

Impacts of elevated temperature and vapour pressure deficit on leaf gas exchange and plant growth across six tropical rainforest tree species

Kali B. Middleby , Alexander W. Cheesman  and Lucas A. Cernusak 

College of Science and Engineering, James Cook University, Cairns, QLD, 4878, Australia

Summary

Author for correspondence:

Kali Middleby

Email: kalimiddleby@gmail.com

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- Elevated air temperature (T_{air}) and vapour pressure deficit (VPD_{air}) significantly influence plant functioning, yet their relative impacts are difficult to disentangle.
- We examined the effects of elevated T_{air} (+6°C) and VPD_{air} (+0.7 kPa) on the growth and physiology of six tropical tree species. Saplings were grown under well-watered conditions in climate-controlled glasshouses for 6 months under three treatments: (1) low T_{air} and low VPD_{air} , (2) high T_{air} and low VPD_{air} , and (3) high T_{air} and high VPD_{air} . To assess acclimation, physiological parameters were measured at a set temperature.
- Warm-grown plants grown under elevated VPD_{air} had significantly reduced stomatal conductance and increased instantaneous water use efficiency compared to plants grown under low VPD_{air} . Photosynthetic biochemistry and thermal tolerance (T_{crit}) were unaffected by VPD_{air} , but elevated T_{air} caused $J_{\text{max}25}$ to decrease and T_{crit} to increase. Sapling biomass accumulation for all species responded positively to an increase in T_{air} , but elevated VPD_{air} limited growth.
- This study shows that stomatal limitation caused by even moderate increases in VPD_{air} can decrease productivity and growth rates in tropical species independently from T_{air} and has important implications for modelling the impacts of climate change on tropical forests.

Introduction

Tropical forests are critically important ecosystems for their role in carbon uptake and water cycling globally (Mitchard, 2018). These ecosystems already operate close to their critical temperature thresholds for photosynthesis, leaving them vulnerable to relatively small increases in temperature (Doughty *et al.*, 2023). As surface temperatures rise world-wide (IPCC, 2022), so does the vapour pressure deficit (VPD_{air}). The VPD_{air} has been increasing since the 1990s and is projected to continue in the coming decades (Fang *et al.*, 2022). High VPD_{air} increases the atmospheric demand for water from plants, leading to atmospheric drought (Grossiord *et al.*, 2020), and can exacerbate soil water deficit. Globally increasing VPD_{air} has resulted in gross primary productivity (GPP) declines partly offsetting the expected CO_2 fertilisation effect (Yuan *et al.*, 2019). Furthermore, there is increasing evidence that hot, dry periods limit tropical tree growth (Bauman *et al.*, 2022a) and drive higher rates of global forest mortality events (Mcdowell *et al.*, 2008; Hammond *et al.*, 2022; Bauman *et al.*, 2022b). Characterising the relative roles of air temperature (T_{air}) and VPD_{air} on net primary productivity and leaf-level gas exchange in trees will allow us to understand better the trade-offs between opening stomata to increase carbon uptake and reduce heat

stress (Sadok *et al.*, 2021), and closing them to conserve water (Medlyn *et al.*, 2011).

The temperature response of net photosynthesis (A_{net}) is a product of both the basal rates and thermal sensitivity of processes such as Rubisco carboxylation (V_{cmax}) and electron transport (J_{max}) in concert with the CO_2 concentration observed inside the leaf (C_i), which is influenced by stomatal conductance (g_s) (Farquhar *et al.*, 1980). Both V_{cmax} and J_{max} , along with their thermal sensitivities acclimate to their environment, generally decreasing with increasing growth temperature (Fürstenau Togashi *et al.*, 2018; Kumarathunge *et al.*, 2019). These changes can be explained by the higher rate of enzyme activity under increased temperatures, causing plants to invest less Nitrogen into photosynthetic enzymes like Rubisco (Prentice *et al.*, 2014). Changes in Nitrogen allocation and, consequently, photosynthetic biochemistry can also develop due to changes in leaf water status from decreased soil moisture (Zhou *et al.*, 2013) or elevated VPD_{air} (Walker *et al.*, 2014; Quebeman & Ramirez, 2016; Yang *et al.*, 2019). While the impact of VPD_{air} has received much less attention, a study in wheat showed that even short-term exposure to elevated VPD_{air} can decrease both V_{cmax} and J_{max} (Fakhet *et al.*, 2021).

The response of A_{net} to warming is also constrained by stomata (Farquhar & Sharkey, 1982). Increasing temperature and VPD_{air} influence the instantaneous rate of g_s , and longer-term

acclimation and adaptation (Jarvis, 1976; Yamori *et al.*, 2014; McAdam & Brodribb, 2015; Kruse *et al.*, 2019). The short-term response of g_s to increasing temperature (and coincident VPD_{air}) follows a peaked pattern with reduced rates below and above their thermal optimum. This behaviour is due to increasing VPD_{air} resulting in stomatal closure (Peak & Mott, 2011) as well as optimal stomatal behaviour (Medlyn *et al.*, 2011). However, there are now numerous reports that g_s can be decoupled from photosynthesis, increasing in response to high temperatures and resulting in cooler leaf temperatures (Slot *et al.*, 2016; Urban *et al.*, 2017; Drake *et al.*, 2018; De Kauwe *et al.*, 2019; Marchin *et al.*, 2023). Long-term warming studies in tropical trees report warmed plants having lower g_s measured under standard conditions than unwarmed plants (Fauset *et al.*, 2019; Crous *et al.*, 2023). However, with VPD_{air} generally not controlled in these experiments, this is likely a response to increasing VPD_{air} rather than a direct response to increasing T_{air} . Supporting this, a meta-analysis by Lopez *et al.* (2021) showed acclimation of plants to reduce g_s in response to increasing VPD_{air}, with implications on carbon uptake and plant growth. This generalised response may help prevent drought-induced cavitation, as a recent study in three temperate species shows that high VPD_{air} resulted in hydraulic damage in species that failed to reduce g_s (Schönbeck *et al.*, 2022). Reduction in g_s as an acclimation response to warming suggests plants may opt to conserve water and avoid hydraulic damage, but at the cost of reduced carbon gain and increased vulnerability to thermal extremes at the leaf level due to reduced evaporative cooling (Blonder *et al.*, 2023).

A change in g_s impacts transpiration and modifies the leaf energy balance (Bonan, 2008). Transpirational cooling, through the maintenance or increase of g_s , may be an important mechanism for avoiding critical leaf temperatures when moisture is not limiting (Lapidot *et al.*, 2019; Deva *et al.*, 2020; Drake *et al.*, 2020; Cook *et al.*, 2021; Blonder *et al.*, 2023), or even despite soil drought (Marchin *et al.*, 2023). This response may depend in part on the sensitivity of stomata to VPD_{air}, which varies between and within species (Grossiord *et al.*, 2020). Tropical species originating from wetter biomes have higher sensitivity and exhibit an isohydric response, limiting water loss by closing stomata (Cunningham, 2004, 2005). According to leaf energy balance theory, all else being equal, this reduction in transpiration will increase T_{leaf} during the hottest part of the day (Bonan, 2008). However, increasing atmospheric VPD_{air} increases the vapour pressure difference between the leaf and air (VPD_L) – increasing the evaporation rate and passively reducing T_{leaf} (Massmann *et al.*, 2019).

The integrated biochemical (V_{cmax25} , J_{max25}) and stomatal (g_s) responses to changes in both temperature and VPD_{air} will shape how trees respond to current and future warming. Characterising the relative impacts of temperature and VPD_{air} is important for tropical forests, given the variation in predicted climatic trajectories (Fang *et al.*, 2022). Additionally, tropical species have higher sensitivities to both elevated T_{air} (Cunningham & Read, 2003; Choury *et al.*, 2022; Crous *et al.*, 2022) and VPD_{air} (Cunningham & Read, 2003; Cunningham, 2004). Despite this, only one study has investigated tropical tree acclimation to VPD_{air} (Cunningham, 2005), and only one study investigating

the temperature response of GPP in tropical trees controlled for the associated increase in VPD_{air} (Smith *et al.*, 2020).

