Environmental controls on light inhibition of respiration and leaf and canopy daytime carbon exchange in a temperate deciduous forest

Mary A. Heskel and Jianwu Tang

The Ecosystems Center, Marine Biological Laboratory, 7 MBL Street, Woods Hole, MA 02543, USA; Department of Biology, Macalester College, 1600 Grand Avenue, Saint Paul, MN 55105, USA; Corresponding author (mheskel@macalester.edu) orcid.org/0000-0003-3227-2978

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Uncertainty in the estimation of daytime ecosystem carbon cycling due to the light inhibition of leaf respiration and photorespiration, and how these small fluxes vary through the growing season in the field, remains a confounding element in calculations of gross primary productivity and ecosystem respiration. Our study focuses on how phenology, short-term temperature changes and canopy position influence leaf-level carbon exchange in Quercus rubra L. (red oak) at Harvard Forest in central Massachusetts, USA. Using leaf measurements and eddy covariance, we also quantify the effect of light inhibition on estimates of daytime respiration at leaf and ecosystem scales. Measured rates of leaf respiration in the light and dark were highest in the early growing season and declined in response to 10-day prior air temperatures ($P<0.01$), evidence of within-season thermal acclimation. Leaf respiration was significantly inhibited by light ($27.1\pm2.82\%$ inhibited across all measurements), and this inhibition varied with the month of measurement; greater inhibition was observed in mid-summer leaves compared with early- and late-season leaves. Increases in measurement temperature led to higher rates of respiration and photorespiration, though with a less pronounced positive effect on photosynthesis; as a result, carbon-use efficiency declined with increasing leaf temperature. Over the growing season when we account for seasonally variable light inhibition and basal respiration rates, our modeling approaches found a cumulative $12.9\%$ reduction of leaf-level respiration and a $12.8\%$ reduction of canopy leaf respiration, resulting in a $3.7\%$ decrease in total ecosystem respiration compared with estimates that do not account for light inhibition in leaves. Our study sheds light on the environmental controls of the light inhibition of daytime leaf respiration and how integrating this phenomenon and other small fluxes can reduce uncertainty in current and future projections of terrestrial carbon cycling.

Keywords: ecosystem respiration, Harvard Forest, light inhibition, phenology, photorespiration, photosynthesis, Quercus rubra.

Introduction

Current model estimates of global terrestrial carbon cycling emphasize the role of northern hemisphere forest ecosystems in acting as carbon (C) sinks (Goodale et al. 2002, Sarmiento et al. 2010, Keenan et al. 2013). These estimates depend on accurate quantification of ecosystem carbon dioxide (CO$_2$) assimilation through photosynthesis and release via autotrophic and heterotrophic respiration to account for climatic and biological influences on how C is cycled and stored in forests. Data acquisition relies on the application of eddy covariance, and/or leaf- or ecosystem-chamber methods, where respiration is measured at night or with darkened chambers and daytime respiratory fluxes are then calculated using temperature as a scaler (Baldocchi 2003, Reichstein et al. 2005, Tang et al. 2008). However, uncertainty in the estimation of daytime ecosystem C cycling due to the light inhibition of leaf respiration and photorespiration remains a confounding element in calculations of gross primary productivity (GPP) and ecosystem respiration ($R_{eco}$) (Heskel et al. 2013, Wohlfahrt and Gu 2015, Hanson et al. 2016). Recent interest in resolving this uncertainty at the ecosystem...
scale is driving examination into new methods of isotopic flux partitioning of eddy covariance measurements (Wehr and Saleska 2015, Wehr et al. 2016) and alternatives to standard modeling approaches to aboveground flux partitioning that emphasize isotopes, leaf age, light inhibition and multiple sources of ecosystem respiratory fluxes (Martínez-García et al. 2017, Oikawa et al. 2017, Wohlfahrt and Galvagno 2017). To fully account for daytime leaf respiration (R) and photorespiration in a deciduous forest system, it is necessary to understand how these fluxes are affected by environmental and biological variability throughout the growing season.

The phenomenon of light inhibition of mitochondrial ‘dark respiration’ (RDark) was first reported in the mid-20th century based on measurements made on algae (Kok 1948), and has since been observed in numerous plant species (Tcherkez et al. 2017a). Light inhibition causes reduced O2 consumption and reduced CO2 efflux in leaves (respiration in the light, or RLight) and is the result of light-induced alterations of many metabolic pathways, including the down-regulation of glycolysis and the reorganization of the tri-carboxylic acid cycle, and can reduce respiratory fluxes by ~25–100%, though many studies show a mean inhibition of ~30% (Budde and Randall 1990, Tcherkez et al. 2005, 2009, 2012, 2017a, Buckley and Adams 2011, Heskel et al. 2013, Kroner and Way 2016).

Currently, an experimentally driven discussion is occurring in the literature that is examining the impacts of, and mechanisms behind, light inhibition of leaf respiration. A recent physiological modeling study suggested that changes in the chloroplast CO2 partial pressure (cC) influence respiratory carbon efflux under low-light conditions by altering photosynthetic quantum yield, and this effect may be largely responsible for the observed lower rates attributed to light inhibition measured via the Kok method (Farquhar and Busch 2017). Buckley et al. (2017) responded to this claim by showing the influence of low-light cC using this method is only one of a few causes of the reduced RLight in developing and mature leaves. Other responsible mechanisms are independent of photosynthesis and occur under normal and low oxygen (O2) conditions, when photorespiration is suppressed, supporting a respiration-controlled mechanism of inhibition. Parallel to these papers, two recent studies further advanced our understanding of RLight with experiments using isotopic gas exchange methods. Gauthier et al. (2018) found rates RLight to be dampened by nearly 50% compared with dark respiration when evaluated by either net CO2 assimilation or net O2 production, using a novel gas exchange system that measures gross and net photosynthesis. Gong et al. (2018) compared measurements of RLight using Lausk and isotopic disequilibrium methods, and while the degree of inhibition varied slightly, respiration was effectively reduced in the light. Approaches from multiple methods and in diverse species indicate suppressed respiration in the light compared with dark fluxes. While the underlying biochemistry influencing the reduced flux of leaf RLight continues to be revealed by studies occurring primarily at the leaf and sub-leaf levels under controlled laboratory conditions (Tcherkez et al. 2017a), it is less clear how RLight occurs in plants grown in intact field conditions, and further, how this small flux impacts whole ecosystem C budgets (Wohlfahrt et al. 2005, Heskel et al. 2013).

Predictive characterization of the environmental and biological controls on light inhibition of leaf R remains an ongoing challenge. The degree on inhibition can be—though not always—impacted by environmental conditions, including drought (Ayub et al. 2011, Crous et al. 2012, Sperlich et al. 2016), elevated CO2 (Shapiro et al. 2004, Ayub et al. 2014), long-term growth temperature (Heskel et al. 2014, McLaughlin et al. 2014) and soil nutrient availability (Heskel et al. 2012, Atkin et al. 2013). The ratio of RLight to RDark can vary across measurement temperatures, with some species showing greater degrees of inhibition at warmer temperatures and other species showing the opposite response (Atkin et al. 2006, Way and Yamori 2014). In addition, the temperature sensitivity of RLight may differ from that of RDark (Atkin et al. 2005, Zaragoza-Castells et al. 2007, McLaughlin et al. 2014, Kroner and Way 2016, Crous et al. 2017b). Differences between species and broadly defined plant functional groups may also underlie the degree of inhibition of RLight across 37 species, R in herbaceous plants is less inhibited by light than in woody plants (Crous et al. 2017a), and inhibition may be less in deciduous than evergreen species (Heskel et al. 2012, 2014). Factors that integrate both the physical environment and leaf development and growth, such as leaf canopy position (Weerasinghe et al. 2014) and seasonality (Crous et al. 2012, Heskel et al. 2014) may exhibit stronger controls on metabolism in the light—for instance, leaf expansion and senescence, which have higher energy requirements may promote lower rates of inhibition. Though the collective evidence of light inhibition of leaf R at the leaf level covers a wide swathe of species and environmental conditions, the observed variability across studies, species and ecosystems limits quantitative predictability for scaling this effect to canopy C exchange. Due to this variability, site-, species- and environment-specific data should be used when possible to make calculations across scales. For this reason, we examine a historically monitored, deciduous temperate forest (Harvard Forest, MA, USA) to test how canopy position, seasonality and temperature impact daytime C fluxes in leaves and the Quercus rubra L–dominated canopy.

