




## LETTER

## High water use in desert plants exposed to extreme heat

Luiza M. T. Aparecido,<sup>1\*</sup>   
 Sabrina Woo,<sup>1</sup> Crystal Suazo,<sup>1</sup>  
 Kevin R. Hultine<sup>2</sup>  and  
 Benjamin Blonder<sup>1,3,4</sup> 

The peer review history for this article is available at <https://publons.com/publon/10.1111/ele.13516>

### Abstract

Many plant water use models predict leaves maximize carbon assimilation while minimizing water loss via transpiration. Alternate scenarios may occur at high temperature, including heat avoidance, where leaves increase water loss to evaporatively cool regardless of carbon uptake; or heat failure, where leaves non-adaptively lose water also regardless of carbon uptake. We hypothesized that these alternative scenarios are common in species exposed to hot environments, with heat avoidance more common in species with high construction cost leaves. Diurnal measurements of leaf temperature and gas exchange for 11 Sonoran Desert species revealed that 37% of these species increased transpiration in the absence of increased carbon uptake. High leaf mass per area partially predicted this behaviour ( $r^2 = 0.39$ ). These data are consistent with heat avoidance and heat failure, but failure is less likely given the ecological dominance of the focal species. These behaviours are not yet captured in any extant plant water use model.

### Keywords

Cowan–Farquhar, functional trait, heat waves, Sonoran desert, stomatal regulation, thermal stress, transpiration, water use efficiency.

Ecology Letters (2020) 23: 1189–1200

## INTRODUCTION

Understanding plant gas exchange is crucial for the prediction of global carbon and water fluxes (Franklin *et al.* 2016). Prolonged, drier and warmer conditions are becoming more common, as are episodic heatwaves (Herrera-Strada & Sheffield 2017; Sévellec & Drijfhout 2018). These effects can lead to reduced plant productivity, increased mortality, and reduction of carbon stocks (McDowell *et al.* 2008).

Many models are based on the hypothesis that leaves maximize net photosynthetic carbon gain ( $A_n$ ) for a fixed level of stomatal conductance ( $g_{sw}$ ) and subsequent water loss from transpiration ( $E_t$ ) (Cowan & Farquhar 1977). Where soil water is not limiting, the ratio between these fluxes is largely governed by vapour pressure deficit ( $D$ ). This theory, and its extensions (here, termed ‘marginal gain’) have been widely adopted (Ball *et al.* 1987; Leuning 1995; Medlyn *et al.* 2011). Other model extensions maximize carbon gain while minimizing the risk of xylem failure (Sperry *et al.* 1998). Generally, this class of model predicts that as thermal stress increases at higher  $D$  and air temperature ( $T_{air}$ ),  $A_n$ ,  $E_t$  and  $g_{sw}$  will all be suppressed and eventually decline to zero, as the cost of water loss exceeds the benefit of carbon gain.

These ‘marginal gain’ models have been implemented in all major terrestrial biosphere models (TBMs), such as ORCHIDEE (Chen *et al.* 2016) and E3SM/FATES (Golaz *et al.* 2019). Empirical evidence supports these models (Wijk *et al.* 2000; Bonan *et al.* 2014; Yang *et al.* 2019; Wu *et al.* 2020). However, the majority of tests come from temperate environments or environments where  $D < 3$  kPa. Model predictions may become inaccurate in high  $D$

conditions (De Kauwe *et al.* 2015; Knauer *et al.* 2015; Grossiord *et al.* 2020). Efforts to model gas exchange at higher  $D$  have often yielded results that are at odds with ‘marginal gain’ predictions (Franks *et al.* 1997; Eamus *et al.* 2008).

Divergence of data from predictions are especially relevant for dryland biomes, which currently cover more than 40% of the Earth’s surface (Huang *et al.* 2016) as well as for other biomes that experience seasonal drought or heatwaves. Thus, there is an empirical knowledge gap between observations and models in hot or dry environments. Misrepresenting the water and carbon contributions of arid lands can lead to poor climate and vegetation distribution predictions. The latter is even more problematic as some of these models depend on parameters such as evapotranspiration rates (Dilts *et al.* 2015) and leaf biomass (Quevedo & Frances 2008) that are directly related to plant physiological performance.

There are two alternate scenarios for leaf gas exchange in thermally extreme environments: one for adaptive ‘heat avoidance’ behaviour, and one for non-adaptive ‘heat failure’ behaviour. Neither scenario is yet included in any major TBM (Rogers *et al.* 2017).

The ‘heat avoidance’ hypothesis highlights a trade-off between thermal and economic functions in leaves. Optimal strategies may change if leaves must avoid thermal stress and mortality at high temperatures, to preserve the possibility of future carbon gain (Urban *et al.* 2017b; Blonder & Michaletz 2018; Griebel *et al.* 2019). When water is transpired, cooling occurs (Crawford *et al.* 2012); when no further cooling is possible, leaf temperature ( $T_{leaf}$ ) will increase, decreasing  $A_n$  through biochemical limitations (Slot & Winter 2016) potentially causing tissue damage and

<sup>1</sup>School of Life Sciences, Arizona State University, 427 E Tyler Mall, Tempe, AZ 85821, USA

<sup>2</sup>Department of Research, Conservation and Collections, Desert Botanical Garden, 1201 N. Galvin Parkway, Phoenix, AZ 85008, USA

<sup>3</sup>Environmental Change Institute, School of Geography and the Environment, University of Oxford, South Parks Road, Oxford OX1 3QY, UK

<sup>4</sup>Department of Environmental Science, Policy, and Management, University of California, Berkeley, CA 94720, USA

\*Correspondence: E-mail: [lmataparecido@gmail.com](mailto:lmataparecido@gmail.com)

leaf mortality. To avoid this outcome, leaves may spend water by increasing  $g_{sw}$  and thus  $E_t$ . This behaviour would occur regardless of the rate of carbon gain, yielding divergent predictions under arid conditions than ‘marginal gain’ predictions. While low water use efficiency (WUE) at high D is already predicted by many ‘marginal gain’ models (due to the increase of  $E_t$  with D dominating downregulation of  $g_{sw}$  with D; Eamus *et al.* 2008), the ‘heat avoidance’ hypothesis proposes that WUE may be lower still, or that high  $E_t$  may occur in the complete absence of  $A_n$ . However, heat avoidance is predicted to be possible under conditions where soil water availability is high enough to meet leaf water demand (Urban *et al.* 2017b).

The ‘heat failure’ hypothesis suggests that minimum leaf conductance may increase with increasing  $T_{leaf}$  regardless of optimal strategy. First,  $g_{sw}$  could increase due to a range of physical effects related to the temperature-dependent fluidity of water (Urban *et al.* 2017b; Cochard 2019). Second, regulation of  $g_{sw}$  may be more challenging at high  $T_{leaf}$  when high pressure gradients occur between leaf air spaces and the atmosphere (arising from temperature-dependent water vapour concentration differences or direct temperature effects), resulting in uncontrolled stomatal opening (Bendix *et al.* 1994). Third, water may also be transpired through the leaf cuticle rather than through the stomata (Kerstiens 1996; Duursma *et al.* 2019). This effect is generally negligible at low temperatures but may be almost half of total conductance in some species at very high  $T_{leaf}$  (Eamus *et al.* 2008; Duursma *et al.* 2019).

Limited evidence supports the possibility of plants decoupling  $g_{sw}$  from  $A_n$  under extreme thermal stress to reduce  $T_{leaf}$  (Teskey *et al.* 2015; Slot & Winter 2016; Drake *et al.* 2018). However, most evidence has come from potted plants from temperate – Amey *et al.* (2012), Urban *et al.* (2017), Bauwer-aerts *et al.* (2013); tropical – Slot *et al.* (2016), Lin *et al.* (2017); and subtropical – Drake *et al.* (2018), von Caemmerer & Evans (2015) regions. Less is known about species from arid regions (Rogers *et al.* 2017), under drought conditions (Anderegg *et al.* 2018), or *in situ* (Vargas & Cordero 2013; Teskey *et al.* 2015; Griebel *et al.* 2019).

Here we assessed the prevalence of alternative plant water use behaviour in hot and dry environments. We made *in situ* measurements of 11  $C_3$  species native to the Sonoran Desert of southwestern Arizona and northwestern Mexico. We combined diurnal measurements of leaf gas exchange and  $T_{leaf}$  with leaf structural traits and micrometeorological data. Data were collected in environments where  $T_{air}$  often reached 43 °C/110 °F and  $T_{leaf}$  reached 50 °C/122 °F. We predicted that alternative water use behaviour would reflect a heat avoidance strategy and would be most prevalent in species: (1) from heat-stressed habitats, (2) that express anisohydric stomatal behaviour and (3) with high construction cost leaves, where the carbon investment of leaf construction might counterbalance a high investment of water.

