

Linking nonstructural carbohydrate dynamics to gas exchange and leaf hydraulic behavior in *Pinus edulis* and *Juniperus monosperma*

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Summary

- Leaf hydraulics, gas exchange and carbon storage in *Pinus edulis* and *Juniperus monosperma*, two tree species on opposite ends of the isohydry–aniso-hydry spectrum, were analyzed to examine relationships between hydraulic function and carbohydrate dynamics.
- Leaf hydraulic vulnerability, leaf water potential (Ψ_l), leaf hydraulic conductance (K_{leaf}), photosynthesis (A), stomatal conductance (g_s) and nonstructural carbohydrate (NSC) content were analyzed throughout the growing season.
- Leaf hydraulic vulnerability was significantly lower in the relatively anisohydric *J. monosperma* than in the more isohydric *P. edulis*. In *P. edulis*, Ψ_l dropped and stayed below 50% loss of leaf hydraulic conductance (P_{50}) early in the day during May, August and around midday in September, leading to sustained reductions in K_{leaf} . In *J. monosperma*, Ψ_l dropped below P_{50} only during August, resulting in the maintenance of K_{leaf} during much of the growing season. Mean A and g_s during September were significantly lower in *P. edulis* than in *J. monosperma*. Foliar total NSC was two to three times greater in *J. monosperma* than in *P. edulis* in June, August and September.
- Consistently lower levels of total NSC in *P. edulis* suggest that its isohydric strategy pushes it towards the exhaustion of carbon reserves during much of the growing season.

Introduction

Widespread occurrences of drought-induced tree mortality throughout the world have been documented over the last several years (Allen *et al.*, 2010; Williams *et al.*, 2013), and nonlethal reductions in growth that are associated with the observed occurrences of tree mortality could represent an even greater drought-induced loss of net ecosystem productivity (Williams *et al.*, 2010). Future climate trends are predicted to cause greater drought-related impacts on forest ecosystems (IPCC, 2013) and we have only a partial understanding of the mechanisms involved in tree response to drought stress. Consequently, our ability to accurately predict changes in forest composition and productivity in response to climate change is limited (Scholze *et al.*, 2006). Continuity of the hydraulic pathway from the roots to the sites of evaporation in the leaf is necessary for the maintenance of leaf hydration, as well as stomatal conductance and photosynthesis. Hydraulic vulnerability is likely to be a key determinant of the survival of trees during extended drought, as well as a dominant factor in determining

future species distributions in response to changing climate conditions (Choat *et al.*, 2012; Johnson *et al.*, 2012; Das *et al.*, 2013). Water transport in the leaf is vulnerable to disruptions brought about by conduit embolism when xylem tension exceeds threshold levels (Tyree & Sperry, 1989). The generally higher vulnerability to embolism of leaves relative to stems often results in regular loss and recovery of leaf hydraulic conductance (K_{leaf}) under nonextreme conditions (Bucci *et al.*, 2003; Johnson *et al.*, 2009). Stomatal conductance is directly linked to K_{leaf} and leaf water potential thresholds for stomatal closure have been shown to be associated with a loss of K_{leaf} (Lo Gullo *et al.*, 2003; Woodruff *et al.*, 2007; Johnson *et al.*, 2009). Although short-term stomatal closure represents a constraint on assimilation, prolonged stomatal closure under conditions of severe drought could lead to the depletion of accessible carbon (C) reserves and the inability to heal injuries, resist pathogens or otherwise sustain life (Waring, 1987). The depletion of C reserves can also have detrimental impacts on the hydraulic status of trees, because the maintenance of turgor during drought requires osmotically active compounds, such as sugars,

and because the repair of embolized xylem conduits is thought to occur by the active transport of sugars into empty conduits followed by passive water movement (Johnson *et al.*, 2012, and references therein).

