Responses of Leaf Water Potential and Gas Exchange to Precipitation Manipulation in Two Shrubs on the Chinese Loess Plateau Postprint

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Abstract

Regulation of leaf gas exchange plays an important role in the survival of trees and shrubs under future climate change. However, the responses of leaf water potential and gas exchange of shrubs in semi-arid areas to the precipitation alteration are not clear. Here, we conducted a manipulated experiment with three levels of precipitation, i.e., a control with ambient precipitation, 50% above ambient precipitation (irrigation treatment), and 50% below ambient precipitation (drought treatment), with two common shrubs, Salix psammophila C. Wang & C. Y. Yang (isohydric plant, maintaining a constant leaf water potential by stomatal regulation) and Caragana korshinskii Kom. (anisohydric plant, having more variable leaf water potential), on the Chinese Loess Plateau in 2014 and 2015. We measured the seasonal variations of predawn and midday leaf water potential (pd and md), two parameters of gas exchange, i.e., light-saturated assimilation (An) and stomatal conductance (gs), and other foliar and canopy traits. The isohydric S. psammophila had a similar An and a higher gs than the anisohydric C. korshinskii under drought treatment in 2015, inconsistent with the view that photosynthetic capacity of anisohydric plants is higher than isohydric plants under severe drought. The two shrubs differently responded to precipitation manipulation. pd, An and gs were higher under irrigation treatment than control for S. psammophila, and these three variables and md were significantly higher under irrigation treatment and lower under drought treatment than control for C. korshinskii. Leaf water potential and gas exchange responded to manipulated precipitation more strongly for C. korshinskii than for S. psammophila. However, precipitation manipulation did not alter the sensitivity of leaf gas exchange to vapor-pressure deficit and soil moisture in these two shrubs. Acclimation to long-term changes in soil moisture in these two shrubs was primarily attributed to the changes in leaf or canopy structure rather than leaf gas exchange. These findings will be useful for modeling

canopy water-carbon exchange and elucidating the adaptive strategies of these two shrubs to future changes in precipitation.

Full Text

Preamble

Responses of Leaf Water Potential and Gas Exchange to Precipitation Manipulation in Two Shrubs on the Chinese Loess Plateau

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Abstract

Regulation of leaf gas exchange plays an important role in the survival of trees and shrubs under future climate change. However, the responses of leaf water potential and gas exchange of shrubs in semi-arid areas to precipitation alteration remain unclear. Here, we conducted a manipulated experiment with three levels of precipitation—ambient precipitation (control), 50% above ambient (irrigation treatment), and 50% below ambient (drought treatment)—on two common shrubs, *Salix psammophila* C. Wang & C. Y. Yang (isohydric plant, maintaining constant leaf water potential via stomatal regulation) and *Caragana korshinskii* Kom. (anisohydric plant, having more variable leaf water potential), on the Chinese Loess Plateau in 2014 and 2015. We measured seasonal variations of predawn and midday leaf water potential (pd and md), two gas exchange parameters (light-saturated assimilation, An, and stomatal conductance, gs), and other foliar and canopy traits.

The isohydric *S. psammophila* had similar An but higher gs than the anisohydric *C. korshinskii* under drought treatment in 2015, inconsistent with the view that photosynthetic capacity of anisohydric plants is higher than isohydric plants under severe drought. The two shrubs responded differently to precipitation manipulation. For *S. psammophila*, pd, An, and gs were higher under irrigation treatment than control, while for *C. korshinskii*, these three variables plus md were significantly higher under irrigation treatment and lower under drought treatment compared to control. Leaf water potential and gas exchange responded more strongly to manipulated precipitation in *C. korshinskii* than in *S. psammophila*. However, precipitation manipulation did not alter the sensitivity of leaf gas exchange to vapor-pressure deficit and soil moisture in either shrub. Acclimation to long-term changes in soil moisture in these two shrubs was primarily attributed to changes in leaf or canopy structure rather than leaf gas exchange. These findings will be useful for modeling canopy water-carbon exchange and elucidating the adaptive strategies of these two shrubs to future precipitation changes.

