

# Interspecific differences in photosynthetic gas exchange characteristics and acclimation to soil moisture stress among shrubs of a semiarid grassland<sup>1</sup>

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**Abstract:** Woody plants generally exhibit more conservative water-use characteristics than herbaceous perennials and annuals, including lower stomatal conductance, net photosynthesis, and transpiration, but higher photosynthetic water-use efficiency. Under soil moisture stress, stomatal conductance usually decreases more than net photosynthesis, resulting in lower ratios of internal to atmospheric CO<sub>2</sub> concentrations and higher photosynthetic water-use efficiency. However, we found that shrubs of a semiarid grassland employed opportunistic water-use strategies, which likely evolved due to the short duration of soil moisture availability. We measured photosynthetic gas exchange, leaf reflectance, and foliar stable carbon isotope composition in *Artemisia cana*, *Prunus virginiana*, *Rhus trilobata*, and *Ribes aureum* in southern Alberta. Under moist conditions, net photosynthesis was very high in *A. cana*, *R. trilobata*, and *R. aureum*, but less so in *P. virginiana*. Declining volumetric soil moisture caused stomatal conductance to decrease in proportion with net photosynthesis in 3 of 4 species (*P. virginiana*, *R. aureum*, and *R. trilobata*). Consequently, ratios of internal to atmospheric CO<sub>2</sub> were unaffected by soil moisture depletion, and photosynthetic water-use efficiency declined. At the leaf level, the normalized difference vegetation index did not vary with season in these species, while the chlorophyll index decreased only slightly and the photochemical reflectance index showed a small mid-season peak. *A. cana* had the lowest stable carbon isotope ratio, highest stomatal conductance, highest late-season net photosynthesis, and highest leaf nitrogen (% dry weight). As soil moisture decreased, *A. cana* exhibited a decline in ratios of internal to atmospheric CO<sub>2</sub> concentrations but no change in photosynthetic water-use efficiency. This study shows that even long-lived, woody species of a semiarid grassland have evolved traits facilitating rapid water-use to maximize carbon gain during brief periods of soil moisture availability.

**Keywords:** Alberta, Great Plains, leaf trait, photochemical reflectance index, stable carbon isotope, water-use efficiency.

**Résumé :** Les plantes ligneuses démontrent en général des caractéristiques d'utilisation de l'eau plus conservatrices que celles des plantes herbacées vivaces et annuelles, y compris des valeurs inférieures de conductance stomatale, de photosynthèse nette et de transpiration, mais une plus grande efficacité photosynthétique d'utilisation de l'eau. Habituellement, lors d'un stress hydrique, la conductance stomatale diminue plus que la photosynthèse nette. Il en résulte des ratios moindres des concentrations internes et atmosphériques de CO<sub>2</sub> et une plus grande efficacité photosynthétique d'utilisation de l'eau. Cependant, nous avons constaté que des arbustes d'une prairie semi-aride employaient des stratégies opportunistes d'utilisation de l'eau, celles-ci ont probablement évolué en raison de la courte durée de disponibilité de l'eau dans le sol. Nous avons mesuré les échanges gazeux photosynthétiques, la réflectance des feuilles et la composition foliaire des isotopes stables du carbone chez *Artemisia cana*, *Prunus virginiana*, *Rhus trilobata* et *Ribes aureum* dans le sud de l'Alberta. En conditions humides, la photosynthèse nette était très élevée chez *A. cana*, *R. trilobata* et *R. aureum*, mais moins chez *P. virginiana*. Une diminution de l'humidité volumétrique du sol a provoqué une réduction de la conductance stomatale proportionnelle à la photosynthèse nette dans 3 des 4 espèces (*P. virginiana*, *R. aureum* et *R. trilobata*). En conséquence, les ratios des concentrations internes et atmosphériques ( $c_i/c_a$ ) de CO<sub>2</sub> sont demeurés inchangés par la perte d'humidité du sol et l'efficacité photosynthétique d'utilisation de l'eau a diminué. Au niveau de feuille, l'indice de végétation par différence normalisée n'a pas varié avec la saison chez ces espèces, alors que l'indice basé sur la chlorophylle a diminué seulement légèrement et l'indice de réflectance photochimique a démontré un léger pic en milieu de saison. *A. cana* avait le plus faible ratio d'isotopes stables de carbone, la conductance stomatale la plus élevée, la photosynthèse nette de fin de saison la plus élevée et les plus grandes concentrations foliaires en azote (% du poids sec). À mesure que l'humidité du sol diminuait, les ratios des concentrations internes et atmosphériques de CO<sub>2</sub> chez *A. cana* déclinaient, mais l'efficacité photosynthétique d'utilisation de l'eau ne changeait pas. Cette étude démontre que même chez des espèces ligneuses de grande longévité d'une prairie semi-aride, des traits ont évolué afin de faciliter l'utilisation rapide de l'eau en vue de maximiser le gain en carbone lors des brèves périodes de disponibilité de l'eau dans le sol.

**Mots-clés :** Alberta, efficacité d'utilisation de l'eau, grandes plaines, indice de réflectance photochimique, isotopes stables du carbone, trait de la feuille.

**Nomenclature:** Kartesz, 1994.

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## Introduction

Plants of semiarid grassland ecosystems frequently experience soil moisture stress as a result of seasonal water budget deficits (Lauenroth & Bradford, 2006), the pulsed nature of summer rainfall (Huxman *et al.*, 2004; Loik *et al.*, 2004; Nippert, Knapp & Briggs, 2006), and high interannual precipitation variability (Wever, Flanagan & Carlson, 2002). Soil moisture stress is, thus, a key control on grassland ecosystem productivity (Flanagan, Wever & Carlson, 2002; Li, Grant & Flanagan, 2004; Li *et al.*, 2005) and on the spatial distribution of plant communities within temperate grassland hillslopes (Coxson & Looney, 1986; Lieffers & Larkin-Lieffers, 1987). To increase the likelihood of survival and reproduction in these semiarid ecosystems, plants have been naturally selected for tolerance or avoidance traits conferring resistance to the deleterious effects of drought.

In arid and semi arid ecosystems, short-lived annuals and herbaceous perennials avoid severe soil moisture stress by completing their life cycles before the onset of drought (Gulluscio & Oesterheld, 2007). These plants exhibit rapid growth and high rates of net photosynthesis at light saturation ( $A_{\max}$ ; Ehleringer, 1993). They also tend to employ a profligate water-use strategy to maximize carbon gain during episodes of moisture availability and to compete with other species for soil water (Zea-Cabrera *et al.*, 2006). Longer-lived, woody plants tend to exhibit more conservative photosynthetic water-use strategies than  $C_3$  grasses, as evidenced by lower ratios between internal and atmospheric  $\text{CO}_2$  concentrations ( $c_i/c_a$ ) and higher stable carbon isotope ratios,  $\delta^{13}\text{C}$  (Smedley *et al.*, 1991; Schuster *et al.*, 1992). This life strategy explains why northern semiarid grasslands display much lower water-use efficiency than Douglas-fir or Aspen forest stands at similar latitudes (Ponton *et al.*, 2006).

Leaf functional traits are also related to photosynthetic capacity and plant water-use strategy. Plants with short-lived foliage of high specific leaf area (SLA;  $\text{cm}^2\cdot\text{g}^{-1}$ ) and high leaf nitrogen (N) concentrations generally exhibit higher net photosynthesis and stomatal conductance than plants with leaves of greater longevity but lower SLA and N concentrations (Reich *et al.*, 1991; Wright *et al.*, 2004; Whitfield, 2006). This pattern holds true regardless of climate zone, plant functional type, and growth form in  $C_3$  plants, with no examples found of species with high  $A_{\max}$  and SLA but long leaf lifespan and low N concentration (Reich *et al.*, 1999). Leaves with low SLA have higher longevity but lower  $A_{\max}$  and photosynthetic nitrogen-use efficiency (PNUE;  $A_{\max}/\text{leaf N}$ , in  $\mu\text{mol}\cdot\text{mol}^{-1}\cdot\text{s}^{-1}$ ; Hikosaka, 2004) due to higher biomass and N allocation to non-photosynthesizing cell wall materials (Reich, Walters & Ellsworth, 1997; He *et al.*, 2006; Shipley *et al.*, 2006). Large, wide leaves also tend to have lower  $A_{\max}$  than small, narrow leaves, because of the large vein networks necessary to supply cells with water and nutrients (Nardini, Gortan & Salleo, 2005).

Although species with greater whole-plant and foliar longevity generally display more conservative water-use strategies than short-lived plants, stomatal conductance ( $g_s$ ),  $A_{\max}$ ,  $c_i/c_a$ , photosynthetic water-use efficiency (PWUE;  $A_{\max}/E$  or  $A_{\max}/g_s$ ), and  $\delta^{13}\text{C}$  are not merely heritable traits. These traits are also affected by environmental conditions.