Piñon pine (*Pinus edulis*) and one-seed juniper (*Juniperus monosperma*) are two coexisting tree species on opposite ends of the isohydric–aniso-hydric spectrum. Plants that are relatively isohydric, such as *P. edulis*, regulate stomatal conductance in a manner consistent with the maintenance of leaf water potential above a minimum set point that avoids hydraulic failure in the xylem of different tissues in the plant (Tardieu & Simonneau, 1998). As soil water potential increases and leaf water potential (Ψ_l) rises above the threshold minimum level, isohydric constraints on stomatal conductance are alleviated. The consequence of this avoidance of hydraulic failure is the subsequent limitation of C assimilation that occurs with stomatal closure. By contrast, plants that are described as being aniso-hydric, such as *J. monosperma*, allow leaf water potential to decrease to a substantially greater degree with decreasing soil water potential. Although this aniso-hydric strategy requires the construction of more embolism-resistant xylem to avoid hydraulic failure during drought (Maherali *et al.*, 2006; Meinzer *et al.*, 2010), the maintenance of stomatal conductance under more negative leaf water potentials allows for continued C assimilation under conditions of more severe water stress. The isohydric behavior of *P. edulis*, however, is associated with higher overall rates of transpiration relative to those of juniper when water availability is high (West *et al.*, 2007).

Pinus edulis and *J. monosperma* represent species that experienced dramatically contrasting levels of mortality during the 2000–2002 drought across the southwestern USA, with *J. monosperma* and *P. edulis* experiencing up to 25% and 95% mortality, respectively (Breshears *et al.*, 2005). The difference in mortality rates of *P. edulis* and *J. monosperma* in response to drought has been hypothesized to be related to their contrasting strategies for the avoidance of tissue desiccation and hydraulic failure, and the subsequent impact of these strategies on their C balance (McDowell *et al.*, 2008). The mean annual temperature in the southwestern USA increased by *c.* 0.8°C during the last century and it is projected to rise by an additional 1.4–4.4°C by the end of this century (USGCRP, 2009), with warming projected to be greatest during the summer growing season. Warming has already contributed to decreases in spring snowpack river flows, and future warming is projected to produce more severe droughts in the region (USGCRP, 2009). Although *P. edulis* and *J. monosperma* are co-occurring species, their contrasting hydraulic strategies contribute to differences in their spatial distribution, with junipers having a greater representation in drier sites and piñon tending to dominate in the more mesic locations within piñon–juniper woodlands (Martens *et al.*, 2001; Mueller *et al.*, 2005). The relative dominance, as well as the very coexistence, of these two species in piñon–juniper woodlands is likely to be substantially impacted by future climate change if current projections hold true. The amount of widespread tree mortality that has recently occurred in this region, the intensity of the projected changes in climate conditions there, and the complexity of the dynamics involved in tree survival and productivity point to the

need to develop a greater mechanistic understanding of the relationships between tree hydraulic behavior and C dynamics.

Although several recent studies have investigated the impact of drought on the hydraulic or C dynamics of trees within the C starvation/hydraulic failure framework (McDowell *et al.*, 2010; Galvez *et al.*, 2011; Klein *et al.*, 2011; Anderegg *et al.*, 2012; Galiano *et al.*, 2012; Gruber *et al.*, 2012; Anderegg & Anderegg, 2013; Hartmann *et al.*, 2013; Sevanto *et al.*, 2014), research to date has yet to illustrate mechanistic relationships between hydraulic vulnerability, assimilation and C storage in the context of isohydric vs aniso-hydric hydraulic strategies. In addition, previous research examining C dynamics and hydraulic failure has generally involved potted plants that are prone to rapid dehydration because of limited soil volume. We know of no research that has examined the relationships between hydraulic function, gas exchange and storage/depletion of C compounds, either *in situ* or in mature trees, in the context of isohydric vs aniso-hydric hydraulic strategies.

The objectives of this study were to determine the extent to which leaf hydraulic function is constrained on a daily and seasonal basis in two co-occurring species that exist on opposite ends of the isohydric vs aniso-hydric spectrum, and to identify any relationships between variations in hydraulic function, gas exchange and nonstructural carbohydrate (NSC) storage as drought stress varies throughout the growing season. We hypothesized that: shoots of *P. edulis* would be more hydraulically vulnerable than those of *J. monosperma*; greater hydraulic vulnerability in *P. edulis* would lead to greater constraints on K_{leaf} as leaf water potential declines, relative to *J. monosperma*; and that the constraints on K_{leaf} in *P. edulis* would, in turn, be associated with lower levels of assimilation and lower levels of C storage relative to *J. monosperma*.