We aimed to disentangle the impacts of T_{air} and VPD_{air} on growth and physiology in well-watered saplings of six tropical rainforest tree species using climate-controlled glasshouse chambers. We hypothesised; (H1) T_{air} and VPD_{air} would have contrasting impacts on plant growth, with increasing T_{air} having a positive effect, and increasing VPD_{air} having a negative effect, (H2) species with higher stomatal sensitivity to VPD_{air} would be more negatively affected by growth at elevated VPD_{air} and (H3) photosynthetic biochemistry (e.g. V_{cmax} and J_{max}) would be impacted both directly by growth at elevated T_{air} and indirectly at elevated VPD_{air} through adjustment to lower operating C_i resulting from stomatal limitations, resulting in altered rates of gas exchange when assessed under common conditions.

Materials and Methods

Typical climate of study region

The Australian Wet Tropics bioregion is part of the humid tropics (Köppen–Geiger climate classification), with a hot-humid wet season from December to March and a mild dry season from April to November. The range of mean VPD in the Australian Wet Tropics bioregion is typical of other tropical wet forests (Bauman *et al.*, 2022a), with a recent paper examining plant eco-hydrological strategies across elevation at two well monitored sites (Binks *et al.*, 2023) showing long-term daily maximum VPD averaging 1.0 kPa at a site in the uplands (elevation 720 m) and 1.3 kPa at a site in the lowlands (elevation 86m). It is important to note the daily maximum VPD at the lowland site rarely exceeded 2.5 kPa over 10 yr of monitoring.

Study species

We examined six species, all woody trees from tropical rainforests of the Australian Wet Tropics: *Atractocarpus fitzalanii* (F.Muell.) Puttock *subsp. fitzalanii* (Rubiaceae), *Buckinghamia celsissima* F.Muell. (Proteaceae), *Endiandra microneura* C.T.White (Lauraceae), *Ficus racemosa* L. (Moraceae), *Melicope elleryana* (F.Muell.) T.G.Hartley (Rutaceae), and *Nauclea orientalis* (L.) L (Rubiaceae). Henceforth, these species are referred to by their genus name only. All plant material was obtained from local nurseries, originating from single source provenances of lowland populations within each species range in northeast Queensland, Australia. Three species (*Atractocarpus*, *Buckinghamia*, and *Endiandra*) are evergreen, late successional species, endemic to Queensland, Australia. The other three species (*Ficus*, *Melicope*, and *Nauclea*) are semi-deciduous, early successional species, more widely distributed with ranges including tropical Asia. These semi-deciduous species had not yet experienced a period of leaflessness.

Experimental setup and growth conditions

All seedlings (<1 yr old) were replanted into 8-l pots containing a high organic matter potting mix augmented with a local

volcanic stone, Quincan, to improve drainage. Before treatments, they were grown in a shade house (75% transmission) for 1 month to recover from possible transplant shock. At the experiment start, plants were placed into one of three glasshouse chambers. Treatments began in December 2022, coinciding with the onset of the Wet season, and lasted 6 months. Due to some limitations on total seedling numbers available, the number of individuals per species placed in each chamber varied from 5 to 11 (median 8). Individuals within each species were distributed so each treatment had similar-sized seedlings at the experiment start, with initial seedling heights ranging from an average (± 1 SD) of 16.4 ± 2.6 cm in *Atractocarpus* to 40.4 ± 4.1 cm in *Melicope*.

We applied three treatments to disentangle the physiological and morphological responses of saplings to realistic changes in growth temperature and VPD_{air} . These treatments included (1) a chamber with a 'low' temperature and low VPD_{air} (i.e. low T_{air} & low VPD_{air}), (2) a chamber with an elevated temperature while maintaining a low VPD_{air} (i.e. high T_{air} & low VPD_{air}), and (3) a chamber with both elevated temperature and elevated VPD_{air} (i.e. high T_{air} & high VPD_{air}) (Table 1; Fig. 1). We maintained a 6°C difference between low and high-temperature chambers, with daytime (09:00 h–15:00 h) T_{air} averaging 26.6 (± 2.9) °C in the low-temperature chamber and 32.5 (± 2.9) °C in the high-temperature chambers (Table 1; Fig. 1). We also maintained a 0.7 kPa difference in the daytime average VPD_{air} of low and high VPD_{air} chambers, with daytime VPD_{air} averaging 0.72 (± 0.32) kPa in the low VPD_{air} chamber at high T_{air} and 1.4 (± 0.59) kPa in the high VPD_{air} chamber (Table 1, Fig. 1). Glasshouse temperatures tracked external conditions measured outside the glasshouse with an offset applied between temperature treatments (Table 1; Fig. 1) to maintain a realistic diel variation. T_{air} was controlled with the glasshouse climate control system, whereas VPD_{air} was controlled by manipulating relative humidity with an ultrasonic humidifier (JDH-03Z; Hangzhou Conloon Electric Co Ltd., Hangzhou, China) in the low VPD_{air} treatments and a dehumidifier (OADE20; Omega Altise, Chatswood, Vic., Australia) in the high VPD_{air} treatment, with their action regulated by the relative humidity seen in each chamber.

We rotated treatments between chambers monthly to mitigate chamber effects. Plants were given slow-release fertilizer (Osmocote Native Formula; ScottsMicacle-Gro, Marysville, OH, USA) at the experiment start and again monthly for all species except *Buckinghamia*, which may be susceptible to phosphorous toxicity (Shane *et al.*, 2004). Initially, all plants were irrigated daily using automated drip-line irrigation and watered to saturation once a week. As plants grew, this increased to automated watering twice

daily with manual watering to saturation twice weekly. Toward the experiment end, we gave extra watering to both high T_{air} treatments to ensure any impacts on plants could be attributed to atmospheric drought rather than soil moisture deficit.

The glasshouse structure utilizes a horizontal internal shade screening (XLS-15F Firebreak, Ludvig Svensson, Kinna, Sweden) to create a homogenous light environment within each chamber. This results in a 50 and 47% transmission of direct and diffuse PAR (i.e. 400–700 nm), as well as 35% transmission of IR load. Temperature and relative humidity in each chamber were recorded at 10 min intervals (RHP-2R2B Temperature and Humidity Probe; Dwyer Instruments, MI City, IN, USA) in the building management system, calibrated against a single humidity and temperature probe (HMP60, Vaisala, Finland).

Leaf temperature

T_{leaf} was monitored throughout the experiment to calculate the offset between leaf and air temperatures (ΔT) and the leaf-to-air vapour pressure difference (VPD_L). Abaxial T_{leaf} measurements were made with thermistors (LT-1T-SD112, Edaphic Scientific Pty Ltd, Port Macquarie, NSW, Australia) with a contact area of *c.* 1 mm² and a stated instrument accuracy <0.15°C. We selected healthy, newly expanded mature leaves positioned horizontally with a slight north-facing tilt to minimise differences in radiation inputs that would impact ΔT . Due to limits on the number of thermistors available, T_{leaf} was monitored on two to three plants of different species per chamber at a time and thermistors were switched between plants after at least 5 d of measurements, resulting in eight measurement rounds. During data collection we ensured that a plant from each treatment of the same species was monitored simultaneously, resulting in a dataset for T_{leaf} from 3 to 5 plants (median 4) per species per treatment.

Gas exchange

Leaf gas exchange in a healthy, recently emerged, mature leaf was measured with a LI-6400 xt portable photosynthesis system equipped with a 2 × 3 cm leaf cuvette with a red-blue LED light source (Li-Cor Inc., Lincoln, NE, USA). For all gas exchange measurements, we measured one leaf per plant, and six plants per species per treatment on fully expanded and sun-exposed leaves, except for *Ficus* under the two low VPD_{air} treatments in which only five plants were available. Gas exchange measurements were performed under common environment conditions to allow for the determination of acclimation between plants grown under

Table 1 Daytime (09:00 h–15:00 h) average treatment conditions over the 6-month experiment.

Treatment	VPD_{air} (kPa)	T_{air} (°C)	RH (%)
Low T_{air} & low VPD_{air}	0.78 ± 0.29	26.6 ± 2.9	78 ± 6.6
High T_{air} & low VPD_{air}	0.72 ± 0.32	32.5 ± 2.9	86 ± 5.6
High T_{air} & high VPD_{air}	1.40 ± 0.59	32.5 ± 2.9	73 ± 9.3

Data represents mean ± 1 SD and is based upon calibrated 10 min data recorded by glasshouse building management system ($n = 6344$ observations per treatment). VPD, vapour pressure deficit.

