

Thermal acclimation of leaf dark respiration of beech seedlings experiencing summer drought in high and low light environments

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Summary Little is known about how environmental factors shape the short- and long-term responses of leaf respiration to temperature under field conditions despite the importance of respiration for plant and stand carbon balances. Impacts of water availability and canopy cover on leaf dark respiration (R) and temperature sensitivity were assessed in beech (*Fagus sylvatica* L.) seedlings in a sub-Mediterranean population. We studied seedlings established within canopy gaps (39% global site factor; GSF) that were subject to either no watering (unwatered plants; UW) or regular watering (2–10% higher volumetric topsoil water content as summer progressed; W plants) and seedlings established beneath the adjacent understorey (12% GSF). Leaf R rose exponentially with diurnal increases in temperature; the same temperature sensitivity (Q_{10} : 2.2) was found for understorey and gap plants, irrespective of watering treatment. Respiration estimated at 25 °C (R_{25}) was lower in the understorey than the gaps and was significantly lower in the unwatered than in the watered gap plants by the end of summer (0.65 versus 0.80 $\mu\text{mol m}^{-2} \text{s}^{-1}$). R_{25} declined with increasing summer temperature in all plants; however, respiration estimated at the prevailing ambient temperature did not change through the summer. There were parallel declines in R_{25} and concentrations of starch and soluble sugars with increasing summer temperature for gap plants. We conclude that seasonal shifts in temperature–response curves of beech leaf R occur in both low- and high-light environments; since leaf R decreased with increasing plant water deficit, such shifts are likely to be greater whenever plants experience summer drought compared to scenarios where plants experience high rainfall in summer.

Keywords: canopy closure, *Fagus sylvatica*, temperature sensitivity, water deficit.

Introduction

A key factor in determining the biosphere's response to global climate change is the impact of the abiotic environment on rates of leaf respiration (R) (Ryan 1991, Valentini et al. 2000, Atkin et al. 2008). Leaf R rises exponentially in response to short-term increases in temperature owing to the temperature dependence of respiratory enzymes. However, the response to temperature can differ over longer time periods as respiratory metabolism acclimates to sustained changes in growth temperature (Atkin and Tjoelker 2003). Thermal acclimation of leaf R has been observed in a growing number of evergreen and broad-leaved deciduous species (Teskey and Will 1999, Tjoelker et al. 1999a, 1999b, Atkin et al. 2000, Bolstad et al. 2003, Loveys et al. 2003, Lee et al. 2005, Bruhn et al. 2007, Campbell et al. 2007, Ow et al. 2008a, 2008b, Tjoelker et al. 2008, 2009, Zaragoza-Castells et al. 2007, 2008). It can result in near-constant rates of average daily leaf R (i.e., homeostasis) in response to seasonal shifts in growth temperature (Atkin et al. 2005). Such reduction in carbon release through respiration plays a role in maintaining a positive carbon balance and probably helps trees to survive in ecosystems where photosynthesis is seasonally impaired (Zaragoza-Castells et al. 2008). Moreover, acclimation is likely to influence global and regional-level terrestrial carbon exchange responses to climate change (Wythers et al. 2005, King et al. 2006, Atkin et al. 2008).

Since pioneering studies on temperature effects on respiration (Wager 1941, Rook 1969, Billings et al. 1971), two types of R acclimation (Types I and II; Atkin and Tjoelker 2003) have been identified in plants. Type I acclimation is underpinned by changes in the short-term temperature dependence of R [i.e., changes in the Q_{10} (the proportional change in leaf R per 10 °C change in temperature)] that result from changes

in substrate supply and/or energy demand (Covey-Crump et al. 2002). Temperature-mediated changes in respiratory capacity and basal rates of R underpin Type II acclimation (Armstrong et al. 2006). There are reports (e.g., Armstrong et al. 2008) of both types of acclimation occurring within individual plants, with Type I acclimation being followed by Type II acclimation following sustained changes in growth temperature. In other studies, only Type II acclimation was observed (e.g., Atkin et al. 2000, Bruhn et al. 2007, Zaragoza-Castells et al. 2008, Tjoelker et al. 2009). Given the importance of thermal acclimation of leaf R for whole-plant and ecosystem CO_2 exchange, further studies are needed that document the relative contribution of Types I and II acclimation, particularly under field conditions. The extent to which acclimation is associated with changes in the underlying chemistry of leaves [e.g., concentrations of soluble substrates and/or tissue nitrogen concentrations; Tjoelker et al. (2009)] under field conditions also needs to be better understood.

To fully predict variations in leaf R under field conditions, an understanding is needed on how other abiotic factors (e.g., drought and shade) influence rates of leaf R at any given reference temperature and how such factors shape responses of leaf R to temperature. When grown under shaded conditions, many plant species exhibit lower rates of leaf R compared to plants grown under high-light conditions (Boardman 1977, Sims and Pearcy 1991, Turnbull et al. 1993, Noguchi and Terashima 1997, Lusk and Reich 2000, Noguchi et al. 2001, Zaragoza-Castells et al. 2007), reflecting a shade-induced reduction in the respiratory capacity (i.e., lower amount of mitochondria and amount/activity of mitochondrial enzymes; Noguchi et al. 2005) and/or the availability of carbohydrates (Sims and Pearcy 1991). Similarly, moderate drought often results in lower rates of leaf R (Lawlor and Cornic 2002, Flexas et al. 2005, Atkin and Macherel 2009), with leaf R sometimes increasing in response to more severe drought (Flexas et al. 2005, Slot et al. 2008). Flexas et al. (2005) suggested that while mild water stress reduces respiratory energy demand and/or substrate supply, more severe stress could enhance demand associated with the activation of tolerance mechanisms. Potentially, shade and drought may also influence the temperature dependence of R . For example, depending on the duration and severity of stress, shade- and drought-induced restrictions of photosynthesis can reduce short-term temperature dependence of R in a short-lived herbaceous plant species (Slot et al. 2008). In contrast, a recent field study has reported little impact of severe shade on the Q_{10} or degree of acclimation in a long-lived evergreen tree species (Zaragoza-Castells et al. 2008). Whether the same is true for deciduous plant species experiencing contrasting growth irradiances and summer drought is, however, not clear.

