradigm suggested that variation in plant respiration is driven by requirements for energy and carbon skeletons for growth and maintenance. Growth respiration is often regarded as being insensitive to temperature (Penning De Vries, 1975; Adu-Bredu *et al.*, 1997; Frantz *et al.*, 2004), ensuring that observed sensitivity to temperature is attributed to maintenance respiration. A logical consequence is that growing leaves should have a different respiratory Q_{10} from that in mature leaves. Comparing Q_{10} of growing and mature leaves could thus provide insights into underlying mechanisms of thermal sensitivity. Current land surface models rely on relationships between respiration of mature leaves and associated morphological and physiological parameters to predict whole-plant respiration (Atkin *et al.*, 2017). However, variation in Q_{10} between growing leaves and mature leaves has not been extensively studied.

Uncertainty in temperature responses of R_L can also be associated with methodology. R_L has been estimated by many methods. Those developed by Kok (1949), Yin *et al.* (2011) and Laisk (1977) are perhaps the most well-known. The reaction–diffusion method (Berghuijs *et al.*, 2019), the ^{13}C isotopic disequilibrium method (Gong *et al.*, 2015, 2018), and the NRH-A method (Fang *et al.*, 2022; Yin & Amthor, 2023) have been less widely used. The Kok method has large uncertainty concerning its two assumptions (Farquhar & Busch, 2017; Tcherkez *et al.*, 2017a; Yin *et al.*, 2020; Yin & Amthor, 2023), namely constant photochemical efficiency of photosystem II (Φ_2) and chloroplast CO_2 concentration (C_c) along the A-I_{inc} curve. Both Φ_2 and C_c have been shown to decline with increasing irradiance (Yin *et al.*, 2011; Berghuijs *et al.*, 2019; Sun *et al.*, 2023). To address these issues, Yin *et al.* (2011) suggested using measurements of Φ_2 via chlorophyll fluorescence to improve R_L estimates (hereafter named 'Yin method'). Recently, Sun *et al.* (2023) developed a new method to account for influences of Φ_2 and C_c (named 'Kok-C_c method'). The Kok-C_c method quantifies C_c with species-specific stomatal-to-mesophyll conductance ratio (i.e. g_s/g_m), allowing changes in C_c to be factored into calculations of R_L. This is particularly important for assessing the temperature responses of R_L because both g_m and C_c show temperature dependence. The Kok-C_c method has not previously been applied in studies of temperature responses of R_L.

Here, our questions are as follows: (1) do R_L and R_{Dk} have similar temperature responses? (2) How does R_L acclimate to the warm season (summer)? (3) Do immature and mature leaves differ in their thermal sensitivity and basal rates of respiration? We used simultaneous gas exchange and chlorophyll fluorescence measurements to study temperature responses of R_L and R_{Dk} in immature and mature leaves of two evergreen tree species, *Castanopsis carlesii* Hay. and *Ormosia henryi* Prain. These are common species in subtropical forests of South China. We measured short-term temperature responses of R_{Dk} and R_L that were estimated by the Kok, the Yin and a recently revised Kok-iterC_c method.

Materials and Methods

Plant material and growth conditions

Two-year-old seedlings of *Castanopsis carlesii* Hay. and *Ormosia henryi* Prain were grown in cylindrical pots (55 cm diameter and 62 cm height). The potted seedlings were placed in an open field at Fujian Sanming Forest Ecosystem National Observation and Research Station (geographical coordinates 26°19′N, 117°36′E), Fujian Province, China. The soil at the site is an oxisol according to the USDA Soil Taxonomy. It has a mid-subtropical monsoon climate with an average annual frost-free period of 350 d, an average annual temperature of 19.1°C (average daytime temperature is 26°C) and precipitation of 1749 mm.

Our experiment employed a factorial design that included species, season, and leaf age class as main factors. The measurements and sampling were carried out in two seasons, from 10 November to 24 November 2021 (winter) and from 28 June to 12 July 2022 (summer). For each of the five selected seedlings (as replicates) of a species, an upper, not fully expanded (immature leaf) and a lower, fully expanded leaf (mature leaf) were selected. In the winter 2021 experiment, the height of the selected seedlings ranged from 0.7 to 1.4 m. In the summer 2022 experiment, the height of the selected seedlings ranged from 0.6 to 1.5 m.

Meteorological data for the 5 d before the measurement date were downloaded from a database website of NASA (power.larc.nasa.gov). Acclimation of respiration should have occurred within a few days of temperature change (Atkin *et al.*, 2000; Bolstad *et al.*, 2003; Lee *et al.*, 2005; Reich *et al.*, 2016). Average temperature for the 5 d before measurement was 14.2°C in winter and 26.7°C in summer. Maximal temperature was 20.6°C in winter and 32.5°C in summer, and minimal temperature was 8.7°C in winter and 21.9°C in summer. The average relative humidity was 78.9% in winter and 85.0% in summer.

Gas exchange and chlorophyll fluorescence measurements

Gas exchange and chlorophyll fluorescence (ChlF) parameters were measured using a photosynthetic system (LI-6800; Li-Cor Inc., Lincoln, NE, USA) equipped with a multiphase flash fluorometer (LI-6800-01A) with a 6 cm^2 leaf chamber. We first measured the temperature responses of photosynthetic parameters under ambient irradiance. Gas exchange and ChlF parameters were measured at four temperatures (20°C, 25°C, 30°C, and 35°C) and at a PPFD of 1200 $\mu mol\ m^{-2}\ s^{-1}$ (i.e. PPFD was equal to or slightly lower than those of the growth environment). CO_2 concentration in the leaf chamber was controlled at 410 $\mu mol\ mol^{-1}$ with an airflow rate of 300 $\mu mol\ s^{-1}$, and air relative humidity was maintained at 70% ± 5%. The measured data were further used to calculate g_m (see below).

Then A-I_{inc} curves (to obtain R_L) were measured, including PPFD steps of 100, 80, 60, 40, 20, and 0 (i.e. light source off) μmol m^{-2} s^{-1} at the four temperatures as described previously. ChlF parameters were recorded at PPFD of 100, 80, 60, and 40 μmol m^{-2} s^{-1} using the multiphase flash method. R_{Dk} was measured in 15–30 min after the light was switched off (for dark adaptation) and all gas exchange measurements were performed during the day. As such, the time lag between R_L and R_{Dk} was relatively short (30 min to 1 h) and consistent between measured leaves. Recent studies reported that plant respiration at constant temperature changes with time during both the day and night (Tcherkez *et al.*, 2003; Bruhn *et al.*, 2022; Faber *et al.*, 2022). The influence of this phenomenon on our results is likely negligible.

Measurements were conducted on leaves of cut branches. Selected branches were cut from the tree and re-cut immediately underwater to restore hydraulic conductivity in early morning. We observed no significant difference in gas exchange parameters between cut and intact branches for *C. carlessii* and *O. henryi* (Supporting Information Fig. S1).

Estimation of leaf respiration in the light

We used three methods to estimate leaf respiration in the light, namely the (original) Kok method (Kok, 1949; $R_{\text{L Kok}}$), the method developed by Yin *et al.* (2011; $R_{\text{L Yin}}$), and a recently developed method by Sun *et al.* (2023) with small modifications ($R_{\text{L Kok-iterCc}}$, see below).

