

Species-Specific soil moisture thresholds and photosynthetic responses in dominant sand-fixing shrubs

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Abstract

Soil moisture is critical to photosynthetic function and seedling establishment in arid ecosystems. We selected three dominant sand-fixing shrubs—*Hedysarum scoparium*, *Caragana korshinskii*, and *Artemisia desertorum*—and measured their photosynthetic and physiological responses across a continuous soil moisture gradient. Gas exchange parameters, light-response traits, and key biochemical indicators were determined for each species. Net photosynthetic rate (P_n), water use efficiency (WUE), and maximum net photosynthetic rate (P_{nmax}) all responded unimodally to soil moisture, with interspecific differences in threshold positions. Stomatal limitation-related soil moisture thresholds were 12.35% for *H. scoparium*, 9.79% for *C. korshinskii*, and 8.77% for *A. desertorum*. P_n saturation occurred at 9.39%, 16.85%, and 13.47% respectively. The highest WUE values were found at 13.21%, 20.96%, and 16.93%. Antioxidant enzymes (SOD, POD, CAT) and osmotic adjustment substances (Pro, SS) showed the same unimodal responses. MDA had an opposite trend. This trend reflects more oxidative damage under both drought and excess moisture conditions. Model integration also identified the soil moisture ranges that balance high photosynthetic productivity and efficient water use. These ranges are 9.39%-13.21% for *H. scoparium*, 16.86%-20.96% for *C. korshinskii*, and 13.47%-16.93% for *A. desertorum*. All three shrubs tolerated drought better than excess soil moisture. Our findings clarify species-specific soil moisture thresholds for major xerophytic shrubs, and offer a physiological foundation for precision irrigation during seedling cultivation and vegetation restoration in arid and semi-arid regions.

1. Introduction

Photosynthesis is the basic link connecting plant carbon gain, biomass formation and ecosystem function. It converts light energy into chemical energy, which directly controls dry matter accumulation and organic synthesis in terrestrial plants[1, 2]. Its rate is set by internal factors like developmental stage and genetic background. External conditions, such as light, temperature and water supply, also affect it[3–5]. Water availability often becomes the main environmental constraint in arid and semi-arid regions. Precipitation is scarce and variable here, so soil moisture becomes a key factor regulating plant growth, survival and vegetation succession[6, 7]. Seedling establishment makes this constraint more obvious[8]. Young plants are very sensitive to soil drying and overheating at this stage. They have shallow roots, limited hydraulic capacity, and weak ability to buffer environmental changes[9]. Impairment of photosynthetic function leads to rapid declines in carbon assimilation, slowed growth, and reduced chances of successful establishment.

Water stress influences photosynthesis through several pathways. When soil water declines, stomata usually close. CO₂ diffusion is then restricted, and carbon assimilation decreases[10, 11]. As stress becomes stronger or lasts longer, non-stomatal limitation also becomes important[12]. Mesophyll metabolism can be inhibited in this stage. Photochemical efficiency may drop, pigments may be lost, oxidative damage may increase, and the photosynthetic apparatus may be impaired[13, 14]. In most cases, these two forms of limitation do not act separately. Their relative importance changes with stress intensity, stress duration, plant functional type, and ontogenetic stage[15, 16]. This pattern may explain

why earlier studies did not identify a single cause of photosynthetic decline under drought. It also indicates that gas exchange data alone are often insufficient. A better approach is to combine photosynthetic traits with biochemical indicators such as chlorophyll content, antioxidant enzyme activity, and osmotic adjustment, so that the physiological basis of stress responses can be identified more clearly[17].

In dryland restoration, the key issue is not simply whether more water can increase photosynthesis. Plant performance often responds to soil moisture in a nonlinear way[7, 18]. Photosynthesis can stay at a high level within a suitable moisture range, but it declines when soil water drops below or rises above critical limits[19]. This threshold-like response is especially important in desert shrub seedlings, because they need to balance carbon gain, water use, and stress tolerance during early establishment. From the perspective of restoration, the most useful target is not only the maximum photosynthetic rate. It is the moisture range that can support both high photosynthetic productivity and efficient water use[20]. This range is closely related to seedling quality, irrigation efficiency, and restoration success under limited water supply[21, 22]. Yet quantitative descriptions of such thresholds remain scarce for dominant sand-fixing shrubs, especially under continuous soil moisture gradients.

Hedysarum scoparium(*H.scoparium*), *Caragana korshinskii*█*C.korshinskii*█, and *Artemisia desertorum*█*A.desertorum*█are dominant sand-fixing shrubs widely used in the arid and semi-arid regions of northern China. These species play important roles in dune stabilization, wind erosion control, and vegetation recovery. They are also well known for their drought tolerance and ecological adaptability [9, 23, 24]. Their seedling performance strongly influences restoration outcomes because failure at the establishment stage can limit subsequent community development. Previous studies have examined aspects of their ecophysiological response to drought, including gas exchange, hormonal regulation, and water use efficiency[17, 20, 25]. Some limitations still remain. Most previous studies used discrete water treatments or focused on short-term diurnal responses[26]. As a result, the quantitative links between photosynthetic traits and continuous soil moisture gradients are still not well defined. Studies have rarely identified the critical moisture thresholds at which photosynthetic decline begins during the seedling stage. Cross-species comparisons are also limited, so it remains difficult to determine whether stomatal or non-stomatal limitation is dominant near these critical points[12, 27].

To fill these gaps, we carried out a controlled pot experiment with seedlings of *H.scoparium*, *C.korshinskii*, and *A.desertorum* under seven soil moisture gradients. e measured photosynthetic light-response parameters, chlorophyll content, and antioxidant physiological traits across the whole moisture range. This study had three aims: (1) quantify the responses of key photosynthetic traits to continuous variation in soil moisture; (2) determine species-specific soil moisture thresholds linked to reductions in photosynthetic function and water use efficiency; and (3) distinguish the dominant physiological constraints on photosynthesis under contrasting water conditions through integrated analyses of gas exchange and biochemical traits. By establishing quantitative relationships between soil moisture and photosynthetic performance, this study provides a mechanistic understanding of drought adaptation in

dominant xerophytic shrubs and a physiological basis for precision water management in seedling cultivation and ecological restoration of arid and semi-arid desert regions.

2. Materials and methods

2.1 Study Area

The study was conducted in the Shapotou National Nature Reserve (37°26'06"-37°37'25" N, 104°55'42"-105°11'54" E), located on the southeastern margin of the Tengger Desert in Ningxia, China. The region lies within a steppe-desert ecotone under a typical temperate continental climate, characterized by low precipitation and high evaporation. The mean annual precipitation is approximately 186.6 mm, concentrated between June and August, whereas the mean annual potential evaporation reaches 3000 mm. The mean annual temperature is 9.6°C, with an average of 59 sandstorm days per year. During the growing season, the volumetric soil water content ranges between 4.13% and 16.15% (Figure. 1). The soil is sandy and nutrient-poor, with total nitrogen 0.2513 g·kg⁻¹, total phosphorus 0.4372 g·kg⁻¹, total potassium 15.9917 g·kg⁻¹, alkaline-hydrolyzable nitrogen 9.6238 mg·kg⁻¹, available phosphorus 6.8208 mg·kg⁻¹, available potassium 21.8907 mg·kg⁻¹, and organic matter 0.9097 g·kg⁻¹. Vegetation is dominated by woody species such as *Populus euphratica* and *Ammopiptanthus mongolicus*, together with shrubs and herbaceous plants including *Agriophyllum squarrosum*, *Stilpnolepis centiflora*, *Leymus secalinus*, and *Salsola collina*.