In northern semiarid grasslands, volumetric soil moisture content ( $\theta_v$ ) is the dominant control on interannual variations in productivity (Flanagan, Wever & Carlson, 2002). While short-lived species with opportunistic water-use strategies dominate these ecosystems, physiological acclimation to low volumetric soil moisture ( $\theta_v$ ), high leaf temperature ( $T_{\text{leaf}}$ ), and high vapor pressure deficit (VPD) also occurs. Specifically, reduced stomatal conductance leads to lower  $E$ ,  $A_{\max}$ , and  $c_i/c_a$  (Grime, 1979; Smith, Shugart & Woodward, 1997; Craine *et al.*, 2002). Over time, reduced  $c_i/c_a$  leads to less negative  $\delta^{13}\text{C}$  (Ehleringer, 1993). Soil moisture-related plant stress also affects leaf pigment concentration and form, resulting in lower reflectance indices, including the chlorophyll index (CI), normalized difference vegetation index (NDVI), and photochemical reflectance index (PRI) (Gamon *et al.*, 1990). Leaf gas exchange,  $\delta^{13}\text{C}$ , and leaf reflectance indices are, thus, useful diagnostic tools with which to assess differences in plant acclimation to water scarcity, but the complex interaction of genetic and abiotic factors affecting these stress indicators must be carefully considered.

Given that woody plants tend to utilize relatively conservative water-use strategies and that soil moisture stress encourages the same, one might expect woody plants growing in semiarid grasslands to survive drought stress through especially high water-use efficiency. On the other hand, woody shrubs and stunted trees of temperate grasslands are sun plants, growing in direct competition with grasses and other low PWUE forbs. Leaves of these shrubs are small, and leaf longevity is constrained by a 6-month growing season and late-summer drought. These factors would tend to promote high  $A_{\max}$  and lower PWUE. McCarron and Knapp (2001) measured photosynthetic gas exchange characteristics of moist, temperate grassland shrubs of the Flint Hills, Kansas, USA. They found that PWUE was very low in *Rhus glabra* and *Prunus americana*, ranging seasonally from 1.0 to 1.7 and 0.8 to 1.3  $\text{mmol CO}_2\cdot\text{mol H}_2\text{O}^{-1}$ , respectively. Similarly, in Inner Mongolia, China, PWUE ranged from 2.0 to 2.5  $\text{mmol CO}_2\cdot\text{mol H}_2\text{O}^{-1}$  in *Artemisia frigida* (Chen *et al.*, 2005; Peng *et al.*, 2007). Although high atmospheric demand is also associated with low PWUE, the consistency of the low PWUE observed in these studies suggests that these grassland species employ profligate water-use strategies, as observed at the ecosystem scale (Ponton *et al.*, 2006; Hu *et al.*, 2008).

Assessment of interspecific differences in photosynthetic water-use strategy, governed by genetics and life history, is not only complicated by short-term acclimation to soil moisture stress, but also by spatial heterogeneity in microclimate and pedoclimate at sites with complex and steep topography. Geomorphological heterogeneity promotes biodiversity by providing spatial variation in slope, aspect, and drainage patterns, thus creating variable local-scale environmental conditions (Nichols, Killingbeck & August, 1998). In the coulee hillslopes of the Oldman River valley of southern Alberta, hydrometeorological heterogeneity has resulted in complex distribution patterns of woody shrub species (Coxson & Looney, 1986).

In this study, plant ecophysiological strategies were assessed in 4 semiarid grassland shrubs (*Artemisia cana*, *Ribes aureum*, *Rhus trilobata* and *Prunus virginiana*), each

occupying distinct aspect positions in the coulee landscape. Two hypotheses were tested. Firstly, we expected coulee shrubs to utilize a plant ecological strategy of profligate water use to maximize carbon gain during brief periods of soil moisture availability. Secondly, we hypothesized that more conservative water use would be exhibited by shrubs of relatively cool, moist microsites (*P. virginiana* and *R. aureum*) than by species growing in open sun and direct competition with opportunistic grasses and forbs (*A. cana* and *R. trilobata*).

## Methods

### SITE DESCRIPTION

The 4 shrubs examined in this study were located in a narrow, WSW- to ENE-oriented drainage channel (coulee) immediately to the west of the Oldman River, in Lethbridge, Alberta, Canada (49° 38' N, 112° 50' W; 873 to 903 m asl). Average annual precipitation and temperature are 386 mm and 5.7 °C at the Lethbridge Airport (Canadian Climate Normals 1971–2000; Environment Canada, 2005), located 5.5 km southwest of the study site. Lethbridge has a strong late spring maximum precipitation pattern, with 32% of precipitation received in May and June. The mixed grassland of the study site is located within the northern portion of the Great Plains ecozone (Samson, Knopf & Ostlie, 2004). Soils are loamy Dark Brown Chernozems (Typic Haploborolls), with somewhat deeper A horizons on north-facing aspects, especially in thickets. The coulee site has never been cultivated or grazed and is undisturbed aside from some encroachment of invasive crested wheat grass (*Agropyron cristatum*) on south-facing aspects. Spatial distributions of coulee species were examined in the Oldman River valley by Coxson and Looney (1986) and Lieffers and Larkin-Lieffers (1987). The key factor controlling the spatial distribution of species in the coulee environment is soil moisture. South-facing slopes are characterized by assemblages of C<sub>3</sub> and C<sub>4</sub> grasses, along with 2 cacti species (CAM metabolism) and sparsely distributed shrubs. A much more diverse community of shrubs, C<sub>3</sub> grasses, and forbs characterizes north-facing slopes.

### MICROCLIMATOLOGICAL MONITORING

Two HOBO microclimate stations (Onset Computer Corporation, Bourne, Massachusetts, USA) were installed at the study site in May 2004. To characterize the range of microclimate and pedoclimate types, the stations were located at mid-slope, with one on the south-facing slope and the other in the northeast-facing thicket. Microclimate data collected included air temperature ( $T_{\text{air}}$ ) and relative humidity (RH), measured at heights of 7.5 cm and 100 cm, incoming photosynthetic photon flux density (PPFD), and –40 cm volumetric soil moisture content, ( $\theta_v$ ). Measurements were recorded every 20 min, as the mean of 10 measurements obtained at 2-min intervals. The 7.5-cm air temperature and relative humidity were measured using the HOBO Temperature/RH Smart Sensor (Onset Computer Corporation, Bourne, Massachusetts, USA). Temperature is accurate to  $\pm 0.7$  °C from 0 °C to 50 °C and RH is accurate to  $\pm 3\%$ , except  $\pm 4\%$  near 100% RH. Air temperature at 100 cm was measured with the HOBO Pro H8 RH/

Temperature Logger (Onset Computer Corporation, Bourne, Massachusetts, USA), which is accurate to  $\pm 0.3$  °C from 0 °C to 50 °C and  $\pm 3\%$  RH, except  $\pm 4\%$  near 100% RH. All  $T_{\text{air}}$ /RH sensors were housed within Gill radiation shields.

PPFD was measured level to ground at the south-facing station with a PPF Smart Sensor (Onset Computer Corporation, Bourne, Massachusetts, USA). PPF sensor accuracy is either  $\pm 5\%$  or  $\pm 5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  ( $+0.75 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}\cdot\text{°C}^{-1}$  above 25 °C), whichever is larger. Volumetric soil moisture was measured with an ECHO Dielectric Aquameter soil moisture probe (Decagon Devices Inc., Pullman, Washington, USA). This sensor is precalibrated for most soil types, including loamy Typic Haploborolls of the study site, although gravimetric soil moisture analyses from nearby slopes of similar aspect in 2005 showed that sensor readings from both the south- and north-facing slopes deviated from gravimetric values by up to  $0.06 \text{ m}^3\cdot\text{m}^{-3}$ . All 100-cm  $T_{\text{air}}$ , 100-cm RH, PPF, and –40-cm  $\theta_v$  data were stored on a HOBO Microstation Datalogger.

### PHOTOSYNTHETIC GAS EXCHANGE MEASUREMENTS

All leaf gas exchange measurements were taken with a portable TPS-1 Photosynthesis System with the 25- × 18-mm PLC6 Automatic Universal Leaf Cuvette (PP Systems, Hertfordshire, UK). System calibration was carried out by the manufacturer in April 2004 (PP Systems, Amesbury, Massachusetts, USA). Gas exchange measurements were taken on 4 shrubs in this study: *Artemisia cana*, *Ribes aureum*, *Rhus trilobata*, and *Prunus virginiana*. Shrubs from distinct microclimatological niches were specifically chosen, so as to characterize a range of water-use strategies and stress acclimation patterns in the temperate grassland coulee environment. At the study site, *P. virginiana* and *R. aureum* are found only on northeast-facing thickets, where measurements were taken, and in the central gully. *Artemisia cana* and *R. trilobata* measurements were taken in their dominant north- and south-facing niches, respectively. Measurements were taken between 18 June and 24 September 2004 from 1100 to 1530 solar time. The mid-June start date ensured that leaves had reached maturity so that the evaluation of late summer drought stress acclimation was not confounded by incomplete development of photochemical capacity.

Five healthy, mature leaves were chosen from each species for gas exchange measurements, and each leaf was from a different plant. Ten  $A_{\text{max}}$  readings were taken on each leaf. One leaf mean was derived from these readings. The 5 leaf means were used to obtain a species mean for each of the 4 species on a given date. On occasion, the number of readings and/or leaf means had to be decreased due to the arrival of cloud cover or haze. To ensure light saturation, all readings were taken under clear sky conditions, but 8.6% of individual readings were removed because of PPF below  $800 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . This ensured that the chamber was properly aligned toward the sun and unobstructed during all measurements. The PPF sensor was located inside the chamber. Eighty-one percent of readings were taken with PPF above  $1200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , and there was no discernable difference between  $A_{\text{max}}$  values recorded above this intensity and those taken with PPF in the 800 to  $1200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  range.