Materials and Methods

Field site

Plant material was collected at the Los Alamos National Laboratory Plant Survival and Mortality (SUMO) site in Los Alamos, NM, USA (35°48'48"N, 106°17'58"W, elevation 2150 m above sea level (asl)). The site is located on the eastern side of the Jemez Mountains on the Pajarito Plateau at the transition zone from ponderosa pine (*Pinus ponderosa*)-dominated forest to piñon–juniper woodland, and is dominated by piñon pine (*Pinus edulis* Engelm.) and juniper (*Juniperus monosperma* (Engelm.) Sarg.), with the occasional ponderosa pine trees at the vicinity of the site and Gambel oak (*Quercus gambelii*) shrubs among the trees. The mean annual temperature (1981–2012) is 11°C with the coldest month, January, averaging –2°C and the warmest month, July, averaging 20°C. Mean annual precipitation is 470 mm, 48% of which falls during the North American Monsoon between July and September. Soils are Hackroy clay loam derived from volcanic tuff (Soil Survey Staff, Natural Resource Conservation Service, US Department of Agriculture; <http://websoilsurvey.nrcs.usda.gov/>, accessed 11 December 2013) with a depth ranging from 40 to 80 cm.

Leaf water potential, leaf hydraulic conductance and vulnerability

Leaf hydraulic conductance was determined using a timed rehydration method described in Brodribb & Holbrook (2003), which involved the use of the following equation based on an analogy between rehydrating a leaf and recharging a capacitor:

$$K_{\text{leaf}} = C \log_e(\Psi_0/\Psi_f)/t \quad (\text{Eqn 1})$$

(C , capacitance; Ψ_0 , leaf water potential before partial rehydration; Ψ_f , leaf water potential after partial rehydration; t , duration of rehydration). Vulnerability curves for both species were developed from diurnal field measurements of K_{leaf} . Leaf hydraulic conductance vulnerability was determined from a sigmoidal trajectory with declining Ψ_1 obtained by fitting a three-parameter sigmoid function [$y = \frac{a}{1 + e^{-(x-\frac{b}{c})}}$]. We specifically avoided subjecting *J. monosperma* shoots to prolonged hydration before K_{leaf} measurements because of the likelihood of artifacts associated with artificially rehydrating this species for hydraulic conductance measurements (see Meinzer *et al.*, 2014). For measurement of K_{leaf} in the field, branches (*c.* 10–20 cm in length) were collected from trees, and foliage was then excised for the determination of Ψ_0 , with no equilibration time. Foliage samples from the same branch were then rehydrated for a period of t seconds and Ψ_f was measured. Distilled water was used for timed rehydration of K_{leaf} samples and water temperature was recorded to correct for changes in viscosity. Values of C were estimated from pressure–volume curves (Scholander *et al.*, 1965; Tyree & Hammel, 1972) using the methods described by Brodribb & Holbrook (2003). Briefly, Ψ_1 corresponding to turgor loss was estimated as the inflection point (the transition from the initial curvilinear, steeper portion of the curve to the more linear, less steep portion) of the graph of Ψ_1 vs relative water content (RWC). The slope of the curve before and following turgor loss provided C in terms of RWC (C_{rwc}) for pre-turgor loss and post-turgor loss, respectively. Pressure–volume curves were developed for both species from branch samples from the same individuals as used for the measurement of K_{leaf} . These samples were excised early in the morning and *P. edulis* branches were re-cut under water in the laboratory and were allowed to rehydrate for at least 4 h before pressure–volume analyses were performed. As a result of artifacts associated with the rehydration of *J. monosperma* for pressure–volume curves (see Meinzer *et al.*, 2014), *J. monosperma* branches were not rehydrated. Pressure–volume curves were created by plotting the inverse of Ψ_1 against RWC with alternate determinations of fresh mass and Ψ_1 repeated during slow dehydration of the twig on the laboratory bench until at least five points were obtained in the linear portion of the curve beyond the turgor loss point. Leaf water potential was measured using a pressure chamber (PMS Instrument Company, Albany, OR, USA). For the normalization of C on a leaf area basis, leaf areas were obtained with a

scanner and ImageJ version 1.27 image analysis software (Abramoff *et al.*, 2004).

Gas exchange

Gas exchange was measured with a portable photosynthesis system equipped with a red and blue LED source and CO₂ injector (LI-6400; Li-Cor, Lincoln, NE, USA) for both species on 19 September 2012. Gas exchange measurements were not conducted during other months. The instrument was zeroed and the chemicals were replaced before use each day. Reference [CO₂] was maintained at 400 ppm, the ambient concentration at the site, and the flow rate was kept at 400 $\mu\text{mol s}^{-1}$. Leaf temperature and cuvette irradiance were matched to ambient conditions. Assimilation (A) and stomatal conductance (g_s) values were normalized by leaf area which was scanned using a leaf area meter (LI-3100, Li-Cor).