2.2 Plant Materials and Experimental Design

Topsoil was collected from the 0–20 cm layer at the southeastern margin of the Tengger Desert, Ningxia, China, air-dried, and sieved through a 2 mm mesh for use as the cultivation substrate. Seeds of *Hedysarum scoparium*, *Caragana korshinskii*, and *Artemisia desertorum* were collected from the same region and brought back for indoor cultivation. After germination and early growth under controlled conditions, uniform seedlings of the three species were selected for the experiment. Leaf materials of the three species were also collected from Ningxia, China, and the voucher specimens were deposited at the Herbarium of Ningxia University under the voucher codes SNXU-ZHX-2022133, SNXU-ZHX-2022134, and SNXU-ZHX-2022135, respectively. Selected seedlings were transplanted into black plastic pots with a radius of 14 cm and a height of 17 cm. Each pot contained 2 kg of substrate on a dry mass basis, and 20 seedlings were maintained in each pot. Seven soil moisture treatments were established at gravimetric water contents of 4%, 6%, 8%, 10%, 15%, 20%, and 25%, and soil moisture was maintained gravimetrically within ± 0.5%. Each species × treatment combination included five biological replicates. The seedlings were cultivated in a controlled-environment chamber at 15–25°C, with a photoperiod of 14 h light and 10 h dark, a light intensity of 11,500 lux, and relative humidity of 60%. Soil moisture was controlled by weighing the pots and replenishing water every two days. After 30 d of treatment, photosynthetic traits and physiological indices were measured.

2.3 Photosynthetic Measurements and Biochemical Analyses

Photosynthetic light-response curves were measured on five uniform seedlings per treatment using a portable photosynthesis system (CFS-3000, Walz, Germany). For each seedling, a fully expanded, healthy leaf from the middle or upper canopy was selected and enclosed in the cuvette. Measurements were performed at 25°C leaf temperature, 380 $\mu\text{mol}\cdot\text{mol}^{-1}$ CO_2 concentration, and constant air flow.

Photosynthetically active radiation (PAR) was set sequentially to 13 levels: 2000, 1800, 1500, 1200, 1000, 800, 500, 300, 200, 100, 50, 25, and 0 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, using an integrated red-blue light source. Leaves were light-adapted at 1200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for ~ 15 min before measurement. The following parameters were recorded: net photosynthetic rate (P_n , $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), stomatal conductance (G_s , $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), intercellular CO_2 concentration (C_i , $\mu\text{mol}\cdot\text{mol}^{-1}$), and transpiration rate (T_r , $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). The P_n -PAR curve was fitted with a rectangular hyperbolic model[28], from which the apparent quantum efficiency (AQY), maximum net photosynthetic rate ($P_{n\text{max}}$, $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), light saturation point (LSP), light compensation point (LCP), and dark respiration rate (R_d) were obtained. Leaf water use efficiency (WUE, $\mu\text{mol}\cdot\text{mmol}^{-1}$) and stomatal limitation (L_s) were calculated as:

$$WUE = \frac{P_n}{T_r}$$

1

$$L_s = 1 - \frac{C_i}{C_a}$$

2

where C_a is the atmospheric CO_2 concentration. For interspecies comparisons, parameters were extracted at PAR of 1200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Following gas exchange measurements, leaves were sampled for physiological analyses. Fresh samples were used for chlorophyll content and electrolyte leakage, while others were flash-frozen in liquid nitrogen and stored at -80°C for antioxidant enzyme and osmolyte assays. All analyses were performed with five biological replicates per treatment. Superoxide dismutase (SOD) activity was determined by the nitroblue tetrazolium (NBT) method; peroxidase (POD) activity by guaiacol colorimetry; catalase (CAT) activity by ultraviolet absorption; malondialdehyde (MDA) content by the thiobarbituric acid (TBA) method; proline (Pro) by the sulfosalicylic acid method; soluble sugar (SS) by the anthrone method; chlorophyll (Chl) by ethanol-acetone extraction; and relative water content (RWC) by the soaking method[29].

2.4 Classification of Soil Moisture Thresholds

Soil moisture thresholds were classified using the threshold-solution method [30, 31], based on the quantitative responses of photosynthetic parameters to soil water content. The lower and upper soil moisture limits for photosynthesis were determined from the fitted response models. The mean soil moisture values corresponding to the integrated averages of P_n and WUE were used to define the thresholds of moderate photosynthetic efficiency and moderate WUE, respectively. These critical values were then combined to establish a comprehensive classification framework using the minimum, maximum, and mean thresholds of both P_n and WUE.

2.5 Statistical Analysis

We used Microsoft Excel 2016 to process all data. SPSS 21.0 was applied to analyze the data. One-way analysis of variance (ANOVA) was used to test differences among water treatments. Duncan's multiple range test was then used for further testing ($\alpha = 0.05$). Key photosynthetic parameters (P_{nmax} , T_r , and WUE) were taken from the fitted response models. Origin 2023 and R version 4.2.1 were used to create and refine figures.

3. Results

3.1 Unimodal Response of Leaf Photosynthesis to Soil Moisture in Three Sand-Fixing Plants

A modified rectangular hyperbola model fitted the light-response curves of net photosynthetic rate (P_n) well in the three sand-fixing species. The coefficients of determination were high ($R^2 > 0.98$). P_n showed a unimodal response to soil moisture, as seen in Figure. 2. The response thresholds had obvious interspecific differences ($P < 0.05$). The mean maximum P_n of *H.coparium* reached $11.24 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ when soil moisture was 10%. Photoinhibition occurred at photosynthetically active radiation (PAR) levels above $1500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ under most water treatments. The only exception was severe drought (4% soil moisture). Notable species-specific patterns were observed. *C.korshinskii* maintained relatively high P_n between 8% and 15% soil moisture but exhibited a sharp decline under severe drought (4–6%), where photoinhibition occurred at $\text{PAR} > 1800 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. In contrast, *A.desertorum* achieved the highest P_n ($22.58 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) at 15% soil moisture and exhibited no photoinhibition across all moisture levels. Moreover, unlike *C. korshinskii*, both *H. scoparium* and *A. desertorum* showed better light-response performance under severe drought (4%) than under waterlogging (25%), indicating greater tolerance to water deficit than to excess moisture.

As summarized in Figure. 3, the apparent quantum efficiency (AQY) of *H.coparium*, *C.korshinskii*, and *A.desertorum* reached their highest values at soil moisture levels of 10%, 15%, and 8%, with values of 0.1805, 0.0987, and 0.2005, respectively ($P < 0.05$). The maximum net photosynthetic rate (P_{nmax}) of all three species also changed in a unimodal way as soil moisture increased, reaching peaks of 11.08, 16.91, and $22.52 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ under 10%, 15%, and 15% soil moisture, respectively. In *H.coparium*, the

LSP and (R_d) both increased first and then decreased as soil moisture rose, while the LCP showed the opposite pattern. The highest R_d value

$1.07 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, appeared at 8% soil moisture, and lower values were found under both waterlogging (25%) and severe drought (4%). In *C.korshinskii*, LSP and R_d also showed single-peak trends, with the maximum LSP ($2284.37 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) at 8% and R_d ($0.99 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) at 10% soil moisture. In contrast, LCP in *C. korshinskii* decreased initially and then increased with further increases in soil moisture. For *A. desertorum*, LSP exhibited a more complex nonlinear trend, whereas both LCP and R_d displayed clear unimodal responses across the soil moisture gradient.

