

Morphological and physiological responses of desert plants to drought stress in a man-made landscape of the Taklimakan desert shelter belt

Congjuan Li^a, Huan Han^{a,b}, Madinai Ablimiti^{a,b}, Ran Liu^{c,*}, Heng Zhang^{a,b}, Jinglong Fan^a

^a National Engineering Technology Research Center for Desert-Oasis Ecological Construction, Xinjiang Institute of Ecology and Geography, Urumqi 830011, China

^b University of Chinese Academy of Sciences, Beijing 100049, China

^c State Key Lab of Desert and Oasis Ecology, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, China

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ABSTRACT

Drought and desertification have become a worldwide ecological problems, which pose new challenges for plant survival and adaptation. Thus, it is of great significance to explore the adaptive strategies of plants to drought stress for the survival and sustainable development of man-made ecosystem in desert. In this study, two drought treatments of control (10-day irrigation cycle) and drought (no irrigation) respectively were used to study the physiological and morphological acclimation of *Haloxylon ammodendron* (C.A. Mey) Bunge and *Calligonum arborescens* Litv, in a man-made landscape of the Taklimakan desert shelter belt. The results showed that not only significant difference for stomatal conductance and water potential was presented in this research for two species, but also the morphological difference. In response to drought, *C. arborescens* produced significantly shorter and thicker leaves, while *H. ammodendron* produced significantly shorter and thinner leaves. Additionally, for *C. arborescens*, non-structural carbohydrates (NSC) were significantly aggregated in the roots ($P < 0.05$), while in *H. ammodendron* NSC exhibited no significant change in leaves, stems, or roots, but significantly accumulated in the whole plant. The different leaf water potential Ψ_l for two species leading to a different WUE. In all, the two species exhibited different acclimation responses to drought, *C. arborescens* with a great higher WUE in drought, but *H. ammodendron* was smaller. Morphological adjustment was the main mechanism by which *C. arborescens* acclimated to drought, and it can be used as a "pioneer" sand-fixation species in deserts if resources enough. In contrast, *H. ammodendron* acclimated to drought by physiological adjustment dominated by stomatal closure, suggesting it could be used in sustainable development of ecological restoration areas.

1. Introduction

Drought-induced tree mortality has become a worldwide ecological problems (Ryan, 2011; Granda and Camarero, 2017), and plants often exhibit strong plasticity and adaptation to drought both at physiological and morphological adjustment (Dai et al., 2015; Xu et al., 2016). In response to increasing drought stress, plant economic spectrum is a trade-off strategy between resource acquisition and conservation at various stages of plant growth, development and reproduction (Freschet et al. 2010). In this case, well-developed root systems and higher root-shoot ratios can help plants scavenge more water (Liu et al., 2016; Zhang et al., 2017), additionally, reduce leaf size, and abscising leaves or apex wither is another morphological strategy (Sack et al., 2013; Bartlett et al., 2016). The leaf economic spectrum (LES) is a tradeoff strategy of leaf resource, which play a vital role in water resources restricted, at one

side of the LES, plant towards resource acquisition strategies, producing low investment, high productivity leaves that support high resource utilization, high tissue turnover, rapid growth, and higher competitiveness at the beginning of resource constraints, at the other side of the LES, plant tends to toward resource conservation strategies that support more conservative resource use, slower growth and slower tissue replacement, ensuring long-term sustainability (Shipley et al. 2006; Cianciaruso et al. 2013). In this case, different plants may take different LES in drought adaptation, and result in different leaf biomass allocation and turnover.

Plant water use strategies can be divided into isohydric and anisohydric according to how much stomatal close regulates water potential in response to drought stress (McDowell, 2011). Isohydric plants close stomata early in drought to reduce water losses and avoid excessively low leaf water potential, while anisohydric plants show less strict

* Corresponding author.

E-mail addresses: licj@ms.xjb.ac.cn (C. Li), liuran@ms.xjb.ac.cn (R. Liu).

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stomatal regulation in response to drought, and their leaf water potentials more directly track the fluctuations in soil water availability and atmospheric moisture demand (Roman et al., 2015; Burkhardt et al., 2016). Isohydic plants follow a conservative water use strategy, while anisohydic plants maximize the utilization efficiency of other resources by using water inefficiently. Therefore, in the more arid habitats, the WUE is higher, and the more conservative water use strategy allows plants to survive and grow. In relatively humid environment, lower WUE and wasteful water use pattern can make plants gain higher productivity (Martinez-Vilalta and Garcia-Fomer, 2017). However, other studies have emphasized many plants are not absolutely isohydic or anisohydic and can exist along a continuum between isohydry and anisohydry (Meinzer et al., 2014; Martinez-Vilalta and Garcia-Fomer, 2017). Therefore, stomatal regulation is a key factor distinguishing how plants respond to drought.

During drought, some species close their stomata rapidly and ultimately suppressing photosynthetic carbon gain. Other species, however, keep their stomata open even under drought in order to maintain photosynthetic carbon gain (Zhao et al. 2020). The extent to which stomatal restriction influences photosynthetic rate is variable among species, and some scholars believe that the responsiveness of stomata to stress is driven more by species-specific differences in stress tolerance and genetic control than it is by photosynthesis (Xu et al., 2016; Digrado et al. 2020). The need for carbon would drive stomata to remain open, but stress tolerance and limiting water loss would cause them to close. The tradeoff between these two can result in different degrees of stomatal restriction due to other hydraulic traits (Klein, 2014). Declines of stomatal conductance g_s with declining leaf water potential (Ψ_{leaf}) are expected to reduce water loss and prevent reaching very negative water potentials in the xylem. Additionally, previous research in the Taklimakan desert has shown not only that the allocation to leaves and roots changed substantially in response to drought, but also that leaf hydraulic conductance (K_{leaf}) can differ dramatically between species during drought (Li et al., 2017; Trueba et al. 2019), because drought-induced hydraulic dysfunction caused the appearance of the water status at wilting or turgor loss point (Bartlett et al., 2016) and the decline of leaf hydraulic conductance (K_{leaf}) (Nardini & Luglio, 2014; Trueba et al. 2019).

Additionally, isohydic species are prone to depletion of non-structural carbohydrates (NSCs) due to stomatal closure under drought, which declines photosynthesis and carbon accumulation. On the other hand, anisohydic species can maintain their stomata open to a certain degree and keep photosynthesis under drought (Clark et al., 2016; Adams et al., 2017). NSC could be an indicator to express isohydic/ anisohydic degree. However, the NSC content for different species has been shown differently during drought (Gaylord et al. 2013; Dickman et al. 2015; Woodruff et al. 2015). Thus, there is an urgent need to examine the change of NSC pools in all plant organs (leaves, stems and roots) are affected by drought in species.

The Taklimakan Desert is the region most seriously affected by drought and desertification in China, and water shortage strongly affects the growth and survival of plants in this region (Li et al., 2017). In particular, desert plants may be challenged by the extreme environment of the Taklimakan Desert (Li et al., 2017). Along the Taklimakan Desert highway Shelter forest (TDHS), two desert plants commonly introduced along the Taklimakan desert highway (TDH) are *Haloxylon ammodendron* (C.A. Mey) Bunge and *Calligonum arborescens* Litv. (Su et al., 2004; Li et al. 2017). In both species the leaves have degenerated into photosynthetic branches (Su et al., 2006; Zhang et al., 2017), but they differ in root architecture, with *H. ammodendron* having well-developed vertical roots of more than 10 m that can access groundwater in its natural habitats (Xu and Li, 2006; Dai et al., 2015) and *C. arborescens* with well-developed horizontal roots that can extend more than 10 m horizontally, and almost no more than 2 m in depth (Li et al., 2015). The root-shoot ratio and the individual biomass of *C. arborescens* are often substantially larger than those of *H. ammodendron*, suggesting

C. arborescens uses more assimilates for construction when resources are sufficient (Li et al. 2017). The physiological response documented that *C. arborescens* was more sensitive to drought stress than *H. ammodendron* because of its preferential belowground allocation of resources and no change in leaf-specific hydraulic conductance (T_r/Ψ_{leaf}) to drought (Li et al. 2017). Additionally, with *H. ammodendron* growing mainly on the inter-dune lowland and Oasis-desert transition zone (Zou et al. 2010), while *C. arborescens* grows mainly on the top of sand dune or on mobile sand dunes, and the two species rarely grow together naturally (Su et al. 2004).

