

# Drought stress ecophysiology of shrub and grass functional groups on opposing slope aspects of a temperate grassland valley

Matthew G. Letts, Davin R.E. Johnson, and Craig A. Coburn

**Abstract:** Plant functional groups with contrasting growth strategies co-occur in semiarid ecosystems. In the northern Great Plains, woody shrubs and grasses interact competitively, with shrubs prevalent on mesic hillslopes. To understand topographic influences on physiological drought acclimation, we measured seasonal photosynthetic water use in C<sub>3</sub> shrubs (*Artemisia cana* Pursh and *Rhus trilobata* Nutt.) and grasses (*Agropyron cristatum* (L.) Gaertn. and *Stipa viridula* Trin.) on north- and south-facing slopes. Relationships between abiotic controls and photosynthesis were similar on both aspects, indicating an absence of long-term photosynthetic acclimation to xeric, south-facing conditions. Acclimatory differences were observed between functional groups. Soil moisture depletion lowered intercellular:atmospheric CO<sub>2</sub> ratio ( $C_i/C_a$ ) and increased intrinsic water-use efficiency ( $WUE_i = A_{max}/g_s$ ) in shrubs, but not grasses. Consequently, between-slope differences in net photosynthesis ( $A_{max}$ ) and stomatal conductance ( $g_s$ ) occurred only in shrubs, with lower values on the south-facing slope. Shrubs also exhibited between-slope differences in the photochemical reflectance index, suggesting drought-related photoprotection. The deuterium:hydrogen ratios of stem water showed that deep water use facilitated late summer growth in shrubs. Consistent with plant distribution, cumulative water use and photosynthesis were higher in shrubs than in grasses on the north-facing slope, but higher in grasses than in shrubs on the south-facing slope. This shows that topographic effects on leaf photosynthetic gas exchange are mediated by physiological acclimation strategy and water source use.

**Key words:** NDVI, remote sensing, rooting depth, sap flow, solar radiation, stable carbon isotope ratio.

**Résumé :** Des groupes fonctionnels de végétaux ayant des stratégies contrastées se retrouvent ensemble dans les écosystèmes semi-arides. Dans le nord des Grandes Plaines, où les arbustes ligneux et les graminées entrent en compétition, les arbustes se retrouvent préférentiellement sur les pentes mésiques. Afin de comprendre l'influence de la topographie sur l'acclimatation physiologique à la sécheresse, nous avons mesuré la consommation saisonnière d'eau par la photosynthèse en C<sub>3</sub> chez des arbustes (*Artemisia cana* Pursh et *Rhus trilobata* Nutt.) et des graminées (*Agropyron cristatum* (L.) Gaertn. and *Stipa viridula* Trin.), sur des pentes exposées au nord et au sud. Les relations entre les variables abiotiques et la photosynthèse sont similaires pour les deux expositions, ce qui indique une absence d'acclimatation à long terme aux conditions xériques des faces sud. En revanche, on observe des acclimations différentes selon les groupes fonctionnels. L'épuisement de l'humidité du sol diminue le ratio de la concentration en CO<sub>2</sub> intercellulaire / atmosphérique ( $C_i/C_a$ ) et augmente l'efficacité intrinsèque d'utilisation de l'eau ( $WUE_i = A_{max}/g_s$ ) chez les arbustes, mais pas chez les graminées. En conséquence, des différences de photosynthèse ( $A_{max}$ ) et conductance stomatique ( $g_s$ ) entre les expositions n'apparaissent que chez les arbustes qui présentent des valeurs moindres sur les pentes exposées au sud. Les arbustes montrent également des différences d'indice de réflectance photochimique selon les pentes, ce qui suggère une augmentation de la photoprotection en réponse à la sécheresse. Les ratios deutérium / hydrogène dans l'eau des tiges indiquent un prélèvement d'eau en profondeur chez les arbustes, ce qui facilite leur croissance en fin d'été. De façon congrue avec la distribution des plantes, la consommation d'eau et la photosynthèse cumulées sont plus importantes chez les arbustes sur la pente exposée au nord, et chez les graminées sur la pente exposée au sud. Ceci démontre que les effets de la topographie sur les échanges gazeux foliaires dépendent de la stratégie d'acclimatation physiologique et de l'utilisation de la ressource en eau.

**Mots-clés :** NDVI, télédétection, profondeur d'enracinement, flux de la sève, radiation solaire, carbone stable.

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## Introduction

Water-use efficiency (WUE), the ratio of net photosynthesis ( $A$ ) to transpiration ( $E$ ), is an important functional char-

acteristic of vascular land plants. Interspecific differences in WUE are governed by photosynthetic capacity and stomatal behaviour (Smedley et al. 1991), but WUE is also affected by environmental conditions, including soil moisture avail-

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M.G. Letts,<sup>1</sup> D.R.E. Johnson, and C.A. Coburn. Department of Geography, Alberta Water and Environmental Science Building, University of Lethbridge, 4401 University Drive, Lethbridge, AB T1K 3M4, Canada.

<sup>1</sup>Corresponding author (e-mail: matthew.letts@uleth.ca).

ability (Letts et al. 2009a) and atmospheric demand (Cohen 1970; Da Silveira et al. 1989). In temperate grasslands, soil moisture is the key determinant of plant productivity (Flanagan et al. 2002), and influences the spatial distribution of plant life forms in complex terrain (Coxson and Looney 1986). Grasses and woody shrubs co-occur competitively in semiarid ecosystems (Wilson 2007; Clarke and Knox 2009), but possess distinct physiological and structural characteristics that influence photosynthetic water use (Jenerette et al. 2009). These functional groups employ contrasting adaptive strategies, with  $C_3$  grasses exhibiting profligate water use (Ponton et al. 2006; Moran et al. 2009) and rapid growth during periods of water abundance (Sherrard and Maherali 2006), and most woody shrubs displaying a conservative strategy that promotes water conservation (Golluscio and Oesterheld 2007). In this study, we show how differences in water source use and physiological drought acclimation alter seasonal patterns of photosynthesis and water use among grasses and shrubs on opposing temperate grassland hillslopes.

### Water-use strategies of woody and herbaceous plants

Physiological water-use strategies of conservatism and opportunism are associated with complementary hydraulic architectural and leaf structural traits. Among shrubs, deep-rooted species maintain higher leaf water potential during drought, but have less efficient water transport and higher leaf mass per unit area (LMA; Bucci et al. 2009). High LMA promotes leaf longevity and WUE, but is associated with low mass-based leaf N content and net light-saturated photosynthesis rate ( $A_{\max}$ ; Wright et al. 2004). Grasses usually have shallower rooting systems that rapidly utilize water and nutrients in the upper soil horizons (Sala et al. 1989; McLaren et al. 2004), thereby reducing drainage to the subsoil (Knoop and Walker 1985). In this way, grasses can reduce the productivity of woody shrubs, including *Artemisia* sp. (Booth et al. 2003). Conversely, woody shrubs show flexibility in source water depth (Dodd et al. 1998; Schwinning et al. 2002; Eggemeyer et al. 2009) and can exclude grasses following fire suppression, resulting in woody plant encroachment into native grasslands (Lett and Knapp 2005).

The ability of competitive interactions between grasses and shrubs to reciprocally reduce productivity depends on the extent to which the depth and timing of water extraction overlap. Vertical water partitioning can facilitate the coexistence of different plant functional groups in semiarid environments, by reducing competition for water resources (Walter 1971). However, partitioning is not always observed (Mordelet et al. 1997), owing to among-species variability in water-use strategy within functional groups (McCarron and Knapp 2001). The stable deuterium isotope composition ( $\delta D$ ) of stem water can be used to assess water source depth where there is seasonal variation in the deuterium:hydrogen (D/H) ratio of precipitation water. In southern Alberta, the mean annual  $\delta D$  of precipitation is approximately  $-136.5\text{‰}$  (Peng et al. 2004), but values exceed  $-120\text{‰}$  in summer and are often below  $-200\text{‰}$  in winter. We predicted that lower stem water  $\delta D$  would be observed in woody shrubs, indicating deeper water source use.

### Measures of photosynthetic water-use efficiency

Water-use efficiency is higher in plants with strong sto-

matial regulation of water loss, but decreases with increasing leaf-to-atmosphere vapour pressure deficit (LAVD; Cohen 1970). Intrinsic water-use efficiency ( $WUE_i = A_{\max}/g_s$ ) is a useful indicator of among-species differences in water use, as it accounts for this effect (ie.,  $E = LAVD \cdot g_s$ ).  $WUE_i$  influences the intercellular concentration of carbon dioxide ( $C_i$ ), with lower intercellular:atmospheric  $CO_2$  concentration ratios ( $C_i/C_a$ ) observed in conservative water users, especially when  $g_s$  decreases strongly in response to moderate drought stress (Ehleringer and Cooper 1988). Low  $C_i/C_a$  is associated with high leaf stable carbon isotope composition ( $\delta^{13}C$ ). The relationship between  $C_i/C_a$  and the  $\delta^{13}C$  of plant material ( $\delta^{13}C_p$ ) can be described as follows (Farquhar et al. 1982, 1989):

$$[1] \quad \delta^{13}C_p = \delta^{13}C_a - a - (b - a) \frac{C_i}{C_a},$$

where  $\delta^{13}C_a$  is the  $\delta^{13}C$  of source atmospheric  $CO_2$  ( $-8.0\text{‰}$ ),  $a$  is the discrimination that occurs during diffusion of  $CO_2$  in air ( $4.4\text{‰}$ ), and  $b$  is the net discrimination that occurs through carboxylation ( $27.0\text{‰}$ ). Leaf  $\delta^{13}C$  is negatively correlated with both  $g_s$  (Lloyd and Farquhar 1994) and volumetric soil moisture ( $\theta_v$ ; Meinzer et al. 1992), but positively correlated with air temperature ( $T_a$ ) among taxa (Jäggi et al. 2002). As a result, leaf  $\delta^{13}C$  is often more enriched in plant specimens from xeric ridges and slopes than from mesic sites (Garten and Taylor 1992; Gong et al. 2007). However, the effect of stomatal closure can be offset by increases in LAVD, so that higher  $\delta^{13}C$  and lower cumulative water use (CWU) per unit carbon gain are not always observed in xeric microclimates (Van de Water et al. 2002; Letts et al. 2009b). We expected to observe lower  $C_i/C_a$  and  $g_s$ , but not necessarily leaf  $\delta^{13}C$ , on the more xeric, south-facing slope and in response to drought stress.