3.2 Gas Exchange, Water Use Efficiency, and Stomatal Behavior Under Varying Soil Moisture

3.2.1 Responses of photosynthesis and transpiration to soil moisture

The responses of net photosynthetic rate (P_n) and transpiration rate (T_r) to soil moisture were well described by quadratic regression models ($P < 0.05$), exhibiting clear unimodal patterns across all three sand-fixing species (Figure. 4). Distinct species-specific thresholds were identified. For *H. scoparium*, the maximum P_n ($9.91 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and T_r ($4.04 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) occurred at 9.39% and 9.58% soil moisture, respectively. The mean P_n ($8.66 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) defined an intermediate-to-high photosynthetic range between 1.47% and 17.32% soil moisture. In *C. korshinskii*, P_n peaked at $17.62 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at 16.86% soil moisture, with an effective range of 10.62% to 23.09%. For *A. desertorum*, maximum P_n ($22.90 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) occurred at 13.47% soil moisture, with an active photosynthetic range from 6.97% and 19.97%. Transpiration rates in *C. korshinskii* and *A. desertorum* also exhibited unimodal responses, reaching peaks of $4.32 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (14.00%) and $8.09 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (14.80%), respectively. Beyond these optima, T_r declined under both excessive drought and waterlogging, indicating that water stress at either extreme suppresses gas exchange efficiency.

3.2.2 Response of Leaf Water Use Efficiency to Soil Moisture in Three Sand-Fixing Plants

The leaf water use efficiency (WUE) of all three sand-fixing species exhibited a clear unimodal response to increasing soil moisture, which was well fitted by quadratic regression models ($P < 0.05$, Figure. 5A). The soil moisture corresponding to the maximum WUE in *H.scoparium* was 13.21%. For *C.korshinskii* and *A.desertorum*, the optimal soil moisture ranges sustaining moderate-to-high WUE were 9.89%-32.04% and 10.40%-23.46%, respectively. *H.scoparium* maintained relatively high WUE within the 10.0%-15.0% range, beyond which efficiency declined as soil moisture increased. The integrated fitted model indicated that *H. scoparium* sustained a moderate-to-high WUE within 7.02%-19.41% soil moisture, highlighting its strong adaptation to intermediate water availability.

3.2.3 Stomatal and non-stomatal responses to soil moisture

Cubic models provided the best fit for the relationships of soil moisture with stomatal conductance (G_s) and intercellular CO_2 concentration (C_i) ($P < 0.05$; Figs. 5B, 6). In all three species, G_s increased with rising soil moisture until it reached a peak, and then it declined, while C_i showed the opposite trend. In *H. scoparium*, when soil moisture decreased from 15.00% to 4.00%, G_s and stomatal limitation (L_s) both declined step by step, and C_i increased clearly at the same time. *C. korshinskii* showed the same pattern within the 4.00%-10.00% range. These results indicate that the increase in net photosynthetic rate under suitable soil moisture was controlled mainly by stomatal regulation. The lowest C_i values, together with the highest stomatal limitation, were found at soil moisture levels of 12.35%, 9.79%, and 8.77% in *H. scoparium*, *C. korshinskii*, and *A. desertorum*, respectively.

3.3 Integrated Physiological Responses of Three Sand-Fixing Plants to Soil Moisture Stress

The antioxidant enzyme activities and osmotic regulation traits of the three sand-fixing species all showed nonlinear responses to soil moisture changes ($P < 0.05$; Fig. 7). The activities of superoxide dismutase (SOD) and peroxidase (POD) followed clear unimodal patterns, rising as soil moisture increased to suitable levels and then falling. Catalase (CAT) activity remained higher in *C. korshinskii* than in *H. scoparium* and *A. desertorum* under all treatments, but CAT activity decreased strongly in all three species under severe drought (4%) and waterlogging (25%). Leaf malondialdehyde (MDA), which reflects lipid peroxidation, first declined and then increased as soil moisture rose. Multiple comparisons showed that *H. scoparium* had lower MDA levels, while *C. korshinskii* had higher levels than the other species ($P < 0.05$), which suggests differences in membrane stability under moisture stress. Proline and soluble sugar (SS) also had unimodal trends. These trends show osmotic adjustment under changing water conditions. Proline reached peak values of 236.62, 392.90, and 83.03 $\mu\text{g}\cdot\text{g}^{-1}$ in *H. scoparium*, *C. korshinskii*, and *A. desertorum* at soil moisture levels of 8%, 6%, and 8%, respectively. Soluble sugar reached maximum values of 34.35, 36.05, and 41.98 $\mu\text{g}\cdot\text{ml}^{-1}$. These maximums appeared at 10%, 15%, and 8% soil moisture respectively. SS content did not differ among species within the moderate moisture range (6%-20%), But variation appeared under severe drought and waterlogging ($P < 0.05$). Chlorophyll content also showed no interspecific differences under low soil moisture. It declined sharply once soil moisture went above 15%. The decline followed the order *C. korshinskii* > *H. scoparium* > *A. desertorum*, which indicates that the three species differed in their sensitivity to excess water. Leaf relative water content (RWC) first decreased and then increased with more soil moisture in *H. scoparium* and *C. korshinskii* ($P < 0.05$). Their RWC ranges were 61.83%-69.39% and 53.56%-67.60% respectively, and no difference existed between the two species. *A. desertorum* had the opposite pattern. Its RWC increased first and then dropped, reaching a maximum of 67.43% at 8% soil moisture.

3.4 Delineating Soil Moisture Availability for Three Sand-Fixing Plants

Based on the quantitative relationships between photosynthetic parameters and soil moisture, soil water availability was classified by determining the lower and upper photosynthetic thresholds using fitted nonlinear models. The mean values of the net photosynthetic rate (P_n) and water use efficiency (WUE), obtained from the integral forms of the fitted equations, were used to define the soil moisture intervals corresponding to moderate photosynthetic efficiency. The moisture ranges associated with the maximum values of P_n and WUE were identified as the optimal “high-yield and high-efficiency” zones. Specifically, these optimal soil moisture ranges were 9.39%-13.21% for *H.scoparium*, 16.86%-20.96% for *C.korshinskii*, and 13.47%-16.93% for *A.desertorum* (Table 1; Figure. 8). These quantitative thresholds delineate species-specific water regimes for achieving efficient carbon assimilation and water utilization, thereby providing a physiological basis for precision irrigation and adaptive species selection in arid-land restoration.