At the southern range of its distribution in Europe, the early establishment of temperate deciduous tree species such as beech (*Fagus sylvatica* L.) is hampered by several abiotic stresses in summer, including soil drying, high air temperatures and high vapour pressure deficits (Robson et al. 2009). Global climate change in southern Europe is likely

to result in further increases in temperature and aridity (Rowel and Jones 2006), further exacerbating reductions in seedling establishment (Sabaté et al. 2002, Bréda et al. 2006, Jump et al. 2006, Piovesan et al. 2008). How rates of leaf R of deciduous tree species respond to differences in growth irradiance, soil moisture and air temperature is likely to influence their recruitment success. It is thus useful that further work be conducted assessing the interactive effects of drought, shade and temperature on the respiratory rates of European deciduous tree species.

In this study, we quantify seasonal variations in daily temperature–response curves of seedling leaf R in a population of beech (*F. sylvatica* L.) growing at the most south-westerly limit of this species in Europe. To investigate whether growth irradiance alters the short- and long-term temperature dependence of R , we compared the response of seedlings beneath the forest canopy and within adjacent canopy gaps. The impact of summer drought on the temperature responses of R was also assessed via comparison of watered and unwatered plants growing in gaps under high irradiance. We tested the following hypotheses: (i) rates of leaf R are lower under drought and shaded conditions, compared to plants growing under well-watered and high irradiance conditions; (ii) drought and shade reduce the short-term temperature dependence of leaf R ; and (iii) irrespective of growth irradiance or water availability, large shifts in the daily temperature–response curve of leaf R occur in response to seasonal changes in growth temperature.

Materials and methods

Study area and experimental design

Our study was carried out in a mixed beech–oak forest (*F. sylvatica* L., *Quercus petraea* [Mattuschka] Liebl. and *Quercus pyrenaica* Willd.) located in the mountain ranges of Madrid province, central Spain (3°30' W, 41°07' N; 1400 m a.s.l.). The climate is sub-Mediterranean, with moderate annual rainfall (958 mm), cold winters (2.3 °C mean temperature in the coldest month) and a 2-month dry/warm period in July–August (precipitation/mean air temperatures: July 27.9 mm/18.8 °C and August 32.6 mm/17.8 °C; data from a meteorological station in the forest operating since 1994). Soils are sandy loam, generally deep, fertile, well-drained and with moderate moisture-holding capacity.

In the spring of 2007, beech (*F. sylvatica* L.) nuts (collected from five mother trees) were sown in two canopy gaps (over a 3 × 2 m² plot within each gap) that were 40 m apart. The gaps had been created by the falling of ancient beech trees. To assess the impacts of increased soil moisture on leaf dark respiration (R), the plots were split into two 1.3 × 2 m² watered (W) and unwatered (UW) subplots separated by a 0.4-m unwatered buffer zone. Plants were watered throughout the summers of 2007 and 2008, with irrigation commencing on June 27th in 2008, the year in which this study was conducted. Irrigation consisted on adding 40 l water m⁻² of ground area every 7–10 days. In addition, to assess the impacts of canopy closure on

leaf *R*, beech nuts were also sown in 2007 in the adjacent understorey of mature beech trees where seedlings experienced reduced irradiance in spring–summer following formation of the overhead canopy. Unfortunately, high mortality of seedlings at the understorey site during the first year resulted in there being insufficient plants to assess the impact of irrigation [for further details, see Robson et al. (2009)]; consequently, data were only available for UW plants at the shaded understorey site. At both the gap and understorey sites, plants were fenced off with netting to protect them from animal browsing, with the area around seedlings being regularly weeded to minimize growth of grass species.

Environmental conditions

Canopy closure was quantified by means of hemispherical photographs. These were taken under diffuse radiation during late summer of 2007 at six points in the understorey and gap sites. Mean annual global site factor [(GSF) i.e., the proportion of global solar radiation at a given location relative to that in the open], was calculated with the canopy analysis software Hemiview 2.1 (Delta-T devices Ltd, USA).

Air temperature and relative humidity at 15 cm height above the ground were monitored at 3-min intervals (Hobo H08-032-08, Onset, Pocasset, MA, USA). The volumetric soil water content at 10 and 30 cm depth was measured five times during the study with a time domain reflectometer (Trase System I, Soil Moisture Equipment, Santa Barbara, CA, USA). In addition, we measured leaf water potential (Ψ_{pd}) in the night with a pressure chamber on 12 gap seedlings per watering treatment (six in June); Ψ_{pd} was measured only in late August in the understorey owing to the small number of leaves on seedlings. Precipitation and air temperature were measured, with a rain gauge (Mod. 52202, Campbell Scientific, Canada Corp. Edmonton, Canada) and a temperature sensor (Mod. 50U-44212, Geonica SA, Spain) above the tree canopy on top of a scaffolding tower.

Leaf development

We monitored apical bud growth and subsequent leaf expansion (via repeated measures of leaf length once or twice a week) from mid-April until when leaf size was constant. We further analysed leaf development in plants growing at the gap site by sampling dry mass per unit area (LMA) at eight time points throughout the growing season in 2008. Due to limited numbers of seedlings, measurements of LMA at the understorey site were restricted to the final harvest in August 2008. As an estimation of senescence, we counted how many of the plants used for measuring gas exchange on the last date (see below) exhibited yellowing leaves in October 2008.