### Effects of slope aspect on photosynthetic water use

Competitive interactions among functional groups are affected by meteorological conditions, including light availability (Peltzer 2001), rainfall timing, and rainfall intensity (Schwinning et al. 2002). In short-statured ecosystems, complex terrain results in spatially variable incident solar radiation ( $K\downarrow$ ), so that soil moisture, near-surface  $T_a$ , and atmospheric demand vary not only seasonally, but also with slope aspect (Zou et al. 2007; Bennie et al. 2008). In grasslands, microclimatic differences produce complex vegetation patterns, but certain grasses and shrubs are found on both north- and south-facing slopes (Liefvers and Larkin-Liefvers 1987). Opposing hillslopes can, thus, be used as treatments, to examine the physiological acclimation of individual species to a wide range of abiotic conditions in a given year.

### Reflectance indices as drought stress indicators

The impacts of drought on vegetation properties can be assessed by computing ratios from remotely sensed reflectance data (Myneni and Williams 1994). Chlorophyll content, leaf N, and photosynthetic capacity increase during leaf development, but decrease well before senescence in response to drought, resulting in lower normalized difference reflectance index (NDVI; Tucker 1979) and chlorophyll index (CI; Gitelson and Merzlyak 1994). At the same time, xanthophyll cycle pigments undergo changes from violaxan-

thin to the more photoprotective form zeaxanthin, resulting in lower photochemical reflectance index (PRI; Peñuelas et al. 1995). Changes in leaf water content can be detected using the water band index (WBI; Peñuelas et al. 1997), which is particularly effective at the canopy scale (Sims and Gamon 2003). We predicted that lower leaf and canopy PRI would be observed on the south-facing slope during drought, because of a greater need for photoprotection. Similarly, we expected lower NDVI, CI, and WBI on the more xeric slope, along with decreasing leaf N and chlorophyll content in response to drought.

### Objectives

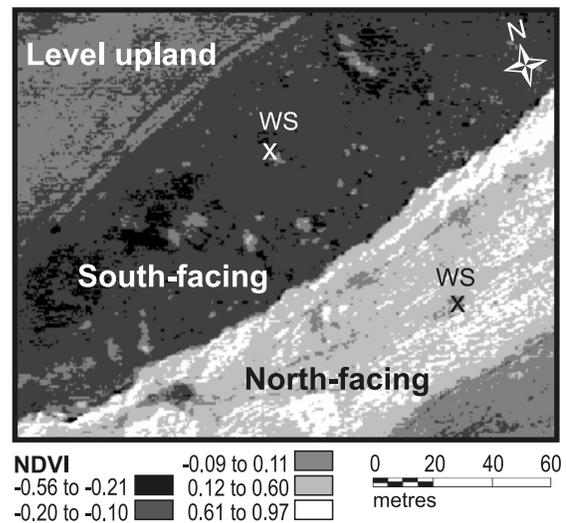
In this study, seasonal patterns of photosynthetic gas exchange, leaf reflectance, and stable isotope composition were measured in two grasses [*Agropyron cristatum* (L.) Gaertn. and *Stipa viridula* Trin.] and two woody shrubs [*Artemisia cana* Pursh and *Rhus trilobata* Nutt.] that naturally occur on north- and south-facing hillslopes of the Oldman River valley, in southern Alberta. The objectives were to determine (i) whether leaf gas exchange and seasonal patterns of growth activity differ between C<sub>3</sub> grasses and shrubs; (ii) whether grass and shrub functional groups respond similarly to the distinct environmental conditions of opposing slopes; and (iii) whether differences in leaf physiology and phenology are related to the depth of water source use. We tested the following hypotheses: (i) WUE is lower in grasses than in woody shrubs; (ii) woody shrubs are more effective than grasses at adjusting  $g_s$  to mediate the effect of higher LAVD on WUE; and (iii) shrubs exhibit deep soil water uptake and extended seasonal activity, resulting in higher CWU than grasses, despite stronger stomatal regulation in response to drought.

## Materials and methods

### Site description

The study was carried out in a narrow coulee of the western uplands of the Oldman River valley at Lethbridge, Alberta (49° 38'N, 112°50'W; 873–903 m a.s.l.; Fig. 1), in the prairie ecozone of the Great Plains (Gauthier and Wiken 2003). The mean annual temperature is 5.7 °C. The region has a late spring precipitation maximum, and a mean annual total of 386 mm (Lethbridge Airport 1971–2000; Environment Canada 2010). Soils are loamy Dark Brown Chernozems (Soil Classification Working Group 1998), with deeper A horizons on the north-facing slope. The north-facing slope consists of diverse C<sub>3</sub> grasses, shrubs, and forbs. The south-facing slope is dominated by C<sub>3</sub> and C<sub>4</sub> grasses, but also supports widely spaced shrubs and cacti (Coxson and Looney 1986). Differences in species composition and environmental conditions on opposing slopes produce contrasting spatial patterns of photosynthetic productivity during drought, as evidenced by higher NDVI on the north-facing slope in summer (Fig. 1). In this study, we isolated the effect of environmental conditions and increased the range of conditions observed in a single year, by measuring seasonal patterns of plant physiological stress in grasses and shrubs that occur on both slopes. Of the two grasses, *S. viridula* was evenly distributed with slope aspect, whereas the invasive species *A. cristatum* was domi-

**Fig. 1.** Spatial pattern of normalized difference vegetation index (NDVI) at the study site, computed from the red and infrared bands of the Multispectral Airborne Remote Sensing System (MARSS), on 5 July 2007. Lighter shades indicate zones of higher photosynthetic activity. WS, weather station.



nant on the south-facing slope, with tufts on the north-facing slope restricted to microscale terracettes. *Rhus trilobata* and *A. cana* occur on both hillslopes, with the former dominant on the south-facing slope and the latter dominant in patches of north-facing terrain.

### Sampling scheme and plant trait measurement

Forty plants were chosen for physiological measurements, including 5 of each species, on both north-facing and south-facing slopes. All specimens were located within 30 m of the weather stations (Fig. 1), except for three north-facing specimens of *A. cristatum*, which were located 100 m west-southwest of the north-facing station, because of a lack of nearby tuft clusters. Shrubs were spaced at least 10 m apart, to reduce the likelihood of clone selection. All specimens had apparently healthy foliage in May. Canopy height and width were determined at the end of the growing season, using a tape measure. The basal diameter of shrubs was measured with digital calipers. Branch increment was determined for 2007 on each shrub as the mean length of five terminal growth units, measured from node scar to tip. Using a light microscope, stomatal density was determined from epidermal leaf peels from five plants per species, grown in a greenhouse, under open-air conditions, diffuse light, and moist soil, during summer 2007.

### Micrometeorological monitoring

Air temperature ( $T_a$ ), atmospheric vapour pressure deficit (VPD), volumetric soil moisture ( $\theta_v$ ), and solar radiation intensity ( $K\downarrow$ ) were monitored using HOBO Microstation Dataloggers (Onset Computer Corp., Bourne, Mass.). Air temperature and VPD measurements were taken at the height of grass and shrub foliage (7.5 cm and 100 cm), with HOBO Temperature/RH Smart Sensors in Gill Radiation Shields. Volumetric soil moisture was measured on the north-facing slope, using ECHO Dielectric Aquameter soil moisture probes (Decagon Devices, Pullman, Wash.) at a

depth of 40 cm. Based on previously measured between-slope differences in  $\theta_v$  (2004 and 2005), south-facing  $\theta_v$  ( $\text{m}^3\cdot\text{m}^{-3}$ ) was estimated as  $0.0133e^{9.287x}$ , where  $x$  is the  $\theta_v$  of the north-facing slope (root mean square error =  $0.024 \text{ m}^3\cdot\text{m}^{-3}$ ;  $r^2 = 0.84$ ). Solar radiation was measured level-to-ground with a HOBO Silicon Pyranometer Smart Sensor. All data were collected from 880 to 898 m a.s.l. Estimates of between-slope differences in daily top-of-atmosphere spectral radiation ( $K\downarrow_{\text{TOA}}$ ) were made by summing hourly values of simulated  $K\downarrow_{\text{TOA}}$  on north-facing and south-facing  $40^\circ$  slopes, using the Iqbal (1983) formulations.

### Photosynthetic gas-exchange measurement

Leaf gas exchange measurements were taken at saturating photosynthetic photon flux density (PPFD  $>1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) during the 2007 growing season, using a TPS-1 Photosynthesis System with a 25 mm  $\times$  18 mm PLC6 Universal Leaf Cuvette (PP Systems, Hitchin, Hertfordshire, UK). Five sun-exposed leaves were chosen from each species with mature and healthy foliage on day of year (DOY) 129, 143, 146, 162, 177, 192, 208, 219, and 264. Each leaf was from a different plant. Ten readings were taken on each leaf, to calculate one leaf mean. Species means for each date were derived from the five leaf means. All measurements were taken between 1100 and 1600 h, alternating among species and between north- and south-facing slopes to minimize time-of-day effects. Following the gas-exchange measurements, leaf area was determined with a LI-COR 3000C Area Meter (LI-COR Biosciences, Lincoln, Nebr.). Leaf samples were then dried for 48 h at  $70^\circ\text{C}$ , and weighed. The leaf area and leaf mass measurements were used to calculate the specific leaf area (SLA;  $\text{cm}^2\cdot\text{g}^{-1}$ ).

### Sap flow and cumulative water use

Sap flow ( $J_s$ ) was measured with SGB25 and SGB19 flow collars, and calculated using Flow32 software (Dynamax, Houston, Tex.). Measurements were taken every 5 min, from DOY 193–203 and DOY 248–258 on three stems of *A. cana* and four stems of *R. trilobata* on the north-facing slope. Following gentle sanding, the stem surface was covered with silicone-based dielectric grease. Aluminium foil was wrapped around the gauges to limit solar heating.

