Studies

Short-term thermal acclimation of dark respiration is greater in non-photosynthetic than in photosynthetic tissues

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Abstract

Thermal acclimation of plant respiration is highly relevant to climate projections; when included in models, it reduces the future rate of atmospheric CO₂ rise. Although all living plant tissues respire, few studies have examined differences in acclimation among tissues, and leaf responses have received greater attention than stems and roots. Here, we examine the short-term temperature acclimation of leaf, stem and root respiration within individuals of eight disparate species acclimated to five temperatures, ranging from 15 to 35 °C. To assess acclimation, we measured instantaneous tissue temperature response curves (14–50 °C) on each individual following a 7-day acclimation period. In leaves and photosynthetic stems, the acclimation temperature had little effect on the instantaneous tissue temperature response of respiration, indicating little to no thermal acclimation in these tissues. However, respiration did acclimate in non-photosynthetic tissues; respiratory rates measured at the acclimation temperature were similar across the different acclimation temperatures. Respiratory demand of photosynthetic tissue increased with acclimation temperature as a result of increased photosynthetic demands, resulting in rates measured at the acclimation temperature that increased with increasing acclimation temperature. In non-photosynthetic tissue, the homeostatic response of respiration suggests that acclimation temperature had little influence on respiratory demand. Our results indicate that respiratory temperature acclimation differs by tissue type and that this difference is the consequence of the coupling between photosynthesis and respiration in photosynthetic, but not non-photosynthetic tissue. These insights provide an avenue for improving the representation of respiratory temperature acclimation in large-scale models.

Keywords: Carbon cycling; climate change; Rₑ; respiratory demand; terrestrial biosphere models; warming.

Introduction

Respiratory carbon release from the land surface is one of the largest fluxes of carbon dioxide (CO₂) between the atmosphere and the Earth’s surface. Respiration by plants makes up about half of this flux (Ciais et al. 2013). As a result, terrestrial biosphere models are highly sensitive to the representation of plant respiratory processes, including respiratory thermal acclimation.
(Atkin et al. 2008; Booth et al. 2012; Slot et al. 2014; Lombardozzi et al. 2015; Smith et al. 2016; Huntingford et al. 2017), a process that may become increasingly important as climates warm globally (Ciais et al. 2013). While thermal acclimation of plant respiration has been observed in a variety of studies, it is still not often included in terrestrial biosphere model simulations (Smith and Dukes 2013), likely due to a poor mechanistic understanding of the response (Atkin et al. 2005).

Thermal acclimation of respiration is defined as a change in the instantaneous response of respiration to temperature as a result of a longer-term change in temperature (Atkin and Tjoelker 2003; Atkin et al. 2005; Smith and Dukes 2013). This commonly results in some combination of a decrease in the slope of the relationship between respiration and temperature and/or a reduction in respiratory rates measured at a common temperature (Atkin and Tjoelker 2003). This effect can dampen respiratory responses to temperature. As such, respiratory rates measured at a tissue temperature identical to the temperature to which the tissue is acclimated are relatively homeostatic across acclimation temperatures (Loveys et al. 2003; Slot and Kitajima 2015). These acclimation responses tend to be stronger in tissues developed at the new temperature or acclimated to the new temperature for a longer period of time (Atkin and Tjoelker 2003).

Acclimation responses likely depend, in part, on changes in maintenance demand that result from changes in temperature (Lambers et al. 1983; Amthor 1984). For instance, increases in temperature may result in increased maintenance demand to support functioning of non-photosynthetic enzymes, reducing the degree of respiratory acclimation observed in response to short-term changes in temperature. In support of this, studies (e.g. Loveys et al. 2003) have found that tissues developed at a new temperature show stronger respiratory acclimation than ones that developed before a change in acclimation temperature (Atkin et al. 2005) and respiratory acclimation in leaves tends to increase with time following the transfer to a new temperature regime (Slot and Kitajima 2015). This suggests that higher maintenance requirements in tissues developed before a temperature change may limit the degree of respiratory acclimation, particularly in leaves. Still, it is unclear how these mechanisms may play out in other plant tissues, such as stems and roots.