Given the known differences in their morphology, physiology, and natural habitats, and the differences in their growth and survival conditions along the TDH (Li et al. 2017), we predicted that the different stomatal regulation and water potential behavior were presented for two species in drought stress. Additionally, two species hold different morphological adaptation strategies to drought. Responses of resource acquisition for *C. arborescens* tends to be higher competitiveness at the beginning of resource constraints, and responses of resource conservation for *H. ammodendron* ensure its sustainability in a long-term.

2. Materials and methods

2.1. Study area

This study was conducted at the hinterland of the Taklimakan Desert (38°56'N, 83°44'E), which is 20 km southwest of the Taklimakan Desert Botanic Garden (TDBG). The Taklimakan Desert is the largest mobile desert in China, which is located in the hinterland of the Tarim Basin. The annual precipitation in this region is no more than 50 mm, while annual pan evaporation is more than 3000 mm, causing the area to be one of the most arid regions in the world (Li et al. 2015). It has been called the "Sea of Death" because so few organisms can exist in such an extreme environment (Li et al. 2015). *H. ammodendron* and *C. arborescens* are two dominated introduced xerophytes in this area preventing sand drift along the Taklimakan Desert Highway and improving environment of nearby oil fields. The experimental base was about 200 m × 400 m in area, the groundwater level of the study area was about 6–8 m, the experimental base was bulldozed before planting in 2009, both the control and drought treatments were at the natural rainfall since the annual rainfall was no more than 50 mm, both studied species used the same irrigation system before April 2021, the irrigation amount was 30 L per plant and the irrigation frequency was 10 days.

2.2. Experiment design

In the experimental base, the spacing between rows and columns was 1 and 3 m, respectively. Saline groundwater (4 g L⁻¹) was extracted from a nearby groundwater well and used for drip-irrigation. We applied two drought treatments from April 2019, these two treatments were 10-day irrigation cycle (control) and no irrigation (drought treatment). The 10-day irrigation cycle is the one currently used on the desert highway, which is based on the annual precipitation of 200 mm for plants in the native habitat. Saline groundwater (4 g L⁻¹) was extracted from a nearby groundwater well and used for drip-irrigation. The same water amount of 35L per plant was used during each irrigation period before the treatment.

2.3. Gas exchange measurements

After almost 3-years treatment (from April 2019 to 2021), diurnal variation 8:00–20:00) in leaf gas exchange was measured with a LI-6400 portable photosynthesis system (LICOR, Lincoln, NE, USA), six sunny days were selected as repetition from July to August 2021. We recorded net photosynthetic rate (P_n , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance (g_s , $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), and leaf transpiration rate (T_r , $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), C_i/C_a also was calculated in this study, where C_i and C_a were the

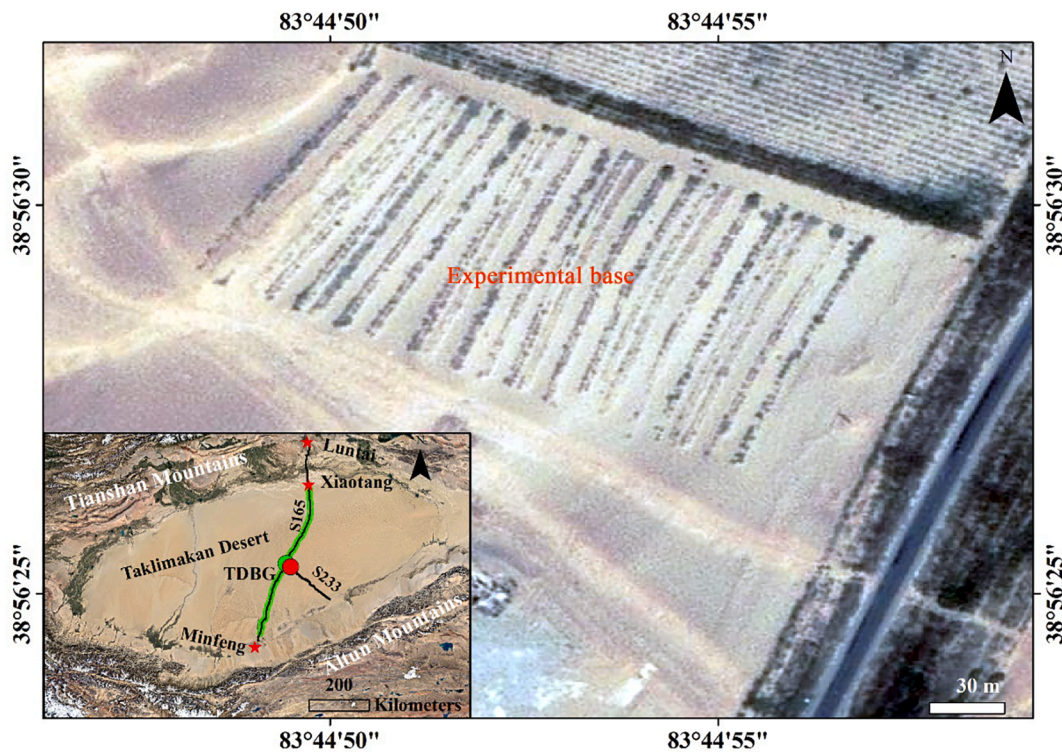


Fig. 1. Map of the Taklimakan Desert highway and the study area. Notes: The green solid point represents the Taklimakan Desert Botanic Garden (TDBG), and the red solid point represents the experimental base. The black line represents the desert highway which crosses the Taklimakan Desert from Luntai to Minfeng. The green lines on both sides of the highway from Xiaotang to Minfeng represent the artificial shelterbelt. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

leaf intercellular and atmospheric CO_2 concentrations, respectively (Larocque, 2002). Details of the measurement were described previously (Xu & Li, 2006). Diurnal P_n and T_r were measured at 2-h intervals from sunrise to sunset (8:00–20:00) during sunny days. The instantaneous WUE ($\text{mmol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$) was calculated as $P_n (\text{mmol CO}_2 \text{ m}^{-2} \text{ s}^{-1})/T_r (\text{mmol m}^{-2} \text{ s}^{-1})$.

2.4. Leaf water characteristics

We measured plant water status as by measuring diurnal variation of leaf water potential (Ψ_l , MPa) at the same days as the gas exchange measured. A Model 1000 Pressure Chamber (PMS Instrument Company, Albany, OR, USA) was used to measure Ψ_l of the two species at the same days as P_n and T_r from sunrise to sunset (6:00–20:00) during sunny days, at 1-h intervals from 6:00–8:00, and 2-h intervals during 8:00–20:00.

2.5. Leaf morphological traits

At the same days as P_n and T_r , a vernier caliper was used to measure the leaf lengths and widths on 30 leaves (collected from more than 5 separate plants) from each species and treatment. 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 65°C for ≥ 48 h to determine dry mass. We calculated specific leaf area (SLA) as leaf area /leaf dry mass, where leaf area was determined using CI-400 CIS image analysis software (Computer Imaging Analysis Software, CID Co., Logan, UT, USA) (Xu et al. 2016; Li et al. 2017).