For the 10 measurement dates, the total number of readings and leaf means were as follows: *A. cana* (421, 46); *P. virginiana* (463, 45); *R. trilobata* (456, 43); *R. aureum* (407, 43). Standard deviations are reported, rather than standard errors, to provide an appropriate indication of variation from the mean. The mean chamber CO<sub>2</sub> concentration during the measurements was 368.0 ± 6.5 ppm, with no seasonal trend.

Projected leaf area was determined using the LI-3100C Area Meter (LICOR Biosciences, Lincoln, Nebraska, USA) at the end of each measurement date. Mean leaf shrinkage was determined on DOY (day of year) 245 by measuring the area of 5 leaves of each species immediately following removal from freshly cut branches and then repeating the measurements on the same leaves placed in sample envelopes for 3 h, to simulate the condition of sampled leaves. On this basis, leaf area increases of 12.4, 6.5, 9.7, and 6.4% were applied to leaves of *A. cana*, *P. virginiana*, *R. aureum*, and *R. trilobata*, respectively.

LEAF REFLECTANCE MEASUREMENTS

Adaxial reflectance readings were acquired on each leaf before A<sub>max</sub> measurements, using a field spectroradiometer (Unispec-SC Spectral Analysis System, PP Systems, Amesbury, Massachusetts, USA), equipped with a bifurcated fibre optic cable (UNI400) and leaf clip (UNI500). On selected dates, reflectance measurements were taken in the absence of A<sub>max</sub> measurements. Within the 400- to 1000-nm range of the Unispec-SC, the spectral resolution was 10 nm full width at half maximum (FWHM), with 16-bit radiometric precision. The leaf clip foreoptic attachment (Gamon & Surfus, 1999) enabled alignment of the probe at 1-mm range and eliminated ambient radiation. Controlled illumination was provided by a built-in halogen light source. All measurements were preceded by dark current scans to subtract instrument noise and by scans of a 99%-reflective polytetrafluoroethylene (PTFE) calibration disk (UNI420) as a white reference. Leaf reflectance was calculated by dividing leaf radiance by the irradiance of the PTFE disk. Measurements were recorded as the mean of 10 scans. Spectral response curves were produced for the full spectral range, and vegetation indices were calculated using 1-nm bandwidths interpolated linearly from the spectroradiometer data. Photochemical reflectance index (PRI; Gamon, Serrano & Surfus, 1997), normalized difference vegetation index (NDVI; Tucker, 1979), and chlorophyll index (CI; Gitelson and Merzlyak, 1994) were determined as follows:

$$NDVI = \frac{r_{800} - r_{670}}{r_{800} + r_{670}} \quad [1]$$

$$PRI = \frac{r_{531} - r_{570}}{r_{531} + r_{570}} \quad [2]$$

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

where *r* is reflectance and subscripts refer to wavelengths in nm. To avoid negative values of PRI, we used the scaled value (Drolet *et al.*, 2005):

$$sPRI = \frac{PRI + 1}{2} \quad [4]$$

LEAF CARBON ISOTOPE (<sup>13</sup>C/<sup>12</sup>C) COMPOSITION

After gas exchange measurements and leaf area analysis, leaf samples were dried at 70 °C for 48 h, weighed, and crushed with a mortar and pestle. Liquid nitrogen was applied to help break leaf fragments into a powder. To determine stable carbon isotope composition and total N content (N<sub>mass</sub>, % dry weight), 1–3 mg samples were weighed into tin cups and combusted in an elemental analyzer (NC2500, CE Instruments, ThermoQuest Italia, Milan, Italy) coupled to a gas isotope ratio mass spectrometer (Delta Plus, Finnigan Mat, Bremen, Germany) operating in continuous flow mode. δ<sup>13</sup>C was calculated from the ratio *R* (<sup>13</sup>CO<sub>2</sub>/<sup>12</sup>CO<sub>2</sub>) as follows:

$$\delta^{13}C = \left[ \frac{R_{sample}}{R_{std}} - 1 \right] \times 1000\text{‰} \quad [5]$$

where *R<sub>std</sub>* refers to the molar ratio of the international standard Pee Dee Belemnite (V-PDB).

PHOTOSYNTHETIC WATER AND NITROGEN-USE EFFICIENCY

Two methods were used to determine leaf photosynthetic water-use efficiency. The first method was based on photosynthetic gas exchange:

$$PWUE = \frac{A}{E} = \frac{c_a \left( 1 - \frac{c_i}{c_a} \right)}{1.6v} \quad [6]$$

where 1.6 is the ratio of the diffusion coefficients of H<sub>2</sub>O and CO<sub>2</sub> and *v* is related to the difference between intracellular and chamber atmospheric water vapour concentrations (*e<sub>i</sub>* – *e<sub>a</sub>*, in Pa) and atmospheric pressure, *P* (kPa), as follows:

$$v = \frac{e_i - e_a}{P} \quad [7]$$

Equation [6] provides an instantaneous measure of PWUE. It is also possible to calculate a more integrated measure of PWUE based on the stable carbon isotope composition of leaf tissue, δ<sup>13</sup>C<sub>*p*</sub>. Farquhar, O’Leary, and Berry (1982) and Farquhar, Ehleringer, and Hubick (1989) demonstrated that the stable carbon isotope composition of leaf material (δ<sup>13</sup>C<sub>*p*</sub>) is related to that of the source atmospheric CO<sub>2</sub> (δ<sup>13</sup>C<sub>*a*</sub>) and to *c<sub>i</sub>*/*c<sub>a</sub>* through the following relation:

$$\delta^{13}C_p = \delta^{13}C_a - a - (b - a) \frac{c_i}{c_a} \quad [8]$$

where *a* is the discrimination during diffusion of CO<sub>2</sub> in air (4.4‰), *b* is the net discrimination during carboxylation (27.0‰), and δ<sup>13</sup>C<sub>*a*</sub> was assumed to be –8‰. By rearranging this formula, *c<sub>i</sub>*/*c<sub>a</sub>* can be estimated from δ<sup>13</sup>C<sub>*p*</sub>. Combining equations [6] and [8], an alternative formulation of PWUE can be derived based on δ<sup>13</sup>C<sub>*p*</sub> and *v* (Ponton *et al.*, 2006):

$$PWUE = \frac{A}{E} = \frac{c_a - c_a \left( \frac{\delta^{13}C_p - \delta^{13}C_a + a}{a - b} \right)}{1.6v} \quad [9]$$

In Equation [9], *c<sub>a</sub>* was determined as the mean CO<sub>2</sub> concentration observed at the Lethbridge Ameriflux

meteorological station during daylight hours of the growth season (0500–2100; May 1 to measurement date) in 2004. These concentrations were used instead of leaf chamber CO<sub>2</sub> concentrations because gas exchange was measured during high light periods, when concentrations were depressed relative to morning hours. Using this method, the *c<sub>a</sub>* value used in the PWUE calculations was 376.8 μmol·mol<sup>-1</sup> at peak season (DOY 180). Photosynthetic nitrogen-use efficiency was determined as the quotient of A<sub>max</sub> and leaf N content (mol N·m<sup>-2</sup>).

STATISTICAL ANALYSIS OF PLANT PHYSIOLOGICAL DATA

Interspecific comparisons of plant ecophysiological variables were carried out for each measurement date, using one-way ANOVA. The null hypothesis for each measurement date was that there was no significant difference among the 4 species for a given physiological variable. The variables considered included A<sub>max</sub>, g<sub>s</sub>, T<sub>leaf</sub>, VPD, *c<sub>i</sub>/c<sub>a</sub>*, N<sub>mass</sub>, area-based leaf N content (N<sub>area</sub>; g m<sup>-2</sup>), NDVI, CI, and sPRI. When the final ANOVA *F*-test indicated significant differences among the means of the 4 species (*P* < 0.05), Tukey tests were undertaken to determine all significant between-species differences (*P* < 0.05). No analysis of *c<sub>i</sub>/c<sub>a</sub>* was carried out for DOY 233 and 268, because leaf senescence had advanced to the point that net CO<sub>2</sub> exchange was near zero or negative in some leaves of *R. aureum*, *R. trilobata*, and *P. virginiana*.

Results

ENVIRONMENTAL CONDITIONS

Mean PPFD increased from 9.9 ± 2.9 MJ·m<sup>-2</sup>·d<sup>-1</sup> in June to 10.8 ± 2.0 MJ·m<sup>-2</sup>·d<sup>-1</sup> in July due to decreased cloud cover, but fell to 8.2 ± 2.8 MJ·m<sup>-2</sup>·d<sup>-1</sup> in August. Both daily maximum T<sub>air</sub> and VPD were higher in July and August than in June for all measurement height and slope aspect combinations (Table I; Figure 1). Soils were moist at the beginning of the study period because of heavier than normal precipitation in May (87.0 mm; Lethbridge Airport, Environment Canada, 2005). Rainfall was higher in June (66.7 mm) and July (64.9 mm) than in August (36.5 mm). Aside from a small increase on DOY 189, -40-cm θ<sub>v</sub> decreased from June to August at the study site, with the most rapid decrease occurring from DOY 190 to 210 (Figure 1).