Respiratory thermal acclimation may differ by tissue type; however, this effect has received little attention in the literature (Atkin and Tjoelker 2003; Smith and Dukes 2013) and terrestrial biosphere models typically simulate stem and root respiration simply as a function of leaf respiration (Atkin et al. 2017). Leaves (e.g. Slot and Kitajima 2015), stems (e.g. Maseyk et al. 2008) and roots (e.g. Jarvis and Burton 2013) have each been observed to acclimate to changes in temperature in previous studies. Nonetheless, comparisons of acclimation among tissue types are rare.

For leaves and photosynthetic stems, acclimation is likely tied to temperature effects on photosynthetic biochemistry (Gifford 2003; Smith and Dukes 2017). Indeed, previous controlled environment (Smith and Dukes 2017) and space-for-time substitution (Atkin et al. 2015) studies have found that the ratio of leaf dark respiration to photosynthetic capacity is similar under different acclimation temperatures. Notably, Smith and Dukes (2017) showed non-homeostatic leaf respiratory responses to five acclimation temperatures in 11 species, an effect that coincided with increases in photosynthetic capacity.

In non-photosynthetic tissues, such as woody stems and roots, one would not expect respiration to be closely linked to photosynthetic processes. Instead, demand for respiratory products in these tissues is more likely to be related to growth and transport (Tjoelker et al. 1999; Covey-Crump et al. 2002; Atkin et al. 2007). If these processes are not influenced by changes in acclimation temperature, one might expect a greater degree of observed acclimation in non-photosynthetic tissues (e.g. as seen by Loveys et al. 2003). Additionally, spectral differences among tissues as well as insolation of roots by soil may result in non-uniform changes in tissue temperatures resulting from a change in air temperature. As acclimation is a response to tissue, not air, temperature, these differences could result in differential acclimation responses among tissues.

In one of the only studies that has examined respiratory temperature acclimation across multiple tissues, Loveys et al. (2003) found greater acclimation of roots than leaves in previously developed, but not newly developed leaves. The authors attributed this effect to the rapid growth and turnover of the roots, such that some portion of the measured roots had developed under the new temperatures (Loveys et al. 2003). However, this may have also been due to an increased demand for respiratory products in leaves due to higher rates of photosynthetic processes, but no change in demand in roots. Stem tissue was not compared to root and leaf tissue. In general, the dearth of studies examining acclimation differences among plant tissues limits our ability to understand and predict how respiratory fluxes will be influenced by temperature.

Here, we examine respiratory thermal acclimation of leaves, stems and roots in response to a short-term (i.e. 7-day) change in temperature in eight species (Betula alleghaniensis, Cucumis sativa, Glycine max, Pinus nigra, Pinus pinaster, Pinus pinea, Pinus sylvestris and Zea mays). We assessed short-term thermal responses to mimic the types of changes that plants may experience over intra-annual timescales and because photosynthesis is known to acclimate over short time periods (Veres and Williams 1984; Battaglia et al. 1996; Atkin et al. 2000; Turnbull et al. 2002; Gunderson et al. 2010). We used a variety of plant species in order to make our results more generalizable across taxa, but did not have reason to expect species to differ in their responses. Individual plants were acclimated to one of five temperatures from 15 to 35 °C and the instantaneous response of respiration to temperature was measured for each tissue from 14 to 50 °C. We hypothesized that acclimation to warmer temperatures would reduce the instantaneous tissue temperature sensitivity of respiration for each tissue. We expected this reduced sensitivity to result in more homeostatic respiratory rates at the acclimation temperatures than would be expected from the instantaneous responses alone. We expected this acclimation to be greater in non-photosynthetic than in photosynthetic tissues because these tissues are not influenced by changes in photosynthetic processes that result from changes in acclimation temperature.

**Methods**

**Growth conditions**

We used species that varied in growth form, including trees (B. alleghaniensis, P. nigra, P. pinaster, P. pinea, P. sylvestris) and crops that included herbaceous species (C. sativa, G. max) and a grass (Z. mays) (Table 1). The individuals were grown from seed in a 50 %/50 % mixture of field soil (sandy loam; pH: 6.9) and potting soil (Sunpro Metro Mix 510; Sunpro Horticulture, Agawam, MA, USA) in 1.9-L pots. Plants were not pot-bound by the end of the experiment. Individuals were germinated and
grown for an initial period in controlled environment glass houses at −25 °C. Relative humidity inside the glass house was 58 % on average over the course of this growing period. The glasshouse was sprayed with reflective paint to reduce the risk of overheating. This also acted to reduce photosynthetically active radiation (PAR). As such, PAR was supplemented using 400-Watt overhead lights, with daily PAR reaching a maximum of ~1500 μmol m−2 s−1. Overhead lights were set to a constant 16/8-h light/dark schedule. Individuals were watered when soil became dry. Individuals were provided fertilizer (Miracle Gro 24-8-16 N-P-K; Scotts Company LLC, Marysville, OH, USA) following initial germination and about every 60 days thereafter to avoid nutrient limitation.