## MATERIALS AND METHODS

### Strategy classification

Three criteria were used to determine whether alternative water use behaviour was occurring (Fig. S1). First, under both

the ‘heat avoidance’ and ‘heat failure’ hypotheses, high water use and relatively low carbon uptake should occur (i.e. low WUE). We therefore carried out linear regressions between  $A_n$  and  $E_t$  and  $g_{sw}$  and reported the regression slopes. Second, under the ‘marginal gain’ hypothesis,  $A_n$  would decrease as a function of decreasing  $g_{sw}$  and  $E_t$  as  $T_{leaf}$  increases, whereas decoupling between  $A_n$  and  $g_{sw}$  would occur under alternate hypotheses. We also reported slope estimates and goodness-of-fit ( $r^2$ ) for relationships among  $A_n$ ,  $E_t$  and  $g_{sw}$  and  $T_{leaf}$ . Third, we assessed the goodness-of-fit of quantitative predictions of the ‘marginal gain’ hypothesis using equations linking observed values of  $E_t$  and  $g_{sw}$  to predicted values based on D, from Medlyn *et al.* (2011) and Lin *et al.* (2015):

$$g_{sw} = g_0 + 1.6 \cdot \left( 1 + \left( \frac{g_1}{\sqrt{D}} \right) \right) \cdot \frac{A_n}{C_a} \quad (1)$$

$$E_t = \frac{A_n \cdot 1.6 \cdot (g_1 \cdot \sqrt{D} + D)}{C_a \cdot P_a} \quad (2)$$

In these equations, D (kPa) is vapour pressure deficit at time of measurement,  $C_a$  is atmospheric  $CO_2$  concentration (= 400  $\mu\text{mol mol}^{-1}$ ) and  $P_a$  is atmospheric pressure (= 96.25 kPa). The model includes two constants,  $g_0$  and  $g_1$ , which were set to  $-0.007$  and  $6.55$ , respectively, based on data for a savanna site (Medlyn *et al.* 2011). Savanna coefficients were chosen as an approximation for a desert environment as no desert data were available.

Alternative water use behaviour was considered to occur when at least two of the three below criteria were met (Fig. S1):

- (1)  $A_n/E_t$  (WUE) and  $A_n/g_{sw}$  (intrinsic water use efficiency; iWUE): regression slopes and coefficient of determination ( $r^2$ ): species with low  $r^2$  and non-significant slopes or  $< 500 \mu\text{mol mol}^{-1}$ ;
- (2)  $E_t$  and  $g_{sw}/T_{leaf}$  regression slopes and  $r^2$ : species with non-negative slopes and low  $r^2$ ;
- (3) Correlation between observed  $E_t$  and  $g_{sw}$  and estimated  $E_t$  and  $g_{sw}$  using eqns 1 and 2: species with low  $r^2$  and  $E_{t\_Predicted} < E_{t\_Observed}$ .

Through these criteria alone, it is not possible to distinguish ‘heat avoidance’ from ‘heat failure’. Doing so would require partitioning the transpiration fluxes into three major components (see Discussion). Hence, hereafter, any species meeting the above criteria are referred to as ‘alternative water use(rs)’.

### Predictors of alternative water use behaviour

We examined which traits predicted alternative water use behaviour across species by regressing thermal strategy indices (i.e. slopes obtained from linear regression between  $A_n$  and  $E_t$  and  $g_{sw}$  per species, and value of  $r^2_{ET}$  from eqns 1 and 2) against species mean values of leaf mass per area (LMA;  $\text{g m}^{-2}$ ), leaf dry matter content (LDMC;  $\text{g g}^{-1}$ ), predawn and midday leaf water potential ( $\psi$ ; MPa) and hydraulic strategy (i.e. isohydric or anisohydric). Details of leaf and hydraulic trait measurements are described in Supporting Text sections ‘Leaf water potentials and hydraulic strategy’ and ‘Leaf structural trait determination’. Additionally, we assessed the



relationship between water use classification indices (regression slopes and  $r^2$ ) and ecological parameters including habitat, leaf habit, stomata distribution and wood anatomy. All of these ecological and morphological parameters were obtained through previously published literature (Table S1).

### Study site and species

Field measurements occurred in 2018 between 4 June and 6 July in natural habitat at the Desert Botanical Garden (DBG) in Phoenix, Arizona, USA (33.46° N, 111.94° W, elevation 330 m). DBG is located in the northern Sonoran Desert and is characterized by hot and dry summers (June mean

maximum  $T_{\text{air}} = 44\text{ }^{\circ}\text{C}$ ; average relative humidity (RH) = 18%), with mean annual precipitation (MAP) totaling  $191\text{ mm yr}^{-1}$ , 35% of which falls during the summer monsoon.

We selected 11 common Sonoran Desert woody plant species (three individuals per species = 33 plants; Fig. 1 and Table S1 for names, abbreviations and authorities). Selection was based on diversity of leaf and canopy architecture (Fig. 1). Selected individuals were similar in size within species, fully exposed to sunlight, and occurred on similar terrain. All species were  $C_3$  with no known CAM expression, in order to focus on species that do not carry out night-time gas exchange (Winter & Holtum 2014).



**Figure 1** Representative photographs and list of species at the Desert Botanical Garden (Phoenix, AZ, USA). Species name abbreviation between parentheses.



## Gas exchange

Three fully expanded, sun-exposed leaves were chosen from each individual ( $n = 99$  leaves) for diurnal gas exchange measurements. The physiological parameters measured were  $A_n$  ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ),  $E_t$  ( $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) and  $g_{\text{sw}}$  ( $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) using a portable photosynthesis system (LI6800, LI-COR Inc., Lincoln, NE, USA). The diurnal measurements were divided into five intervals: 5 AM–7 AM (pre-dawn and dawn); 8 AM–10 AM (early morning); 12 PM–2 PM (midday); 4 PM–6 PM (late afternoon); and 7:30 PM–10 PM (night). We measured three individuals per day, re-measuring individual leaves. Plants that easily lost their leaves had neighbouring leaves chosen for subsequent measurements. For more details on LI6800 measurement settings, refer to *Supporting Text* ('LI6800 sampling details').

All days had  $T_{\text{air}} > 35^\circ\text{C}$  with no rain or clouds (Fig. S2). Plants received limited irrigation during the study period. However, if plants received irrigation on the day of measurement, we postponed the measurements to another day when shallow soils were superficially dry [i.e. volumetric water content at 10 cm depth  $< 15\%$  using a soil moisture meter (FieldScout TDR150, Spectrum Technologies, Aurora, IL); Table S2]. Sampled plants had rooting depths likely well beyond the depth of our soil moisture measurements, and as a result could have had access to deep soil moisture.

## Environmental variables

$T_{\text{air}}$  ( $^\circ\text{C}$ ) and RH (%) were measured at DBG every 30 s and averaged at 30-min intervals using a weather station (S-THB-M008, Onset, Bourne, MA). Values of  $T_{\text{air}}$  and RH were used to calculate D (Monteith & Unsworth 1990). D values were consistent with LI6800 point measurements ( $D_{\text{Leaf}}$ , kPa).

$T_{\text{leaf}}$  ( $^\circ\text{C}$ ) was measured using an infrared camera (A615, FLIR, Nashua, NH, USA;  $640 \times 480$  pixels, accuracy  $\pm 1^\circ\text{C}$ ). Two thermal images per sampled plant were taken before every gas exchange period at 1–2 m from the target leaves ( $n = 10$  images per plant per day).  $T_{\text{leaf}}$  was estimated from each thermal image as the mean value within a polygon enclosing each of three leaves per image (Schindelin *et al.* 2012).

## RESULTS

### Prevalence of alternative water use

We identified seven marginal gain species and four alternative water users (Table S1). Alternative water use was supported in *Qutu*, *Rhov* and *Vaca*, as each fulfilled all three criteria, as well as in *Dovi*, which fulfilled two criteria, since  $E_t$  of this species remained constant throughout 8 AM and 4 PM even though  $A_n$  did not decline as sharply as other species within this group (34% reduction of  $A_n$  compared to  $58 \pm 7\%$  of the other three species, between 8 AM and 12 PM). Classification was based on the evidence below.

Criterion 1: marginal gain species with  $A_n/E_t$  regressions with slopes above 1000 and  $P < 0.05$ , included *Chli*, *Enfa*, *Latr*, *Olte*, *Pofr*, *Prve* and *Sich* species ( $n = 7$ ). Alternative water users with slopes below 1000 and  $P > 0.05$  (*ns*),

included *Dovi*, *Qutu*, *Rhov* and *Vaca* species ( $n = 4$ ; Figs 2a,d and S3, Table S3). Exceptions to these criteria were made for *Enfa* (slope = 853) and *Latr* ( $P = 0.15$ ) since both species did not completely suppress  $A_n$  during midday (Fig. S3) and maintained a higher  $E_t$  than the other species over the same period (thus we did not observe decoupling between  $A_n$  and  $g_{\text{sw}}$ ). All species had  $r^2 > 0.50$  for the correlation of  $A_n$  and  $g_{\text{sw}}$  ( $P < 0.05$ ; Table S3, Fig. 2d), except *Rhov*. In most species,  $E_t$  and  $g_{\text{sw}}$  were coupled ( $r^2 > 0.60$  and  $P < 0.01$ ), except *Dovi*, *Prve*, *Rhov* and *Vaca* with *Prve* being the only species among those not classified as an alternative water user.