Cumulative water use (CWU) was estimated for the above dates, using data for  $E$  and  $J_s$ . Because gas exchange measurements were taken between 1100 and 1600 h, mean  $J_s$  was first calculated for this period, and reported in  $\text{g}\cdot(\text{cm stem})^{-2}\cdot\text{hr}^{-1}$ . For all 24 h periods, the observed  $J_s$  values for each 5-min interval were then divided by the 1100–1600 mean, to estimate proportional sapflow relative to the gas exchange measurement period. To obtain CWU, these proportions were multiplied by the transpiration rate determined for each date, and expressed in  $\text{L}\cdot(\text{m leaf area})^{-2}\cdot\text{d}^{-1}$ . To determine the daily  $E$  values used in these calculations, a linear scale was applied to interpolate between the  $E$  observations on each gas exchange measurement date.

### Leaf reflectance measurement

Adaxial leaf and canopy reflectance were measured with a field spectroradiometer (Unispec-SC, PP Systems) on DOY 127, 143, 155, 162, 177, 192, and 208. Leaf and canopy reflectance indices follow seasonal changes in the physiology

of healthy leaves. Canopy reflectance also responds to changes in leaf area index and collective leaf health. Measurements were taken on the same five plants of each species selected for photosynthesis measurement. Mean NDVI, CI, PRI, and WBI were determined as follows:

$$[2] \quad \text{NDVI} = \frac{r_{800} - r_{670}}{r_{800} + r_{670}},$$

$$[3] \quad \text{CI} = \frac{r_{750} - r_{705}}{r_{750} + r_{705}},$$

$$[4] \quad \text{PRI} = \frac{r_{531} - r_{570}}{r_{531} + r_{570}},$$

and

$$[5] \quad \text{WBI} = \frac{r_{900}}{r_{970}},$$

where  $r$  is reflectance and subscripts refer to wavelengths in nanometres. Leaf reflectance was measured with a bifurcated fibreoptic cable (UNI400) and leaf clip (UNI500), and canopy measurements were taken at a height of 1 m above the vegetation target, using a straight cable with  $28^\circ$  angular field of view. White and dark reference scans preceded each measurement.

### Airborne remote sensing

An aerial NDVI image of the study site (Fig. 1) was taken with the six-band Multispectral Airborne Remote Sensing System (MARSS), at solar noon on DOY 186, using 100 nm wide spectral bands, centred at 650 nm (red) and 800 nm (near infrared). NDVI was calculated by eq. 2, with infrared and red image bands substituted for the indicated wavelengths. The MARSS system consisted of six Pulnix TM-540 cameras with enhanced infrared capability. Lenses of 8 mm focal length were used at an altitude of 500 m to produce an image with 25 cm resolution. To assign image gain relationships and produce reliable ground reflectance measurements, the camera system was calibrated with a variety of known targets (e.g., concrete, asphalt, and water) that were also measured with ground-based spectroradiometers.

### Stable carbon and hydrogen isotope composition

Leaf samples were collected for  $\delta^{13}\text{C}$  analysis from *A. cana* (DOY 129, 192, and 219), *A. cristatum* (DOY 129, 146, 162, and 179), *R. trilobata* (DOY 146, 192, and 219), and *S. viridula* (DOY 129, 146, 177, and 192). For each of these dates, analysis was performed on the same 20 leaves (5 per species) used in photosynthesis measurements. Samples from each leaf were crushed into a powder, using a mortar, pestle, and liquid nitrogen. To determine leaf  $\delta^{13}\text{C}$  (‰) and leaf N (%), 6 mg samples were combusted in an NC2100 elemental analyzer (CE Instruments, Elantech, Lakewood, N.J.), coupled to an Optima mass spectrometer (VG Isotech, Cheshire, UK).

To obtain plant water samples for  $\delta\text{D}$  analysis, stems 45 mm in length and 8 mm in diameter were cut from the two shrub species (DOY 166, 194, 222, 236, and 264) and

the grass *S. viridula* (DOY 166 and 194) on both hillslopes. For *S. viridula*, several 45 mm-long cuttings were obtained from living, non-photosynthetic tissue near the contact point between the base of the blades and the stem. From both slope aspects, three cuttings per species were placed in glass tubes sealed with rubber stoppers and paraffin wax. Stem samples were frozen before water extraction by cryogenic vacuum distillation. The H<sub>2</sub>O samples were then transferred to a glass tube with a rubber stopper and sealed with wax. Rainwater was also collected for analysis. All  $\delta$ D data were obtained by high precision laser spectroscopy (Lis et al. 2008), at the National Hydrology Research Centre (University of Saskatchewan). Stable isotope composition ( $\delta^{13}\text{C}$  and  $\delta$ D) is expressed in delta notation, from the ratio,  $R$  ( $^{13}\text{CO}_2$ : $^{12}\text{CO}_2$  or D/H) as:

$$[6] \quad \delta = \left[ \frac{R_{\text{sample}}}{R_{\text{std}}} - 1 \right] \cdot 1000 \%,$$

where  $R_{\text{std}}$  is the molar ratio of the international standards [Pee Dee Belemnite for  $\delta^{13}\text{C}$  (Craig 1957), and Vienna standard mean ocean water for  $\delta$ D (VSMOW; de Laeter et al. 2003)].

### Statistical analysis

For each species and date, between-slope differences in photosynthetic gas exchange characteristics, reflectance, leaf N,  $\delta^{13}\text{C}$ , and  $\delta$ D were assessed using Student's  $t$  tests. Analysis of variance (ANOVA) was used to assess overall between-slope differences in seasonal patterns of gas exchange, reflectance, and fluorescence variables. The analysis was performed with DOY input as the repeated measure, slope aspect specified as a fixed effect, aspect  $\times$  DOY interaction included, and post-hoc evaluation of differences assessed by Tukey's HSD test.

## Results

### Plant and leaf traits

The two shrub species were both short statured (Table 1). *Rhus trilobata* had a greater horizontal than vertical extent, and did not differ in size between slopes. *Artemisia cana*, a narrower plant with more vertically oriented shoots, was considerably larger on the north-facing slope. Branch increment was higher on the north-facing slope and was higher in *A. cana* than *R. trilobata*. The grasses *A. cristatum* and *S. viridula* grew in tufts with maximum heights of  $61 \pm 2$  cm and  $75 \pm 5$  cm, respectively.

Specific leaf area (SLA) was highest in the grass *A. cristatum* and decreased with DOY in all species (Table 2). The thin, white-haired leaves of *A. cana* were amphistomatous, as were leaves of both grasses. Of these species, *S. viridula* had the highest stomatal density. Abaxial stomatal density was also high in the hypostomatous, compound leaves of *R. trilobata*.

### Environmental conditions on contrasting slope aspects

Consistent with the higher incident solar radiation on the south-facing slope, higher maximum near-surface air temperature ( $T_a$ ) occurred on the south-facing slope from July to September, and higher near-surface VPD was observed in all months (Table 3). Differences were largest on dates with

high maximum  $T_a$ , because these were usually days with intense spectral radiation (Fig. 2). In contrast with the near-surface differences, neither maximum  $T_a$  nor maximum VPD differed significantly between slope aspects at a height of 1 m. Volumetric soil moisture ( $\theta_v$ ) decreased seasonally from near  $0.2 \text{ m}^3\text{-m}^{-3}$  in May, to asymptotic minima below  $0.1 \text{ m}^3\text{-m}^{-3}$  in late summer (Table 3).

The growth season of 2007 was warmer and drier than the historical climate mean. From 1 May to 30 September the mean maximum 2 m  $T_a$  was  $24.4 \text{ }^\circ\text{C}$ , or  $1.8 \text{ }^\circ\text{C}$  warmer than the 1971–2000 mean. Total precipitation from May to September was 177 mm, or 71% of the 1971–2000 mean (Environment Canada 2010). The deviation from climate normals was the result of very warm ( $2.8 \text{ }^\circ\text{C}$  above normal) and dry (30% of normal precipitation) conditions from June to August. July 2007 remains the warmest and driest month in the 1938–2010 Lethbridge Airport climate record, with a mean daily maximum  $T_a$  of  $31.2 \text{ }^\circ\text{C}$  ( $5.6 \text{ }^\circ\text{C}$  above normal) and total precipitation of 2.0 mm.

### Photosynthetic gas exchange at peak season

Net PPFD-saturated photosynthesis ( $A_{\text{max}}$ ) and stomatal conductance ( $g_s$ ) differed at peak season among species, but not between slope aspects (Table 4). On the south-facing slope, peak  $A_{\text{max}}$  and  $g_s$  were higher in the grasses than in the hypostomatous shrub *R. trilobata*. Peak  $A_{\text{max}}$  was also higher in *A. cana* than in *R. trilobata* on the south-facing slope. On the north-facing slope, peak  $A_{\text{max}}$  and  $g_s$  did not differ among species, except for a tendency for higher  $A_{\text{max}}$  in the grass *S. viridula* than in *R. trilobata*. Leaf  $\delta^{13}\text{C}$  was highest in *R. trilobata* on both slope aspects, and did not differ between slopes at peak season, except for a tendency for higher  $\delta^{13}\text{C}$  in leaves of *A. cana* growing on the south-facing slope (\*,  $P < 0.10$ ).

### Seasonal patterns of leaf reflectance

Seasonal patterns of leaf reflectance differed between shrubs and grasses. Leaf NDVI showed very little seasonal change in the shrubs, aside from a slight decline during spring in *A. cana*, which had the lowest values, because of its reflective leaf hairs (Fig. 3a). In the grass species, NDVI also remained relatively constant before declining sharply upon dormancy in July, first in *A. cristatum* and later in *S. viridula*. Leaf CI decreased slightly with DOY in *A. cana*, but reached maximum values in July in *R. trilobata*, despite intensifying drought. In the grasses, CI declined slightly with DOY before dormancy. No consistent between-slope differences were observed in either greenness index, although maximum values were slightly higher in north-facing specimens of *A. cristatum*. Leaf PRI decreased with DOY in the two grass species, and to a lesser extent in the shrub *A. cana*. By contrast, maximum values of PRI were observed in July in *R. trilobata*. Leaf PRI generally did not differ between slope aspects in the grasses, but became lower in south-facing specimens of the two shrub species in late summer.

At the canopy scale, NDVI and CI increased in shrubs during spring, as leaf area increased (Fig. 3b). Maximum values did not differ significantly between slope aspects, but both indices became much lower in shrubs of the south-facing slope in response to progressive summer drought.