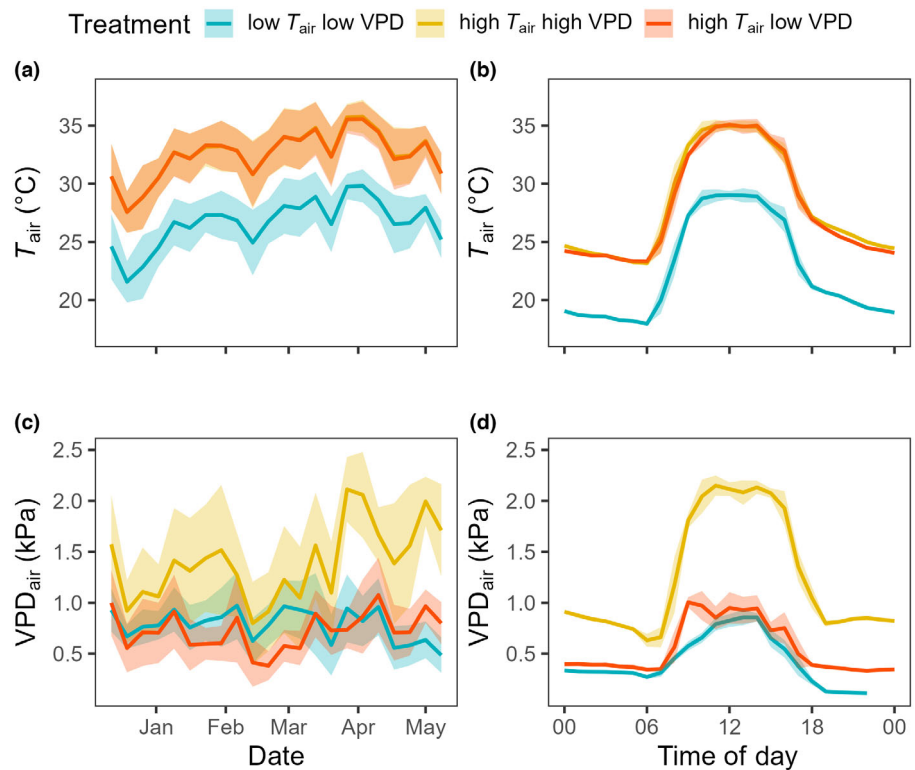


Fig. 1 Growth conditions during the experiment. Weekly averages of T_{air} (a) and VPD_{air} (c) during the day (09:00 h–15:00 h) throughout the growth experiment, and hourly averages of T_{air} (b) and VPD_{air} (d) for an example day on 29/04/2023. Solid lines represent means, and the shaded region represents one SD. Colours represent different treatments but note in panels (a, b) they overlap for the two high T_{air} treatments. VPD, vapour pressure deficit.

different environmental conditions. Plants were taken out of their growth chambers and into an antechamber where they acclimated for 1 h before measurements. The room temperature averaged 28°C during measurements and had a transparent glass roof so plants were exposed to solar radiation. The licor cuvette was set to a block temperature of $28 \pm 0.05^{\circ}\text{C}$ and VPD_{air} was controlled at $1 \pm 0.1 \text{ kPa}$ for all plants. CO_2 concentration in the cuvette was set to 400 ppm, PAR to $1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$, and flow rate to $500 \mu\text{mol s}^{-1}$ unless stated otherwise. Once gas exchange rates stabilised (minimum 15 min), 10 measurements were taken, 30 s apart. These were averaged to get a plant-level mean for A_{net} , g_s , and intrinsic water use efficiency ($i\text{WUE} = A_{\text{net}}/g_s$). The same gas exchange data were also used to determine g_1 (Medlyn *et al.*, 2011), with g_1 fit for each plant using the ‘fitBBs’ function in the PLANTECOPHYS package (Duursma, 2015), without fitting the intercept term g_0 .

Following these measurements, on the same leaf, photosynthetic CO_2 response curves ($A-C_i$) were performed to obtain estimates of the maximum rates of RuBisCO carboxylation (V_{cmax}) and the maximum rates of electron transport (J_{max}) and their ratio. V_{cmax} and J_{max} were fitted using the ‘fitacis’ function in the PLANTECOPHYS package in R (Duursma, 2015). Each curve was inspected for outliers. The curve for one plant could not be fit using the default method, so was removed from analysis. Model fitting was done on each curve. To account for the control over block temperature instead of T_{leaf} (T_{leaf} varied by up to $c. 2^{\circ}\text{C}$), the fitted parameters V_{cmax} and J_{max} were normalised to 25°C using temperature response parameters from Kelly (2014).

To determine if the impact of elevated VPD_{air} on growth was related to species-level stomatal sensitivity to VPD_{air} , we

characterised this in plants grown under the high T_{air} & low VPD_{air} treatment. Stomatal insensitivity to VPD (Φ) is a normalized response seen in transpiration across a change in VPD. It represents an index to examine stomatal responses to an imposed VPD change (Franks & Farquhar, 1999). Theoretically, the index ranges from 0, if stomata were to close completely following a change in VPD (while also assuming $g_{\text{min}} = 0$), to 1, which would occur if there was no change in g_s following a change in VPD. We calculated Φ by examining steady-state transpiration in leaves when exposed to a step change in VPD from 1 to 2 kPa in plants ($n=6$) grown under high T_{air} and low VPD_{air} . Leaf-level gas exchange in well-watered plants with a T_{leaf} of 28.0 ± 0.1 , PAR of $1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$, and VPD_L of 1 kPa ($1.02 \pm 0.02 \text{ kPa}$) was allowed to equilibrate before data were logged every 30 s for 5 min. Then, a gradual ($c. 5 \text{ min}$ ramping) change in VPD_L was imposed by increasing the proportion of air routed through the drierite until a VPD_L of $2 \pm 0.04 \text{ kPa}$ was achieved. At this point, gas exchange was monitored, and once stable, data was once again logged every 30 s for 5 min. Since Φ will depend upon the incremental change in VPD experimentally applied, we first made minor adjustments to measured transpiration via extrapolation to standardise the VPD increment to exactly 1.0 kPa in all cases as per Franks & Farquhar (1999).

Minimum conductance

Leaf minimum conductance (g_{min}) was determined using the mass loss of detached leaves (MLD) (Percy *et al.*, 1989; Sack & Scoffoni, 2007). For this, one fully expanded mature leaf per

plant was sampled early in the morning before plants received full sunlight. Leaves were the same physiological stage as those used for leaf gas exchange. Petioles were sealed with parafilm, and leaves were placed into a ziplock bag within another opaque bag to keep samples in the dark. Leaves were taken immediately to the lab, scanned, weighed, and set up in the drying chamber. The drying chamber (with heating element off) contained two additional small fans directed upwards to increase air movement and allowed up to 16 leaves to be hung from the upper rail. A temperature–humidity sensor (HT-3015, Lutron Electronic Enterprise Co. Ltd., Taipei, Taiwan) recorded conditions during leaf drying. T_{air} and relative humidity in the drying chamber averaged $26.9 \pm 1.57^\circ\text{C}$ and $53.6 \pm 4.7\%$, respectively. Fresh leaf mass was measured every 30 min with a 4-point balance until there were sufficient points (minimum 8) to observe the linear portion of the mass loss curve. To calculate g_{min} , we determined the transpiration rate (mass loss over time) divided by VPD, with VPD calculated per the Tetens equation (Monteith & Unsworth, 2013).

Photosynthetic heat tolerance

Photosynthetic heat tolerance was assessed using the rise of minimum fluorescence (F_0) with increasing temperature. Leaves were the same physiological stage as those used for leaf gas exchange and g_{min} . Leaves were dark adapted for 30 min then placed in a temperature-controlled chamber (3010-GWK1 Gas-Exchange Chamber, Walz; Heinz Walz GmbH, Effeltrich, Germany) connected to a portable gas exchange system (LiCor 6400xt; LiCor Inc., Lincoln, NE, USA). A Chl fluorometer (PAM-2000, Walz; Heinz Walz GmbH) measured fluorescence every 60 s with the fibre-optic sensor secured on the glass lid of the chamber. T_{leaf} was recorded using the Walz thermocouple, which has a stated accuracy of $\pm 0.2^\circ\text{C}$. The initial chamber temperature was set to 30°C , and once F_0 was stable, leaves were heated at 1°C per min until T_{leaf} reached 60°C . Leaves were kept in the dark during temperature ramping. This method is commonly used to determine the critical temperature (T_{crit}) threshold at which photosynthetic efficiency of PS-II begins to decline (Schreiber, 1986), which increases with growth temperature (Zhu *et al.*, 2018). Here, T_{crit} is the breakpoint separating the slow and fast rise phases of the F_0 vs T_{leaf} curve, determined from breakpoint regression using the SEGMENTED v.1.6-2 package in R (Mugge, 2003). We determined T_{crit} on one leaf per plant for six plants per treatment on all six species, except for *Ficus* in the two low VPD_{air} treatments, which only had 5 plants available (106 curves).

