

# Soluble sugar drives plant leaf turgor, restricts plant growth, and regulates plant hydraulic capacity of *Haloxylon ammodendron* to different groundwater level

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## Abstract

Anthropogenic withdraw of groundwater and climatic drought results in the decline of groundwater depth that, in turn, severely limits the water availability for phreatophytic vegetation in arid regions. In this study, a small xeric, phreatophytic tree *Haloxylon ammodendron* (C.A. Mey.) was investigated to understand the influence of depth to groundwater (DGW) on hydraulic traits and on the trade-off between drought tolerance and leaf area increment. A suite of traits including leaf water potential, pressure–volume (P–V) curves, Huber value, assimilation branch growth, and osmotic regulation substance were measured across five sites with DGW ranges from 3.45 to 15.91 m. Our results indicate that *H. ammodendron* was subject to greater water stress with increasing DGW, as indicated by decreased predawn ( $\Psi_{pd}$ ) & midday ( $\Psi_{md}$ ) branch water potential. We also found that growth rate declined as Huber value increased with increasing DGW in the early growing season (EGS). Solute sugar, as a major osmotic substance, drives decreases in osmotic potential at full turgor, and thus constrains assimilation branch growth with increasing DGW in EGS. Therefore, osmotic adjustment accompanied with water potential regulation ( $\Psi_{pd}$ - $\Psi_{md}$ ) and plasticity of Huber value allows this phreatophyte to absorb water from deeper soil layers and tolerate drought. However, these adaptive adjustments cannot fully compensate for nonoptimal water conditions as growth rate continued to decrease as DGW increased in EGS and even became negative in the late growing season (LGS) at almost all sites. Our results provide an insight into how *H. ammodendron* responds and adapts to changes DGW in a region experiencing hydrological and climatic drought. Greater depth of groundwater had a significant effect on *H. ammodendron* and may have similar effects for other non-riparian phreatophytic plants in arid regions.

## Introduction

Arid regions occupy approximately 30% of the Earth's land surface and are occupied by almost 1 billion people (Yin et al., 2013; Yin et al., 2015). The ecosystems in these regions are substantially more fragile than most owing to dry infertile soil, resulting in poor vegetation cover (J. Huang, Yu, Guan, Wang, & Guo, 2016; Reynolds et al., 2007). Native vegetation plays an important role in keeping these environments suitable for socioeconomic development and human habitation by providing natural protection against desertification and reducing poverty and food insecurity (Reynolds et al., 2007; Schreckenberget al., 2006). Because of low rainfall accompanied by high evapotranspiration in these inland regions, groundwater is often an important water source for natural vegetation, agricultural practices, domestic use, and public drinking (Fan, Li, & Miguez-Macho, 2013; F. Huang, Zhang, & Chen, 2019). In recent years, the vegetation of inland groundwater-dependent terrestrial ecosystems (GDEs) has been increasingly recognized for its ecological and socio-economic values (Froend & Sommer, 2010; Thomas et al., 2006). However, anthropogenic withdrawal of groundwater for expanding agricultural and domestic use, coupled with climate change-related drought (Ashraf et al., 2017; Taylor et al., 2013), had lead to the depletion of groundwater reserves and resulted in

groundwater decline, hence severely limiting water availability for inland vegetation (Glazer & Likens, 2012; Orellana, Verma, Loheide, & Daly, 2012). GDEs are at risk of degrading, which has become of increasing concern, due to uncertainties about how vegetation will respond to changing groundwater conditions on short and long term timescales (Antunes, Chozas, et al., 2018; Antunes, Díaz Barradas, et al., 2018).

Plant functional traits are important in determining differential tree mortality in response to drought (O'Brien et al., 2017), and trait-based methods have proven effective in identifying changes to water availability due to precipitation gradients or altitudinal variations (Rosado, Joly, Burgess, Oliveira, & Aidar, 2016). However, these methods have been applied to tropical rainforest plants where rainfall is plentiful; whereas, woody, perennial phreatophytes that are supported by shallow groundwater aquifers (Wu, Zheng, Li, & Xu, 2019; Yin et al., 2015; Zhou, Zhao, & Zhang, 2017) make up the majority of natural vegetation in arid regions (Sommer & Froend, 2011; Thomas, 2014). The resilience of these ecosystems to changing hydrological and climatic conditions depends largely on the capacity of phreatophytic plants to cope with reduced groundwater availability (Hultine et al., 2020). Understanding the physiology of woody phreatophytes to decreases in groundwater level is considered to be a major unresolved question in GDEs (Eamus, Zolfaghar, Villalobos-Vega, Cleverly, & Huete, 2015; Orellana et al., 2012). Previous studies have focused on groundwater fluctuation (flood inundations, experimental alterations, or distances to main river channels) on desert riparian forest performance (Li et al., 2019; Pan, Chen, Chen, Wang, & Ren, 2016). However, the effects of variation in groundwater on non-riparian phreatophytes remains unclear (Wu, Zheng, Yin, et al., 2019), despite the affected areas being very large (Cooper et al., 2006).

Access to groundwater plays an important role in determining plant function and survival in GDEs (Froend & Sommer, 2010; Zolfaghar, Villalobos-Vega, Cleverly, & Eamus, 2015). Depth to groundwater (DGW) further influences leaf water relations, hydraulic properties, growth, productivity, survival, and species composition (Gries et al., 2003; Griffith, Rutherford, Clarke, & Warwick, 2015; Yang, Li, Li, & He, 2019; Zolfaghar et al., 2014). Plasticity in hydraulic architecture plays a central role in adapting to differences in water availability (Tyree & Ewers, 1991; Zolfaghar, Villalobos-Vega, Zeppel, & Eamus, 2015). By affecting the rate of water flow through xylem, hydraulic architecture potentially influences water potential ( $\Psi$ ), stomatal conductance, rate of photosynthesis and growth (Awad, Barigah, Badel, Cochard, & Herbette, 2010; Carter & White, 2009; Zeppel & Eamus, 2008). Huber value, xylem vulnerability to embolism, and hydraulic safety margins are key components of hydraulic architecture (Carter & White, 2009; Hubbard, Ryan, Stiller, & Sperry, 2001). Understanding how these traits change across gradients of water availability (DGWs) is important for predicting the fates of xeric phreatophytes facing groundwater decline (Antunes, Díaz Barradas, et al., 2018). Currently, few studies have assessed the response of intraspecific hydraulic architecture to variations in DGW (Garrido et al., 2020; Lucani, Brodribb, Jordan, & Mitchell, 2019).

Osmotic adjustment protects against declining water availability by counteracting turgor loss (Burgess, 2006; Cushman, 2001; Si, Feng, Yu, Zhao, & Li, 2015). Pressure–volume (P-V) analyses have revealed that osmoregulation increases drought resistance as DGW increases (Zolfaghar, Villalobos-Vega, Cleverly, et al., 2015). By increasing and maintaining higher levels of intracellular compatible solutes, osmotic adjustment enhances the capacity for turgor maintenance (Cater, 2011; Gebre, Tschaplinski, Tuskan, & Todd, 1998; Nolan et al., 2017) via active accumulation of in/organic solutes in cells in response to decreases in the cellular environment's  $\Psi$ . Declining cell osmotic potential ( $\pi$ ) further attracts water into cells maintaining turgor pressure (Cater, 2011; Si et al., 2015). However, attributes that confer the capacity to tolerate water stress (lower saturated turgor) may limit growth potential under favorable water conditions (Chen et al., 2015; Fernandez & Reynolds, 2000; Leuschner, Wedde, & Luebbe, 2019). Increasing DGW limits the growth of woody phreatophytes (Gries et al., 2003; J. Li et al., 2013), but how do groundwater-dependent woody phreatophytes survive hydrological drought conditions and what drives that osmoregulation (Kroeger, Zerzour, & Geitmann, 2011)? To our knowledge, the effects of increasing DGW on plant growth through cell turgor regulation has never been tested.