**Table 1.** Structural traits of the 20 sampled shrubs, in September 2007.

Characteristic	Aspect	<i>Artemisia cana</i>	<i>Rhus trilobata</i>
Plant height (cm)	North facing	82±5***	71±7
	South facing	57±5	68±12
Canopy width (cm)	North facing	108±11***	281±35*
	South facing	64±5	232±21
Basal stem diameter (mm)	North facing	44±4***	25±2
	South facing	15±2	26±3
Branch increment (mm)	North facing	108±19**	78±7**
	South facing	78±9	48±10

**Note:** Values represent means ± SE;  $N = 5$  for each combination of species and aspect. Significant between-slope differences are indicated as follows: \*,  $P < 0.10$ ; \*\*,  $P < 0.05$ ; and \*\*\*,  $P < 0.01$ .

**Table 2.** Shrub and grass leaf traits, including leaf size, specific leaf area, and stomatal density.

Characteristic	Origin	Shrubs		Grasses	
		<i>Artemisia cana</i>	<i>Rhus trilobata</i> *	<i>Agropyron cristatum</i>	<i>Stipa viridula</i>
Leaf size (cm <sup>2</sup> )	North facing	1.9±0.1	2.8±0.1	5.7±0.6	7.4±0.9
	South facing	1.3±0.1	2.4±0.2	4.7±0.6	7.1±1.8
SLA <sub>max</sub> (cm <sup>2</sup> ·g <sup>-1</sup> )	North facing	111.9±4.9	118.5±1.7	180.1±7.3	92.2±14.6
	South facing	123.4±8.2	115.1±3.2	153.8±15.0	113.0±12.6
SLA <sub>min</sub> (cm <sup>2</sup> ·g <sup>-1</sup> )	North facing	67.5±9.2	73.5±1.5	128.5±13.7	79.6±10.2
	South facing	68.3±1.6	75.5±4.1	118.4±12.9	74.4±3.5
Stomata·mm <sup>-2</sup>	Abaxial	117±10	193±20	125±7	191±74
	Adaxial	104±11	0±0	93±48	147±12

**Note:** Leaf size and specific leaf area were measured in study specimens of contrasting slope aspects. Stomatal density was calculated from leaves of greenhouse-grown plants taken from the study site. SLA<sub>max</sub>, spring maximum specific leaf area; SLA<sub>min</sub>, summer minimum specific leaf area. Values represent means ± SE;  $N = 5$  for each combination of species and aspect for leaf size and SLA, and also for each combination of species and leaf surface for stomatal density.

\**R. trilobata* leaves are compound, and the leaf size indicated is that of the middle (largest) leaf.

Higher NDVI and CI were also observed on the north-facing slope in grasses. This between-slope difference increased over time in *S. viridula* and decreased with DOY in *A. cristatum*, an invasive species with earlier completion of annual growth cycles (Vaness and Wilson 2007). As observed at the leaf scale, canopy PRI was higher in shrubs of the north-facing slope in late summer. Canopy PRI did not differ between slope aspects in grasses, but decreased with DOY in *A. cristatum* well before dormancy.

Broad agreement was observed between canopy and aerial NDVI, especially on the north-facing slope. Airborne NDVI was lower than canopy values on the south-facing slope, where the canopy is more open. This is likely due to the effects of background soil and dead biomass. The aerial image accurately revealed patches of elevated productivity on the south-facing slope, wherever there were shrubs or hydrologically favourable, southeast-facing terrain (Fig. 1).

### Photosynthetic activity during drought

Photosynthesis continued through late September in the two shrubs, although  $A_{\max}$  temporarily reached zero on the south-facing slope in July and August (Fig. 4). Significant between-slope differences in seasonal patterns of photosynthetic gas exchange were observed in shrubs, but not grasses. Between May and late June,  $A_{\max}$  became much lower in shrubs of the south-facing slope, because of strong reductions in  $g_s$  that led to lower  $E$ , despite increasing LAVD. On the north-facing slope,  $\theta_v$  was higher, while leaf

temperature ( $T_l$ ) and LAVD were lower. Under these conditions,  $g_s$  and  $E$  peaked in late June, so that  $A_{\max}$  increased in *R. trilobata*, and was maintained at high levels in *A. cana*, despite decreasing leaf N content.

In contrast with the late season rebound observed in shrubs,  $A_{\max}$  decreased rapidly with DOY in grasses of both slope aspects, until dormancy was reached in July. Net photosynthesis declined slightly later in *S. viridula* than in *A. cristatum*, in which  $g_s$  increased until early June. However,  $A_{\max}$  still declined under conditions of increasing LAVD and decreasing leaf N (Fig. 4). For the growing season as a whole, shrubs exhibited higher  $A_{\max}$ ,  $g_s$ , and  $E$  on the north-facing slope (repeated measures ANOVA;  $P < 0.01$ ; Table 5). Of the two grasses, no between-slope differences were observed in *A. cristatum*, while *S. viridula* exhibited somewhat higher overall  $A_{\max}$  on the north-facing slope ( $P = 0.05$ ).

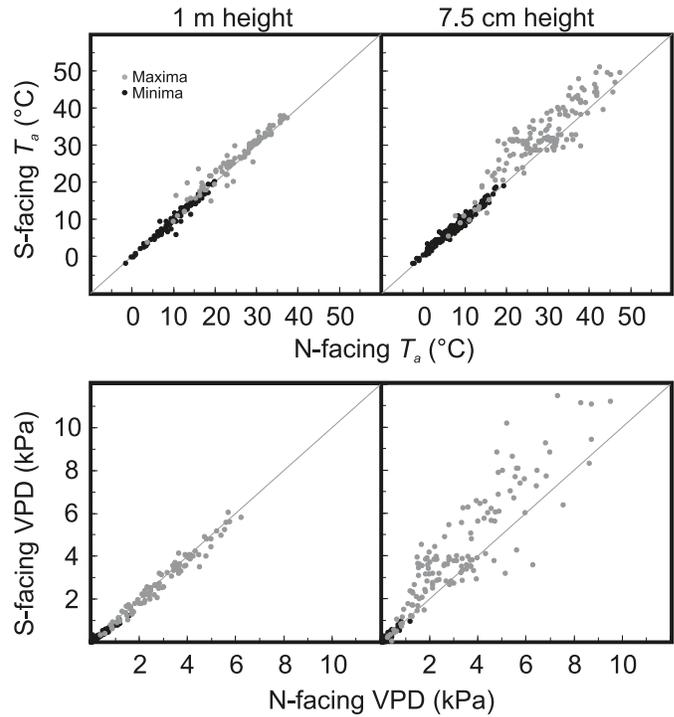
### Water-use efficiency

Water-use efficiency (WUE;  $A_{\max}/E$ ) decreased with LAVD in a logarithmic pattern that did not differ among species or between slope aspects (Fig. 5a). No relationship was observed between intrinsic water-use efficiency (WUE<sub>i</sub>;  $A_{\max}/g_s$ ) and LAVD in shrub species, and only weak, negative relationships were observed in the grass *S. viridula*, and in grasses overall ( $r^2 = 0.31$ ). In contrast with the effect of LAVD, WUE was poorly correlated with  $\theta_v$  and WUE<sub>i</sub> decreased logarithmically with  $\theta_v$  in shrubs, but not grasses

**Table 3.** Comparison of environmental conditions on north- and south-facing slopes

Variable	Aspect	May	June	July	August	September	Overall
$K_{\downarrow TOA}$	North facing	26.5 (0.71)	32.3 (0.82)	30.4 (0.79)	20.9 (0.61)	5.7 (0.21)	23.2 (0.63)
	South facing	37.1 (1.00)	36.4 (0.92)	36.4 (0.95)	37.1 (1.08)	37.1 (1.37)	36.8 (1.06)
$T_a$ (1 m)	North facing	19.2±5.1	24.1±5.0	31.6±3.9	25.1±5.2	19.6±7.1	25.3±7.4
	South facing	20.3±5.1	25.0±4.9	32.4±3.8	26.4±5.0	20.8±7.3	26.4±7.3
$T_a$ (7.5 cm)	North facing	23.6±6.9	27.8±6.3	39.7±4.1	30.1±5.0	22.6±8.1	28.6±9.0
	South facing	26.4±7.3	30.0±4.1	42.4±5.8**	35.6±5.2***	28.0±10.4**	32.2±9.3***
VPD (1 m)	North facing	1.8±0.8	2.2±0.8	4.1±1.1	3.2±1.1	2.2±1.2	3.0±1.4
	South facing	2.0±0.8	2.4±0.9	3.9±1.1	2.7±1.1	2.1±1.2	2.9±1.4
VPD (7.5 cm)	North facing	2.1±1.0	2.5±1.1	6.1±1.6	4.0±1.4	2.3±1.5	3.3±2.0
	South facing	2.6±1.2*	3.2±0.9**	7.6±2.5***	5.1±1.7***	3.6±2.3**	4.3±2.6***
$\theta_v$ (-40 cm)	North facing	0.23±0.02	0.18±0.03	0.13±0.00	0.11±0.00	0.10±0.00	0.10±0.00
	South facing	0.18±0.02	0.11±0.02	0.07±0.00	0.06±0.00	0.06±0.00	0.06±0.00

**Note:**  $K_{\downarrow TOA}$ , daily top-of-atmosphere direct solar radiation, expressed in  $\text{MJ m}^{-2}\text{d}^{-1}$  on a  $40^\circ$  slope of the indicated aspect, and, in parentheses, as a proportion of  $K_{\downarrow TOA}$  incident on a level plane;  $T_a$ , maximum air temperature ( $^\circ\text{C}$ ); VPD, maximum atmospheric vapour pressure deficit (kPa);  $\theta_v$ , volumetric soil moisture ( $\text{m}^3\text{m}^{-3}$ ). \*, \*\*, and \*\*\*, indicate significantly higher  $T_a$  or VPD values than on opposing slope aspects at  $P < 0.10$ ,  $P < 0.05$ , and  $P < 0.01$ , respectively (Student's  $t$  test). Values represent means  $\pm$  SD.

**Fig. 2.** Scatterplots of north- vs. south-facing maximum and minimum daily air temperature ( $T_a$ ) and vapour pressure deficit (VPD) during the 2007 growing season, compared to a 1:1 line.