Leaf morphology

Leaf traits were measured on all plants using a composite sample of 4–8 leaves per plant, including leaves used for T_{crit} , g_{min} and gas exchange measurements. We measured leaf fresh weight (g) using a 2-point balance, and scanned leaves (CanoScan, LiDE220; Canon Inc., Tokyo, Japan). IMAGEJ was used to calculate leaf area (cm^2) and effective leaf width (cm) – defined as the diameter of the largest circle that fits within the leaf lamina.

Samples were oven-dried at 70°C for 3 d to obtain dry weight (g). We calculated specific leaf area (SLA, $\text{m}^2 \text{kg}^{-1}$), and leaf dry matter content (LDMC, mg g^{-1}). Petioles were not removed, and for species with compound leaves (*Melicope*), leaflets were treated as single leaves.

Biomass

Seedling height and diameter at the root collar were measured at the beginning and end of the experiment. Additionally, at the end of the experiment, all plants were harvested, partitioned, and dried (70°C until constant mass) to get the dry biomass of leaf, stem, and roots. To analyse whether species-level stomatal sensitivity to VPD could explain the different biomass responses to VPD across species, we calculated the response ratio of biomass to VPD as the average biomass of the high T_{air} low VPD_{air} treatment divided by the average biomass of the high T_{air} high VPD_{air} treatment for each species.

Data analysis

All analyses and graphical representations were performed using R v.4.2.2. To determine if 6 months of treatment duration had significantly impacted plant growth (total biomass), plant physiology (A_{net} , g_s , iWUE, g_1 , g_{min} , T_{crit} , $V_{\text{cmax}25}$, $J_{\text{max}25}$, and their ratio) and leaf morphology (SLA, LDMC, and leaf width), we ran a series of two-way ANOVA with species, treatment, and their interaction as explanatory variables, and type III sums of squares, with contrasts set to ‘contr.sums’. To ensure assumptions of normality were met we cube root transformed g_s , and log transformed iWUE, g_{min} , SLA, and the ratio of $J_{\text{max}25}$ to $V_{\text{cmax}25}$. Pairwise comparisons of treatments were assessed using the R package ‘EMMEANS’ (Lenth, 2022).

To examine if the species-level trait, Φ (stomatal insensitivity to VPD) could explain observed differences in plant growth under elevated temperature but different VPD_{air} (i.e. high T_{air} low VPD_{air} and high T_{air} high VPD_{air}) we examined the correlation between Φ and the response ratio of biomass to VPD_{air} using ordinary least squares regression.

To examine the impact of treatments on daytime (i.e. 09:00 h–15:00 h) VPD_L and ΔT across treatments, we fit a linear mixed effects model with either ΔT or VPD_L as the response variable, and species, treatment, and their interaction as fixed effects. We also included incoming radiation as a continuous covariate, to account for varying radiation within and across days. To account for the measurement design, plant ID nested within the measurement round was included as a random effect. Pairwise comparisons for treatments were assessed using the R package EMMEANS (Lenth, 2022).

Results

Impacts on plant biomass

There were significant differences in total dry biomass across species and treatments, but their interaction was not significant

(Table 2). For plants grown under low VPD_{air}, biomass was higher in plants grown under higher T_{air}, with the increase in biomass averaging +28.9% across species and ranging from +18.1 to +45.5% (Table 2; Fig. 2a). For plants grown under high T_{air}, biomass was lower in plants grown under high VPD_{air} than low VPD_{air}, with biomass –11.1% lower in the high VPD_{air} compared to low VPD_{air} when averaging across species, and ranging between +3.0 to –20.5% (Table 2; Fig. 2a).

We found no evidence for a correlation between species-level stomatal sensitivity to VPD and the response ratio of biomass to elevated VPD ($P > 0.05$; Fig. 2b).

Acclimation of leaf-level gas exchange

There was a statistically significant interaction between species and treatment on A_{net}, g_s, and iWUE (Table 2). For most species, we found no evidence that growth temperature (at low VPD_{air}) affected A_{net}, g_s, or iWUE measured under standard conditions ($P > 0.05$; Table 2; Fig. 3). The exception was *Nauclea*, where A_{net} was +40% higher ($t(88) = 3.883, P < 0.001$), g_s was +281% higher ($t(88) = 7.497, P < 0.0001$), and consequently, iWUE was –67% lower ($t(88) = -6.552, P < 0.0001$), for plants grown under high T_{air} than low T_{air} (Table 2; Fig. 3). We found evidence that elevated growth VPD_{air} (at high T_{air}) reduced g_s in *Melicope* ($t(88) = -4.855, P < 0.0001$), *Nauclea* ($t(88) = -5.024, P < 0.0001$), and *Buckinghamia* ($t(88) = -2.507, P < 0.05$; Table 2; Fig. 3). In addition, iWUE was significantly higher for plants grown under high VPD_{air} than low VPD_{air}; for *Melicope* ($t(88) = 4.282, P < 0.001$), *Nauclea* ($t(88) = 4.583, P < 0.0001$), and *Buckinghamia* ($t(88) = 2.459, P < 0.05$; Table 2; Fig. 3). Although average A_{net} was lower for plants grown under elevated VPD_{air} than low VPD_{air} for most species (Supporting Information Table S1), statistically significant differences were observed only for *Melicope* ($t(88) = -2.440, P < 0.05$; Table 2; Fig. 3). When averaged across species this represented an –11% reduction in A_{net}, a

– 41% reduction in g_s, and a +58% increase in iWUE for plants grown under elevated VPD_{air} than those grown under low VPD_{air} at the same growth temperature.

The trait g₁ averaged 4.6 (Range 0.8 to 15.5) across all species and treatments and was significantly affected by the interaction between species and treatment (Table 2; Fig. S1). *Post hoc* analysis revealed similar trends to iWUE, with g₁ higher (indicating lower iWUE) in plants grown under high T_{air} compared to low T_{air} (at low VPD_{air}) for both *Melicope* ($t(88) = 2.648, P < 0.05$) and *Nauclea* ($t(88) = 7.429, P < 0.0001$) (Table 2; Fig. S1). VPD_{air} also impacted g₁, with plants grown under high VPD_{air} having a lower g₁ than those grown under low VPD_{air} for all species (Table S1), but statistically significant differences observed only for *Melicope* ($t(88) = -4.350, P < 0.001$) and *Nauclea* ($t(88) = -5.801, P < 0.0001$). In *Buckinghamia*, there was weak evidence for differences between the two VPD_{air} treatments under high T_{air} ($t(88) = -2.352, P = 0.054$), and additionally, g₁ in the high VPD_{air}, high T_{air} treatment was also significantly different than g₁ in the low VPD_{air}, low T_{air} treatment ($t(88) = -3.658, P < 0.01$) (Table 2; Fig. S1).

The average species g_{min} was 0.58 mmol m⁻² s⁻¹ and ranged from 0.04 to 3.54 mmol m⁻² s⁻¹ across all species and treatments (Table 2; Fig. S1). There was a significant interaction between species and treatment on g_{min} (Table 2; Fig. S1). For plants grown under low VPD_{air}, g_{min} decreased with elevated T_{air} for *Nauclea* ($t(88) = -2.418, P < 0.05$) but increased with elevated T_{air} for *Buckinghamia* ($t(88) = 2.541, P < 0.05$) and *Atractocarpus* ($t(88) = 2.312, P = 0.0593$) (Table 2; Fig. S1). For plants grown under high T_{air}, no species had significantly different g_{min} between plants grown under the low and high VPD_{air} treatments ($P > 0.05$).

Acclimation of photosynthetic biochemistry

Most species did not acclimate V_{cmax25} in response to treatments (Table 2; Figs 4, S2). However, J_{max25} was –14.9% lower on

Table 2 Two-way ANOVA results of treatment effects of physiology and biomass in six tropical woody species.