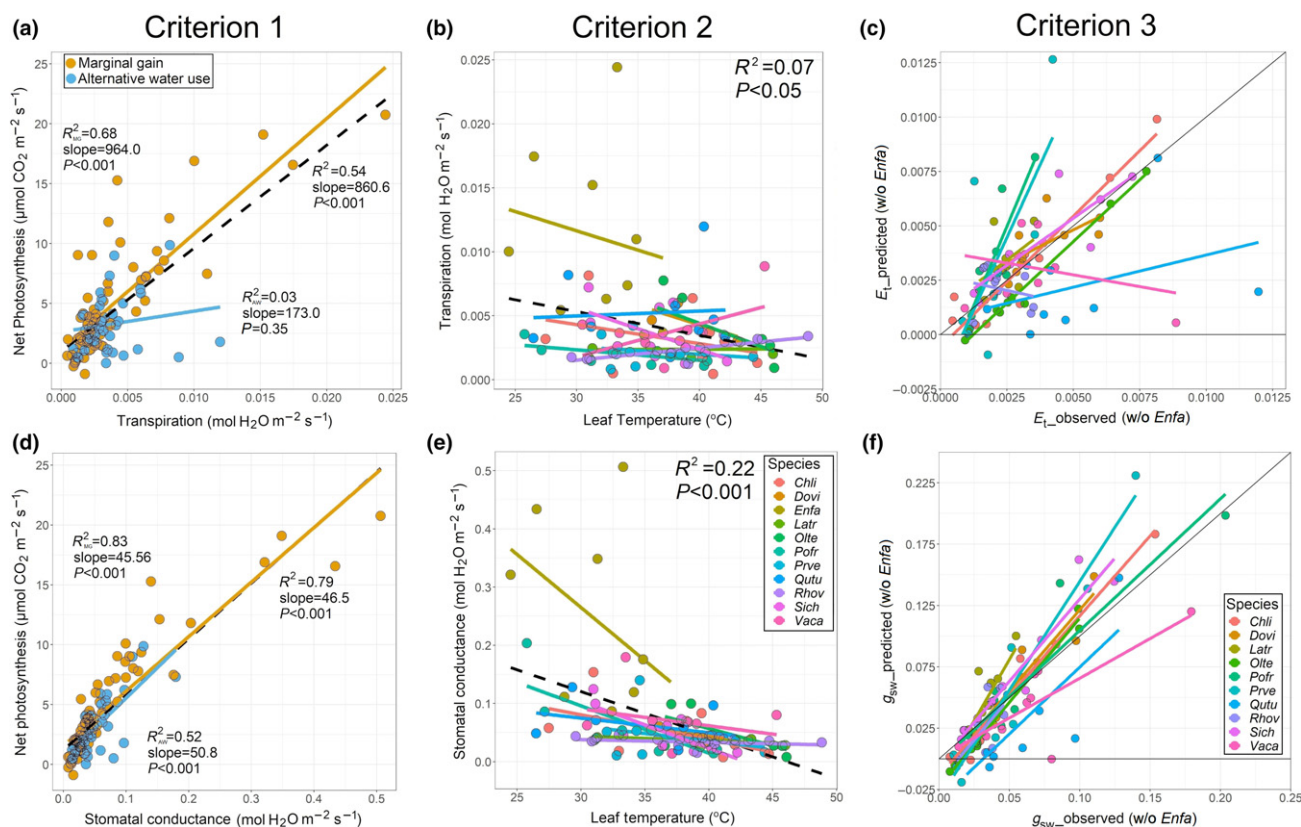
Criterion 2: Alternative water use species exhibited some of the strongest relationships between  $E_t$  or  $g_{\text{sw}}$  and  $T_{\text{leaf}}$  (Fig. 2b,e; Table S3), with exception of *Qutu* ( $r^2 < 0.10$ ). Overall, the relationship between  $E_t$  or  $g_{\text{sw}}$  and  $T_{\text{leaf}}$  was mostly non-significant and had  $r^2 < 0.50$ , and  $g_{\text{sw}}$  was better correlated to  $T_{\text{leaf}}$  than  $E_t$  ( $r^2_{g_{\text{sw}}} = 0.22$ ,  $P < 0.001$ ;  $r^2_{E_t} = 0.07$ ,  $P < 0.05$ ). However,  $g_{\text{sw}}$  declined with increases in  $T_{\text{leaf}}$  for all species, but not enough to prevent  $E_t$  from increasing for three alternative water users (*Qutu*, *Rhov*, *Vaca*) and one marginal gain species (*Latr*).

Criterion 3: For most species, the  $E_{t,\text{predicted}}$  from eqn 1 and  $E_{t,\text{observed}}$  correlations were strong ( $P < 0.05$ ), with exception of *Dovi*, *Latr*, *Qutu*, *Rhov* and *Vaca* ( $P > 0.05$ ; Fig. 2c,f and Table S3). Besides *Qutu*, the model proposed by Medlyn *et al.* (2011) and Lin *et al.* (2015) closely estimated  $g_{\text{sw}}$  to the observed rates, with  $r^2 > 0.50$  ( $P < 0.05$ ) and slopes close or above 1. However, these relationships were highly dependent on the time of day and the inclusion of *Enfa* in the data set. The estimation of  $E_t$  and  $g_{\text{sw}}$  using eqns 1 and 2 yielded poor estimates when compared to the observed  $E_t$  and  $g_{\text{sw}}$  rates of all species ( $r^2_{E_t} = 0.61$ ,  $P < 0.001$ ;  $r^2_{g_{\text{sw}}} = 0.51$ ,  $P < 0.001$ ), with model predictions on average 36 and 60% smaller than the observed  $E_t$  and  $g_{\text{sw}}$  rates respectively. These failures were not solely caused by inappropriate values of  $g_0$  and  $g_1$  in predictions, as the predicted and observed values were not linearly correlated.

### Predictors of heat avoidance

Alternative water using behaviour was predicted by higher leaf construction costs (supporting Prediction 2; Table S4). Species with higher LMA had lower  $A_n/E_t$  slope coefficients (Criterion 1;  $r^2 = 0.39$ ,  $P < 0.05$ ; Fig. 3a) and lower  $r^2_{E_t}$  (Criterion 3; LDMC -  $r^2 = 0.60$ ,  $P > 0.01$ ; Fig. 3d). However, alternative water using behaviour was not related to hydraulic strategy, as of half alternative water users and 57% of marginal gain species were anisohydric (Figs S4 and S5).

Additionally, alternative water use behaviour was also dependent on species' habitat ( $r^2 = 0.77$ ,  $P < 0.05$ ), leaf habit ( $r^2 = 0.47$ ,  $P < 0.05$ ) and stomata distribution ( $r^2 = 0.41$ ,  $P < 0.05$ ; Fig. 4a,c). All species classified as alternative water users were evergreen, and occurred naturally in mountain and canyons at higher elevations. All other species mostly occurred in lower deserts, were deciduous and were amphistomatous. Wood anatomy and leaf water potential ( $\psi_{\text{pd}}$ ,  $\psi_{\text{md}}$  and  $\Delta\psi$ ; Table S4, Figs S5 and S6) had little to no relationship with water use behaviour of the studied species, even though these traits are nominally linked to drought-tolerance.



**Figure 2** Criterion 1 – (a and d) Relationship between daytime photosynthesis ( $A_n$ ), transpiration rates ( $E_t$ ), and stomatal conductance ( $g_{sw}$ ) per water use classification (blue = alternative water user; orange = marginal gain); Criterion 2 – (b and e) relationship between  $T_{leaf}$  and  $E_t$ , and  $g_{sw}$ ; and Criterion 3 – (c and f) daytime relationship between observed and predicted  $E_t$  and  $g_{sw}$  per species, excluding *Enfa*. Statistical results for each species are in Table S2; inclusion of *Enfa* and night-time estimates of  $E_t$  and  $g_{sw}$  are shown in Figure S7.

### ‘Marginal gain’ model breakdowns

The marginal gain model performed poorly for morning and evening estimates of  $E_t$  and  $g_{sw}$  (Fig. S7). The model also did not predict positive  $E_t$  and  $g_{sw}$  during night-time and predawn periods. The model also yielded weak fits, especially for the evening ( $r^2_{E_t, 7PM} = 0.16$ ,  $P < 0.05$ ;  $r^2_{g_{sw}, 7PM} = 0.16$ ,  $P < 0.05$ ). When disregarding morning and evening values, the model explained 78% of the variance for  $g_{sw}$  ( $P < 0.001$ ) and 59% for  $E_t$  ( $P < 0.001$ ). When excluding *Enfa* from the data set,  $E_t$  estimates were further reduced and the explained variance was only 23% ( $P < 0.001$ ; Fig. S7c), while  $g_{sw}$  was similar with and without *Enfa* in the analysis (74%,  $P < 0.001$ ; Fig. S7d).  $E_t$  was poorly estimated at midday ( $r^2_{E_t, 12PM} = 0.09$ ,  $P = 0.12$ ), afternoon ( $r^2_{E_t, 4PM} = 0.19$ ,  $P < 0.05$ ) and morning ( $r^2_{E_t, 8AM} = 0.36$ ,  $P < 0.001$ ). These poor estimates of  $E_t$  reflect the distinct and decoupled manner to which  $E_t$  increased when  $A_n$  and  $g_{sw}$  concurrently decreased with rising D (Fig. 5; Figs S8–S11).