Additional daytime fluxes confound estimates of autotrophic and total ecosystem respiration measured at the canopy scale. Photorespiration, like mitochondrial R, releases CO2 during conditions when light is present and generally increases with measurement temperature, due to a decline in the CO2/O2 specificity of Rubisco as leaf temperatures increase (Brooks and Farquhar 1985, Sharkey 1988). However, like RLight, the small flux of
photorespiration can contribute to the overestimation of GPP at the canopy scale and is often not considered when calculating ecosystem C fluxes using eddy covariance data (Wohlfart and Gu 2015, Hanson et al. 2016). Daytime leaf fluxes may be further confounded by an additional source of C: xylem-transported CO₂, which may originate in non-leaf sources and is carried through vascular tissue to leaves and released simultaneously with leaf-derived photorespiration and mitochondrial respiration (Stutz et al. 2017).

In deciduous forest systems, leaf metabolism and the resulting fluxes of CO₂ are regulated across the growing season by the energetic demands of development, growth and senescence. Daily and seasonal variability in leaf microclimate of light, temperature and humidity also drive these processes through the canopy, and integrating these fluxes at the ecosystem scale poses modeling and experimental challenges. Recent applications of continuous monitoring of C isotopes, carbonyl sulfide and solar-induced chlorophyll fluorescence at Harvard Forest seek to track canopy GPP through the growing season, accounting for changes in phenology (Commane et al. 2015, Yang et al. 2015, 2017, Wehr et al. 2016). These studies provide valuable information about stomatal conductance (Wehr et al. 2017) and the relationship of light to canopy C exchange (Yang et al. 2015, 2017). However, there remain limitations in the correlations of these approaches with estimates of gross canopy photosynthesis through the growing season; the inclusion of controlled, leaf-level quantification of Rₜᵢₕₑ and photorespiration provide new estimates of C fluxes in the light, and may improve modeling of canopy and ecosystem C exchange.

We aim to quantify leaf-level daytime C exchange in Q. rubra and evaluate the influence of leaf canopy position, phenology and temperature during the growing season at Harvard Forest, MA. We hypothesize that leaves will be significantly inhibited throughout the growing season, with the lowest degree of inhibition in the early and late growing season during periods leaf expansion and senescence, and that the resulting leaf-level net C exchange will be altered by measurement temperature. We also seek to correlate photosynthetic parameters with parameters of light respiration, to examine how these related processes may co-vary. Collectively, this study aims to quantify environmental and biological controls of daytime leaf C exchange in a closely monitored temperate forest, with the goal of providing both insight and utility for current and future calculations of Rₑₑₒᵣₒᵣ.

### Materials and methods

#### Field site and sampling

Our experiment was conducted at Harvard Forest (42.538 N, 72.171 W), a mixed temperate forest in central Massachusetts that is 70–100 years old. Within Harvard Forest, sampling occurred in the general footprint of the Hardwood Walk-Up Tower, where Q. rubra (red oak) is the dominant species, and Acer rubrum (red maple) and Fagus grandifolia (American beech) are common. Sampling for gas exchange measurements occurred at four periods representing different phenological stages of the 2015 growing season (Table 1; see Figure S1 available as Supplementary Data at Tree Physiology Online). Micrometeorological data were collected continuously at the top of the tower above the canopy (28 m) as well as at 18 m (representing a mid-canopy environment below the crown), including air temperature (°C), photosynthetically active radiation (PAR, mol m⁻²⋅d⁻¹), precipitation and relative humidity (%), and were provided courtesy of the Harvard Forest Long Term Ecological Research station. Values of vapor pressure deficit (VPD, kPa) were calculated by converting relative humidity using the saturated vapor pressure (kPa) based on measurements of air temperature. Table 1 reports the ranges of micrometeorological data measured during the four sampling periods, and seasonal variability in these data is shown in SI Figure 1.

Leaves of Q. rubra were sampled from individual trees (n = 4–5) located around the Hardwood Walk-Up Tower at two different heights: top of the canopy (~25 m), with leaves sun-lit and unshaded during the day, and mid-canopy, at 15–20 m from the ground, ~5 m below the top of canopy branches, with leaves shaded through the day; conditions at the top- and mid-canopy through the growing season are shown in SI Figure 1. To maximize replication and allow for temperature control, all gas exchange measurements occurred in a laboratory setting. Thus, small branches containing multiple leaves (30–50 cm) at the canopy heights were removed from trees with pruners and then re-cut while stem ends were submerged in water to minimize potential embolism during transport to the lab. Sampled shoots were kept in water-pics, with cut ends submerged in water and under well-lit laboratory conditions prior to gas exchange measurements. Measurements occurred directly after collection and transport to the lab (~30 min) to minimize time after sampling.

### Table 1. Environmental conditions at Harvard Forest for the four 2015 sampling intervals during the growing season.

<table>
<thead>
<tr>
<th>Sampling period (date and DOY)</th>
<th>Air T range (°C)</th>
<th>VPD range (kPa)</th>
<th>Precipitation (mm)</th>
<th>Avg. PAR range (mol m⁻²⋅d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>May 28 (148)–June 1 (152)</td>
<td>6.0–26.6</td>
<td>0.06–0.58</td>
<td>42.3</td>
<td>10.0–50.5</td>
</tr>
<tr>
<td>July 14 (195)–July 16 (197)</td>
<td>10.2–25.9</td>
<td>0.38–0.70</td>
<td>0</td>
<td>22.6–27.1</td>
</tr>
<tr>
<td>Aug. 24 (236)–Aug. 25 (237)</td>
<td>17.1–27.2</td>
<td>0.30–0.44</td>
<td>21.6</td>
<td>22.8–45.3</td>
</tr>
<tr>
<td>Sept. 23 (266)–Sept. 25 (268)</td>
<td>7.6–23.9</td>
<td>0.36–0.49</td>
<td>0</td>
<td>32.6–41.0</td>
</tr>
</tbody>
</table>
Gas exchange measurements

CO₂ fluxes of photosynthesis and respiration of sampled leaves were measured using an infrared gas analyzer (IRGA; LI-6400XT, LI-COR, Lincoln, NE, USA). Leaves were enclosed in the IRGA cuvette at high light conditions (~1200 μmol m⁻² s⁻¹ PAR) to activate photosynthesis, and maintained until photosynthesis and stomatal conductance reached stable rates. For the entire measurement, leaf shoots were kept in water. Measurement conditions of the cuvette were set to ambient CO₂ (400 ppm), and relative humidity was maintained at 40–60%. CO₂ fluxes were measured to create a light response curve under 26 levels of PAR (μmol m⁻² s⁻¹): 1500, 1200, 800, 400, 200, 100, and then for every 5 μmol m⁻² s⁻¹ between 100 and 0. Readings were recorded at each light level after a stabilization interval of 90 s. This range of PAR encompasses the range of light experienced by Q. rubra leaves during the growing season. After the final measurement point of the light response curve at 0 PAR, leaves were kept in the darkened cuvette for 10 min. After 10 min, we logged a measurement, assuming no post-illumination respiration enhancement and photorespiration were occurring, and that the CO₂ flux could be fully attributed to dark mitochondrial respiration ($R_{Dark}$). Measurements of $R_{Dark}$ taken too quickly (<5 min) after illumination can provide inaccurate, higher values due to what is referred to as ‘post-illumination burst’; waiting a bit longer for $R_{Dark}$ allows for a stable reading. Net photosynthetic rate ($A_{net}$) was recorded at each level of PAR. Diffusion of CO₂, which was on average ~5% of the measured flux, through the gasket into the cuvette was accounted for according correction equations in the LI-6400XT manual.

