Contrasting responses of two C₄ desert shrubs to drought but consistent decoupling of photosynthesis and stomatal conductance at high temperature

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A B S T R A C T

How desert shrubs respond to drought and heat remains poorly understood. We investigated how drought affected the morphology and physiology of two C₄ species with different root architecture, *Calligonum arborescens* and *Haloxylon ammodendron*, in the Taklamakan Desert. We measured leaf traits, chlorophyll fluorescence and temperature responses of photosynthesis after ~3 years of experimental drought and asked: (1) Do both species adjust similarly to drought? (2) Does transpirational cooling increase at high temperature, and if so, how is this affected by drought? Drought plants of *H. ammodendron* had shorter and thinner leaves than controls, and lower chlorophyll content, photochemical efficiency, and electron transport rate. In contrast, *C. arborescens* leaves became shorter and thicker. Drought reduced maximum photosynthesis by 63 % and 21 % in *H. ammodendron* and *C. arborescens*, respectively. The optimum temperature did not change significantly in *H. ammodendron* and decreased by ~2 °C in *C. arborescens*. Surprisingly, above 40–45 °C, stomatal conductance (g_{sw}) and transpiration increased—even under drought stomata partially reopened. This consistent uncoupling of photosynthesis and g_{sw} suggests that widely-implemented stomatal optimization models may poorly reflect high-temperature behaviors in dryland ecosystems. Our study established the basis for predicting the eco-physiological responses of C4 species in hyper-arid ecosystems to climate change.

1. Introduction

Global mean annual temperatures are predicted to increase by 0.7–4.0 °C during this century (Lee et al., 2021), and this warming will increase atmospheric drought. At the same time, rainfall patterns are changing, increasing the frequency and intensity of soil droughts in many areas (Singh et al., 2013; Knapp et al., 2015). The ability to acclimate to atmospheric and soil drought is thus critical for most species (DeSoto et al., 2020). Acclimation can be physiological, morphological, and biochemical (Feng et al., 2018). Stomatal control is considered the main physiological mechanism for avoiding irreversible damage due to dehydration (Choat et al., 2018; Bhusal et al., 2020), while biochemical changes include reduction in chlorophyll content and

adjustment of pigment composition, osmotic adjustments, and a reduction in the electron transport capacity (Gholamin and Khayat-nezhad, 2011; Turner, 2018). Morphological adjustments that improve drought tolerance include changes in leaf size and thickness, while (facultative) deciduousness can be an effective drought-avoidance strategy (Basu et al., 2016; Pritzkow et al., 2021). Severe droughts may cause widespread plant mortality and reduce plant productivity, which may feedback to accelerating CO₂ accumulation rates in the atmosphere (Ciais et al., 2013; Peters et al., 2018). Understanding whether and how plants adjust to extreme conditions is thus critical for predicting how anthropogenic climate change will affect global carbon dynamics.

Desert and arid systems comprise approximately one-third of the

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land surface and global warming has already increased dryland area since the 1950s (Li et al., 2019). Some semi-arid regions contribute far more to carbon cycling than previously thought (Cleverly et al., 2013), highlighting the significance of understanding these systems in the context of global climate change. Many dryland species have architectural and morphological adaptations to withstand high temperature and water limitation (Ehleringer, 1985), including extensive root systems, epidermal appendages that increase reflectance (e.g., trichomes, cuticular and wax crystals), leaf angles that reduces incident irradiance, elevation of leaves from the hot soil surface, and small and thick leaves with high boundary layer conductance (Leigh et al., 2012; Groom et al., 2004; Xu et al., 2020; Feng et al., 2022). Despite their ability to withstand harsh environmental conditions, desert vegetation may be particularly vulnerable to climate change because they already are already operating near the thermal and physiological limits of what most organisms can withstand (Hamerlynck and Huxman, 2009; Curtis et al., 2014, 2016). Understanding the impact of climate change on the physiology and morphology of vegetation in desert environments will provide a reference for understanding effects of extreme climate conditions.

The response of photosynthesis to temperature is a central facet of plant response to climate (Lin et al., 2012). Understanding the temperature sensitivity of photosynthesis improves predictions for plant productivity and terrestrial carbon flux models under global warming (Jagadish et al., 2021; Sadok et al., 2021). Due to the harsh environment and limited access of many deserts, there is very little research on the response of desert vegetation to temperature. This leaves a significant gap in our understanding of ecosystem response to future temperature extremes (Curtis et al., 2014). C4 photosynthesis, with its carbon concentrating mechanism (CCM), and high water use efficiency (WUE), is considered an adaptation to warm climates, where C4 species achieve their highest diversity (Watcharamongkol et al., 2018). Because of their adaptive advantages, some C4 plants can form dominant populations in harsh desert environments (Xu et al., 2007) – whether they can maintain this in the future will depend on their capacity to operate under conditions of more extreme atmospheric drought and temperatures.

The optimal temperature for photosynthesis (T_{opt}) of C3 plants is usually close to ambient temperature (Slot and Winter, 2017a; Kumarathunge et al., 2019; Hernández et al., 2020), with T_{opt} of C4 plants typically being moderately higher because the CCM reduces photorespiration (Sage and Kubien, 2007). However, for long periods during the day, leaf temperatures exceed optimal conditions for carbon assimilation due to radiative heating, especially in upper-canopy foliage (Sharkey, 2005; Mau et al., 2018). Leaf temperatures can exceed air temperature by as much as 18–20 °C in tropical forest (Fauset et al., 2018) and desert plants (Smith, 1978), and regularly exceed 40 °C (Smith, 1978; Slot et al., 2016; Tiwari et al., 2021). Excessive leaf temperature can damage and ultimately kill leaves (Krause et al., 2010). In the short term, transpirational cooling can be an effective means to reduce this risk. For example, transpiration kept heat-stressed leaves 7.5 °C cooler than predicted by a photosynthetic model (Drake et al., 2018). However, this requires a lot of water, which is potentially risky for plants under drought conditions.