2.6. Non-structural carbohydrates (NSC) allocation

Samples for non-structural carbohydrate (NSC; soluble sugars and starch) analysis were taken from 5 plants from each species and

treatment. Different organs (leaves, stems, and roots) of each individual were sampled separately. Root samples were taken from roots with a diameter of <2 mm. All samples were oven dried at 65°C and ground to pass through a 40-mesh sieve. The detailed procedures followed Hansen & Møller (1975) and Haissig & Dickson (1979). Soluble sugars were measured at $\lambda = 625$ nm after a colorimetric reaction with anthrone, and starch concentrations were measured at $\lambda = 450$ nm following a reaction with dianisidine. Absorbance of carbohydrates was determined using an ultraviolet spectrophotometer (UV2550, Japan). Total NSC was calculated as the sum of soluble sugars and starch.

2.7. Leaf anatomical structures

Fresh leaves were excised and immediately fixed in FAA (70% ethanol: glacial acetic acid: 40% formalin = 18: 1: 1, v/v). Following a standard dehydration in a graded ethanol-xylene series, samples were infiltrated through xylene-olefin mixtures and cured in pure olefin (Zheng and Gu, 1994). Transverse sections, $10 \mu\text{m}$ thick, were made on a Leica Rotary Microtome 2235 (Leica, Germany). Semi-thin sections were double-stained using safranin and fast solid green. The slices were stained in safranin for 2 h, then washed and decolorized with 70% alcohol, then stained with solid green for 6–20 s and decolorized with anhydrous ethanol. Slices were observed with an Olympus CX41 microscope (Olympus, Japan), and images were obtained using an Olympus DP70 imaging system (Olympus, Japan) (Wen et al. 2011).

2.8. Soil moisture measurement

The soil was sampled five times at each treatment using an auger at depths of 0–5, 5–10, 10–20, 20–30, 30–40, 40–60, 60–80, 80–100 and 100–120 cm for *C. arborescens*, and 0–5, 5–10, 10–20, 20–30, 30–40, 40–60, 60–80, 80–100, 100–120, 120–140, 140–160, 160–180, 180–200, 200–220, 220–240, 240–260, 260–280, 280–300 for

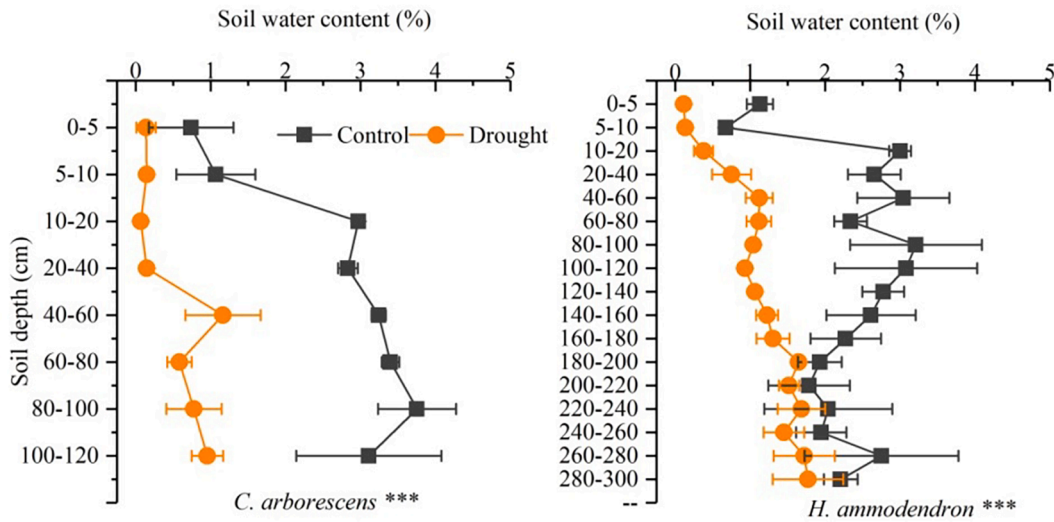


Fig. 2. Soil water content under different irrigation treatments of *C. arborescens* and *H. ammodendron*. The *** presents significant differences between the different irrigation treatments at $P < 0.001$.

H. ammodendron respectively since the main roots distributed at different layers. The soil water content was determined by oven-drying method (Li et al. 2015).

2.9. Statistical analyses

All variables were analyzed using standard statistical analyses. The nonparametric test methods were used to determine the differences in mean soil water contents, gas exchange parameters, and metrics of water status, morphological traits, and NSC. Figures were prepared with Origin 2021 (Origin LAB Corp., Northampton, MA, USA). Light compensation points (LCP) and the light saturation point (LSP), dark respiration rate (R_d), and maximum net photosynthetic rate (P_{max}) were obtained by fitting a Farquhar non-linear hyperbolic model of P_n against PPFD at high light levels ($\geq 200 \mu\text{mol m}^{-2} \text{s}^{-1}$):

$$P_n = \frac{\Phi^*Q + P_{max} - \sqrt{(\Phi^*Q + P_{max})^2 - 4\Phi^*Q^*k^*P_{max}}}{2k} - R_d$$

where P_n = net photosynthetic rate; Φ = apparent quantum yield; Q = effective photosynthetic radiation incident to the leaf; P_{max} = maximum net photosynthetic rate; R_d = dark respiration rate; and k = curvature of the light response curve. Fig. 1.

3. Results

3.1. Soil water content

According to the root depth of the two plants, two depths of 0–120 cm and 0–300 cm were selected for *C. arborescens* and *H. ammodendron* respectively. The drought treatment significantly reduced soil water content by no more than 2%, especially in the range of 10–120 cm for *C. arborescens* and 10–180 cm for *H. ammodendron* (Fig. 2) ($P < 0.001$). In the drought treatment, soil water content in the 20–120 cm was lower under for *C. arborescens* than it was under *H. ammodendron* (Fig. 2).

3.2. Light response curve

Drought stress significantly reduced the photosynthetic rate for both species. Under drought stress, the P_{max} of *H. ammodendron* was $6.20 \mu\text{mol m}^{-2} \text{s}^{-1}$, which was significantly lower than that of control plants ($13.04 \mu\text{mol m}^{-2} \text{s}^{-1}$). For *C. arborescens*, the P_{max} under drought treatment was $15.32 \mu\text{mol m}^{-2} \text{s}^{-1}$, which was significantly lower than that of control plants ($21.00 \mu\text{mol m}^{-2} \text{s}^{-1}$) ($P < 0.05$). In addition, with increasing drought stress, the LSP for *H. ammodendron* was only $1411.80 \mu\text{mol m}^{-2} \text{s}^{-1}$ and *C. arborescens* was $1847.09 \mu\text{mol m}^{-2} \text{s}^{-1}$. Which is much less than that of the control respectively. While, the LCP presented a different trend with the drought intensify, the LCP for *C. arborescens*

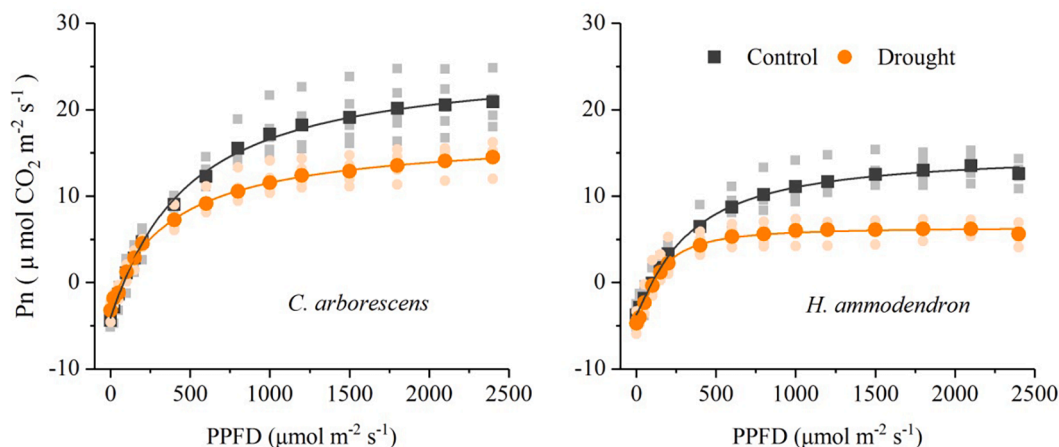


Fig. 3. Light response curves of the two species under different water conditions.