With the Kok method, a linear regression was fitted to the data of A vs I_{inc} above the breakpoint (which is usually near the light compensation point), and the intercept of this regression was $R_{\text{L Kok}}$. Linear regressions were performed using data of the PPFD levels of 40, 60, 80, and 100 μmol m^{-2} s^{-1}, which were above the breakpoint and in the linear range of the A-I_{inc} curve by visual inspection. The Kok method uses A measured at low irradiance where A should be limited by the light-dependent electron transport rate (Yin *et al.*, 2009). According to the equation of electron transport-limited photosynthesis (Farquhar *et al.*, 1980; Yin *et al.*, 2011), A is described as:

$$A = f_{\text{aet}}\Phi_2\rho_2\alpha I_{\text{inc}}\frac{1-\Gamma^*/C_c}{4+8\Gamma^*/C_c} - R_L \qquad \text{Eqn 1}$$

where f_{aet} is the fraction of electron transport for photosynthesis, Φ_2 is the photochemical efficiency of PSII, ρ_2 is the fraction of absorbed irradiance partitioned to PSII, α is the absorptance by leaf photosynthetic pigments, and I_{inc} is incident irradiance. We define γ as:

$$\gamma = \frac{1-\Gamma^*/C_c}{4+8\Gamma^*/C_c} \qquad \text{Eqn 2}$$

so, Eqn 1 becomes:

$$A = \gamma f_{\text{aet}}\Phi_2\rho_2\alpha I_{\text{inc}} - R_L \qquad \text{Eqn 3}$$

In the Kok method, both Φ_2 and γ are assumed unchanged with I_{inc}.

Second, we used the Yin method, which incorporates variation in Φ_2 estimated from chlorophyll fluorescence measurements (Yin *et al.*, 2011). The Yin method estimates R_L by plotting A vs $\Phi_2 I_{\text{inc}}$:

$$A = (\gamma f_{\text{aet}}\rho_2\alpha)\Phi_2 I_{\text{inc}} - R_{\text{L Yin}} \qquad \text{Eqn 4}$$

Φ_2 is calculated as:

$$\Phi_2 = (F'_m - F'_s)/F'_m \qquad \text{Eqn 5}$$

where F_s is the steady-state fluorescence in the light and F'_m is the maximal fluorescence during short saturating pulses of light. This method assumes that γ is constant, which is not true under photorespiratory conditions (Yin *et al.*, 2014).

Finally, a modified Kok method, the Kok-C_c method suggested by Sun *et al.* (2023), treats C_c and γ in the above formula as variables to estimate R_L. A is calculated as:

$$A = (f_{\text{aet}}\rho_2\alpha)\gamma\Phi_2 I_{\text{inc}} - R_{\text{L Kok-Cc}} \qquad \text{Eqn 6}$$

In this method, A was plotted against $\gamma\Phi_2 I_{\text{inc}}$ and $R_{\text{L Kok-Cc}}$ was obtained as the intercept of the linear relation (note that the values of f_{aet}, ρ_2, and α are not required here for calculations since they form the slope in Eqn 6). Here, Γ^* and C_c needed to be estimated for calculating γ (Eqn 2). Γ^* is the C_c-based CO$_2$ compensation point, and its value was estimated from leaf temperature (T; Brooks & Farquhar, 1985):

$$\Gamma^* = 42.7 + 1.68(T-25) + 0.012(T-25)^2 \qquad \text{Eqn 7}$$

While C_c was calculated as:

$$C_c = C_i - A/g_m \qquad \text{Eqn 8}$$

g_m for each point in each Kok curve individually was calculated from leaf- and temperature-specific g_s/g_m values. We assume that g_s/g_m is conservative for a leaf at a given temperature as supported by the analysis of (Ma *et al.*, 2021), and this assumption leads to much smaller errors than ignoring variation in γ or assuming a constant g_m (Sun *et al.*, 2023). To obtain the leaf- and temperature-specific g_s/g_m values, g_m was estimated at ambient light intensity of the growing environment by using the variable J method, which is based on the equation of RuBP regeneration-limited photosynthesis (Farquhar *et al.*, 1980; Harley *et al.*, 1992):

$$g_m = \frac{A}{C_i - \frac{\Gamma^*[J+8(A+R_L)]}{J-4(A+R_L)}} \qquad \text{Eqn 9}$$

where J is estimated from ChlF parameters as $J = \rho_2\alpha\Phi_2 I_{\text{inc}}$. $\rho_2 = 0.5$ and $\alpha = 0.843$ were used as suggested by the manufacturer of LI-6800.

In the calculation of Sun *et al.*, 2023, $R_{\text{L Yin}}$ was used to calculate g_m given that the estimated g_m using Eqn 9 is not very sensitive to R_L and thus inputting different R_L values had very small effects on the estimated $R_{L\ \text{Kok-Cc}}$. Here, we provided a modified Kok-iterC_c method using an iterative process. We (1) calculated the initial g_m value using Eqn 9 by assuming $R_L = R_{Dk}$ (we used measured R_{Dk}), (2) used the g_s/g_m value to calculate g_m for each point in the Kok curve, (3) calculated γ for each point in the Kok curve using Eqns 2, 8, (4) performed a linear fit using the value of $\gamma\Phi_2 I_{inc}$ and A, and the intercept of this equation (see Eqn 6) gives the initial value of $R_{L\ \text{Kok-iterCc}}$. The initial $R_{L\ \text{Kok-iterCc}}$ value was inserted into Eqn 9 to calculate a new g_m value, which was further used to update the $R_{L\ \text{Kok-iterCc}}$ value. This iterative calculation continues until the estimated R_L is stable, that is when the difference in $R_{L\ \text{Kok-iterCc}}$ between two consecutive iterations is $< 0.01\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$ (3–5 iterations are sufficient for our data). An Excel tool (Methods S1) has been created to illustrate the calculation process for $R_{L\ \text{Kok-iterCc}}$, and the dataset of our experiment (Dataset S1) has been provided.

According to (Harley *et al.*, 1992), $\mathrm{d}C_c/\mathrm{d}A$ is a useful proxy for controlling the quality of g_m estimations using the variable J method:

$$\mathrm{d}C_c/\mathrm{d}A = 12\Gamma^* J/[J - 4(A + R_L)]^2 \qquad \text{Eqn 10}$$

Harley *et al.* (1992) showed that the variable J method became too sensitive to small errors when $\mathrm{d}C_c/\mathrm{d}A$ is far higher than 50 and suggested using the data with $\mathrm{d}C_c/\mathrm{d}A$ within the range of 10–50. We found that all $\mathrm{d}C_c/\mathrm{d}A$ values obtained at the ambient light intensity were within this range with an average value of 21.8, while $\mathrm{d}C_c/\mathrm{d}A$ was typically higher than 50 with many cases higher than 100 when data were obtained at low light levels of the Kok curve. This supports the use of ambient or subambient irradiance to estimate g_m, but very low irradiance levels should be avoided.