### Acclimation treatments

The time between germination and transfer to growth chambers differed by species. For trees, this period was ~6 months. As such, all trees were juveniles and ranged from ~30 to 50 cm in height. For annual species, this time period was ~1–2 months. In all cases, species were transferred before the production of reproductive tissues.

After germination in the glass houses and the initial growth period, individuals were transferred to environmentally controlled growth chambers (Conviron E15; Controlled Environments Inc., North Branch, MN, USA) for a 7-day acclimation period. Chambers were set to either 15, 20, 25, 30 or 35 °C (acclimation temperature or Tₐ; see Table 1 for the number of individuals of each species acclimated to each temperature). Relative humidity was set to 50 %, lights were set to a 16/8-h light/dark schedule with lights increasing in intensity (25 % every 15 min) during the first hour and decreasing in intensity (25 % every 15 min) during the last hour of the 16-h light period. Photosynthetically active radiation was ~1470 μmol m−2 s−1 during peak hours inside the chamber. Plants in the chamber were provided water when soil became dry. Each individual was acclimated to only one of the five acclimation temperatures.

### Gas exchange measurements

Following the 7-day acclimation treatment, instantaneous temperature response curves were developed for leaf (Rₐ,leaf), stem (Rₐ,stem) and root (Rₐ,root) dark respiration (R₀). To build these curves, R₀ measurements were taken at tissue temperatures of 14, 23, 32, 41 and ~50 °C using two LiCor 6400 portable photosynthesis systems running simultaneously (LiCor Biosciences, Lincoln, NE, USA) with a standard 3 cm x 2 cm chamber and attached light source turned off. The cuvette was sealed with putty to ensure there were no leaks. The lack of leaks was confirmed by leak tests (i.e. blowing on the chamber) during each measurement. All measurements proceeded inside the chamber following a 1-h dark adaptation period prior to the first measurement and during which the entire plant was placed in a dark growth chamber. The chamber was kept dark throughout the course of the measurements. Both the cuvette and growth chamber temperatures were adjusted to alter tissue temperatures. Measurements were made successively at progressively warmer temperatures from 14 to ~50 °C. Full temperature responses of leaves, then stems and then roots were recorded. For root measurements, roots were carefully removed from soil to reduce breakage and measured while attached to the plant. Tissue temperatures were measured using the internal thermocouple on the LiCor 6400, ensuring that tissues were in contact with the thermocouple during measurement. The warmest tissue temperature was set to the maximum temperature attainable by the machine and thus varied by individual (mean ± SE: leaf = 44.49 ± 0.013 °C, stem = 45.36 ± 0.012 °C, root = 45.14 ± 0.011 °C). For each temperature setting, we took 30 measurements over 30 s after matching the infrared gas analyzers. The average of these was used for analysis. One individual per portable photosynthesis system was measured each day for a total of two individuals measured per day. Note that Rₐ,stem was not measured for Z. mays due to the thickness of the stems. Rₐ,root was measured for all other species.

Prior to, but on the same day as, all respiratory measurements, net photosynthesis (A) by intercellular CO₂ (C) curves were taken on each individual. To build these curves, A/C, measurements were taken at leaf temperatures of 14, 23, 32, 41 and ~50 °C using the LiCor 6400 portable photosynthesis instruments (LiCor Biosciences, Lincoln, NE, USA). Both cuvette and growth chamber temperatures were adjusted to alter leaf temperatures. Responses were measured first at the temperature at which the plant was grown. This measurement was made to ensure stomata were open and responding to changes in CO₂ and was discarded prior to analysis. Measurements were then made successively at progressively warmer temperatures from 14 to ~50 °C. Leaf temperatures were measured using the internal thermocouple on the LiCor 6400. The warmest leaf temperature was set to the maximum temperature attainable by the machine and thus varied by individual (mean ± SE: 44.31 ± 0.10 °C). Light inside the chamber was set to a saturating rate of 1200 μmol m−2 s−1. Humidity inside the leaf chamber was maintained at ~60 %, but was occasionally lower at high temperatures. In those cases, water was added to the flow path by adding water (~< 5 mL) to the soda lime to achieve the highest level of humidity possible. A/C curves were generated using leaf chamber CO₂ values of (in order): 400, 300, 200, 100, 50, 400, 400, 600, 800, 1000, 1200, 1500 and 2000 μmol mol−1 CO₂ for C₃ species and 400, 300, 200, 100, 50, 0, 400, 400, 600 and 800 μmol mol−1 CO₂ for C₄ species. Photosynthetic parameters, including the maximum rate of