Gas exchange measurements

We measured CO_2 exchange of attached, fully expanded leaves using an infrared gas analyser system (LI-6400;

Li-Cor Inc., NE, USA). Measurements of leaf *R* were made at the prevailing ambient air temperature [as described in previous studies: Atkin et al. (2000), Griffin et al. (2002) and Zaragoza-Castells et al. (2008)] and relative humidity (typically 40–60%); an atmospheric CO_2 concentration of 400 p.p.m. CO_2 (using the built in CO_2 mixer); and a flow rate of $300 \mu\text{mol s}^{-1}$. On each visit to the field site, four to six replicate plants (see below) were used for measurements of leaf *R*, using one south-facing leaf per plant. To assess how *R* varied in response to diurnal changes in temperature, we repeatedly measured CO_2 efflux rates in darkness (using the same region of each leaf) for each plant several times over the course of three consecutive days. Measurements were made at 1–2-h intervals, during the night and daytime (to expand the temperature range over which *R* in the dark was measured). For daytime measurements, entire plants were darkened for 30 min prior to measurement to avoid post-illumination transients (Azcón-Bieto and Osmond 1983, Atkin et al. 1998). To avoid substrate depletion, every plant was exposed to ambient light for at least 1 h between measurements of dark-adapted *R* (Azcón-Bieto 1992). We also measured stomatal conductance (g_s) and net photosynthesis (P_n) in the same leaves under saturating irradiance (600 and $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ for understorey and gap plants, respectively), at a CO_2 concentration of 400 p.p.m. and a leaf temperature of 25°C (controlled using the Licor 6400 temperature control system). Measurements of P_n were made in the mid-late morning.

Sampling of leaf gas exchange was made in June (18–20), July (16–18), early August (3–5) and late August (20–22) in 2008. In most cases, measurements were made using six replicate plants (except in June when four replicate plants were measured). At the gap site, different W and UW plants were used on each occasion; however, the limited number of UW plants at the understorey site necessitated that the same plants be used on each occasion. All measurements were made on leaves from the single spring flush of growth (no additional leaves were produced during the growing season in understorey plants). Most gap plants started to produce a second growth flush in mid-July; although we continued to take measurements only on leaves from the first flush, six plants with either one or two growth flushes were sampled in early and late August.

Dry mass and chemical analysis

For gap-grown W and UW plants, the same leaves used for Ψ_{pd} and leaf gas exchange measurements were harvested, oven dried for 2 days at 70°C and then weighed. Thereafter, leaves were ground for determination of nitrogen concentration by the Kjeldhal procedure. Soluble sugars and starch were extracted and measured as described by Loveys et al. (2003).

The values of dry mass of the leaf portion contained in the chamber were used to express leaf *R* and P_n of the gap-grown W and UW plants (for each sampling date) on a dry-mass basis (in addition to the area-based measurements made with

the Licor 6400). Because it was not possible to destructively harvest understorey leaves through the summer (due to the limited numbers of plants), understorey leaves were only collected at the last sampling date (20 August); consequently, while estimates of understorey leaf R expressed on an area basis were obtained throughout the summer, expressing rates of R per leaf dry mass was only possible for the last harvest date at the understorey site.

Temperature–response curves

The response of leaf R to diurnal changes in temperature was explored by using Q_{10} values for plants growing at the gap site (where leaves experienced a large diurnal range of temperatures). Firstly, we explored whether the Q_{10} of gap-grown plants varied with measuring temperature; we plotted data points of $\log R$ versus temperature of each plant and explored the adjustment to linear and polynomial models. Linear models fitted well to data (mean $R^2 = 0.94$), indicating that an average Q_{10} value was adequate to describe the short-term temperature sensitivity of R (Atkin et al. 2005). Because of this, exponential curves were then fitted to individual plants' R versus temperature data according to:

$$R_T = R_{\text{RefT}} e^{kT} \quad (1)$$

where R_T is the predicted rate of R at any given measuring temperature, and R_{RefT} is the rate of R at a reference temperature. Using non-linear least squares regression methods with the software Statistica 6 (Statsoft Inc., Tulsa, USA), we estimated R_{RefT} at a reference temperature of 0 °C and k , a temperature coefficient. Values of k were then used to estimate the Q_{10} of individual plants according to:

$$Q_{10} = e^{10k}. \quad (2)$$

Finally, we used Eq. (1) to estimate R at 25 °C (R_{25}) and at the mean ambient temperature averaged for the 7-day period preceding gas exchange measurements (R_{amb}).

Due to fewer observations and the more limited temperature range experienced by plants in the understorey, it was not possible to calculate individual replicate Q_{10} , R_{25} and R_{amb} values for these plants. Rather, we calculated Q_{10} , R_{25} and R_{amb} for each sampling date using pooled data of all replicate plants on each sampling date.

Statistical analyses

To test for significant differences (at $P < 0.05$) in the variables studied at the gap site among dates and watering treatments, data were subjected to analysis of variance (ANOVA) using general linear models. The models tested for the main effects of date (3 df), watering (1 df) and gap replication (1 df) and the interaction between date and watering. Following a significant effect of date, means were compared with Tukey's multiple comparisons tests. In addition, we used ANOVA to test

for significant differences between watered and unwatered plants in the gaps on individual dates. The model included watering and gap replication as fixed factors. In August, for variables Q_{10} , R_{25} and R_{amb} , we examined the effect of growth flush (1 df) with ANOVA. Since the number of growth flushes had no effect on leaf respiration (of first-flush leaves), nor on respiration responses to watering, we present the data pooled for growth flushes within watering treatments. Variables were transformed when necessary to attain a normal distribution.

For each sampling date, we used analysis of covariance to examine differences in R between the understorey and the gap site (for unwatered plants only), setting the leaf temperature as a covariate. Comparisons were made for the range of temperatures common to both sites. All analyses were performed with the software Statistica 6 (Statsoft Inc., Tulsa, USA).