Chemical analyses

Samples were immediately placed on dry ice in a cooler in the field and later stored in a -70°C freezer before being micro-waved for 90 s to stop all enzymatic activity. They were then oven dried for 72 h at 65°C , and ground to a fine powder. Dried and ground samples of needles were analyzed for the content of sucrose, glucose + fructose, starch and total NSC. Water was added to the powdered samples and NSC was extracted from the solutions by heating them in steam for 1.5 h. The concentration of free glucose was determined photometrically on a 96-well microplate photometer (Multiskan FC; Thermo Scientific, Waltham, MA, USA) after enzymatic conversion of glucose to gluconate-6-phosphate. Photometric analysis was based on the absorbance of samples at 340 nm in solution with reference to the absorbance of a glucose reference solution. Samples were analyzed both before and after enzymatic treatments of sucrose digestion by invertase for 45 min and starch digestion by amyloglucosidase overnight. Glucose + fructose content was determined from photometric analysis of sample solutions with no enzymatic treatment. Sucrose content was determined by subtracting the glucose + fructose content from the photometric analysis of the glucose concentration of sample solutions following invertase enzyme treatment. Total NSC was determined from the amyloglucosidase reaction mixture, which contains the original concentrations of free glucose and fructose, plus glucose and fructose liberated from starch and sucrose. Inclusion of sucrose standards in each set of samples subjected to amyloglucosidase treatment indicated that amyloglucosidase hydrolyzed sucrose as well as starch. The starch content was determined by subtracting the glucose content of the sample solution following invertase enzyme treatment from the total NSC content. All NSC values are presented as percent dry matter.

Sampling and statistical analyses

Field sample collection and measurements were performed on 7 June 2011, 15 and 16 May 2012, 15, 16 and 24 August 2012, and

Table 1 Pre-dawn and midday leaf water potential (Ψ_l) for *Juniperus monosperma* and *Pinus edulis* during May, August and September, morning (AM) and midday assimilation (A) during September, and whether or not diurnal values of Ψ_l dropped below P_{50}

	Species	Pre-dawn Ψ_l (MPa)	Midday Ψ_l (MPa)	AM A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Midday A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	AM g_s ($\text{mmol m}^{-2} \text{s}^{-1}$)	Midday g_s ($\text{mmol m}^{-2} \text{s}^{-1}$)	P_{50} reached during diurnal?
May	<i>J. monosperma</i>	−0.87 (0.02)*	−2.10 (0.06)	—	—	—	—	No
May	<i>P. edulis</i>	−0.78 (0.03)*	−2.10 (0.07)	—	—	—	—	Yes
August	<i>J. monosperma</i>	−3.82 (0.41)**	−4.17 (0.25)**	—	—	—	—	Yes
August	<i>P. edulis</i>	−1.23 (0.21)**	−1.99 (0.35)**	—	—	—	—	No
September	<i>J. monosperma</i>	−2.14 (0.19)*	−2.67 (0.13)*	5.02 (0.035)	9.83 (1.5)*	50.1 (4.6)	54.6 (8.0)*	Yes
September	<i>P. edulis</i>	−1.25 (0.09)*	−1.88 (0.12)*	4.4 (0.073)	4.59 (1.5)*	44.9 (1.1)	20.8 (6.4)*	Yes

Asterisks indicate significant differences between species within a given month: *, $P \leq 0.05$; **, $P \leq 0.001$. Values in parentheses are standard errors. Water potential measurements were made on four individuals per species in May, and six individuals in August and September. Gas exchange measurements were made on six individuals per species in September.