Slope aspect had little effect on 100-cm T<sub>air</sub> and VPD, but near-surface (7.5 cm) T<sub>air</sub> and VPD were much higher on the south-facing slope (Table I). At this measurement height, the greatest differences were observed in July, when

mean daily maximum T<sub>air</sub> was 14.0 °C warmer and VPD was 3.3 kPa higher on the south-facing slope than within the northeast-facing thicket. Mean daily maximum T<sub>air</sub> was 6.6 to 9.3 °C warmer at 7.5 cm than at 100 cm on the south-facing slope, while VPD was 0.8 to 1.8 kPa higher. By contrast, mean daily maximum T<sub>air</sub> was 2.9 to 4.1 °C cooler at 7.5 cm than at 100 cm in the northeast-facing thicket, while VPD was about 0.2 kPa lower (Table I). θ<sub>v</sub> was lower on the south-facing slope (0.04–0.21 m<sup>3</sup>·m<sup>-3</sup>) than on the northeast-facing slope (0.13–0.26 m<sup>3</sup>·m<sup>-3</sup>; Figure 1).

At Lethbridge Airport, average daily mean T<sub>air</sub> average maximum T<sub>air</sub> and average minimum T<sub>air</sub> were 16.2, 24.0, and 8.4°C during the summer months (June to August) of 2004. These values are 0.4–1.3 °C cooler than the 30-y (1971–2000) climate means of 17.2, 24.4, and 9.7 °C, respectively (Environment Canada, 2005). June to August rainfall was 12.5 mm above the 30-y mean of 155.6 mm.

VARIATION IN PLANT CHARACTERISTICS AND PLANT DISTRIBUTION

Three of the 4 shrubs in this study (*A. cana*, *R. aureum*, and *R. trilobata*) were relatively short plants with small leaves and low total leaf area per plant (Table II). By contrast, *P. virginiana* was 3 m in height and had substantially larger leaves and total plant leaf area than the other species. Rooting depth was not assessed, but we observed multiple tap roots of *A. cana* and *R. trilobata* extending beyond a depth of 2 m at a nearby site of soil slumping. Average specific leaf area (SLA) ranged from 76.7 to 126.9 cm<sup>2</sup>·g<sup>-1</sup> and was significantly lower in *R. trilobata* than in *R. aureum* and *P. virginiana* (single factor ANOVA, *F* (3, 15) = 7.74, *P* = 0.002, *post hoc* Tukey test; Table II).

*Artemisia cana* leaves are covered with white, reflective hairs. As a consequence, reflectance indices that quantify leaf “greenness” or chlorophyll content (NDVI and CI) were lower in *A. cana* than in the other species (Table II; Figure 2). Reflectance indices remained quite constant in *A. cana* throughout the growing season. In *P. virginiana*, *R. aureum*, and *R. trilobata*, reflectance indices varied only within a small range before senescence, with CI trending slightly downward with DOY and sPRI reaching its maximum value near DOY 205.

At the peak of the growing season (DOY 180), N<sub>mass</sub> differed among species (single factor ANOVA, *F* (3, 15) = 6.87, *P* < 0.01), with significantly lower values in *R. trilobata* than in the thicket species *P. virginiana* and *R. aureum* (*post hoc* Tukey, *P* < 0.05; Table II). No significant differences were observed in N<sub>area</sub> on DOY 180. Leaf N<sub>mass</sub> decreased in the 4 species from mid-July onward, with *R. trilobata*

TABLE I. Mean daily maximum T<sub>air</sub> and VPD on the south-facing and northeast-facing slopes at heights of 7.5 cm and 100 cm. For comparison, mean daily maximum T<sub>air</sub> and VPD are also listed for Lethbridge Airport (200 cm, flat). \*June data for the northeast-facing aspect at 100 cm are based on DOY 174 – 182 only. T<sub>air</sub> and VPD for south-facing slopes during this period were 23.6 ± 2.9 °C and 1.34 ± 0.45 kPa. Errors are standard deviations.

Instrument site		Mean daily maximum T <sub>air</sub> (°C)			Mean daily maximum VPD (kPa)		
height	Aspect	June	July	August	June	July	August
7.5 cm	S	27.7 ± 5.2	36.4 ± 6.2	33.5 ± 8.0	2.05 ± 0.87	3.91 ± 1.49	3.50 ± 1.73
	NE	18.3 ± 3.3	22.4 ± 3.4	21.9 ± 4.2	0.52 ± 0.40	0.65 ± 0.47	0.98 ± 0.93
100 cm	S	21.1 ± 4.0	27.1 ± 4.6	25.5 ± 5.5	1.27 ± 0.54	2.08 ± 0.80	1.88 ± 0.87
	NE	23.5 ± 2.7*	26.5 ± 4.5	24.8 ± 5.3	1.19 ± 0.40*	1.83 ± 0.73	1.71 ± 0.60
200 cm	Flat	20.6 ± 3.9	26.2 ± 4.6	25.0 ± 5.2	1.40 ± 0.56	2.20 ± 0.91	2.00 ± 0.99

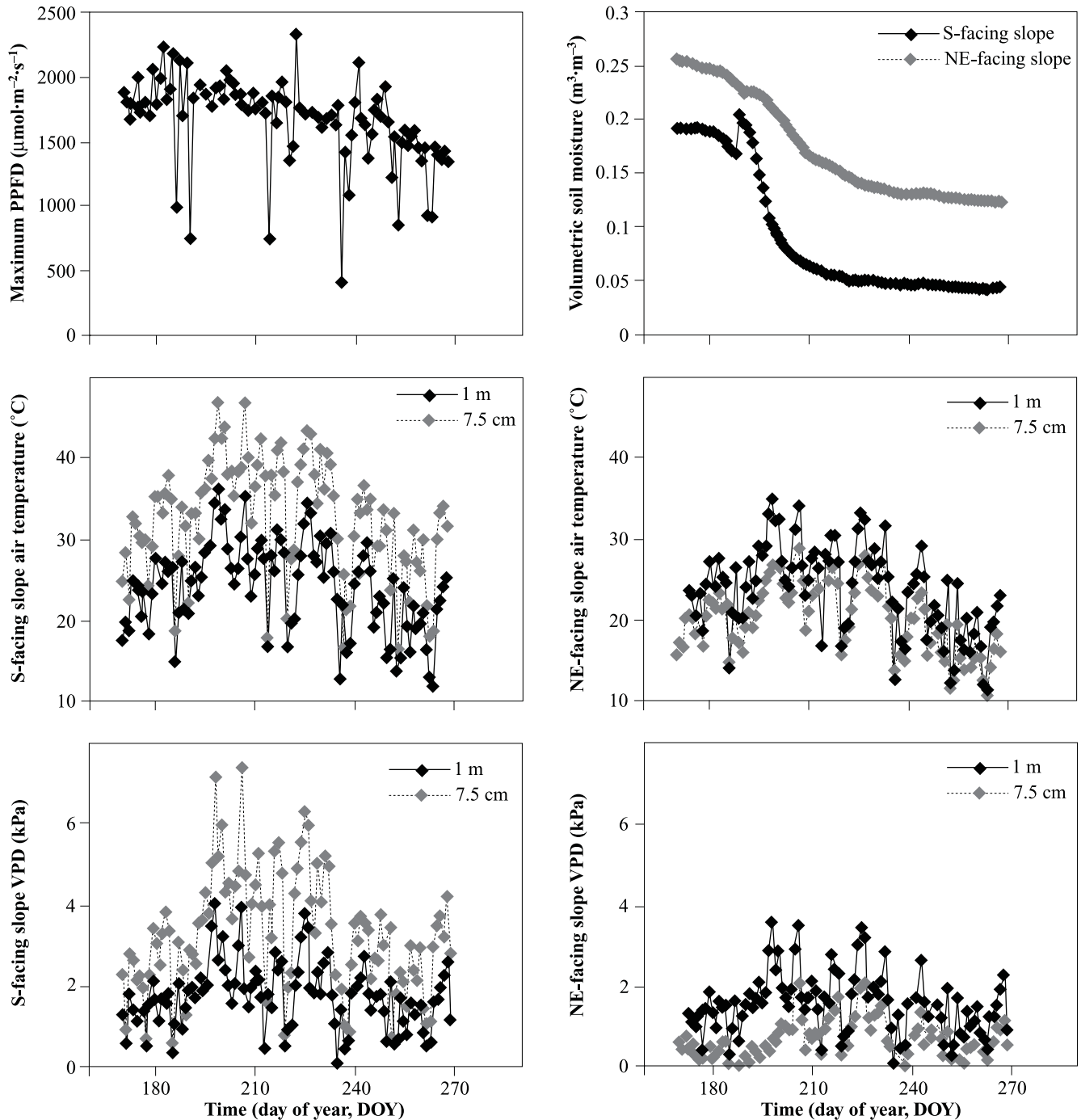


FIGURE 1. Microclimatic comparison of the open, south-facing and partially shaded, northeast-facing sites, from DOY 170 – 269. Daily maxima are shown for PPFD,  $T_{air}$  and VPD, whereas the daily mean is displayed for  $\theta_v$ .

having the lowest  $N_{mass}$  of all species on DOY 170, 202, 215, 218 and 233 (single factor ANOVA, *post hoc* Tukey,  $P < 0.05$ ). However, *R. trilobata* and *A. cana* had lower SLA than *P. virginiana* and *R. aureum*. Consequently, maximum  $N_{area}$  was near or above  $3 \text{ g}\cdot\text{m}^{-2}$  in *R. trilobata* and *A. cana*, respectively, but not in the thicket species *R. aureum*, and *P. virginiana*. Furthermore, SLA decreased linearly in *A. cana* from  $100.6 \text{ cm}^2\cdot\text{g}^{-1}$  to  $71.0 \text{ cm}^2\cdot\text{g}^{-1}$  between DOY 170 and DOY 233 ( $r^2 = 0.57$ ), so  $N_{area}$  did not decrease with DOY as it did in the other 3 species (Figure 3). In

*A. cana*,  $N_{area}$  exceeded levels observed in *P. virginiana* and *R. aureum* by DOY 215 and also surpassed those of *R. trilobata* by DOY 233 (single factor ANOVA, *post hoc* Tukey,  $P < 0.05$ ).