*Haloxylon ammodendron* (C.A. Mey.), a small non-riparian, xeric phreatophytic tree, is endemic to desert regions of Asia and Africa (Thomas, 2014; G.-Q. Xu, McDowell, & Li, 2016). Our early research showed

several morphological adjustments (decreases in assimilation-related branch areas and assimilation-related branch growth rates) that are important drought acclimation strategies to ensure normal photosynthesis and survival of *H. ammodendron* during summer droughts (H. Xu, Li, Xu, & Zou, 2007). As a succulent and xero-halophytic shrub, osmotic adjustments are also important for *H. ammodendron*'s success in a drought environment, as mediated through  $\text{Na}^+$ , soluble sugar, and proline (Lu et al., 2019; Zheng, Xu, Li, & Wu, 2019). Moreover, during the extreme summer drought period, *H. ammodendron* uses deeper water sources, while the depth of the water absorption deepens as groundwater depth declines (Wu, Zheng, Li, et al., 2019). Therefore, absorbing water from deeper soils may influence water budgets, hydraulic properties, and growth rates at the leaf and branch levels (Canham, Froend, & Stock, 2009; Zolfaghar et al., 2014). Yet, the influence of DGW on hydraulic properties and the trade-off between drought tolerance and growth are not clear. Here, we compared xeric phreatophytes under the same meteorological conditions that provided an opportunity to compare intra-specific variation in hydraulic architecture across sites having differential access to groundwater. The aims of this study were to: 1) elucidate differences in hydraulic performance across sites with different DGW, 2) assess drought resistance at greater DGW, and 3) reveal the major active osmoregulation compounds responsible for influencing growth in *H. ammodendron*.

## Materials and methods

### Site description

The current study was performed in the southern edge of the Gurbantunggut Desert near the Fukang Station of Desert Ecology, Chinese Academy of Science (44°17'N, 87deg56'E). This region has a continental, arid climate with a dry, hot summer and cold winter. The minimum air temperature is -42.2degC in winter, and the maximum air temperature is 44.2degC in summer. The annual mean temperature and annual precipitation average are 6.6degC and 164 mm, respectively, with an annual pan evaporation of approximately 1,000 mm. The stable snow cover can last 100–150 d with depth of 25 cm in winter, which then quickly melts and recharges soil moisture. Therefore, plants in this area experience frequent water deficits in the later growing season (Xu, Yu, & Li, 2017).

From the southern edge of the Gurbantunggut Desert to the interior, topography defines a spatial gradient of depth to groundwater (Wu, Zheng, Yin, et al., 2019). Along this gradient, five sites with different groundwater depths were selected (Fig. 1). We used a hand-held laser rangefinder to determine the DGW every month (Deli Tools Co., Ltd, Ningbo, Zhejiang, China). All the sites have been protected from logging since their declaration as “Desert public welfare forests” in the 2000s. The location coordinates of the sampling site is shown in Table 1. The straight-line distance is 15 km from the outmost site to the innermost site. The soil in the desert has a sandy texture (1.3% clay, 13.7% loam, and 85% sand). In such a short distance, we assumed that precipitation patterns were the same. The contrasting hydrological conditions of groundwater were ideal for studying the influence of DGW on the hydraulic properties and growth of *H. ammodendron*.

### Measurement protocols

#### Πρεδawn ανδ μιδδαψΨ

The predawn and midday  $\Psi$  of leaf ( $\Psi_{pd}$  and  $\Psi_{md}$ , respectively) were measured once a month from May to September 2019 using a model 3005 pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA) to evaluate the water states of the plants. In the field, eight healthy and similar-sized individuals at each site were selected and marked, and from each of them, one distal twig with leaf attached was sampled and the water state measured immediately after the twig was cut. The  $\Psi_{pd}$  and  $\Psi_{md}$  values were measured 20 min before sunrise and at solar noon, respectively. In accordance with our previous diurnal measurements, the  $\Psi_{md}$  was considered the minimum leaf  $\Psi$  (G. Q. Xu & Li, 2008). The  $\Psi$  difference ( $\Psi_{pd} - \Psi_{md}$ ) was also calculated.

### Measurement of assimilation branch surface area

At each site across the DGW, 10 branches were selected and labeled on each of the five sampled plants.

All foliage on each branch was photographed using a  $6 \times 108$  pixel digital camera (EOS550D, Canon Inc., Japan), at 10-d intervals, from the beginning to the end of the experimental period. The total leaf surface area of each branch was calculated from the images using CI-400 CIAS software (Computer Imaging Analysis Software, CID Co., Logan, UT, USA). Seasonal changes in leaf area per branch were recorded (H. Xu et al., 2007). The daily relative growth rate of leaves was calculated as the change in leaf area during the intervals between the two measurements. The calculation formula is as follows:

$$, (1)$$

where  $S_t$  represents the leaf area at the time  $t$ ;  $S_{t+1}$  represents the assimilation branch area at the next measurement time,  $t+1$ , and  $\Delta t$  represent the interval time between the two measurements.

### Pressure–volume traits

Pressure–volume (P-V) measurements were carried out during spring (from the end of May to the beginning of June) and late summer (at the end of August) to assess leaf water relations. P-V measurements were generated for a minimum of four twigs using the bench-drying method in accordance with Tyree and Hammel (Tyree & Hammel, 1972). Within each site, five trees were selected, and one terminal woody branch of each tree was excised (leaf attached) and immediately recut under deionized water at 6:00 AM to remove any air emboli. The newly recut ends of the branches were rehydrated in deionized water for more than 4 h with entire branch and assimilating organs covered in black plastic bags to facilitate full rehydration (Arndt, Irawan, & Sanders, 2015). One leaf of each woody branch was cut, and its turgid weight measured using a digital balance (0.0001 g resolution). Then, the corresponding  $\Psi$  was measured using the model 3005 pressure chamber. If this measurement was less than -0.5 MPa, then the twig was discarded, because it had not fully rehydrated. The leaves were left on a bench at room temperature ( $\sim 22^\circ\text{C}$ ) to slowly dehydrate. This process was repeated several times until the correlation between  $1/\Psi$  and fresh weight formed a straight line consisting of at least four measurements with  $R^2 > 0.98$ . The twig was then oven-dried at  $70^\circ\text{C}$  for 1 week (until a constant weight was reached) and then weighed to determine the relative water content (RWC) as follows:

$$\text{RWC (\%)} = 100 \times (\text{FW-DW})/(\text{TW-DW}), (2)$$

where FW represents the fresh weight of the twig; DW represents the dry weight; and TW represents the turgid weight. The  $\pi$  at full turgor ( $\pi_{100}$ ) and turgor loss point ( $\Psi_{\text{TLP}}$ ) were derived from the curve of  $1/\Psi$  against RWC using a P-V curve-fitting method available online (<http://landflux.org>) based on Schulte and Hinckley (Schulte & Hinckley, 1985).

### Huber value

Huber values, i.e., sapwood-to-leaf area ratios, were measured both at the beginning of June and the end of August. Five healthy adult trees were selected from each field sample point, and the new assimilating twigs of the current year were taken as the first-order branches. We collected one third-order branch from the southern upper part of the canopies of the selected trees at each site. The areas of the assimilating branches were scanned (Epson Perfection 2400 Photo, Seiko Epson Corp., Japan). The cross sections of the branches were dyed with 1% aqueous acid-fuchsin, and the stained sapwood areas were measured using digital calipers.