To examine the influence of leaf temperature on CO₂ exchange, the above-described light response curves were measured in leaves maintained at three temperatures, ~15°C, ~25°C, ~35°C at four points across the growing season. Values represent means and standard of deviation.

Figure 1. Dark (a–c) and light (d–f) respiration of leaves from top and mid-canopy of Q. rubra. Leaves were measured at three controlled temperatures (15°C, 25°C, 35°C) at four points across the growing season. Values represent means and standard of deviation.
~35 °C. To do this in the lab setting, we temperature controlled the entire IRGA cuvette and replicate leaf by setting them in a small refrigerator set to 15 °C, an incubator set to 35 °C, or in ambient lab conditions (~20–25 °C). The IRGA block temperatures were also set to the corresponding temperatures (15, 25, 35 °C) to further maintain leaf temperatures. As leaves were enclosed in the IRGA cuvette for the entirety of the light curve measurement, temperature and relative humidity impacts of the surrounding conditions of the refrigerator and incubator could be monitored and controlled. Each response curve of three temperature levels took ~3 h—a minimum of 45 min (light curve) plus acclimation and transfer time of ~10 min. Leaves were first measured under cool, then ambient, then warm measurement conditions. Leaf temperatures were measured and recorded throughout the light response curve measurement with the copper-constantin thermocouple associated with the IRGA cuvette chamber that is in contact with the underside of the contained leaf.

Estimating respiration in the light and photosynthetic parameters

Respiration in the light for replicate leaves at each measurement temperature were estimated using the Kok method (Kok 1948), to ensure the greatest number of replicates in field conditions across the growing season. For a current comparison of the different methods for estimating light inhibition, see Tcherkez et al. (2017b). A regression line was fit to the section of the light response curve between the identified ‘breakpoint’ and linear segment of the light-response curve (~100–150 μmol m⁻² s⁻¹ PAR). The y-intercept of this regression is the estimated apparent rate of respiration in the light (R Light). To retrieve more accurate estimates of the rate of R Light, we needed to account for changes in intercellular [CO2] (c_i) that occur with decreasing PAR. To do this, we adjusted values of R Light obtained via the fitting of the regression described above to a constant c_i value, using calculations described by Kirschbaum and Farquhar (Kirschbaum and Farquhar 1987). In this approach, rates of R Light are adjusted so that the intercept of photosynthetic electron transport (J) and irradiance is minimized according to (Farquhar and von Caemmerer 1982):

\[ J = \frac{[4 \times (A_{net} + R_{light})] \times (c_i + 2\Gamma^*)]}{(C_i - \Gamma^*)} \]  

(1)

where Γ^* is the CO2 compensation point in the absence of R_Light. We express the inhibition of respiration as the proportion of R Light to R Dark (R Light/R Dark), with values ranging between 0 and 1; a low value represents a high degree of inhibition, and a high value represents low inhibition.

An additional goal of this study was to relate photosynthetic parameters to the degree of inhibition by light in leaves, and how this may vary across the growing season and with canopy height. The high-resolution light curve described in the above section maintained a constant reference CO2 value of 400 ppm, and saturating light value of 1500 μmol m⁻² s⁻¹ PAR; from each replicate curve, photosynthetic parameters were calculated. Specifically, this study focuses on the values of light-saturated photosynthetic C assimilation (A sat), photosynthetic electron transport (J sat), described above), and rates of oxygenation (V O sat) and carboxylation (V C sat) of Rubisco at saturating irradiance (~1500 μmol m⁻² s⁻¹ PAR). The rates of V O sat and V C sat were calculated according to:

\[ V_c = \frac{2}{3} \times \left[ \left( \frac{J}{4} \right) - \left( A_{net} + R_{Light} \right) \right] \]  

(2)

\[ V_c = \frac{1}{3} \times \left[ \left( \frac{J}{4} \right) - 2 \left( A_{net} + R_{Light} \right) \right] \]  

(3)

To assess the simultaneous relative rates of gross carbon assimilation (‘true photosynthesis’, or carboxylation) to net carbon assimilation, we also calculate a metric of leaf-level carbon-use efficiency (CUE) from parameters based on the light response curves using both R Light and R Dark, fluxes according to the following equation (based on von Caemmerer and Farquhar 1981, Gifford 2003, Wohlfahrt and Gu 2015):

\[ \text{CUE}_\text{Light} = \frac{A_{net}}{A_{gross}} = \frac{V_c - 0.5V_c - R_{Light}}{V_c} \]  

(4)

\[ \text{CUE}_\text{Dark} = \frac{A_{net}}{A_{gross}} = \frac{V_c - 0.5V_c - R_{Dark}}{V_c} \]  

(5)

Modeling respiration

The three measurement temperatures at which light response curves were conducted (~15, 25 and 35 °C) provide information on how both respiratory and photosynthetic parameters vary with temperatures experienced by the leaves during the growing season. The light response curves require ~45 min each, and we prioritized replication over increased measurement temperatures, though this limited the data points available to produce a higher-resolution temperature response of R Light. Response curves of higher resolution show that a log-polynomial response best represents how R Dark responds to measurement temperature (O’Sullivan et al. 2013, Heskel et al. 2016), log-linear models may best describe lower-resolution temperature response curves (curves with fewer than five measurement points), as shown in Kroner and Way (2016), Reich et al. (2016) and Wei et al. (2017). For these reasons, fluxes of R Dark and R Light were modeled as a log-linear response of T Leaf for each replicate leaf. Q10 values that represent the temperature sensitivity of R Dark and R Light across the three temperature points were calculated as:

\[ Q_{10} = 10^{\frac{\Delta T}{10}} \]  

(6)
where $k$ is the slope of log-transformed respiration as a response to $T_{\text{Leaf}}$.

To examine the cumulative impact of light inhibition on leaf respiration fluxes across the growing season, we applied both ‘bottom up’ and ‘top down’ modeling approaches. The ‘bottom up’ approach calculated leaf-level values of $R_{\text{Dark}}$ based on the temperature response determined by the log-linear fit described above. For the ‘bottom up’ approach, we focus on the impacts of seasonality on fluxes and inhibition, not the influence of canopy position and for this reason use mean values of $R_{\text{Dark}}$ that include both sampling heights. As there was no significant difference of the temperature response fit parameters from data collected at different measurement periods, heights as well as between $R_{\text{Dark}}$ and $R_{\text{Light}}$ (see Results), we applied the mean $R_{\text{Dark}}$ $Q_{10}$ value of 2.13 ($n = 34$) to model the temperature response of $R_{\text{Dark}}$. A goal of this exercise was to integrate seasonally sensitive parameters of $R_{\text{Dark}}$ and inhibition, though measurement constraints only allowed four monthly collection periods. To produce seasonally sensitive values of $R_{\text{Dark}}$ and inhibition, we modeled rates with a second order polynomial fit, allowing us to populate unmeasured dates through the season (see Figure S2 available as Supplementary Data at Tree Physiology Online). Seasonally variable inhibition values are then applied to temperature-modeled $R_{\text{Dark}}$ during daylight hours (i.e., $PAR > 25 \mu mol$ photons m$^{-2}$ s$^{-1}$), with values based on its seasonally sensitive model. We also compare the seasonally variable inhibition with a fixed value of inhibition for the whole season (21.4%), which was calculated as the mean of both sampling heights and all months at 25 °C. Above-canopy air temperature and PAR values are from the Harvard Forest EMS tower in 2015 (Munger and Wofsy 2017).