Results

Environmental conditions and plant water status

Spring 2008 was relatively wet and cold for the study area. We recorded 382 mm of rain from 10 April to 1 July (data before 10 April not available). This is the largest amount of rain recorded from 1 April to 1 July in the 14 years prior to 2008 (Figure 1a); mean air temperature was 10.3 °C over this period (1 °C lower than 14-year average; measured over the canopy top). Summer 2008 was dry, with 41 mm of rain during July and August (31% lower than average) but slightly colder than average (18 versus 18.3 °C). Although there was substantial day-to-day variation in daily maximum and minimum temperatures, the general trend was for temperatures to progressively increase from late spring to mid August. Maximum temperatures at the gap site were substantially higher than those at the understorey site; there was very little difference in the minimum temperatures recorded at the two sites. As a result, a larger diurnal range of temperature was achieved at the gap site (≈ 20 °C) than at the understorey site (≈ 10 °C) (Figure 1a).

Radiation received by gap plants was significantly higher ($P < 0.001$) [GSF = 0.39 ± 0.05 (equivalent to 10.8 ± 1.1 mol photons $\text{m}^{-2} \text{day}^{-1}$ of photosynthetically active radiation in summer)] than that in the understorey [GSF = 0.12 ± 0.01 (2.9 ± 0.2 mol photons $\text{m}^{-2} \text{day}^{-1}$)]. There was no difference in radiation received by W and UW plants at the gap site ($P > 0.25$).

Volumetric soil water content averaged from 10- and 30-cm depths declined through the summer, with the decline being less in watered (W) soils at the gap site compared to unwatered (UW) soils at the gap and the understorey sites (Figure 2a). Although irrigation at the gap site had very little effect on pre-dawn water potential (Ψ_{pd}) through early-mid-summer (Figure 2b), Ψ_{pd} was significantly lower in UW plants than their W counterparts by late August ($P < 0.001$).

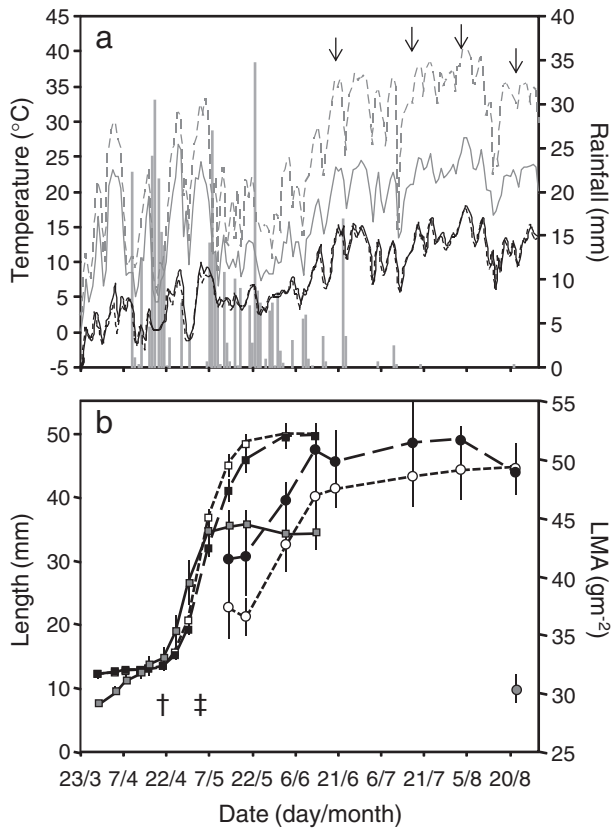


Figure 1. (a) Temporal variations in daily rainfall (vertical bars, mm) and maximum and minimum daily temperatures (°C) at the gap and understorey sites. For temperature data, grey and black lines are used to show maximum and minimum values, respectively, and dashed and solid lines are used to show values at the gap and understorey sites, respectively. Arrows show the dates of physiological measurements. (b) Changes in bud/leaf length of unwatered (open squares) and watered (closed squares) gap-site plants and understorey (grey squares) plants; leaf mass per area (LMA, gm⁻²) of unwatered (open circles) and watered (closed circles) gap-site plants on eight sampling dates and of understorey plants at the end of the experiment (grey circles). Error bars show 1 SE [$n = 30$ for bud/leaf length ($n = 18$ in the understorey)) and $n = 6$ for LMA (12 in the last two dates for gap plants)]. The dagger and double dagger indicate the dates when half of sampled plants broke buds in the understorey and gap sites, respectively.

Phenology

In 2008, apical buds of understorey plants were slowly enlarging from the end of March until 22 April, when bud break had occurred in half of the monitored plants; at that time point, no bud break had occurred at the gap site (Figure 1b). Once bud break occurred at the gap site, leaf expansion continued until early May in the understorey plants and late May in the gap plant. The timing of bud break, bud/leaf growth rate and final leaf size were similar in UW and W gap-site grown plants.

Dry mass per unit leaf area at the gap site increased, while leaves were expanding, and continued to increase rapidly just after the lamina had completely expanded (Figure 1b); subsequent variations in LMA were not statistically significant ($P =$

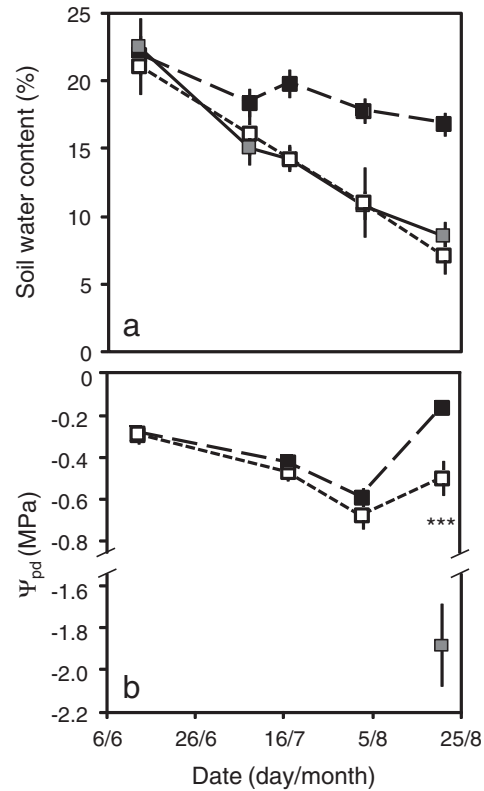


Figure 2. (a) Temporal evolution of volumetric soil water content (%) at the understorey (grey symbols), watered (closed symbols) and unwatered (open symbols) gap sites. (b) Pre-dawn leaf water potential (Ψ_{pd} , MPa) in the understorey plants at the end of the experiment and in the watered (W) and unwatered (UW) gap plants on four sampling dates; symbols as in (a). Error bars show 1 SE ($n = 6$ for soil water content and $n = 6-12$ for Ψ_{pd}). Significance of differences between W and UW gap plants within a date are indicated as *** $P < 0.001$, ** $P < 0.01$ and * $P \leq 0.05$.