### Determination of inorganic ions and organic solutes in plant tissues

The green assimilating branches of *H. ammodendron* were collected at the five sites in May and July and oven-dried at  $70^\circ\text{C}$  for at least 48 h to a constant mass. The dried samples were ground in a ball mill MM400 (Retsch, Germany) into they were uniformly fine powders and freeze dried for preservation. For the extraction of soluble sugar and starch, we followed Anderegg et al., 2012. After the digestion of sucrose and starch, the concentrations of each was measured using the phenol-sulfuric acid method and a UV spectrophotometer (colorimetric method, UV-2401PC, Shimadzu Corporation, Japan). A ninhydrin colorimetric method was used for proline concentration determinations with a UV spectrophotometer (Abraham,

Hourton-Cabassa, Erdei, & Szabados, 2010; Song et al., 2006). The  $\text{Na}^+$  concentrations were determined using pulsed flame photometer detection (Song et al., 2006; Jenway Ltd., UK).

### Data analyses

Owing to the contrasting soil-water conditions and water-use strategies along the DGW gradient in spring and summer (Wu, Zheng, Li, et al., 2019), all data were grouped into two growth stages, namely the early growing season (EGS) from April to June, which included spring and early summer, and late growing season (LGS) from July to September, which included late summer and autumn. Descriptive statistics were used to calculate the means and standard errors of each subset. A linear mixed effect model (LMEM) was used to evaluate the effects of growth stage on the hydrological drought (different depth to groundwater) response of the studied species using “lme4” package (Bates et al., 2014) and running on 3.6.3 version on R. Differences among hydraulic traits,  $\pi_{100}$ ,  $\Psi_{\text{TLP}}$ , osmotic substance contents, assimilation branch areas, and growth rates at different sites within the same growing season were also tested using one-way analyses of variance (ANOVA) with Tukey’s-HSD post-hoc test. To compare the differences of traits in the EGS and LGS, independent-sample *t*-tests were used. Linear regressions were carried out between hydraulic properties, P-V curve parameters, Huber values, growth rates, and DGWs. Figures were prepared using Origin 8.0 (Origin Lab Corp., Northampton, MA, USA) and R ver. 3.6.3 (R development Core Team 2016). Data analyses were conducted using R ver. 3.6.3 and IBM SPSS Statistics software (Ver. 22, Armonk, NY, USA).

### 3. Results

#### Λεαφ $\Psi$ ανδ Π-ο τραιτς

The mixed models revealed that growth stage significantly influenced the relationship between DGW and  $\Psi_{\text{pd}}$ ,  $\Psi_{\text{md}}$ ,  $\Psi_{\pi\delta} - \Psi_{\mu\delta}$  ( $F = 52.34, P < 0.001; F = 61.32, P < 0.001; F = 18.05, P < 0.001$ , respectively). There were significant differences in the average  $\Psi_{\text{pd}}$  and  $\Psi_{\text{md}}$ , as well as in the  $\Psi_{\pi\delta} - \Psi_{\mu\delta}$  values among the five sites during both the EGS and LGS (Fig. 2). There were also significant differences between the two growth stages within the same site (Fig. 3). Except the  $\Psi_{\text{pd}}$  during the LGS, branch  $\Psi$ s ( $\Psi_{\text{pd}}$  and  $\Psi_{\text{md}}$ ) and  $\Psi_{\text{pd}} - \Psi_{\text{md}}$  significantly decreased as DWG increased (Fig. 4). The assimilating branch  $\Psi$  values at  $\pi_{100}$  and  $\Psi_{\text{TLP}}$  were significantly different among the sites (Fig. 5). The  $\pi_{100}$  ranged between -3.74 MPa (shallowest DGW) and -4.59 MPa (deepest DGW; EGS). The  $\Psi_{\text{TLP}}$  ranged between -4.14 MPa (shallowest DGW) and -5.00 MPa (deepest DGW; EGS). The  $\pi_{100}$  values ranged between -3.80 MPa (shallowest DGW) and -4.82 MPa (deepest DGW; LGS). The  $\Psi_{\text{TLP}}$  ranged between -4.21 MPa (shallowest DGW) and -5.14 MPa (deepest DGW; LGS) (Fig. 5). There were no significant differences in  $\pi_{100}$  and  $\Psi_{\text{TLP}}$  between the two growth periods at the same site (data not show here). The  $\Psi_{\text{md}}$  values were generally less negative than the leaf  $\Psi$  at zero turgor. The mixed models revealed that growth stage had no significant influence on the relationship between DGW and  $\pi_{100}$ ,  $\Psi_{\text{TLP}}$  ( $F = 55.80, P < 0.001; F = 60.97, P < 0.001$ , respectively). However, there was a decreasing trend of  $\Psi$  at  $\pi_{100}$  and  $\Psi_{\text{TLP}}$  along with groundwater depth irrespective of the growth stage (Fig. 6).

#### Growth rates and Huber values

Decreasing DGW significantly influenced the seasonal patterns of assimilation area per branch, especially during the EGS (Fig. 7). The average assimilation area accumulation per branch at each trial day was greater in the shallow DGW sites than the deeper DGW sites during the EGS (Fig. 7a). The growth rate of the branch assimilation area was greater in the shallow DGW sites than the deeper DGW sites in the same trial phase during the EGS (Fig. 7a and b). The branch assimilation areas and growth rates were not significantly different among sites on the same trial day during the LGS (Fig. 7a and b); growth rate slowed and even became negative during the LGS as a result of summer droughts (Fig. 7a and b). The mixed models revealed that growth stage had a significant influence on the relationship between DGW and growth rate & Huber values ( $F = 5.07, P < 0.01; F = 6.23, P < 0.01$ , respectively). We saw growth rates in assimilation branches decrease as DGW increased during EGS (Fig. 8a) but not LGS (Fig. 8b). Huber values increased as DGW increased during the EGS (Fig. 8c) but not during the LGS (Fig. 8d).

## Variation in organic solutes and inorganic ions

Growth stage was significant ( $P = 0.097$ ) in the relationship between DGW and soluble sugars ( $F = 7.64$ ,  $P < 0.01$ ). There were no correlation between soluble sugar concentrations and increasing DGW during both EGS and LGS. Concentrations of soluble sugars in the assimilation branches of *H. ammodendron* were lower at the shallowest DGW site compared to the deepest DGW site during both growth stages. The concentrations of soluble sugars in the assimilation branches within the shallowest site were also significantly different between the EGS and LGS (Table 1). Growth stage significantly affected DGW and starch ( $F = 13.65$ ,  $P < 0.001$ ). There were no correlations between starch concentration and increasing DGW during either EGS or LGS. Starch concentrations were significantly different among sites having contrasting DGW levels. Starch concentrations were high at the site with the shallowest DGW compared to the site with the deepest DGW (Table 1). Growth stage was significant in the relationship between DGW and proline ( $F = 13.52$ ,  $P < 0.001$ ). There were no correlation between proline and increasing DGW during either EGS or LGS. Proline during the whole growth season ranged between  $10.44 \pm 1.34$  and  $1.70 \pm 0.08$  (Table 1). Season had significant influence on the relationship between DGW and  $\text{Na}^+$  concentration ( $F = 37.40$ ,  $P < 0.001$ ).  $\text{Na}^+$  concentration was higher during LGS than EGS (Table 1). There were no trends between  $\text{Na}^+$  and DGW during EGS or LGS.