Calculation of thermal sensitivity and basal rate of leaf respiration

The temperature response of respiration can be described by the Q_{10} or Arrhenius functions (Atkin & Tjoelker, 2003; Kruse *et al.*, 2011; O'Sullivan *et al.*, 2013; Slot & Kitajima, 2015). These two types of models usually predict very similar respiration rates (Reich *et al.*, 2016). We used the Q_{10} model to calculate the Q_{10} values of R_{Dk}, $R_{L\ \text{Kok}}$, $R_{L\ \text{Yin}}$, and $R_{L\ \text{Kok-iterCc}}$, for each leaf for the 15°C temperature range. The R_T was calculated as:

$$R_T = R_{T_{ref}} Q_{10}^{\frac{T - T_{ref}}{10}} \qquad \text{Eqn 11}$$

where R_T is the respiration rate measured at the temperature T and R_{Tref} is the respiration rate at the reference temperature (set as 25°C in this study, in line with the mean annual daytime temperature). Using the logarithm, Eqn 11 can be transformed:

$$\mathrm{Ln}R_T = \mathrm{Ln}R_{25} + \frac{T - 25}{10}\mathrm{Ln}Q_{10} \qquad \text{Eqn 12}$$

Fitting Eqn 12 to the $\mathrm{Ln}R_L$ (or $\mathrm{Ln}R_{Dk}$) data for each leaf at different temperatures, we obtained $\mathrm{Ln}Q_{10}$ as the slope and $\mathrm{Ln}R_{25}$ as the intercept of the linear relation. Basal respiration rate (R_{25Dk}, $R_{25\ \text{Kok}}$, $R_{25\ \text{Yin}}$, and $R_{25\ \text{Kok-iterCc}}$) was taken as the measured values at 25°C.

Plant sampling and leaf trait parameters

After gas exchange measurements, leaves were harvested and photographed. The leaf surface area was calculated using IMAGEJ software (www://imagej.nih.gov/ij/). Leaves were dried to constant weight in an oven at 70°C (before this, the leaves were placed in an oven at 105°C for 1 h to stop enzymatic activity), after which the dry mass of the leaves was weighed. These data were used to calculate the specific leaf area (SLA, leaf area/dry mass) of each leaf.

Nitrogen (N) content of the leaf samples was measured using an elemental analyser (VARIO ELIII, Elementar Analysensysteme GmbH, Hanau, Germany), and the N content per unit leaf area ($\mathrm{N_{area}}$, g m^{-2}) was calculated. Using the $\mathrm{N_{area}}$, the measured area-based respiration rates were also expressed per gram N.

Statistical analysis

Three-way ANOVA was used to determine the effects of species (*C. carlesii* and *O. henryi*), seasons, and leaf age classes and their interactions on leaf photosynthetic parameters, basal respiration rate, thermal sensitivity, and leaf traits. Statistical analysis was performed using SPSS (v. 25.0; SPSS, Chicago, IL, USA).

Results

Effects of leaf age and season on photosynthetic parameters and leaf traits

A_{25}, g_s, and g_m did not vary significantly with seasons and leaf age of the two tree species. There was a significant interaction between season and species on A_{25}, g_s, and g_m. The A_{25}, g_s, and g_m of *C. carlesii* were higher in winter, while those parameters of *O. henryi* were higher in summer (Table 1; Fig. S2). *C. carlesii* has a higher SLA and a lower $\mathrm{N_{area}}$ than *O. henryi* (Table 1; Fig. 1). SLA and $\mathrm{N_{area}}$ varied significantly with seasons, and higher SLA (+9%) and lower $\mathrm{N_{area}}$ (−24%) in summer than in winter were found. SLA and $\mathrm{N_{area}}$ did not differ significantly between immature and mature leaves, although the $\mathrm{N_{area}}$ of immature leaves tended to be lower than that of mature leaves (Table 1; Fig. 1). g_s/g_m was not strongly affected by measurement temperature, but significantly influenced by leaf age and season and interaction of leaf age and species, being higher in immature leaves and higher in summer (Figs S2, S3). Nevertheless, g_s and g_m were positively correlated and g_s/g_m ranged from 0.59 to 1.17 with a mean value of 0.72 (Figs S2, S3).

Temperature response of respiration estimated by different methods

R_{Dk} and R_L showed a similar exponential increasing trend with leaf temperature (Figs 2, S4). Differences in temperature sensitivity

Table 1 ANOVA tests of photosynthetic parameters and traits of immature and mature leaves of two tree species (*Castanopsis carlessii* and *Ormosia henryi*) measured in summer and winter.

	Significance (*P*-value)					
Parameter	Species	Season	Age	*Spe* × *Sea*	*Spe* × *Age*	*Sea* × *Age*
$R_{25\ \mathrm{Dk}}$	0.416	<**0.001**	**0.002**	0.066	0.468	0.098
$R_{25\ \mathrm{Kok}}$	0.684	<**0.001**	**0.032**	0.805	0.677	**0.033**
$R_{25\ \mathrm{Yin}}$	0.304	<**0.001**	**0.049**	0.342	0.905	0.444
$R_{25\ \mathrm{Kok\text{-}iterCc}}$	0.319	<**0.001**	**0.041**	0.753	0.958	0.109
$R_{25\ \mathrm{Kok\text{-}iterCc}}/R_{25\ \mathrm{Dk}}$	0.582	**0.048**	0.878	0.714	0.773	0.848
$Q_{10\ \mathrm{Dk}}$	**0.028**	0.341	**0.015**	0.792	0.784	0.512
$Q_{10\ \mathrm{Kok}}$	0.331	**0.003**	**0.003**	0.771	0.127	0.632
$Q_{10\ \mathrm{Yin}}$	0.084	0.178	**0.015**	0.205	0.350	0.733
$Q_{10\ \mathrm{Kok\text{-}iterCc}}$	0.512	0.164	<**0.001**	0.085	**0.048**	0.310
A_{25}	0.545	0.443	0.542	**0.001**	0.805	0.485
g_s	0.333	0.177	0.837	**0.004**	0.607	0.629
g_m	**0.006**	0.116	0.075	**0.005**	0.126	0.337
SLA	<**0.001**	<**0.001**	0.541	**0.040**	0.182	0.082
N_{area}	<**0.001**	<**0.001**	0.223	0.090	0.491	0.306

Parameters include leaf respiration rate in the darkness at 25°C ($R_{25\ \mathrm{Dk}}$, μmol m^{-2} s^{-1}), leaf respiration rate in the light at 25°C estimated using three methods Kok ($R_{25\ \mathrm{Kok}}$), Yin ($R_{25\ \mathrm{Yin}}$), and Kok-iterC_c ($R_{25\ \mathrm{Kok\text{-}iterCc}}$, μmol m^{-2} s^{-1}), respectively, temperature sensitivity of respiration rates ($Q_{10\ \mathrm{Dk}}$, $Q_{10\ \mathrm{Kok}}$, $Q_{10\ \mathrm{Yin}}$, and $Q_{10\ \mathrm{Kok\text{-}iterCc}}$), the ratio of leaf respiration rate in the light (calculated by the Kok-iterC_c method) to respiration rate in the darkness ($R_{25\ \mathrm{Kok\text{-}iterCc}}/R_{25\ \mathrm{Dk}}$), net photosynthesis rate (A_{25}, μmol m^{-2} s^{-1}), mesophyll conductance (g_m, mol m^{-2} s^{-1}), stomatal conductance to CO$_2$ (g_s, mol m^{-2} s^{-1}) at 25°C, specific leaf area (SLA, cm^2 g^{-1}), and N content per unit area (N_{area}, g m^{-2}). *Spe*, species; *Sea*, Season; *Age*, Age. Significant differences are shown in bold ($P < 0.05$).