PHOTOSYNTHETIC GAS EXCHANGE CHARACTERISTICS

$A_{max}$  peaked in late June in all 4 species but decreased steadily throughout July and August (Figure 3).  $A_{max}$  was lower in *P. virginiana* than in all other species during June and early July (DOY 170, 175, and 180; Table III).

TABLE II. Shrub structural characteristics and peak growth season (DOY 180) leaf traits. Errors are standard deviations. Numbers in parentheses indicate the sample size. \**Rhus trilobata* grows in clusters of several individuals, but individual dimensions are shown. \*\*Leaves of *R. trilobata* are compound and consist of three leaflets. The area of a single leaflet is shown.

Characteristic	<i>Artemisia cana</i>	<i>Prunus virginiana</i>	<i>Ribes aureum</i>	<i>Rhus trilobata</i>
Plant height (m)	0.6 ± 0.1 (10)	3.0 ± 0.4 (10)	1.7 ± 0.4 (10)	0.6 ± 0.2 (10)
Plant LA (m <sup>2</sup> )	0.3 (2)	1.1 (2)	0.4 (2)	0.2* (2)
Area/leaf (cm <sup>2</sup> )	2.8 ± 0.9 (5)	13.2 ± 6.8 (5)	8.1 ± 4.8 (5)	1.6 ± 0.8** (5)
SLA (cm <sup>2</sup> ·g <sup>-1</sup> )	100.6 ± 10.8 (5)	116.2 ± 9.7 (5)	126.9 ± 25.4 (5)	76.7 ± 8.9 (4)
Leaf N <sub>mass</sub> (%)	2.4 ± 0.1 (5)	2.8 ± 0.2 (5)	2.7 ± 0.4 (5)	2.0 ± 0.2 (4)
NDVI	0.28 ± 0.04 (5)	0.92 ± 0.01 (5)	0.87 ± 0.05 (5)	0.90 ± 0.03 (4)
CI	0.14 ± 0.02 (5)	0.49 ± 0.03 (5)	0.43 ± 0.06 (5)	0.44 ± 0.04 (4)
sPRI	0.50 ± 0.00 (5)	0.47 ± 0.02 (5)	0.50 ± 0.02 (5)	0.47 ± 0.01 (4)

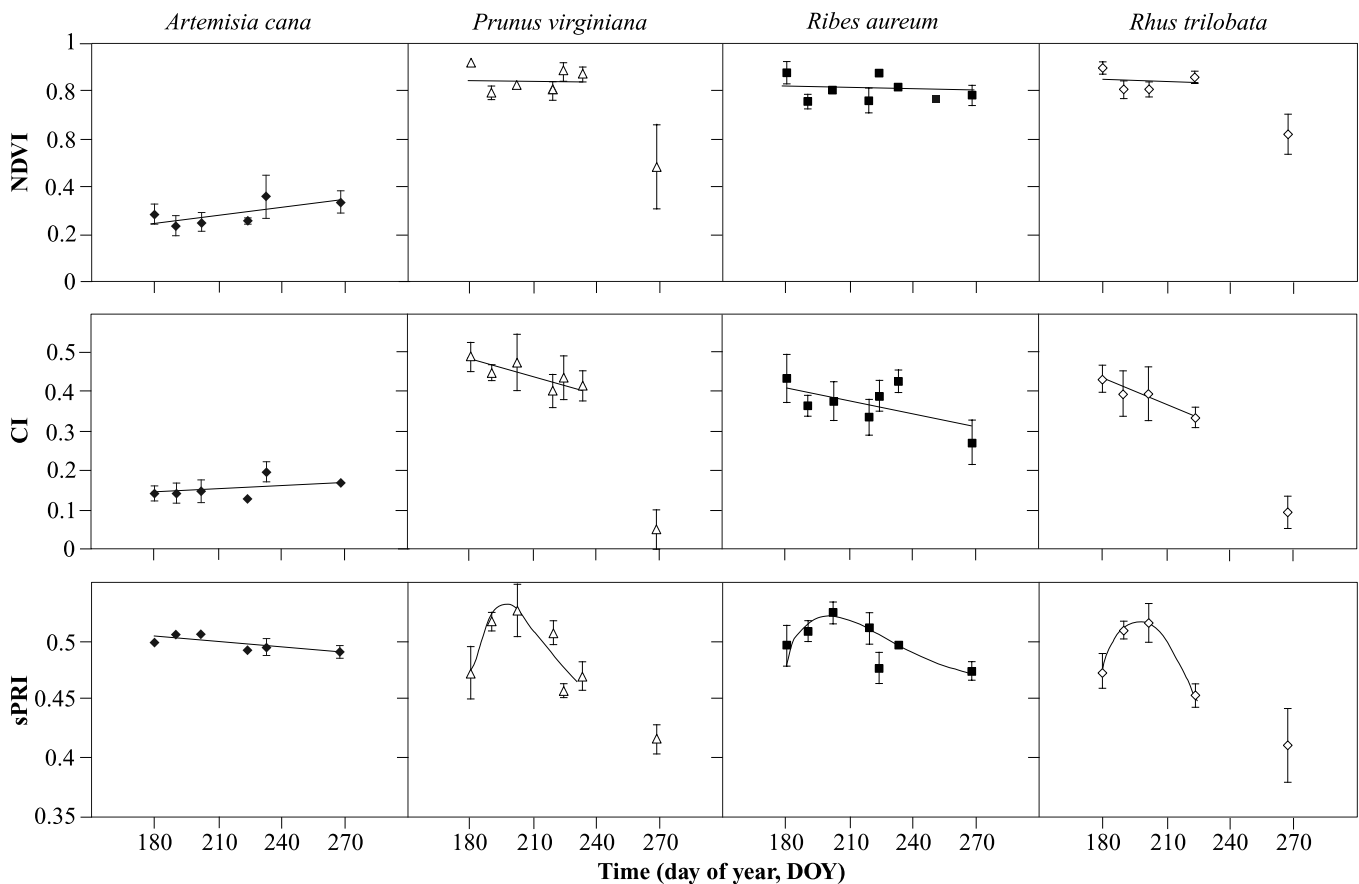


FIGURE 2. Seasonal patterns of NDVI, CI, and sPRI. Values represent the mean ± standard deviation ( $n = 5$ ).

No significant differences were observed on DOY 185, but *A. cana* and *R. aureum* both exhibited higher  $A_{\max}$  than *P. virginiana* and *R. trilobata* by mid-season (DOY 202). The seasonal decrease in  $A_{\max}$  was least pronounced in *A. cana*, which had the highest rate of all species on DOY 218, 224, and 233.  $A_{\max}$  was also higher in *A. cana* than in *P. virginiana* or *R. trilobata* on DOY 268. By DOY 233,  $A_{\max}$  had decreased by 74%, 98%, 101%, and 94% in *A. cana*, *P. virginiana*, *R. aureum*, and *R. trilobata*, respectively.

Stomatal conductance decreased from DOY 180 to 233 in all species, but higher late-season values were maintained in *A. cana*, which had the highest  $g_s$  of the 4 species on June 18 (DOY 170) and again throughout August and September (DOY 218, 224, 233, and 268; single fac-

tor ANOVA, *post hoc* Tukey,  $P < 0.05$ ). Daily mean  $A_{\max}$  increased exponentially with  $g_s$  in all species ( $r^2 = 0.96, 0.93, 0.96$  and  $0.88$  for *P. virginiana*, *R. aureum*, *R. trilobata*, and *A. cana*, respectively). The close and broadly proportional association between  $g_s$  and  $A_{\max}$  in *P. virginiana*, *R. aureum*, and *R. trilobata* resulted in little seasonal change in  $c_l/c_a$ . Although the highest late-season  $g_s$  was observed in *A. cana*,  $g_s$  fell proportionally more than  $A_{\max}$  from late June to August in this species. As a result,  $c_l/c_a$  decreased linearly in *A. cana* from DOY 170 to 233 (Figure 3).