Variable	Error df	Treatment		Species		Treatment:Species value_treatment: species	
		df	F	df	F	df	F
A _{net}	88	2	5.17**	5	48.08***	10	2.57**
$\sqrt[3]{g_s}$	88	2	22.09***	5	42.55***	10	6.17***
Log ₁₀ (iWUE)	88	2	19.57***	5	30.32***	10	4.61***
g ₁	88	2	21.03***	5	16.62***	10	5.73***
V _{cmax25}	87	2	1.12	5	27***	10	1.37
J _{max25}	87	2	12.86***	5	17.96***	10	0.78
Log ₁₀ (J _{max25} /V _{cmax25})	87	2	12.21***	5	48.72***	10	0.83
T _{crit}	88	2	26.69***	5	22.63***	10	1.76
Log ₁₀ (g _{min})	88	2	0.1	5	64.94***	10	2.37*
Total biomass	125	2	29.33***	5	74.34***	10	1.7
Leaf dry matter content	125	2	1.71	5	181.69***	10	1.08
Log ₁₀ (specific leaf area)	125	2	1.69	5	193.61***	10	0.92
Leaf width	125	2	4.73*	5	271.16***	10	5.05***

DF means degrees of freedom. Significance denoted with '***', '**', and '*', are significant at $P < 0.001$, $P < 0.01$, $P < 0.05$, respectively. Bold entries are significant to $P < 0.05$.

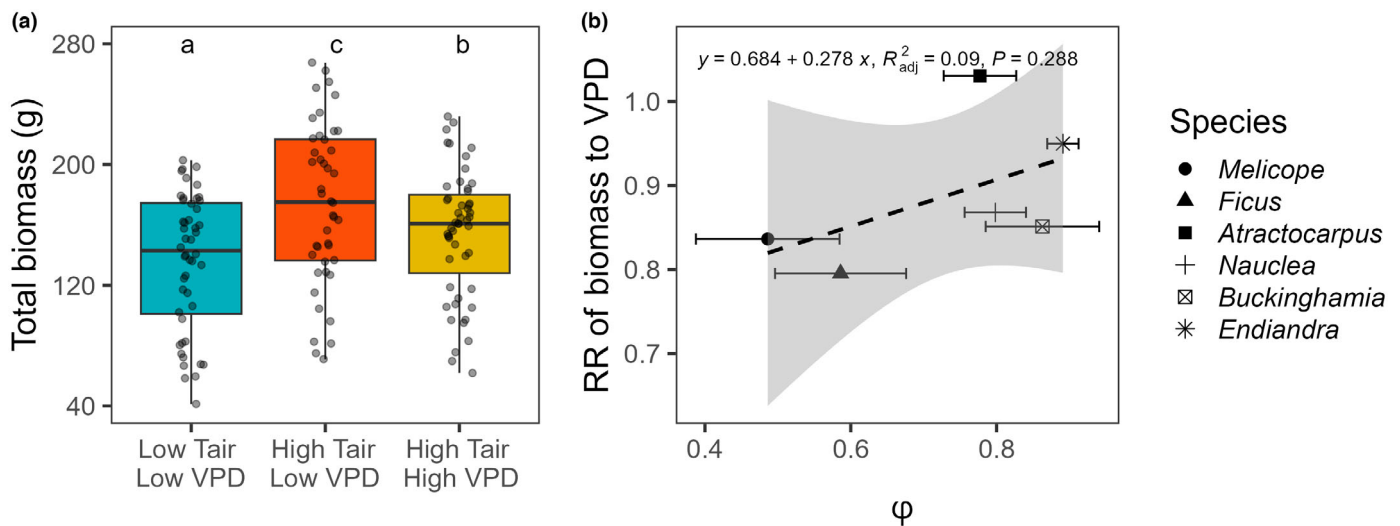


Fig. 2 Impact of treatments on total dry biomass accumulation (a) and correlation between stomatal insensitivity to vapour pressure deficit (VPD) and the response ratio (RR) of biomass to elevated VPD_{air} (b). Each point in panel a represents a plant measurement. Box and whisker plots show the median, the 25th and 75th percentile, along with 1.5× the interquartile range. Colours represent the different treatments. Significant differences among treatments from Tukey *Post Hoc* results ($P < 0.05$) are denoted with letters. In (b) species with higher stomatal sensitivity to VPD have lower values on the x axis. Different species are represented with different shapes. Each point represents a species average and SE for stomatal insensitivity to VPD, Φ ($n = 5-6$ per species), and the response ratio (RR) of biomass to VPD_{air} ($n = 5-10$ per species). The RR was calculated as the mean biomass of the high T_{air} low VPD_{air} treatment divided by the mean biomass of the high T_{air} high VPD_{air} treatment. Grey shaded region represents the confidence interval (0.95) around the regression line, and the dashed line is the ordinary least squares regression. VPD, vapour pressure deficit.

average in plants grown under high T_{air} compared to T_{air} (range -29.5 to -3.4%) (Tables 2, S1; Figs 4, S2). Similarly, the ratio between J_{max25} and V_{cmax25} was -11.3% lower on average in plants grown under high T_{air} than plants grown under low T_{air} but the same VPD_{air} (range -21.5 to -3.4%) (Tables 2, S1; Fig. 4). No statistically significant differences were associated with VPD_{air} treatments (Table 2; Fig. 4).

Acclimation of leaf thermal tolerance

T_{crit} averaged 47.4°C (range 41.7 to 51.0°C) across all species and treatments (Figs 4, S3). T_{crit} averaged 46.3°C (range 41.7 to 49.8°C) in the low T_{air} low VPD_{air} treatment, 48.1°C (range 45.7 to 51.0°C) in the high T_{air} low VPD_{air} treatment, and 47.6°C (range 44.6 to 50.1°C) in the high T_{air} high VPD_{air} treatment. Elevated T_{air} significantly affected T_{crit} (Table 2), leading to a $+0.31^\circ\text{C}$ (range 0.06 to 0.57°C) increase in T_{crit} per $^\circ\text{C}$ rise in T_{air} (Fig. 4). Most species also had a slightly higher T_{crit} for plants grown under low VPD_{air} than high VPD_{air} (average difference 0.8°C , range -0.2 to 1.8°C ; Fig. S3). However, this was not statistically significant (Table 2; Fig. 4).

Acclimation of leaf morphology

Leaf dry matter content and SLA were not affected by the treatments ($P > 0.05$, Table 2; Figs 4, S3), whereas leaf width in *Atractocarpus* and *Nauclea* was (Table 2; Fig. S1). In *Atractocarpus*, plants grown under high T_{air} had narrower leaf widths on average than those grown under low T_{air} for both the low VPD_{air} treatment ($t(125) = -5.935$, $P < 0.0001$), and the high

VPD_{air} treatment ($t(125) = -4.789$, $P < 0.0001$) (Table 2; Fig. S1). In *Nauclea*, the opposite trend was observed, with plants grown under low VPD_{air} having wider leaf widths when grown at high T_{air} compared to low T_{air} ($t(125) = 2.484$, $P < 0.05$). In *Nauclea*, leaf width was also impacted by the VPD_{air} treatment, with narrower leaf widths observed for plants grown under high VPD_{air} compared to low VPD_{air}, at the same high T_{air} ($t(125) = -3.743$, $P < 0.001$) (Table 2; Fig. S1).

Impacts of treatment conditions on T_{leaf} and VPD_L

Daytime T_{leaf} was warmer than T_{air} , with ΔT ($T_{leaf} - T_{air}$) averaging $+1.1^\circ\text{C}$ (range -1.3 to 4.3°C) across all species and treatments (Fig. 5). ΔT was significantly higher for plants growing under low VPD_{air} than high VPD_{air}, with ΔT averaging 1.3 and 0.7°C in the low and high VPD_{air} treatments, respectively (Fig. 5; Table 3). Despite these differences in T_{leaf} between treatments, there were still significant differences in VPD_L between VPD_{air} treatments, with VPD_L averaging 1.3 and 1.9 kPa in the low and high VPD treatments, respectively (Fig. 5; Table 3). There were no significant differences in VPD_L between low and high T_{air} treatments, growing under low VPD_{air} (Fig. 5; Table 3). Thus, the pattern of VPD_L across treatments was similar to the pattern of VPD_{air}.