The regulation of CO_2 and H_2O exchange under extreme temperature remains a key uncertainty. The decrease in photosynthesis above the optimum in C3 plants is generally ascribed to decreasing stomatal conductance (Lin et al., 2012; Slot and Winter, 2017b) or electron transport rate (Vårhammar et al., 2015). Theory predicts that the optimal stomatal behavior is one that minimizes leaf transpiration while maximizing photosynthetic carbon uptake, which is the basis of many models (Cowan and Farquhar, 1977). Stomatal optimization models predict that as vapor pressure deficit (VPD) increases at higher air temperature (T_{air}), stomatal conductance (g_{sw}), transpiration (E), and photosynthesis (A) will all be suppressed and eventually declined to zero, as the cost of water loss exceeds the benefit of carbon gain, i.e., A and E are coupled via g_{sw}. However, A and g_{sw} may decouple under extreme temperature, leading to increase in transpiration in the absence of CO_2 fixation (Blonder and Michaletz, 2018). Such decoupling has been reported in several recent studies at the leaf (Teskey et al., 2015; Slot et al., 2016; Urban et al., 2017; Drake et al., 2018; Aparecido et al., 2020; Sadok et al., 2021; Marchin et al., 2021) and ecosystem level (Krich et al., 2022), but such stomatal behavior is not captured in water use models (Aparecido et al., 2020; Blonder et al., 2023). These studies challenge existing models, but most evidence comes from potted C3 plants from temperate (Urban et al., 2017), tropical (Slot et al., 2016) and subtropical regions (Drake et al., 2018), and less is known about species from arid regions (Rogers et al., 2017; Aparecido et al., 2020), in the field, and under drought conditions (Anderegg et al., 2018). Whether C4 plants adapted to the hot desert environment likewise exhibit decoupling of stomatal conductance and photosynthesis at high temperatures is currently unknown.

We carried out an almost-3-year long drought experiment in the Taklamakan Desert, where T_{air} often reaches 45 °C and annual precipitation is less than 50 mm. We used planted individuals of two C4 shrub species with distinctly different root architecture, *Haloxylon ammodendron* and *Calligonum arborescens*; *H. ammodendron* has very deep roots, whereas *C. arborescens* has much shallower roots that extend laterally across a large area (Xu and Li, 2006; Wu et al., 2019). In their natural environment these species rarely occur together (Li et al., 2017). We combined measurements of leaf gas exchange, chlorophyll fluorescence, and leaf morphology and chemistry. We first asked what physiological and morphological responses to drought the two species exhibited, hypothesizing that:

1. The two species adopt different drought acclimation strategies, given the known differences in root architecture and the fact that they appear to occupy different niches in their native range.

We then tested the hypothesis that:

2. Decoupling of water use and carbon gain also occurs in desert C4 plants at high temperatures, but drought will inhibit this strategy of transpirational cooling.

2. Material and methods

2.1. Study sites and tree species

This experiment was conducted in the Taklamakan Desert, China (38°58′15.27″N, 83°39′34.69″E, 30 m above sea level) (Fig. 1). This is an extremely arid area where annual rainfall averages < 50 mm and annual pan evaporation is > 3000 mm. The mean air temperature is 12 °C, with a daily maximum of 45.6 °C in August (Fig. S1) and a minimum of − 22.2 °C in January, and mean annual wind speed and maximum instantaneous wind speed are 2.5 and 20 m·s⁻¹, with more than 130 days for sand-shifting. Such harsh conditions mean that almost nothing grows here, hence the nickname "Dead Sea". The Taklamakan Desert Highway Shelterbelt (TDHS) was built through the Taklamakan Desert. The soil is typical sandy soil with low nutrient content (Li et al., 2015). Several saline-alkali and drought-tolerant species of the genera *Haloxylon Bunge, Calligonum Linn, and Tamarix Linn* have been introduced since 1997, and saline groundwater with drip-irrigation was used for an artificial shelterbelt. Gradually, *Haloxylon ammodendron* and *Calligonum arborescens* have become the two dominant species along the TDHS (Li et al., 2017).

Our experiment was conducted on *H. ammodendron* and *C. arborescens* plants at an experimental plot near the TDBG. They are both drought tolerant shrubs with C4 photosynthesis (Xu et al., 2007; Liang et al., 2013). Both species have big root systems, but while roots of mature *H. ammodendron* plants can penetrate to 10 m below the soil surface and reach the groundwater, roots of *C. arborescens* mainly extend horizontally, by 10–20 m, remaining relatively shallow at less than 3 m depth (Xu and Li, 2006; Wu et al., 2019). *H. ammodendron* is native to the inter-dune lowland and the flat slope of dunes of the Gurbantunggut Desert, which is wetter and more nutrient rich than the Taklamakan
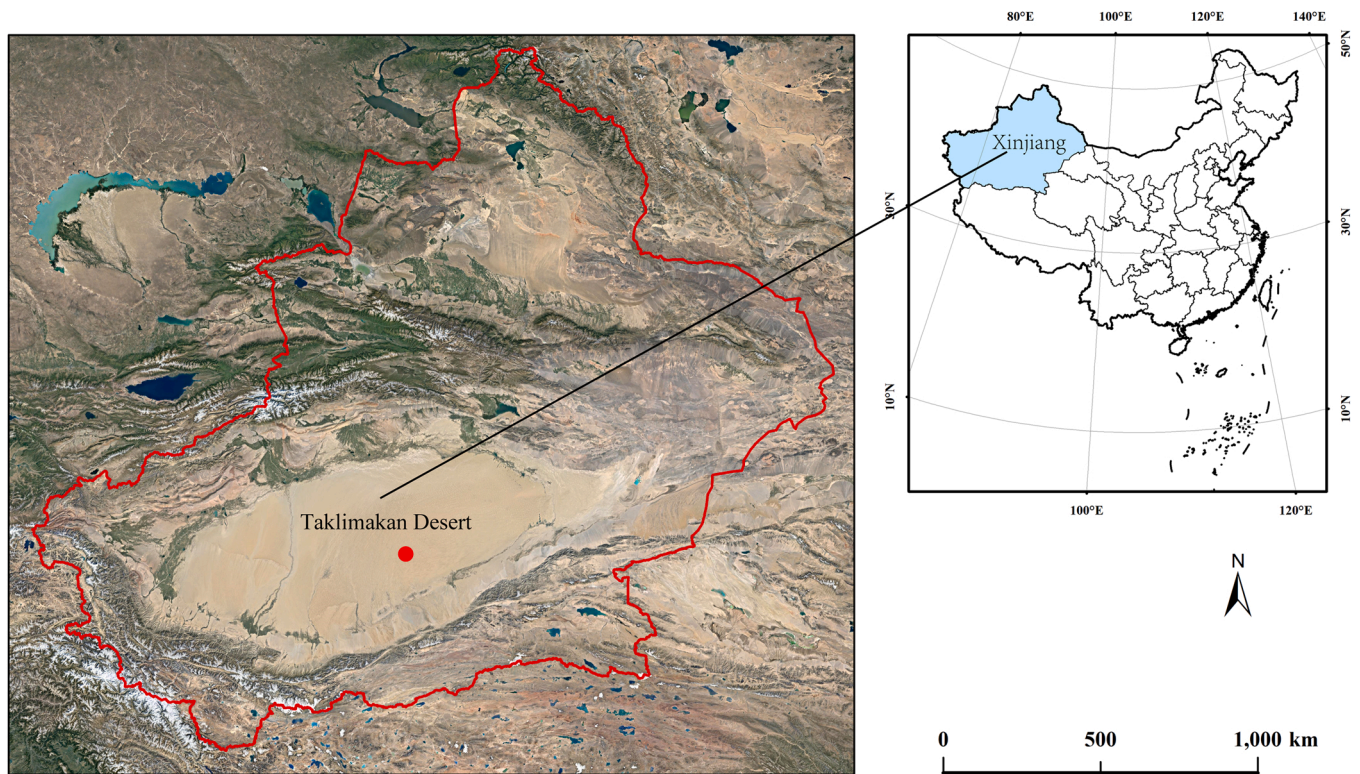
Fig. 1. Sketch map of the study area. We studied *Haloxylon ammodendron* and *Calligonum arborescens* under different treatments during the plant growth season in August 2021, in the Taklamakan Desert.