Table 1

Comparison of photosynthetic characteristics (P_{max} —photon-saturated photosynthetic rate; Φ —apparent quantum yield; LSP—light saturation point; LCP—light compensation point; R_d —dark respiration rate) of *C. arborescens* and *H. ammodendron* in different water conditions.

Species	Treatment	LCP ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	LSP ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Φ	P_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	R_d ($\text{mmol m}^{-2} \text{s}^{-1}$)	Determination coefficient
<i>C. arborescens</i>	Control	81.51 a	2826.63 a	0.056	21.00 a	4.01	0.999
	Drought	56.13b	1847.09b	0.048	15.32b	2.47	0.999
<i>H. ammodendron</i>	Control	95.98 a	2356.09 a	0.048	13.04 a	3.84	0.999
	Drought	108.96b	1411.80b	0.072	6.20b	5.03	0.998

Notes, the different lowercase letter represent significant differences between the different irrigation treatments at $P < 0.05$ level for the same species.

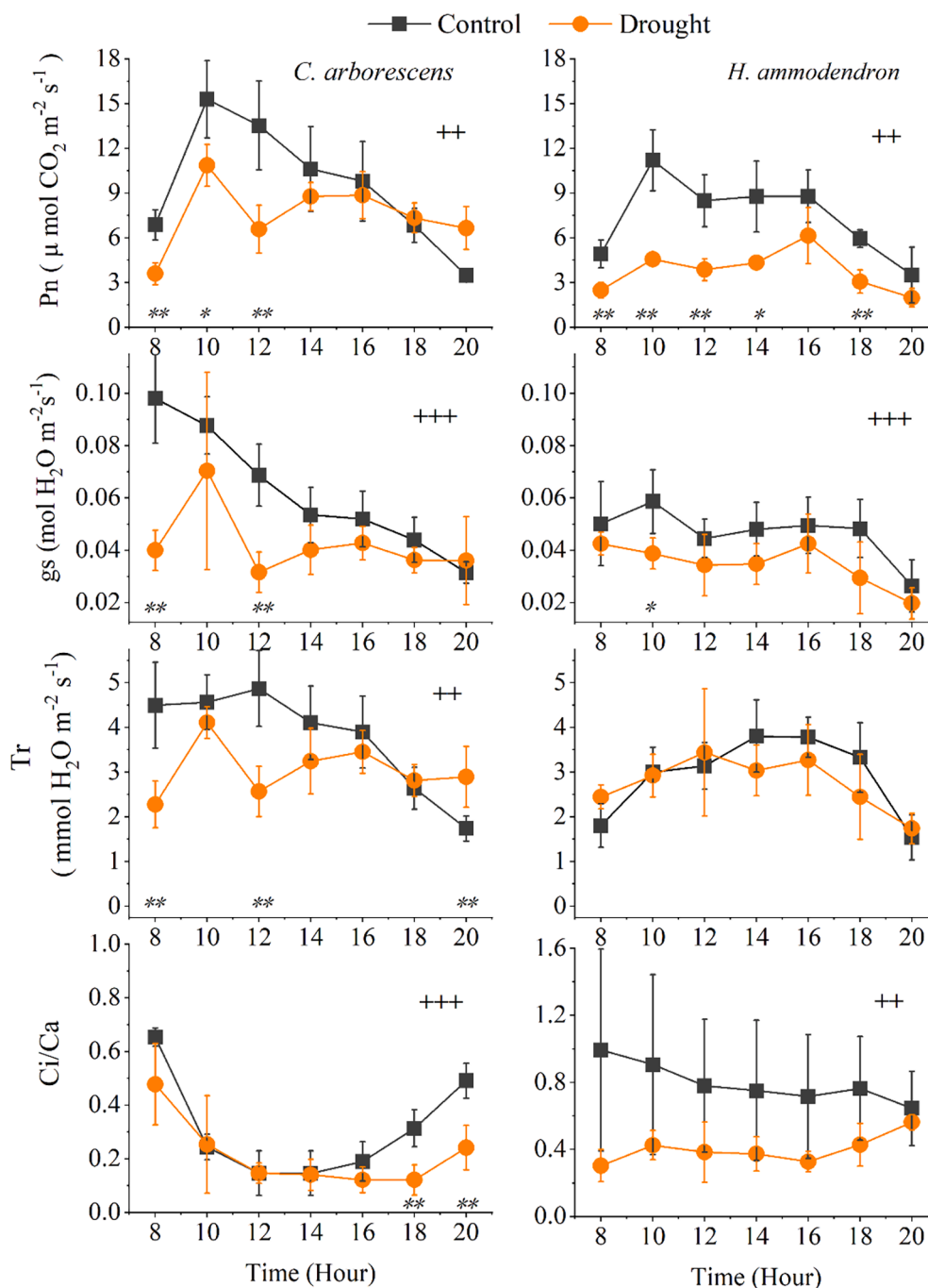


Fig. 4. Diurnal variation of gas exchange of *C. arborescens* and *H. ammodendron* under different water conditions. The vertical bars represent the standard errors of the mean, and “**”, “***” and “****” represent significant differences at the same time of the day between the different irrigation treatments at $P < 0.05$, $P < 0.01$ and $P < 0.001$, the “+” and the “++” present significant difference between diurnal pattern respectively.

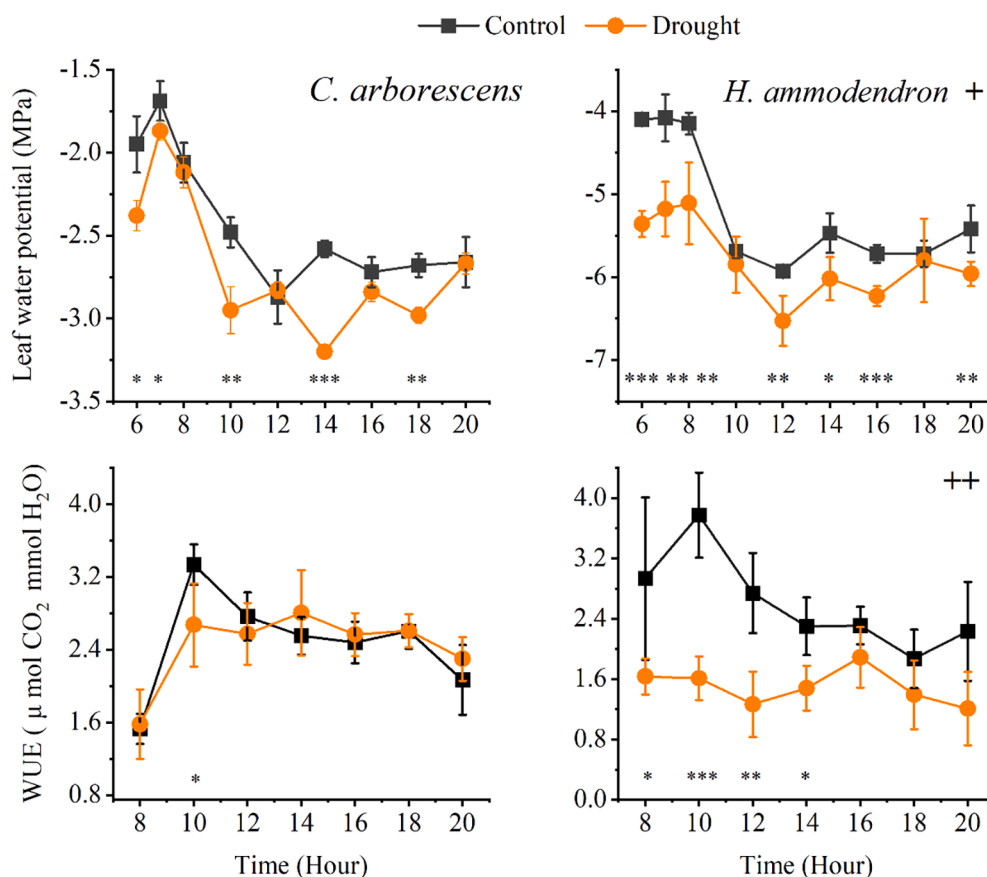


Fig. 5. the diurnal variation of leaf water potential (Ψ_l) of *C. arborescens* and *H. ammodendron* under different water conditions. The vertical bars represent the standard errors of the mean, and “*”, “**” and “***” represent significant differences at the same time of day between the different irrigation treatments at $P < 0.05$, $P < 0.01$ and $P < 0.001$, the “+” and the “++” present significant difference between diurnal pattern.