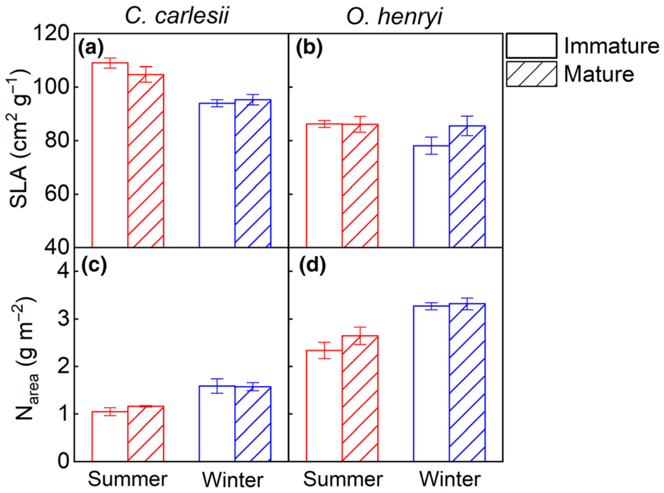

Fig. 1 Specific leaf area (SLA, cm^2 g^{-1}) and N content per unit area (N_{area}, g m^{-2}) of immature (clear) and mature (diagonals) leaves of *Castanopsis carlesii* (a, c) and *Ormosia henryi* (b, d). Data are shown as mean ± SE ($n = 4$).

were observed using various estimation methods (Fig. 3). The Kok method provided higher Q_{10} of R_L as compared to the Yin and Kok-iterC_c methods (Fig. 4). Mean values of the two species were 3.51 ± 0.22 for $Q_{10\ \mathrm{Kok}}$, 2.62 ± 0.20 for $Q_{10\ \mathrm{Yin}}$ and 2.47 ± 0.15 for $Q_{10\ \mathrm{Kok\text{-}iterCc}}$. This difference between methods was attributed to the fact that both Φ_2 and C_c decreased with the increase of I_{inc} along the Kok curve in all species. Such a decrease was influenced by leaf temperature (Figs S5, S6).

The light-to-dark respiration ratio (R_L/R_{Dk}) was not significantly affected by leaf temperature, but was influenced by R_L estimation methods (Fig. 5). The Kok method yielded a significantly lower R_L/R_{Dk} (0.61 ± 0.02) compared with the Yin (0.68 ± 0.02) and the Kok-iterC_c (0.81 ± 0.02) methods (Fig. 5). $R_{25\ \mathrm{Kok\text{-}iterCc}}/R_{25\ \mathrm{Dk}}$ did not change with species or leaf age but significantly changed with season (Table 1), being higher in winter than in summer.

Effects of leaf age and season on basal respiration rate (R_{25}) and temperature sensitivity (Q_{10})

The basal respiration rate of mature leaves was significantly lower than that of immature leaves, which was the case for R_{Dk} and R_L for both seasons (Table 1; Fig. 6). Combining the data of the two tree species and seasons, $R_{25\ \mathrm{Dk}}$ and $R_{25\ \mathrm{Kok\text{-}iterCc}}$ of mature leaves were 30% and 32% lower than immature leaves. Season had a significant impact on the basal respiration rates with a higher $R_{25\ \mathrm{Dk}}$, $R_{25\ \mathrm{Kok}}$, $R_{25\ \mathrm{Yin}}$, and $R_{25\ \mathrm{Kok\text{-}iterCc}}$ in winter than in summer (Table 1; Fig. 6). Pooling of data on leaf age classes of both species, mean $R_{25\ \mathrm{Dk}}$ and $R_{25\ \mathrm{Kok\text{-}iterCc}}$ increased by 65% and 107% in winter than in summer. Expressed as per gram N, $R_{25\ \mathrm{Dk}}$, $R_{25\ \mathrm{Kok}}$, $R_{25\ \mathrm{Yin}}$, and $R_{25\ \mathrm{Kok\text{-}iterCc}}$ were also significantly affected by leaf age and season (Fig. S7), and the magnitude of responses were comparable for leaf area- and N content-based respiration rates.

Leaf age but not season significantly affected Q_{10} (Table 1). $Q_{10\ \mathrm{Dk}}$, $Q_{10\ \mathrm{Kok}}$, $Q_{10\ \mathrm{Yin}}$, and $Q_{10\ \mathrm{Kok\text{-}iterCc}}$ of mature leaves were 0.62, 1.17, 0.92, and 0.99 higher (absolute difference) than that of immature leaves (Fig. 3), respectively. Combining all data, there was a significantly negative correlation between $R_{25\ \mathrm{Dk}}$ and $Q_{10\ \mathrm{Dk}}$ ($r^2 = 0.24$; $P < 0.01$), between $R_{25\ \mathrm{Kok}}$ and $Q_{10\ \mathrm{Kok}}$ ($r^2 = 0.29$; $P < 0.01$), between $R_{25\ \mathrm{Yin}}$ and $Q_{10\ \mathrm{Yin}}$ ($r^2 = 0.26$; $P < 0.01$), and between $R_{25\ \mathrm{Kok\text{-}iterCc}}$ and $Q_{10\ \mathrm{Kok\text{-}iterCc}}$ ($r^2 = 0.24$; $P < 0.01$; Fig. S8).