Decreasing  $g_s$  led to reduced  $E$ , so that  $[T_{\text{leaf}} - T_{\text{air}}]$  and leaf VPD rose from DOY 170 to 233. Mean  $T_{\text{leaf}}$  values, computed from the means on all measurement dates, were  $29.0 \pm 4.1$  °C (*A. cana*),  $31.6 \pm 3.5$  °C (*P. virginiana*),

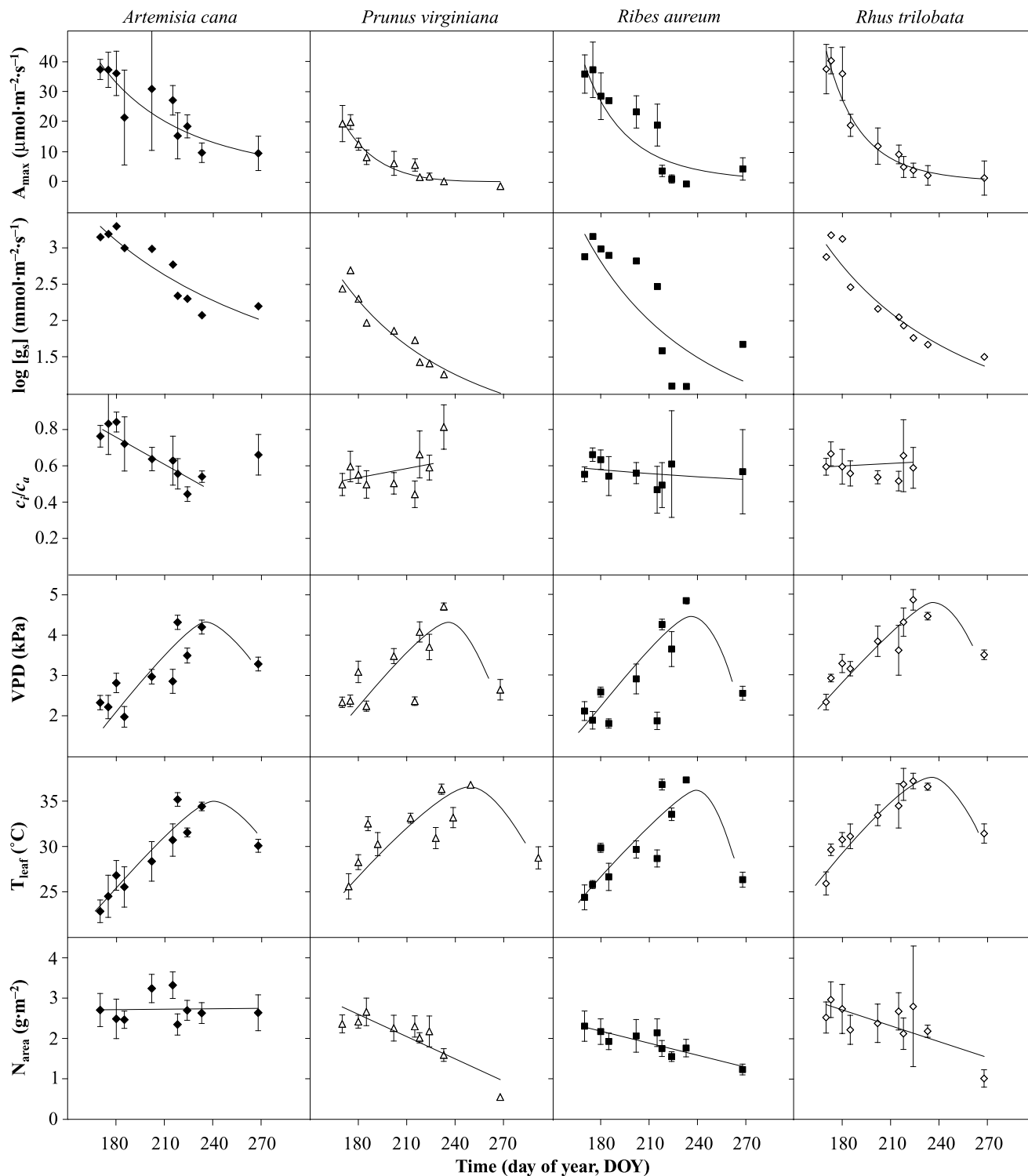


FIGURE 3. Seasonal trends in  $A_{\max}$ ,  $g_s$ ,  $c_i/c_a$ , leaf VPD,  $T_{\text{leaf}}$  and  $N_{\text{area}}$  in *A. cana*, *P. virginiana*, *R. aureum*, and *R. trilobata*. Values represent the mean  $\pm$  standard deviation.  $c_i/c_a$  values were removed when near zero or when negative net  $\text{CO}_2$  exchange resulted in values exceeding unity.

$29.9 \pm 4.6$  °C (*R. aureum*), and  $32.7 \pm 3.6$  °C (*R. trilobata*).  $T_{\text{leaf}}$  was cooler in *A. cana* than in all other species on DOY 180, 224, and 233, while  $T_{\text{leaf}}$  was warmer in *R. trilobata* than in all other species on DOY 215 and 224 (single factor ANOVA, *post hoc* Tukey,  $P < 0.05$ ). Mean  $E$  values, determined from the means on all measurement dates, were

$10.3 \pm 6.0$   $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (*A. cana*),  $2.6 \pm 2.6$   $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (*P. virginiana*),  $4.3 \pm 3.6$   $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (*R. aureum*), and  $5.8 \pm 3.9$   $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (*R. trilobata*). *Artemisia cana* displayed the highest  $E$  of all species on DOY 170, 180, 215, 218, 224, 233, and 268 (single factor ANOVA, *post hoc* Tukey,  $P < 0.05$ ).

TABLE III. Summary of significant daily interspecific differences in  $A_{\max}$ , from one-way ANOVA ( $P < 0.05$ ) and *post hoc* Tukey tests to determine the species pairs with significant differences ( $P < 0.05$ ). AC = *A. cana*, PV = *Prunus virginiana*, RA = *Ribes aureum*, and RT = *Rhus trilobata*. The Tukey test results for DOY 268 fell on the  $P = 0.05$  boundary with rounding to the nearest decimal point.

DOY	MS	<i>n</i>	df	ANOVA <i>F</i> -statistic	<i>P</i> -value	Tukey <i>T</i> -statistic ( $P < 0.05$ )	Interspecific comparison	Tukey trellis	Extreme ( $P < 0.05$ )
170	409, 44	17	3, 13	9.4	0.001	12.0	AC > PV RA > PV RT > PV	20.9 16.4 18.0	Low = PV
175	336, 41	20	3, 16	8.3	0.002	11.1	AC > PV RA > PV RT > PV	17.3 17.3 20.3	Low = PV
180	585, 47	19	3, 15	12.5	< 0.001	12.4	AC > PV RA > PV RT > PV	23.4 15.9 23.3	Low = PV
202	331, 56	19	3, 15	5.9	0.007	13.5	AC > PV RA > PV	17.2 17.0	
215	438, 23	20	3, 16	19.5	< 0.001	8.7	AC > PV AC > RT RA > RT RA > PV	21.4 17.9 9.7 13.2	
218	186, 19	20	3, 16	10	0.001	7.8	AC > PV AC > RA AC > RT	13.7 11.6 10.3	High = AC
224	325, 6	19	3, 15	53.5	< 0.001	4.5	AC > PV AC > RA AC > RT	16.6 17.4 14.5	High = AC
233	79, 6	14	3, 10	12.4	0.001	5.5	AC > PV AC > RA AC > RT	9.5 10.4 7.5	High = AC
268	108, 20	20	3, 16	5.5	0.008	8.0	AC > PV AC > RT	10.8 8.2	

Volumetric soil moisture content ( $\theta_v$ ) was a major control on seasonal patterns of leaf gas exchange characteristics.  $A_{\max}$ ,  $\log [g_s]$ , *E*, and photosynthetic nitrogen-use efficiency (PNUE;  $A_{\max}/\text{leaf N}$ , in  $\mu\text{mol}\cdot\text{mol}^{-1}\cdot\text{s}^{-1}$ ) were strongly and positively correlated with  $\theta_v$  in all species (Figure 4). However,  $A_{\max}$  fell to near-zero values at much higher  $\theta_v$  in the thicket shrubs (*P. virginiana* or *R. aureum*) than in *R. trilobata* and *A. cana*, which maintained positive  $A_{\max}$  throughout the summer.

#### INDICATORS OF WATER-USE EFFICIENCY

Among-species differences in photosynthetic water-use strategies were assessed through an examination of water-use indicators on fully developed, healthy leaves in the absence of soil moisture stress (Table IV). On DOY 180, late enough in the growing season to ensure full foliar development, but before the onset of drought, average  $\delta^{13}\text{C}$  was more negative in *A. cana* than in all other species. Accordingly,  $c_i/c_a$ , calculated either from gas exchange or from  $\delta^{13}\text{C}$ , was higher in *A. cana* than in *P. virginiana*, *R. aureum*, and *R. trilobata*, while PWUE (*A/E*;  $\text{mmol CO}_2\cdot\text{mol H}_2\text{O}^{-1}$ ) was lower. PWUE (*A/E*) and  $c_i/c_a$  values (from  $\delta^{13}\text{C}$ ) were similar to those determined by photosynthetic gas exchange, both in relative and absolute terms (Table IV).

Consistent with the higher  $c_i/c_a$  of *A. cana*, mean  $\delta^{13}\text{C}$ , computed from daily averages, was more negative in *A. cana* ( $-29.34 \pm 0.37\text{‰}$ ) than in *P. virginiana* ( $-25.71 \pm 0.36\text{‰}$ ), *R. aureum* ( $-26.17 \pm 0.78\text{‰}$ ), and *R. trilobata* ( $-24.97 \pm 0.34\text{‰}$ ).  $\delta^{13}\text{C}$  was not significantly

related to either DOY or  $\theta_v$  in *A. cana*, *P. virginiana*, or *R. aureum* (not shown). In *R. trilobata*,  $\delta^{13}\text{C}$  was weakly and linearly correlated with DOY ( $r^2 = 0.51$ ), rising from a minimum of  $-25.68 \pm 0.80\text{‰}$  (DOY 180) to a maximum of  $-24.44 \pm 1.20\text{‰}$  (DOY 268).