(Fig. 5b). Similarly,  $C_i/C_a$  decreased in response to low  $\theta_v$  in shrubs, but no change was observed in grasses (Fig. 6). Without tight stomatal control of water loss in response to drought,  $A_{\max}$  decreased at least as steeply as  $g_s$  in the grasses.

Stable carbon isotope composition ( $\delta^{13}\text{C}$ ) was higher in *R. trilobata* than in *A. cana* or the two grass species (Tables 4 and 5). There was a tendency for slightly higher  $\delta^{13}\text{C}$  on south-facing slopes, but differences were not significant on individual dates ( $t$  tests,  $P > 0.05$ ) or for the season as a whole (repeated measures ANOVA,  $P > 0.05$ ; Table 5). Whereas stomatal behaviour varied between functional groups, there was no clear within-species evidence of between-slope differences in the fundamental relationships between photosynthetic gas exchange characteristics and either LAVD or  $\theta_v$  (Fig. 6).

### Cumulative water use

Seasonal patterns of sapflow ( $J_s$ ) were consistent with variations in leaf transpiration ( $E$ ). In both *A. cana* and *R. trilobata*, daytime  $J_s$  (1100–1600 h) and  $E$  were higher in July than in September on the north-facing slope (Fig. 7). In mid-July (DOY 193–203), daytime  $J_s$  decreased by 52% and 49% in *A. cana* and *R. trilobata*, respectively. This is consistent with 52% and 27% decreases in  $E$ , at saturating PPFD intensity, between DOY 192 and 208 (Fig. 4). In September (DOY 248–258), a trend of increasing  $J_s$  was observed in *A. cana*, but not *R. trilobata* (DOY 248–258). Similarly,  $E$  increased following September rains in *A. cana*, but not *R. trilobata*. By this time, strong afternoon depression of  $J_s$  was observed in *R. trilobata*, whereas the daytime pattern remained more parabolic in *A. cana* (Fig. 7).

**Table 4.** Net PPFD-saturated leaf photosynthesis rates ( $A_{\max}$ ;  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ), stomatal conductance ( $g_s$ ;  $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) and stable carbon isotope composition ( $\delta^{13}\text{C}$ ; ‰) of two grasses and two shrubs at peak season.

Variable	Aspect	Shrubs			Grasses			ANOVA	
		<i>A. cana</i>	<i>R. trilobata</i>	<i>A. cristatum</i>	<i>S. viridula</i>	F	P	$P < 0.05^a$	
$A_{\max}$	North facing	19.4±3.0	16.8±3.0	19.1±2.7	32.4±6.3	3.1	0.06	No differences	
	South facing	22.1±2.4	11.0±0.7	21.1±2.2	22.9±3.8	3.7	0.04	SV,ACr,AC>RT	
$g_s$	North facing	440±98	264±40	322±86	424±122	0.8	0.49	No differences	
	South facing	299±5	138±29	326±31	361±70	4.2	0.03	SV,ACr>RT	
$\delta^{13}\text{C}$	North facing	-29.3±0.4*	-26.9±0.6	-29.1±0.6	-28.2±0.4	3.4	0.05	AC>RT	
	South facing	-27.7±0.5	-26.4±0.5	-28.6±0.4	-27.9±0.4	6.7	0.00	ACr>RT	

**Note:** \*,  $P < 0.10$ . For  $A_{\max}$  and  $g_s$ , peak season was defined as the DOY with highest  $A_{\max}$  for each species. Because of the integrative nature of leaf  $\delta^{13}\text{C}$ , values are reported for dates beyond peak season (DOY 162 for grasses and DOY 192 for shrubs).  $N = 5$  for each combination of species and aspect. AC, *Artemisia cana*; ACr, *Agropyron cristatum*; RT, *Rhus trilobata*; SV, *Stipa viridula*.

<sup>a</sup>Tukey's HSD post hoc test.

Using daily courses of  $J_s$  to define hourly changes in  $E$ , mean daily cumulative water use (CWU) of fully sunlit leaves was estimated at  $3.7 \text{ L}\cdot(\text{m leaf area})^{-2}\cdot\text{d}^{-1}$  in *A. cana* and  $4.1 \text{ L}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  in *R. trilobata* on north-facing slopes in early July (DOY 193–203). Because the daytime pattern was approximately parabolic, these values are proportional to observed  $E$  for the two species for the same dates. This was the only portion of the growth season and slope aspect for which  $E$  was slightly higher in *R. trilobata* than *A. cana* (Fig. 4). In September (DOY 248–258), strong patterns of late afternoon  $g_s$  depression were observed in *R. trilobata*, but not *A. cana*. Estimated CWU during this period was  $2.2 \text{ L}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  in *A. cana*, but only  $0.7 \text{ L}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  in *R. trilobata*, with an increase over time in the former. Assuming that the proportionality of photosynthetic carbon gain and water use is approximately constant among species below PPFD saturation, and using a 12-week growing season for grasses and a 22-week growing season for shrubs, the mean shrub and grass  $A_{\max}$  and  $E$  values of Table 5 indicate that total leaf photosynthesis was approximately 15% higher in shrubs and grasses on a per-unit area basis on the north-facing slope, while  $E$  was approximately 42% higher. On the south-facing slope, total estimated leaf photosynthesis was 24% higher in grasses than in shrubs, while transpiration was 28% higher.

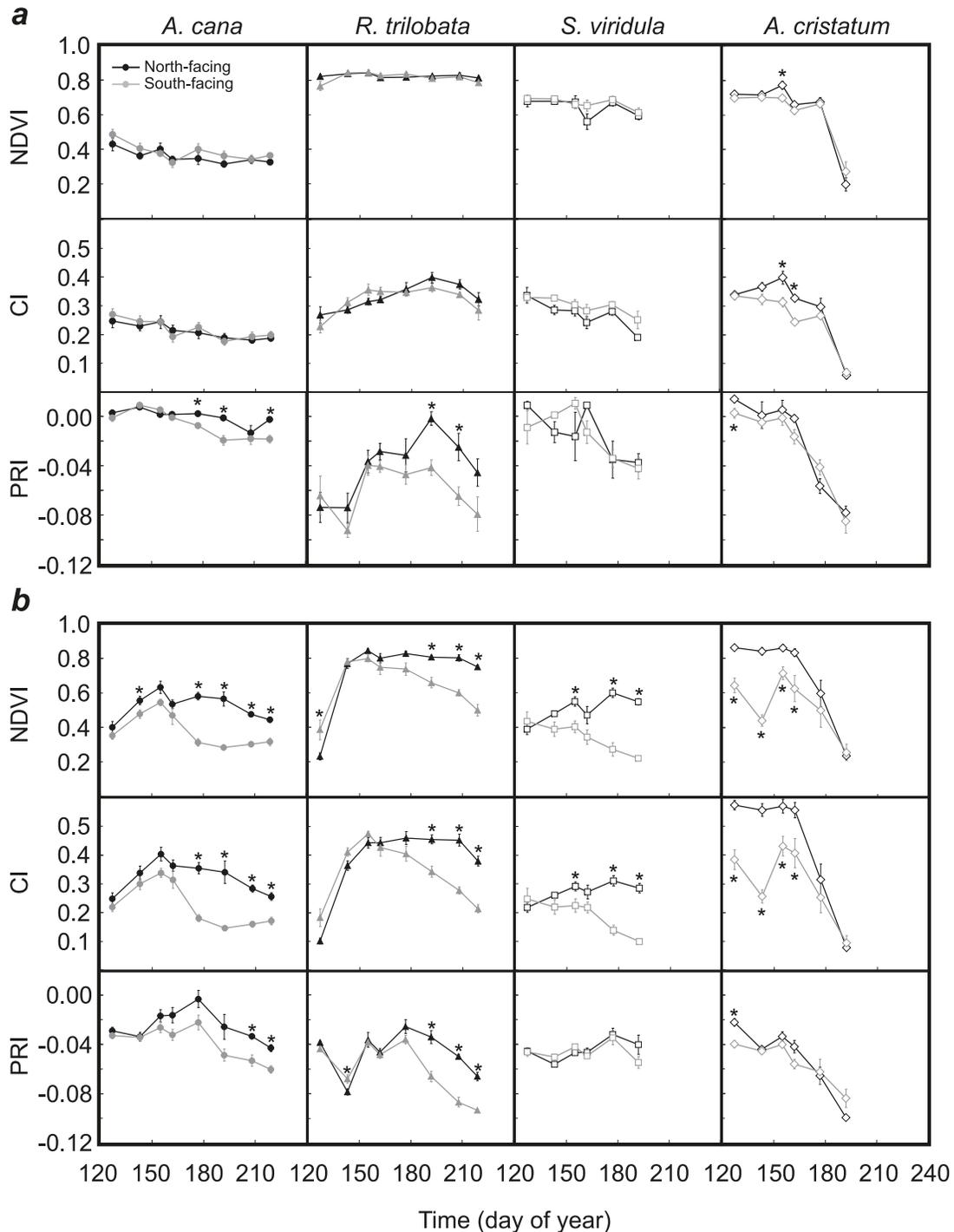
### Water source use

Stem water  $\delta\text{D}$  was similar to that of rainwater in June. As drought progressed, the stem water  $\delta\text{D}$  of shrubs decreased relative to rainwater (Fig. 8). By contrast,  $\delta\text{D}$  did not decrease from DOY 166–194 in the grass species *S. viridula*. Lower stem water  $\delta\text{D}$  was observed in *R. trilobata* shrubs on the south-facing slope (Table 5), with values as low as  $-172.2 \pm 3.6\text{‰}$  during the most intense portion of the drought (DOY 222). At the site of a coulee slump event in south Lethbridge, soils were exposed to a depth of 2.15 m, revealing roots of *A. cana* and *R. trilobata* throughout this depth range. Both species exhibited vigorous lateral and vertical root growth, but *R. trilobata* produced a particularly dense assortment of tap roots, in addition to roots and rhizomes several metres in length, roughly parallel to the slope. *A. cana* roots exhibited extensive clonal propagation upon exposure.