Desert, and *C. arborescens* mainly grows on sand dunes in the Russian Federation and Central Asia.

2.2. Experimental design and sampling

One-year-old seedlings were planted in 2011 and they were regularly watered by drip irrigation to facilitate their establishment (Fig. S6). Because the crown width of adult plants exceeds 2 m, seedlings were planted 3 m apart. The two treatments were separated by > 20 m to prevent droughted plants from receiving irrigation. Within treatment, the two species were spaced by ~10 m. For each treatment 5–10 plants were selected, with a base diameter of about 8 cm. For detailed soil properties, see Li et al. (2015).

Starting in March 2019, control plants were irrigated once every 10 days with ~27 L using the drip-irrigation system, and irrigation was stopped altogether for the drought group. After 3 growing seasons, the measurements were carried out in August 2021. For each treatment, physiological parameters were measured on at least 6 sun-exposed leaves from different individuals, to ensure the independence of the samples. All leaves were measured at heights between c. 1.3–1.6 m (mid-upper plant canopy). After in situ gas exchange and fluorescence measurements (see below), the measured leaves and a sufficient number of adjacent leaves were collected for functional trait analysis.

Soil samples were collected using a T-handle auger next to the marked plants at 17 depths (0–5, 5–10, 10–20 cm, and down to 3 m with a sampling interval of 20 cm.), with 3 replicates per soil layer for each species at each treatment. The maximum depth of soil sampling of *C. arborescens* was 120 cm because of their shallower root system. After weighing, the soil samples were dried in tin cups at 105 °C for 24 h to obtain the mass water content (w, %). A PMS Pressure Chamber (PMS Instrument Company, Albany, NY, USA) was adopted to measure predawn (05:00 h) and midday (14:00 h) leaf water potential. Five replicates were determined for different treatments of each species.

2.3. Measurement protocol

2.3.1. Photosynthetic temperature response

The selected leaves were healthy, fully developed, and sun exposed. The narrow leaves were carefully arranged parallel to one another avoiding overlap, and clamped into the 6800–01 F fluorescence leaf chamber (Licor, Lincoln, NE, USA). The enclosed leaf area was calculated from the photographs using CI-400 CIAS software (CID Co.), following protocols described in Xu and Li (2006).

Photosynthetic temperature response was measured in situ over as wide an ambient temperature range as possible. The same process was described in detail in Feng et al. (2022). Sample [CO_2] was maintained at 400 ppm by using the automatic CO_2 injection system. Irradiance at the leaf surface was controlled at 1500 μmol photons m^{-2} s^{-1} to simulate the surroundings, which nearly approaches the light saturation point while not high enough to cause photoinhibition of photosynthesis (Fig. S5).

We analyzed the temperature response of the composite data from repeated measurements of ≥ 6 sets of leaves per species per treatment. After changing the block temperature in Licor 6800, we logged the data after A and g_{sw} had stabilized, which may take up to 10 min at higher temperatures. Where necessary, measurements of a given species and treatment were spread across multiple days, but because the weather at the study site is typically very consistent from one day to the next, we pooled the data collected on different days.

Photosynthetic temperature response was fitted following Gunderson et al. (2010) as:

$$A(T) = A_{Opt} - b(T - T_{Opt})^2 \tag{1}$$

where A_{opt} is the rate of photosynthesis at T_{opt} and b determines the width of the curve. To estimate the high-temperature CO_2 compensation point (T_{max}) and its standard error we also fitted the data following Cunningham and Read (2002) as:

$$A(T) = b \times \left(T_{leaf} - T_{min}\right) \times \left(1 - e^{c \times \left(T_{leaf} - T_{max}\right)}\right) \qquad (2)$$

where T_{min} is the low-temperature CO_2 compensation point, and b and c are constants.

2.4. Chlorophyll fluorescence and electron transport rate

All fluorescence measurements were performed with Multi Phase Flash Fluorometer (MPF). We determined maximum quantum efficiency (F_v/F_m) on dark adapted leaves before dawn. We set flash modulation rate as 250 kHz and 250 ms duration MultiPhase flashes of 10000 μmol photons $m^{-2} s^{-1}$ intensity. During the measurements of the temperature response of photosynthesis, chlorophyll fluorescence was simultaneously measured. ETR was calculated as $ETR = \Phi_{II} \times PPDF \times 0.5$ (Genty et al., 1989). Quantum yield of PSII was calculated as $\Phi_{II} = 1 - F_s / F_{m'}$. Non-photochemical quenching was calculated as $NPQ = (F_m - F_{m'}) / F_{m'}$, using a MultiPhase flash type (Maxwell and Johnson, 2000).

2.5. Morphological and physiological traits

Leaf lengths and widths for 30 leaves (collected from 7 or 8 separate plants) from each species and treatment were determined using a vernier caliper. Leaves were collected before dawn and fresh mass was recorded, then the leaves were placed in distilled water for more than 12 h to determine saturated mass, and finally the leaves were oven-dried at 60 °C for ≥ 48 h to determine dry mass (N = 5). CIS image analysis software was adopted to determine leaf area. Specific leaf area (SLA) was calculated as leaf area /leaf dry mass and leaf dry mater content (LDMC) as leaf dry mass/leaf saturated weight. Chlorophyll determination and calculation refer to previous methods with an ultraviolet spectrophotometer (Thermo Fisher Scientific, AQ8100, USA) (Gao, 2006) (N = 5). Collected samples were also used for the measurement of non-structural carbohydrate (NSC). Wood samples were taken from mid-and upper canopy branches nearly 1 cm in diameter and root samples were taken from roots with a diameter of less than 2 mm, collected at ~60–100 cm depth. The same process was described in detail in Feng et al. (2022).