### Table 1. Number of individuals sampled per species per acclimation temperature for dark respiration and, in parentheses, dark respiration and photosynthesis. Tₛ = acclimation temperature. *Stem respiration was not measured for Z. mays.

| Species                     | Tₛ = 15 °C | Tₛ = 20 °C | Tₛ = 25 °C | Tₛ = 30 °C | Tₛ = 35 °C | Average |
|-----------------------------|------------|------------|------------|------------|------------|---------|
| Betula alleghaniensis       | 1 (1)      | 3 (3)      | 0 (0)      | 3 (3)      | 1 (1)      | 1.6 (1.6) |
| Cucumis sativa             | 3 (3)      | 2 (2)      | 1 (1)      | 1 (1)      | 2 (2)      | 1.8 (1.8) |
| Glycine max                 | 5 (2)      | 2 (0)      | 1 (1)      | 6 (6)      | 1 (1)      | 3 (2)   |
| Pinus nigra                 | 2 (2)      | 3 (3)      | 3 (2)      | 2 (2)      | 2 (2)      | 2.4 (2.2) |
| Pinus pinaster              | 1 (1)      | 1 (1)      | 3 (1)      | 2 (2)      | 4 (4)      | 2.2 (1.8) |
| Pinus pinea                 | 1 (1)      | 1 (1)      | 2 (2)      | 1 (1)      | 0 (0)      | 1 (1)   |
| Pinus sylvestris            | 1 (1)      | 1 (1)      | 2 (2)      | 3 (3)      | 3 (3)      | 2 (2)   |
| Zea mays*                   | 3 (3)      | 2 (2)      | 2 (1)      | 4 (0)      | 3 (0)      | 2.8 (1.2) |
| Average                     | 2.1 (1.8)  | 1.9 (1.6)  | 1.8 (1.3)  | 2.8 (2.3)  | 2 (1.6)    | 2.1 (1.7) |
Rubisco carboxylation ($V_{\text{max,acc}}$), the maximum rate of electron transport for Ribulose-1,5-bisphosphate regeneration ($V_{\text{max}}$) and, for C₄ species, the maximum rate of phosphoenol pyruvate carboxylation ($V_{\text{ppm}}$), were fit for each A/C ratio curve using the ‘plantcophys’ package in R (Duursma 2015), following Smith et al. (2009). We used planned contrasts to compare slopes of photosynthetic versus non-photosynthetic tissues to the acclimation temperatures following our original hypothesis. This was done using t-ratio tests using the ‘contrast’ function in the ‘emmeans’ package (Lenth 2018). Using the least squared mean slope and intercept values for the relationship between the response variables and $T_a$, we were able to calculate fitted $R_d$ values at different tissue temperatures ($T$). Specifically, we calculated $R_d$ at $T_a = T_r$. To assess the degree of acclimation for each, we used the ‘Homeostasis Method’ (Loveys et al. 2003). This involved calculating the ratio of $R_d$ at $T_a = T_r$ to $R_d^{\text{25}}$ at $T_r = 25^\circ \text{C}$ for plants acclimated to 15, 20, 30 and 35 °C, which we refer to as $\text{Acclim}_{\text{Homo}}$. For ease of comparison, $R_d^{\text{25}}$ at $T_r = 25^\circ \text{C}$ was used in the denominator when calculating $\text{Acclim}_{\text{Homo}}$, for plants acclimated to 15 and 20 °C and $R_d^{\text{35}}$ at $T_r = 25^\circ \text{C}$ was used in the numerator when calculating $\text{Acclim}_{\text{Homo}}$, for plants acclimated to 30 and 35 °C. As such, in all cases, $\text{Acclim}_{\text{Homo}}$ values of 1 indicate homoeostatic $R_d$ rates, with values further from 1 indicating progressively less homeostasis. We used plants acclimated to 25 °C as the reference because this was the temperature at which the plants were germinated and grown prior to being placed in the growth chamber.