### Discussion

Water availability is the primary factor limiting plant growth in semiarid ecosystems (Flanagan et al. 2002; Chen et al. 2005). In temperate grasslands, plants must cope with high interannual precipitation variability (Yuan et al. 2009), to ensure growth and survival to reproductive maturity. Contrasting adaptive strategies have evolved in response to these conditions and are sorted by life form. Our results show that common  $\text{C}_3$  grasses and shrubs respond differently to drought in a northern semiarid ecosystem. Shrubs allowed  $C_i/C_a$  to decrease to promote  $\text{WUE}_i$  as soil moisture ( $\theta_v$ ) declined, whereas grasses exhibited no such acclimation. Net PPFD-saturated photosynthesis,  $E$ ,  $g_s$ , and PRI were lower on the south-facing slope in shrubs at midsummer, whereas grasses exhibited no between-slope differences before early dormancy. The dis-

**Fig. 3.** Seasonal patterns of (a) leaf reflectance and (b) canopy reflectance, including the normalized difference reflectance index (NDVI), chlorophyll index (CI), and photochemical reflectance index (PRI). Daily leaf or canopy reflectance values represent the mean and standard error of five measurements. Asterisks (\*) indicate significant between-slope differences (Student's *t* tests; *P* < 0.05).

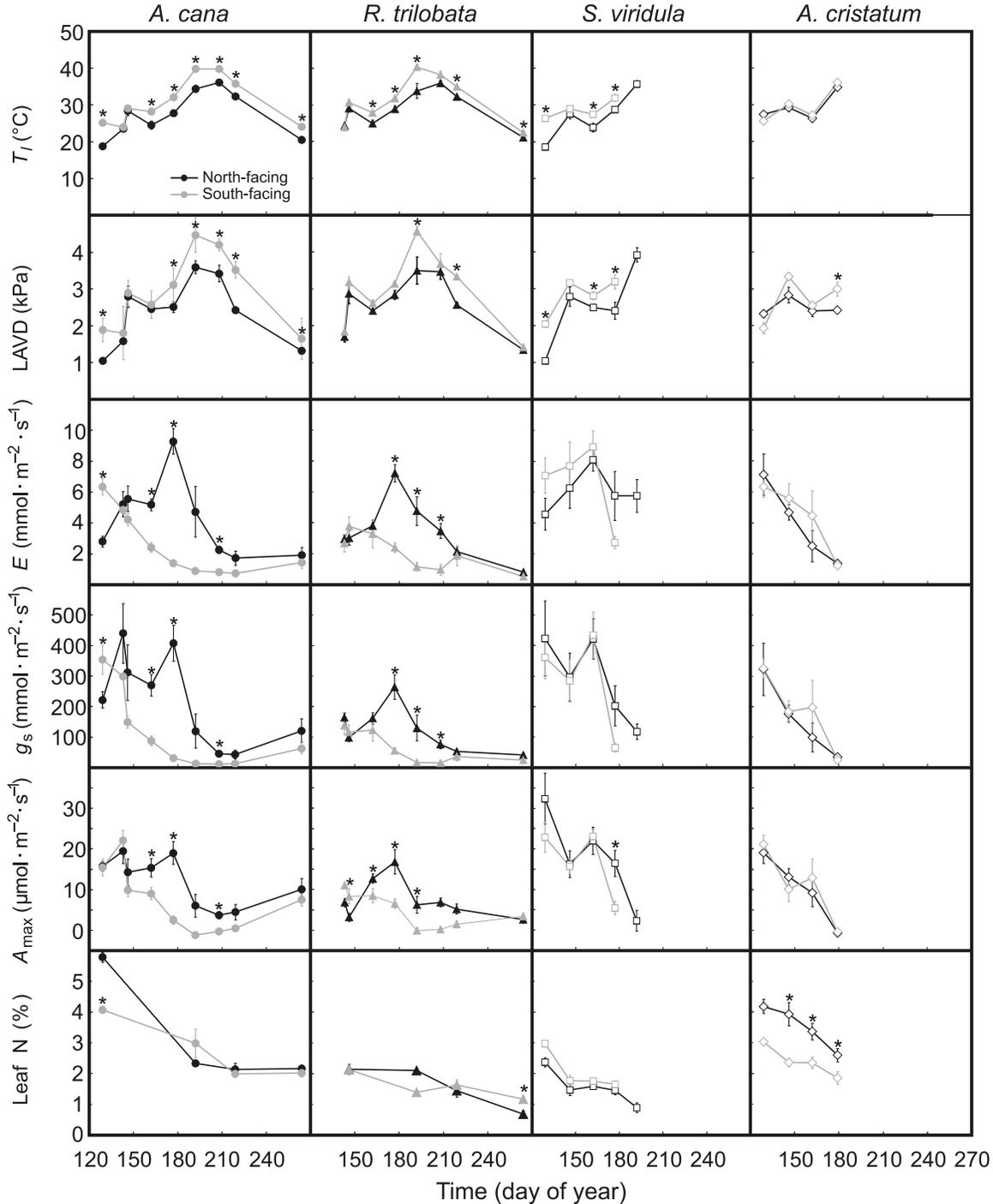


tinct photosynthetic water-use strategies of these two life forms complemented plant structural characteristics. Shrubs used deep water resources and strong physiological acclimation to increase drought tolerance, while grasses avoided drought by maximizing gas exchange rates and completing the annual growth cycle during a short period of water availability.

#### Seasonality of leaf gas exchange

The contrasting acclimatory and structural characteristics of woody shrubs and grasses explain differences observed in the seasonality of photosynthetic gas exchange on opposing slope aspects in a drought year. Grasses exhibited high photosynthetic activity ( $A_{max}$ ,  $g_s$ , and  $E$ ) and reflectance-based indicators of photosynthetic capacity (CI and PRI) in

**Fig. 4.** Seasonal patterns of leaf chamber environmental conditions [leaf temperature ( $T_l$ ) and leaf-to-atmosphere vapour pressure deficit (LAVD)], photosynthetic gas exchange characteristics [transpiration ( $E$ ), stomatal conductance ( $g_s$ ), and net PPFD-saturated photosynthesis ( $A_{max}$ )], and leaf N content. Data points represent the mean and standard error of five measurements. Asterisks (\*) indicate significant between-slope differences (Student's  $t$  tests;  $P < 0.05$ ).



spring, when  $\theta_v$  was high. By contrast, peak photosynthetic activity did not occur in shrubs of the north-facing hillslope until June, nearly 2 months after the highest values of  $\theta_v$  were observed at a depth of 40 cm. Furthermore, leaf CI and PRI increased through early July in *R. trilobata*, and both shrubs showed strong increases in canopy scale NDVI, CI, and PRI. This demonstrates that shrubs had developed

the leaf area and photosynthetic capacity required to maximize carbon gain by the time that competition from grasses had been strongly reduced by dormancy.

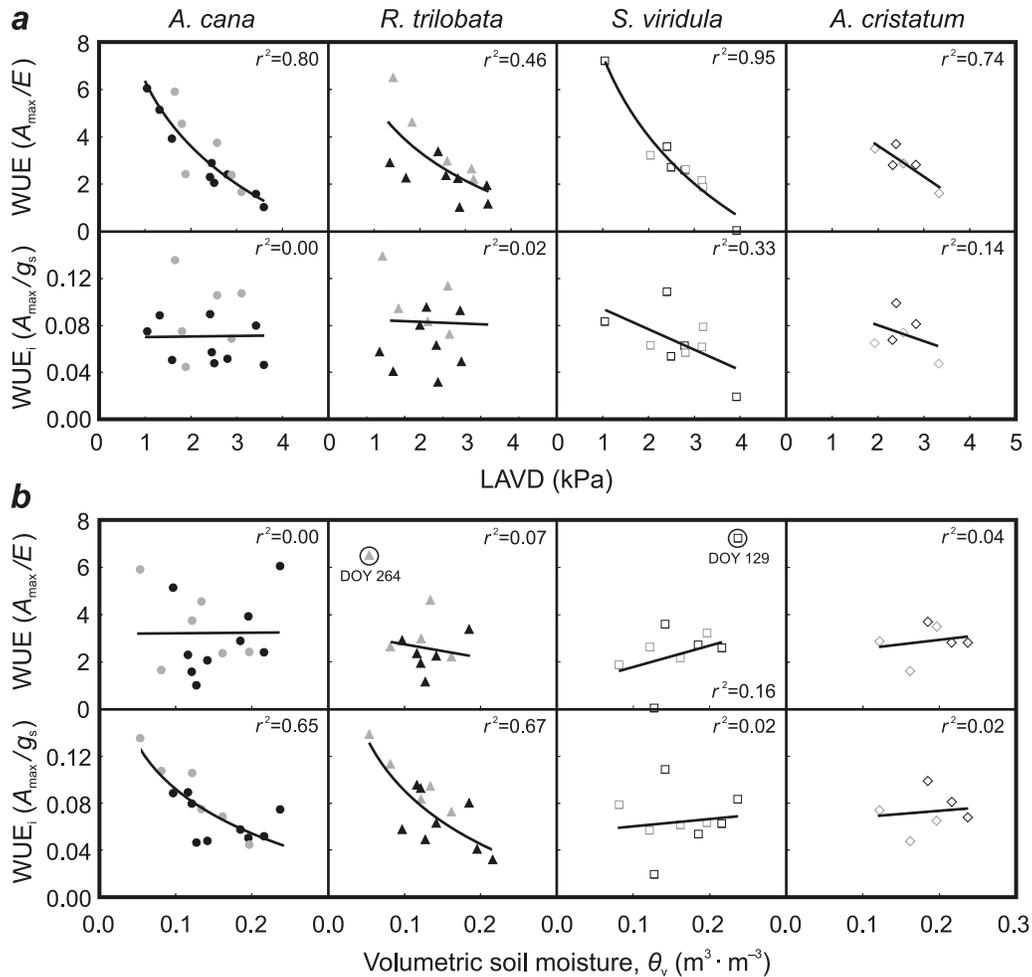
Seasonal decreases in shrub stem water  $\delta\text{D}$  indicate that the moisture enabling leaf survival through extreme drought conditions was derived from deep water sources supplied substantially by cold season precipitation. This was espe-

**Table 5.** Results of repeated measures analysis of variance (ANOVA) on photosynthetic gas exchange characteristics ( $A_{\max}$ ,  $g_s$ , and  $E$ ), including between-slope differences and slope  $\times$  day of year (DOY) interaction.