0.795). This suggests that leaves were fully expanded and structurally developed by first sampling date for leaf gas exchange. Finally, leaf senescence appeared to occur earlier in plants growing in the gap compared to those in the understorey, as indicated by the higher percentage of plants exhibiting yellowing leaves on 16 October 2008 (58% and 33% in gap and understorey plants, respectively). In any case, it is unlikely that senescence modified leaf physiology and chemistry at the end of August.

Leaf gas exchange and chemistry

For all four measurement periods (June, July and early and late August), rates of respiratory CO₂ release increased exponentially with ambient temperature (Figure 3), with rates of leaf R being lower in plants grown at the understorey site than their gap-grown counterparts (analysis of covariance over a common span of measuring temperatures showed significantly lower rates at the understorey site irrespective of the sampling period; Figure 3a–d; all $P < 0.001$). At the gap site in June, little difference in rates of leaf R was exhibited by W and UW plants (Figure 3a). However, at later dates,

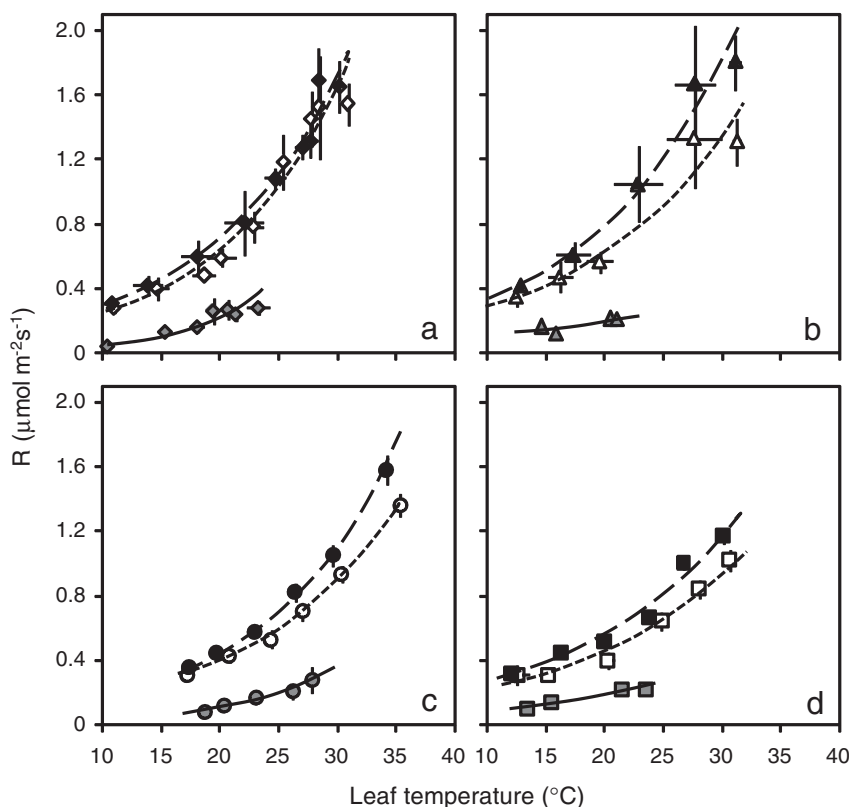


Figure 3. Relationship between leaf respiration (R) and temperature for plants on four sampling dates [June (a), July (b), early August (c) and late August (d)]. Data shown are for the unwatered understorey (grey symbols, solid lines) and unwatered gap plants (open symbols, dotted lines) as well as for the watered gap plants (closed symbols, dashed lines). Error bars show 2 SE ($n = 6-12$). Lines correspond to exponential models fitted to data.

rates of leaf R were generally lower in UW gap plants (compared to their W counterparts) particularly at higher measuring temperatures (Figure 3b–d).

To further explore the impact of growth irradiance and watering regime on leaf R , we calculated rates of leaf R at a common temperature of 25 °C (R_{25}) and at the prevailing ambient temperature of the previous 7 days leading up to measurement of leaf R (R_{amb}). Figure 4a shows that predicted rates of area-based R_{25} decreased as average daily air temperatures increased from June to late August, with the R_{25} of gap plants being significantly lower in UW plants than their W counterparts. The relationships were strong ($R^2 > 0.70$), but they were not significant because only four temperatures (dates) were available. Moreover, R_{25} values were consistently lower in plants grown in the understorey compared to those at the gap site (Figure 4a); the same was true when R_{25} was expressed on a dry-mass basis (8.6 versus 13.1 $\text{nmol g}^{-1} \text{s}^{-1}$ for understorey and unwatered gap plants, respectively). In contrast to the changes in area-based R_{25} , predicted rates of area-based R_{amb} were rather insensitive to changes in average daily temperature (Figure 4b).

Predicted rates of R_{25} and R_{amb} on a leaf dry-mass basis in the gap site showed the same response to seasonal shifts in temperature as values expressed on an area basis (not shown). Mass-based R_{25} was significantly lower in UW plants than

their W counterparts in early August (11.3 ± 0.4 versus $13.1 \pm 0.2 \text{ nmol g}^{-1} \text{s}^{-1}$; $P < 0.001$) and late August (13.1 ± 0.6 versus $16.5 \pm 0.4 \text{ nmol g}^{-1} \text{s}^{-1}$; $P < 0.001$). Taken together, these data strongly suggest that rates of leaf R at the gap site are consistently lower in plants growing on drier soils than their well-watered counterparts.