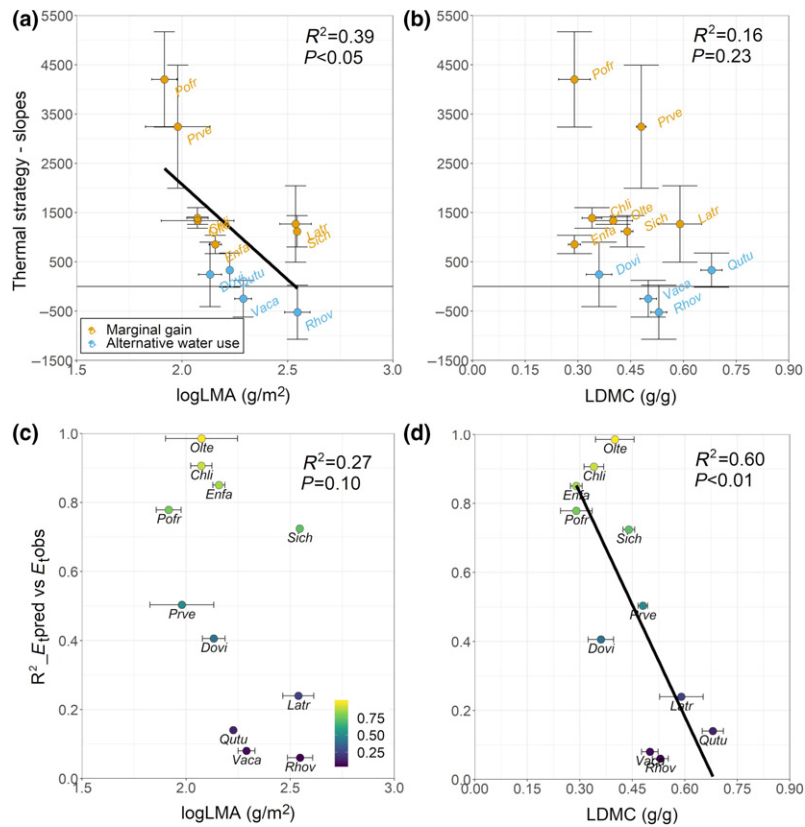
## DISCUSSION

This study is a potential proxy for future environmental change in aridlands, and for environments facing more extreme heatwaves. Under conditions of high daytime D

(> 6 kPa),  $T_{air}$  (> 40 °C) and low RH (< 20%) (Figs S2, S12–S15), we found evidence that heat avoidance or heat failure is common in many desert plant species. We also found that species with high construction cost leaves were more likely to have alternative water use behaviour. These results provide empirical evidence from natural environments that current gas exchange models are in some cases insufficient for predicting plant water use under hot and dry conditions.

### Heat avoidance or heat failure?

Results from the present study showed that 37% of desert species expressed gas exchange patterns of high water loss relative to net carbon assimilation, consistent with either heat avoidance or heat failure. Although data from this study do not allow us to directly test whether non-‘marginal gain’ species fall under the definition of heat avoidance or heat failure, our results seem most consistent with heat avoidance. In this study, the environmental conditions, while extreme globally, were locally within the range of typical summer conditions. Nevertheless, alternative water use might occur in other biomes or under episodic heatwave conditions. It is also possible that plants in this study extended their thermal tolerance threshold, thus leading to reductions of  $A_n$  as a result of increased mitochondrial respiration, increased photorespiration, reduced electron transport



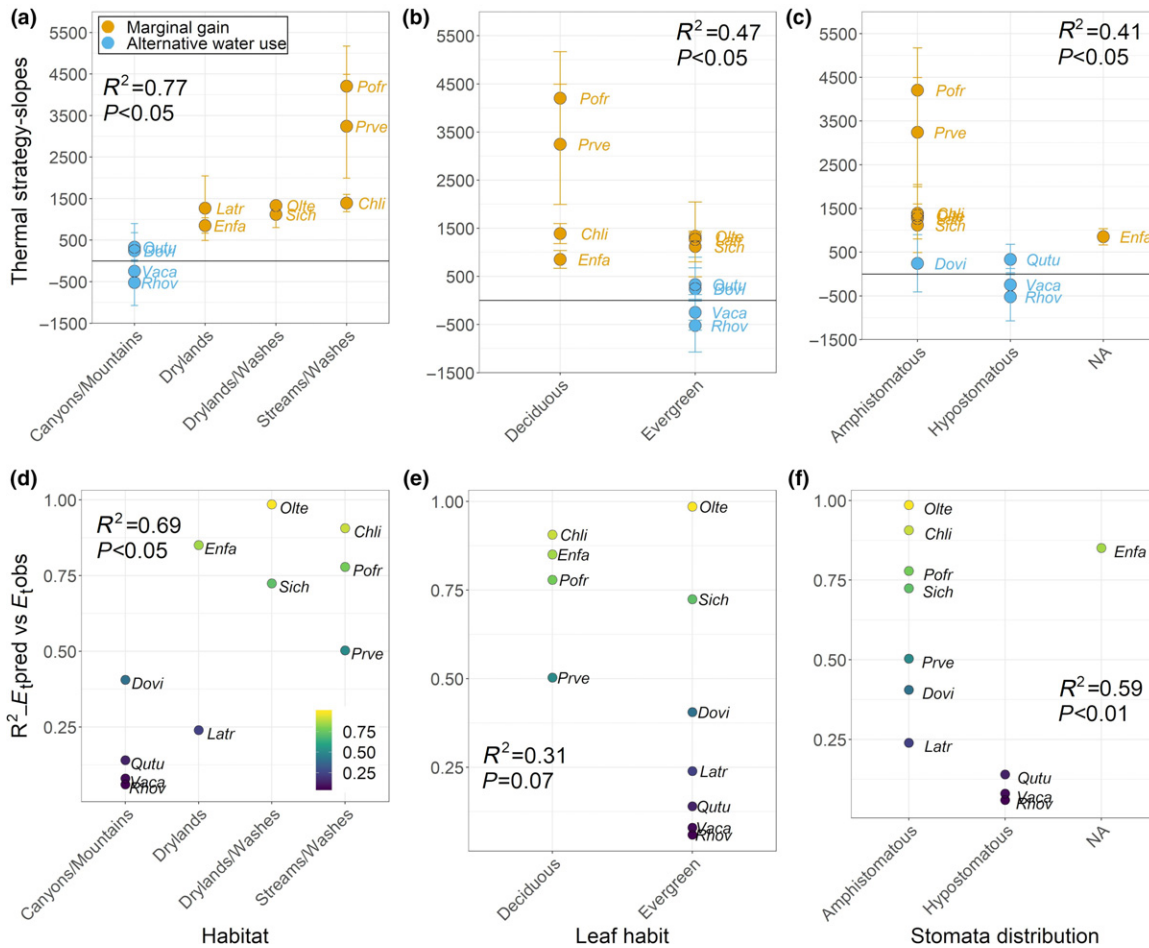
**Figure 3** Plant trait (leaf mass per area – LMA and leaf dry matter content – LDMC) relationship with thermal water use behaviour categories and model goodness-of-fit parameters. (a and b)  $A_n$  vs.  $E_t$  slope coefficients of each water use classification; and (c and d)  $r^2_{E_t}$  obtained through the Medlyn *et al.* (2011) and Lin *et al.* (2015) model fits for each species. Only significant regression lines are shown ( $\alpha = 0.05$ ).

capacity of Photosystem II or a combination of all three. Thermal tolerances of various desert species (including half of species sampled in our current study) were found to be between 45 and 52 °C (Downton *et al.* 1984; Knight & Ackerly 2003; Gallagher 2014), with most species having the potential of recovering ~90% of initial  $A_n$  and  $E_t$  rates. Although some of the observed  $T_{leaf}$  values were above  $T_{air}$  or not significantly cooler than  $T_{air}$  at peak evaporative cooling capacity (Figs S12–S14), we speculate that they transpire just enough to not exceed thermal tolerance thresholds (i.e. lethal temperatures; Blonder & Michaletz 2018), or temperatures would have been even higher in the absence of transpiration.