The ‘top down’ estimation of the cumulative impact of light inhibition of leaf respiration on $R_{\text{Eco}}$ used eddy covariance flux data from the 2015 growing season and relies on partitioning estimates for calculations (Munger and Wofsy 2017). First, $R_{\text{Eco}}$ was divided into ‘early’ (DOY 131–171), ‘mid’ (DOY 172–269) and ‘late’ (DOY 270–304) season. These temporal divisions were determined by inflection points, where the slope of average net ecosystem exchange (NEE) changed from baseline pre-leaf-out to greatly positive, and in autumn, where the slope became sharply negative during senescence across the 2015 growing season. Early season was marked by an increasing slope of NEE, mid-season was marked by a non-increasing or decreasing mean slope of NEE, and late season was marked by a sharp decline of NEE that parallels leaf senescence. The temporal segments of $R_{\text{Eco}}$ were then partitioned into leaf- ($R_{\text{Eco,leaf}}$) and non-leaf ($R_{\text{Eco,non-leaf}}$) sources based on a previous study at Harvard Forest (Giasson et al. 2013). We defined early-, mid- and late-season proportional contribution of leaf dark respiration to be 45%, 27% and 8% of $R_{\text{Eco}}$, respectively, based on findings from Harvard Forest published in Giasson et al. (2013) and discussion with R. Wehr (personal communication). After this coarse partitioning, seasonally variable inhibition values were applied during daylight (PAR > 25 $\mu mol$ m$^{-2}$ s$^{-1}$) with the same values as the ‘bottom up’ approach, creating a ‘top down’, whole-canopy estimate of light-inhibited leaf respiration ($R_{\text{Eco,leaf,ln}}$). These estimates could then be used to calculate new ‘inhibited’ values of $R_{\text{Eco}}$ that account for altered daytime fluxes:

$$R_{\text{Eco,day}} = R_{\text{Eco,leaf,ln}} + R_{\text{Eco,non-leaf}}$$

### Leaf traits and chemistry

After gas exchange measurements were completed, the area (cm$^2$) of individual measured leaves was measured using a belt-based leaf area meter (LI-3100C Area Meter, LI-COR). Leaves were then placed in labeled paper enveloped and dried at 65 °C for a minimum of 48 h, after which dry mass was measured. To measure percent C and nitrogen, dried leaves were ground using a ball mill, and ground leaf material was analyzed with an elemental analyzer (FLASH 2000, Thermo Scientific, Waltham, MA, USA). Collectively, these measurements allow for gas exchange and leaf chemical content to be expressed on area and mass bases.

### Statistical analyses

The physiological and leaf trait variables measured and calculated in our study tested for normality prior to all comparisons, and natural-log-transformed when necessary to meet standards of statistical tests. A multiple-factor ANOVA was applied to test for significant variance and interactions between measurement temperatures (15, 25 and 25 °C), canopy heights (top and mid) and sampling month during the growing season (May/June, July, August and September). Post-hoc Tukey’s tests were applied after ANOVA analysis to examine differences within factor groups. Linear models were applied to determine regression relationships between variables (as in Figures 2, 3 and 5; and see Figure S2 available as Supplementary Data at Tree Physiology Online), and details of the regression equations are reported. All analyses were conducted using R (R Development Core Team).

### Results

#### Photosynthetic rates controlled by height, seasonal timing and temperature

The processes that comprise photosynthesis, as measured and calculated in our study, were differentially affected by environmental factors. Across the growing season, light-saturated rates of carbon assimilation ($A_{\text{sat}}$) were highest in leaves measured at roughly ambient conditions (−25 °C) and lowest in leaves measured at the highest cuvette temperature (−35 °C). The difference between rates at warmed and ambient conditions, considering all leaves, was significant (Table 2), though there were no differences when compared with the cooler (−15 °C) measurement temperature. Values of $A_{\text{sat}}$ were generally higher.
in top-of-canopy leaves ($P = 0.07$), and did not vary significantly across measurement months (Tables 2 and 3).

The rate of carboxylation of Rubisco under saturating light ($V_{csat}$) was more sensitive than $A_{sat}$ to environmental growth conditions and seasonal timing. $V_{csat}$ was higher in top-canopy compared with mid-canopy leaves ($P < 0.005$, Tables 2 and 3), and a seasonal pattern is observed, with rates highest in early-season (May/June) and late-season (September) leaves compared with leaves measured in July ($P < 0.05$). In the early season, the highest rates are observed at lower measurement temperatures, but as the season progresses, the highest rates are observed at higher measurement temperatures (Table 2).

Rates of oxygenation of Rubisco at saturating light ($V_{osat}$) were significantly lower under the cooler measurement temperature ($P < 0.001$, Tables 2 and 3), with rates at ambient and warmer temperatures statistically similar ($P = 0.73$). Similar to patterns observed in $V_{csat}$, $V_{osat}$ was higher in top-canopy leaves compared with mid-canopy leaves, and while rates were generally higher in May/June and September compared with mid-season measurements made in July and August, these differences were
not significant (Tables 2 and 3). Similar to \( V_{\text{c sat}} \), highest rates of \( V_{\text{o sat}} \) were observed in lower measurement temperatures in the early season, but at higher temperatures in the mid- and late-growing season (Table 2). The rates of electron transport at saturating light, \( J_{\text{sat}} \), were highest in top-canopy leaves compared with mid-canopy \((P < 0.01)\), and under ambient measurement temperature compared with cooler and warmer cuvette temperatures \((P < 0.05)\). As with \( V_{\text{c sat}} \) and \( A_{\text{sat}} \), rates of \( J_{\text{sat}} \) were also highest in early- and late-season leaves, and similarly lower in leaves measured in mid-season (Tables 2 and 3).

**Respiration in the dark and light**

Dark respiration was greatest in early-season (May/June), top-canopy leaves (Figure 1, Table 4) measured at the warmest temperature \((-35^\circ C)\). Across all measurement temperatures and both canopy heights, rates of respiration declined significantly \((P < 0.001)\) from early season to mid-season (July and August) measurements (which were statistically similar), and increased from mid-season to late-season measurements (September; \(P < 0.001\)). Within each measurement period except July, top-canopy leaves had higher rates of respiration than mid-canopy leaves \((P < 0.01; \text{Figure 1})\).

Respiration in the light varied with environmental variables in similar patterns to those of \( R_{\text{Dark}} \) (Table 4). Highest rates of \( R_{\text{Light}} \) were measured in leaves sampled in the early season (May/June) at the top of the canopy (Figure 1). As observed with \( R_{\text{Dark}} \), rates of \( R_{\text{Light}} \) decreased between the first measurements in May/June and July \((P < 0.001)\), maintained similar low rates in August \((P = 0.61)\), then increased in the last measurement in September under the 25 \(^\circ C\) measurement temperature \((P < 0.01; \text{Figure 1, Table 4})\). Measurement temperature significantly increased rates of \( R_{\text{Light}} \) (Figures 1 and 2, Table 4). Differences in \( R_{\text{Light}} \) between canopy levels were significant considering all measured leaves (Table 4); within each measurement period, only leaves measured in July showed no significant
Table 2. Leaf-level photosynthetic parameters, including light-saturated photosynthetic rate ($A_{sat}$), carboxylation rate at saturating light ($V_{c,sat}$), oxygenation rate of Rubisco at saturating light ($V_{o,sat}$) and regeneration rate of RuBP at saturating light ($J_{sat}$), for each sampling interval at three measurement temperatures (Avg. Meas. $T$), in Q. rubra leaves sampled from the top- and mid-canopy levels ($n = 4–5$).