Due to poor germination, death and equipment malfunctioning, the number of individuals per species per acclimation temperature differed. On average 2.1 individuals per species per acclimation temperature were measured. This sample size was the result of a choice to maximize the number of species used to assess the generality of our hypotheses, which were not species-specific, but rather tissue-specific. To match this hypothesis, we included acclimation temperature as a continuous variable in our models and did not include any interaction terms with species. Thus, the low number of species per acclimation temperature was not an issue for our models. Table 1 shows the number of individuals per species per acclimation temperature used for the analysis. The mixed-model analyses of variance used here are robust for handling unbalanced designs (Zuur et al. 2009).

All data used for the analyses described here can be found at [https://github.com/SmithEcophysLab/tissue_respiration](https://github.com/SmithEcophysLab/tissue_respiration) (doi: 10.5281/zenodo.3445384).

**Results**

The basal rate of dark respiration ($a$)

The parameter, $a$, that describes the rate of $R_d$ at a tissue temperature of 0 °C was not detectably influenced by tissue type, the temperature at which the plants were acclimated (acclimation temperature; $T_a$), or the interaction between the two factors ($P > 0.05$ in all cases; Table 2). Post hoc analyses of slopes did indicate a marginally significant increase in a rates with $T_a$ for roots ($P = 0.054$; Table 3; Fig 1), but no effect for other tissue types ($P > 0.10$ in all cases; Table 3; Fig 1). A planned contrast found no difference between slopes of the $a$–$T_a$ relationship between photosynthetic and non-photosynthetic tissue ($t_{13,21} = 1.79, P > 0.05$). The $a$ value did differ by species ($P < 0.05$; Table 2). Post hoc comparisons indicated that the only statistically different ($P < 0.05$) species combination was between the species with the lowest $a$ rates ($P. pinaster$; estimated
Table 2. Results from mixed-model analysis of variance testing thermal acclimation of instantaneous temperature response parameters across tissue types. *P values less than 0.05 are indicated in bold. $T_a$ = acclimation temperature, $a$ corresponds to the exponential rate of $R_d$ at 0 °C (µmol g$^{-1}$ s$^{-1}$), $b$ is a parameter describing the change in rates with temperature at temperatures near 0 °C and $c$ is a parameter describing the change in this increase with increasing temperature. $R_{d,acc}/V_{cmax,acc}$ is the ratio of dark respiration to the maximum rate of Rubisco carboxylation at tissue temperatures equal to the acclimation temperature.

| Species | $T_a$ | Tissue | $a$ | SE | df | $b$ | SE | df | $c$ | SE | df | $R_{d,acc}/V_{cmax,acc}$ | SE | df | $P$ |
|---------|-------|--------|-----|----|----|-----|----|----|-----|----|----|----------------------------|----|----|-----|
| Species | $T_a$ | Tissue | $a$ | SE | df | $b$ | SE | df | $c$ | SE | df | $R_{d,acc}/V_{cmax,acc}$ | SE | df | $P$ |
| Species | $T_a$ | Tissue | $a$ | SE | df | $b$ | SE | df | $c$ | SE | df | $R_{d,acc}/V_{cmax,acc}$ | SE | df | $P$ |

Table 3. Slopes of the response of the instantaneous temperature response parameters to $T_a$.* Slope indicates the least squared mean slope of the relationship between the parameter (i.e. $a$, $b$ or $c$) and $T_a$. The SE is the standard error of the least squared mean slope. Degrees of freedom (df) were estimated using Kenward-Roger approximation. The $t$-ratio test examined whether the slopes were significantly different from 0. Values with $P$ values less than 0.05 and 0.1 are indicated in bold and italics, respectively.