Variable	Species	Between-slope differences (LSM $\pm$ SE)				Slope $\times$ DOY interaction		
		North-facing	South-facing	$F$ ratio	$P$	df	$F$ ratio	$P$
$A_{\max}$ ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	AC	12.0 $\pm$ 0.7	7.3 $\pm$ 0.7	23.8	<0.01*	8	3.1	<0.01*
	RT	7.5 $\pm$ 0.5	4.9 $\pm$ 0.5	12.7	<0.01*	7	7.2	<0.01*
	ACr	10.1 $\pm$ 1.3	10.9 $\pm$ 1.3	0.2	0.66	3	0.6	0.61
	SV	21.8 $\pm$ 1.7	16.8 $\pm$ 1.7	4.2	0.05*	3	1.6	0.22
$g_s$ ( $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	AC	221 $\pm$ 15	114 $\pm$ 15	25.5	<0.01*	8	4.8	<0.01*
	RT	123 $\pm$ 8	66 $\pm$ 8	24.3	<0.01*	7	5.2	<0.01*
	ACr	158 $\pm$ 25	183 $\pm$ 24	0.5	0.47	3	0.5	0.67
	SV	336 $\pm$ 38	286 $\pm$ 38	0.9	0.36	3	0.4	0.77
$E$ ( $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	AC	4.3 $\pm$ 0.2	2.6 $\pm$ 0.2	34.7	<0.01*	8	11.9	<0.01*
	RT	3.5 $\pm$ 0.2	2.1 $\pm$ 0.2	27.3	<0.01*	7	7.0	<0.01*
	ACr	3.9 $\pm$ 0.5	4.4 $\pm$ 0.4	0.6	0.45	3	0.9	0.44
	SV	6.2 $\pm$ 0.6	6.6 $\pm$ 0.6	0.3	0.59	3	2.2	0.11
NDVI	AC	0.358 $\pm$ 0.008	0.377 $\pm$ 0.008	2.7	0.10	8	1.1	0.40
	RT	0.817 $\pm$ 0.005	0.813 $\pm$ 0.005	0.3	0.56	8	1.4	0.21
	ACr	0.624 $\pm$ 0.011	0.611 $\pm$ 0.011	0.8	0.38	5	1.8	0.14
	SV	0.643 $\pm$ 0.011	0.668 $\pm$ 0.011	2.4	0.13	5	0.9	0.49
CI	AC	0.211 $\pm$ 0.005	0.215 $\pm$ 0.005	0.3	0.56	8	0.5	0.87
	RT	0.321 $\pm$ 0.008	0.314 $\pm$ 0.008	0.4	0.52	8	1.0	0.47
	ACr	0.298 $\pm$ 0.007	0.259 $\pm$ 0.007	18.4	<0.01*	5	3.1	0.02*
	SV	0.269 $\pm$ 0.008	0.300 $\pm$ 0.007	7.9	0.01*	5	0.8	0.58
PRI	AC	-0.002 $\pm$ 0.001	-0.007 $\pm$ 0.001	17.2	<0.01*	8	3.6	<0.01*
	RT	-0.044 $\pm$ 0.004	-0.062 $\pm$ 0.004	12.0	<0.01*	8	1.3	0.27
	ACr	-0.019 $\pm$ 0.003	-0.024 $\pm$ 0.003	1.8	0.18	5	1.3	0.27
	SV	-0.014 $\pm$ 0.004	-0.014 $\pm$ 0.004	0.0	0.94	5	1.8	0.14
WBI	AC	1.025 $\pm$ 0.001	1.012 $\pm$ 0.001	89.5	<0.01*	8	9.9	<0.01*
	RT	1.029 $\pm$ 0.001	1.022 $\pm$ 0.001	66.9	<0.01*	8	29.4	<0.01*
	ACr	1.010 $\pm$ 0.001	1.015 $\pm$ 0.001	8.2	0.01*	5	2.9	0.02*
	SV	1.025 $\pm$ 0.001	1.020 $\pm$ 0.001	4.3	0.04*	5	2.9	0.02*
$\delta^{13}\text{C}$ (‰)	AC	-28.54 $\pm$ 0.21	-28.23 $\pm$ 0.18	1.2	0.29	3	8.0	<0.01*
	RT	-26.71 $\pm$ 0.22	-26.25 $\pm$ 0.20	2.4	0.13	3	0.5	0.70
	ACr	-28.56 $\pm$ 0.29	-28.52 $\pm$ 0.29	0.0	0.93	3	0.6	0.60
	SV	-27.68 $\pm$ 0.21	-27.75 $\pm$ 0.21	0.1	0.81	3	2.4	0.08
$\delta\text{D}$ (‰)	AC	-127.1 $\pm$ 3.2	-128.9 $\pm$ 3.0	0.2	0.68	4	2.9	0.06
	RT	-129.8 $\pm$ 2.5	-145.2 $\pm$ 3.0	15.5	<0.01*	4	7.1	<0.01*
	SV	-124.2 $\pm$ 4.9	-130.7 $\pm$ 4.3	—	—	—	—	—

**Note:** For grass species, data were omitted beyond the last DOY of positive  $A_{\max}$  on either slope. Significant differences at  $P < 0.05$  are denoted by an asterisk (\*).  $N = 5$  for each species on each date. LSM, least squares mean; SE, standard error; AC, *Artemisia cana*; RT, *Rhus trilobata*; ACr, *Agropyron cristatum*; SV, *Stipa viridula*.

**Fig. 5.** The effect of (a) leaf-to-atmosphere vapour pressure deficit (LAVD) and (b) volumetric soil moisture content ( $\theta_v$ ) on water-use efficiency (WUE;  $A/E$ , in  $\text{mmol}\cdot\text{mol}^{-1}$ ) and intrinsic water-use efficiency (WUE<sub>i</sub>;  $A/g_s$ , in  $\text{mmol}\cdot\text{mol}^{-1}$ ) in shrubs and grasses of north-facing (black) and south-facing (grey) slopes. Data are not shown for dates with near-zero or negative  $A_{\text{max}}$ . Dates with very low LAVD (DOY 264 and 129) were not used in the calculation of  $\theta_v$  vs. WUE trend lines for *Rhus trilobata* and *Stipa viridula*. Data points shown are the mean of five measurements.



cially true in the case of *R. trilobata*, which had the lowest  $\delta D$ . Deep water access is important for woody shrubs, because of the ability of grasses to inhibit shrub growth by rapidly reducing soil moisture within their rooting depth (Clary et al. 2004). Fast acquisition of soil moisture from nutrient-rich A horizons may also present an advantage to grasses in terms of nitrogen capture, which is essential for maximizing photosynthetic capacity (Sala et al. 1989). However, *Artemisia* spp. tend to possess substantial rooting in both shallow and deep soils. This allows conservation of deep water by focusing water uptake in shallow layers when it is available, and extends the growth period during drought (Schwinning et al. 2002). Enhanced shallow root water uptake could explain the higher spring leaf N, stem water  $\delta D$ , peak  $E$ , and extended leaf display of *A. cana*, compared with *R. trilobata*. *Artemisia cana* also has a relatively open canopy and does not exclude grasses as effectively as *R. trilobata*. A higher density of shallow roots may, therefore, serve to improve competition for water resources with co-occurring grasses. To test these hypotheses, stem water  $\delta D$  should be collected from *A. cana* following

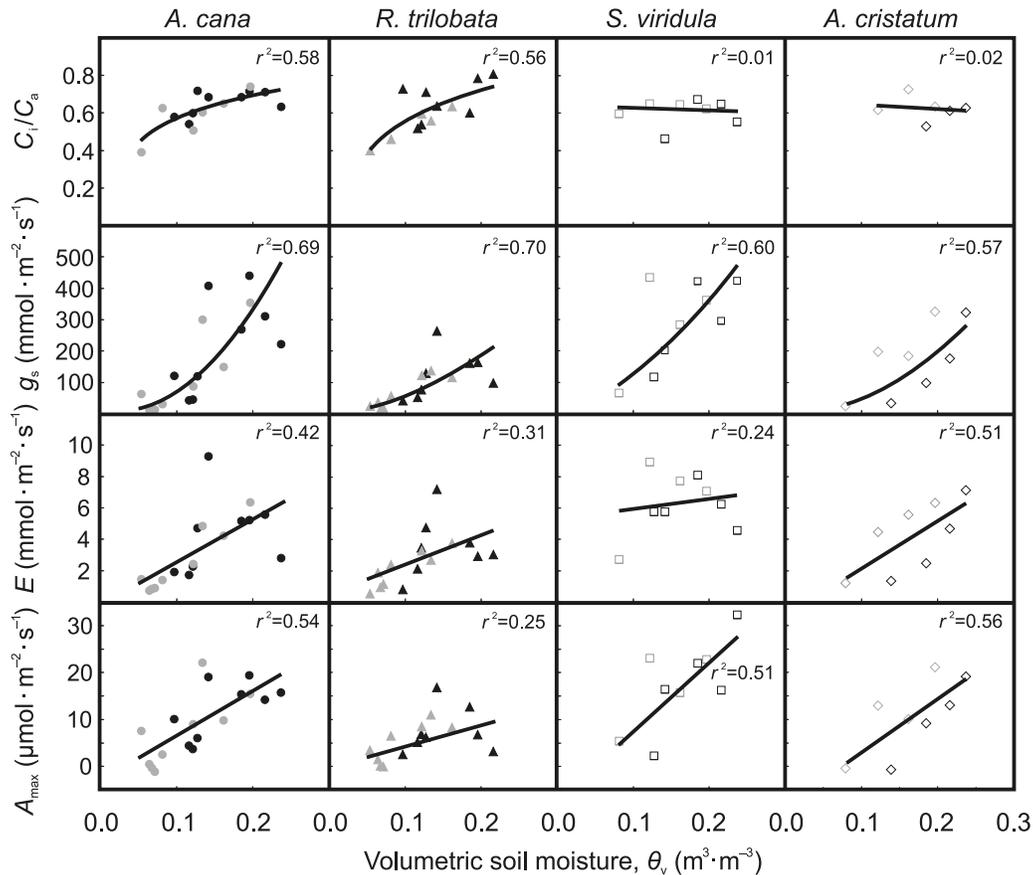
pulse rainfall events in late summer, and competition and removal studies should be carried out to examine the impact of grass standing crop on the growth of shrub species varying in shading and rooting characteristics.