Keywords: drought; irrigation; leaf water potential; gas exchange; acclimation

Introduction

Precipitation is one of the most important factors controlling primary productivity in terrestrial ecosystems. Ecosystems in areas with low mean annual precipitation are predicted to be most susceptible to projected precipitation changes associated with climatic warming. The wind-water erosion crisscross region on the Chinese Loess Plateau experiences the most intensive soil and water losses in the world. Mean annual precipitation in this area is about 400 mm, while mean annual evaporation exceeds 1500 mm, making water scarcity the primary factor limiting plant growth and survival. Large-scale coal and oil mining have exacerbated water resource shortages by depleting groundwater sources, and some plants already show dieback symptoms. Understanding how projected precipitation variations affect the physiology, growth, and productivity of local vegetation is therefore essential.

Plants alleviate drought threats in the short term by regulating stomatal closure, leading to classification into two drought strategies based on stomatal regulation: isohydry and anisohydry. Isohydric plants maintain leaf water potential (leaf) by rapidly closing stomata during drought, thereby decreasing stomatal conductance (gs) and photosynthetic assimilation. In contrast, anisohydric plants decrease leaf during drought, which may increase vulnerability to hydraulic failure. Anisohydric plants often close stomata later, thus maintaining higher gs and carbon assimilation under severe drought. This framework has been extensively used to explain drought-induced tree mortality, yet some experimental results contradict it. For example, daily gs decreased more in anisohydric sunflower than in isohydric maize under severe water deficit, and gs and photosynthesis were similar at the end of a dry period for co-existing isohydric and anisohydric Mediterranean woody species. This pattern has also been reported in different *Vitis* cultivars. These results challenge the traditional view that anisohydric species maintain higher gas exchange under severe drought. Furthermore, isohydric *Pinus edulis* exhibited chronic embolism, whereas anisohydric *Juniperus monosperma* had very little embolism, refuting the hypothesis that anisohydric species are more vulnerable to hydraulic failure than isohydric species. Garcia-Forner et al. (2016) suggested that iso/anisohydric regulation of leaf may be independent of leaf gas exchange dynamics or the degree of hydraulic/carbon limitations under drought, necessitating simultaneous measurements of leaf water potential and gas exchange rather than inferring gas exchange or hydraulic traits solely from iso/anisohydric behavior.

Long-term acclimation to limited water supply is important for plant growth and survival. Experimental precipitation manipulation provides a way to conduct controlled studies of acclimation under long-term drought, improving our understanding of adaptive strategies under projected future climate conditions. Acclimation may involve adjustments in plant morphology, physiology, anatomy, growth, and carbon partitioning among organs. Photosynthesis is crucial in this process, and effects of long-term drought on photosynthetic acclimation have been studied. For example, in evergreen *Quercus ilex*, net light-saturated assimilation rate (An), gs, mesophyll conductance of CO_2, maximum carboxylation rate, and maximum electron transport rate all decreased when predawn leaf was reduced through throughfall manipulation, but functional relationships between these parameters and leaf were unaffected by water treatments. In contrast, An and gs for *Q. ilex* from mesic habitats decreased more rapidly than in dry habitats. In a mixed forest in southwestern USA, An under full irrigation, specific leaf hydraulic conductance, and leaf at zero assimilation decreased with precipitation for both piñon and juniper, and leaf gas exchange acclimated to precipitation levels in both species. Long-term water stress can also invoke acclimatory responses that decrease photosynthetic capacity sensitivity to drought in xeric but not riparian *Eucalyptus* species. These variations suggest that functional photosynthetic acclimation may be influenced by climatic and soil conditions and can vary among species/populations.