<table>
<thead>
<tr>
<th>Month</th>
<th>Canopy height</th>
<th>Avg. Meas. $T$ (°C)</th>
<th>$A_{sat}$ ($\mu$mol m$^{-2}$ s$^{-1}$)</th>
<th>$V_{c,sat}$ ($\mu$mol m$^{-2}$ s$^{-1}$)</th>
<th>$V_{o,sat}$ ($\mu$mol m$^{-2}$ s$^{-1}$)</th>
<th>$J_{sat}$ ($\mu$mol m$^{-2}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>May/June</td>
<td>Top</td>
<td>21.5 ± 3.5</td>
<td>13.76 ± 1.61</td>
<td>14.27 ± 1.89</td>
<td>3.06 ± 0.79</td>
<td>69.34 ± 9.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24.5 ± 0.4</td>
<td>8.85 ± 2.39</td>
<td>8.76 ± 2.76</td>
<td>2.27 ± 0.77</td>
<td>44.13 ± 13.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>33.0 ± 1.7</td>
<td>9.48 ± 2.31</td>
<td>10.07 ± 1.86</td>
<td>3.51 ± 0.80</td>
<td>54.34 ± 10.37</td>
</tr>
<tr>
<td></td>
<td>Mid</td>
<td>20.7 ± 3.7</td>
<td>11.35 ± 3.40</td>
<td>11.37 ± 4.84</td>
<td>2.31 ± 1.32</td>
<td>54.75 ± 24.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24.4 ± 0.7</td>
<td>10.93 ± 2.83</td>
<td>11.72 ± 3.40</td>
<td>3.29 ± 1.19</td>
<td>60.03 ± 17.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35.6 ± 0.4</td>
<td>7.43 ± 1.59</td>
<td>7.32 ± 1.95</td>
<td>2.82 ± 0.82</td>
<td>40.53 ± 10.83</td>
</tr>
<tr>
<td>July</td>
<td>Top</td>
<td>16.9 ± 0.9</td>
<td>10.58 ± 4.98</td>
<td>8.22 ± 6.52</td>
<td>1.22 ± 0.95</td>
<td>37.76 ± 29.87</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25.7 ± 0.2</td>
<td>10.19 ± 6.96</td>
<td>12.68 ± 3.16</td>
<td>4.22 ± 3.15</td>
<td>64.79 ± 26.28</td>
</tr>
<tr>
<td></td>
<td>Mid</td>
<td>35.3 ± 0.4</td>
<td>6.87 ± 3.73</td>
<td>6.29 ± 4.52</td>
<td>2.88 ± 2.56</td>
<td>36.67 ± 28.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16.7 ± 0.7</td>
<td>9.42 ± 0.74</td>
<td>9.40 ± 4.77</td>
<td>0.76 ± 0.80</td>
<td>22.64 ± 22.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25.0 ± 0.6</td>
<td>13.54 ± 4.59</td>
<td>7.29 ± 6.34</td>
<td>2.41 ± 2.17</td>
<td>38.79 ± 34.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35.4 ± 0.2</td>
<td>5.64 ± 3.29</td>
<td>4.66 ± 3.47</td>
<td>2.22 ± 1.96</td>
<td>27.55 ± 21.67</td>
</tr>
<tr>
<td>Aug.</td>
<td>Top</td>
<td>16.1 ± 1.0</td>
<td>9.81 ± 5.69</td>
<td>9.27 ± 5.80</td>
<td>1.47 ± 0.92</td>
<td>42.94 ± 26.86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25.1 ± 2.0</td>
<td>13.28 ± 3.87</td>
<td>13.64 ± 3.79</td>
<td>4.47 ± 0.95</td>
<td>72.44 ± 18.90</td>
</tr>
<tr>
<td></td>
<td>Mid</td>
<td>33.7 ± 0.6</td>
<td>11.95 ± 6.25</td>
<td>13.85 ± 6.56</td>
<td>5.59 ± 2.76</td>
<td>77.75 ± 37.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16.7 ± 1.0</td>
<td>5.01 ± 3.30</td>
<td>4.40 ± 3.58</td>
<td>0.64 ± 0.57</td>
<td>20.15 ± 16.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25.6 ± 0.2</td>
<td>7.64 ± 2.83</td>
<td>5.13 ± 4.45</td>
<td>1.60 ± 1.45</td>
<td>26.92 ± 23.60</td>
</tr>
<tr>
<td>Sept.</td>
<td>Top</td>
<td>34.8 ± 1.2</td>
<td>11.06 ± 1.26</td>
<td>4.63 ± 6.71</td>
<td>2.32 ± 3.69</td>
<td>27.81 ± 41.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.0 ± 0.1</td>
<td>8.91 ± 5.39</td>
<td>7.15 ± 6.55</td>
<td>1.31 ± 1.17</td>
<td>33.84 ± 30.83</td>
</tr>
<tr>
<td></td>
<td>Mid</td>
<td>23.5 ± 0.4</td>
<td>14.25 ± 4.09</td>
<td>13.25 ± 8.42</td>
<td>3.82 ± 2.48</td>
<td>68.28 ± 43.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35.8 ± 0.3</td>
<td>9.80 ± 4.23</td>
<td>12.10 ± 5.15</td>
<td>5.70 ± 2.92</td>
<td>71.18 ± 32.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.1 ± 0.0</td>
<td>7.79 ± 5.09</td>
<td>7.27 ± 5.89</td>
<td>0.98 ± 0.70</td>
<td>33.00 ± 26.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23.8 ± 0.3</td>
<td>12.00 ± 1.64</td>
<td>10.01 ± 7.40</td>
<td>3.72 ± 0.83</td>
<td>69.09 ± 13.93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35.8 ± 0.1</td>
<td>9.99 ± 0.91</td>
<td>11.97 ± 0.78</td>
<td>5.79 ± 0.30</td>
<td>71.02 ± 4.17</td>
</tr>
</tbody>
</table>

Table 3. Summary of ANOVA for photosynthetic parameters presented in Table 2, $A_{sat}$, $V_{c,sat}$, $V_{o,sat}$ and $J_{sat}$, in relation to canopy height (top or mid), three levels of measurement temperature (Avg. $T$) and measuring month (May/June, July, August, September). $P$ and $F$-ratio values are reported, with statistical significance denoted with asterisks ($*P < 0.05, **P < 0.01$).

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>$A_{sat}$</th>
<th>$V_{c,sat}$</th>
<th>$V_{o,sat}$</th>
<th>$J_{sat}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$F$</td>
<td>$P$</td>
<td>$F$</td>
<td>$P$</td>
</tr>
<tr>
<td>Height</td>
<td>3.776</td>
<td>0.06</td>
<td>9.779</td>
<td>**</td>
</tr>
<tr>
<td>Meas. $T$</td>
<td>3.182</td>
<td>*</td>
<td>1.399</td>
<td>0.25</td>
</tr>
<tr>
<td>Month</td>
<td>0.748</td>
<td>0.53</td>
<td>2.847</td>
<td>*</td>
</tr>
<tr>
<td>Height x Meas. $T$</td>
<td>0.581</td>
<td>0.56</td>
<td>0.051</td>
<td>0.95</td>
</tr>
<tr>
<td>Height x Month</td>
<td>1.110</td>
<td>0.35</td>
<td>2.389</td>
<td>0.08</td>
</tr>
<tr>
<td>Meas. $T$ x Month</td>
<td>3.459</td>
<td>**</td>
<td>1.876</td>
<td>0.09</td>
</tr>
<tr>
<td>Height x Meas. $T$ x Month</td>
<td>0.847</td>
<td>0.54</td>
<td>0.711</td>
<td>0.64</td>
</tr>
</tbody>
</table>

The difference between top- and mid-canopy rates of $R_{Light}$ ($P = 0.78$), compared with all other periods ($P < 0.01$). Comparison of the slope and intercept of the temperature response of $R_{Light}$ found no difference amongst measurement months and canopy positions (Figure 2). For $R_{Dark}$, measurement month affected the intercept of the temperature response, with early- and late-season values higher than mid-season, but unaffected by canopy height; neither factor impacted the slope (see Figure S2 available as Supplementary Data at Tree Physiology Online). The resulting $Q_{10}$ values for $R_{Light}$ and $R_{Dark}$ showed no significant differences between the canopy levels, measurement months, or light or dark respiration (Table 5), though it should be noted $Q_{10}$ values are based on response curves with limited resolution (three measurement temperatures). Both $R_{Light}$ and $R_{Dark}$ at 25 °C responded in a similar declining pattern to 10-day prior temperatures (Figure 3); there were no significant differences in the respective slopes.