decreased from 81.51 to 56.13 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and *H. ammodendron* increased from 95.98 to 108.96 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 3 and Table1).

3.3. The diurnal process of photosynthesis

The drought treatment had significant effects on net photosynthetic rate (P_n) ($P < 0.01$), stomatal conductance (g_s) ($P < 0.001$), and C_i/C_a ($P < 0.001$) for *C. arborescens* respectively (Fig. 4). However, for *H. ammodendron* the P_n ($P < 0.01$), g_s ($P < 0.001$) and C_i/C_a ($P < 0.001$) were significantly reduced by drought, but no significant difference for T_r . Additionally, both species showed midday depression of gas exchange between 12:00 to 14:00 hrs. The P_n , g_s , and T_r for *C. arborescens* was lowest at 12:00 hrs.

3.4. Water status and water use efficiency

The predawn water potential at 6:00 hrs were significantly higher than that of and midday water potential at 14:00 hrs for both species at each treatment ($P < 0.05$). Additionally, striking differences of leaf water potential (Ψ_l) were presented at 6:00, 7:00, 10:00; 14:00 and 18:00 hrs for *C. arborescens* and 6:00, 7:00, 8:00, 12:00; 14:00 and 16:00 hrs for *H. ammodendron*. In both species, Ψ_l was lower after 10:00 hrs than earlier in the morning, and the lowest Ψ_l occurred at 12:00 hrs for *H. ammodendron*, consistent with midday depression of gas exchange, the diurnal Ψ_l at control and drought were -2.41 MPa and -2.64 MPa respectively for *C. arborescens*, which is great higher than that of *H. ammodendron* (-5.14 MPa for control and -5.78 MPa for drought). Moreover, there was no difference in diurnal patterns of either Ψ_l or WUE for *C. arborescens* ($P > 0.05$), but there were significant differences between treatments for both Ψ_l and WUE of *H. ammodendron* ($P < 0.05$

and $P < 0.01$).

3.5. Leaf morphological characteristics

The morphological characteristics of branches differed significantly between the two treatments, with photosynthetic branches appearing dry and obviously defoliate for *C. arborescens* (Figs. 6, 7). Both species exhibited shorter leaves in drought than in control treatments ($P < 0.001$), with leaves of *C. arborescens* being more than 6.5 times longer in control plants than in droughted plants, and leaves of *H. ammodendron* being 4 times longer in control plants than in droughted plants (Fig. 7). Drought stress caused leaves of *C. arborescens* to be thicker in diameter than in control plants ($P < 0.001$), though *H. ammodendron* leaves were thinner in diameter in droughted plants than in control plants ($P < 0.001$) (Fig. 7). In short, drought caused leaves to be smaller in diameter and shorter in length for *H. ammodendron* with little defoliation. These morphological changes in response to drought led to an insignificant increase in SLA of *H. ammodendron* ($P > 0.05$), while SLA of *C. arborescens* decreased significantly under drought stress ($P < 0.05$).

3.6. Allocation of non-structural carbohydrates (NSC)

Drought stress caused species-specific changes in soluble sugars, starch, and non-structural carbohydrates. Drought led to a significant increase in starch and non-structural carbohydrates for whole plants of *H. ammodendron* ($P < 0.05$), while soluble sugars increased but not significantly ($P > 0.05$). In *C. arborescens*, soluble sugars ($P < 0.001$), starch ($P < 0.05$) and NSC ($P < 0.05$) were significantly higher in roots of droughted plants, but they exhibited no significant changes in stems and leaves ($P > 0.05$). In *H. ammodendron*, soluble sugars, starch, and

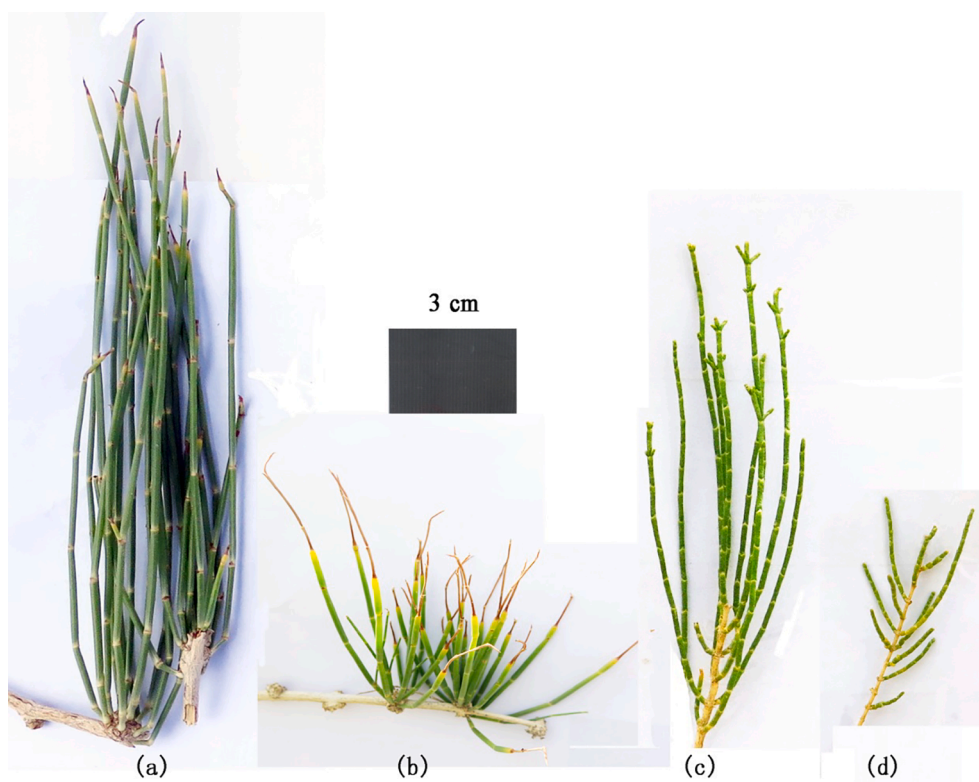


Fig. 6. Leaf morphological characteristics under different watering treatments: (a) *C. arborescens* in control and (b) drought, and *H. ammodendron* in (c) control and (d) drought treatments.