Salix psammophila C. Wang & C. Y. Yang (Salicaceae) and *Caragana korshinskii* Kom. (Fabaceae) are two typical deciduous shrubs in the wind-water erosion crisscross region on the Chinese Loess Plateau. Both play important roles in controlling soil erosion and blocking sand invasion. *S. psammophila* is important for energy and paper industries, while *C. korshinskii* is economically valuable as a highly productive forage shrub. Effects of short-term drought on seedling water relations and photosynthesis have been studied in these species, but little is known about responses of mature plants to long-term drought and precipitation variation. We therefore conducted a precipitation manipulation experiment with three levels (ambient control, 50% above ambient (irrigation), and 50% below ambient (drought)) on these shrubs to address three questions: (1) Are An and gs higher in *C. korshinskii* than in *S. psammophila* under severe drought due to the anisohydric behavior of *C. korshinskii* and isohydric behavior of *S. psammophila*? (2) Does shallow-rooted *S. psammophila* respond more strongly than *C. korshinskii* to higher precipitation, and is it severely inhibited under drought treatment? (3) Since xeric species photosynthetically acclimate better than riparian species to long-term drought, does drought-resistant *C. korshinskii* photosynthetically acclimate better than drought-vulnerable *S. psammophila*?

2.1 Study Area

The study was conducted in the Liudaogou Watershed of Shenmu County, Shaanxi Province, China (38°46 -38°51 N, 110°21 -110°23 E; 1081.0-1273.9 m a.s.l.), located in a typical wind-water erosion crisscross region on the Chinese Loess Plateau. Mean annual precipitation is 437 mm, with 70% falling between July and September. Annual mean temperature is 8.4°C, with an accumulated temperature $ 10°Cof3248°C, 153frost − freedays, totalannualsolarradiationof5922MJ/m^{2}$, and mean annual sunshine duration of 2836 h. The area has mainly loamy, sandy, and silt-dammed soils. Vegetation includes plants common in loessial and desert regions; typical woody plants are trees such as *Populus simonii* and *Salix matsudana*, and shrubs such as *S. psammophila*, *C. korshinskii*, *Amorpha fruticosa*, and *Hedysarum scoparium*.

2.2 Experimental Design

We selected two adjacent stands of healthy, uniformly growing *S. psammophila* and *C. korshinskii* on a flat hilltop in the watershed as experimental sites. Plants were established 30 years ago at densities of 1600 and 2100 individuals/hm^2 for *S. psammophila* and *C. korshinskii*, respectively. Soil at the sites was classified as typical sandy soil. Average canopy size and height were 3.71 ($±0.14)mand3.04(±0.07)mfor ∗ S.psammophila∗, and1.78(±0.08)mand2.24(±$0.21) m for *C. korshinskii*, respectively.

The experimental design consisted of nine randomly arranged 50 m^2 plots, with three replicates of three treatments per stand. Treatments were: (1) ambient control receiving normal precipitation, (2) drought treatment with 50% less precipitation, and (3) irrigation treatment with 50% more precipitation. We selected these precipitation amounts based on mean annual precipitation over the last 30 years and the precipitation gradient (200-600 mm) within the distribution range of the two shrubs on the Chinese Loess Plateau. For the drought treatment, 50% of precipitation was diverted by transparent V-shaped troughs mounted on a steel frame at 20.0 cm intervals. The steel frame was 2.5 m above the canopy and supported by ten steel posts inserted 0.5 m into the soil and fixed with concrete. Intercepted precipitation was collected and diverted by a PVC trough beside the frame, stored in a large tank, and filtered for use as irrigation water. Photosynthetic photon flux density at the canopy position was generally 90%-95% of that in the control, and ground-level temperature was 1°C-4°C lower during the growing season. For the irrigation treatment, water was delivered from the tank using a solar-powered pump after rainfall events through eight equally spaced sprinklers mounted about 2.5 m above the canopy. Precipitation <5 mm was not collected by the troughs, so approximately 45% of precipitation was diverted for irrigation treatment. Vertical asbestos sheets were buried to 60 cm depth between plots to restrict lateral water movement in shallow soil. Three neutron-probe access tubes were installed to 3 m depth in each replicate for periodic soil moisture monitoring. Experimental setups were installed on 11 May 2014 for *C. korshinskii* and 21 May 2014 for *S. psammophila*, with no rainfall between installations.

Three representative plants centrally located within each plot were selected as target shrubs for physiological measurements. leaf, gas exchange, and leaf area index (LAI) were determined for these 27 plants of each species during the 2014 and 2015 growing seasons, while foliar dry mass per unit area and foliar δ^{13}C were measured only during the 2015 growing season.