Variability of light inhibition and its influence on leaf daytime carbon cycling

Light significantly inhibited rates of leaf $R$ (Figure 3), though the degree of inhibition did not vary significantly with environmental or seasonal change. The relationship of $R_{Light}$ to $R_{Dark}$ was similar...
in early and late measurement months, which exhibited lower rates of inhibition than the two mid-season months (Figures 4 and 5). The ratio of \(R_{\text{Light}}\) to \(R_{\text{Dark}}\) did not vary with canopy height or measurement temperature (Table 4). There was a seasonal trend in \(R_{\text{Light}}/R_{\text{Dark}}\), with higher rates of inhibition occurring in the middle of the growing season, and less inhibition exhibited in the early and late season (see Figure S2 available as Supplementary Data at Tree Physiology Online). Considering both top- and mid-canopy leaves, the mean value of \(R_{\text{Light}}/R_{\text{Dark}}\) at the \(-25^\circ\)C measurement point was \(0.95 \pm 0.18\) in May/June, \(0.49 \pm 0.32\) in July, \(0.66 \pm 0.45\) in August and \(0.85 \pm 0.27\) in September. The most inhibition by light is observed in the mid-season, with the lowest levels of inhibition observed in the early- and late-season leaves (Figure 5; see Figure S2 available as Supplementary Data at Tree Physiology Online). We also found a significant linear relationship between the degree of light inhibition in leaves measured at \(25^\circ\)C and the 10-day prior mean temperature experienced by the leaves in the forest pre-sampling (see Figure S3 available as Supplementary Data at Tree Physiology Online), with generally lower inhibition occurring at lower forest temperatures.

Table 4. Summaries of ANOVA for respiration and carbon use efficiency in light and dark relation to factors of canopy height, measurement temperature (Meas. T) and measuring month (May/June, July, August, September). \(P\) and \(F\)-ratio values are reported, with statistical significance denoted with asterisks (*\(P < 0.05\), **\(P < 0.01\) and ***\(P < 0.001\)).

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>(R_{\text{Light}})</th>
<th>(R_{\text{Dark}})</th>
<th>(R_{\text{Light}}/R_{\text{Dark}})</th>
<th>(\text{CUE}_{\text{Light}})</th>
<th>(\text{CUE}_{\text{Dark}})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(F)</td>
<td>(P)</td>
<td>(F)</td>
<td>(P)</td>
<td>(F)</td>
</tr>
<tr>
<td>Height</td>
<td>34.263</td>
<td>***</td>
<td>44.031</td>
<td>***</td>
<td>0.023</td>
</tr>
<tr>
<td>Meas. T</td>
<td>44.961</td>
<td>***</td>
<td>113.695</td>
<td>***</td>
<td>0.922</td>
</tr>
<tr>
<td>Month</td>
<td>50.670</td>
<td>***</td>
<td>74.913</td>
<td>***</td>
<td>4.103</td>
</tr>
<tr>
<td>Height \times Meas. T</td>
<td>8.131</td>
<td>***</td>
<td>8.794</td>
<td>***</td>
<td>0.984</td>
</tr>
<tr>
<td>Height \times Month</td>
<td>2.558</td>
<td>0.06</td>
<td>1.760</td>
<td>0.16</td>
<td>0.974</td>
</tr>
<tr>
<td>Meas. T \times Month</td>
<td>9.430</td>
<td>***</td>
<td>17.611</td>
<td>***</td>
<td>1.544</td>
</tr>
<tr>
<td>Height \times Meas. T \times Month</td>
<td>0.312</td>
<td>0.93</td>
<td>0.556</td>
<td>0.76</td>
<td>0.266</td>
</tr>
</tbody>
</table>

Table 5. \(Q_{10}\) values of \(R_{\text{Dark}}\) and \(R_{\text{Light}}\) (mean and standard deviation) for each month and both canopy levels. Comparison by ANOVA found no differences between \(Q_{10}\) values of \(R_{\text{Dark}}\) and \(R_{\text{Light}}\) across all measurements (\(F = 0.740, P = 0.394\)), and no significant differences of \(Q_{10}\) values amongst sampling month or canopy height calculated from \(R_{\text{Dark}}\) and \(R_{\text{Light}}\).

<table>
<thead>
<tr>
<th>Month</th>
<th>Canopy height</th>
<th>(Q_{10}) of (R_{\text{Dark}})</th>
<th>(Q_{10}) of (R_{\text{Light}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>May/June</td>
<td>Top</td>
<td>2.53 ± 1.35</td>
<td>2.44 ± 1.31</td>
</tr>
<tr>
<td></td>
<td>Mid</td>
<td>2.50 ± 0.58</td>
<td>2.08 ± 0.55</td>
</tr>
<tr>
<td>July</td>
<td>Top</td>
<td>2.32 ± 0.46</td>
<td>2.76 ± 0.66</td>
</tr>
<tr>
<td></td>
<td>Mid</td>
<td>2.09 ± 0.62</td>
<td>1.68 ± 0.29</td>
</tr>
<tr>
<td>Aug.</td>
<td>Top</td>
<td>2.13 ± 0.47</td>
<td>1.84 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>Mid</td>
<td>1.58 ± 0.49</td>
<td>1.34 ± 0.86</td>
</tr>
<tr>
<td>Sept.</td>
<td>Top</td>
<td>2.24 ± 0.57</td>
<td>1.84 ± 0.76</td>
</tr>
<tr>
<td></td>
<td>Mid</td>
<td>1.67 ± 0.18</td>
<td>1.81 ± 0.87</td>
</tr>
</tbody>
</table>

An objective of this study was to assess daytime C fluxes and potential relationships between individual processes and environmental controls. Considering all replicate measurements, \(R_{\text{Light}}\) varied significantly with \(V_{\text{Csat}}\) and \(V_{\text{Osat}}\) though the predictive capabilities of these relationships were not strong (Figure 6). Values of \(R_{\text{Light}}/R_{\text{Dark}}\) also varied significantly with \(V_{\text{Csat}}\) and \(V_{\text{Osat}}\). The stronger positive relationship being between \(R_{\text{Light}}/R_{\text{Dark}}\) and \(V_{\text{Csat}}\) (Figure 6). Carbon-use efficiency, a variable that integrates multiple co-occurring fluxes, was calculated to include \(R_{\text{Light}}\) or \(R_{\text{Dark}}\) to assess the influence of light inhibition on C exchange. Measurements of \(\text{CUE}_{\text{Light}}\) were highest in the 15°C measurement, when rates of respiration and photorespiration were lowest, and declined as measurement temperature increased (see Figure S4 available as Supplementary Data at Tree Physiology Online; Table 4). Early-season (May/June) rates of \(\text{CUE}_{\text{Light}}\) were the lowest of all measurement months due to high rates of respiration in early-season leaves, and significantly lower compared with rates measured in September (\(P < 0.034\)). There were no significant differences between the other measurement months. In contrast, \(\text{CUE}_{\text{Dark}}\) did not vary across months, though shared the same response to measurement temperature (see Figure S4 available as Supplementary Data at Tree Physiology Online; Table 4). The highest values of \(\text{CUE}_{\text{Dark}}\) were observed in at the coldest measurement temperature (see Figure S4 available as Supplementary Data at Tree Physiology Online), driven by relatively lower rates \(R_{\text{Light}}\) and \(V_{\text{Osat}}\).

**Scaling daytime respiration**

To quantify whole-season impacts of inhibition, we applied two approaches to scaling respiration: ‘bottom-up’ and ‘top-down’, as described in depth in the Materials and methods. The results of these modeling exercises are shown in Figure 7. Starting with seasonally variable leaf-level measurements of \(R_{\text{Dark}}\) (see Figure S2 available as Supplementary Data at Tree Physiology Online), we modeled fluxes based on the short-term temperature response of \(R\) to hourly measurements of air temperature and an application of seasonally variable and fixed inhibition terms.
during daylight across the growing season (Figure 7a). The higher basal rates of $R_{\text{Dark}}$ in the early and late season drove the ‘u’-shape response; even as higher temperature occurred mid-season, this is also when the lowest basal rates of $R_{\text{Dark}}$ were measured (see Figure S2 available as Supplementary Data at Tree Physiology Online, Figure 7a). Inhibition was weakest in early and late season, when values of $R_{\text{Dark}}$ were highest, and this led to a diminished effect of seasonally variable inhibition compared with the fixed inhibition term. Based on our ‘bottom-up’ model, the application of fixed rate of light inhibition resulted in a reduction of 12.9% in cumulative leaf respiratory fluxes across the growing season, compared with a 8.2% reduction when seasonally variable inhibition was applied.