2.6. Curve fitting and statistical analyses

To fit Eqs. 1 and 2, we used the 'nls_multstart' function in the 'nls. multstart' package version 1.0.0. (Padfield and Matheson, 2018) in R.0.3. This allowed us to derive estimates of the mean and standard error of the parameters of interest, T_{opt}, A_{opt}, and T_{max}. To estimate 95 % confidence intervals of the fitted curves we used bootstrapping (500 iterations) using the 'modelr' package (Wickham, 2017). The temperature response of E and ETR, and their 95 % confidence intervals, were fitted with generalized additive models using cubic regression splines fit with restricted maximum likelihood in the "mgcv" package (Wood, 2017). The temperature response of g_{sw} was fitted with "loess" method. Linear regressions were analyzed with the "lm" function. Treatments and species difference in leaf N and pigment content, and F_v/F_m were determined using one-way ANOVA with the 'aov' function, normality and homogeneity were checked to ensure data comparability. When data could not be normalized, a Wilcoxon rank test was used. We also used the "scheirerRayHare" function in the rcompanion package (Klasson, 2020) to do a two-way analysis on species × treatment. All analyses were performed in R, version 4.0.3.

3. Results

The drought treatment significantly reduced soil water content, especially in the range of 0–2 m (Fig. 2). In the drought treatment there was a tendency towards lower soil water content in the 20–100 cm range near C. arborescens plants than near H. ammodendron plants (Fig. 2). Despite low soil water content, neither species exhibited signs of wilting (FXL, LR and LCJ. Personal observation).

3.1. Physiological and morphological traits

Drought significantly reduced relative water content in both species (ANOVA, $P < 0.01$) (Table 1), and specific leaf area in C. arborescens (ANOVA, $P < 0.01$). The leaf water potential of both species was significantly more negative in the drought treatment (Fig. 2). Leaf dry matter content and nitrogen concentrations were not significantly affected by drought.

Drought affected the NSC distribution of the two species, especially in C. arborescens. The content of soluble sugar (ANOVA, $P < 0.001$) and NSC (ANOVA, $P < 0.05$) in the roots of C. arborescens increased significantly in the drought treatment, while no significant difference was detected in H. ammodendron (Fig. S7).

Leaves of H. ammodendron and C. arborescens showed two different forms of morphological adjustment to drought: leaf diameter and length of H. ammodendron decreased significantly (ANOVA, $P < 0.001$), resulting in thinner and shorter leaves under drought. In contrast, leaf length of C. arborescens decreased significantly (Wilcoxon, $P < 0.001$), but the leaf diameter increased (ANOVA, $P < 0.001$), resulting in thicker and shorter leaves. Compared with H. ammodendron, the morphological change of C. arborescens was more pronounced (Fig. 3).
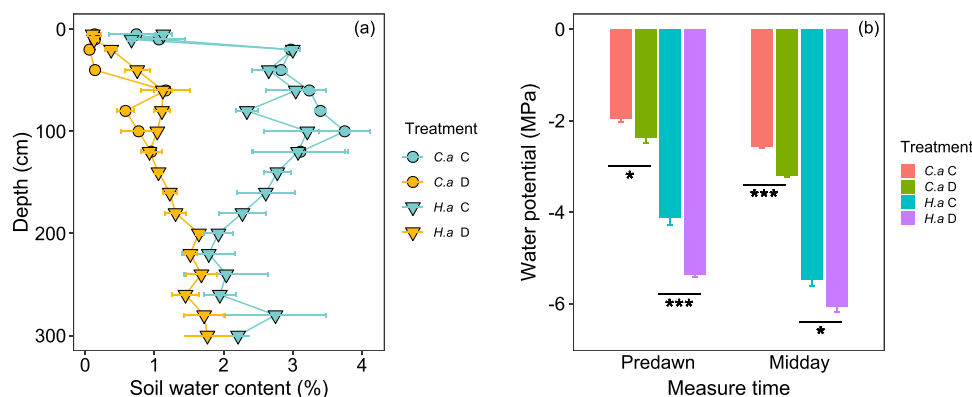

Fig. 2. Vertical distribution of soil water content (a) and leaf water potential (b) at the H. ammodendron (triangles) and C. arborescens habitats (circles) in the study area during the growing season of 2021. Orange represents drought and teal represents control. Data are presented as the mean± 1 standard error (n = 3 (a); n = 5 (b)).

Table 1

Leaf traits of plants under different treatment conditions. Means (SEM) of n = 5 replicates are shown. Traits include specific leaf area (SLA), leaf dry matter content (LDMC), relative water content (RWC) and nitrogen (N) content.

Species	Treatment	SLA	LDMC	RWC	N content
		cm² /g		%	mg/g
C.a.	Drought	**71.1 (3.1)****	0.235 (0.008)	**75.0 (0.2)*****	11.6 (0.6)
C.a.	Control	93.7 (5.7)	0.218 (0.013)	85.0 (1.2)	10.3 (0.7)
H.a.	Drought	119.8 (5.9)	0.213 (0.008)	**82.0 (0.9)****	13.7 (0.5)
H.a.	Control	107.3 (4.8)	0.228 (0.004)	88.0 (0.9)	15.3 (1.7)

Asterisks indicate significant drought effects * P < 0.05; * * P < 0.01; * ** P < 0.001. SLA: Specific leaf area; LDMC: Leaf dry matter content. H.a.: H. ammodendron; C.a.: C. arborescens.
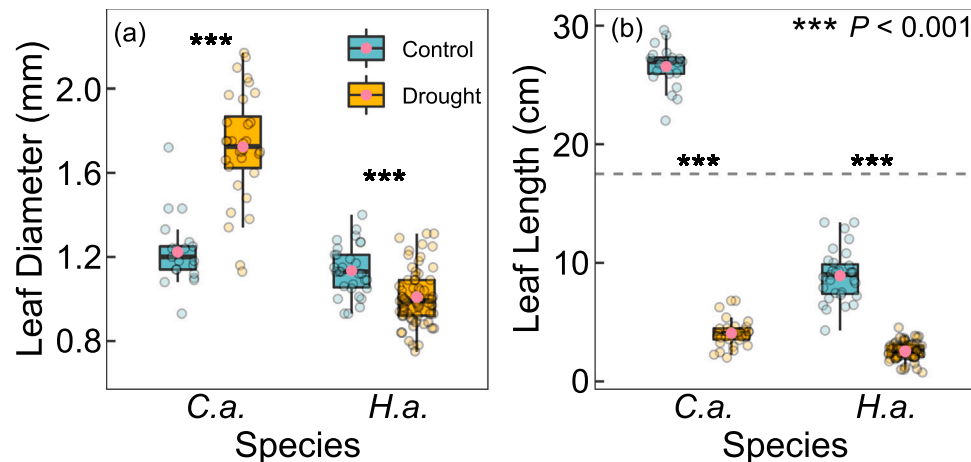

Fig. 3. Leaf diameter (a) and length (b) across treatments, along with photographic illustrations (*H.a.*: (c, d); *C.a.*: (c, d). The red dot represents the average value (n~30). *H.a.*: *H. ammodendron*; *C.a.*: *C. arborescens*. The center line of the box plots represents the median, the lower and upper hinges represent the first and third quartiles, and whiskers indicate the interquartile range.