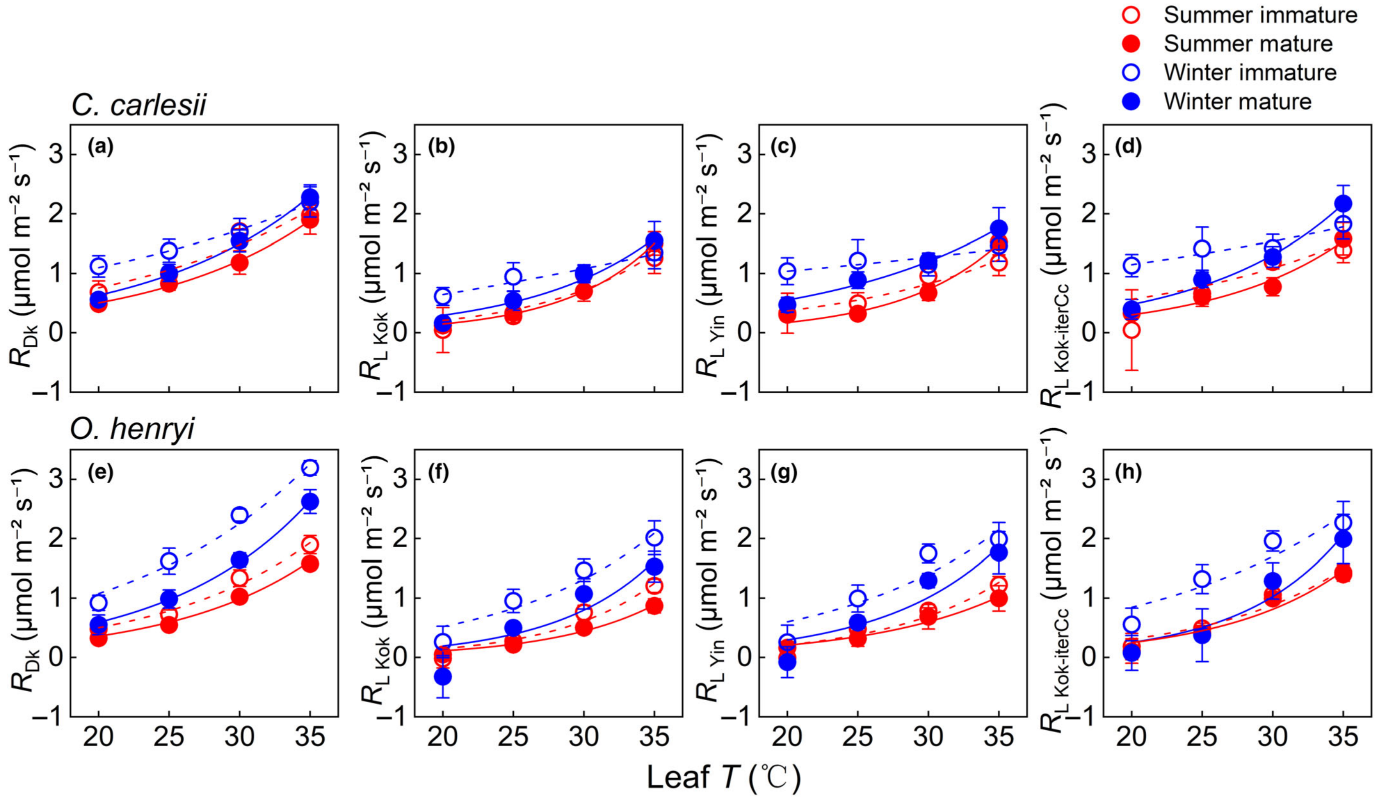

Fig. 2 Temperature response of leaf respiration in light and darkness (R_L and R_{Dk}, μmol m^{-2} s^{-1}) in summer (red points and lines) and winter (blue points and lines) for *Castanopsis carlesii* (a–d) and *Ormosia henryi* (e–h). Three methods including the Kok (b, f), Yin (c, g) and Kok-iterC_c method (d, h) were used to estimate R_L. Solid points represent mature leaves, and open points represent immature leaves. Solid and dashed lines represent regressions (Eqn 11) fitted to data from mature and immature leaves, respectively. Data are shown as mean ± SE ($n = 3$–5).

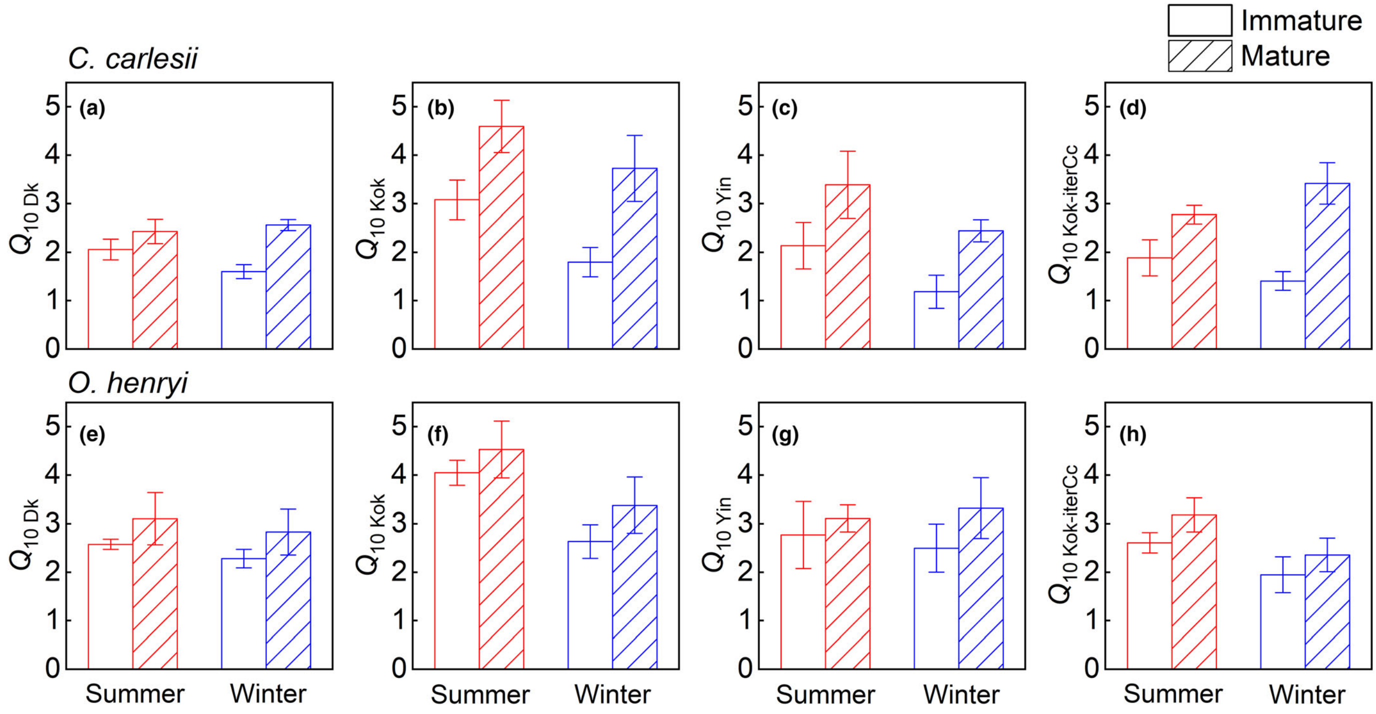

Fig. 3 Leaf temperature sensitivity (Q_{10}) of immature (clear) and mature (diagonals) leaves of *Castanopsis carlesii* (a–d) and *Ormosia henryi* (e–h). Data were measured in summer (red) and winter (blue). Q_{10} of respiration in the darkness is $Q_{10\ Dk}$, and Q_{10} of respiration in the light is $Q_{10\ Kok}$, $Q_{10\ Yin}$, and $Q_{10\ Kok\text{-}iterCc}$. Data are shown as mean ± SE ($n = 4$–5).

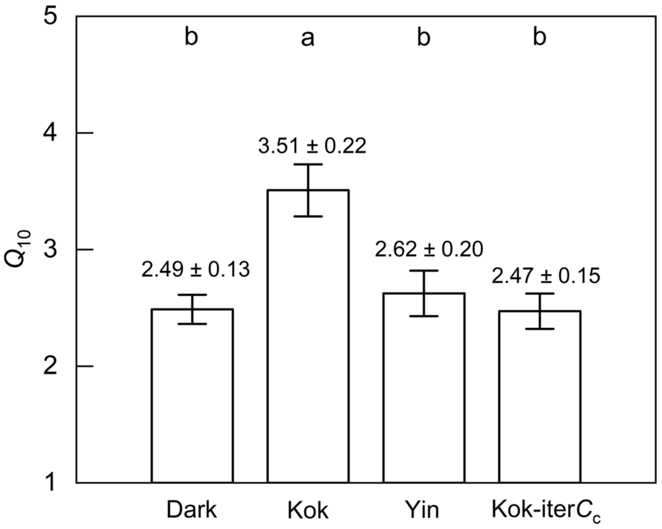

Fig. 4 Comparison of temperature sensitivity (Q_{10}) of respiration in the darkness (Dark) and respiration in the light estimated using three methods, the Kok, Yin, and Kok-iterC_c methods. Data were obtained from *Castanopsis carlesii* and *Ormosia henryi*. Data are shown as mean ± SE ($n = 32$–33), and different letters indicate significant differences between means.