No significant differences were found in Q_{10} values among any of the treatments, irrespective of growth irradiance or watering regime (Figure 4c). Thus, the seasonal decrease in R_{25} in all treatments (Figure 4a) resulted from decreases in the basal rate of leaf R (i.e., from differences in the elevation of temperature–response curves) rather than decreases in temperature sensitivity of leaf R . The average Q_{10} value of all data pooled was 2.2 (calculated for the temperature range 13.5–25.5 °C common to both understorey and gap plants).

For both watering treatments in the gap, summer variation in the concentration of starch (Figure 4d) and soluble sugars (Figure 4e) tracked shifts in the prevailing 7-day average air temperature. The change in R_{25} with temperature mirrored that of starch and soluble sugars but not that of nitrogen (Figure 4a, d–f). The concentration of starch and soluble sugars showed a positive relationship with R_{25} across dates (Table 1). For gap-grown plants, starch concentrations were slightly lower in UW plants than their W counterparts in August when air temperatures were high (Figure 4d). Water-

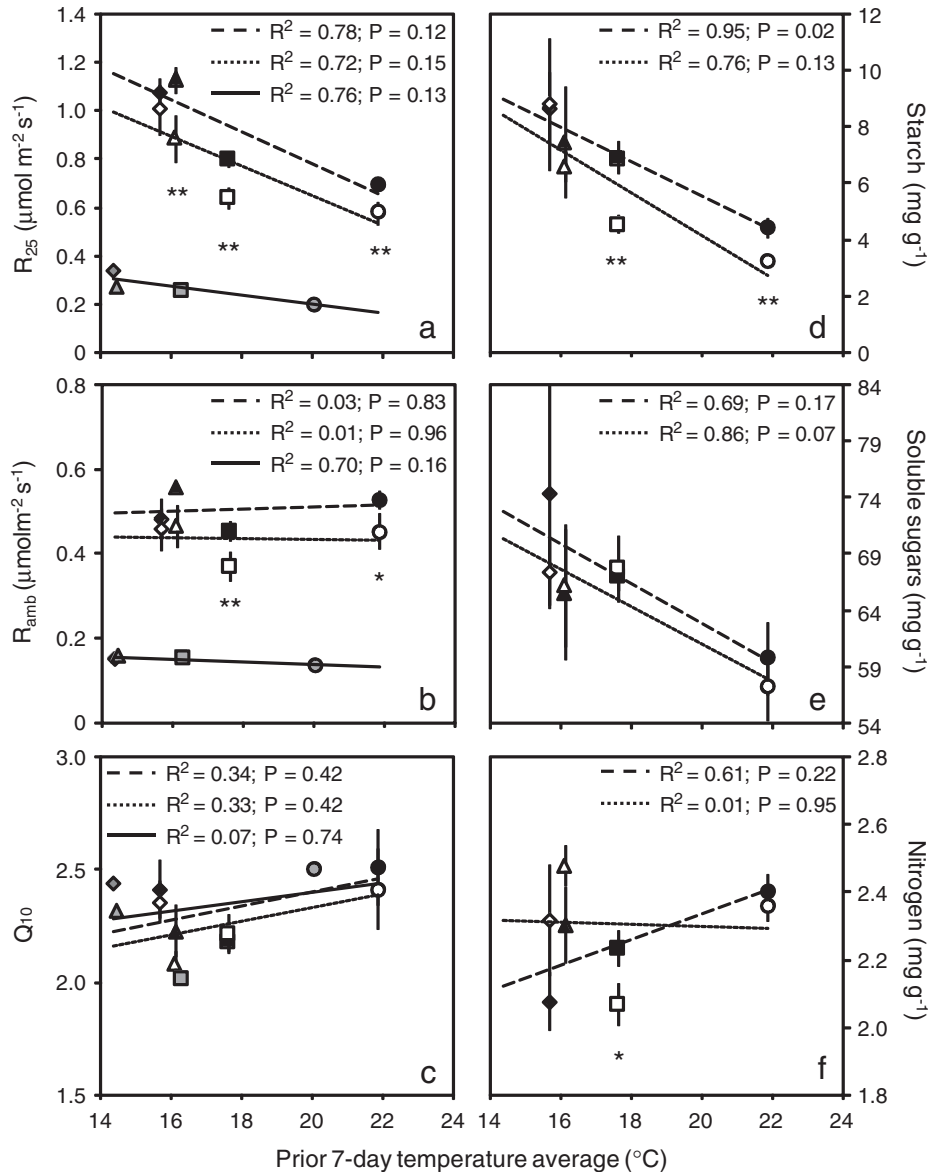


Figure 4. Variation of physiological and chemical traits with respect to mean ambient temperatures averaged for the 7-day period preceding measurements in June (diamonds), July (triangles), early August (circles) and late August (squares). (a) Estimated rates of leaf respiration (R , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) at a common measuring temperature of 25 °C (R_{25}); (b) estimated rates of leaf R at the 7-day average temperature (R_{amb}); (c) the Q_{10} of leaf R (i.e., the proportional increase in leaf R per 10 °C short-term rise in temperature); (d) starch concentration (mg g^{-1} dry mass); (e) soluble sugar concentration (mg g^{-1} dry mass); and (f) leaf total nitrogen concentration (mg N g^{-1} dry mass). Data shown are for the unwatered (UW) understory (grey symbols, solid lines) and UW gap plants (open symbols, dotted lines), as well as for the watered (W) gap plants (closed symbols, dashed lines). Error bars show 1 SE ($n = 6\text{--}12$ for variables of respiration, and $n = 6$ for variables of chemistry). Significance of differences between W and UW gap plants within a date are indicated as *** $P < 0.001$, ** $P < 0.01$ and * $P \leq 0.05$. The coefficients of determination and P values of the linear models are shown.

ing did not, however, have any effect on the concentrations of soluble sugars in any date, and it only did on nitrogen in late August (Figure 4e and f).