11 and 13 September 2012. Measurements and sampling for K_{leaf} , Ψ_l , A and NSC were performed over diurnal time periods starting around sunrise and continuing through late afternoon. Pre-dawn and midday measurements (Table 1) were the initial measurement round and the afternoon measurement round closest to 13:00 h, respectively. Only fully developed foliage that was no older than 1 yr was measured or sampled. All individuals were in open areas and sunlit branches/leaves on the south side of trees were chosen for measurement. K_{leaf} measurements were made on four individuals per species in May, four to six individuals per species in August and six individuals per species in September. To provide a clearer picture of the trend in K_{leaf} with changing Ψ_l , the K_{leaf} data in the vulnerability curves (Fig. 1) were grouped (binned) over water potential ranges of c. 0.2–0.8 MPa to produce mean K_{leaf} and Ψ_l values. Each bin contained between five and 27 measurements and a total of 103–106 shoots were used to create each curve. The sigmoid regression curves, however, were fitted through individual data points. This reduces the correlation coefficient, but avoids the inflation of regression correlation as a result of the binning of the data. For example, r^2 values for sigmoid curve fits to binned data would be 0.99 and 0.95 for *P. edulis* and *J. monosperma*, respectively. Rounds of gas exchange measurements were made during September on six individuals per species approximately every 90 min, and one shoot per tree was measured repeatedly throughout the day. NSC data represent samples from four trees per species in June 2011, two trees per species in May 2012 and seven trees per species in August and September 2012. Diurnal sampling for NSC analyses was only conducted during May, August and September 2012. All statistical analyses were performed using R Statistical Software (Foundation for Statistical Computing, Vienna, Austria). Physiological characteristics were analyzed using two-way t -tests. Student's t -test was used unless variances between populations were unequal based on Bartlett's test, in which case Welch's t -test was used. All comparisons were considered to be significant at the 95% confidence level.

Results

The vulnerability of leaf hydraulic conductance was drastically different in the two species with contrasting stomatal control

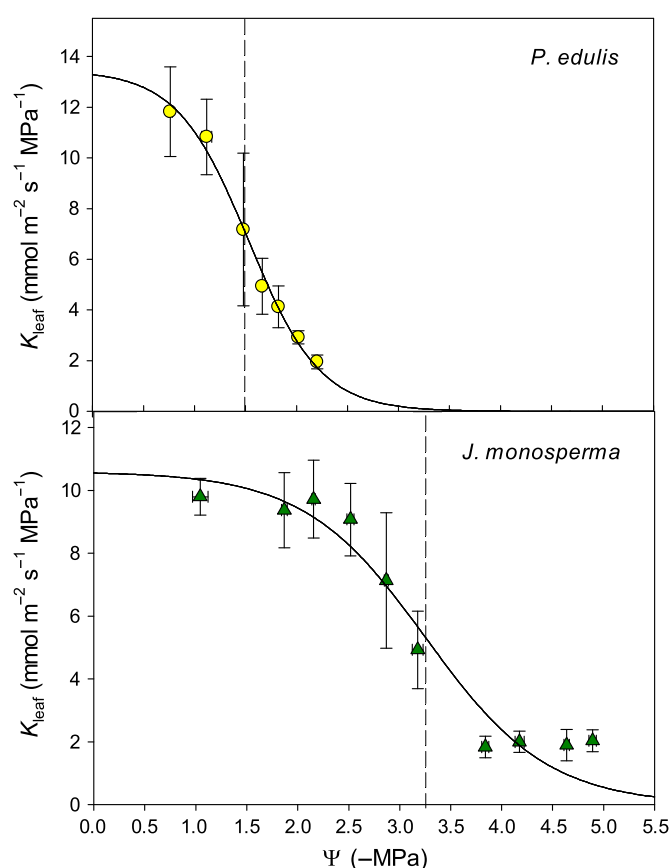


Fig. 1 Leaf hydraulic conductance (K_{leaf}) for *Pinus edulis* and *Juniperus monosperma*. The decline in K_{leaf} with progressively more negative leaf water potential (Ψ_l) represents K_{leaf} vulnerability. Dashed vertical lines represent 50% loss of leaf hydraulic conductance (P_{50}), which occurred at $\Psi_l = -3.2$ MPa for *J. monosperma* and $\Psi_l = -1.54$ MPa for *P. edulis*. $n = 10$ trees per species. Error bars, $\pm$ SE.

strategies (Fig. 1). *Pinus edulis* leaves showed a 50% loss in conductance (P_{50} , vertical dashed lines in Fig. 1) at -1.54 MPa (95% confidence interval, -1.38 to -1.66 MPa), whereas *J. monosperma* had a P_{50} value that was more than twice as negative as that of *P. edulis* at -3.2 MPa (95% confidence interval, -2.9 to -3.6 MPa). The sigmoid functions fitted to leaf hydraulic

conductance data yielded r^2 values of 0.43 for *P. edulis* and 0.37 for *J. monosperma* for the dependence of K_{leaf} on Ψ_1 .