#### Effects of slope aspect on leaf gas exchange

Shrubs and grasses responded differently to the lower  $\theta_v$ , higher LAVD, and higher leaf temperature of the south-facing slope. Stomatal conductance,  $A_{\text{max}}$ ,  $E$ , and PRI were lower on the south-facing slope in the shrubs, but no between-slope differences were observed in the grasses, aside from a slight extension of the growth period in *S. viridula*. These observations are consistent with the hypothesis that woody species minimize drought impacts through stomatal regulation (Jenerette et al. 2009), whereas standing crop responds more in grasses (Köchy and Wilson 2004).

Leaf NDVI and CI did not show consistent, directional differences between slope aspects. The same was true of PRI in grasses, but PRI became lower on the south-facing slope in shrubs, as drought intensified (Fig. 3a). This suggests that shrubs responded to photoinhibitory conditions on

**Fig. 6.** Relationship between volumetric soil moisture content ( $\theta_v$ ) and variables related to photosynthetic water-use efficiency, including the ratio of intercellular to atmospheric CO<sub>2</sub> concentrations ( $C_i/C_a$ ), stomatal conductance ( $g_s$ ), transpiration ( $E$ ), and net PPFD-saturated photosynthesis rate ( $A_{\max}$ ). Data from plants of the north- and south-facing slopes are shown in black and grey, respectively. Data points represent the mean of five measurements.



the south-facing slope by increasing the ratio of carotenoids to chlorophyll pigments in sun-exposed leaves, as part of an overall strategy to maintain leaf vigour through the summer. Given the lack of seasonal change of NDVI and CI in apparently healthy leaves, the late summer decrease in canopy greenness indices in shrubs of south-facing slopes (Fig. 3b) is likely the result of (i) partial, drought-induced senescence and (ii) dormancy of background grasses and forbs (see Fig. 1). Similarly, lower canopy NDVI and CI in south-facing grasses resulted from a higher proportion of dormant leaf area. The decrease in shrub canopy PRI on south-facing slopes suggests reduced net ecosystem exchange of carbon dioxide, based on recent results in semi-arid grasslands (Black and Guo 2008). The absence of between-slope differences in leaf or canopy PRI in grasses is consistent with a lack of drought stress acclimation in a plant with a growth strategy of drought avoidance rather than tolerance.

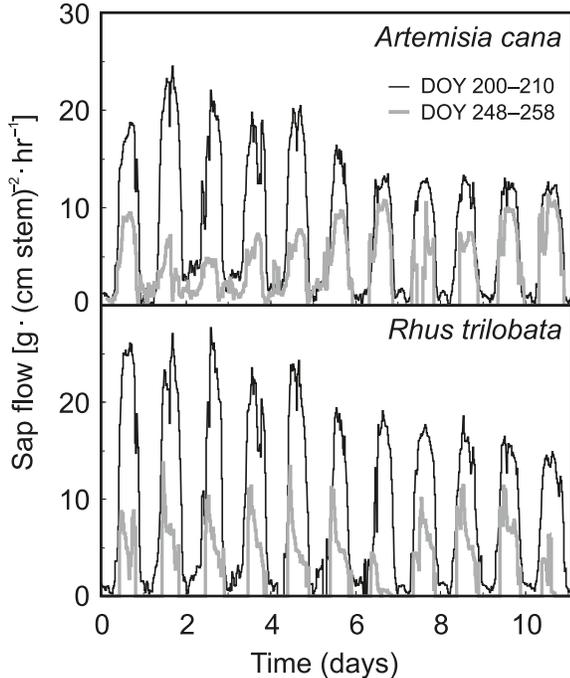
#### Water use by shrubs and grasses

Shrubs and grasses are considered to form distinct plant functional groups in terms of stable carbon isotope composition, with grasses exhibiting lower  $\delta^{13}\text{C}$  (Smedley et al. 1991; Chen et al. 2005). On this basis, the shrub *R. trilobata* was a more conservative water-user than either

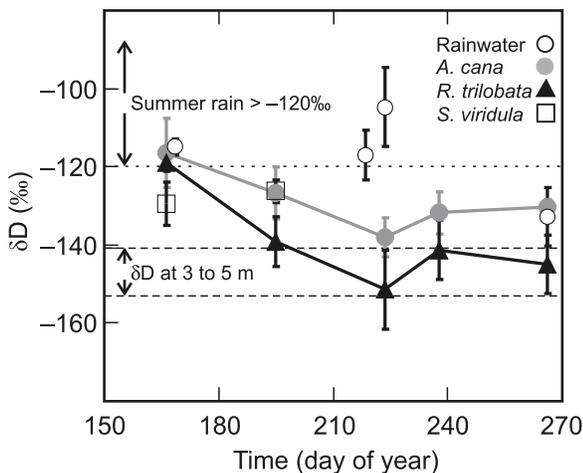
grass species, but *A. cana* exhibited very low WUE at optimal  $\theta_v$  (Letts et al. 2009a), resulting in low  $\delta^{13}\text{C}$  (Tables 4 and 5). Nevertheless, in response to drought stress, both shrubs showed a similar stomatal regulation pattern, which likely evolved to limit water loss and enable survival through many successive growing seasons. In both *A. cana* and *R. trilobata*, progressive reductions in  $\theta_v$  resulted in stronger reductions in  $g_s$  than  $A_{\max}$ , so that  $\text{WUE}_i$  ( $A_{\max}/g_s$ ) increased (Fig. 5b) and  $C_i/C_a$  declined (Fig. 6). Water-use efficiency ( $A_{\max}/E$ ) decreased with LAVD regardless of  $\theta_v$ . Unlike the pattern observed in shrubs,  $C_i/C_a$  and  $\text{WUE}_i$  were unaffected by soil moisture in grasses. The lack of  $C_i$  regulation in response to drought demonstrates the opportunistic growth and water use strategy of these  $\text{C}_3$  grasses.

Integrated over the growing season, estimated CWU and  $A$  were higher in shrubs than in grasses on the north-facing slope and higher in grasses than in shrubs on the south-facing slope. This suggests a competitive advantage for the dominant life form of each slope aspect and also explains a paradox regarding plant water use in  $\text{C}_3$  grasses and shrubs of northern semi-arid ecosystems. On a leaf area basis, shrubs showed greater relative performance in cool, moist microsites, despite stomatal control mechanisms favouring water-use efficiency during extreme drought. By contrast,  $\text{C}_3$  grasses showed higher productivity relative to

**Fig. 7.** Mean sap flow rates ( $J_s$ ) of three *Artemisia cana* and four *Rhus trilobata* shrubs in July (DOY 193–203) and September (DOY 248–258).



**Fig. 8.** Seasonal patterns of stable deuterium composition ( $\delta D$ ) in rainwater, and in stem water of the shrubs *Artemisia cana* and *Rhus trilobata*, and the grass *Stipa viridula* on the north-facing hill-slope. Typical values for summer rainfall (Peng et al. 2004) and for soil water at 3–5 m depth (Hendry 1988) are shown. Each data point represents the mean and standard error of three stems or water samples.



shrubs on the warmer, drier, south-facing slopes, despite their inability to withstand extreme summer drought. The reason for this apparent contradiction is that the low  $\theta_v$  and high LAVD on the xeric slope more than offset the benefit of using stomatal regulation to extend the period of active photosynthesis in deep-rooted plants. Phenotypic traits conferring low WUE and drought avoidance have previously been shown to increase fitness in certain species within desert (Donovan et al. 2007) and grassland ecosys-

tems (Zea-Cabrera et al. 2006). However, seasonal and between-slope differences in photosynthetic water-use vary among species and functional groups, as well as among years with contrasting environmental conditions. For example, in the Oldman River valley, water-use efficient CAM species [*Opuntia polyacantha* Haw. and *Escobaria vivipara* (Nutt.) Buxbaum] and  $C_4$  grasses [*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths] co-occur with  $C_3$  grasses and shrubs on the south-facing slope. This shows that conservative water use can also be suitable to xeric terrain in temperate grasslands. Coexistence of life forms with distinct water use strategies is likely related to temporal and spatial resource partitioning (Peters 2002). Furthermore, interannual differences in rainfall timing and intensity can alter plant performance among life forms (Gao and Reynolds 2003). Therefore, while this study reveals how distinct physiological drought acclimation mechanisms altered seasonal and between-slope differences in photosynthetic water use between two functional groups, our results are specific to the species examined and to progressive drought conditions. Further research is needed to assess the generality of these relationships among  $C_3$  grasses and woody shrubs in response to drought.

## Conclusions

In the northern Great Plains, shrub and grass life forms show contrasting leaf physiological responses to drought stress, which likely evolved because of the distinct requirements of plants differing in rooting depth, phenology, and growth season duration. Because of their need to tolerate successive growing seasons in a biome with high interannual precipitation variability, we hypothesized that two shrubs of a northern semiarid grassland would exhibit deep soil water uptake, high WUE, and strong stomatal regulation in response to drought stress.

Stable hydrogen isotope composition confirmed a deep source of stem water in both shrubs, but especially in *R. trilobata*. Leaf  $\delta^{13}C$  was higher in *R. trilobata* than in grasses, but *A. cana* also had low  $\delta^{13}C$ , indicating profligate water use. Slope aspect had no effect on fundamental species-specific relationships between environmental controls (LAVD and  $\theta_v$ ) and photosynthetic gas exchange characteristics, suggesting an absence of long-term drought acclimation on the south-facing slope. The key difference between the two plant functional groups was related to the behaviour of intrinsic water-use efficiency in response to decreasing  $\theta_v$ . Low  $\theta_v$  had no impact on  $WUE_i$  or  $C_i/C_a$  in grasses, but shrubs responded to decreasing  $\theta_v$  by lowering  $C_i/C_a$  through strong reductions in  $g_s$ , so that  $WUE_i$  increased. Along with deep rooting, this enabled shrubs to tolerate severe drought conditions, resulting in much higher cumulative water use and net photosynthesis on the north-facing slope. By contrast, the profligate water-use strategy of grasses permitted higher CWU and  $A$  than shrubs on the south-facing slope. These results demonstrate that topographic effects on photosynthetic water use are governed by complex interactions between physiological drought acclimation strategies, plant structural characteristics, and the timing of moisture availability.

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