Discussion

We utilised three climate-controlled chambers to disentangle the relative roles of T_{air} (at low VPD) and VPD_{air} (at high T_{air}) on integrated growth, leaf-level gas exchange, photosynthetic

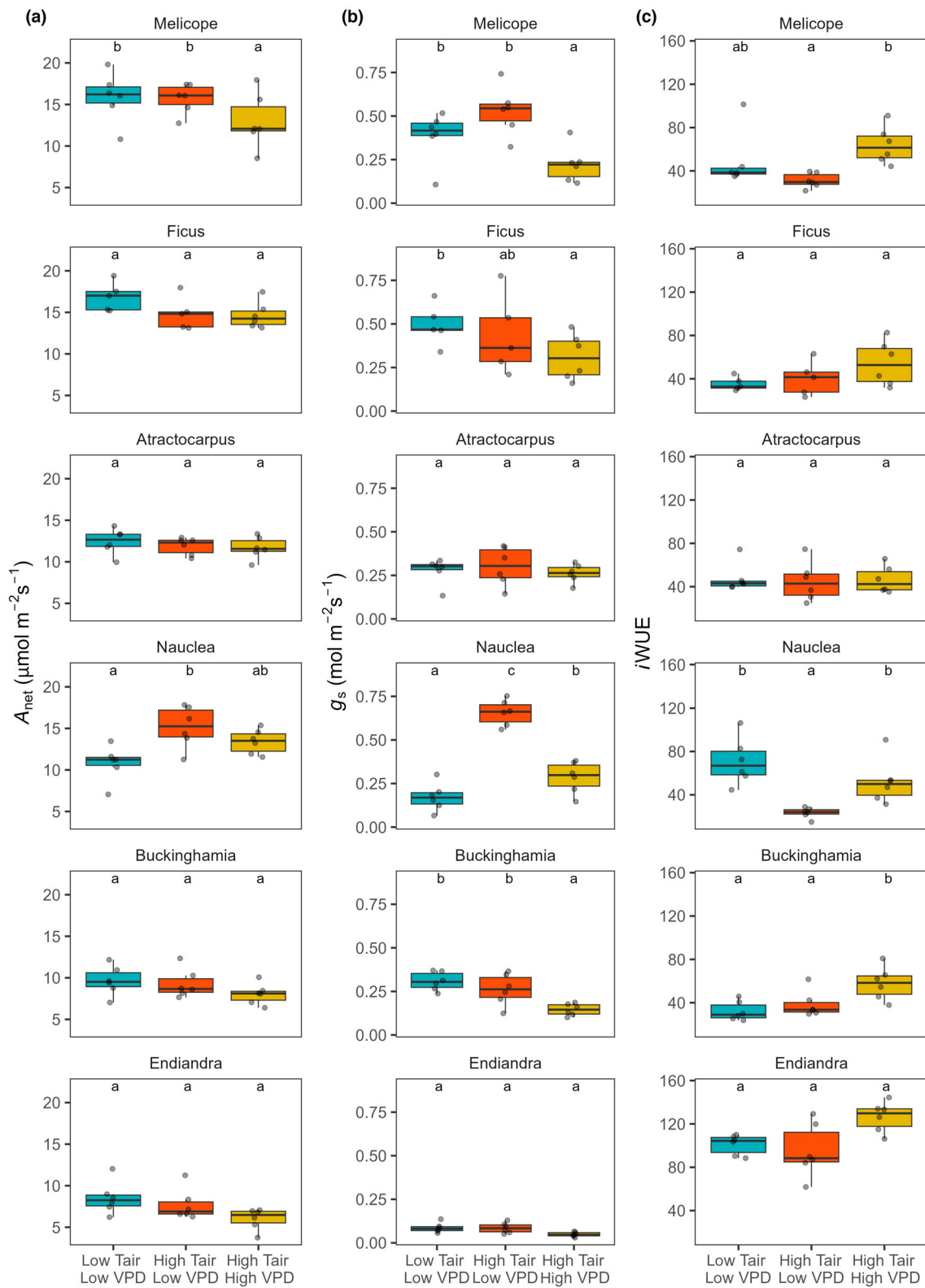


Fig. 3 Treatment impacts on gas exchange measured under common conditions for net photosynthesis, A_{net} (a), stomatal conductance, g_s (b), and intrinsic water use efficiency, iWUE (c). Species are ordered in decreasing stomatal sensitivity to vapour pressure deficit (VPD) (top panel is high sensitivity, bottom panel is low sensitivity). Box and whisker plots show the median, the 25th and 75th percentile, along with 1.5× IQR. Colours represent the different treatments. Significant differences between treatments from Tukey *Post hoc* results ($P < 0.05$) denoted with letters. Note that g_s and iWUE were transformed for analysis, but their untransformed values are presented here. VPD, vapour pressure deficit.

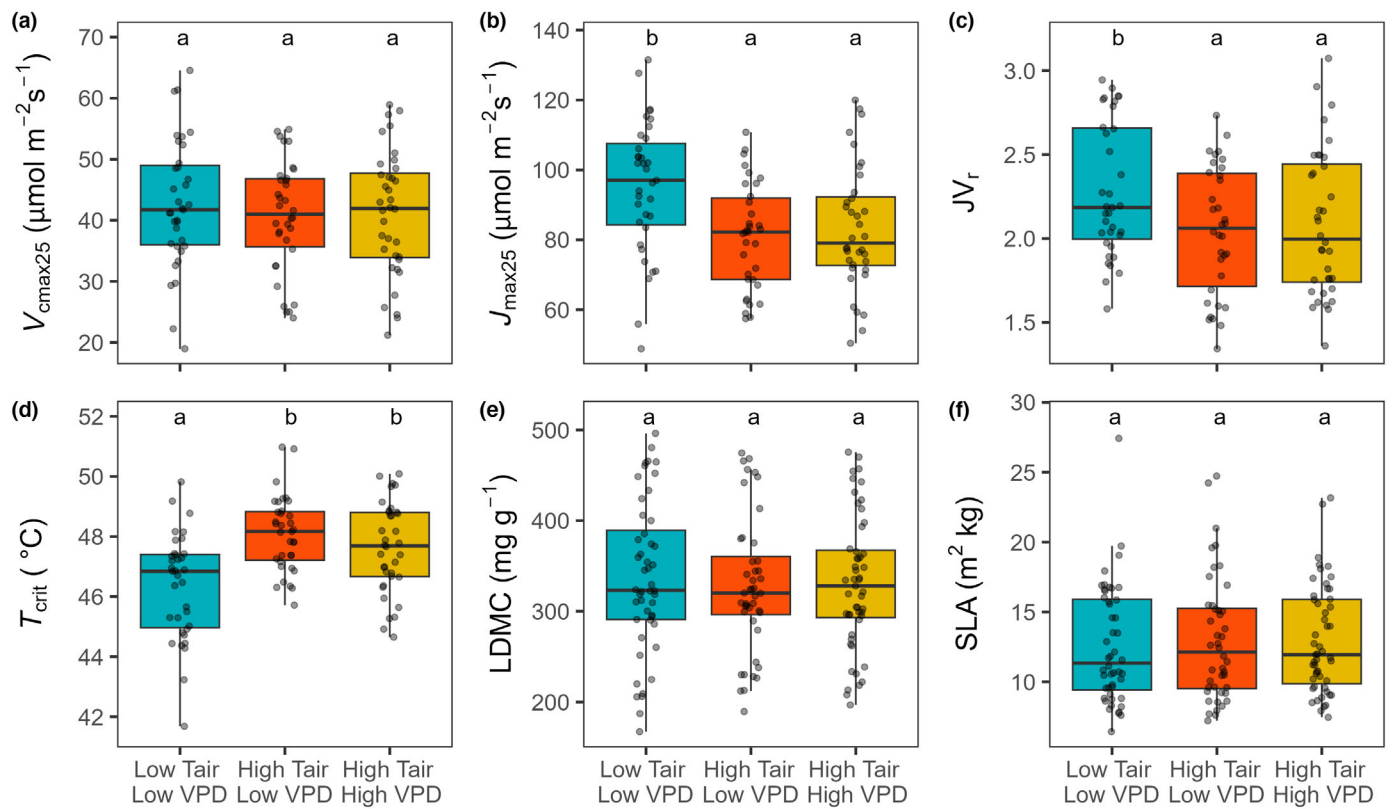


Fig. 4 Treatment impacts representing long-term acclimation for V_{cmax25} (a), J_{max25} (b), the ratio of J_{max25} to V_{cmax25} , Jv_r (c), T_{crit} (d), leaf dry matter content (LDMC), (e) and specific leaf area, SLA, (f) across all species. Each point represents a plant measurement. Box and whisker plots show the median, the 25th and 75th percentile, along with $1.5 \times$ IQR. Colours represent the different treatments. Significant differences between treatments from Tukey Post Hoc results ($P < 0.05$) denoted with letters. Note that Jv_r and SLA were transformed for analysis, but their untransformed values are presented here. VPD, vapour pressure deficit.