3.2. PSII photochemical efficiency and photosynthetic pigments

Maximum photochemical efficiency (F_v/F_m) was moderately, but statistically significantly lower in drought than in the control plants of *H. ammodendron* (wilcox.test, W = 27, P < 0.001), but not in *C. arborescens* ($F_{1,24} = 1.6$, P = 0.22) (Fig. 4).

In leaves of droughted *H. ammodendron*, Chl a ($F_{1,8}$=18.1, P = 0.003), total chlorophyll content ($F_{1,8} = 12$, P = 0.009), and the Chl a / Chl b ratio were significantly lower than in control plants ($F_{1,8} = 20.4$, P = 0.002), while the content of Chl b and carotenoid did not differ. In *C. arborescens*, there were no significant differences in pigment content or pigment ratios due to the drought treatment (Fig. 5). The Chl/ Carotenoid ratio in *C. arborescens* was significantly higher than in *H. ammodendron* (Fig. S3).

3.3. Temperature responses of photosynthesis parameters

The drought treatment significantly reduced the maximum photosynthetic rate of *H. ammodendron* and *C. arborescens*. A_{opt} decreased by 63 % and 21 % in *H. ammodendron* and *C. arborescens*, respectively. At around 25 °C, photosynthesis of *H. ammodendron* was significantly decreased due to drought, but this was not the case for *C. arborescens*. T_{opt} of *H. ammodendron* in the drought treatment was 30.9 °C, which was higher than T_{opt} of the control group (27.5 °C), but the estimate for the controls was not well constrained due to the small number of observations below T_{opt}, and the 95 % confidence intervals of the two groups overlapped (Fig. 6). T_{opt} of droughted *C. arborescens* was 33.3 °C, which was 2 °C lower than the T_{opt} of 35.3 °C in the control group. Regardless of treatment, T_{opt} tended to be lower in *H. ammodendron* than in *C. arborescens* (Fig. 6 and Table 2). The temperature response curve of *H. ammodendron* was narrower in the drought than in the control treatment, as indicated by the smaller temperature range over which 80 % of A_{opt} could be achieved (Table 2). In *C. arborescens*, in contrast, the curve was wider in the drought treatment (Table 2). Net photosynthesis approached 0 at ~50 °C, with the exception of droughted *C. arborescens*, for which T_{max} equaled 57.6 °C (Table 2)—but this was a poorly-constrained estimate (SEM = 3.6 °C).

Transpiration rate increased slowly as the temperature increased above 25 °C. Above 35 °C, the transpiration rate of *H. ammodendron*
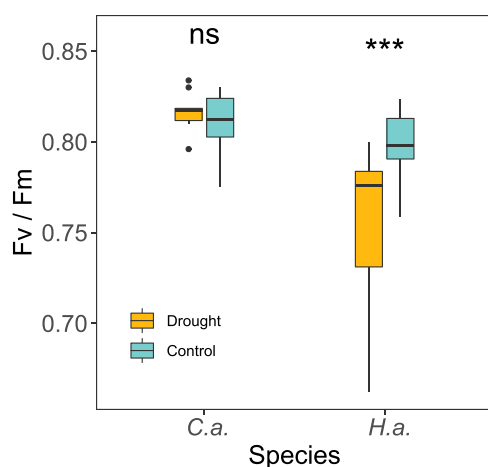

Fig. 4. Maximum photochemical efficiency (*Fv/Fm*) among different treatments. (ns: no significant difference; *** P < 0.001, n ≥ 10). The center line of the box plots represents the median, the lower and upper hinges represent the first and third quartiles, and whiskers indicate the interquartile range. *H.a.*: *H. ammodendron*; *C.a.*: *C. arborescens*.
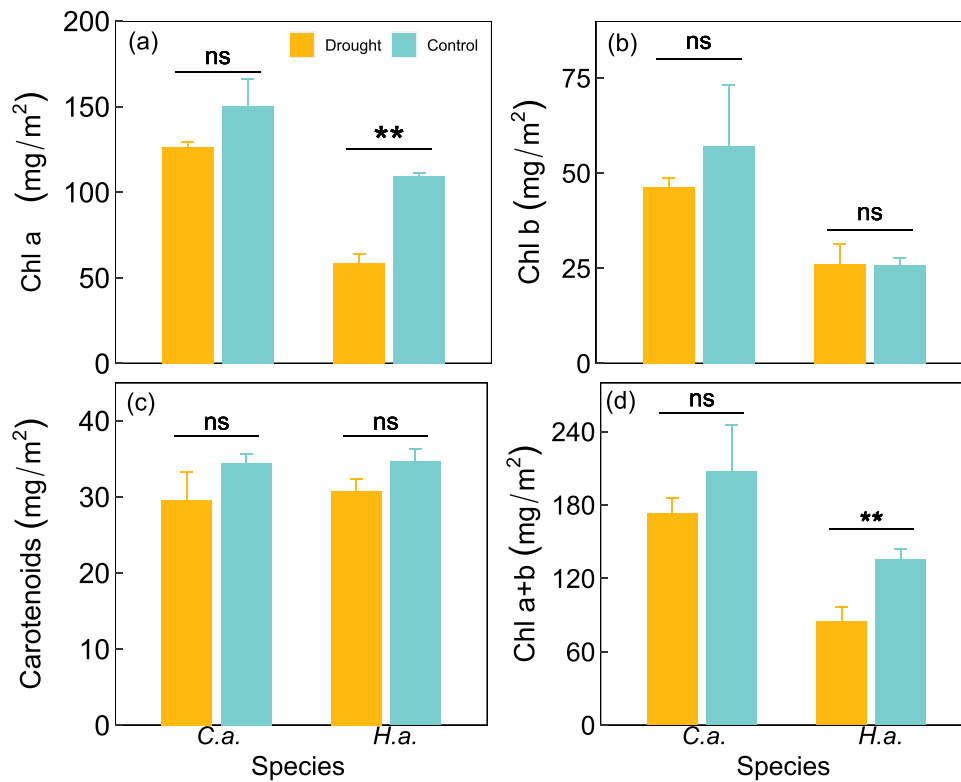
Fig. 5. Photosynthetic pigment content (Chl a (a); Chl b (b); Carotenoids (c); Chl a+b (d)) among different treatments (ns: no significant difference; ** P < 0.01). *H. a.*: *H. ammodendron*; *C.a.*: *C. arborescens*. Data are presented as the mean± 1 standard error (n = 5).
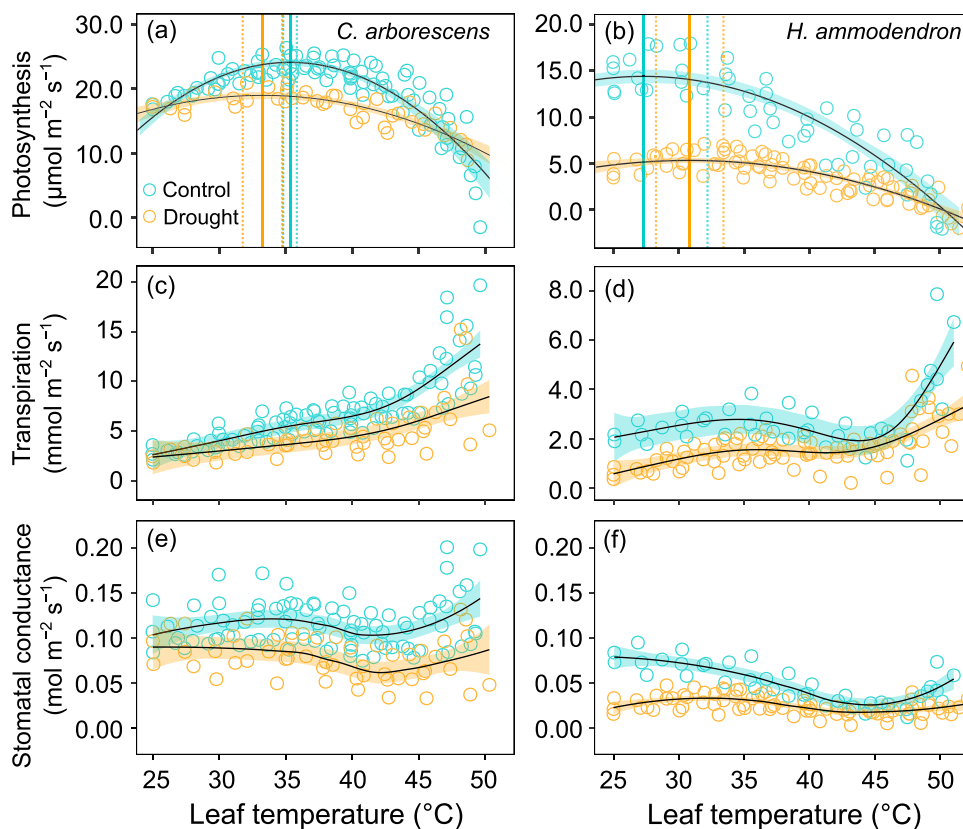