In response to declining  $\theta_v$ , PWUE decreased in *R. aureum*, *R. trilobata*, and *P. virginiana* but not in *A. cana* (Figure 4). *Artemisia cana* had the lowest peak PWUE in June (DOY 170 and 180; ANOVA, *post hoc* Tukey,  $P < 0.05$ ; Table IV) but maintained consistent PWUE throughout the growing season. In this species,  $g_s$  regulated  $c_i/c_a$ , such that *E* and  $A_{\max}$  decreased proportionately in response to soil moisture stress and high VPD.

## Discussion

It is well established that woody plants tend to exhibit more conservative water-use strategies than grasses (Schuster *et al.*, 1992; Ponton *et al.*, 2006), which rapidly use soil moisture during brief periods of availability. However, we observed that woody species of a northern semiarid grassland ecosystem also exhibited low PWUE when  $\theta_v$  was high and VPD was low. Although low PWUE was characteristic of all 4 species, seasonal patterns of photosynthetic uptake varied among shrubs occupying distinct microclimatological niches. These differences can be explained not only by environmental conditions, but also by photosynthetic water-use strategy, plant functional traits, and nitrogen economy.

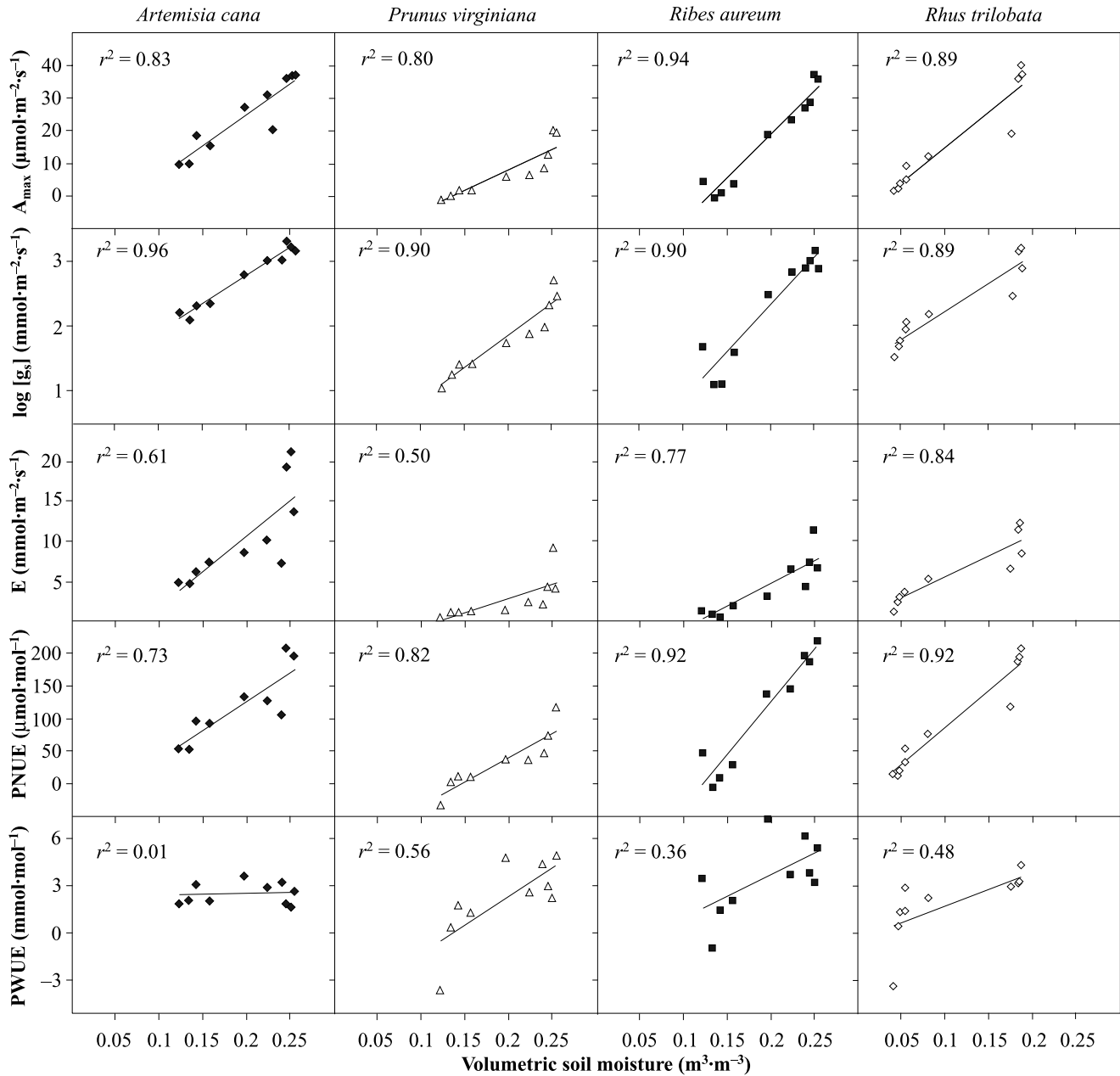


FIGURE 4. Relationships between  $\theta_v$  and  $A_{max}$ ,  $g_s$ ,  $E$ , PNUE, and PWUE.

ENVIRONMENTAL CONDITIONS PROMOTE LOW PWUE

In the grasslands of southern Alberta, rainfall and  $\theta_v$  usually peak in June, while  $T_{air}$  and VPD peak in late July and August (Figure 1). Consequently, it is advantageous for both annuals and perennials to photosynthesize at a high rate early in the summer to complete growth, development, and reproduction cycles before the onset of drought and heat stress. To maximize  $A_{max}$  in a high light environment,  $g_s$  must be sufficiently high for maintenance of optimal  $c_i$ . Inevitably, this promotes  $E$ . However, transpiration is not solely controlled by  $g_s$ , but also by VPD. The 4 shrubs of this study exhibited very high  $g_s$  only during the relatively moist and cool period of early summer. This strategy may maximize net carbon gain in a semiarid environment, facilitating very high  $A_{max}$  before high VPD exacerbates the

problem of soil moisture deficit, by increasing  $E$  for a given rate of  $g_s$ .

The low PWUE observed in this study is consistent with the “tragedy of the commons” hypothesis in plant water use (Zea-Cabrera *et al.*, 2006). Each plant exhibited profligate water use, but this was most apparent in *A. cana*. This widely spaced species is dominant on north-facing slopes and grows among a variety of opportunistic species, including  $C_3$  grasses (*Stipa viridula*, *Koeleria cristata*, and *Agropyron* spp.), diverse forbs, and small shrubs. The remaining species exhibited much higher peak season PWUE (Table IV), but  $A_{max}$ ,  $g_s$ , and  $E$  were lowest in *P. virginiana*, which establishes denser thickets that exclude grasses at moist northeast-facing sites. Of the 2 species with intermediate  $E$  and  $g_s$ , *R. aureum* grows at the thicket edge,

TABLE IV. Average peak growth season  $\delta^{13}\text{C}$  (‰),  $c_i/c_a$  (from gas exchange),  $c_i/c_a$  (from  $\delta^{13}\text{C}$ ), PWUE (from gas exchange), PWUE (from  $\delta^{13}\text{C}$ ) ( $\text{mmol}\cdot\text{CO}_2\cdot\text{mol}\cdot\text{H}_2\text{O}^{-1}$ ) in *Artemisia cana*, *Prunus virginiana*, *Ribes aureum*, and *Rhus trilobata*. All data and leaf samples were obtained on DOY 180. Errors indicate the standard deviation ( $n = 5$  for each species-indicator pair).

Water-use indicator	<i>Artemisia cana</i>	<i>Prunus virginiana</i>	<i>Ribes aureum</i>	<i>Rhus trilobata</i>
$\delta^{13}\text{C}$ (‰)	$-29.39 \pm 0.86$	$-25.58 \pm 0.70$	$-25.98 \pm 1.37$	$-25.49 \pm 0.77$
$c_i/c_a$ (gas exchange)	$0.84 \pm 0.05$	$0.55 \pm 0.05$	$0.63 \pm 0.05$	$0.60 \pm 0.10$
$c_i/c_a$ (from $\delta^{13}\text{C}$ )	$0.75 \pm 0.04$	$0.58 \pm 0.03$	$0.60 \pm 0.06$	$0.58 \pm 0.03$
PWUE (gas exchange)	$1.93 \pm 0.51$	$3.00 \pm 0.44$	$3.87 \pm 0.53$	$3.17 \pm 0.38$
PWUE (from $\delta^{13}\text{C}$ )	$1.92 \pm 0.32$	$2.94 \pm 0.23$	$3.35 \pm 0.57$	$2.77 \pm 0.22$

between shrubs and grasses, while *R. trilobata* is the dominant shrub on the warmer and drier south-facing slopes, where it grows not only among  $C_3$  grasses (*Agropyron cristatum*), but also among more water-use-efficient  $C_4$  grasses (e.g., *Bouteloua gracilis*) and cacti (*Opuntia polyacantha* and *Mamillaria vivipara*). Overall, this pattern is consistent with the hypothesis that a low PWUE growth strategy may allow woody plants to compete effectively for soil moisture with  $C_3$  grasses and other low PWUE annuals in temperate semiarid grasslands.