2.3 Environmental Variables

Environmental variables were continuously monitored at a nearby field microclimatic station. Solar radiation (Rs) was measured using an Apogee20 PRY-P pyranometer (CS300, Apogee Instruments Inc., Logan, USA). Air temperature and relative humidity (RH) were measured using a temperature and RH probe (HMP155A, Vaisala Com., Helsinki, Finland). Wind speed was measured using an anemometer (Met One, Met One Instrument, Inc., OR, USA), and precipitation was measured using a tipping-bucket rain gauge (TE525MM, Texas Electronics, Texas, USA). Data were recorded on CR1000 data loggers (Campbell Scientific, Shepshed, UK) every 10 s, with averages or totals stored every 10 min. Vapor-pressure deficit (VPD, kPa) was calculated as the difference between saturated vapor pressure (es) and actual vapor pressure (ea), where es (Pa) was calculated using Murray' s formula:

$$es = 610.78 \times \exp\left(\frac{t}{t + 238.3} \times 17.2694\right)$$

where t is temperature (°C) and RH is relative humidity (%).

2.4 Measurements of Leaf Water Potential and Soil Moisture

Predawn (pd) and midday (md) leaf water potentials were measured on sunny days at approximately 20-day intervals during the 2014 and 2015 growing seasons using a pressure chamber (PMS 1000, PMS Instruments, Corvallis, USA). Two to three healthy mature leaves on current-year twigs from the south-facing side of each plant (6–9 leaves per plot) were measured on each date. Leaves for pd and md measurements were collected at 05:00–06:00 (LST, local standard time) and 12:00–14:00, respectively.

Soil moisture at 0.2-3.0 m depth was measured in each block at 20-cm intervals using a neutron probe (CNC 503, Beijing Chaosheng Technology Co. LTD, Beijing, China) approximately every three weeks. The neutron probe was calibrated in situ by saturating ground near the experimental sites and periodically measuring soil moisture using both neutron probe and gravimetric methods at different depths. The relationship between probe and gravimetric measurements was used to infer soil moisture. Surface soil moisture (0–20 cm) was measured gravimetrically.

2.5 Measurements of Gas Exchange, Mass per Unit Area, and δ^{13}C of Leaves

We measured two leaf gas exchange parameters, An and gs, using a portable gas exchange system (LI-6400, Li-Cor Inc., Lincoln, USA) on two mature leaves from current-year twigs on the south-facing side of three plants per block and species, on the same dates as leaf measurements. An and gs were measured from 09:00 to 12:00 on sunny days, with the light source set to a saturating photosynthetic photon flux density of 1800 mol/($m^2 \cdot s$). Ambient temperature, relative humidity, and CO_2 concentration ranged from 7.4°C–27.2°C, 14.1%–62.8%, and 376–402 mol/mol, respectively, depending on measurement date and time. After each measurement, leaves enclosed in the chamber were collected for projected area determination via flatbed scanner imaging and ImageJ software (US National Institutes of Health, Bethesda, USA).

Following gas exchange measurements, leaf samples were pooled on 29 May and 24 August 2015 with other mature leaves from current-year twigs for leaf mass per unit area (LMA) and foliar δ^{13}C measurements. Leaves were oven-dried at 75°C for 48 h for dry mass determination. LMA was calculated as the ratio of dry mass to projected area. Ground dried leaves were analyzed for δ^{13}C using a continuous-flow isotope ratio mass spectrometer (DeltaPlus, Finnigan, Germany). δ^{13}C was calculated as:

$$\delta^{13}C = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000$$

where R is the isotope ratio (^{13}C/^{12}C), and Rsample and Rstandard are the isotope ratios of the sample and standard, respectively. Isotope values are expressed in delta notation relative to the VPDB (Vienna Peedee Belemnite) standard. Standard deviation for replicate analyses was \$±\$0.3‰.