## Discussion

Predicted droughts and anthropogenic water intensify groundwater limitation, particularly for groundwater-dependent ecosystems arid environments (Ashraf et al., 2017; Challis, Stevens, McGrath, & Miller, 2016). Yet, our ability to predict the impacts of groundwater changes on these ecosystems is still poor. Increasing depth of groundwater (DGW) alters the soil water supply by dramatically reducing the water availability (Garrido et al., 2020; Hultine et al., 2020). Water absorption occurs deeper as groundwater depths decline, which increases the water transport distance along with the DGW (Wu, Zheng, Li, et al., 2019). Here, DGW was the most important driver of ecophysiological adjustments for a small, xeric phreatophytic tree. Increasing DGW negatively affected the hydraulic properties and growth rate of *H. ammodendron* (Fig. 4, Fig. 6 and Fig. 7). Effective osmotic adjustments (accumulating organic compounds to tolerate low assimilating branch  $\Psi$  and facilitate water absorption from deeper soil layers) decreases the cell turgor, which constrained leaf growth (Fig. 8 and Fig. 9). The decline in the growth rate, in turn, led to a Huber value adjustment to increase the water supply of a unit of sapwood area (Fig. 9). The switching of water sources (Wu, Zheng, Li, et al., 2019), osmotic regulation, and plasticity in Huber value, however, did not sufficiently compensate for the impact of drought stress on the physiological performance as reflected in decreased growth rates in response to DGW during the EGS and even a negative growth rate at most sites during the LGS. Therefore, more attention should be given to the influence of groundwater level decline on plant health in natural ecosystems, especially the most vulnerable arid, desert ecosystems mainly dominated by phreatophytic species.

We divided the growing season into early and late phases to reflect contrasting meteorological conditions in different growth stages. In our research area, snow usually covers the land, with a maximum depth of 20 cm, from late November to late March of the next year (H.-F. Zhou, Zheng, Zhou, Dai, & Li, 2012). Due to the seasonal recharge from snowmelt, the soil water content was higher in spring and early summer but quickly depleted in the dry, hot mid/late summer (Tiemuerbieke et al., 2018; Wu, Zheng, Li, et al., 2019). Therefore, *H. ammodendron* experiences different upper soil water conditions during the growing season despite the pattern of upper soil water content variation being the same across sites with different DGW values. Thus, it is necessary to distinguish the EGS and LGS, which may help clarify the impact of groundwater on hydraulic traits and growth. Additionally, annual growth rhythms may influence the impact of DGW on plants. Future studies should distinguish dynamic changes in leaf growth caused by soil water supply conditions.

## The influence of DGW on hydraulic traits

The impact of groundwater depth on the water available to *H. ammodendron* was evident from leaf  $\Psi_{pd}$  values experienced by trees over shallow and deep groundwater (Fig. 2, Fig. 3, Fig. 4). The decrease in the

twig  $\Psi_{pd}$  suggested reduced water availability, implying that *H. ammodendron* was under more severe water stress at deeper groundwater sites. The decline in  $\Psi$  as DGW increased had been previously reported (Wu, Zheng, Li, et al., 2019) and was consistent with the study by Cater (2011).  $\Psi_{pd} - \Psi_{md}$  values also increased as the DGW increased (Fig. 4), which we anticipated because the xylem transport distance increases at a greater groundwater depth. Lower leaf  $\Psi_s$  and greater  $\Psi_{pd} - \Psi_{md}$  values are required for water uptake (Gries et al., 2003).

Prior analyses of leaf P-V traits in multiple species across gradients of aridity indicate that  $\pi_{100}$  and  $\Psi_{TLP}$  are robust proxies for important components of drought tolerance (Bartlett, Scoffoni, & Sack, 2012; Bartlett et al., 2014).  $\Psi_{TLP}$  indicates the capacity of a plant to maintain cell turgor pressure during dehydration, which is strongly predictive of the plant's response to drought (Zhu et al., 2018). In our currently study,  $\Psi_{TLP}$  decreased as DGW increased (Fig. 5 and 6), which indicates an increasing drought resistance as DGW increases. These results are consistent with previous studies in which water stress was induced by groundwater level decline (Pan et al., 2016; Zolfaghar, Villalobos-Vega, Cleverly, et al., 2015). However, the  $\pi_{100}$  was lower than the average  $\Psi_{pd}$  during the EGS and LGS in our current study, which is inconsistent with previous results (Zheng et al., 2019). This inconsistency may be attributed to the long-term average of  $\Psi_{pd}$  during the EGS lowering the  $\Psi_{pd}$  values corresponding to the period when the P-V curve measurements were taken or to the absorption of atmospheric water or dew water in the early morning, which lowers  $\Psi_{pd}$  values (Gong, Lu, He, Sarkar, & Yang, 2019).

### How plants respond to increases in DGW and its impact on their growth rates

Osmotic adjustment is an important drought-tolerance mechanism that offsets turgor loss through the accumulation and maintenance of soluble substances in cells (Cushman, 2001). Leaf or stem succulence facilitates osmotic adjustment by regulating internal ion concentrations in many xerophytic shrubs (Ogburn & Edwards, 2010). Variation in osmotic solute levels under different groundwater depths (Si et al., 2015), and thus, the corresponding osmotic regulation, have been reported in a few case studies (Pan et al., 2016; Zolfaghar, Villalobos-Vega, Cleverly, et al., 2015). Here, osmotic adjustments were evident by decreases in  $\Psi$  at  $\pi_{100}$  and  $\Psi_{TLP}$  as DGW increased (Fig. 6).  $\text{Na}^+$  has been reported as an important inorganic osmoregulatory substance used by *H. ammodendron* to cope with summer drought (Kang, Duan, Wang, Zhao, & Yang, 2013). Higher  $\text{Na}^+$  concentration during LGS compared to EGS may facilitate this xeric species's resistance to summer drought (Table 1). However,  $\text{Na}^+$  concentration did not correlate to increasing DGW during either the EGS or LGS, which may indicate that the accumulation of  $\text{Na}^+$  was not a key factor in sustaining negative  $\pi_{100}$  as DGW increased.

The levels of organic compounds, such as soluble sugar and proline, increase under water-stress conditions and are, therefore, potentially important contributors to osmotic adjustment (Hong, Lakkineni, Zhang, & Verma, 2000; Si et al., 2015). In our current study, the proline concentration was lower during LGS than EGS, and thus, it did not increase the plant's tolerance to summer droughts. Previous studies have proposed that proline does not play an important role in osmoregulation for *H. ammodendron* (Song et al., 2006). Soluble sugar concentration was higher at the deepest DGW site compared to the shallowest DGW site during both EGS and LGS (Table 1), indicating that sugar concentration increased along with DGW. Additionally, sugar concentration correlated with  $\pi_{100}$  as DGW increased during EGS. Thus, we ascertained that soluble sugars act as important osmotic substances for decreasing  $\pi_{100}$  (Fig. 9a). The use of these substances, in turn, decreases the growth rate as DGW increases (Fig. 8a and Fig. 9c). Decreasing  $\pi_{100}$  limited the expansion of plant cell walls during leaf cellular growth (Passioura & Fry, 1992). Limited studies have investigated such an apparent correlation between leaf turgor and leaf growth rates (Kroeger et al., 2011), and fewer have investigated the effects of physiological processes, including those involving osmotic solutes, on decreasing  $\pi_{100}$ , which then constrains growth. Here, we are the first to display such a correlation.