#### PLANT FUNCTIONAL TRAITS AND WATER-USE STRATEGY

At the peak of the growth season, *A. cana* had the lowest PWUE, most negative  $\delta^{13}\text{C}$ , and highest  $c_i/c_a$ . In contrast to grass species and other shrubs, however, this profligate early season water use did not prevent *A. cana* from possessing the highest  $A_{\text{max}}$  and  $g_s$  of all species in August. The impact of high E on  $\theta_v$  is decreased by wide between-plant spacing, low total leaf area (Table II), growth on relatively moist north-facing slopes, and deep roots. Furthermore, leaves of *A. cana* are narrow and pubescent. Narrow leaves inhibit boundary layer growth, resulting in lower  $T_{\text{leaf}}$  under high irradiance than in broader leaves (Schuepp, 1993). Reflective leaf hairs act to reflect a portion of incoming radiation that would otherwise be absorbed while still allowing for penetration of saturating PPFD. Along with evaporative cooling due to high E, these characteristics likely explain why *A. cana* had the lowest mean  $T_{\text{leaf}}$  of the 4 shrubs in this study. The maintenance of higher late summer  $A_{\text{max}}$  was also related to nitrogen economy. The PNUE of all 4 species declined during July and August due to the  $g_s$ -mediated decrease in  $A_{\text{max}}$  (Figure 4). However, in *A. cana*, the maintenance of high leaf  $N_{\text{area}}$  (Figure 3) prevented further decreases in photosynthetic capacity.

*Prunus virginiana*, *R. aureum*, and *R. trilobata* experienced more severe late-season drought stress than *A. cana* despite higher early season PWUE. In these 3 species,  $A_{\text{max}}$  decreased to very low values in August, in part due to stomatal closure. A cessation of net photosynthesis was observed in some green leaves by DOY 233. In contrast with *A. cana*, for which reflectance patterns were obscured by white leaf hairs, sPRI peaked on DOY 205 in these species as the leaves matured but then decreased as VPD rose under limiting soil moisture conditions. This sPRI trend suggests that the ratio of carotenoid to chlorophyll pigments increased in these species to protect the leaves from photoinhibition (Stylinski, Gamon & Oechel, 2002). Neither the greenness indices (NDVI and CI) nor leaf N measures were correlated with peak season  $A_{\text{max}}$  on a between-species basis. Compared to the other glabrous species, *P. virgini-*

*ana* had the lowest  $A_{\text{max}}$ , despite similar NDVI, CI, and  $N_{\text{area}}$  (Figure 3) and higher  $N_{\text{mass}}$  (Table II; Figure 3). The relatively low peak season  $A_{\text{max}}$  and PNUE (Figure 3) of *P. virginiana* may be the result of its larger leaves and whole-plant leaf area (Table II). Nardini, Gortan, and Salles (2005) demonstrated that larger leaves with lower hydraulic efficiency tend to be produced in species that experience a lower PPFD environment. Saplings and subcanopy leaves of *P. virginiana* grow in a more shaded environment than the other species in this study.

Although water-use indicators were similar in *P. virginiana*, *R. aureum*, and *R. trilobata*, these species differed in terms of drought tolerance. *Ribes aureum* and *R. trilobata* showed similar magnitudes and seasonal patterns of  $A_{\text{max}}$ , but these patterns occurred with much lower  $\theta_v$  in *R. trilobata*. *Rhus trilobata* may be more drought tolerant than *R. aureum* and *P. virginiana*, because of its vigorous and deep rooting network. Higher drought resistance may also be conferred by its thicker, waxier, and smaller leaves, providing protection from water loss and high  $T_{\text{leaf}}$ . The relatively high late-season gas exchange rates of *A. cana* were similarly indicative of higher drought tolerance than *P. virginiana* and *R. aureum*. This may explain why a few stunted specimens of *A. cana* can also be found on south-facing slopes, whereas *P. virginiana* and *R. aureum* are confined to north-east-facing slopes and gully sites. To improve understanding of the relative use of summer precipitation and groundwater by coulee shrubs, future research should include (i) analysis of seasonal patterns of deuterium:hydrogen ratios in xylem sap and (ii) assessment of rooting structure, density and depth in each species.

#### INTERSPECIFIC DIFFERENCES IN STOMATAL BEHAVIOUR

Although all 4 shrubs showed high photosynthetic capacity and low PWUE, they differed in terms of stomatal behaviour. In *P. virginiana*, *R. aureum*, and *R. trilobata*, stomatal conductance was regulated to maintain  $c_i/c_a$  within a constant range. In response to soil moisture stress,  $g_s$  decreased in close association with  $A_{\text{max}}$ , and PWUE declined, due to the effect of increasing VPD on E (Figure 4). *Artemisia cana* exhibited an exceptionally high capacity for  $g_s$  and E when  $\theta_v$  was high and had the highest  $c_i/c_a$  and lowest PWUE in June. As  $\theta_v$  decreased,  $g_s$  declined more than  $A_{\text{max}}$  in *A. cana*, allowing its high  $c_i/c_a$  values to decrease to the range observed in the other species. Transpiration and  $A_{\text{max}}$  decreased proportionately in response to soil moisture stress and increasing VPD, so that PWUE was maintained within a constant range. Two drought stress acclimation strategies are thus apparent, with  $g_s$  regulated to maintain constant PWUE in *A. cana*

and  $g_s$  regulated to maintain constant  $c_i/c_a$  in *P. virginiana*, *R. aureum*, and *R. trilobata*.

#### HIGH PHOTOSYNTHETIC CAPACITY IN WOODY DECIDUOUS SHRUBS

The similarity of PWUE ( $A_{\max}/E$ ) values from photosynthetic discrimination (Equation [9]) and gas-exchange methods (Equation 6) reinforces the validity of the Ponton *et al.* (2006) model and suggests that the microclimatic conditions of measurement dates were representative of the season. However, during a relatively moist and cool period in late June,  $A_{\max}$  was substantially higher than previously reported for the genera examined, ranging from  $20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  in *P. virginiana* to  $40 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  in *R. trilobata*. Maximum photosynthetic rates of up to  $34 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  have been reported in annual species of *Artemisia* (Wang *et al.*, 2008), but  $A_{\max}$  was  $18\text{--}23 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  in the woody species *A. tridentata* (Gillespie & Loik, 2004; Hill, Willson & Smith, 2005) and just  $15 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  in *A. frigida* (Peng *et al.*, 2007). Peak  $A_{\max}$  was  $16.8\text{--}17.7 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  in *Rhus glabra* (Larson, 1998; McCarron & Knapp, 2001),  $11.1\text{--}12.9 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  in *Prunus* spp. (*ibid.*), and  $21 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  in *R. aureum* (D. A. Sims, pers. comm.). With respect to the global relationship between  $N_{\text{mass}}$  and  $A_{\text{mass}}$  (GLOPNET; Wright *et al.*, 2004), the coulee shrubs of this study were among the most photosynthetically productive and nitrogen-rich species at the peak of the growth season (Figure 5). Mass-based net photosynthesis ( $A_{\text{mass}}$ ) ranged from  $143 \text{nmol}\cdot\text{g}^{-1}\cdot\text{s}^{-1}$  in *P. virginiana* to  $365 \text{nmol}\cdot\text{g}^{-1}\cdot\text{s}^{-1}$  in *R. aureum*, and  $A_{\text{mass}}:N_{\text{mass}}$  ratios were high in *A. cana*, *R. aureum*, and *R. trilobata*. High photosynthetic rates and low PWUE represent adaptive characteristics that may have evolved to maximize net carbon gain in northern semiarid grasslands, where adequate soil moisture is usually short-lived.

The short duration of soil moisture availability is a key control on the productivity of northern semiarid grasslands.

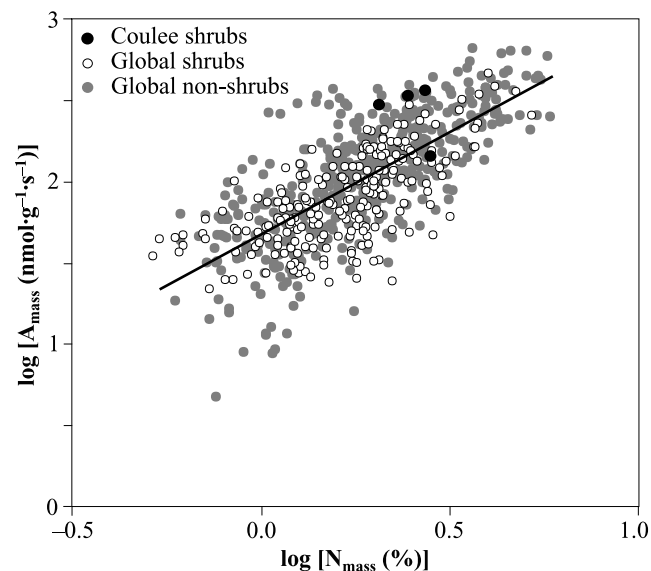


FIGURE 5. Relationship between mass-based nitrogen content ( $N_{\text{mass}}$ ) and net photosynthesis ( $A_{\text{mass}}$ ) in leaves of coulee shrubs, as compared with other shrubs and non-shrubs at a global scale. Global data were obtained with permission from the Global Plant Trait Network (GLOPNET; Wright *et al.*, 2004).

The evolution of plant traits that promote tolerance or avoidance of drought conditions is, therefore, essential for survival in these ecosystems. Whereas woody plants typically exhibit more conservative water-use strategies than annuals and herbaceous plants, we found that 4 shrubs of a southern Alberta grassland used profligate water-use strategies to maximize  $A_{\max}$  during a brief period of soil moisture availability.

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