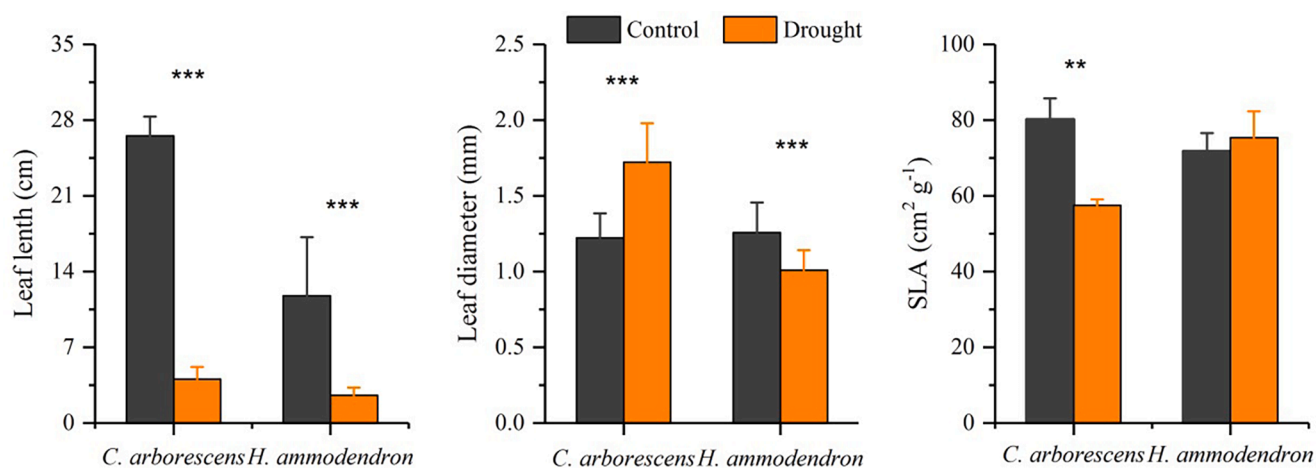


Fig. 7. Quantitative differences in leaf morphology of *C. arborescens* and *H. ammodendron* under different watering treatments. The vertical bars represent the standard errors of the mean, and “***”, “****” and “*****” represent significant differences between the different irrigation treatments at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

non-structural carbohydrates in leaves, stems, and roots were higher under drought but not significantly ($P > 0.05$), though drought induced significantly higher starch and NSC contents of whole plants compared to controls ($P < 0.01$).

3.7. Leaf anatomical structure characteristics

Drought lead to differences in leaf anatomy in both *C. arborescens* and *H. ammodendron*, with their cross-sections changing in shape from round to oval. The photosynthetic branch of *C. arborescens* became thicker (Fig. 9), and *H. ammodendron* became thinner (Fig. 10). Cell structure of leaves were intact for both species, but drought altered the

shape and number of vascular bundles (VB), the number of crystal cells (Fig. 10), and the number of mucous cells in *C. arborescens* (Fig. 9).

4. Discussion

The two species studied here exhibited shifts in morphology, internal anatomy, physiology, and biochemistry in response to drought, though the two species exhibited differently responded to drought. The “Efficiently” and “Safely” adaptation strategies to drought stress were presented for *C. arborescens* and *H. ammodendron* respectively (Fig. 11). The different between morphology and physiology and the decoupling of stomatal regulation and water potential behavior are the main causes of

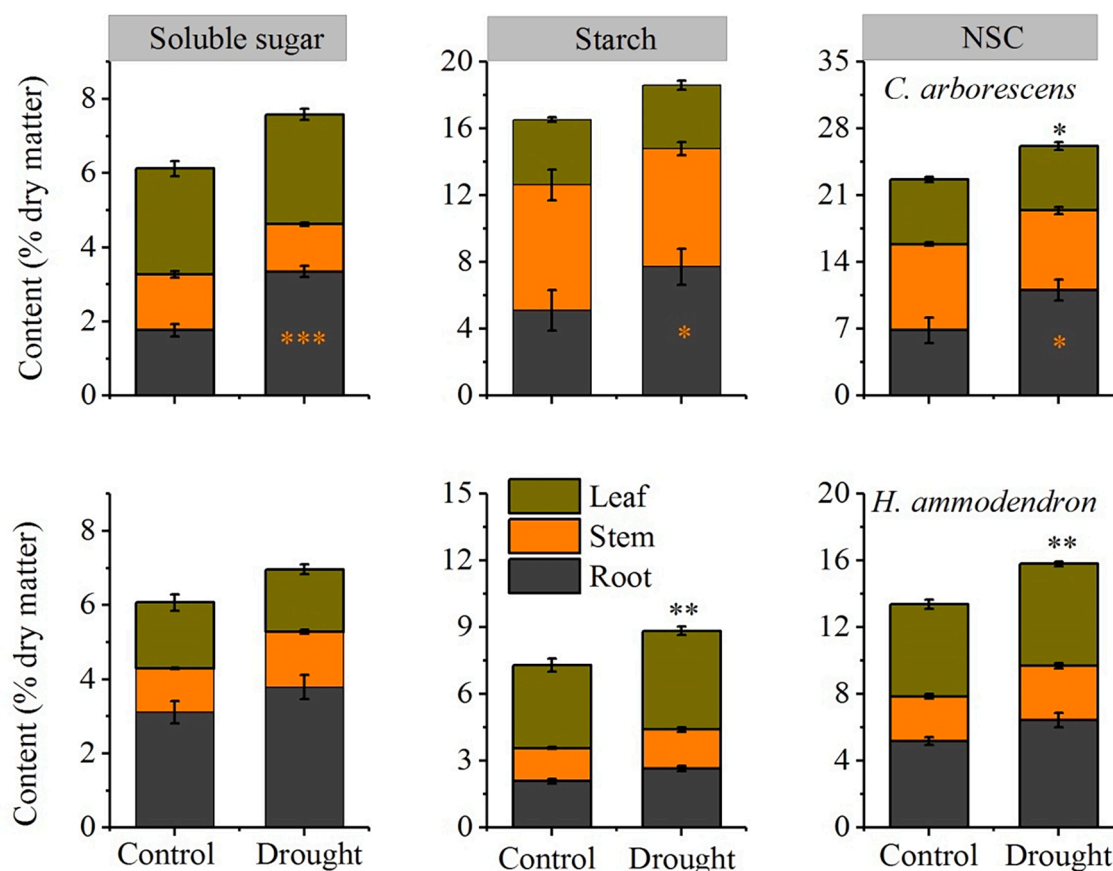


Fig. 8. Non-structural carbohydrates (NSC = Soluble sugar and starch) of *C. arborescens* and *H. ammodendron* under different water conditions. The vertical bars represent the standard errors of the mean, and “*”, “**” and “***” represent significant differences between the different irrigation treatments at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

morality. “Efficiently” plants are characterized by high levels of resource acquisition and rapid growth at the resource-rich period, but they risk decline when resources are scarce. “Safely” plants, on the other hand, can tolerate resource limitations and balance between water use and carbon assimilation. Understanding these differences between species is important for understanding resource allocation in plants, plant responses to climate change, and the sustainable development of ecological restoration in environments with severe water shortage.

4.1. Physiological responses to drought

The effects of drought stress on plants involve a variety of physiological and biochemical responses. Stomatal restriction can reduce transpirational water loss and prevent xylem embolism. Though the two species exhibited different physiological responses to drought, *H. ammodendron* showed obvious stomata adjustment behavior (stoma closure) under drought stress, but keep a higher T_r (Fig. 4, Fig. 11). In *C. arborescens*, changes in stomatal conductance under drought had significant effect on photosynthetic capacity and T_r , resulting in a high WUE under drought (Fig. 4, Fig. 11). Stomatal regulation coordinates the relationship between photosynthetic carbon assimilation and transpirational water loss (López-Calcano et al. 2020; Taylor et al. 2020), and can lead to high resistance to drought stress, such as in *H. ammodendron*.

Regulation of water loss can vary among species along a spectrum of isohydry to anisohydry. Isohydric plants have sensitive stomata that close rapidly in response to drought in order to maintain relative stability in water potential, but this rapid stomatal closure limits photosynthesis (Oliva et al. 2014; Meinzer et al., 2014; Ratzmann et al. 2019).