Figure 5 shows summer fluctuations in stomatal conductance (g_s), light-saturated photosynthesis (P_n) measured at 25 °C and the balance between R_{25} and P_n (R_{25}/P_n) with respect to previous 7-day average air temperature. UW plants at the understory and gap sites both exhibited lower g_s values than W plants in the gap (Figure 5a). Higher rates of P_n were

associated with the higher g_s in the W gap plants (Figure 5b). Differences in g_s and P_n between W and UW plants exhibited along summer were statistically significant in early August (both $P < 0.001$) and just marginally so in July (P values are 0.067 for g_s and 0.013 for P_n) (Figure 5a and b). Irrespective of the growth irradiance or watering regime (at the gap site), rates of P_n were highest in July. For gap-grown plants, P_n was lowest in June and early August for W and UW plants, respectively (Figure 5b). The lowest rates of P_n were

Table 1. Summary of correlation and regression analyses between leaf biochemical variables and either leaf respiration at 25 °C (R_{25}) or the temperature sensitivity of respiration (Q_{10}) for watered and unwatered gap plants ($n = 44$). Starch and Q_{10} were log transformed to attain a normal distribution.

	R_{25}		Q_{10}	
	r	R^2	r	R^2
Starch	0.68	0.46**	0.13	0.02 ^{ns}
Soluble sugars	0.37	0.14*	0.13	0.02 ^{ns}
Nitrogen	0.02	0 ^{ns}	0.03	0 ^{ns}

* $P \leq 0.05$.

** $P < 0.001$.

^{ns} $P > 0.05$.

exhibited by UW understorey plants in late summer. At the gap site, watering regime had little effect on the balance between respiratory CO_2 release and photosynthetic CO_2 uptake, even if R_{25}/P_n values were significantly higher in UW plants than W plants in early August (Figure 5c). R_{25}/P_n values were generally lower in understorey plants than gap-grown plants in June and July, whereas there was little difference (between understorey and gap plants) in R_{25}/P_n values in late August (due to equipment failure, understorey data are not available for early August).

To further explore the relationship between leaf R and plant water status, we plotted rates of area-based leaf R_{25} against the corresponding Ψ_{pd} values for individual replicates of gap W and UW plants in August 2008 (when there was maximal difference in the volumetric soil water content values between W and UW treatments; Figure 2a). Figure 6 shows that values of R_{25} decreased with decreasing Ψ_{pd} , further supporting the suggestion that the onset of plant water deficit at the gap site is associated with a decline in leaf R .

Discussion

Drought and shade-mediated changes in leaf R

Our study sought to determine the impacts of soil moisture and growth irradiance on seasonal shifts in the temperature response of leaf R of beech seedlings growing in south-western Europe. We hypothesized that, as a result of decreases in substrate supply, rates of leaf respiration at a common temperature (R_{25}) and the daily temperature dependence of R would both be lower in plants experiencing summer drought, particularly in plants experiencing the combination of summer drought and shade. While strong support was found for a drought- and shade-mediated decrease in R_{25} (Figures 3 and 4a), we observed no change in the daily temperature dependence of leaf R (i.e., Q_{10} values were similar; Figure 4c) among the treatments, which supports the findings of recent studies showing no link between the Q_{10} of leaf R and substrate availability (Hartley et al. 2006, Zaragoza-Castells et al. 2008). Similarly, the differences in R_{25} exhibited by watered and unwatered plants at the gap site were not asso-

ciated with a difference in the concentration of soluble sugars (although starch concentrations did differ significantly between W and UW plants; Figure 4d). Thus, differences in R_{25} between watered and unwatered plants were most likely the result of differences in the basal rate of R with the latter being underpinned by differences in respiratory capacity. To date, no study has investigated the effect of drought on respiratory capacity. However, there is growing evidence that differences in respiratory capacity (and basal rates of R) exhibited by plants grown at different temperatures or irradiances are associated with differences in the amount of mitochondria and amount/activity of mitochondrial enzymes (Noguchi et al. 2005, Armstrong et al. 2006). Thus, it seems likely that lower respiratory capacity underpinned the lower rates of R_{25} exhibited by understorey plants compared to their gap-grown counterparts.

By reducing overall rates of leaf R , understorey plants would be better able to maintain a positive whole-plant carbon balance. In addition to restrictions in carbon gain due to deep shade, plants in the understorey in late August faced a more severe water deficit than in the gaps (Figure 2b), as previously found in the study area for this and other tree species (Rodríguez-Calcerrada et al. 2008, Robson et al. 2009). Given that shade per se is likely to result in reduced rates of leaf R (e.g., Lusk and Reich 2000) and that respiration rates were lower in gap plants that were drought stressed (Figure 3), it is likely that shade and drought combine to further reduce rates of leaf R in the understorey. Probably caused by intense water deficit, though, the proportionally larger decline in P_n than R_{25} in the understorey plants through summer resulted in the increase of R_{25}/P_n at the end of August. Combined stresses of low light and soil drought promoted carbon starvation and probably contributed to the high mortality of seedlings in the understorey (Robson et al. 2009). Because P_n did not decline through summer under mild and moderate water deficits in W and UW gap plants, respectively, declining rates of leaf respiration as summer temperature increased resulted in a tendency of R_{25}/P_n to decrease.