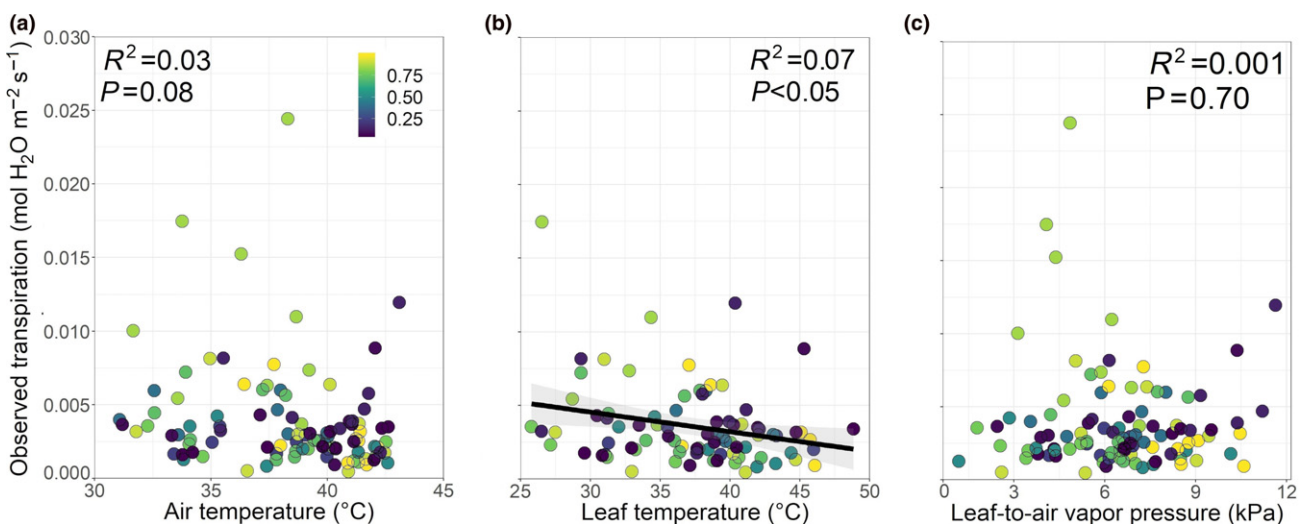
Heat tolerance could also preclude alternate water use behaviour. While high tolerance is documented in many CAM species that do not transpire during daytime conditions (e.g. Cactaceae; Smith *et al.* 1984), it appears less common in the  $C_3$  species studied here. High transpiration may be evolutionarily more easily achieved or lower cost than other strategies. While drought deciduousness or CAM photosynthesis could enable temporal avoidance of stressful environmental conditions (Cushman & Borland 2002; Tomlinson *et al.* 2013; Winter & Holtum 2014), many arid-adapted species do not deploy these strategies to cope with extreme aridity since they require large amounts of energy and carbon to produce organic acids or new leaves. CAM photosynthesis, dense trichomes, higher reflectance, increased thickness and

higher waxiness (Ehleringer & Mooney 1978; Smith 1978; Ehleringer 1988; Schuster *et al.* 2016) all have been identified as adaptive leaf traits in desert species to cope with the extreme conditions during harsh summers. However, because these traits are metabolically costly, some species might instead increase investment of water loss for evaporative cooling (i.e. saving carbon through respiration reduction and increase of Rubisco efficiency; Carmo-Silva *et al.* 2015) than investing carbon in leaves that might be permanently lost during heatwaves. This perspective is consistent with prior findings that species with tolerance to high  $T_{leaf}$  also express low minimum midday leaf conductance ( $g_{min}$ ), and subsequently reduced evaporative cooling (Schreiber 2001; Schuster *et al.* 2016).

In the present study, plants that adopted an alternative water use behaviour were mostly evergreen and had higher LMA, which suggests that these species prioritize tissue preservation instead of allocating carbon towards new leaf production after leaf mortality induced by heat stress. Desert and evergreen plants tend to have higher LMA and higher construction costs than deciduous plants and plants from more mesic biomes (Poorter *et al.* 2009), possibly due to the effects of lower water availability (de la Riva *et al.* 2016a) or high carbon investment in defensive traits against leaf desiccation (de la Riva *et al.* 2016b). The loss of leaves in response to extreme heat could result in a significant long-term carbon



**Figure 4** Variation in water use classification by species' habitat, leaf habit and stomatal distribution. Colours represent the thermal water use behaviour category (orange = marginal gain; blue = heat avoidance). Non-significant relationships with other traits are shown in the supporting information (Figures S5 and S6).



**Figure 5** Comparison between observed  $E_i$  rates when related to climatic parameters. Colour scale is based on the  $r^2_{ET}$  obtained between daytime observed and predicted  $E_i$  rates for each species (e.g. species with stronger fits are shown in yellow and lighter green colours). (a)  $T_{air}$  ( $^{\circ}C$ ), (b)  $T_{leaf}$  ( $^{\circ}C$ ), and (c)  $D_{leaf}$  (kPa). Analyses include all species and only daytime values (8 AM–4 PM). Only statistically significant regression lines are shown ( $\alpha = 0.05$ ). For the comparison between observed and predicted  $g_{sw}$ , see Figure S10; and Table S1 for  $r^2_{ET}$  values for each species.



deficits and potentially reduced plant growth, which could be avoided if  $T_{\text{leaf}}$  were tightly regulated.

### Implications for water availability

Heat avoidance may not be possible for extended temporal intervals in arid regions that are limited by soil moisture. For example, if we assume a leaf area index of only 1, whole-canopy water use in *Enfa* – the species with the highest  $E_t$  and  $g_{\text{sw}}$  in our study (Fig. S3) – extrapolated over the entire summer would exceed the incoming amount of precipitation ( $191 \text{ mm yr}^{-1}$ ) by a factor of three. However,  $E_t$  and  $g_{\text{sw}}$  rates in *Enfa* also were similar to rates previously reported for this species under well-watered and natural conditions (Smith & Nobel 1977; Ehleringer & Mooney 1978; Ehleringer 1988; Pockman & Sperry 2000).

Thus it instead seems likely that heat avoidance would only occur under high soil water availability (Urban *et al.* 2017b; Drake *et al.* 2018). All individuals in this study did receive supplemental irrigation. The four potential alternative water users in the present study all naturally occur along mountain slopes, and deep valley-bottom regolith where extensive mountain front recharge likely results in considerably higher soil moisture relative to mean annual precipitation that falls on the landscape. Rooting depths of *Qutu* have been reported to extend below 9 m, and rooting depths of a *Rhus* species closely related to *Rhov*, have been reported to extend below 13 m (Canadell *et al.* 1996). The deep roots would allow these species to forage for water throughout an extremely large volume of soil that in turn, could support high midday  $E_t$  rates during periods of high evaporative demand.

### Implications for models

The decoupling of  $E_t$  and  $g_{\text{sw}}$  from  $A_n$  and  $D$  results in significant underestimations of water fluxes when using current ‘marginal gain’ stomatal regulation models, for example, Medlyn *et al.* (2011). These biases would then scale up to ecosystem-level predictions in TBMs, with potentially major consequences for predicting climate change impacts especially in aridlands.

The overall implication is that current models do not effectively describe many species’ responses to extreme conditions. Models may need revision for plants occurring in hot and dry environments or those exposed to episodic heatwaves. Our trait-based results provide some preliminary insights into which species are mostly likely to follow the ‘marginal gain’ hypothesis, but the number of species predicted to be ‘alternative water users’ is large enough that further model development is likely necessary.

Importantly, the mathematical assumption of the portable photosynthesis system we used and most theory, is  $E_t = g_{\text{sw}} \cdot D$ . This is based on the assumption that water diffuses from internal air spaces through stomata and then into a boundary layer (Jones 1992). Regardless of this assumption, the values of  $A_n$  and  $E_t$  estimated from the system can be easily validated as they are estimated directly from the measured gas fluxes. However,  $g_{\text{sw}}$  is inferred based on this model, meaning that reported values should be interpreted with some

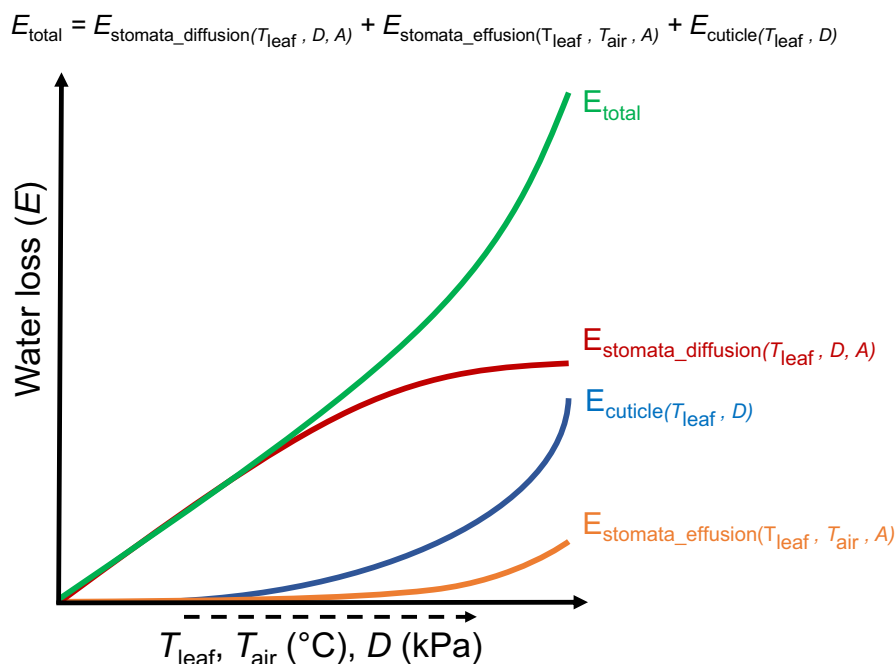
caution. More strongly, in a mixed model regression analysis, we found that  $E_t$  and  $g_{\text{sw}}$  depend statistically not only on  $D$  but also on  $T_{\text{leaf}}$  (Fig. S16). Thus there is a direct temperature effect on water transport that requires further explanation (see Fig. S17 for  $T_{\text{leaf}}$  effects on WUE and iWUE). There are several processes that would lead to model failure.