Table 1
Classification of soil water availability thresholds for three types of sand-fixing plants

Grading of soil water availability	Threshold grade of relative soil water content		
	<i>H.scoparium</i>	<i>C.korshinskii</i>	<i>A.desertorum</i>
No-yield and no-efficiency water	< 0.00%, > 31.77%	< 1.68%, > 32.03%	< 0.00%, > 33.93%
Low-yield and low-efficiency water	0.00%~1.47%, 19.41%~31.77%	1.68%~6.06%, 23.46%~33.93%	0.00%~6.97%,
Medium-yield and low-efficiency water	1.47%~7.02%, 17.32%~19.41%	6.06%~10.62%, 23.09%~32.03%	6.97%~10.40%, 19.97%~23.46%
Medium-yield and medium-efficiency water	7.02%~9.39%, 13.21%~17.32%	10.62%~16.86%, 20.96%~23.09%	10.4%~13.47%, 16.93%~19.97%
High-yield and high-efficiency water	9.39%~13.21%	16.86%~20.96%	13.47%~16.93%

4. Discussion

Seedling establishment is the most vulnerable stage of vegetation recovery in arid lands, and water availability is the dominant constraint at this stage[32]. In restoration practice, drought is often treated as the main threat, and increased water supply is therefore assumed to improve seedling performance. Our results challenge this assumption. Along the continuous soil moisture gradient, all three sand-fixing shrubs showed clear unimodal responses in photosynthetic performance. P_n , WUE, P_{nmax} , AQY, and

several related traits all rose to a maximum and then declined. The decline under excessive moisture was often as strong as, or stronger than, that under severe drought. In *H. scoparium* and *A. desertorum*, soil moisture at 4% led to less functional inhibition than at 25%. This pattern has clear ecological significance. For xerophytic seedlings, excess water may bring higher physiological cost than water shortage. The seven continuous moisture levels in this study helped identify this pattern. Many previous studies used only a few separate water treatments. They thus reported general trends instead of exact turning points[33]. Our experimental design showed the nonlinear response more directly. It also made it possible to quantify threshold positions, instead of estimating them indirectly. This is especially important for dryland species. Photosynthesis across the full water gradient is controlled by linked changes in stomatal behavior, biochemical activity, and photochemical stability[7, 13, 18].

We can better explain this response by combining the analysis of gas exchange features, light response indexes and physiological and biochemical indicators. Photosynthetic capacity mainly dropped due to stomatal adjustment under mild water deficit conditions[34]. Plant G_s dropped to slow down transpiration and reduce carbon assimilation, while the photosynthetic structure of leaves still worked normally in this period. Plants adopt such physiological changes to reduce water consumption and adapt to water-deficit environments. Many studies have confirmed that woody plants can cope with mild drought stress through stomatal movement. Leaf water condition and cell turgor control will trigger stomatal closure to slow down drought damage in the early stress period[35, 36]. The main restrictive factors for plant photosynthesis will change when soil water content goes beyond the critical range suitable for plant growth. Non-stomatal factors play a key role in limiting photosynthesis under extreme drought and waterlogging conditions[27, 37]. Plants show lower AQY and LSP, along with higher LCP in such environments. Chl content decreases and MDA builds up in leaf cells at the same time. The activities of antioxidant enzymes and the content of osmotic regulation substances rise under mild water stress. These physiological indexes will drop continuously as stress conditions become harsher. Plants will start self-protection mechanisms to relieve oxidative damage in the early stage of stress, and these regulatory mechanisms cannot maintain normal cell metabolism with continuous stress. The restriction of carbon dioxide diffusion is not the only factor affecting photosynthesis in this period. Abnormal chloroplast structure, damaged photosynthetic reaction system, pigment loss and weakened carbon fixation efficiency also occur in stressed leaves[38, 39].

Desert shrubs often experience clear physiological constraints when water is excessive. These species are widely recognized for strong drought resistance, but their sensitivity to over-watering has received much less attention[40]. In our study, the shrubs showed greater physiological tolerance to drought than to long-term water saturation. This pattern may be linked to long-term adaptation to arid environments. Plants in dry regions have evolved effective strategies to maintain physiological stability under water deficit, such as strict stomatal control, osmotic adjustment, and hydraulic safety mechanisms[9]. By comparison, tolerance to long-term waterlogging and hypoxia has rarely been a key target of natural selection. Excessive soil moisture typically reduces root oxygen availability, inhibits root respiration, disturbs underground metabolism, and increases leaf oxidative stress[19]. The rapid reduction in photosynthetic capacity observed at 25% soil water content supports this explanation. This finding also

has practical implications. Over-irrigation during seedling culture may cause greater damage than appropriate water limitation, even for species widely recognized as drought-tolerant[19, 41]. In short, providing more water for desert seedlings does not always improve performance. Instead, excessive water may cause physiological function to exceed the optimal hydrological range.

A second major finding is that the soil moisture level supporting maximum carbon assimilation did not coincide with the level supporting maximum water-use efficiency. Maximum P_n occurred at 9.39% soil moisture in *H. scoparium*, 16.86% in *C. korshinskii*, and 13.47% in *A. desertorum*, whereas peak WUE occurred later along the gradient, at 13.21%, 20.96%, and 16.93%, respectively. This separation is physiologically meaningful. For desert seedlings, persistence does not come from maximizing carbon gain alone[42]. This pattern relies on the balance between carbon gain and water loss. Under mild water stress, WUE can increase. Transpiration may decrease more than photosynthesis in part of the response range, which leads to this increase. Stress beyond this range restricts photosynthesis too much. WUE then decreases. The moisture condition with the highest P_n is not always the most efficient. This finding also changes how optimal water supply is understood in restoration practice. We should not target a single moisture point where photosynthesis peaks. The key is the range that maintains relatively high carbon gain and efficient water use together. Recent synthesis studies point out WUE should be seen as part of a broader water-use strategy, not a single isolated trait[22]. Our results provide direct support for that principle at the seedling stage.

Our threshold-based analysis identified moisture ranges that allowed both high photosynthetic productivity and efficient water use: 9.39%-13.21% for *H. scoparium*, 16.86%-20.96% for *C. korshinskii*, and 13.47%-16.93% for *A. desertorum*. These suitable moisture windows were not the same among species. *H. scoparium* showed its best performance under relatively dry conditions, while *C. korshinskii* was better suited to a wetter environment. *A. desertorum* fell between the two in moisture demand, but it maintained the highest photosynthetic capacity. These species-level differences suggest different water-use strategies in these desert shrubs[9, 22], and they also reflect niche separation during seedling establishment. In the same desert community, coexistence is therefore shaped not by similar water requirements, but by different physiological responses to the same water limitation. This view is consistent with earlier work showing that drought-tolerant *Caragana* species maintain a balance between hydraulic safety and hydraulic efficiency[9]. Our results further expand this perspective by indicating that photosynthetic and hydraulic thresholds may vary greatly even among restoration shrubs growing together.

This study helps define the key transition points at which plant physiological responses move from adaptive regulation to functional decline. Under drought conditions, we found soil moisture thresholds linked to stomatal limitation. They were 12.35% for *H. scoparium*, 9.79% for *C. korshinskii*, and 8.77% for *A. desertorum*. These values mark the point where drought responses change from reversible stomatal adjustment to irreversible physiological injury, as reported in earlier drought studies[27, 35]. The photosynthetic system is more likely to suffer biochemical damage and photoinhibition when soil moisture falls below these thresholds. Assessing seedling drought resistance under field conditions with

a threshold-based method is more practical and reliable than using single traits alone[22]. This framework can also guide more precise management in sandland restoration and nursery production. Irrigation in arid regions should not only focus on adding more water. It should keep soil moisture within species-specific suitable ranges. These ranges protect photosynthetic function and support stable carbon assimilation.