Discussion

Thermal sensitivity of leaf respiration in light and darkness

Our study showed that temperature response curves and Q_{10} of R_L and R_{Dk} were similar, and this result was confirmed by the improved methods of R_L: the Yin and Kok-iterC_c methods. Previous studies found that R_L had a higher (Way *et al*., 2019; Fang *et al*., 2022) or lower (Atkin *et al*., 2005a; Zaragoza-Castells *et al*., 2007; Crous *et al*., 2017) temperature sensitivity than R_{Dk}. In principle, dissimilar temperature sensitivity of R_L and R_{Dk} (Gulías *et al*., 2002; Way *et al*., 2019) is expected due to their different metabolic pathways and the noncyclic nature of the tricarboxylic acid pathway (TCAP) under illumination (Tcherkez *et al*., 2009, 2012). Furthermore, photorespiration could also affect respiratory metabolisms although the mechanisms remain speculative (Tcherkez *et al*., 2008, 2012; Griffin & Turnbull, 2013; Abadie *et al*., 2016; Gong *et al*., 2017; Abadie & Tcherkez, 2019).

R_L/R_{Dk} was not significantly affected by leaf temperature, which reflects the similar temperature sensitivity of R_L and R_{Dk}. Consistently, insignificant leaf temperature effect on R_L/R_{Dk} was found in *Triticum aestivum* (Fang *et al*., 2022). Increased R_L/R_{Dk} with increasing leaf temperature was found in two spruce species (Way & Sage, 2008; Kroner & Way, 2016) and *Eucalyptus tereticornis* (Way *et al*., 2019) using the Kok method. However, recent studies suggest caution in interpreting the results from the Kok method as theoretical and experimental uncertainties have been demonstrated (Farquhar & Busch, 2017; Gauthier *et al*., 2020; Yin *et al*., 2020; Sun *et al*., 2023). In our study, significant differences in Q_{10} of R_L and R_{Dk} were not detectable for the measured species, suggesting that a generic Q_{10} value for R_{Dk} could be used to model the temperature response of R_L for these species.

Temperature response of R_L estimated by different methods

Our study finds an overestimation (*c*. 37%) of Q_{10} (R_L) via the Kok method compared with the Yin and Kok-iterC_c methods. Such an overestimation comes from the assumption of constant Φ_2 and γ (driven by changes in C_c) with irradiance across the Kok curve. This assumption seems to be incorrect since both Φ_2 and C_c decreased with increasing I_{inc} in both species, which is in agreement with previous reports (Yin *et al*., 2020; Sun *et al*., 2023). Since there is presently no evidence for changes in f_{aet}, ρ_2, or α with I_{inc} along the Kok curve, the Kok-iterC_c method (Eqn 6) has addressed potential issues with important variables involved in the linear extrapolation. In particular, changes in Φ_2 and C_c with I_{inc} were accounted for and in effect,

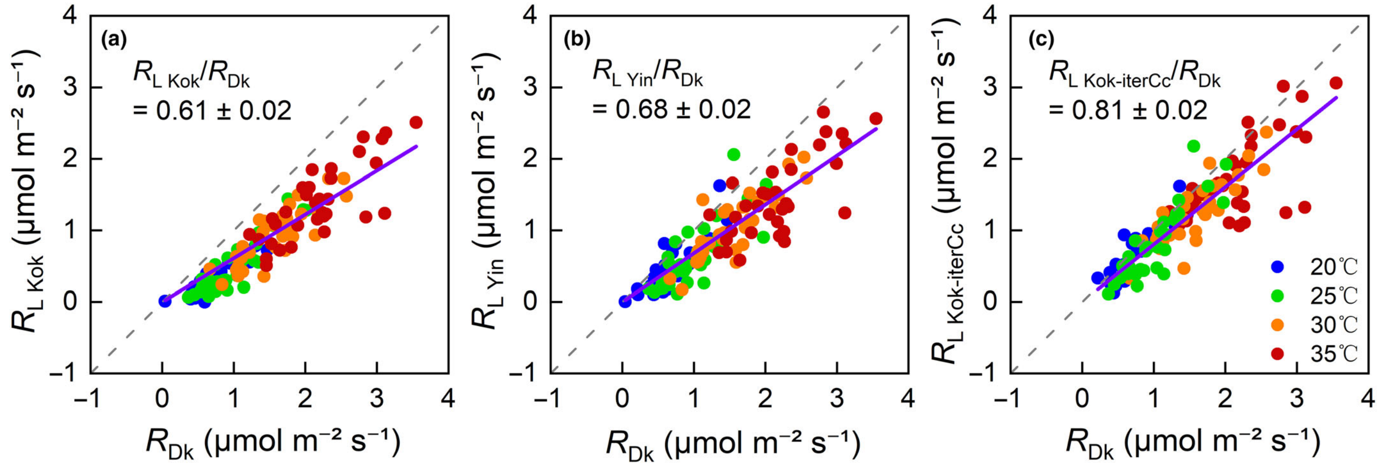

Fig. 5 Relationships between leaf respiration in light and darkness (R_L and R_{Dk}, μmol m^{-2} s^{-1}) measured at different leaf temperatures. R_L was estimated using the Kok method (a), the Yin method (b), and the Kok-iterC_c method (c), being denoted as $R_{L\ Kok}$, $R_{L\ Yin}$, and $R_{L\ Kok\text{-}iterCc}$, respectively. Purple solid lines indicate a fit to all data, and the grey dashed lines represent 1 : 1 line. Data were obtained from *Castanopsis carlesii* and *Ormosia henryi* measured in two different seasons. Mean R_L/R_{Dk} (±SE, $n = 126$–128) was calculated from all data.