Fig. 6. Photosynthesis (a,b), transpiration rate (E) (c,d) and stomatal conductance (g_{sw}) (e,f) of *C. arborescens* (left panels) and *H. ammodendron* (right panels) and its dependence on leaf temperature (T_{leaf}). Curves in a and b were fitted with Eq. 1, with 95 % confidence intervals based on bootstrapping with 1000 iterations. T_{opt} is indicated in solid lines for control (cyan) and drought (orange) plants; dashed lines indicate 95 % confidence intervals of $T_{opt.}$ In c–f, shaded areas represent the 95 % confidence intervals of fitted temperature trends. Cyan represents the control group and orange represents drought treatment (n = 6–9).

Table 2

Temperature response parameters of photosynthesis (mean ± SEM. n = 5–10) for *H. ammodendron* and *C. arborescens* plants grown control and drought conditions. Parameter values were derived from Eq. 1 (T_{opt}, A_{opt}, A_{T80}) and 2 (T_{max}).

Species	Treatment	T_{opt}	A_{opt}	T_{A80}	T_{max}
		°C	µmol m^{-2} s^{-1}	°C	°C
C.a.	Drought	33.3 (0.7)	19.0 (0.3)	22.3 – 44.2	57.6 (3.6)
C.a.	Control	35.3 (0.3)	24.1 (0.3)	27.6 – 43.1	50.7 (0.3)
H.a.	Drought	30.9 (1.3)	5.3 (0.2)	22.3 – 39.6	50.3 (0.5)
H.a.	Control	27.5 (2.4)	14.4 (0.6)	17.4 – 37.6	49.8 (0.7)

decreased a little in the control group, but not in the drought group. When the temperature reached ~44 °C, the transpiration rate increased rapidly. The transpiration rate of *C. arborescens* increased gradually with the increase of temperature, and beyond ~40–43 °C, the transpiration rate increased rapidly. Compared with the control group, the drought treatment had a more moderate response to temperature changes. The transpiration rate of *H. ammodendron* tended to be much lower than that of *C. arborescens*, and the drought treatment group was significantly lower than the control group for much of the temperature range, as indicated by the non-overlapping 95% confidence intervals of the fitted curves. The instantaneous water use efficiency (WUE), calculated as the ratio of photosynthesis over transpiration, decreased with increasing temperature, but did not differ between drought and control plants, nor between species (Fig. S4).

Stomatal conductance (g_{sw}) of control plants of *H. ammodendron* decreased slowly with the increase of temperature, but then increased again beyond ~44 °C. g_{sw} of droughted *H. ammodendron* remained almost unchanged, even at extreme temperatures. g_{sw} of *C. arborescens* appeared to peak around 35 °C, followed by a slow decrease until ~40 °C, after which it increased again. In general, g_{sw} of *C. arborescens* was higher than that of *H. ammodendron*, and g_{sw} of the drought treatment group was lower than that of the control group.

At low temperature (~25 °C), T_{leaf} of *C. arborescens* was higher than T_{air} due to radiative heating (Fig. S2). As the temperature increased, ΔT gradually decreased until at very high temperature (~50 °C), T_{leaf} was lower than T_{air}, by 2.5 °C, and 1.4 °C in control and drought plants, respectively. The decrease in T_{leaf} relative to T_{air} with increasing temperature was associated with an increase in transpiration rate (Fig. S2). In contrast, T_{leaf} in *H. ammodendron* did not show obvious cooling effect (ΔT< 0 °C) at high temperature (Fig. S2), despite a steep increase in transpiration at high temperature and VPD (Fig. 6 and S8).