Despite the difference in leaf hydraulic vulnerability, diurnal values of shoot water potential were similar between *P. edulis* and *J. monosperma* during the month of May (Fig. 2a,b, Table 1). By August, however, substantial differences in diurnal patterns of shoot water potential had developed (Fig. 2c,d). During August, mean pre-dawn and midday shoot water potentials were significantly lower in *J. monosperma* than in *P. edulis* (Table 1). Differences in diurnal water potentials during September were less pronounced than in August, although they were still significant (Table 1, Fig. 2e,f).

As expected, diurnal patterns of K_{leaf} appeared to be highly coordinated with diurnal patterns of Ψ_1 . Changes in K_{leaf} were particularly well coordinated with the P_{50} values of each species, with K_{leaf} tending to decline abruptly when Ψ_1 dropped below P_{50} (Fig. 2). To more directly compare relationships between Ψ_1 and K_{leaf} across species, diurnal K_{leaf} values were normalized by

dividing each K_{leaf} value by the corresponding maximum K_{leaf} for each species (maximum K_{leaf} = mean of 10 highest values) and reported as relative K_{leaf} (Fig. 3). Excluding one mean value that occurred during the late afternoon in May, and was therefore strongly influenced by the low light level, there were a total of 17 rounds of measurements for both Ψ_1 and K_{leaf} during May, August and September. In order to assess how isohydric vs anisohydric behavior influenced potential C gain, we analyzed diurnal values of Ψ_1 and K_{leaf} to evaluate the relative tendency of each species to exist above or below P_{50} , and the extent to which being above or below P_{50} impacted K_{leaf} (Fig. 3). In *J. monosperma*, 65% of the mean K_{leaf} diurnal values occurred when the mean Ψ_1 was above (less negative than) P_{50} and 47% had a mean K_{leaf} index value of 0.5 or greater (Fig. 3). Conversely, only 18% of the mean K_{leaf} diurnal values for *P. edulis* occurred when the mean Ψ_1 was above (less negative than) P_{50} and only 18% had a mean K_{leaf} index value of 0.5 or greater (Fig. 3). The substantially greater tendency of *P. edulis* to experience Ψ_1 below P_{50} , and to