| Tissue | Photosynthetic | Slope | SE | df | t-ratio | $P$ | Slope | SE | df | t-ratio | $P$ | Slope | SE | df | t-ratio | $P$ |
|--------|---------------|-------|----|----|--------|----|-------|----|----|--------|----|-------|----|----|--------|----|
| Leaf   | Yes           | -0.027| 0.030| 219.7| -0.91 | 0.361| 0.002| 0.002| 221.9| 1.17 | 0.244| -0.000041| 0.000032| 221.9| -1.29 | 0.200|
| Stem   | Yes           | 0.002| 0.053| 222.9| 0.04 | 0.968| 0.001| 0.004| 222.6| 0.42 | 0.675| -0.000033| 0.000056| 222.6| -0.59 | 0.555|
| Stem   | No            | 0.060| 0.043| 222.7| 1.38 | 0.168| -0.005| 0.003| 222.7| -1.87 | 0.062| 0.000083| 0.000046| 222.7| 1.82 | 0.071|
| Root   | No            | 0.058| 0.030| 219.7| 1.94 | 0.054| -0.005| 0.002| 221.9| -2.56 | 0.011| 0.000094| 0.000032| 221.9| 2.99 | 0.003|

The thermal response of the instantaneous rate of dark respiration near 0 °C (b)

There was an interaction between tissue type and $T_a$ for the parameter, $b$, that describes the instantaneous response of $R_d$ to temperature near a tissue temperature of 0 °C ($P < 0.05$; Table 2). Post hoc analyses of slopes indicated that root $b$ decreased with $T_a$ ($P < 0.05$; Table 3; Fig. 1), and that non-photosynthetic stem $b$ similarly decreased with $T_a$, but this response was only marginally significant ($P = 0.062$; Table 3; Fig. 1). The post hoc slope analysis indicated that leaf and photosynthetic stem $b$ were not significantly influenced by $T_a$ ($P > 0.10$; Table 3; Fig. 1). These results suggested that non-photosynthetic tissue $b$ was more responsive to $T_a$ than photosynthetic tissue $b$, an effect confirmed by a planned contrast showing that slopes of the relationship between $b$ and $T_a$ differed between photosynthetic and non-photosynthetic tissue ($t_{res} = 2.96$; $P < 0.01$). The $c$ parameter did not differ by species ($P > 0.10$; Table 2).

Modelled thermal acclimation of dark respiration

We assessed thermal acclimation of dark respiration for each tissue type by modelling the instantaneous response for each $T_a$ assessed in the study (i.e. 15, 20, 25, 30 and 35 °C). We did this using the least squared mean slope and intercept values for the relationship between parameters $a$, $b$ and $c$ and $T_a$ from the mixed-model analysis of variance (Table 3) to calculate parameter values at $T_a$ values of 15, 20, 25, 30 and 35 °C. We also calculated modelled respiration rates at $T_a$ equal to the tissue temperature. These calculations showed that instantaneous responses to tissue temperature were strongest in photosynthetic tissues, leaves in particular, and dampened in non-photosynthetic tissue, roots in particular (Fig. 2).

Homeostasis of dark respiration under varying temperatures

This difference in thermal acclimation between photosynthetic and non-photosynthetic tissue was also apparent in $Acclim_{times}$ values (Table 4). Values closer to 1 indicate a greater degree of homeostasis in respiration rates. Leaves and photosynthetic stems had average $Acclim_{times}$ values of 0.58 and 0.55, respectively, while root and non-photosynthetic stems had values of 0.89 and 0.71, respectively (Table 4). This effect was driven by greater homeostasis at warmer temperatures in non-photosynthetic tissue. In fact, across all tissue types, $Acclim_{times}$ tended to be furthest from 1 in plants acclimated to 15 °C (average $= 0.48$; Table 4). This suggests that low acclimation temperatures tended to reduce $R_d$ regardless of tissue type.