5. Conclusion

Water availability influences seedling survival and establishment in arid regions. Our results show that more water supply does not keep improving plant physiological performance. *H. scoparium*, *C. korshinskii*, and *A. desertorum*, all coped with severe drought better than long-term soil waterlogging. This pattern shows excess soil moisture limits seedling growth more than drought does in typical desert xerophytes. Photosynthetic traits changed with soil water in a clear nonlinear way. P_n , WUE, and related physiological traits all had typical unimodal patterns. This means stable physiological function was maintained only in a narrow range of suitable moisture conditions. Under mild drought or slight water excess, plants mainly used stomatal regulation to adapt to the changing environment. When soil moisture went beyond critical thresholds, non-stomatal inhibition of photosynthesis became more important. This shift was accompanied by stronger oxidative damage, lower chlorophyll content, and injury to the photosynthetic apparatus. The critical moisture thresholds for stomatal limitation were 12.35% in *H. scoparium*, 9.79% in *C. korshinskii*, and 8.77% in *A. desertorum*.

Notably, the soil moisture corresponding to peak photosynthetic carbon assimilation differed distinctly from that supporting optimal water-use efficiency. The maximum P_n appeared at 9.39%, 16.85% and 13.47% for the three species, while the highest WUE occurred at relatively higher moisture levels of 13.21%, 20.96% and 16.93%. By comprehensively coupling photosynthetic carbon fixation and water utilization characteristics, we determined the species-specific suitable moisture windows: 9.39%-13.21% for *H. scoparium*, 16.86%-20.96% for *C. korshinskii*, and 13.47%-16.93% for *A. desertorum*. These interspecific variations clearly reflect differentiated water-use strategies among sympatric desert shrubs, and accurately mark the critical transition boundary from reversible stomatal acclimation to irreversible photophysiological damage. Overall, this study clarifies the physiological adaptive mechanisms of typical desert seedlings under variable moisture conditions, and supplies reliable quantitative references for precise nursery irrigation, reasonable species matching and ecological vegetation restoration in arid and semi-arid ecosystems.

Declarations

Ethics approval and consent to participate

This study did not involve human participants, human data, or animal subjects. Seeds and soil samples used in this research were collected from the southeastern margin of the Tengger Desert, Ningxia, China, and did not involve any endangered or protected species. The plant materials were formally identified by

Prof. Xue Zhang and Prof. Lei Wang, School of Ecology Environment, Ningxia University. The collection and use of plant materials complied with relevant institutional, national, and international guidelines and legislation. No specific permits were required for the collection of the materials used in this study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Author Contribution

Z.Q.Y., L.W., and X.Z. conceived and designed the study. X.Y.L., N.L., J.X.S., Y.J.Z., B.Z., J.S.N., and R.Z. performed the field investigation and sampling. Z.Q.Y. and X.Y.L. performed the physiological characterization. L.W., X.Z., B.Z., and R.Z. provided constructive comments on the results and discussion. Z.Q.Y. and X.Z. wrote and edited the manuscript. All authors discussed the results and approved the final manuscript.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding authors on reasonable request.

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Figures

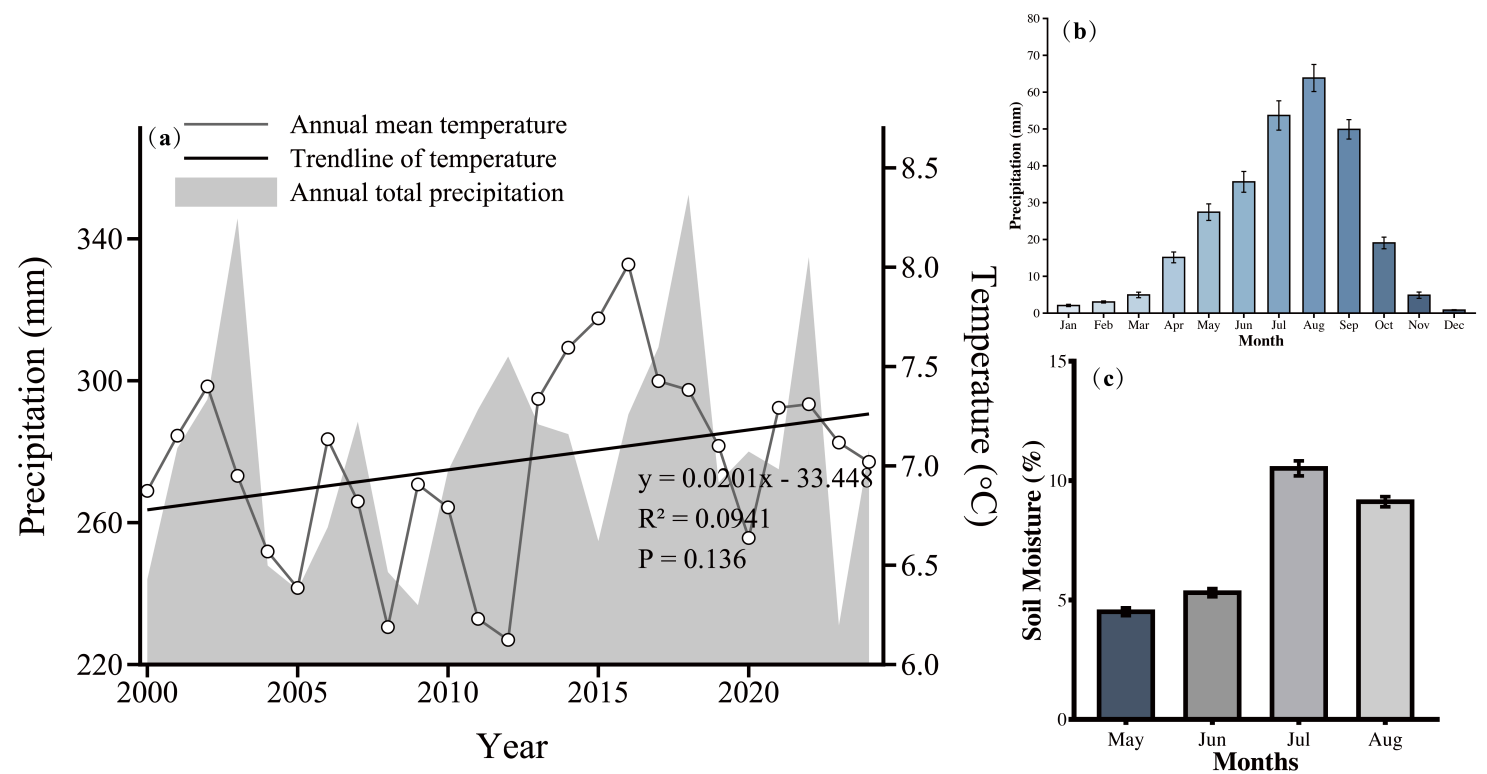


Figure 1

Climate and soil moisture conditions at the study site, including (a) annual total precipitation, annual mean temperature, and the temperature trend from 2000 to 2024; (b) mean monthly precipitation; and (c) mean soil moisture during the growing season (May–August) from 2020 to 2024.