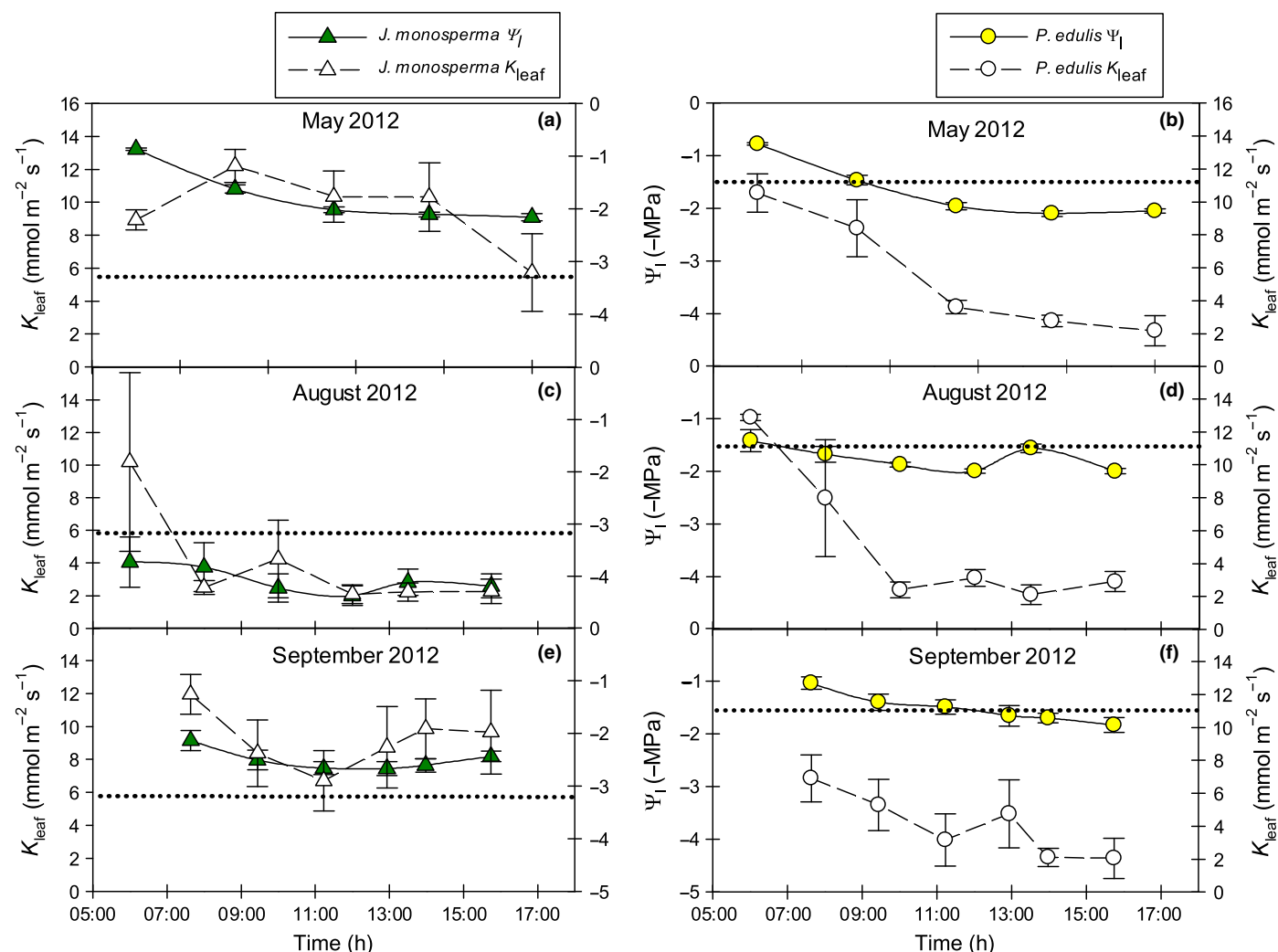


Fig. 2 Diurnal patterns of leaf hydraulic conductance (K_{leaf}) represented by open symbols and leaf water potential (Ψ_1) represented by closed symbols for *Juniperus monosperma* (a, c, e), represented by triangles, and *Pinus edulis* (b, d, f), represented by circles. Horizontal dashed lines represent Ψ_1 corresponding to 50% loss of leaf hydraulic conductance (P_{50}). Measurements were made on four individuals per species in May and August and six individuals per species in September. Error bars, $\pm$ SE.

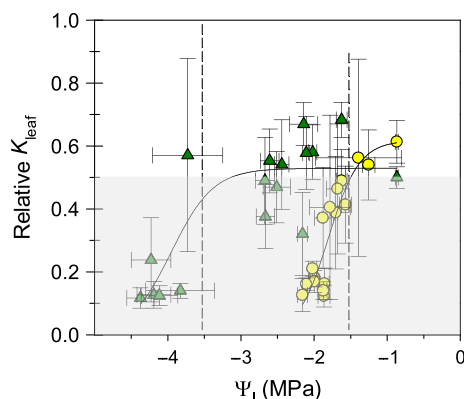


Fig. 3 Mean relative values of leaf hydraulic conductance (K_{leaf}) for *Pinus edulis* (yellow circles) and *Juniperus monosperma* (green triangles) plotted against leaf water potential (Ψ_l). Values of both are from diurnal measurements. Dashed vertical lines represent 50% loss of leaf hydraulic conductance (P_{50}). The shaded portion represents the area in which K_{leaf} is below an index value of 0.5. Measurements were made on four individuals per species in May and six individuals per species in August and September. Error bars, $\pm$ SE.

exhibit relative K_{leaf} values below 0.5, suggests that the species generally functioned within a lower leaf hydraulic safety margin, and that it was more hydraulically constrained during the growing season.

Diurnal patterns of A and g_s during September were markedly different between species (Fig. 4). Although initial morning measurements were similar between species, subsequent mean A and g_s values were all higher for *J. monosperma* than *P. edulis*. Both A and g_s began to drop immediately following initial measurements for *P. edulis* and continued to drop throughout the day, whereas they continued to rise for *J. monosperma* until late morning/early afternoon. By midday, the mean A value of *J. monosperma* was approximately twice that of *P. edulis* ($P=0.002$; Table 1, Fig. 4) and the mean g_s value was over 2.5 times higher than that of *P. edulis* ($P=0.008$; Table 1, Fig. 4). Subsequent afternoon and late afternoon measurements continued to show A and g_s values for *J. monosperma* that were more than twice those of *P. edulis* (Fig. 4).