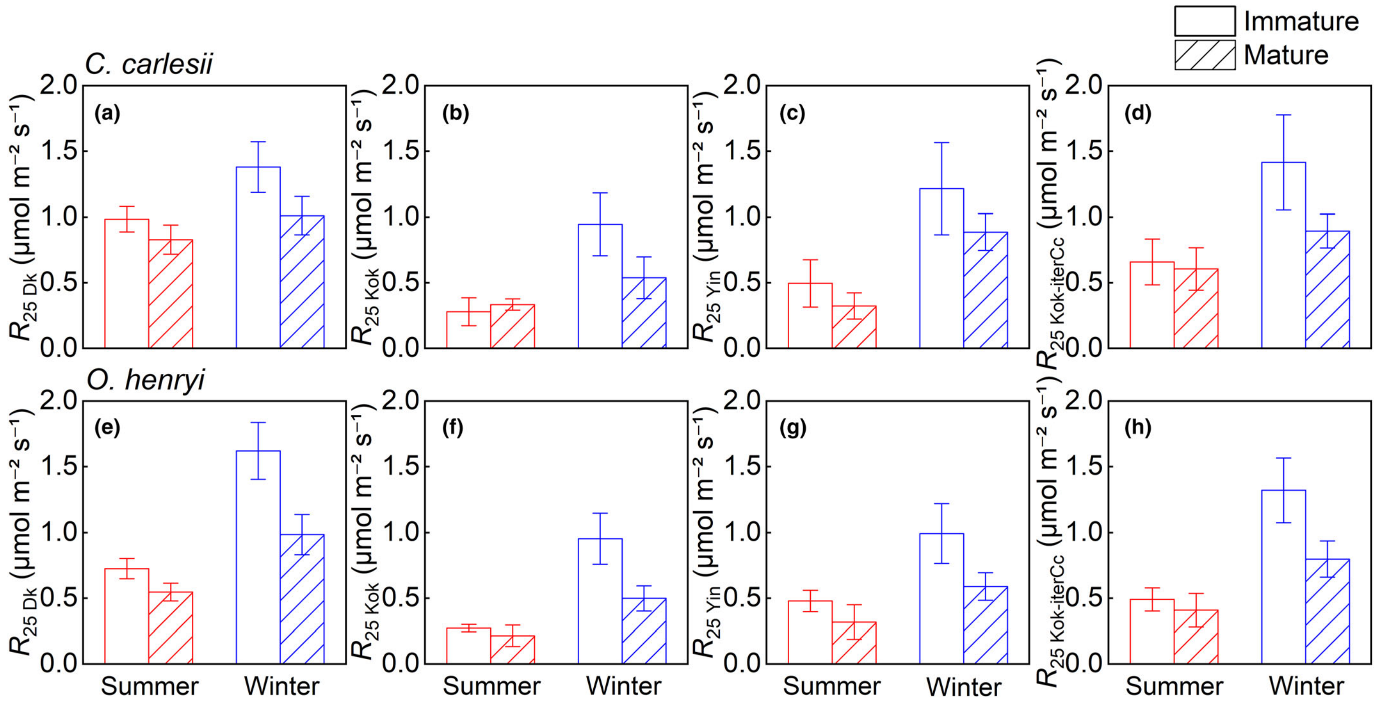

Fig. 6 Leaf respiration rates at 25°C of immature (clear) and mature (diagonals) leaves of *Castanopsis carlesii* (a–d) and *Ormosia henryi* (e–h). Data are shown for leaf respiration rate in the darkness at 25°C ($R_{25\ \mathrm{Dk}}$, μmol m^{-2} s^{-1}) and respiration rate in the light at 25°C ($R_{25\ \mathrm{Kok}}$, $R_{25\ \mathrm{Yin}}$, and $R_{25\ \mathrm{Kok\text{-}iterCc}}$, μmol m^{-2} s^{-1}) measured in summer (red) and winter (blue). Data are shown as mean ± SE ($n = 4$–5).

these two variables were found to be affected by measurement temperature, which in turn modulates the estimated temperature sensitivity using the classical Kok method and the Yin method.

Furthermore, our study showed that the Kok method underestimates R_L and overestimates the inhibition of respiration by light, which is in line with recent studies (Fang *et al.*, 2022; Sun *et al.*, 2023). The mean apparent inhibition of respiration ($1\text{-}R_{\mathrm{L\ kok}}/R_\mathrm{Dk}$) of our two tree species was 0.39, which is comparable to the reported apparent inhibition rate of trees, for example *Eucalyptus saligna* (0.4; Ayub *et al.*, 2011), *Eucalyptus globulus* (0.31; Crous *et al.*, 2017), and *Picea glauca* (0.2; Schmiege *et al.*, 2023), herbs, for example *Triticum aestivum* (0.29; Griffin & Turnbull, 2013). However, the actual inhibition ($1\text{-}R_{\mathrm{L\ Kok\text{-}iterCc}}/R_\mathrm{Dk}$) was only 0.19 in tree leaves. That is, the inhibition of respiration contributed by 49% (=0.19/0.39) to the apparent Kok effect (the rest being due to Φ_2/C_c effects), supporting a significant light inhibition of respiration in tree leaves. Consistently, Sun *et al.* (2023) found that the mean apparent inhibition of respiration ($1\text{-}R_{\mathrm{L\ kok}}/R_\mathrm{Dk}$) was 0.23 while the actual inhibition of respiration ($1\text{-}R_{\mathrm{L\ Kok\text{-}Cc}}/R_\mathrm{Dk}$) reduced to 0.06 in sunflower and wheat plants grown in growth chambers.

It is worth noting that we used an iterative process to improve the Kok-C_c method suggested by Sun *et al.* (2023). When estimating R_L, assumptions must be made on g_m and vice versa, as both parameters appear in the equation of electron transport-limited photosynthesis (Eqn 1). Sun *et al.* (2023) used $R_{\mathrm{L\ Yin}}$ to calculate g_m given that the estimated g_m using Eqn 9 is not very sensitive to R_L values. In addition, varying $g_\mathrm{s}/g_\mathrm{m}$ by ±0.4 had a very small influence on the sensitivity of γ with respect to irradiance ($\mathrm{d}\gamma/\mathrm{d}I_\mathrm{inc}$) – a factor that impacts directly $R_{\mathrm{L\ Kok\text{-}Cc}}$ (or $R_{\mathrm{L\ Kok\text{-}iterCc}}$) estimates. Similarly, applying a different Γ^*-temperature relation suggested by Crous *et al.* (2013) has a negligible effect on the temperature response of $R_{\mathrm{L\ Kok\text{-}iterCc}}$ (Fig. S9). Our approach used R_Dk instead of $R_{\mathrm{L\ Yin}}$ as an initial input parameter and shows good agreement in estimated R_L compared with the estimates using Sun *et al.* (2023) approach (Figs S10, S11). Furthermore, it required less than five rounds of iterative calculation to reach a stable R_L value (Fig. S11), meaning that $R_{\mathrm{L\ kok\ Cc}}$ was not very sensitive to small variations in g_m (Sun *et al.*, 2023). In principle, this iterative approach should also improve g_m estimation by Eqn 9. Importantly, Fang *et al.* (2022) used the NRH-A model of Yin & Struik (2009) to estimate R_L and g_m simultaneously based on the gas exchange and ChlF data across a range of low I_inc. The Kok-iterC_c method is based on the same considerations as Fang *et al.* (2022) but differs in implementation and data use. In particular, the NRH-A method estimates the average g_m along the Kok curve, while the Kok-iterC_c approach relies on the variable J method and the assumption of constant $g_\mathrm{s}/g_\mathrm{m}$ to estimate g_m at each I_inc level. Notably, the results from the NRH-A (Fang *et al.*, 2022), the Kok-C_c (Sun *et al.*, 2023), and the Kok-iterC_c methods (this study) all demonstrated that R_L has been underestimated by the Kok and the Yin methods, highlighting the need to account for γ (or Γ^*/Cc) effects.

Growing leaves have lower thermal sensitivity of respiration than mature leaves

Our study shows that growing leaves have a lower Q_{10} of R_L and R_Dk than mature leaves. Previous study showed that Q_{10} of R_Dk in growing leaves is lower than in mature leaves (Frantz *et al.*, 2004), while comparisons have not been performed for R_L. This finding can be explained by the growth-maintenance paradigm (Azcón-Bieto *et al.*, 1983; Atkin & Cummins, 1994; Amthor, 2000), whereby growth respiration is mostly determined by the cost of biosyntheses while observed Q_{10} is mostly due to the temperature response of maintenance respiration (Penning De Vries, 1975; Frantz *et al.*, 2004). That is, changes in contributions of growth vs maintenance components of respiration are likely the main reason for the lower Q_{10} of growing leaves compared with mature leaves. Also, Q_{10} of respiration is influenced by a range of physiological factors, including the balance between enzyme capacity, substrate availability, and adenylate control over mitochondrial activity (Hoefnagel *et al.*, 1998), each of which could change with leaf expansion (but related observations are limited).

We also found that growing leaves had a higher basal respiration rate than mature leaves, both in light and in darkness. Similar results were obtained in *Vicia faba* (Buckley *et al.*, 2017), *Heteromeles arbutifolia* and *Lepechinia fragrans* (Villar *et al.*, 1995). This effect is probably linked to the higher demand for ATP through maintenance processes and the higher mitochondria number per unit cell volume in young leaves (Armstrong *et al.*, 2006). The developmental dependence of leaf respiration has important implications for respiratory models. Respiratory models could be improved by accounting for leaf developmental effects, namely different R_{Tref} and Q_{10} values should be used for growing (immature) and mature leaves separately, and the relative contributions of leaf age class (developmental stage) to total respiration should be quantified. Using parameters (R_{Tref} and Q_{10}) derived from mature leaves only would lead to errors in predicting whole-plant respiration, and the magnitude of the error likely depends on developmental and phenological stages of plants.