First, water may also be transpired through the leaf cuticle rather than through the stomata (Kerstiens 1996). This effect is generally negligible at low temperatures (Schuster *et al.* 2016; Duursma *et al.* 2019) but may be almost half of total conductance at  $T_{\text{leaf}} > 50 \text{ }^\circ\text{C}$  (Eamus *et al.* 2008; Cochard 2019; Duursma *et al.* 2019). Second, minimum leaf conductance has been shown to increase with  $T_{\text{leaf}}$ , due to a range of physical effects related to the temperature-dependent fluidity of water and increase in mesophyll conductances (Urban *et al.* 2017a; Urban *et al.* 2017b; Cochard 2019). Third, regulation of  $g_{\text{sw}}$  may be more challenging at high  $T_{\text{leaf}}$ , when pressure gradients occur between leaf air spaces and the atmosphere (arising from temperature-dependent water vapour concentration differences or direct temperature effects; Cernusak *et al.* 2018; Grossiord *et al.* 2020), and if this pressurization then leads to uncontrolled stomatal opening (Brix *et al.* 1992; Steinberg 1996; Cochard 2019). The vapour concentration effect could lead to pressurizations of approximately 4 kPa at  $45 \text{ }^\circ\text{C}$  (Supporting text – ‘Effect of temperature increases on closed stomata’). Internal leaf pressurizations have been reported of 1–2 kPa (Brix *et al.* 1992; Steinberg 1996; Arkebauer *et al.* 2001), but it seems doubtful that stomata could remain closed at much higher pressures. Fourth, damage to electron transport capacity of Photosystem II and other photo-biochemical processes could occur at high  $T_{\text{leaf}}$  that affect carbon assimilation but not water loss (Jiao & Grodzinski 1996; Slot & Winter 2016). Our field experiments were not designed to directly assess the relative contributions of these processes, but will be critical to advance simultaneous measurements of each in future studies.

Thus, the assumption of  $E_t = g_{\text{sw}} \cdot D$  will no longer be fully valid if  $E_t$  depends on  $T_{\text{leaf}}$  through the above mechanisms (Grossiord *et al.* 2020). High  $g_c$  is likely to occur when  $T_{\text{leaf}}$  is high, due to temperature-dependence of cuticular permeability (i.e. wax barriers; Schreiber 2001). Non-diffusive transport is likely to occur when the specific humidity of water vapour is high (i.e. when the water vapour is non-negligible molar fraction of the air), in which there is significant momentum transfer and thus directional flow from water transport (Kowalski 2017). Additionally, if leaf pressurization occurs at high temperatures, then there may also be further effusive transport occurring as stomata spontaneously open to equalize pressure differences. None of these processes are captured by the LI6800’s  $g_{\text{sw}}$  calculations (LI-COR 2018). More importantly, none of these processes are yet widely incorporated into gas exchange models (Kowalski 2017; Cochard 2019; Duursma *et al.* 2019).

To determine whether any of these processes may have influenced our conclusions, we took a heuristic approach, in which we assume an uncertainty in  $g_{\text{sw}}$  estimates that increases nonlinearly with  $T_{\text{leaf}}$ , taking a value of  $\pm 1\%$  at  $T_{\text{leaf}} = 10 \text{ }^\circ\text{C}$ ,  $\pm 5\%$  at  $T_{\text{leaf}} = 25 \text{ }^\circ\text{C}$  to  $\pm 40\%$  at  $T_{\text{leaf}} = 52 \text{ }^\circ\text{C}$ . These values are chosen based on prior simulations of the importance of





**Figure 6** Illustration of hypothesized alternative transpiration pathways ( $E$ ) in a leaf in response to increases in leaf temperature ( $T_{\text{leaf}}$ ), air temperature ( $T_{\text{air}}$ ) and/or vapour pressure deficit ( $D$ ) increase (dashed arrow indicates that all three variables follow the same increasing trend).  $E_{\text{stomata\_diffusion}}$  is transpiration through water diffusing from stomata (red);  $E_{\text{cuticle}}$  is cuticular transpiration (blue);  $E_{\text{stomata\_effusion}}$  is water loss through effusion from the stomata at high temperatures (orange);  $E_{\text{total}}$  is the sum of all components described (green). Parameters subscript between parentheses indicate drivers of that specific  $E$  component ( $A_n$  refers to photosynthesis).

$g_c$  (~40%; Eamus *et al.* 2008) and  $g_{\text{sw,effusion}}$  (9%; Haynes 2014; Kowalski 2017) at the highest temperature extremes. These simulations are likely overestimating the magnitude of these potential processes, as there is evidence that some desert plant cuticles can withstand thermal stress without impacting cuticle integrity and functioning (Schuster *et al.* 2016; Bueno *et al.* 2019). Regardless, we found that the  $g_{\text{sw}}$  uncertainty did not change the fundamental conclusion that the ‘marginal gain’ model did not fit the data well in the heat avoidance/heat failure species (Fig. S18).

Development of high temperature gas exchange theory, in which  $E_t$  depends not only on  $g_{\text{sw}}$  and  $D$ , but also directly on  $T_{\text{leaf}}$  and  $T_{\text{air}}$ , is beyond the scope of this empirical study. It seems necessary to adapt current gas exchange models from  $E = E_{\text{stomata,diffusion}}(D, A) = g_{\text{sw}}(D, A) \cdot D$  to a more general partitioning in terms of multiple fluxes, as  $E = E_{\text{stomata,diffusion}}(T_{\text{leaf}}, D, A) + E_{\text{stomata,effusion}}(T_{\text{leaf}}, T_{\text{air}}, A) + E_{\text{cuticle}}(T_{\text{leaf}}, D)$  (Fig. 6). Such a partitioning would be challenging, as there would no longer be a single leaf conductance value that summarizes transport against a single gradient. This partitioning would also be difficult to measure with field-based gas exchange measurements that cannot easily separate transport processes. However, these advances are critical for more accurate modelling of high temperature gas exchange in plants and thus for TBMs.

Moreover, optimality models would also need to be extended to predict stomatal responses given the conflicting demands of marginal gain and heat avoidance, as well as the possibility of heat failure. Such theory remains to be developed. One immediate ‘workaround solution’ might be to assume that  $E_t$  always follows marginal gain theory, but also

necessarily increases due to temperature effects. This could be implemented in eqn 1 by assuming that the  $g_0$  coefficient is an increasing function of  $T_{\text{leaf}}$ , that is, implicitly capturing some of the effects of  $E_{\text{effusion}}$  and  $E_{\text{cuticle}}$  (Duursma *et al.* 2019). Such a perspective is supported by other studies that have found temperature effects on this parameter (Duursma *et al.* 2019; Wu *et al.* 2020).

## CONCLUSION

This study highlights the limitations of current theory in describing plant gas exchange at high temperatures under field conditions. It remains unknown how frequently this alternative water use behaviour might be occurring in other environments and for other taxa. Further studies should quantify this strategy variation at broader scales, from potted plants to experimental common gardens to naturally occurring plants in the wild. This study also highlights the need for further development of gas exchange models that can capture the full range of processes and plant responses to extreme environments. Advancing such models will provide more robust predictions for aridlands and for biomes experiencing heatwaves and drought.

## ACKNOWLEDGEMENTS

This project was supported by grants from the John Fell Oxford University Press (OUP) Research Fund (163/049) and the British Ecological Society (SR17/100228). BB was supported by a UK Natural Environment Research Council

fellowship (NE/M019160/1). BB and LA were supported by Arizona State University's School of Life Sciences. Undergraduate students were supported by the Western Alliance to Expand Student Opportunities (WAESO, V18UR027/V2018ur0034) under a National Science Foundation cooperative agreement (HRD-1619524). The Desert Botanical Garden's Raul Puente-Martinez and Kimberlie McCue granted access to their living collections, permitted the gas exchange measurements, leaf collection and equipment setup throughout the Garden and allowed use of the research facility. Research technician Dan Koepke provided valuable technical assistance throughout the measurement period. Debra Page Baluchat the ASU Keck Imaging Lab, assisted with the stomatal imaging. We also thank Martijn Slot and Sean Michaletz for their valuable scientific input towards the manuscript. Thoughtful feedback from three anonymous reviewers greatly improved the manuscript.