The plasticity of Huber values is important for plants to respond to variations in groundwater depth and to sustain the homeostasis of leaf water use and water budget (Carter & White, 2009). Here, Huber values increased along with DGW during the EGS (Fig. 8c). Huber values were lower for trees over shallow groundwater ( $8.19 \times 10^{-4}$ ) than for trees over deep groundwater ( $13.14 \times 10^{-4}$ ; Fig. 8c) during the EGS,

which resulted in an increased capacity of stems to transport water to leaves (Carter & White, 2009). These results are consistent with those of other studies on the same or closely related species across climatic gradients (Addington et al., 2006; Canham et al., 2009; Magnani, Grace, & Borghetti, 2002). Furthermore, decreased growth rate was marginally significant to the increase in Huber values with increasing DGW during EGS (Fig. 9e) as similarly reported for *Prosopis tamarugo* (Garrido et al., 2020). Therefore, as an important strategy to hydrological drought induced by a greater DGW, osmotic adjustment may constrain growth but also facilitate the plasticity of Huber values and help xeric trees buffer suboptimal water-supply conditions. However, these adjustments cannot fully compensate for the effects of the hydrological drought, as shown by decreased growth rates, even becoming negative during the LGS, at almost all sites.

## Conclusion

For *Haloxyylon ammodendron*, a small xeric phreatophytic tree, we showed that increasing DGW significantly worsened water conditions. We postulate turgor loss traits to be adaptive for osmoregulation due to their decrease with increasing DGW. Consequently, an osmotic adjustment that accompanied  $\Psi$  regulation ( $\Psi_{pd} - \Psi_{md}$ ) and Huber value plasticity helps phreatophytes absorb water from deeper soil layers and tolerate drought. Soluble sugars, as major components of osmoregulation, drove the decline in osmotic potential at full turgor; thereby, constraining assimilating branch growth during the early growing season (EGS). However, these adaptive adjustments did not fully compensate for worsened water conditions as growth rate continued to decrease during EGS and resulted in negative growth rates during the late growing season at almost all sites. Our results provide insight into how *H. ammodendron* responds and adapts to changes in DGW in a region experiencing hydrological and climatic drought. Greater depth to groundwater had a significant effect on this small, xeric phreatophytic tree's survival and growth.

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## Author contributions statement

GQX and LST conceived this study; GQX. and JM designed the experiments; XJM. performed the experiments and analyzed the samples; JM provided technical support in the field; GQX and XJM. process the data. The manuscript was composed by GQX with constructive suggestions and carefully editing by LST; all the authors contributed to writing and revising the manuscript.

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

- Abraham, E., Hourton-Cabassa, C., Erdei, L., & Szabados, L. (2010). Methods for Determination of Proline in Plants. In R. Sunkar (Ed.), *Plant Stress Tolerance: Methods and Protocols* (Vol. 639, pp. 317-331).
- Addington, R. N., Donovan, L. A., Mitchell, R. J., Vose, J. M., Pecot, S. D., Jack, S. B., . . . Oren, R. (2006). Adjustments in hydraulic architecture of *Pinus palustris* maintain similar stomatal conductance in xeric and mesic habitats. *Plant, Cell & Environment*, *29* (4), 535-545. doi:10.1111/j.1365-3040.2005.01430.x
- Anderegg, W. R. L., Berry, J. A., Smith, D. D., Sperry, J. S., Anderegg, L. D. L., & Field, C. B. (2012). The roles of hydraulic and carbon stress in a widespread climate-induced forest die-off. *Proceedings of the National Academy of Sciences of the United States of America*, *109* (1), 233-237. doi:10.1073/pnas.1107891109
- Antunes, C., Chozas, S., West, J., Zunzunegui, M., Diaz Barradas, M. C., Vieira, S., & Máguas, C. (2018). Groundwater drawdown drives ecophysiological adjustments of woody vegetation in a semi-arid coastal ecosystem. *Global Change Biology*, *24* (10), 4894-4908. doi:doi:10.1111/gcb.14403

- Antunes, C., Díaz Barradas, M. C., Zunzunegui, M., Vieira, S., Pereira, Â., Anjos, A., . . . Máguas, C. (2018). Contrasting plant water-use responses to groundwater depth in coastal dune ecosystems. *Functional Ecology*, *32* (8), 1931-1943. doi:doi:10.1111/1365-2435.13110
- Arndt, S. K., Irawan, A., & Sanders, G. J. (2015). Apoplastic water fraction and rehydration techniques introduce significant errors in measurements of relative water content and osmotic potential in plant leaves. *Physiologia Plantarum*, *155* (4), 355-368. doi:10.1111/ppl.12380
- Ashraf, B., AghaKouchak, A., Alizadeh, A., Baygi, M. M., Moftakhari, H. R., Mirchi, A., . . . Madani, K. (2017). Quantifying Anthropogenic Stress on Groundwater Resources. *Scientific Reports*, *7* . doi:10.1038/s41598-017-12877-4
- Awad, H., Barigah, T., Badel, E., Cochard, H., & Herbette, S. (2010). Poplar vulnerability to xylem cavitation acclimates to drier soil conditions. *Physiologia Plantarum*, *139* (3), 280-288. doi:10.1111/j.1399-3054.2010.01367.x
- Bartlett, M. K., Scoffoni, C., & Sack, L. (2012). The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: a global meta-analysis. *Ecology Letters*, *15* (5), 393-405. doi:10.1111/j.1461-0248.2012.01751.x
- Bartlett, M. K., Zhang, Y., Kreidler, N., Sun, S., Ardy, R., Cao, K., & Sack, L. (2014). Global analysis of plasticity in turgor loss point, a key drought tolerance trait. *Ecology Letters*, *17* (12), 1580-1590. doi:10.1111/ele.12374
- Bates D., Maechler M., Bolker B., Walker S., Christensen R. H. B., Singmann H., et al. (2014). Package 'lme4'. Vienna: R Foundation for Statistical Computing
- Burgess, S. S. O. (2006). Facing the challenge of seasonally dry environments. *Physiologia Plantarum*, *127* (3), 339-342. doi:10.1111/j.1399-3054.2006.00726.x
- Canham, C. A., Froend, R. H., & Stock, W. D. (2009). Water stress vulnerability of four Banksia species in contrasting ecohydrological habitats on the Gngangara Mound, Western Australia. *Plant, Cell & Environment*, *32* (1), 64-72. doi:10.1111/j.1365-3040.2008.01904.x
- Carter, J. L., & White, D. A. (2009). Plasticity in the Huber value contributes to homeostasis in leaf water relations of a mallee Eucalypt with variation to groundwater depth. *Tree Physiology*, *29* (11), 1407-1418. doi:10.1093/treephys/tpp076
- Cater, M. (2011). Osmotic adaptation of *Quercus robur* L. under water stress in stands with different tree density - relation with groundwater table. *Dendrobiology*, *65* , 29-36.
- Challis, A., Stevens, J. C., McGrath, G., & Miller, B. P. (2016). Plant and environmental factors associated with drought-induced mortality in two facultative phreatophytic trees. *Plant and Soil*, *404* (1-2), 157-172. doi:10.1007/s11104-016-2793-5
- Chen, L.-Y., Shi, D.-Q., Zhang, W.-J., Tang, Z.-S., Liu, J., & Yang, W.-C. (2015). The Arabidopsis alkaline ceramidase TOD1 is a key turgor pressure regulator in plant cells. *Nature Communications*, *6* . doi:10.1038/ncomms7030
- Cooper, D. J., Sanderson, J. S., Stannard, D. I., & Groeneveld, D. P. (2006). Effects of long-term water table drawdown on evapotranspiration and vegetation in an arid region phreatophyte community. *Journal of Hydrology*, *325* (1-4), 21-34. doi:10.1016/j.jhydrol.2005.09.035
- Cushman, J. C. (2001). Osmoregulation in plants: Implications for agriculture. *American Zoologist*, *41* (4), 758-769. doi:10.1668/0003-1569(2001)041[0758:oiipifa]2.0.co;2
- Eamus, D., Zolfaghar, S., Villalobos-Vega, R., Cleverly, J., & Huete, A. (2015). Groundwater-dependent ecosystems: recent insights from satellite and field-based studies. *Hydrology and Earth System Sciences*, *19* (10), 4229-4256. doi:10.5194/hess-19-4229-2015