Seasonal changes in the temperature response of respiration

Acclimation is another potential mechanism for changes in Q_{10}. Previous studies reported that growth in a warmer environment/-season led to a decrease in Q_{10} (Tjoelker *et al.*, 2001; Crous *et al.*, 2022) or a decrease in R_{Tref} (Crous *et al.*, 2011; Slot *et al.*, 2014; Slot & Kitajima, 2015; Reich *et al.*, 2016) when acclimation occurred. In our study, the acclimation of respiration was associated with a decline in R_{25} but not in Q_{10}. Crous *et al.* (2022) suggested that the decrease in basal respiration is usually found in leaves developed under new, warmer conditions and the change in R_{25} is likely related to changes in enzyme capacity (Atkin & Tjoelker, 2003). In another study, Crous *et al.* (2017) found that R_{25} was higher in the warm season (R_L was assessed using the Laisk method), and they argued that seasonal differences in leaf %N played a role. Accordingly, leaf nitrogen content is commonly used to predict basal respiration, because of the positive leaf N-respiration relationship found in many species (Atkin *et al.*, 2015).

Here, leaf N content could explain the seasonal effect on R_{25}, as winter leaves had higher N_{area} along with higher basal respiration rate, and R_{25} showed a positive correlation with N_{area} across seasons (Table 1; Figs 1, S12). Changes in N content have long been considered to explain respiratory temperature acclimation (Tjoelker *et al.*, 1999). This is because respiration is strongly influenced by the concentration of key enzymes and cofactors, which make up a large proportion of total N in leaves (Atkin & Tjoelker, 2003; Atkin *et al.*, 2005a,b; Abadie *et al.*, 2017). Both global meta-analysis and experimental studies indicated that leaf N content decreased with the increased temperature (Reich & Oleksyn, 2004; Kroner & Way, 2016). Organisms may have higher protein concentrations at lower temperatures to maintain biosynthesis rates given that ribosome degradation is more sensitive to temperature than ribosome synthesis (Woods *et al.*, 2003). On the contrary, the significantly higher R_{25} of immature (growing) leaves is not explained by the N status since immature leaves had slightly lower N_{area} compared with mature leaves, which is in line with the observed delay in chlorophyll production after leaf expansion (Croft *et al.*, 2014). Expressing respiration rate per unit N content did not eliminate the age effect (Figs S7, S12), indicating that the impact of leaf age was not driven by N nutrition. Our results highlighted the difference in the basal respiration-N content relationship between immature and mature leaves, which should be considered in respiration models.

Interestingly, a significant negative correlation between Q_{10} and R_{25} was found when combining all data across leaf age, season, and species (Fig. S8). This relationship was associated with leaf development, that is with higher Q_{10} but lower R_{25} in mature leaves, compared with immature leaves. Furthermore, the growing season also played a role since the warmer season led to reduced R_{25} but increased Q_{10} in most cases. The negative correlation between thermal sensitivity and basal respiration rate could be beneficial for plants to acclimate and maintain an optimal carbon balance under warming. To our knowledge, this relationship has not been reported before. Whether this negative relationship applies to other species and conditions needs to be verified, and the associated physiological mechanisms need to be explored.

Conclusions

Explicit modelling of leaf respiration in the light has become an emerging issue for predicting terrestrial carbon cycle. Using improved Kok methods, we found that leaf respiration in light and darkness had similar temperature sensitivity (as indicated by Q_{10}), and consequently, the inhibition of respiration by light was not significantly influenced by leaf temperature. The Kok method overestimated the inhibition of respiration by light and Q_{10} of R_L, highlighting that Φ_2 and γ (or Γ^*/C_c) should be accounted for when studying the temperature response of R_L. Acclimation of respiration to seasonal temperature was driven by basal respiration in summer (Q_{10} being unchanged) and was modulated by leaf nitrogen content. We found that growing leaves had lower Q_{10} of R_L and R_{Dk} than mature leaves, supported by the results of two tree species (dicots) grown in an open field. This conclusion should be further validated in other species since there is a far-reaching implication for carbon cycle models that fail to account for leaf development-associated changes in temperature sensitivity could lead to errors in estimating respiration rate from plant to landscape scales. This could be of particular importance when estimating respiratory carbon losses at the phenological phases where fast leaf area development takes place, such as spring in temperate forests.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (NSFC 32120103005, 31870377, and 32201277), and the Natural Science Foundation of Fujian Province, China (2023J01289). The staff of Fujian Sanming Forest Ecosystem National Observation and Research Station are acknowledged for their help in maintaining the experiment. Improvement of the paper was greatly aided by the comments made by Belinda Medlyn and the anonymous referees. GT thanks the support of the Region Pays de la Loire and Angers Loire Metropole via the grant Connect Talent Isoseed.

Competing interests

None declared.

Author contributions

XYG and XW designed and planned the research. DMZ, LL, ZY and QL performed the experiments. DMZ, WTM and YRS analysed the data. DMZ and XW wrote the first draft. GT, MAA, YY and XYG discussed the implications and contributed to the revision.

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Data availability

All data that support the findings of this study are included in the published article and its Supporting Information.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Dataset S1 Gas exchange data.

Fig. S1 Comparison of net photosynthesis rate (A), stomatal conductance to CO_2 (g_s) and A/g_s between intact and cut branches of *Castanopsis carlesii* and *Ormosia henryi*.

Fig. S2 Temperature response of net photosynthesis rate, stomatal conductance to CO_2, and mesophyll conductance in summer and winter for *Castanopsis carlesii* and *Ormosia henryi*.

Fig. S3 Relationships between stomatal conductance to CO_2 and mesophyll conductance.

Fig. S4 Relationships between natural log of leaf respiration rates and $(T-25)/10$.

Fig. S5 Photochemical efficiency of photosystem II, chloroplastic CO_2 concentration, and γ in response to incident irradiance at different leaf temperatures for *Castanopsis carlesii*.

Fig. S6 Photochemical efficiency of photosystem II, chloroplastic CO_2 concentration, and γ in response to incident irradiance at different leaf temperatures for *Ormosia henryi*.

Fig. S7 Respiration rate per gram N at 25°C of immature and mature leaves of *Castanopsis carlesii* and *Ormosia henryi*.

Fig. S8 Relationships between respiration rates at 25°C and temperature sensitivity (Q_{10}) of *Castanopsis carlesii* and *Ormosia henryi*.

Fig. S9 Temperature response of leaf respiration rate in the light calculated by the Kok-iterC_c method using different Γ^*-temperature relations.

Fig. S10 Relationship between R_L and g_m calculated by the new iterative method and by the Sun *et al.* (2023) method.

Fig. S11 Comparison of leaf respiration rate in the darkness and leaf respiration rate in the light estimated using four methods Kok, Yin, Sun, and Kok-C_c by new iterative process (results of the first six iterative calculations are given).

Fig. S12 Relationships between leaf respiration in light and darkness at 25°C and leaf N_{area}.

Methods S1 An Excel tool for calculating $R_{L\ \mathrm{Kok\text{-}iterCc}}$.