2.6 LAI

LAI was monitored for three plants in each replicated block using an LAI-2200 Plant Canopy Analyzer (LI-COR Biosciences, Lincoln, USA). Three measurements from three directions at 120° intervals were taken at half the projected canopy size between 05:30 and 08:00 on sunny days. These measurements were taken three times each growing season.

2.7 Data Analyses

Treatment effects on soil moisture, pd, md, An, gs, LMA, δ^{13}C, and LAI on each sampling date were tested using one-way analysis of variance (ANOVA), with individual plots as replicates (n=3) for each species. Tukey post-hoc tests identified differences among treatments when ANOVA indicated significant effects. Independent t-tests detected differences between species in pd, md, An, and gs for each treatment during the 2015 growing season. Analysis of covariance (ANCOVA) compared regressions between gs and An, between VPD and An or gs, and between pd and An or gs among treatments for each species.

We modeled sensitivities of gas exchange parameters to VPD and pd as follows:

$$g_s = -m \times \ln(VPD) + n$$

where m is the sensitivity of An or gs to VPD, and n is the corresponding value of An or gs at VPD=1 kPa (Oren et al., 1999). Additionally:

$$A_n = a \times \exp(b \times \Psi_{pd})$$

where x is pd, b is the sensitivity of An or gs to pd, and a is the corresponding value of An or gs when x approaches 0 (Zhou et al., 2016). Standardized major axis regression compared differences in m or b between species using SMATR (Standardised Major Axis Tests and Routines) 2.0 software (Falster et al., 2006).

3.1 Climatic Variables and Soil Moisture

Mean annual precipitation was 439.2 mm in 2014 and 388.4 mm in 2015, with growing season precipitation of 373.3 mm in 2014 and 305.6 mm in 2015 [Figure 1: see original paper]. Compared to multi-year means (1980-2014), precipitation in 2014 was nearly average, but 65 mm lower during the growing season and 45 mm lower annually in 2015, indicating 2014 was a normal year while 2015 was relatively dry. Mean daytime VPD was higher in 2015 (1.46 kPa) than in 2014 (1.24 kPa), but mean daily solar radiation did not differ significantly between seasons (20.7 MJ/($m^2 \cdot d$) in 2014 and 20.3 MJ/($m^2 \cdot d$) in 2015).

Soil moisture for all treatments was lower in 2015 than in 2014 for both species [Figure 2: see original paper], as expected. Soil moisture was relatively low under all treatments at the end of June and August in both years, corresponding to low precipitation during these periods. Treatments did not significantly affect soil moisture at the end of June and August for either species. However, soil moisture changed significantly during other months. For *S. psammophila*, soil moisture was generally lower under drought treatment and higher under irrigation treatment than control during the 2014 growing season and 2015 rainy season, except in June–August 2014 when drought treatment soil moisture did not significantly decrease compared to control, possibly because the experimental setup had been installed recently.

3.2 Leaf Water Potential

For *S. psammophila*, pd during the growing seasons generally ranged from -0.1 to -0.5 MPa, except at the end of June when pd reached its lowest values (-0.6 MPa in 2014 and -0.7 MPa in 2015) [Figure 3: see original paper]. pd was significantly affected by treatments on two of nine sampling dates in 2014 ($P<0.05$) and five of eleven dates in 2015 ($P<0.05$). pd responded more to irrigation than drought treatment, especially on dates when treatments had significant effects [FIGURE:3; TABLE:1].

For *C. korshinskii*, pd ranged from -0.3 to -1.3 MPa during the two growing seasons. pd under control and irrigation treatments remained relatively stable throughout both growing seasons, but decreased significantly under drought treatment during the late growing season in both years [Figure 3: see original paper]. Seven of twelve pd measurements in 2014 and all ten measurements in 2015 were significantly influenced by treatments ($P<0.05$). pd was significantly lower under drought treatment and higher under irrigation treatment during both growing seasons, particularly on dates when treatments significantly affected pd [FIGURE:3; TABLE:1].