- Fan, Y., Li, H., & Miguez-Macho, G. (2013). Global Patterns of Groundwater Table Depth. *Science*, *339* (6122), 940-943. doi:10.1126/science.1229881
- Fernandez, R. J., & Reynolds, J. F. (2000). Potential growth and drought tolerance of eight desert grasses: lack of a trade-off? *Oecologia*, *123* (1), 90-98. doi:10.1007/s004420050993
- Froend, R., & Sommer, B. (2010). Phreatophytic vegetation response to climatic and abstraction-induced groundwater drawdown: Examples of long-term spatial and temporal variability in community response. *Ecological Engineering*, *36* (9), 1191-1200. doi:https://doi.org/10.1016/j.ecoleng.2009.11.029
- Garrido, M., Bown, H., Ayamante, J., Orell, M., Sanchez, A., & Acevedo, E. (2020). The adjustment of Prosopis tamarugo hydraulic architecture traits has a homeostatic effect over its performance under descent of phreatic level in the Atacama Desert. *Trees-Structure and Function*, *34* (1), 89-99. doi:10.1007/s00468-019-01899-2
- Gebre, G. M., Tschaplinski, T. J., Tuskan, G. A., & Todd, D. E. (1998). Clonal and seasonal differences in leaf osmotic potential and organic solutes of five hybrid poplar clones grown under field conditions. *Tree Physiology*, *18* (10), 645-652.
- Glazer, A. N., & Likens, G. E. (2012). The Water Table: The Shifting Foundation of Life on Land. *Ambio*, *41* (7), 657-669. doi:10.1007/s13280-012-0328-8
- Gong, X.-W., Lu, G.-H., He, X.-M., Sarkar, B., & Yang, X.-D. (2019). High Air Humidity Causes Atmospheric Water Absorption via Assimilating Branches in the Deep-Rooted Tree Haloxylon ammodendron in an Arid Desert Region of Northwest China. *Frontiers in Plant Science*, *10* . doi:10.3389/fpls.2019.00573
- Gries, D., Zeng, F., Foetzki, A., Arndt, S. K., Bruelheide, H., Thomas, F. M., . . . Runge, M. (2003). Growth and water relations of Tamarix ramosissima and Populus euphratica on Taklamakan desert dunes in relation to depth to a permanent water table. *Plant Cell and Environment*, *26* (5), 725-736. doi:10.1046/j.1365-3040.2003.01009.x
- Griffith, S. J., Rutherford, S., Clarke, K. L., & Warwick, N. W. M. (2015). Water relations of wallum species in contrasting groundwater habitats of Pleistocene beach ridge barriers on the lower north coast of New South Wales, Australia. *Australian Journal of Botany*, *63* (7), 618-630. doi:10.1071/bt15103
- Hong, Z. L., Lakkineni, K., Zhang, Z. M., & Verma, D. P. S. (2000). Removal of feedback inhibition of Delta(1)-pyrroline-5-carboxylate synthetase results in increased proline accumulation and protection of plants from osmotic stress. *Plant Physiology*, *122* (4), 1129-1136. doi:10.1104/pp.122.4.1129
- Huang, F., Zhang, D., & Chen, X. (2019). Vegetation Response to Groundwater Variation in Arid Environments: Visualization of Research Evolution, Synthesis of Response Types, and Estimation of Groundwater Threshold. *International Journal of Environmental Research and Public Health*, *16* (10). doi:10.3390/ijerph16101849
- Huang, J., Yu, H., Guan, X., Wang, G., & Guo, R. (2016). Accelerated dryland expansion under climate change. *Nature Climate Change*, *6* (2), 166+. doi:10.1038/nclimate2837
- Hubbard, R. M., Ryan, M. G., Stiller, V., & Sperry, J. S. (2001). Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. *Plant Cell and Environment*, *24* (1), 113-121. doi:10.1046/j.1365-3040.2001.00660.x
- Hultine, K. R., Froend, R., Blasini, D., Bush, S. E., Karlinski, M., & Koepke, D. F. (2020). Hydraulic traits that buffer deep-rooted plants from changes in hydrology and climate. *Hydrological Processes*, *34* (2), 209-222. doi:10.1002/hyp.13587
- Kang, J., Duan, J., Wang, S., Zhao, M., & Yang, Z. (2013). Na compound fertilizer promotes growth and enhances drought resistance of the succulent xerophyte Haloxylon ammodendron. *Soil Science and Plant Nutrition*, *59* (2), 289-299. doi:10.1080/00380768.2012.763183

- Kroeger, J. H., Zerzour, R., & Geitmann, A. (2011). Regulator or Driving Force? The Role of Turgor Pressure in Oscillatory Plant Cell Growth. *Plos One*, *6* (4), e18549. doi:10.1371/journal.pone.0018549
- Leuschner, C., Wedde, P., & Luebbe, T. (2019). The relation between pressure-volume curve traits and stomatal regulation of water potential in five temperate broadleaf tree species. *Annals of Forest Science*, *76* (2). doi:10.1007/s13595-019-0838-7
- Li, E., Tong, Y., Huang, Y., Li, X., Wang, P., Chen, H., & Yang, C. (2019). Responses of two desert riparian species to fluctuating groundwater depths in hyperarid areas of Northwest China. *Ecohydrology*, *12* (3), e2078. doi:10.1002/eco.2078
- Li, J., Yu, B., Zhao, C., Nowak, R. S., Zhao, Z., Sheng, Y., & Li, J. (2013). Physiological and morphological responses of *Tamarix ramosissima* and *Populus euphratica* to altered groundwater availability. *Tree Physiology*, *33* (1), 57-68. doi:10.1093/treephys/tps120
- Lu, X.-P., Gao, H.-J., Zhang, L., Wang, Y.-P., Shao, K.-Z., Zhao, Q., & Zhang, J.-L. (2019). Dynamic responses of *Haloxylon ammodendron* to various degrees of simulated drought stress. *Plant Physiology and Biochemistry*, *139*, 121-131. doi:10.1016/j.plaphy.2019.03.019
- Lucani, C. J., Brodribb, T. J., Jordan, G., & Mitchell, P. J. (2019). Intraspecific variation in drought susceptibility in *Eucalyptus globulus* is linked to differences in leaf vulnerability. *Functional Plant Biology*, *46* (3), 286-293. doi:10.1071/fp18077
- Magnani, F., Grace, J., & Borghetti, M. (2002). Adjustment of tree structure in response to the environment under hydraulic constraints. *Functional Ecology*, *16* (3), 385-393. doi:10.1046/j.1365-2435.2002.00630.x
- Nolan, R. H., Tarin, T., Santini, N. S., McAdam, S. A. M., Ruman, R., & Eamus, D. (2017). Differences in osmotic adjustment, foliar abscisic acid dynamics, and stomatal regulation between an isohydric and anisohydric woody angiosperm during drought. *Plant, Cell & Environment*, *40* (12), 3122-3134. doi:10.1111/pce.13077
- O'Brien, M. J., Engelbrecht, B. M. J., Joswig, J., Pereyra, G., Schuldt, B., Jansen, S., . . . Macinnis-Ng, C. (2017). A synthesis of tree functional traits related to drought-induced mortality in forests across climatic zones. *Journal of Applied Ecology*, *54* (6), 1669-1686. doi:https://doi.org/10.1111/1365-2664.12874
- Ogburn, R. M., & Edwards, E. J. (2010). The Ecological Water-Use Strategies of Succulent Plants. In J. C. Kader & M. Delseny (Eds.), *Advances in Botanical Research, Vol 55* (Vol. 55, pp. 179-225).
- Orellana, F., Verma, P., Loheide, S. P., II, & Daly, E. (2012). MONITORING AND MODELING WATER-VEGETATION INTERACTIONS IN GROUNDWATER-DEPENDENT ECOSYSTEMS. *Reviews of Geophysics*, *50*. doi:10.1029/2011rg000383
- Pan, Y., Chen, Y., Chen, Y., Wang, R., & Ren, Z. (2016). Impact of groundwater depth on leaf hydraulic properties and drought vulnerability of *Populus euphratica* in the Northwest of China. *Trees*, *30* (6), 2029-2039. doi:10.1007/s00468-016-1430-5
- Passioura, J. B., & Fry, S. C. (1992). TURGOR AND CELL EXPANSION - BEYOND THE LOCKHART EQUATION. *Australian Journal of Plant Physiology*, *19* (5), 565-576. doi:10.1071/pp9920565
- Reynolds, J. F., Stafford Smith, D. M., Lambin, E. F., Turner, B. L., Mortimore, M., Batterbury, S. P. J., . . . Walker, B. (2007). Global desertification: Building a science for dryland development. *Science*, *316*(5826), 847-851. doi:10.1126/science.1131634
- R Development Core Team, 2016. R: A Language and Environment for Statistical Computing. R Found. Stat. Comput. Vienna, Austria. doi:10.1038/sj.hdy.6800737.
- Rosado, B. H. P., Joly, C. A., Burgess, S. S. O., Oliveira, R. S., & Aidar, M. P. M. (2016). Changes in plant functional traits and water use in Atlantic rainforest: evidence of conservative water use in spatio-temporal scales. *Trees*, *30* (1), 47-61. doi:10.1007/s00468-015-1165-8