Our results indicated that *C. arborescens* exhibits a not absolutely isohydric behavior, while *H. ammodendron* exhibits an anisohydric behavior (Figs. 4, 5 and 11), resulting in a decoupling of stomatal conductance and T_r , and leading to a smaller WUE for *H. ammodendron*. Because stomata have a limited capacity to regulate water loss and sustained drought exacerbates the imbalance between water transport and transpiration through stomata and cuticles (Rodríguez-Calcerrada et al. 2017). Moreover, plants can exhibit variation between the extreme isohydry or extreme anisohydry (Ratzmann et al. 2019), and the same species may shift its strategy between being isohydric and being anisohydric due to changes in ontogenetic stage or environmental conditions (Klein 2014). Additionally, isohydry or anisohydry is based on short time scales (i.e. diurnal regulation of gas exchange and water potential), and it is believed that rapid stomatal responses can maintain the stability of leaf water potential for a long time to drought (Martinez-Vilalta and Garcia-Fomer, 2017; Garcia-former et al., 2016).

4.2. Morphological adjustments in response to drought

Drought also caused changes in many characteristics that may improve leaf water retention, such as a reduction in leaf size, more well developed palisade mesophyll, depressed stomata, and leaf senescence (Sack et al., 2013; Li et al., 2017). Changes in leaf shape (i.e. from round to oval in cross-section) may limit sunlight interception and prevent overheating during drought (Leigh et al. 2012). However, the two species also differed in some morphological responses to drought. Leaves of *H. ammodendron* tended to become thinner and smaller (Figs. 7-8), this was consistent with “efficiently” strategy plants tends to toward resource conservation strategies that support more conservative resource use,

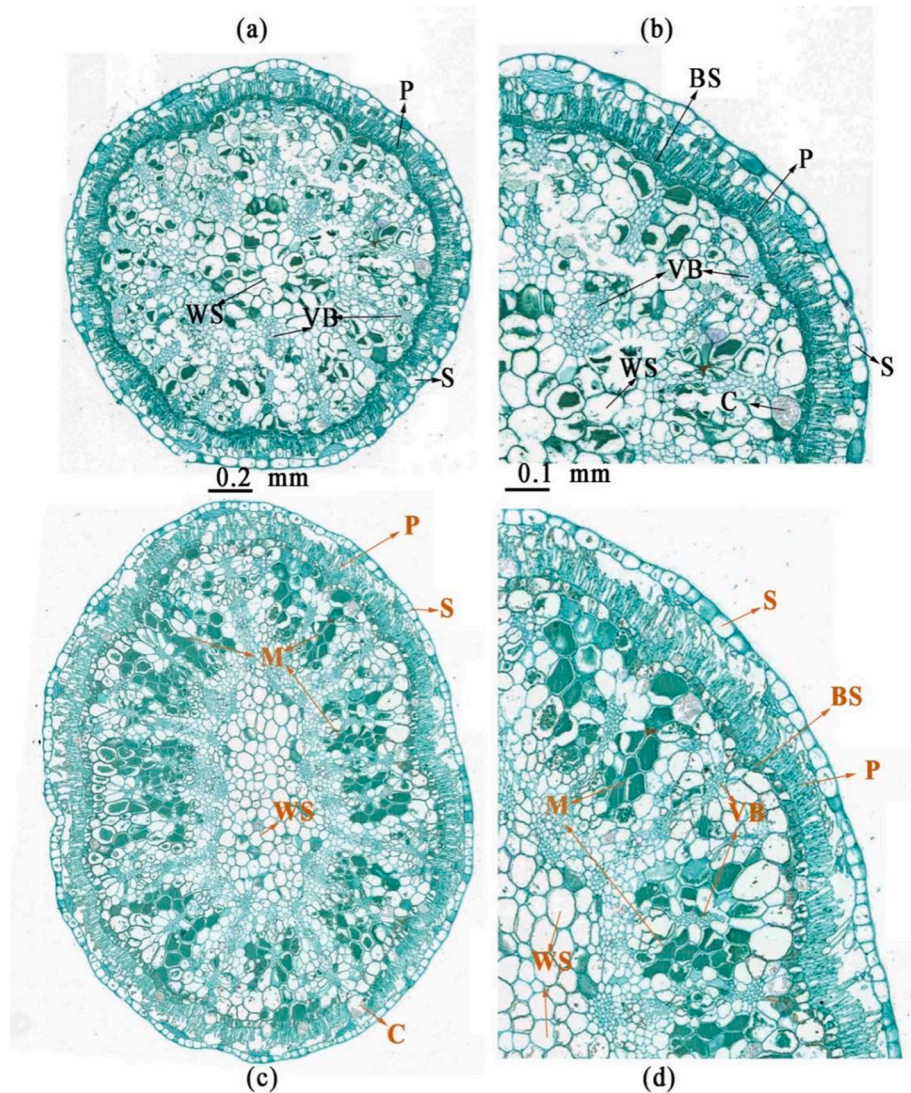


Fig. 9. Transverse sections of photosynthetic leaf organs of *C. arborescens* under different water conditions: (a) and (b) were in control, (c) and (d) were in drought. Abbreviations: BS, bundle sheath; C, crystal; VB, vascular bundle; WS, water-storage tissue; P, Palisade tissue, M, Myxocyte cell; same as follows.

slower growth and slower tissue replacement, ensuring long-term sustainability (Shipley et al. 2006; Cianciaruso et al. 2013). These morphological adaptations may be the result of the physiological accommodation, as stomatal closure will inevitably limit plant growth (Lu et al. 2021). However, under drought the photosynthetic branches of *C. arborescens* Litv became thicker and “shorter”, the “shorter” is slightly withered at the apex (Figs. 7-8), which can be documented by that the leaf functions as a safety valve to protect the hydraulic pathway from water stress-induced dysfunction to a larger extent than the dose the stem (Wright et al., 2004). It is thought that terminal organs, such as leaves, which are relatively short-lived compared to stems and more easily replaced may be more vulnerable to embolism and thus may be preferentially sacrificed in order to prevent embolism from occurring in more costly and more long-lived structures, such as stems (Pivovarov et al., 2014). Additionally, under arid conditions, some species have apparent over-investment in leaf venation to compensate for the adverse effect that the thicker leaves have on photosynthesis (de Boer et al. 2016).

In addition, crystal abundance was significantly higher under drought for *H. ammodendron* (Fig. 10), perhaps to reduce the accumulation of harmful salts (Du et al., 2019; Wen et al. 2021). More myxocytes abundance in *C. arborescens* Litv in response to drought may improve cell osmotic potential and increase water absorption and water

holding capacity (Fig. 9). These morphological differences between the species in response to drought are consistent that *H. ammodendron* being more salt tolerant than *C. arborescens*.

4.3. NSC allocation traits

Carbohydrates can be converted from one form to another to meet the needs of plants for metabolism, growth, and defense (Herrera-Ramírez et al., 2020). Non-structural carbohydrates (such as soluble carbon and starch) are important substances involved in plant metabolism and help to couple the carbon and water cycles (Hartmann & Trumbore 2016). Because the synthesis, transformation, and transportation of carbohydrates require water, the maintenance of the hydraulic transportation system is also inseparable from carbohydrates (McDowell 2011, Savi et al. 2016). Plants tend to maximize carbon assimilation and minimize carbon consumption during drought, and such carbon balance plays an important role in sustaining life (McDowell, 2011). We observed similar patterns in this study as well, but some differences also appeared between the two species, with drought leading to higher NSC concentrations in roots of *C. arborescens*. Since water limitations to P_n are just one way can prompt a variable response in NSCs, which also depending on existing NSC pools preceding drought, and the impact of drought on rates of P_n , growth, and respiration both at