The ratio of dark respiration to photosynthetic capacity

The ratio of dark respiration to the maximum rate of Rubisco carboxylation at the tissue temperature equal to $T_a$ ($R_{d,acc}/V_{cmax,acc}$)
depended on $T_a$ in some tissue types, but not others ($P < 0.01$; Table 2). Post hoc analyses of slopes indicated that root and non-photosynthetic stem $R_{d,acc}/V_{max,acc}$ decreased with $T_a$ ($P < 0.05$ in both cases; Fig. 3), but that neither leaf nor photosynthetic stem $R_{d,acc}/V_{max,acc}$ was influenced by $T_a$ ($P > 0.05$ in both cases; Fig. 3). These results suggest that non-photosynthetic tissue $R_{d,acc}/V_{max,acc}$ was more responsive to $T_a$ than photosynthetic tissue $R_{d,acc}/V_{max,acc}$. This conclusion was supported by a planned contrast showing that slopes of the relationship between $R_{d,acc}/V_{max,acc}$ and $T_a$ differed between photosynthetic and non-photosynthetic tissue ($t_{154} = -2.00; P < 0.05$). The $R_{d,acc}/V_{max,acc}$ varied by species ($P < 0.01$; Table 2). Post hoc comparisons found that the only species that differed significantly in $R_{d,acc}/V_{max,acc}$ ($P < 0.05$) was that with the highest ratio ($P. pinea$; estimated marginal mean $R_{d,acc}/V_{max,acc}$ across tissue types $= 0.041$) and that with lowest ratio ($Z. mays$; estimated marginal mean $R_{d,acc}/V_{max,acc}$ across tissue types $= 0.010$).

**Discussion**

Here, we asked: As is commonly assumed by large-scale models (e.g. Oleson et al. 2013), is thermal acclimation of dark respiration ($R_d$) similar across tissue types (leaves, stems and...
roots? And, if not, then why do tissue types differ? Using eight species across four diverse plant functional types, we found that thermal acclimation in response to a short acclimation period (7 days) was apparent in non-photosynthetic tissues, but was not observed in photosynthetic tissues. This pattern is consistent with the results found by Loveys et al. (2003), who found Acclim_{th} values of leaves and roots that were nearly equivalent to the results found here. Our stem results provide further insight into the mechanisms driving this response and suggest that photosynthetic tissues have reduced short-term thermal down-regulation of dark respiration, thus decreasing Acclim_{th} values. Photosynthetic data taken on the same individuals showed that increases in T_a resulted in an increase in maximum rates of Rubisco carboxylation (V_{cmax}) and electron transport (J_e) (Smith and Dukes 2017). This effect likely increased respiratory demand for photosynthetic processes, which may have limited any respiratory down-regulation.

Past work has shown that dark respiration acclimation of leaves is primarily driven by acclimation of V_{cmax} (Wang et al. 2018). Indeed, V_{cmax} has been used as a proxy to model leaf dark respiration for decades (Farquhar et al. 1980; Collatz et al. 1991). Our results support the idea that dark respiration of photosynthetic tissue is highly correlated to V_{cmax} as evidenced by the fact that R_{d,acc}/V_{cmax,acc} ratios were similar across all acclimation temperature treatments in photosynthetic tissues. As such, V_{cmax} may be a suitable proxy for simulating dark respiration thermal acclimation of photosynthetic tissue in large-scale models.

However, our results suggest that dark respiration is less sensitive to changes in acclimation temperature in non-photosynthetic tissue than in photosynthetic tissue. This was particularly true for acclimation temperatures between 20 and 35 °C. At these temperatures, rates of dark respiration were relatively homeostatic (Acclim_{th} values ranging from 0.74 to 1.07). This may have been because growth and maintenance demands remained similar across these temperatures in these tissues, coupled with reduced respiratory temperature limitation at these temperatures. Indeed, at 15 °C Acclim_{th} values tended to drop in all tissues, indicating that this low acclimation temperature may have induced some degree of temperature limitation to dark respiration.

The strong acclimation responses of non-photosynthetic tissue are consistent with previous stem and root respiratory thermal acclimation studies (e.g. Maseyk et al. 2008; Jarvis and Burton 2013). Our results do not fully clarify the drivers of the observed response. Our results do, however, suggest that non-photosynthetic tissue respiration cannot be modelled using photosynthetic tissue responses, as is commonly done (e.g. Oleson et al. 2013), as this approach may overestimate non-photosynthetic tissue respiration at high temperatures. Further work is necessary to understand the mechanisms driving temperature responses of non-photosynthetic dark respiration in order to reliably simulate this process in models.