- Schreckenber, K., Awono, A., Degrande, A., Mbosso, C., Ndoye, O., & Tchoundjeu, Z. (2006). DOMESTICATING INDIGENOUS FRUIT TREES AS A CONTRIBUTION TO POVERTY REDUCTION. *Forests, Trees and Livelihoods*, *16* (1), 35-51. doi:10.1080/14728028.2006.9752544
- Schulte, P. J., & Hinckley, T. M. (1985). A COMPARISON OF PRESSURE-VOLUME CURVE DATA-ANALYSIS TECHNIQUES. *Journal of Experimental Botany*, *36* (171), 1590-1602. doi:10.1093/jxb/36.10.1590
- Si, J., Feng, Q., Yu, T., Zhao, C., & Li, W. (2015). Variation in *Populus euphratica* foliar carbon isotope composition and osmotic solute for different groundwater depths in an arid region of China. *Environmental Monitoring and Assessment*, *187* (11). doi:10.1007/s10661-015-4890-y
- Sommer, B., & Froend, R. (2011). Resilience of phreatophytic vegetation to groundwater drawdown: is recovery possible under a drying climate? *Ecohydrology*, *4* (1), 67-82. doi:doi:10.1002/eco.124
- Song, H., Feng, G., Tian, C. Y., & Zhang, F. S. (2006). Osmotic adjustment traits of *Suaeda physophora*, *Haloxylon ammodendron* and *Haloxylon persicum* in field or controlled conditions. *Plant Science*, *170* (1), 113-119. doi:10.1016/j.plantsci.2005.08.004
- Taylor, R. G., Scanlon, B., Doell, P., Rodell, M., van Beek, R., Wada, Y., . . . Treidel, H. (2013). Ground water and climate change. *Nature Climate Change*, *3* (4), 322-329. doi:10.1038/nclimate1744
- Thomas, F. M. (2014). Ecology of Phreatophytes. In U. Lüttge, W. Beyschlag, & J. Cushman (Eds.), *Progress in Botany: Vol. 75* (pp. 335-375). Berlin, Heidelberg: Springer Berlin Heidelberg.
- Thomas, F. M., Foetzki, A., Arndt, S. K., Bruelheide, H., Gries, D., Li, X., . . . Runge, M. (2006). Water use by perennial plants in the transition zone between river oasis and desert in NW China. *Basic and Applied Ecology*, *7* (3), 253-267. doi:https://doi.org/10.1016/j.baae.2005.07.008
- Tiemuerbieke, B., Min, X.-J., Zang, Y.-X., Xing, P., Ma, J.-Y., & Sun, W. (2018). Water use patterns of co-occurring C-3 and C-4 shrubs in the Gurbantonggut desert in northwestern China. *Science of the Total Environment*, *634* , 341-354. doi:10.1016/j.scitotenv.2018.03.307
- Tyree, M. T., & Ewers, F. W. (1991). THE HYDRAULIC ARCHITECTURE OF TREES AND OTHER WOODY-PLANTS. *New Phytologist*, *119* (3), 345-360. doi:10.1111/j.1469-8137.1991.tb00035.x
- Tyree, M. T., & Hammel, H. T. (1972). The Measurement of the Turgor Pressure and the Water Relations of Plants by the Pressure-bomb Technique. *Journal of Experimental Botany*, *23* (1), 267-282. doi:10.1093/jxb/23.1.267
- Wu, X., Zheng, X.-J., Li, Y., & Xu, G.-Q. (2019). Varying responses of two *Haloxylon* species to extreme drought and groundwater depth. *Environmental and Experimental Botany*, *158* , 63-72. doi:10.1016/j.envexpbot.2018.11.014
- Wu, X., Zheng, X.-J., Yin, X.-W., Yue, Y.-M., Liu, R., Xu, G.-Q., & Li, Y. (2019). Seasonal variation in the groundwater dependency of two dominant woody species in a desert region of Central Asia. *Plant and Soil* . doi:10.1007/s11104-019-04251-2
- Xu, G.-Q., McDowell, N. G., & Li, Y. (2016). A possible link between life and death of a xeric tree in desert. *Journal of Plant Physiology*, *194* , 35-44. doi:https://doi.org/10.1016/j.jplph.2016.02.014
- Xu, G.-Q., Yu, D.-D., & Li, Y. (2017). Patterns of biomass allocation in *Haloxylon persicum* woodlands and their understory herbaceous layer along a groundwater depth gradient. *Forest Ecology and Management*, *395* , 37-47. doi:10.1016/j.foreco.2017.03.037
- Xu, G. Q., & Li, Y. (2008). Rooting depth and leaf hydraulic conductance in the xeric tree *Haloxylon ammodendron* growing at sites of contrasting soil texture. *Functional Plant Biology*, *35* (12), 1234-1242. doi:10.1071/fp08175

Xu, H., Li, Y., Xu, G., & Zou, T. (2007). Ecophysiological response and morphological adjustment of two Central Asian desert shrubs towards variation in summer precipitation. *Plant Cell and Environment*, *30* (4), 399-409. doi:10.1111/j.1365-3040.2006.001626.x

Yang, Z., Li, W., Li, X., & He, J. (2019). Quantitative analysis of the relationship between vegetation and groundwater buried depth: A case study of a coal mine district in Western China. *Ecological Indicators*, *102*, 770-782. doi:https://doi.org/10.1016/j.ecolind.2019.03.027

Yin, L., Zhou, Y., Ge, S., Wen, D., Zhang, E., & Dong, J. (2013). Comparison and modification of methods for estimating evapotranspiration using diurnal groundwater level fluctuations in arid and semiarid regions. *Journal of Hydrology*, *496*, 9-16. doi:https://doi.org/10.1016/j.jhydrol.2013.05.016

Yin, L., Zhou, Y., Huang, J., Wenninger, J., Zhang, E., Hou, G., & Dong, J. (2015). Interaction between groundwater and trees in an arid site: Potential impacts of climate variation and groundwater abstraction on trees. *Journal of Hydrology*, *528*, 435-448. doi:10.1016/j.jhydrol.2015.06.063