#### AUTHORSHIP

LA collected and analyzed the data and wrote the manuscript; BB and KH wrote the manuscript and provided scientific input. BB conceived the project and obtained funding. LA, CS and SW conducted fieldwork, and processed the samples and data. The authors declare no conflict of interest.

#### DATA ACCESSIBILITY STATEMENT

The data supporting these results are archived in Dryad (<https://doi.org/10.5061/dryad.j9kd51c8h>).

#### REFERENCES

- Ameye, M., Wertin, T., Bauweraerts, I., McGuire, M., Teskey, R. & Steppe, K. (2012). The effect of induced heat waves on *Pinus taeda* and *Quercus rubra* seedlings in ambient and elevated CO<sub>2</sub> atmospheres. *New Phytol.*, 196, 448–461.
- Anderegg, W.R.L., Wolf, A., Arango-Velez, A., Choat, B., Chmura, D.J., Jansen, S. *et al.* (2018). Woody plants optimise stomatal behaviour relative to hydraulic risk. *Ecol. Lett.*, 21, 968–977.
- Arkebauer, T.J., Chanton, J.P., Verma, S.B. & Kim, J. (2001). Field measurements of internal pressurization in *Phragmites australis* (Poaceae) and implications for regulation of methane emissions in a midlatitude prairie wetland. *Am. J. Bot.*, 88, 653–658.
- Ball, J.T., Woodrow, I.E. & Berry, J.A. (1987). A model predicting stomatal conductance and its contribution to the control of photosynthesis under different environmental conditions. In *Progress in Photosynthesis Research* (ed Biggins, J.). Martinus-Nijhoff Publishers Dordrecht, Netherlands, pp. 221–224.
- Bauweraerts, I., Wertin, T.M., Ameye, M., McGuire, M., Teskey, R. & Steppe, K. (2013). The effect of heat waves, elevated [CO<sub>2</sub>] and low soil water availability on northern red oak (*Quercus rubra* L.) seedlings. *Glob. Change Biol.*, 19, 517–528.
- Bendix, M., Tornbjerg, T. & Brix, H. (1994). Internal gas transport in *Typha latifolia* L. and *Typha angustifolia* L. I. Humidity-induced pressurization and convective throughflow. *Aquat. Bot.*, 49, 75–89.
- Blonder, B. & Michaletz, S.T. (2018). A model for leaf temperature decoupling from air temperature. *Agric. For. Meteorol.*, 262, 354–360.
- Bonan, G.B., Williams, M., Fisher, R.A. & Oleson, K.W. (2014). Modeling stomatal conductance in the earth system: linking leaf water-use efficiency and water transport along the soil-plant-atmosphere continuum. *Geosci. Model Dev.*, 7, 2193–2222.
- Brix, H., Sorrell, B.K. & Orr, P.T. (1992). Internal pressurization and convective gas flow in some emergent freshwater macrophytes. *Limnol. Oceanogr.*, 37, 1420–1433.
- Bueno, A., Alfarhan, A., Arand, K., Burghardt, M., Deininger, A., Hedrich, R. *et al.* (2019). Effects of temperature on the cuticular transpiration barrier of two desert plants with water-spender and water-saver strategies. *J. Exp. Bot.*, 70, 1613–1625.
- Canadell, J., Jackson, R.B., Ehleringer, J.R., Mooney, H.A., Sala, O.E. & Schulze, E.D. (1996). Maximum rooting depth of vegetation types at the global scale. *Oecologia*, 108, 583–595.
- Carmo-Silva, E., Scales, J.C., Madgwick, P.J. & Parry, M.A. (2015). Optimizing Rubisco and its regulation for greater resource use efficiency. *Plant, Cell Environ.*, 38, 1817–1832.
- Cernusak, L.A., Ubierna, N., Jenkins, M.W., Garrity, S.R., Rahn, T., Powers, H.H. *et al.* (2018). Unsaturation of vapour pressure inside leaves of two conifer species. *Nat. Sci. Rep.*, 8(7667), 1–7.
- Chen, Y., Ryder, J., Batrikov, V., McGrath, M.J., Naudts, K., Otto, J. *et al.* (2016). Evaluating the performance of land surface model ORCHIDEE-CAN v1.0 on water and energy flux estimation with a single- and multi-layer energy budget scheme. *Geosci. Model Dev.*, 9, 2951–2972.
- Cochard, H. (2019). A new mechanism for tree mortality due to drought and heatwaves. *bioRxiv*, 531632.
- Cowan, I.R. & Farquhar, G.D. (1977). Stomatal functioning in relation to leaf metabolism and environment. *Symp. Soc. Exp. Biol.*, 31, 471–505.
- Crawford, A.J., McLachlan, D.H., Hetherington, A.M. & Franklin, K.A. (2012). High temperature exposure increases plant cooling capacity. *Curr. Biol.*, 22, R396–R397.
- Cushman, J.C. & Borland, A.M. (2002). Induction of Crassulacean acid metabolism by water limitation. *Plant, Cell Environ.*, 25, 295–310.
- De Kauwe, M.G., Zhou, S., Medlyn, B.E., Pitman, A.J., Wang, Y., Duursma, R.A. *et al.* (2015). Do land surface models need to include differential plant species responses to drought? Examining model predictions across a mesic-xeric gradient in Europe. *Biogeosciences*, 12, 7503–7518.
- Dilts, T.E., Weisberg, P.J., Dencker, C.M. & Chambers, J.C. (2015). Functionally relevant climate variables for arid lands: a climatic water deficit approach for modelling desert shrub distributions. *J. Biogeogr.*, 42, 1986–1997.
- Downton, W.J.S., Berry, J.A. & Seemann, J.R. (1984). Tolerance of photosynthesis to high temperature in desert plants. *Plant Physiol.*, 74, 786–790.
- Drake, J.E., Tjoelker, M.G., Varhammar, A., Medlyn, B.E., Reich, P.B., Leigh, A. *et al.* (2018). Trees tolerate an extreme heatwave via sustained transpirational cooling and increased leaf thermal tolerance. *Glob. Change Biol.*, 24, 2390–2402.
- Duursma, R.A., Blackman, C.J., Lopéz, R., Martin-StPaul, N.K., Cochard, H. & Medlyn, B.E. (2019). On the minimum leaf conductance: its role in models of plant water use, and ecological and environmental controls. *New Phytol.*, 221, 693–705.
- Eamus, D., Taylor, D.T., Macinins-Ng, C., Shanahan, S. & Silva, L. (2008). Comparing model predictions and experimental data for the response of stomatal conductance and guard cell turgor to manipulations of cuticular conductance, leaf-to-air vapour pressure difference and temperature: feedback mechanisms are able to account for all observations. *Plant Cell Environ.*, 31, 269–277.
- Ehleringer, J.R. (1988). Comparative ecophysiology of *Encelia farinosa* and *Encelia frutescens*. I. Energy balance considerations. *Oecologia*, 76, 553–561.
- Ehleringer, J.R. & Mooney, H.A. (1978). Leaf hairs: effects on physiological activity and adaptive value to a desert shrub. *Oecologia*, 37, 183–200.
- Franklin, J., Serra-Diaz, J.M., Syphard, A.D. & Regan, H.M. (2016). Global change and terrestrial plant community dynamics. *Proc. Natl Acad. Sci.*, 113, 3725–3734.