Our results may have been related to the length of our acclimation time period. We chose a 7-day acclimation period to simulate short-term (i.e. intra-annual) variation in temperature that these plants might experience in the field. Slot and Kitajima (2015) used a meta-analysis to examine the T_a thermal acclimation of leaves, and found that acclimation increased with increasing duration of the experimental treatment. Thus, the leaves (and potentially other tissues) in our experiment may not have had time to fully adjust to the changed conditions. Additionally, the lack of leaf dark respiration acclimation observed in the photosynthetic tissue in our study contrasts with strong acclimation responses seen in longer-term studies of leaves (Heskel et al. 2016; Reich et al. 2016). However, this does not explain the acclimation seen by non-photosynthetic tissue in our study and, indeed, those studies did not report data on photosynthetic capacity (e.g. V_{cmax}), which may have helped to explain the respiration acclimation observed. Further studies that examine the timescale of respiratory temperature acclimation across multiple tissue types would support a more refined representation of acclimation in large-scale models.

While the b and c parameters defining the shape of the temperature response curve did not differ by species, the a parameter defining the basal rate did show species specificity. This indicates that the species in this study did not show variation in the shape of the acclimation response, but did vary in the magnitude of their respiratory rates. These results were not surprising given previous reports of wide variation in basal respiration rates among species (e.g. Reich et al. 2007; Atkin et al. 2015; Heskel et al. 2016; Smith and Dukes 2018). The goal of our study was not to determine differences across species, but rather to use multiple species to broadly examine tissue-specific acclimation responses, which was reflected in the design of our statistical models in that species by T_a interactions were not included. Nonetheless, the low number of species used here

### Table 4

Calculated homeostasis (Acclim_{th}) values for each tissue at each acclimation temperature (T_a). All values are in relation to R_A values at T_a = 25 °C. Values closer to 1 indicate a greater degree of homeostatic acclimation. T_a = acclimation temperature; Ps = photosynthetic.

| T_a  | Leaf | Ps stem | Non-Ps stem | Root | Average |
|------|------|---------|-------------|------|---------|
| 15 °C| 0.45 | 0.42    | 0.45        | 0.62 | 0.48    |
| 20 °C| 0.67 | 0.65    | 0.74        | 0.87 | 0.73    |
| 30 °C| 0.70 | 0.67    | 0.86        | 1.00 | 0.81    |
| 35 °C| 0.52 | 0.47    | 0.82        | 1.07 | 0.72    |
| Average | 0.58 | 0.55 | 0.72 | 0.89 | 0.68 |

Figure 3. The effect of acclimation temperature (T_a) on the ratio of dark respiration to the maximum rate of Rubisco carboxylation at tissue temperature equal to the acclimation temperature (R_{d,acc}/V_{cmax,acc}) for each tissue type leaf, photosynthetic (Ps) stem, non-Ps stem and root values are indicated by pink squares, red circles, grey circles and blue triangles, respectively. Leaf and root points are jittered along the x-axis by −0.8 and 0.8 °C, respectively, to improve visibility. Significant (P < 0.05) slopes are shown with solid lines, with colours corresponding to tissue type (i.e. black = non-Ps stem, blue = root). Slope values are least squared means from the mixed-model analyses of variance. The line equations are y = e^{−0.063x−2.94} for black (i.e. non-photosynthetic stem) and blue (i.e. root) lines, respectively. Data are plotted on a log scale.
was a limitation of our study and future work should build upon these results and examine whether tissue-specific temperature acclimation varies by species or plant type.

Taken as a whole, our results support the idea that respiration processes in models need to be timescale- and tissue-dependent. While we provide the data necessary to parameterize such statistical models (https://github.com/SmithEcophysLab/tissue_respiration), we suggest that these data instead be used to test and develop more mechanistic models of plant \( R \). Our results, coupled with those from previous studies mentioned above, suggest some core principles acting to drive respiration responses and acclimation to temperature. First, respiratory processes, under many conditions, are likely driven by demand for respiratory products. Respiration in plants acts to support processes such as enzyme turnover, carbohydrate export and growth (Amthor 1984) and many models, at least at the leaf scale, are already designed based on this principle and are capable of acting at timescales longer than instantaneous (i.e. they include acclimation) (Atkin et al. 2017). This mechanism could be extended to non-photosynthetic tissues, which, as we show in this study, are likely to acclimate differently than photosynthetic tissues.

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Contributions by the Authors

N.G.S., G.L. and J.S.D. designed the study. N.G.S. and G.L. carried out the research and performed the analyses. N.G.S., G.L. and J.S.D. wrote the manuscript.

Conflict of Interest

None declared.

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Data Accessibility

All data and analysis scripts can be accessed at https://github.com/SmithEcophysLab/tissue_respiration (doi: 10.5281/zenodo3445384).

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