Consistent with net photosynthesis, the electron transport rate was higher in *C. arborescens* than in *H. ammodendron* and the drought treatment had a stronger effect in the latter species (Fig. 7). Electron transport rate peaked at 40 °C in *C. arborescens* regardless of treatment,
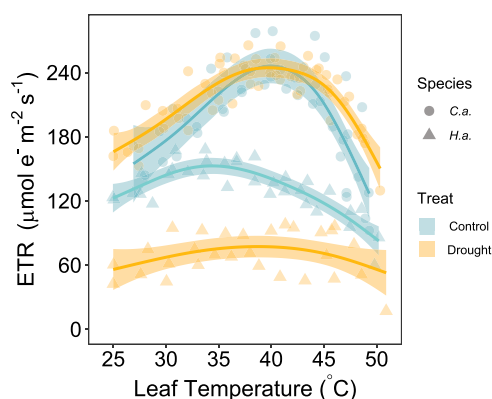
whereas T_{opt} was lower in control (33.5 °C) than in droughted plants (38.5 °C) in *H. ammodendron*, for which the curve was generally very flat (Fig. 7).

4. Discussion

We investigated morphological and physiological adjustments to drought in two C4 shrubs in the Taklamakan desert, highlighting different adaptive strategies to tolerate extreme conditions. Significant morphological and physiological adjustments occurred in the *H. ammodendron*, while *C. arborescens* alleviated the physiological changes (especially the relative stability of photosynthetic system) through an extremely effective morphological adjustment. The decoupling of photosynthesis and stomatal conductance also existed in both C4 woody shrubs at extremely high temperatures, as previously reported for C3 species in other ecosystems. Furthermore, drought weakened, but did not fully prevent this decoupling in either species.

4.1. Morphological and physiological adjustment

Desert plants typically have small leaves with thin boundary layers, reducing the risk of overheating (Wright et al., 2017). *C. arborescens* and *H. ammodendron* are both drought-adapted species with needle-like leaves. Under drought, leaves of both species became significantly shorter. This further reduces boundary layer resistance and may compensate for the potential heating effect that results from reduced stomatal conductance and potential transpirational cooling under drought. Increases in diameter helped *C. arborescens* reduce maximum T_{leaf} by increasing the thermal mass of the leaves and thereby increasing the time it takes for the leaves to heat up and reach critically high temperatures (Fig. S2) (Leigh et al., 2012).

The morphological adjustment to drought was more pronounced in *C. arborescens* than in *H. ammodendron*, which may contribute to its ability to maintain comparatively high photosynthesis rates (Fig. 6). SLA decreased with drought in *C. arborescens*, as it commonly does (Marron et al., 2003; Nautiyal et al., 2002; Kumarathunge et al., 2020), reducing the surface area per leaf over which water can be lost. However, the lower SLA increased leaf N per unit area, potentially supporting the relatively high rates of photosynthesis in drought plans despite reduced stomatal conductance. In *H. ammodendron*, SLA was not affected by drought, but physiological adjustments were observed instead.

A reduction of photosynthetic pigments under drought can reduce damage to the photosystem when more light gets absorbed than can get processed photochemically (Nikolaeva et al., 2010; Gholamin and Khayatnezhad, 2011). At the same time, changes in pigment composition can increase reflectivity and thereby help reduce leaf heating (Muller et al., 2021). In our study, catorenoid content was not significantly reduced by drought in either species. Chlorophyll content of *H. ammodendron* decreased significantly under drought, but there was no change in *C. arborescens* (Fig. 5). The photosynthetic system of *C. arborescens* is relatively stable (Figs. 4 and 7), and drought had only moderate effects on its photosynthetic physiology (Fig. 6), and this is consistent with the pigment results. In contrast, in *H. ammodendron* drought had stronger impacts on physiological traits than morphological ones. This conflicts with reports from the less dry Gurbantonggut desert, where *H. ammodendron* exhibits strong morphological responses to drought (Xu et al., 2007). There was no evidence for osmotic adjustment in leaves of either species based on the analyses of sugar concentrations, but roots of droughted *C. arborescens* plants accumulated significantly more soluble sugars than controls. Similar increases in root soluble sugars during drought have been shown for crop species, including C4 *Zea mays* L (Mohammadkhani and Heidari, 2008; Du et al., 2020), suggesting a role for roots in osmotic adjustment in *C. arborescens*. Although the effect was small, the reduced F_v/F_m in droughted *H. ammodendron* indicates that these plants in the current experiment experienced some sustained physiological stress. *ETR* also decreased

Fig. 7. Electron transport rate (*ETR*) among different treatments. Shaded areas indicate 95% confidence intervals of the fitted curves. *H.a.*: *H. ammodendron*; *C. a.*: *C. arborescens*.

significantly, as did net photosynthesis. Reduced photosynthetic pigment content reduced the amount of solar radiation harvested, lower PSII quantum yield and *ETR* further limited potential photosynthesis rates, and combined with a reduction in stomatal conductance, Aopt was significantly reduced relative to control plants. While *C. arborescens* maintains its photosynthetic potential through morphological adjustment, *H. ammodendron* mainly relies on physiological regulation: the dissipation of excitation energy at the chloroplast level through processes other than photosynthetic C-metabolism is an important defense mechanism, which is accompanied by down-regulation of photochemistry and photosynthetic capacity and, in the longer term, growth (Chaves et al., 2002).

Drought did not strongly improve instantaneous water use efficiency, and the water use efficiency of the two species was almost the same (Fig. S4). Xu et al. (2007) noted that stomatal control of *H. ammodendron* was weak when water was abundant. While water was not abundantly available in the current study, our control treatment was effectively a moderate irrigation treatment—maintaining the conditions under which the plants were established in this extremely dry environment, and the drought treatment represented ambient, < 50 mm of rain per year, conditions.

In such extreme environmental conditions of soil and meteorological drought, solar radiation typically considerably exceeds the requirements of photosynthesis, especially when electron transport rate capacity is downregulated, which causes photoinhibition associated with PS II. Excess radiation needs to be dissipated to limit the production of reactive oxygen species (ROS) that can damage the photosynthetic apparatus and ultimately cause cell death. This excess energy can be dissipated through NPQ or photochemical pathways such as photorespiration (Müller et al., 2001; Erel et al., 2015). In this study, both species showed effective morphological adjustments and thus improved heat dissipation capacity. In addition, NPQ was elevated in droughted relative to control *C. arborescens* (Fig S9), which may contribute to the maintenance of effective quantum yield and relatively high rates of gas exchange in this species. Several studies also found plants increased NPQ under drought conditions (Guan et al., 2004; Naumann et al., 2007). In contrast, NPQ of *H. ammodendron* slightly decreased during drought. Petsas and Grammatikopoulos (2009) showed that NPQ in Mediterranean shrub species *Phlomis fruticosa* increased under mild drought stress but decreased under severe drought stress; and we infer that these differences may be related to the photoprotective mechanism of chlorophyll loss in the current study (Fig. 5).