Midday leaf water potential (md) for *S. psammophila* ranged from -0.6 to -1.6 MPa during the two growing seasons and decreased during the late growing season in 2015 [Figure 3: see original paper]. Three of nine md measurements in 2014 and four of eleven in 2015 were significantly affected by treatments ($P<0.05$). md was lower under drought treatment in 2014 and higher under irrigation treatment in 2015 compared to control on dates when treatments significantly affected md [FIGURE:3; TABLE:1].

For *C. korshinskii*, md ranged from -1.3 to -2.8 MPa during the two growing seasons [Figure 3: see original paper]. Three of twelve md measurements in 2014 were significantly affected by treatments ($P<0.05$). Nine of ten md measurements in 2015 were significantly affected ($P<0.05$), with highest md under irrigation treatment, followed by control, and lowest under drought treatment [FIGURE:3; TABLE:2].

pd and md were generally lower for *C. korshinskii* than for *S. psammophila* in both years [FIGURE:3; TABLE:2]. There was no significant correlation between pd and md for *S. psammophila* [Figure 4a: see original paper], but pd was positively correlated with the water potential gradient (Δ = pd - md, $P<0.05$) [Figure 4b: see original paper]. For *C. korshinskii*, pd was positively correlated with md ($P<0.05$) [Figure 4c: see original paper], but not significantly correlated with the water potential gradient [Figure 4d: see original paper].

3.3 Leaf Gas Exchange

Gas exchange parameters in *S. psammophila* fluctuated seasonally, with An and gs much lower at the end of June and in mid-to-late August during both growing seasons [Figure 5: see original paper]. These decreases corresponded to low precipitation and soil moisture during these periods [FIGURE:1, FIGURE:2]. Only one An and two gs measurements among eight in 2014 were significantly affected by treatments ($P<0.05$) [Figure 5: see original paper]. Four An and three gs measurements among eight in 2015 were significantly affected ($P<0.05$) [Figure 5: see original paper]. An and gs responded more to irrigation treatment on sampling dates when treatments had significant effects [FIGURE:5; TABLE:1, TABLE:2].

For *C. korshinskii*, An peaked in mid-to-late August during both growing seasons. Treatments did not significantly affect An or gs in 2014 [Figure 5: see original paper]. All eight An and gs measurements were significantly affected by treatments in 2015 ($P<0.05$), with highest values under irrigation treatment, followed by control, and lowest under drought treatment [FIGURE:5; TABLE:1, TABLE:2].

An and gs responded more strongly to precipitation manipulation in 2015 than in 2014 for both species [Figure 5: see original paper]. During the 2015 growing season, An and gs were higher for *C. korshinskii* than for *S. psammophila* under irrigation treatment, but An was similar and gs was even lower for *C. korshinskii* under drought treatment , indicating that *C. korshinskii* did not have higher photosynthetic capacity than *S. psammophila* under severe drought. Response patterns and magnitudes differed between species: *S. psammophila* responded clearly to irrigation but seldom to drought, while *C. korshinskii* responded strongly to both irrigation and drought relative to control [TABLE:1, TABLE:2]. Response magnitude for both treatments was larger for *C. korshinskii* than for *S. psammophila* . Precipitation treatments did not affect the linear relationship between An and gs for either species [Figure 6: see original paper].

3.4 Sensitivities of Leaf Gas Exchange to VPD and pd

Treatment and VPD or pd did not significantly interact with An and gs for either species ($P>0.05$) in ANCOVA, indicating that relationships between leaf gas exchange and VPD or pd did not differ among treatments. When data from all treatments were pooled, leaf gas exchange decreased with increasing VPD and decreasing pd, and decreased faster for *S. psammophila* than for *C. korshinskii* ($P<0.05$) [FIGURE:7, FIGURE:8], indicating higher sensitivity to VPD and pd in *S. psammophila*.

An and gs were highest at pd = -0.2 MPa for *S. psammophila* and at pd = -0.4 MPa for *C. korshinskii*. The pd at 50% loss of maximum An was -0.39 MPa for *S. psammophila* and -1.25 MPa for *C. korshinskii*, with corresponding pd at 50% loss of maximum gs of -0.42 MPa for *S. psammophila* and -1.21 MPa for *C. korshinskii*. These lower pd values corresponding to 50% losses of maximum An and gs in *C. korshinskii* suggest its photosynthetic apparatus was more drought-resistant than that of *S. psammophila*.