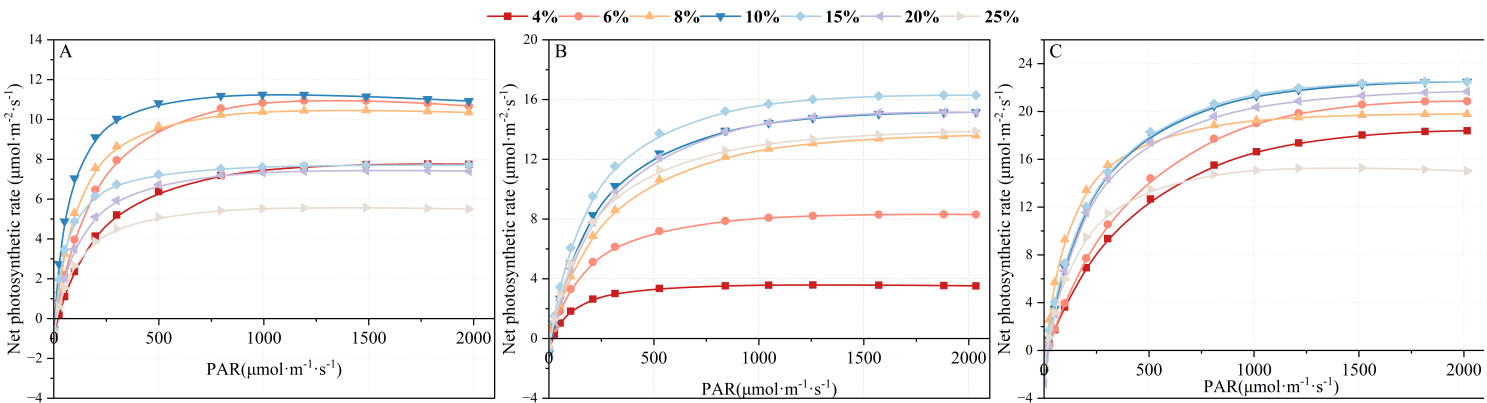


Figure 2

Light-response curves of net photosynthetic rate (P_n) in the leaves of three sand-fixing species under different soil moisture conditions. (A) *H. scoparium*; (B) *C. korshinskii*; (C) *A. desertorum*. Symbols represent observed data, and solid lines indicate model-fitted curves based on the modified rectangular hyperbola function.

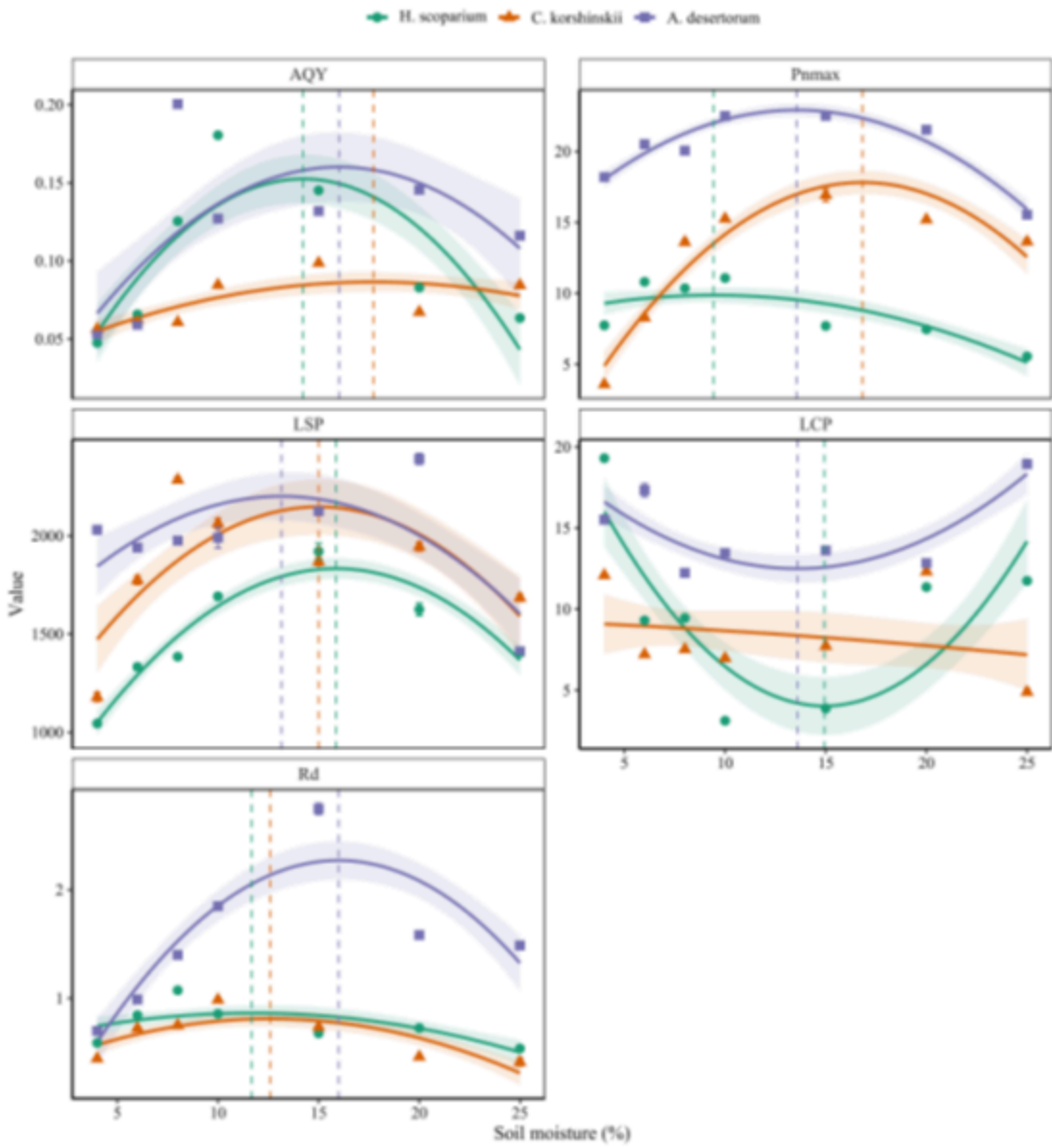


Figure 3

Changes in apparent quantum efficiency (AQY), maximum net photosynthetic rate (P_{nmax}), light saturation point (LSP), light compensation point (LCP), and dark respiration rate (R_d) of three sand-fixing shrubs along a soil moisture gradient.