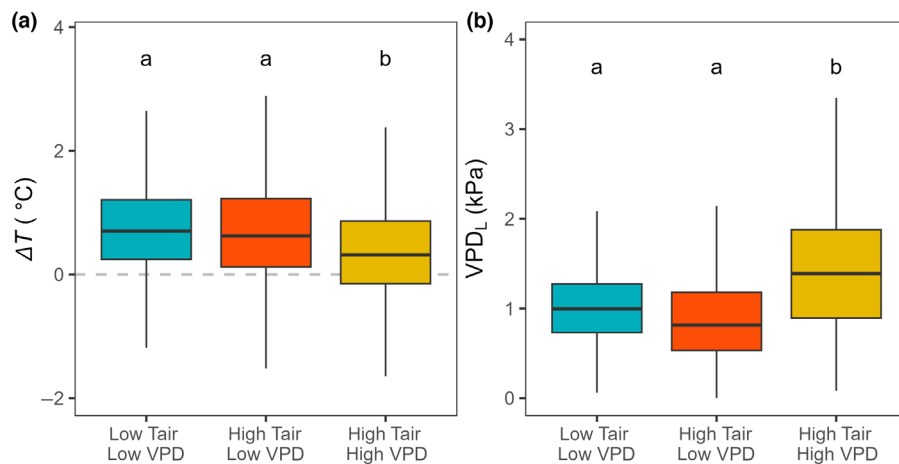


Fig. 5 Distribution of (a) leaf-air temperature differences (ΔT) and (b) leaf-air vapour pressure difference (VPD_L) in the different treatments for six species. Underlying data are from 10-min averages, filtered to only include measurements during the daytime (09:00 h–15:00 h). Letters denote significant differences between groups from pairwise comparisons. VPD, vapour pressure deficit.

capacity, and thermal tolerance in saplings of six tropical tree species under well-watered conditions. We found that plant biomass increased under higher T_{air} , but that this positive impact was partially counteracted by elevated VPD_{air} . Contrary to our expectation, variation in the response ratio of accumulated biomass to VPD across species was not associated with stomatal sensitivity to VPD, but the analysis was limited by a relatively small number of species. Our results also show that different physiological traits

acclimated to either T_{air} or VPD_{air} , with J_{max25} and thermal tolerance affected by T_{air} , and g_s and $iWUE$ affected by VPD_{air} .

Interactive effects of T_{air} and VPD_{air} on growth

Tropical tree growth increased in response to increasing growth temperature when considering nonextreme temperatures and nonlimiting soil moisture, similar to other studies (Lin

Table 3 Differences in ΔT and VPD_L during the growth experiment.

Parameter	df	ΔT		VPD_L	
		F	P	F	P
Treatment	2	11.44	< 0.001	65.69	< 0.0001
Species	5	7.68	< 0.0001	2.56	< 0.05
Radiation	1	57.16	< 0.0001	93.45	< 0.0001
Treatment: species	10	0.72	ns	0.77	ns

Results are from a linear mixed model to assess impacts of treatment, species, and their interaction on ΔT and VPD_L . The model included radiation as a covariate and plant nested within round as a random effect (1round/plant). VPD, vapour pressure deficit.

et al., 2010; Cheesman & Winter, 2013; Ramesh *et al.*, 2023). Although we tested a 6°C difference in growth temperatures, the absolute temperature of the high T_{air} treatment in our study was relatively conservative, with conditions comparable to those experienced by our focal species during summer across their lowland distributions in the Australian Wet Tropics. Our study also revealed concurrent increases in VPD_{air} counteracted the stimulation of plant growth by increased growth temperatures. This supports recent findings that tropical tree growth and productivity is highly sensitive to increasing VPD (Smith *et al.*, 2020; Bauman *et al.*, 2022a), likely due to stomatal limitations on photosynthesis (Binks *et al.*, 2023).

With the low number of species-replicates in our current study, we could not find a positive correlation between species-level sensitivity of stomata to VPD and plant biomass response ratio to elevated VPD_{air} (Fig. 2b). However, it should be noted the two species (*Melicope* and *Ficus*) with the greatest reduction of biomass under elevated VPD also had the highest stomatal sensitivity to VPD, highest SLA, and fastest growth rates, whereas the species that were least affected by VPD (*Atractocarpus* and *Endiandra*) had amongst the least sensitive stomata, the lowest SLA, and slowest overall growth rates. This could indicate differential responses of fast and slow-growing species to changes in VPD, as reported by Bauman *et al.* (2022a), who showed fast-growing species typically had more negative growth responses to VPD anomalies than slower growing species. Further work would be needed to determine which functional trait drives the observed difference in VPD response across species. It should be noted that while we did not test if the stomatal sensitivity to VPD itself acclimated across treatments, other studies report a negative correlation between increasing VPD and stomatal sensitivity to VPD (Binks *et al.*, 2023).

Reduction of leaf conductance with warming is a response to elevated VPD_{air} , but not T_{air}

Warming experiments often find lower g_s in leaves grown under higher temperatures than controls (Carter *et al.*, 2021; Choury *et al.*, 2022; Crous *et al.*, 2023). However, with temperature and VPD_{air} often covarying, it is not always clear whether this response is to increasing growth temperature or the associated increase in VPD_{air} . We expected plants grown under high T_{air} would acclimate their physiology to reduce g_s only if also exposed to high VPD_{air} . While our results support this, an

exceptional species in our study was *Nauclea*, which had a large increase in g_s and A_{net} under higher T_{air} . We are unsure of reasons why gas exchange was affected by growth temperature in this species and not others, but worth noting is that species is found in particularly wet, swampy environments. For the other species in our study, g_s was only affected by VPD_{air} . It appears tropical woody species reduce g_s in response to increasing VPD_{air} to maintain leaf water status and maintain hydraulic function, even at the expense of reduced carbon uptake. This contrasts with results from a similar study performed on three temperate species (Schönbeck *et al.*, 2022), which found no acclimation of g_s in response to elevated VPD, and as a result, plants reduced leaf water potential and showed signs of hydraulic dysfunction. These differences between species from temperate and tropical biomes are not surprising considering tropical species are generally more isohydric than temperate species (Cunningham, 2004).

We assessed leaf-level gas exchange under standard temperature and VPD_{air} to determine if plants had acclimated leaf function to their growth conditions. Gas exchange in these species acclimated more to long-term VPD_{air} than long-term T_{air} , with our results showing tropical trees grown under higher VPD_{air} conditions shift to a more conservative water use strategy (higher iWUE) even without soil moisture deficit. This was driven by declining g_s rather than an increase in assimilation rate. This could be significant for modelling g_s , as the stomatal slope parameter (g_1), commonly used to represent dynamic changes in g_s in ecosystem models (Medlyn *et al.*, 2011) is inversely related to iWUE. Although there has been discussion about the plasticity of g_1 as it pertains to soil moisture (Héroult *et al.*, 2013; Wu *et al.*, 2020), and across elevation where temperature and VPD covary (Mujawamariya *et al.*, 2023), there have been no studies showing the plasticity of g_1 in plants acclimating directly to altered VPD. We show some tropical trees may modify g_1 in response to both higher VPD_{air} (g_1 decreasing in response) and when VPD is constant, higher T_{air} (g_1 increasing in response). These contrasting effects of T_{air} and VPD_{air} on acclimation of g_1 may account for the lack of acclimation to growth temperature observed in some studies (Mujawamariya *et al.*, 2023). Furthermore, it is important to note the species variation not just in mean g_1 , but in their range, with *Endiandra* varying relatively little (range 2.3) and *Nauclea* varying greatly (range 14). Further effort to disentangle the effects of temperature and VPD in a greater range of species would be helpful to establish both the

generality of our observations, and potential drivers of species variation in acclimation capacity.