3.5 LMA, δ^{13}C, and Canopy LAI

Leaf δ^{13}C and LMA in *S. psammophila* during the 2015 growing season were significantly affected by treatments ($P<0.05$), with lower δ^{13}C and LMA under irrigation treatment than control. However, treatments did not significantly affect δ^{13}C or LMA in *C. korshinskii* . Drought significantly decreased LAI in *S. psammophila* in 2015 and in *C. korshinskii* in both seasons, while irrigation increased LAI in *C. korshinskii* during the middle growing season in 2015 [Figure 9: see original paper].

4.1 Strategies of Stomatal Regulation and Carbon Assimilation in Two Shrubs

S. psammophila is an isohydric plant, whereas *C. korshinskii* is anisohydric, based on seasonal changes in leaf, daily stomatal regulatory capacity, and stem hydraulic vulnerability. Seasonal fluctuations in pd and md were small in *S. psammophila*, and pd and md were not significantly affected by drought and were uncorrelated for most sampling dates [Figure 3: see original paper]. The significant correlation between pd and water potential gradient suggests *S. psammophila* could maintain pd while adjusting its water potential gradient when soil water potential decreased, behaving as a typical isohydric plant. In contrast, seasonal fluctuations in pd and md and drought effects on these variables were larger for *C. korshinskii* than for *S. psammophila* [Figure 2: see original paper]. The significant linear regression between pd and md, but lack of correlation between pd and water potential gradient, suggests md decreased as soil water potential decreased, indicating anisohydric behavior.

An and gs were higher for *C. korshinskii* than for *S. psammophila* under irrigation treatment, perhaps due to higher leaf N content (1.84% for *C. korshinskii* vs. 1.05% for *S. psammophila*) in this leguminous species. However, An was similar and gs was lower for *C. korshinskii* than for *S. psammophila* under severe drought , inconsistent with the expectation of higher An and gs in anisohydric plants under extreme drought. This pattern has been reported in various crops, woody species, and *Vitis* cultivars, further suggesting that iso/anisohydric behavior based on leaf regulation may be difficult to associate with gas exchange behavior.

4.2 Responses of leaf and Gas Exchange to Precipitation Manipulation

Both shrubs' leaf and gas exchange parameters responded to precipitation manipulation, but response patterns differed between species. *S. psammophila* showed asymmetrical responses to irrigation and drought treatments: pd, An, and gs were higher under irrigation than control but similar between drought and control treatments. In contrast, *C. korshinskii* responded symmetrically: all variables were higher under irrigation and lower under drought compared to control [FIGURE:3, FIGURE:5; TABLE:1, TABLE:2]. These different patterns may result from hydraulic constraints, particularly root distribution and acclimation of water uptake.

S. psammophila has shallow roots not exceeding 1.5 m depth but spreading horizontally up to 3 times the canopy size, restricting water uptake to shallow soil layers and making leaf and gas exchange very sensitive to irrigation. Stronger stomatal regulation in isohydric *S. psammophila* may have limited responses of leaf, An, and gs to drought on most measurement dates. *C. korshinskii* has a taproot system with dense lateral roots that can reach >6.0 m depth in 12-15-year-old plants. A hydrogen isotope experiment in the Ulanbuh Desert found *C. korshinskii* acquired water mainly from 0–30 cm and 60–90 cm depths during late autumn, indicating water uptake from both shallow and deep layers. This versatile water acquisition strategy likely accounts for the symmetrical responses of leaf, An, and gs to irrigation and drought treatments.