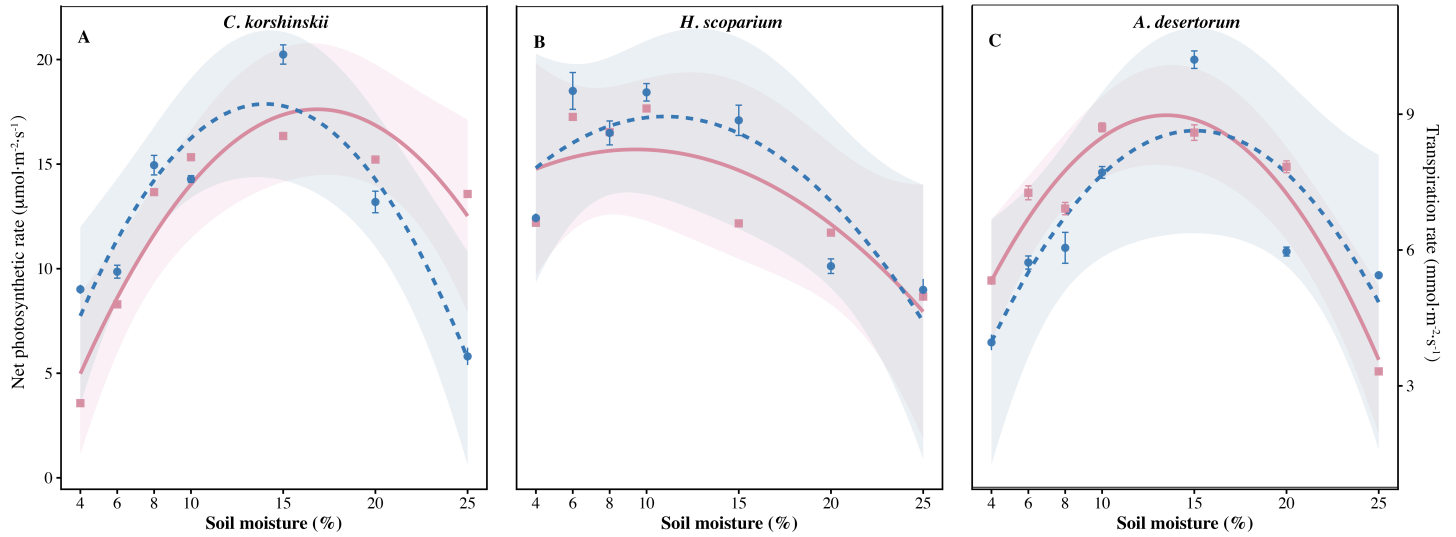


Figure 4

Response curves of net photosynthetic rate (P_n) and transpiration rate (T_r) to soil moisture in leaves of three sand-fixing shrubs. (A) *C. korshinskii*; (B) *H. scoparium*; (C) *A. desertorum*.

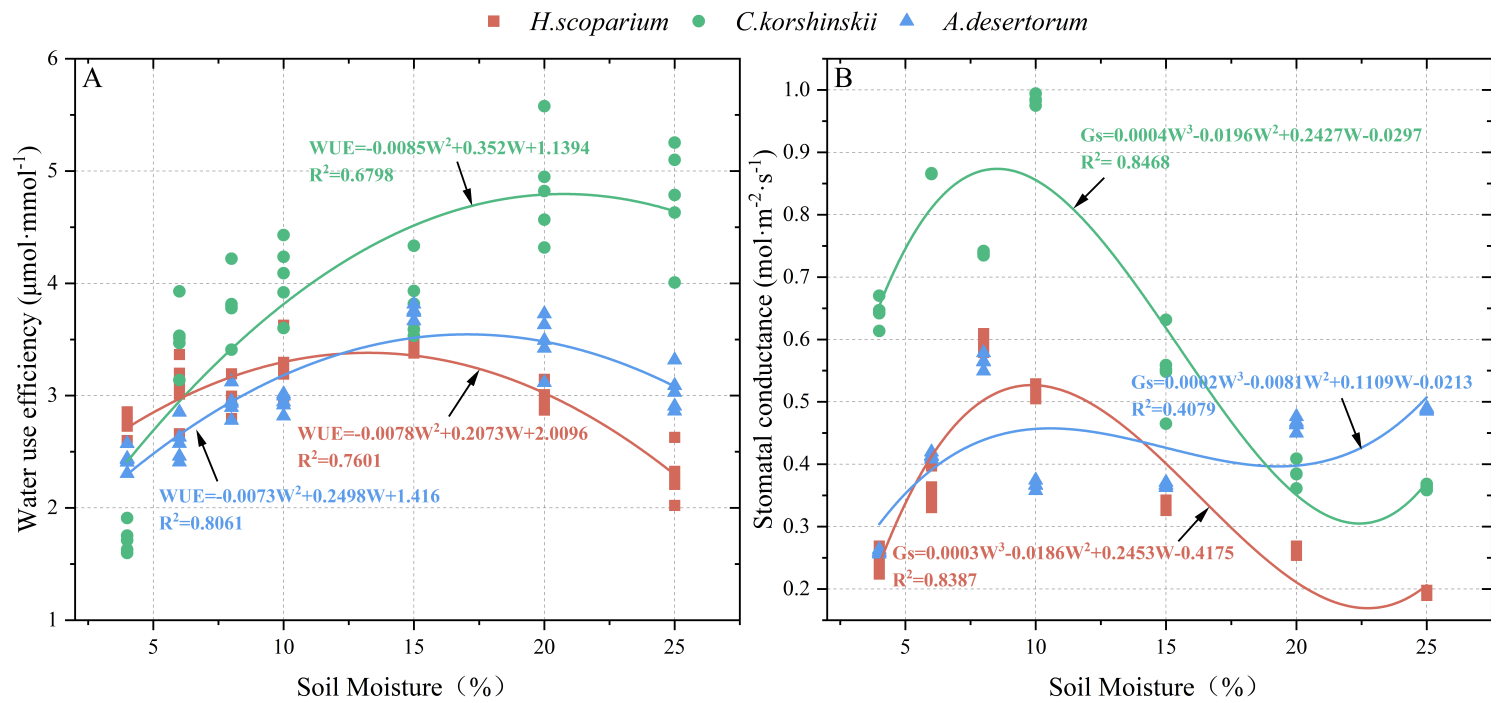


Figure 5

Soil moisture response curves of (A) leaf water use efficiency (WUE) and (B) stomatal conductance (G_s) in three sand-fixing plant species. Data points represent measured values, and solid lines denote fitted regression curves. Red: *H. scoparium*; green: *C. korshinskii*; blue: *A. desertorum*.

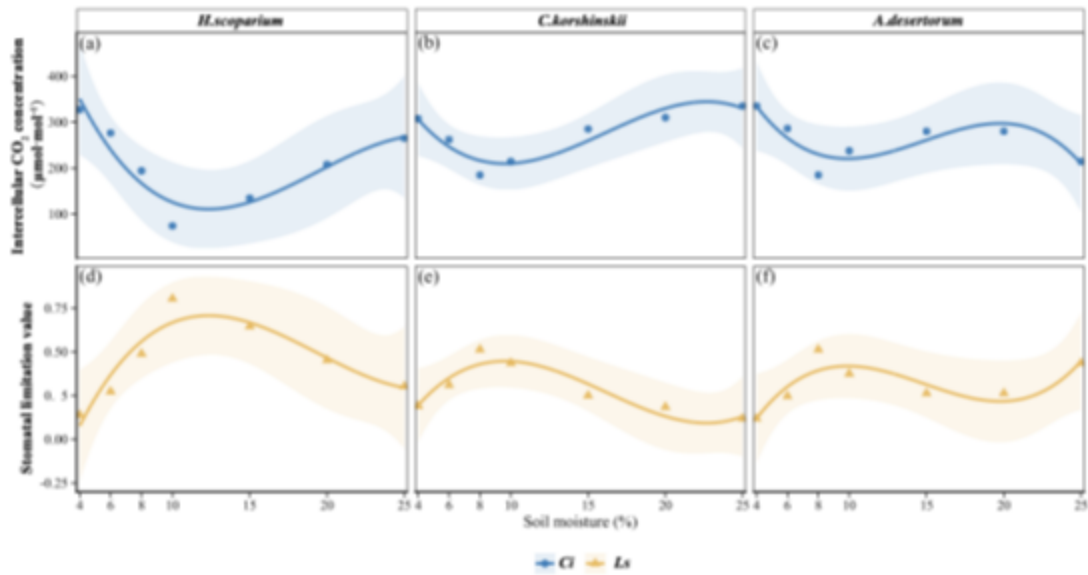


Figure 6

Soil moisture-induced response curves of intercellular CO₂ concentration (C_i) and stomatal limitation (L_s) in three sand-fixing plant species. (A) *H.scoparium*; (B) *C.korshinskii*; (C) *A.desertorum*. Solid lines indicate fitted cubic regression curves.