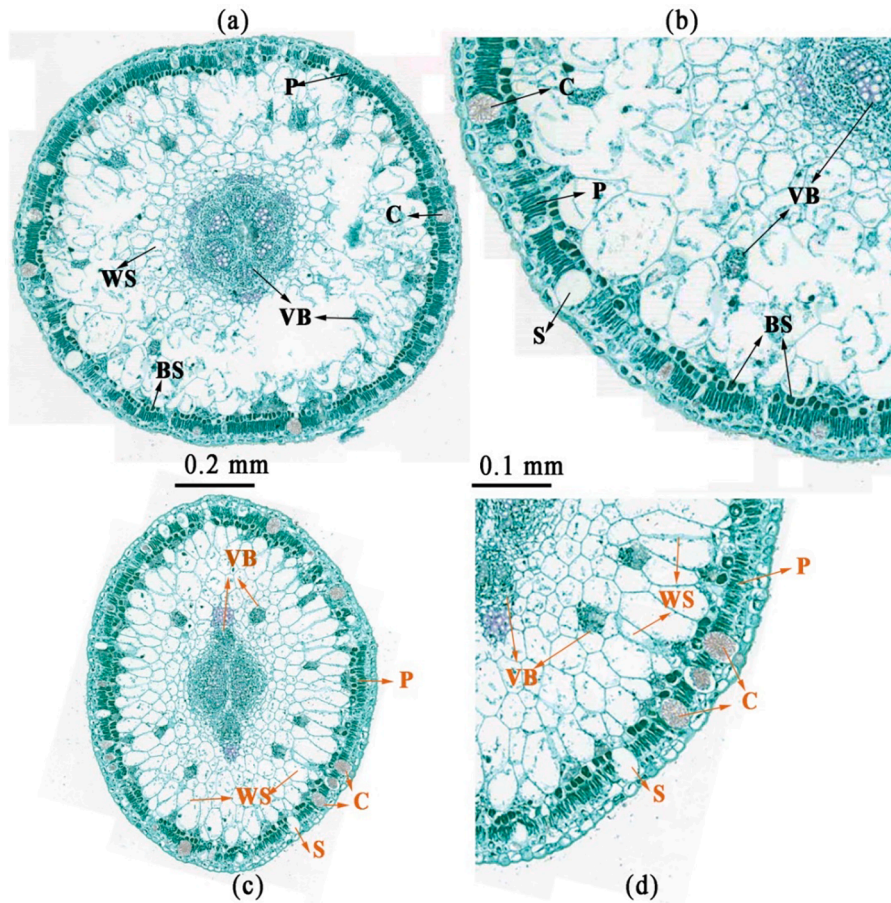


Fig. 10. Transverse sections of leaf organs of *H. ammodendron* under different water conditions, (a) and (b) were in control, (c) and (d) were in drought. Abbreviations: BS, bundle sheath; C, crystal; VB, vascular bundle; WS, water-storage tissue; P, Palisade tissue, M, Myxocyste cell; same as follows.

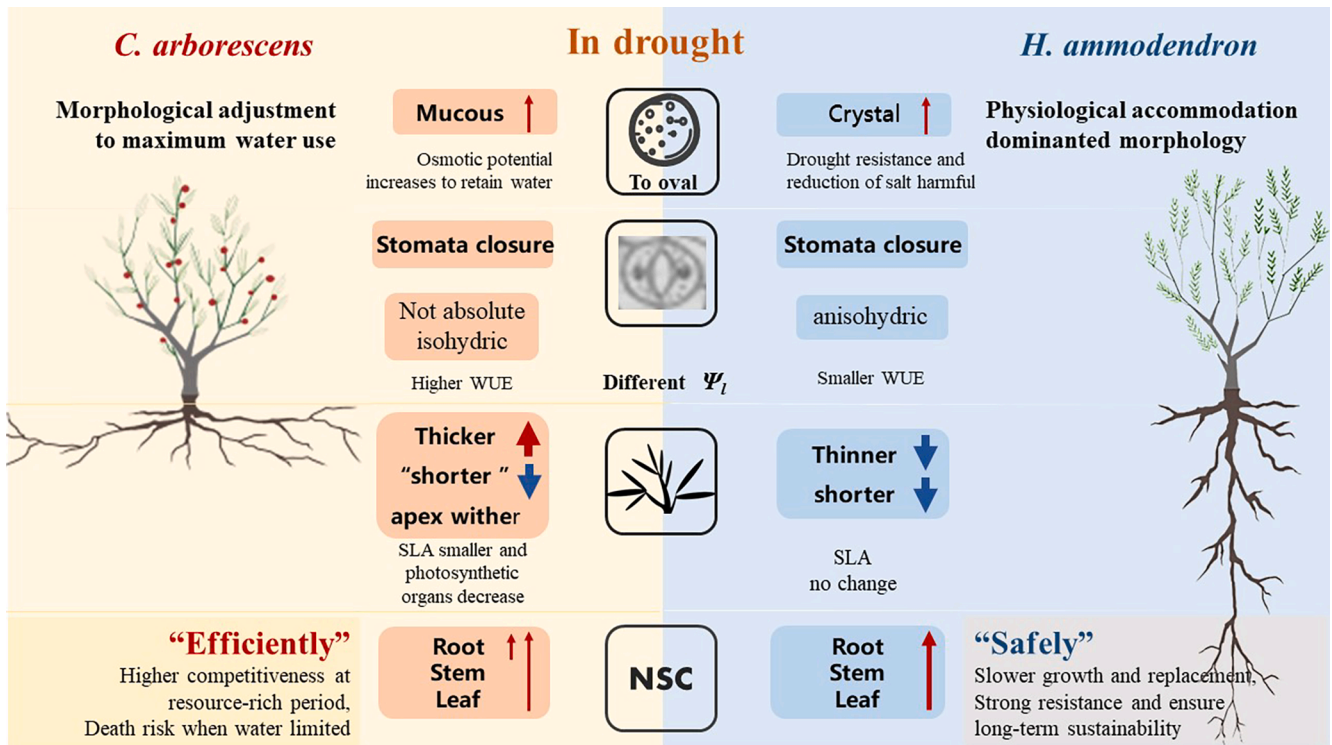


Fig. 11. Conceptual diagram of *C. arborescens* and *H. ammodendron* responses to drought stress in the Taklimakan Desert.

the above- and belowground organs (Hartmann & Trumbore, 2016).

NSC accumulation in the fine root may be helpful for root cells to provide more energy to absorb water from soil through active transport (Zhang et al. 2017). Additionally, such a strategy could lead to leaf withering at the apex and dieback if drought continues or intensifies, potentially resulting in insufficient NSC in photosynthetic organs and exacerbating the risk of death (Mitchell et al. 2013; Hartmann and Trumbore, 2016, Greenwood et al. 2017). The NSC of *H. ammodendron*, in contrast, remained constant among organs, the NSCs accumulate slightly amounts roots, stems and leaves, lead to the significant NSCs accumulation in whole plant, This balanced allocation of NSC at different organs is a relatively economical strategy indicative of a “conservative” strategy (Li et al. 2020).

5. Conclusions

This study showed that *C. arborescens* maintains stomatal conductance in order to obtain a higher water use efficiency in drought, with morphological adjustments being the main responses to drought stress. Together, these patterns suggest *C. arborescens* has an “efficiently” strategy and could be used as a “pioneer” sand-fixation species in desert areas, but it may also face the risk of death if resources become limited. *H. ammodendron*, however, exhibited physiological adjustment dominated by stomatal regulation of water loss, consistent with a “safely” strategy that enables resistance to drought stress, indicating it can be used as in sustainable development of ecological restoration areas.

CRedit authorship contribution statement

Congjuan Li: Formal analysis, Funding acquisition, Methodology, Writing – original draft, Writing – review & editing. **Huan Han:** Data curation, Formal analysis, Investigation. **Madinai Ablimiti:** Investigation, Data curation. **Ran Liu:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing. **Heng Zhang:** Investigation, Data curation. **Jinglong Fan:** Methodology

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.

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