The ‘top-down’ calculations were derived from eddy covariance flux measurements of $R_{\text{Eco}}$, which we partitioned into leaf and non-leaf sources, thereafter applying a light inhibition reduction to the leaf-based sources of respiration (resulting in $R_{\text{Eco, leaf,in}}$). The application of seasonally variable light inhibition resulted in a 12.8% reduction of cumulative modeled leaf-based respiratory efflux (Figure 7b). Applying daytime inhibition to leaf-derived $R_{\text{Eco}}$ showed a variable effect through the growing season, with the most pronounced effect observed mid-season (see Figure S5 available as Supplementary Data at Tree Physiology Online). When non-leaf $R$ is added to inhibited leaf-based $R$ fluxes to quantify $R_{\text{Eco,day}}$, we found only a 3.68% reduction in ecosystem scale $R$ compared with ‘un-inhibited’ $R_{\text{Eco}}$, considering cumulative fluxes (Figure 7b). Within-season acclimation to thermal conditions was not considered in the seasonal models.

Leaf functional traits

Leaf SLA was highest in top-canopy and in early-season leaves (see Tables S1 and S2 available as Supplementary Data at Tree Physiology Online), and values of SLA declined with growing season progression. Leaf LMA, a converse trait to SLA responded in an opposite manner—the highest values were observed in mid- and late-season leaves, and in top-canopy leaves (see Tables S1 and S2 available as Supplementary Data at Tree Physiology Online). Leaf %C followed a similar seasonal pattern to LMA with some slight variation: lowest values were measured in the early and late season (i.e., May/June and September), with highest values in the mid-growing season; all months were different ($P < 0.001$) except for May/June and September ($P = 0.13$). Top-canopy leaves exhibited higher values of %C than mid-canopy values ($P < 0.001$; see Tables S1 and S2 available as Supplementary Data at Tree Physiology Online).

Discussion

Temperate forests of the Northeast and Midwest of North America act as a large component of the continent’s terrestrial C
sink, thus understanding the ecological and physiological drivers of C exchange and reducing uncertainty in estimates and projections of regional ecosystem productivity remain prioritized research objectives (Fan et al. 2011, Gough et al. 2016). However, of the numerous field-grown species measured for both photosynthetic parameters and $R_{\text{Light}}$, only a handful fall under the broad plant functional category of temperate deciduous broadleaf trees (Atkin et al. 2013, Sun et al. 2014, Sperlich et al. 2016, Turnbull et al. 2017). The design of our study focused on the influences of seasonality, short-term temperature and canopy height on leaf-fluxes in $Q.\ rubra$ in Harvard Forest. The resulting data show the impact of seasonal controls on daytime leaf-level C cycling in a common canopy species. Our findings suggest significant light inhibition of leaf $R_l$ and the potential for this inhibition to impact estimates of daytime whole ecosystem respiratory flux.

Environmental controls on daytime leaf carbon fluxes

Intra-season variability of photosynthesis in deciduous species can be influenced by a range of environmental and biological elements, including leaf expansion, canopy microenvironment, herbivory and short-term changes in precipitation, VPD, air temperature and absorbed PAR, and experimentally distinguishing these overlapping influences can be especially challenging in field-based studies of forest species (Mooney et al. 1981, Bassow and Bazzaz 1998). Across the growing season, we observed a shift in the light-saturated rates of photosynthetic parameters ($A_{\text{sat}}$, $V_{\text{C sat}}$, $V_{\text{O sat}}$ and $J_{\text{sat}}$) exhibited by top-canopy leaves: highest rates occurred at cooler measurement temperatures in early-season measurements (May/June), but in late-season measurements (August, September), the highest rates are observed at warmer measurement temperatures. This shift may suggest potential photosynthetic acclimation over the growing season in multiple processes, resulting in higher light-saturated rates in the late season (Way and Yamori 2014). Across all measurement temperatures, average values of both $A_{\text{sat}}$, $V_{\text{C sat}}$ and $J_{\text{sat}}$ were similar or lower in the mid-July than in the late May/early June sampling period. This trend diverges from reported early-to-mid season increases in photosynthetic parameters in deciduous species from the same site (Bassow and Bazzaz 1998, Yang et al. 2017). Sampling scheduling did not allow for us to measure leaves over the summer solstice to test the influence of maximum photoperiod on photosynthesis, which can assert stronger control than maximum growth temperatures (Bauerle et al. 2012), though $Q.\ rubra$ may be less sensitive to photoperiod than other species considering phenological cues (Laube et al. 2014, Way and Montgomery 2015). The short- and long-term temperature responses of $R_{\text{Light}}$ remain a source of uncertainty when considering daytime leaf C fluxes (McLaughlin et al. 2014, Way and Yamori 2014, Way et al. 2015, Slot and Winter 2017, Crous et al. 2017b). In $Q.\ rubra$, $R_{\text{Light}}$ increased with measurement temperature from $-15$ to $35^\circ$, and there were no significant impacts of seasonal timing or canopy position on the temperature response. Our data suggest a similar short-term temperature response between $R_{\text{Light}}$ and $R_{\text{Dark}}$, with no differences observed in either $Q_{10}$ or the response to the 10-day prior growth temperature. Kroner and Way (2016) found higher $Q_{10}$ values for $R_{\text{Light}}$ than $R_{\text{Dark}}$ in $Picea abies$, an evergreen conifer; it is possible that leaf age and/or growth form may mediate the temperature sensitivity of $R_{\text{Light}}$. We found the highest rates of $R_{\text{Light}}$ occurred in leaves sampled in the early season at the warmest measurement temperature. High rates of $R_{\text{Dark}}$ in the early season are generally attributed to the high demand for energy and C skeletons associated with growth (Xu and Griffin 2006), especially in expanding leaves of deciduous species, and this pattern has also been observed in $R_{\text{Light}}$ (Crous et al. 2012, Heskel et al. 2014). Increasing $R_{\text{Light}}$ with measurement temperature also led to the lowest CUE at the highest temperatures. Mid-summer estimates of CUE were greater than early season, a pattern also observed in field grown $Quercus ilex$ under drought and control conditions (Sperlich et al. 2016). While our study did not explicitly examine the impact of
warmed growth conditions to assess acclimation, there is evidence of intra-seasonal adjustments to longer-term thermal conditions: both \( R_{\text{Dark}} \) and \( R_{\text{Light}} \) of a common reference temperature declined with increasing 10-day average growth temperature. This decline in respiration with intra-season changes in growth temperature recalls similar trends observed in evergreen and deciduous species (Ow et al. 2010, Heskel et al. 2014), and suggests intra-season (days to weeks) thermal acclimation may control leaf respiratory fluxes as much or more than same day temperature variability (Vanderwel et al. 2015, Smith and Dukes 2017). The flexibility in leaf respiratory fluxes in the light and dark due to short-term, within-season acclimation can have a pronounced impact on terrestrial C exchange (Lombardozzi et al. 2015, Smith et al. 2015, Huntingford et al. 2017).

Canopy position mediates the light environment of a leaf, and the resulting intra-canopy resource allocation and function (Mooney et al. 1981, Delong and Doyle 1985, Hirose and Werger 1987). We found significant influence of canopy position through the growing season in Q. rubra with respect to many, but not all, physiological and functional traits. Two traits most commonly associated with canopy optimization, \( \text{A}_{\text{sat}} \) and \( \% \text{N} \), did not differ significantly in leaves representing top- and mid-canopy positions, though \( \text{A}_{\text{sat}} \) was slightly lower in mid-canopy leaves. Respiration in the light has only previously been reported at multiple canopy heights in a tropical forest in Northern Queensland on evergreen broadleaf trees, in which top-canopy leaves released more carbon than lower-canopy leaves (Weerasinghe et al. 2014).