Zeppel, M., & Eamus, D. (2008). Coordination of leaf area, sapwood area and canopy conductance leads to species convergence of tree water use in a remnant evergreen woodland. *Australian Journal of Botany*, *56* (2), 97-108. doi:10.1071/bt07091

Zheng, X.-J., Xu, G.-Q., Li, Y., & Wu, X. (2019). Deepening Rooting Depths Improve Plant Water and Carbon Status of a Xeric Tree during Summer Drought. *Forests*, *10* (7). doi:10.3390/f10070592

Zhou, H.-F., Zheng, X.-J., Zhou, B., Dai, Q., & Li, Y. (2012). Sublimation over seasonal snowpack at the southeastern edge of a desert in central Eurasia. *Hydrological Processes*, *26* (25), 3911-3920. doi:10.1002/hyp.8402

Zhou, H., Zhao, W., & Zhang, G. (2017). Varying water utilization of *Haloxylon ammodendron* plantations in a desert-oasis ecotone. *Hydrological Processes*, *31* (4), 825-835. doi:10.1002/hyp.11060

Zhu, S.-D., Chen, Y.-J., Ye, Q., He, P.-C., Liu, H., Li, R.-H., . . . Cao, K.-F. (2018). Leaf turgor loss point is correlated with drought tolerance and leaf carbon economics traits. *Tree Physiology*, *38* (5), 658-663. doi:10.1093/treephys/tpy013

Zolfaghar, S., Villalobos-Vega, R., Cleverly, J., & Eamus, D. (2015). Co-ordination among leaf water relations and xylem vulnerability to embolism of *Eucalyptus* trees growing along a depth-to-groundwater gradient. *Tree Physiology*, *35* (7), 732-743. doi:10.1093/treephys/tpv039

Zolfaghar, S., Villalobos-Vega, R., Cleverly, J., Zeppel, M., Rumman, R., & Eamus, D. (2014). The influence of depth-to-groundwater on structure and productivity of *Eucalyptus* woodlands. *Australian Journal of Botany*, *62* (5), 428-437. doi:10.1071/bt14139

Zolfaghar, S., Villalobos-Vega, R., Zeppel, M., & Eamus, D. (2015). The hydraulic architecture of *Eucalyptus* trees growing across a gradient of depth-to-groundwater. *Functional Plant Biology*, *42* (9), 888-898. doi:10.1071/fp14324

## TABLES

Table 1 The contents of osmotic substances in assimilating *Haloxylon ammodendron* branches

Growing Season	Sites	Sample location	DGW (m)	Na <sup>+</sup> (%)	Proline (%)	Sugars (%)	Starch (%)
EGS	S1	44°24'54" N, 87deg55'01" E	3.45	1.47±0.05a*	0.06±0.003b*	3.81±0.11b*	2.43±0.03a*
	S2	44°24'32" N, 87deg55'22" E	9.08	1.52±0.22a*	0.06±0.006b*	4.83±0.12b	2.64±0.19a*
	S3	44°23'45" N, 87deg55'34" E	10.47	1.71±0.07a*	0.10±0.013a*	4.23±0.40b	2.38±0.13a

Growing Season	Sites	Sample location	DGW (m)	Na <sup>+</sup> (%)	Proline (%)	Sugars (%)	Starch (%)
LGS	S4	44°25'37" N, 87deg54'30" E	13.27	1.23±0.29a*	0.06±0.47b*	4.22±0.23b	2.83±0.15a*
	S5	44°30'35" N, 87deg53'50" E	15.91	1.35±0.10a*	0.05±0.003b	8.37±0.61a	1.80±0.22b
	S1	44°24'54" N, 87deg55'01" E	3.45	2.38±0.23a*	0.03±0.007a*	5.89±0.60b*	1.52±0.11b*
	S2	44°24'32" N, 87deg55'22" E	9.68	2.60±0.06a*	0.03±0.002a*	4.98±0.26b	1.81±0.14ab*
	S3	44°23'45" N, 87deg54'34" E	16.57	2.23±0.02a*	0.03±0.003a*	5.15±0.55b	2.01±0.07a
	S4	44°25'37" N, 87deg54'30" E	15.27	2.59±0.12a*	0.02±0.001b*	4.83±0.37b	1.76±0.14ab*
	S5	44°30'35" N, 87deg55'15" E	15.91	2.74±0.37a*	0.04±0.004a	9.69±0.47a	1.69±0.08ab

Note: Data are the means ± SEs shown with significant differences among sites as assessed using Tukey's HSD test ( $P < 0.05$ ). Values followed by the same letter were not significantly different among sites. Values with an asterisk (\*) were significantly different among the two growth stages at the same site. Results are presented for both early growing season (denoted as EGS,  $n = 5$ ) and late growing season (denoted as LGS,  $n = 5$ ).

## FIGURE LEGENDS

**Figure 1** . Location of the five study sites.

**Figure 2** .Box plots of twig water potential in *Haloxylon ammodendron* across five sites differing in depth-to-groundwater (DGW) at early (EGS) and late (LGS) growing season. The global assessment of a statistical difference among the sites with different DGW was conducted using ANOVA. Different letters represent significant differences among different DGWs as assessed using Tukey's HSD test ( $P < 0.05$ ).

**Figure 3** .Twig water potential in *Haloxylon ammodendron*between the early (EGS) and late growing season (LGS) with the same depth-to-groundwater (DGW). To compare the difference between twig water potential in the EGS and LGS at every site with contrasting DGW, independent-samples  $t$ -tests were used.

**Figure 4** . Relationships between twig water potential and depth-to-groundwater (DGW) of *Haloxylon ammodendron* in early growing season (EGS; a, c and e) and late growing season (LGS; b, d and f). Blue lines indicate linear trends of DGW changes of turgor pressure, and shallow blue bands represent 95% confidence intervals.

**Figure 5** . Twig water relation traits in *Haloxylon ammodendron* across five sites differing in depth-to-groundwater (DGW) at early (EGS; a and c) and late (LGS; b and d) growing season. The global assessment of a statistical difference among the sites with different DGW was conducted by ANOVA. Different letters represent significant differences among the different DGWs as assessed using Tukey's HSD test ( $P < 0.05$ ).

**Figure 6** . Relationships between twig P-V parameters and depth-to-groundwater (DGW) of *Haloxylon ammodendron* in the early growing season (EGS; a and c) and late growing season (LGS; b and d). Values are means ± SEs ( $n = 5$ ).

**Figure 7** . Seasonal changes in leaf area per branch (a) and growth rate of branch area (b) of *Haloxylon ammodendron* at five sites with different depth-to-groundwater (DGW). Error bars represent standard errors of means. Asterisks (\*) indicate significant differences among the five sites with different DGWs on the same trial day or during the same growth period at  $P = 0.05$ .

**Figure 8** . Relationship between growth rates, Huber values, and depth-to-groundwater (DGW) of *Haloxylon ammodendron* during the early growing season (EGS; a and c) and the late growing season (LGS; b and d). Blue lines indicate linear trends of DGW changes of growth rate and HV, and shallow blue bands represent 95% confidence intervals.

**Figure 9** . Relationship between soluble sugars and full turgor ( $\pi_{100}$ ; a and b), between  $\pi_{100}$  and growth rate (c and d), and between growth rate and Huber value (e and f) of *Haloxylon ammodendron* during the early growing season (EGS; a, c and e) and the late growing season (LGS; b, d and f). Values are means  $\pm$  SEs (n = 5–10). Blue lines indicate linear trends between soluble sugar and full turgor, between full turgor and growth rate, and between growth rate and Huber Value. Dashed blue lines represent 95% confidence intervals of linear trendlines.