Water limitation from soil moisture deficit and higher VPD can cause a reduction in g_{\min} (Fanourakis *et al.*, 2013; Duursma *et al.*, 2019; Schönbeck *et al.*, 2022); however, in our study, g_{\min} was not impacted by VPD_{air}. The mean daytime differences in T_{air} and VPD_{air} used in our study are within the range likely experienced by these species across their home-range distributions, which could explain our contrasting findings to Schönbeck *et al.* (2022), where g_{\min} decreased with increasing VPD_{air} only at the highest growth temperature used in their study. In addition, while other studies held diel growth conditions constant during their experiments (Fanourakis *et al.*, 2013; Duursma *et al.*, 2019; Schönbeck *et al.*, 2022), we allowed temperature, and thereby VPD_{air}, to vary. The influence of dynamic VPD_{air} and acclimation to its range on g_{\min} is poorly understood.

Two target species showed acclimation of g_{\min} to growth T_{air} , with values higher for those grown under the warmer T_{air} treatments. This response may allow plants to avoid lethal temperatures under heat waves (Schuster *et al.*, 2016; Slot *et al.*, 2021) at the risk of increased vulnerability to hydraulic failure (Cochard, 2019). Although this contrasts with some reports of reduced g_{\min} in response to long-term increases in temperature (Duursma *et al.*, 2019), g_{\min} is highly variable and responses are species-specific (Duursma *et al.*, 2019). In addition, other studies did not control for VPD_{air}, so the long-term response of g_{\min} to growth temperature is still poorly understood.

Photosynthetic capacity acclimated to elevated T_{air} , but not VPD

Our results reveal photosynthetic biochemistry acclimated to elevated T_{air} , but not VPD_{air}. We expected $V_{\text{cmax}25}$, $J_{\text{max}25}$, or their ratio to change given the internal CO₂ concentration, C_i , will likely decrease at a higher VPD_{air} due to stomatal closure. This change in operating C_i could be expected to induce a change in either $V_{\text{cmax}25}$ or $J_{\text{max}25}$, such that co-limitation of photosynthesis at the new operating C_i is maintained (Wang *et al.*, 2017). Despite observing a reduction in g_s in response to elevated growth VPD_{air}, this did not translate into an associated impact on $V_{\text{cmax}25}$ or $J_{\text{max}25}$, nor on A_{net} . There are very few studies reporting acclimation of photosynthetic biochemistry directly in response to growth VPD, with limited research done on tomato (Zhang *et al.*, 2018), *Prosopis juliflora* (Shirke, 2004), and wheat (Fakhet *et al.*, 2021), which report reduced rates of apparent V_{cmax} or Nitrogen allocation to V_{cmax} in response to short-term increases of VPD_{air}. Substantially more studies report how growth VPD_{air} affects A_{net} (Lopez *et al.*, 2021), with no change in photosynthetic rates between plants grown under low and high VPD when measured at common conditions. Our sample size might not have been high enough to detect statistically significant differences in A_{net} , despite rates being lower on average in the high VPD_{air} treatment. However, our results on g_s indicate changes in A_{net} were likely due to stomatal limitation rather than changes in biochemistry. While we show a limited ability of tropical species to acclimate their biochemistry to growth VPD_{air},

further research is greatly needed to determine the effect in plants of contrasting biomes.

Growth temperature induced changes in $J_{\text{max}25}$ and the ratio of $J_{\text{max}25}$ to $V_{\text{cmax}25}$, but not $V_{\text{cmax}25}$. This is consistent with results from other studies (Kumarathunge *et al.*, 2019) showing limited acclimation of $V_{\text{cmax}25}$, but a reduction of $J_{\text{max}25}$ and the ratio of $J_{\text{max}25}$ to $V_{\text{cmax}25}$ in response to higher growth temperatures. Current meta-analyses suggest the positive correlation between the optimum temperature of photosynthesis and growth temperature observed in mature trees (Kumarathunge *et al.*, 2019) to be driven by decreasing $J_{\text{max}25}$ with increasing growth temperature, rather than changes in $V_{\text{cmax}25}$ (Hikosaka *et al.*, 2006).

Impact of VPD on leaf temperatures

Daytime ΔT observed during our growth experiment was lower than reported in the field (Rey-Sanchez *et al.*, 2017; Fauset *et al.*, 2018). This is perhaps due to the glasshouse shade sail reducing radiation inside the glasshouse and may account for the lack of variation in T_{crit} between high and low VPD_{air} treatments. In our study, T_{crit} increased 0.32°C per 1°C rise in T_{air} , comparable with the 0.34°C found by Zhu *et al.* (2018). But with the difference in ΔT between the low and high VPD_{air} treatments being < 1°C, we would not expect to find large differences in T_{crit} due to VPD_{air}.

Increasing VPD has a complex impact on leaf energy balance, with the higher diffusion of water across the stomatal pore, and stomatal closure having opposing effects on latent heat flux (Gu *et al.*, 2006). This results in a nonlinear relationship between transpiration and VPD. Identifying when one process dominates over the other is critical to understanding how plant functioning will be impacted by warming. In our experiment, for plants grown at high T_{air} , those exposed to a higher VPD_{air} had a smaller or more negative ΔT . This was due to the higher atmospheric demand for water response to increasing VPD dominating over the decreased g_s response. Similarly, Massmann *et al.* (2019) demonstrated, using species traits combined with energy balance theory, that plant species from tropical biomes are likely to exhibit a positive transpiration trend in response to increasing VPD. In the range of VPD_{air} observed in our study, we found increasing VPD_{air} will not likely exacerbate heat stress experienced by tropical plants, so long as soil moisture is not limiting to transpiration. However, future work should explore whether acclimation to high VPD_{air} (i.e. reduced g_s) result in differences in ΔT when exposed to the same environmental conditions.

While we discuss the implications of our results broadly, caution should always be made when extrapolating findings from controlled glasshouse experiments conducted on saplings to mature trees in the field. Mature trees can have different hydrological strategies (Ryan & Yoder, 1997) to well-watered saplings and may therefore respond differently to changes in VPD. In addition, our glasshouse used light reducing shade sails to ensure light was distributed evenly to avoid leaf scorching. While sun and shade leaves can show different stomatal sensitivities to VPD (Hernández *et al.*, 2020), this is often when comparing extremes

in illumination (e.g. 5–20% full sunlight). By contrast, in our experiment direct PAR was only reduced by 50% from full tropical sunlight. If moderately reduced radiation during growth did impact stomatal response to VPD, this would mean our experiment underestimates the impact of VPD on plant growth in mature plants exposed to full sun.

Conclusion

In this study we demonstrate how both T_{air} (independent of VPD_{air}) and VPD_{air} (at high T_{air}) have direct impacts on leaf gas exchange and growth in a range of tropical tree species. Under future climate change, understanding the relative role changing temperature and VPD will have on plant growth is of critical importance to predicting the fate of tropical forests and global carbon cycling. Across the six species tested, we show how reduced conductance in response to elevated VPD led to reduced productivity. Further work across a broader range of species and growth conditions would be needed to establish the generality of this, and whether species-level stomatal sensitivity to VPD can help to predict long-term growth trends among species.

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Competing interests

None declared.

Author contributions

All authors, KBM, AWC and LAC were involved in design of the experiments. KBM carried out the experiment with help from AWC. KBM analysed the data and wrote the manuscript. All authors contributed to discussion of results and editing of the final manuscript.

ORCID

Lucas A. Cernusak  <https://orcid.org/0000-0002-7575-5526>

Alexander W. Cheesman  <https://orcid.org/0000-0003-3931-5766>

Kali B. Middleby  <https://orcid.org/0000-0002-9323-0870>

Data availability

The authors declare that data supporting the findings of this study are available within the paper and its [Supporting Information](#) files.

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Supporting Information

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

Fig. S1 Treatment impacts on acclimation of g_1 , leaf minimum conductance, g_{min} and leaf width for each species.

Fig. S2 Treatment impacts on $V_{\text{cmax}25}$, $J_{\text{max}25}$ and their ratio, JVR for each species.

Fig. S3 Treatment impacts on thermal tolerance, T_{crit} , specific leaf area, specific leaf area, and leaf dry matter content for each species.

Table S1 Table with trait means and one SD for each species, treatment, and trait studied.

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