The asymmetrical response pattern of *S. psammophila* resembles that of isohydric *P. edulis* in southwestern USA, while the symmetrical pattern for *C. korshinskii* differs from anisohydric *J. monosperma*, whose photosynthetic capacity was similar in irrigation and control plots but significantly lower under drought. These differences between anisohydric species may reflect greater reliance on deep soil water in *J. monosperma* than in *C. korshinskii.*

C. korshinskii responded more strongly than *S. psammophila* to precipitation manipulation, outperforming *S. psammophila* under irrigation but being severely inhibited under drought . Higher leaf N content in *C. korshinskii* due to N-fixation may enhance photosynthetic capacity under irrigation in this nutrient-limited area. High infiltration capacity of sandy soils may also allow rainwater to quickly reach deep soil layers inaccessible to shallow-rooted *S. psammophila.* Our previous data on shrub growth also demonstrated larger seasonal aboveground biomass increase for *C. korshinskii* than for *S. psammophila* under irrigation but smaller under drought, supporting our gas exchange data. Greater plasticity in leaf and gas exchange for *C. korshinskii* may increase its fitness in highly variable environments, contributing to its wide distribution on the Chinese Loess Plateau. Stricter stomatal regulation and more conservative gas exchange responses to drought in *S. psammophila* may enhance adaptation to drought and desert areas, suggesting different strategies for long-term drought adaptation between the two shrubs.

4.3 Acclimation of Leaf Gas Exchange to Long-term Alteration of Water Supply

Sensitivities of leaf An and gs to VPD or pd did not differ among treatments for either species, suggesting VPD and precipitation manipulation did not alter functional relationships between leaf gas exchange and water stress. These findings are consistent with reports for evergreen trees *Q. ilex* and *Arbutus unedo* in Mediterranean Macchia ecosystems, indicating two years of soil moisture variation did not acclimate photosynthetic gas exchange. However, studies of *Q. ilex* across precipitation gradients, piñon and juniper under precipitation manipulation in southwestern USA, and xeric *Eucalyptus* species have reported acclimatory responses in photosynthetic capacity sensitivity to long-term drought. These contradictions may arise from differences in species, developmental stage (seedling, sapling, mature tree), and water stress severity.

Long-term structural adjustment may alleviate drought effects on photosynthesis and gs. Precipitation manipulation influenced LMA in *S. psammophila*, which was lower under irrigation treatment. Higher photosynthetic rates and lower integrated water-use efficiency (lower foliar δ^{13}C) under irrigation than control in *S. psammophila* may therefore be partly attributed to changes in foliar structural properties, as leaves with lower LMA generally have higher An and lower water-use efficiency. LMA in *C. korshinskii* was not significantly affected by treatments, so leaf gas exchange responses to irrigation and drought likely resulted from factors other than foliar structural changes. LAI was significantly lower under drought than control for both shrubs and partially higher under irrigation in 2015 [Figure 9: see original paper], indicating acclimatory responses in canopy structure. Changes in soil-plant system hydraulic conductance may be responsible for altered leaf gas exchange under precipitation manipulation, suggesting foliar and canopy properties may regulate root water uptake and hydraulic transport, thereby affecting photosynthesis in these shrubs.

Finally, while this study identified mature shrub responses to projected precipitation variation in a natural environment, experiments were conducted for only two years. Long-term experiments are needed to characterize drought resistance and resilience of these fragile ecosystems under projected climate change.

5 Conclusions

Two consecutive years of precipitation manipulation did not alter stomatal control strategies in the two shrubs. An was similar between isohydric *S. psammophila* and anisohydric *C. korshinskii*, and gs was even higher in *S. psammophila* under severe drought, inconsistent with the view that anisohydric plants maintain higher An and gs under extreme drought. Leaf water potential and gas exchange parameters (pd, md, An, and gs) in *S. psammophila* were most strongly influenced by irrigation treatment, while those in *C. korshinskii* were significantly influenced by both irrigation and drought treatments. leaf and gas exchange showed greater plasticity to precipitation treatments in *C. korshinskii*. Precipitation manipulation did not alter leaf gas exchange sensitivity to VPD or soil moisture in either species. Acclimation to soil moisture changes in both species was primarily due to changes in foliar structure (e.g., LMA) and/or canopy structure (e.g., LAI) rather than photosynthetic gas exchange. These findings may be useful for modeling canopy water-carbon exchange and elucidating adaptive strategies of these two shrubs to future precipitation changes.

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Note: Figure translations are in progress. See original paper for figures.