Thermal acclimation under shade and drought

We also hypothesized that irrespective of growth irradiance and/or water supply, leaf R would exhibit seasonal shifts in daily temperature–response curves (i.e., thermally acclimate). Our results point to thermal acclimation having occurred in all plants, irrespective of growth irradiance and/or soil moisture availability. Both gap and understorey plants exhibited downward shifts in temperature–response curves and decreases in predicted rates of R_{25} during the onset of summer (Figures 3 and 4). Importantly, such changes were exhibited in both watered and unwatered gap plants, suggesting that the shifts in temperature–response curves occurred in response to changes in daily average temperature rather than soil moisture. Moreover, although variations in leaf R and acclimation are often closely associated with changes in tissue N concentration (Ryan 1995, Bolstad et al. 2003, Xu and Griffin 2006,

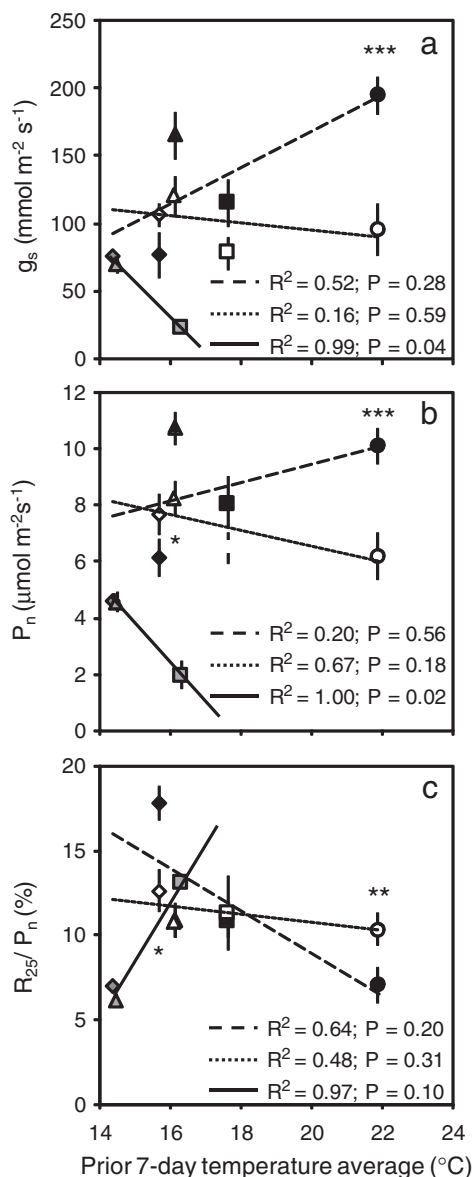


Figure 5. Variation of physiological traits with respect to mean ambient temperatures averaged for the 7-day period preceding measurements in June (diamonds), July (triangles), early August (circles) and late August (squares). (a) Stomatal conductance (g_s ; $\text{mmol m}^{-2} \text{s}^{-1}$); (b) light-saturated net photosynthesis (P_n ; $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$); and (c) balance between leaf respiration and net photosynthesis at 25 °C (R_{25}/P_n , expressed on a percentage basis). Data shown are for the unwatered (UW) understorey (grey symbols, solid lines) and UW gap plants (open symbols, dotted lines) as well as for the watered (W) gap plants (closed symbols, dashed lines). Error bars show 1 SE ($n = 6$). Significance of differences between W and UW gap plants within a date are indicated as *** $P < 0.001$, ** $P < 0.01$ and * $P \leq 0.05$. The coefficients of determination and P values of the linear models are shown.

Reich et al. 2008, Tjoelker et al. 2008), there was no relationship between seasonal variations in R_{25} and N concentration in our study (Figure 4f), showing that declines in tissue N concentration could not explain the decline in rates of leaf R . Rather, parallel variations in soluble sugars, starch and

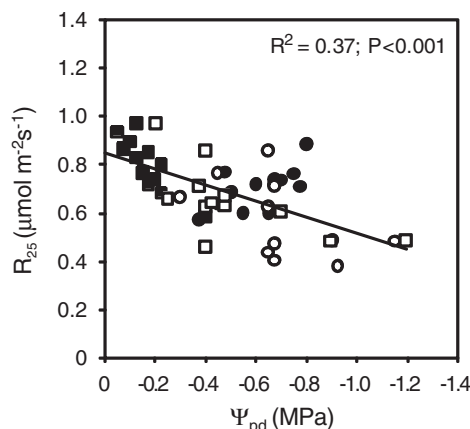


Figure 6. Relationships between values of leaf respiration at a common measuring temperature of 25 °C (R_{25}) for gap-site grown plants in August and pre-dawn water potential (Ψ_{pd} , MPa). Symbols shown: unwatered gap plants (open symbols) and watered gap plants (closed symbols) for measurements in early August (circles) and late August (squares). The coefficient of determination and P value of a linear model fitted to all data are shown.

R_{25} occurred throughout the summer. Direct effects of temperature on the availability of soluble sugars could mediate thermal acclimation of leaf R (Lee et al. 2005), but the lack of changes in Q_{10} values (which would be expected if substrates became limiting) points to acclimation being mainly controlled by changes in the basal rate of leaf R . Similar to previous field studies (Atkin et al. 2000, Bruhn et al. 2007, Tjoelker et al. 2009), our results point to Type II acclimation being responsible for the dynamic seasonal shift in respiration rates during summer, with the downward shift in daily temperature–response curves and R_{25} values being underpinned by a decrease in the basal rate of leaf R rather than in the Q_{10} of leaf R (Figure 4). This resulted in near-perfect homeostasis of R_{amb} (Figure 4b).

It seems likely that the onset of summer drought may accentuate the downward adjustment in rates of R exhibited by leaves as they thermally acclimate to hot conditions in summer. While it was clear that drought reduced respiration rates of beech, we suggest that leaf water deficit had a proportionally lesser impact on seasonal changes in R than long-term changes in temperature because the decline in R_{25} from June to early August was 2.5-fold higher than the difference in R between W and UW plants in late August, under equal variations in Ψ_{pd} between dates and treatments, respectively (≈ 0.35 MPa).

Conclusion

In conclusion, we observed complete acclimation of leaf respiration to increasing summer temperatures in beech plants growing beneath the canopy and within canopy gaps subject to contrasting watering regimes. Although the parallel decreases in starch, soluble sugars and leaf respiration at a common measuring temperature of 25 °C suggest that there may

have been a direct substrate limitation associated with respiratory acclimation, the absence of changes in Q_{10} values points to acclimation being primarily controlled by changes in respiratory capacity. Increasing drought could contribute such acclimation, as enhanced water deficit reduced leaf respiration in gap-grown plants.

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