A main objective of our study was to quantify the degree of light inhibition of \( R \) across the growing season and at different measurement temperatures and canopy heights. Across all sampling and measurement conditions, we found significant inhibition by light and a seasonally mediated change in the degree of the inhibitory effect. Early and late-season leaves exhibited generally lower inhibition (higher \( R_{\text{Light}}/R_{\text{Dark}} \) values) than mid-season (July, August), potentially to allow for higher \( R \) during periods of leaf expansion and senescence, periods when leaf energy demand may outstrip available photosynthetic energy supply (Heskel et al. 2014). The late-season relaxation of inhibition that we observe at the leaf level in Harvard Forest was also reported at the ecosystem scale through isotopic flux partitioning methods: Wehr et al. (2016) found lower reductions in August and September compared with earlier season measurements. Unlike the actual respiratory fluxes, \( R_{\text{Light}}/R_{\text{Dark}} \) did not vary with canopy height or measurement temperature. The lack of significant variation in degree of light inhibition with canopy height agrees was also found in tropical trees (Weerasinghe et al. 2014). Considering multiple studies of different plant functional types, there is no apparent common short-term temperature response of \( R_{\text{Light}}/R_{\text{Dark}} \) (Way and Yamori 2014): \( R_{\text{Light}}/R_{\text{Dark}} \) increases with temperature in Picea mariana and Betula nana (Way and Sage 2008, McLaughlin et al. 2014), decreases with Plantago species and Eriophorum vaginatum (Atkin et al. 2006, McLaughlin et al. 2014), does not change in B. nana grown under warming (McLaughlin et al. 2014) and shows no discernable pattern in Q. ilex (Zaragoza-Castells et al. 2007). The positive relationship between inhibition and the 10-day prior mean temperature suggests a potential longer-term thermal influence on this phenomenon, and assessing different temporal scales of environmental variables on inhibition may contribute to a greater understanding of its control.

Previous experiments that manipulated \( \text{CO}_2/\text{O}_2 \) conditions identified consistent relationships between \( R_{\text{Light}} \) and photorespiration in wheat (Griffin and Turnbull 2013), cocklebur (Tcherkez et al. 2008) and common bean (Gauthier et al. 2018): under low \( \text{O}_2 \), photorespiration and \( R_{\text{Light}} \) (and as a result \( R_{\text{Light}}/R_{\text{Dark}} \) declined. Our data reveal positive linear relationships between \( R_{\text{Light}}/R_{\text{Dark}} \) and \( \text{Vo}_{\text{sat}} \) across the growing season, a pattern also observed in Eucalyptus globulus leaves (Crous et al. 2017b). Recent gas exchange and isotopic labeling studies suggest a bottleneck of unrecycled amino acids and the resulting photorespiratory demand for glutamate enhance nitrogen metabolism and stimulate not only rates of \( R_{\text{Light}} \) but also \( \text{CO}_2 \) assimilation under normal (i.e., not low) \( \text{O}_2 \) conditions (Abadie et al. 2017, Tcherkez et al. 2017b, Busch et al. 2018). It should be noted, though our study examines ‘small’ leaf-level fluxes of \( \text{C} \) (that are assumed to be leaf-originated), there is evidence based on isotopic labeling that a proportion of daytime \( \text{C} \) efflux may be derived from root sources and carried to leaves through xylem (Stutz et al. 2017).

Pursuing the convergence of daytime \( \text{C} \) release, nitrogen assimilation and root-to-leaf transport of non-leaf-originated \( \text{CO}_2 \), and how these processes co-vary with phenology and environmental change may prove to be a valuable research direction for understanding leaf-to-canopy daytime \( \text{C} \) cycling.

**Daytime respiration and ecosystem carbon fluxes**

Bridging observed nuances of leaf-level processes to whole forest ecosystem fluxes, and doing so across growing season-shaped environmental and biological influences, poses challenges when creating models of \( \text{C} \) exchange. In our study, we confront this challenge by isolating a handful of the controlling environmental (temperature, light) and biological (canopy position, phenology) factors. In modeling daytime \( R \) across the growing season at the leaf and ecosystem scales, we applied two approaches to examine the influence of light inhibition on \( \text{C} \) efflux. The ‘bottom-up’ model used seasonally variable reference values of \( R_{\text{Dark}} \), driven by air temperature, and applied seasonally variable values of inhibition during daylight hours, resulting in a 8.2% cumulative reduction in leaf \( \text{C} \) efflux across the entire growing season, which fits within the range of reported values for arctic plants (6–49% reduction when including inhibition) (McLaughlin et al. 2014). The application of a fixed value of inhibition resulted in a higher reduction of leaf respiration overall (12.9%), implying...
that fixed values do not capture the limited inhibition in the early and late season, when rates of leaf $R$ are generally high.

Scaling the impact of light inhibition to ecosystem fluxes requires the quantitative dissembling and modeling of eddy covariance data. In the ‘top-down’ approach, we applied seasonally variable partitioning values to quantify leaf and non-leaf sources of C fluxes and then applied a seasonally variable inhibition term to leaf fluxes during daytime, resulting in a seasonal cumulative reduction just under 12.8%. The degree of overestimation in canopy respiration is lower than a modeled value (20.4%) from leaf-level measurements made in a deciduous forest in China (Sun et al. 2014). Ecosystem-scale studies have also identified a reduction in daytime total respiration fluxes using a range of eddy covariance-based methods (Wohlfahrt et al. 2005, Jassal et al. 2007, Bruhn et al. 2011, Wehr et al. 2016). The variability in the effect of inhibition on total $R_{\text{Eco}}$ across the season is likely related to the relative contribution of leaves to total $R_{\text{Eco}}$, as leaves comprise a greater proportion of the total flux, the impact of inhibition may be more significant. In single-species mesocosms, the contribution of leaf daytime respiration to total ‘ecosystem’ respiration can be as great as 50–60% (Gong et al. 2017). However, values of leaf contribution to total ecosystem fluxes in forest systems are often reported to be much lower, can vary widely, and are sure to be influenced by environment and phenology (Tang et al. 2008, Hermle et al. 2010, Giasson et al. 2013).

Conclusions

Based on our leaf-level measurements of a dominant canopy tree, Q. rubra, across the growing season at two canopy positions and under three measurement temperatures, we present a multi-faceted characterization of daytime C exchange in a temperate deciduous forest. We also present two approaches and the resulting models of seasonal autotrophic respiration that apply the light inhibition of respiration to the leaf and canopy scale. Collectively, the data show a seasonally influenced light inhibition of leaf respiration that impacts many metrics of leaf and forest carbon cycling: a decrease in leaf CUE, decreased seasonal cumulative leaf respiration using leaf-based and eddy covariance-derived data, and decreased ecosystem respiration. Our field study focuses primarily on course categories of controls on physiology—canopy height as a proxy for light, temperature, and wind microenvironment, and seasonal time points that capture phenology of forest environmental and leaf development. There remains a need to better understand the metabolic intricacies and biochemical networks that control leaf metabolism in the light, especially in leaves across developmental stages that are exposed to the stochastic setting of a forest canopy, and not a controlled setting. Our study adds to the growing body of literature on metabolic underpinnings and environmental controls of the light inhibition of leaf $R$, which collectively urge for incorporation in studies of ecosystem C exchange. We suggest integrating seasonally variable daytime fluxes when calculating $R_{\text{Eco}}$ and gross photosynthetic assimilation of forest canopies and believe the integration of these variables may lead to more robust estimates of canopy carbon uptake when applying methods such as solar-induced fluorescence and carbonyl sulfide. Small, environmentally sensitive leaf fluxes such as $R_{\text{Light}}$ do not fit easily into the eddy covariance-driven narrative of ecosystem C exchange and longer-term C storage in forests. However, integrating these small fluxes, and importantly, their response to long- and short-term environmental change, are likely to reduce uncertainty in current and future projections of terrestrial C cycling.

Supplementary Data

Supplementary Data for this article are available at Tree Physiology Online.

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Conflict of interest

None declared.

References