4.2. Photosynthetic decoupling and transpirational cooling

Photosynthesis and stomatal conductance should be strongly coupled under most environmental conditions, and there is plenty of theoretical and empirical support for this (Cowan and Farquhar, 1977; Lin et al., 2015). However, our experiment showed that when the temperature was close to 50 °C, photosynthesis was almost zero but g_{sw} did not decrease as predicted by stomatal optimization theory. Instead, g_{sw} increased or maintained at a relatively high level. This result is consistent with other resent studies (Ameye et al., 2012; van Gorsel et al., 2016; Slot et al., 2016; Urban et al., 2017; Drake et al., 2018; Marchin et al., 2021), including in arid deserts (Aparecido et al., 2020; Feng et al., 2022). The fact that the decoupling is observed only at temperatures that approach lethal thresholds, suggests that latent cooling of leaves by transpiration is an important component to plant response to extreme temperatures (Drake et al., 2018).

Increased permeability of leaf cuticles at high temperature could potentially contribute to increased water loss at high temperature, but both species in our study have thick cuticles, thought to be effective barriers to non-stomatal water loss (Su 2010; Yan et al., 2007). Slot et al. (2021) have shown that the temperature-stimulated increase in cuticle conductance is insufficient to explain the observed increase in conductance in tropical tree seedlings, and likewise in the current study the

increase in conductance is almost certainly due to stomatal opening.

The increase in transpiration at high temperatures helps to dissipate heat, as a strategy enabling 'heat avoidance' (Mathur et al., 2014; Aparecido et al., 2020). Stocker (1976) observed that *Ziziphus lotus* (L.) Lam. expressed high rates of transpiration between noon and 4 p.m. in the Sahara Desert, which he interpreted as a strategy to avoid 'heat death', and Lange (1959) distinguished what he called 'over-temperature' and 'under-temperature' species in the Sahel, where the former were conservative water users that tolerated high temperatures, and the latter were species that prevented high temperatures through high water use. Aparecido et al. (2020) observed a similar situation in the Sonoran Desert. In our study, both species showed a strong increase in transpiration at high temperature, but thermal regulation was stronger in the morphologically plastic but shallow-rooted *C. arborescens* than in the physiologically plastic, deep-rooted *H. ammodendron* (Fig. S2), as was the absolute transpiration rate. In all these cases, a clear trade-off between leaf thermal safety and water loss is presented. When leaf temperatures approach critical levels that lead to irreversible leaf damage and necrosis, transpirational cooling to preserve the likelihood of future carbon gain is clearly advantageous (Sadok et al., 2021), but the increased water consumption further aggravate drought when the soil moisture is limited (Marchin et al., 2021). Interestingly, a large proportion of the identified reports indicates that high-temperature-triggered increase in transpiration rate takes place particularly under water-stressed environments, in plants adapted to xeric or desert environments (see Table 1 in Sadok et al., 2021), like the C4 plants in this study. Desert vegetation coverage is usually low, and soil surface temperatures may exceed 70 °C (Buxton, 1924). Kolb and Robberecht (1996) found seedlings with high stomatal conductance survived better during dry conditions with very hot soil surface temperatures, because high transpiration reduced basal stem temperature by as much as 30 °C by heat convection in rapidly moving water. Therefore, regardless of whether high transpiration primarily prevents leaves of stems from overheating, we speculate that transpirational cooling may be widespread in perennial desert plants as an adaptive strategy for plant survival.

4.3. Differences in temperature response curves

It is advantageous for net photosynthesis to be optimized at the plants' typical ambient temperature (Slot and Winter, 2017b). Plants can adapt to different environments through the adjustment of T_{opt} and *A*. The low T_{opt} for *H. ammodendron* is thus surprising. One possibility is that this species maximizes carbon uptake in the morning hours before temperatures and VPD get too high, and therefore a T_{opt} corresponding with moderate morning temperatures instead of extreme afternoon temperatures is advantageous. However, if peak photosynthetic activity is adjusted to diel patterns in temperature, VPD, and water availability, one would expect T_{opt} to decrease with drought, but instead, T_{opt} was higher for droughted than control plants. In *C. arborescens* drought did cause a decrease in T_{opt}, consistent with C3 species *Eucalyptus tereticornis* (Kumarathunge et al., 2020). The proximate explanation for low T_{opt} of *H. ammodendron* is the low T_{opt} of electron transport rate (Fig. 7), but to find a satisfactory ultimate explanation further study is required.

Between T_{opt} and ~40 °C the decrease in photosynthesis in the current study was paralleled by a decrease in stomatal conductance, consistent with studies on C3 species (Slot and Winter, 2017a; b). However, we did not find strong evidence of stomatal limitation when temperatures approached T_{max}. In C4 plants, decreases in photosynthesis at high temperature have been assigned to Rubisco inactivation (Crafts-Brandner and Salvucci, 2002), and reduced activity of bundle sheath enzymes (Boyd et al., 2015). Here, instead, the decline in electron transport rate above 40 °C appears to be an important factor in the continuing decline of photosynthesis at high temperatures. This is consistent with observations by Oberhuber and Edwards (1993) that temperature-dependent change in CO_2 fixation of C4 plants scaled

closely with electron transport rate. Decreasing electron transport rate may be caused by leaky thylakoid membranes or thermally labile links in the electron transport chain (June et al., 2004; Wise et al., 2004). This suggests that in these C4 plants the temperature effect on photosynthesis has indirect and direct effects shaping the temperature response curve, with an initial indirect effect of warming—stomatal closure in response to increased VPD—causing a decrease in net photosynthesis above T_{opt}, followed by a direct effect of temperature – deceasing electron transport rate – causing photosynthesis to drop down to zero at very high temperatures.

5. Conclusions

Morphological and physiological adjustments to drought enabled plants to optimize their performance under extreme conditions – understanding such acclimation strategies under extreme environments provides a valuable reference in the context of climate change and the increase in frequency and intensity of extreme events to which plants are exposed. Our study provides clear evidence for decoupling of photosynthesis and water use under high temperature conditions, lending support for alternative water use strategies. Even in water stressed conditions, partial stomatal closure or stomatal reopening resulted in high or increasing stomatal conductance and transpiration while carbon gain was approaching zero. Combined with existing research, this challenges the currently widely used stomatal optimization models. This phenomenon might be more prevalent in dryland ecosystems than currently assumed, and to ensure accurate representation of plant water use in Earth system models, stomatal behavior at high-temperature is a high-priority research topic for the coming decade.

CRediT authorship contribution statement

FXL, LCJ and LR developed the original research questions and designed the study, FXL collected the field samples, performed all physiological measurements and, in close collaboration with LMQ and ZH. MS and FXL carried out data analyses, FXL and RL wrote the first version of the manuscript, which was intensively discussed and revised by MS. All authors contributed to the interpretation of the results.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envexpbot.2023.105295.

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