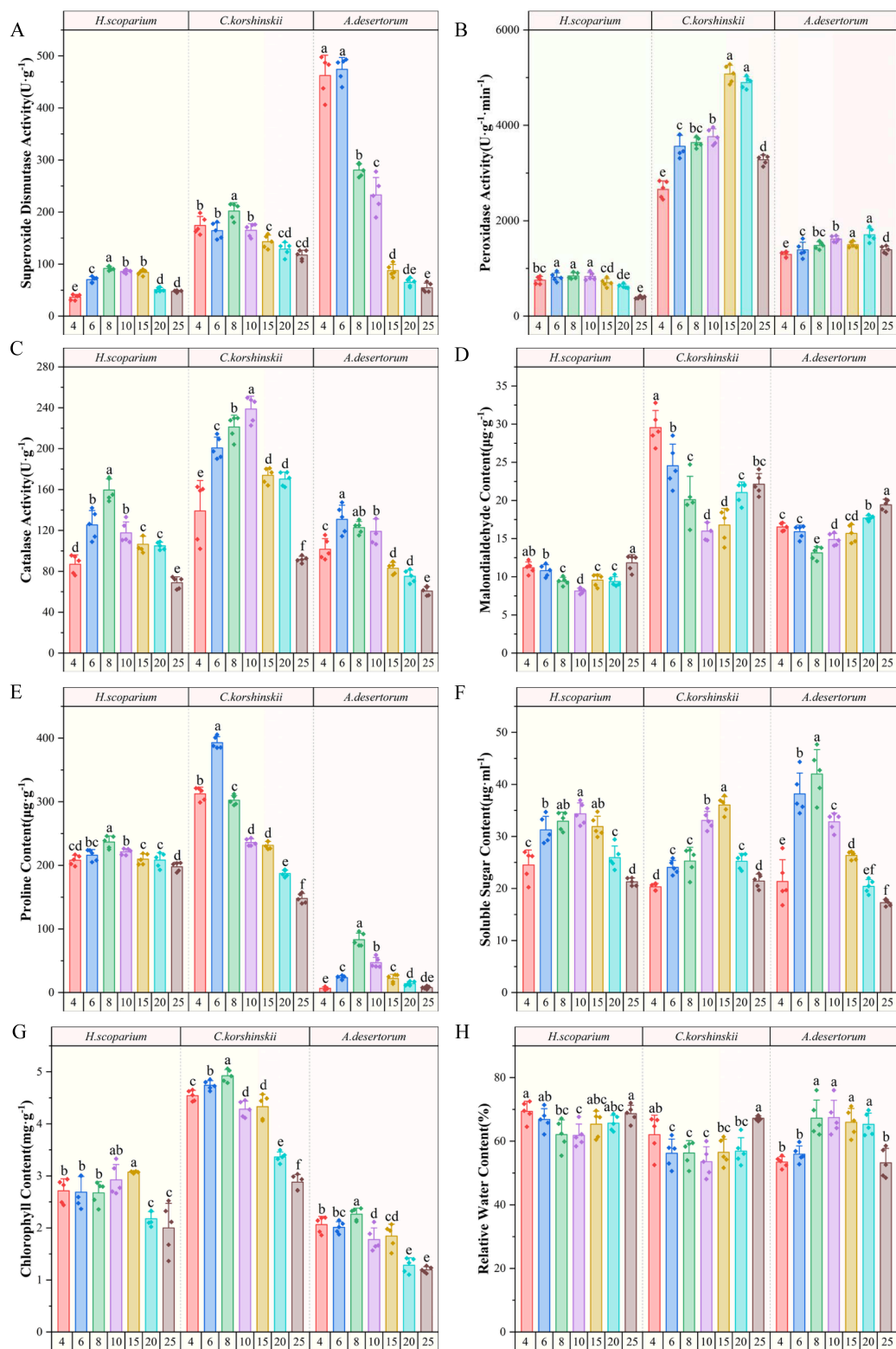


Figure 7

Effects of soil moisture variation on physiological and biochemical traits in three sand-fixing shrub species. (A) Superoxide dismutase (SOD) activity; (B) peroxidase (POD) activity; (C) catalase (CAT) activity; (D) malondialdehyde (MDA) content; (E) proline (Pro) content; (F) soluble sugar (SS) content; (G) chlorophyll (Chl) content; and (H) leaf relative water content (RWC). Different lowercase letters indicate

significant differences among soil moisture treatments within each species ($P < 0.05$, one-way ANOVA). Data are presented as mean $\pm$ SD ($n = 5$).

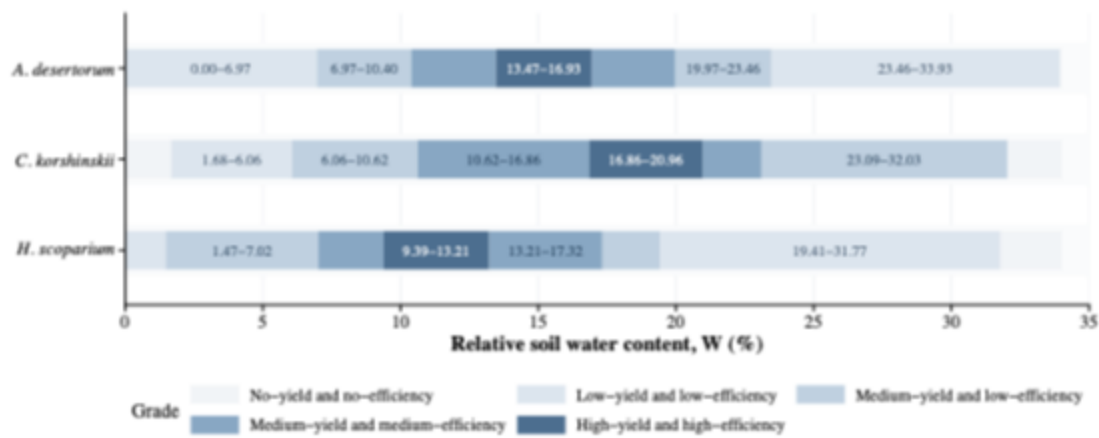


Figure 8

Soil water availability thresholds of three sand-fixing shrubs based on relative soil water content