- Franks, P.J., Cowan, I.R. & Farquhar, G.D. (1997). The apparent feedforward response of stomata to air vapour pressure deficit: information revealed by different experimental procedures with two rainforest trees. *Plant Cell Environ.*, 20, 142–145.
- Gallagher, D.W. (2014). *Photosynthetic Thermal Tolerance and Recovery to Short Duration Temperature Stress in Desert and Montane Plants: A Comparative Study*. California Polytechnic State University, San Luis Obispo, CA, p. 39.
- Golaz, J., Caldwell, P.M., Van Roekel, L.P., Petersen, M.R., Tang, Q., Wolfe, J.D. *et al.* (2019). The DOE E3SM Coupled Model version 1: Overview and evaluation at standard resolution. *J. Adv. Model. Earth Syst.*, 11, 2089–2129.
- Griebel, A., Bennett, L.T., Metzen, D., Pendall, E., Lane, P.N.J. & Arndt, S.K. (2019). Trading water for carbon: sustained photosynthesis at the cost of increased water loss during high temperatures in a temperate forest. *bioRxiv*. <https://doi.org/10.1101/760249>
- Grossiord, C., Buckley, T.N., Cernusak, L.A., Novick, K.A., Poulter, B., Siegwolf, R.T.W. *et al.* (2020). Plant responses to rising vapor pressure deficit. *New Phytol.* <https://doi.org/10.1111/nph.16485>
- Haynes, W.M. (2014). *CRC Handbook of Chemistry and Physics*. CRC Press, Boca Raton, LA.
- Herrera-Strada, J.E. & Sheffield, J. (2017). Uncertainties in future projections of summer droughts and heat waves over the contiguous United States. *J. Clim.*, 30, 6225–6246.
- Huang, J., Ji, M., Xie, Y., Wang, S., He, Y. & Ran, J. (2016). Global semi-arid climate change over last 60 years. *Clim. Dyn.*, 46, 1131–1150.
- Jiao, J. & Grodzinski, B. (1996). The effect of leaf temperature and photorespiratory conditions on export of sugars during steady-state photosynthesis in *Salvia splendens*. *Plant Physiol.*, 111, 169–178.
- Jones, H.G. (1992). *Plants and Microclimate: A Quantitative Approach to Environmental Plant Physiology*, 2nd edn. Press Syndicate of the University of Cambridge, Cambridge, UK.
- Kerstiens, G. (1996). Cuticular water permeability and its physiological significance. *J. Exp. Bot.*, 47, 1813–1832.
- Knauer, J., Werner, C. & Zaehle, S. (2015). Evaluating stomatal models and their atmospheric drought response in a land surface scheme: a multi-biome analysis. *Biogeosciences*, 12, 1894–1911.
- Knight, C.A. & Ackerly, D.D. (2003). Evolution and plasticity of photosynthetic thermal tolerance specific leaf area and leaf size: congeneric species from desert and coastal environments. *New Phytol.*, 160, 337–347.
- Kowalski, A.S. (2017). The boundary condition for vertical velocity and its interdependence with surface gas exchange. *Atmos. Chem. Phys.*, 17, 8177–8187.
- Leuning, R. (1995). Critical appraisal of a coupled stomatal-photosynthesis model for C3 plants. *Plant Cell Environ.*, 18, 339–357.
- LI-COR (2018). *Using the LI-6800*. Portable Photosynthesis System, Lincoln, NE.
- Lin, Y., Medlyn, B.E., Duursma, R.A., Prentice, I.C., Wang, H., Baig, S. *et al.* (2015). Optimal stomatal behaviour around the world. *Nat. Clim. Chang.*, 5, 459–464.
- Lin, H., Chen, Y., Zhang, H., Fu, P. & Fan, Z. (2017). Stronger cooling effects of transpiration and leaf physical traits of plants from a hot dry habitat than from a hot wet habitat. *Funct. Ecol.*, 31, 2202–2211.
- McDowell, N., Pockman, W.T., Allen, C.D., Breshears, D.D., Cobb, N., Kolb, T. *et al.* (2008). Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytol.*, 178, 719–739.
- Medlyn, B.E., Duursma, R.A., Eamus, D., Ellsworth, D.S., Prentice, I.C., Barton, C.V.M. *et al.* (2011). Reconciling the optimal and empirical approaches to modelling stomatal conductance. *Glob. Change Biol.*, 17, 2134–2144.
- Monteith, J.L. & Unsworth, M.H. (1990). *Principles of Environmental Physics*. Edward Arnold, London, UK.
- Pockman, W.T. & Sperry, J. (2000). Vulnerability to xylem cavitation and the distribution of Sonoran Desert vegetation. *Am. J. Bot.*, 87, 1287–1299.
- Poorter, H., Niinemets, U., Poorter, L., Wright, I.J. & Villar, R. (2009). Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytol.*, 182, 565–588.
- Quevedo, D.I. & Frances, F. (2008). A conceptual dynamic vegetation-soil model for arid and semiarid zones. *Hydrol. Earth Syst. Sci.*, 12, 1175–1187.
- de la Riva, E.G., Olmo, M., Poorter, H., Ubersa, J.L. & Villar, R. (2016a). Leaf mass per area (LMA) and its relationship with leaf structure and anatomy in 34 Mediterranean woody species along a water availability gradient. *PLoS ONE*, 11, e0148788.
- de la Riva, E.G., Tosto, A., Perez-Ramos, I.M., Navarro-Fernandez, C.M., Olmo, M., Anten, N.P.R. *et al.* (2016b). A plant economics spectrum in Mediterranean forests along environmental gradients: is there coordination among leaf, stem and root traits? *J. Veg. Sci.*, 27, 187–199.
- Rogers, A., Medlyn, B.E., Dukes, J.S., Bonan, G., von Caemmerer, S., Dietze, M.C. *et al.* (2017). A roadmap for improving the representation of photosynthesis in Earth system models. *New Phytol.*, 213, 22–42.
- Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T. *et al.* (2012). Fiji: an open-source platform for biological-image analysis. *Nat. Methods*, 9, 676–682.
- Schreiber, L. (2001). Effect of temperature on cuticular transpiration of isolated cuticular membranes and leaf discs. *J. Exp. Bot.*, 52, 1893–1900.
- Schuster, A., Burghardt, M., Alfarhan, A., Bueno, A., Hedrich, R., Leide, J. *et al.* (2016). Effectiveness of cuticular transpiration barriers in a desert plant at controlling water loss at high temperatures. *AoB Plants*, 8, plw027.
- Sévellec, F. & Drijfhout, S.S. (2018). A novel probabilistic forecast system predicting anomalously warm 2018–2022 reinforcing the long-term global warming trend. *Nat. Commun.*, 9, 1–12.
- Slot, M. & Winter, K. (2016). The effects of rising temperature on the ecophysiology of tropical forest trees. In: *Tropical Tree Physiology* (eds Goldstein, G. & Santiago, L.S.). Springer, Switzerland, pp. 385–412.
- Slot, M., Garcia, M.N. & Winter, K. (2016). Temperature response of CO<sub>2</sub> exchange in three tropical tree species. *Funct. Plant Biol.*, 43, 468–478.
- Smith, W.K. (1978). Temperatures of desert plants: another perspective on the adaptability of leaf size. *Science*, 201, 614–616.
- Smith, W.K. & Nobel, P.S. (1977). Influences of seasonal changes in leaf morphology on water-use efficiency for three desert broadleaf shrubs. *Ecology*, 58, 1033–1043.
- Smith, S.D., Didden-Zopf, B. & Nobel, P.S. (1984). High-temperature responses of North American cacti. *Ecology*, 643–651.
- Sperry, J., Adler, F.R., Campbell, G.S. & Comstock, J.P. (1998). Limitation of plant water use by rhizosphere and xylem conductance: results from a model. *Plant Cell Environ.*, 21, 347–359.
- Steinberg, S.L. (1996). Mass and energy exchange between the atmosphere and leaf influence gas pressurization in aquatic plants. *New Phytol.*, 134, 587–599.
- Teskey, R., Werten, T., Bauweraerts, I., Ameye, M., McGuire, M. & Steppe, K. (2015). Responses of tree species to heat waves and extreme heat events. *Plant Cell Environ.*, 38, 1699–1712.
- Tomlinson, K.W., Poorter, L., Sterck, F.J., Borghetti, F., Ward, D., Bie, S. *et al.* (2013). Leaf adaptations of evergreen and deciduous trees of semi-arid and humid savannas on three continents. *J. Ecol.*, 101, 430–440.
- Urban, J., Ingwers, M.W., McGuire, M. & Teskey, R. (2017a). Stomatal conductance increases with rising temperature. *Plant Signaling Behavior*, 12, 1–3.
- Urban, J., Ingwers, M.W., McGuire, M.A. & Teskey, R. (2017b). Increase in leaf temperature opens stomata and decouples net photosynthesis from stomatal conductance in *Pinus taeda* and *Populus deltoides* × *nigra*. *J. Exp. Bot.*, 68, 1757–1767.
- Vargas, G.G. & Cordero, S.R.A. (2013). Photosynthetic responses to temperature of two tropical rainforest tree species from Costa Rica. *Trees*, 27, 1261–1270.



- Von Caemmerer, S. & Evans, J.R. (2015). Temperature responses of mesophyll conductance differ greatly between species. *Plant Cell Environ.*, 38, 629–637.
- Wijk, M.T., Dekker, S.C., Bouten, W., Bosveld, F.C., Kohsiek, W., Kramer, K. *et al.* (2000). Modeling daily gas exchange of a Douglas-fir forest: comparison of three stomatal conductance models with and without a soil water stress function. *Tree Physiol.*, 20, 115–122.
- Winter, K. & Holtum, J.A.M. (2014). Facultative crassulacean acid metabolism (CAM) plants: powerful tools for unravelling the functional elements of CAM photosynthesis. *J. Exp. Bot.*, 65, 3425–3441.
- Wu, J., Serbin, S.P., Ely, K.S., Wolfe, B., Dickman, L.T., Grossiord, C. *et al.* (2020). The response of stomatal conductance to seasonal drought in tropical forests. *Glob. Change Biol.*, 26, 823–839.
- Yang, J., Duursma, R.A., De Kauwe, M.G., Kumarathunge, D., Jiang, M., Mahmud, K. *et al.* (2019). Incorporating non-stomatal limitation

improves the performance of leaf and canopy models at high vapour pressure deficit. *Tree Physiol.*, 39, 1961–1974.

#### SUPPORTING INFORMATION

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

Editor, Josep Penuelas

Manuscript received 18 March 2020

First decision made 22 March 2020

Manuscript accepted 25 March 2020