There were significant contrasts in the concentrations of NSC constituents between species that varied throughout the growing season (Fig. 5, Table 2). Differences in mean total NSC between species were least pronounced, and nonsignificant, at the beginning of the growing season in May ($P=0.35$). Although total NSC declined in both species from May until August, total NSC content was significantly greater in *J. monosperma* than in *P. edulis* in June ($P=0.0067$), August ($P=0.0053$) and September ($P=0.00028$; Fig. 5a, Table 2). Starch concentrations also declined in both species from May until August, the majority of which presumably represents the depletion of reserves for the development of new tissues. Although sucrose content also declined in *P. edulis* from May until August, it remained relatively constant in *J. monosperma*, such that, by August, there was a pronounced difference in sucrose content between species ($P<0.000001$), which persisted through September ($P<0.000001$). Glucose and fructose contents were significantly

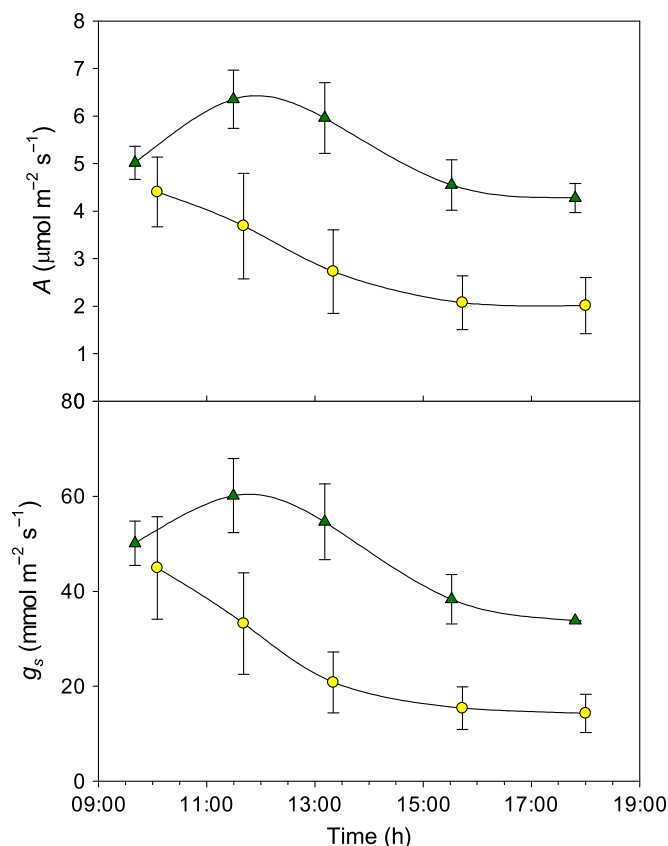


Fig. 4 Mean values of assimilation (A) and stomatal conductance (g_s) for *Pinus edulis* (yellow circles) and *Juniperus monosperma* (green triangles) plotted against time. n = six trees per species. Error bars, $\pm$ SE.

lower in *J. monosperma* than in *P. edulis* during May ($P=0.0018$). By June, however, this had reversed ($P<0.00001$).

Although the magnitude of depletion and recovery of NSC was far less pronounced over diurnal periods than seasonally, there were significant diurnal changes in NSC components for both species during different periods of the growing season (Fig. 6). During May, fluctuations in sucrose occurred in *P. edulis* as well as a midday drop, followed by a recovery, in starch. Glucose and fructose also increased significantly in *P. edulis* over the course of the day (Fig. 6a). No significant changes in any NSC constituent occurred over the course of the day during August in either species (Fig. 6b). Total NSC during May and August was not significantly changed over the course of the day in either species. However, in September, total NSC was significantly greater in both *J. monosperma* and *P. edulis* at the end relative to the beginning of the day ($P<0.0001$ and $P<0.013$, respectively; Fig. 6c). The September increase in total NSC in *J. monosperma* appeared to be a result of an increase in starch ($P=0.0059$), whereas the corresponding increase in *P. edulis* appeared to be caused by an increase in sucrose ($P=0.0078$) and glucose/fructose ($P=0.004$).

Across species a number of different NSC constituents were negatively correlated with midday Ψ_l (i.e. with more negative minimum Ψ_l , NSC constituents increased in percent content; Fig. 7). The degree to which NSC was correlated with midday Ψ_l

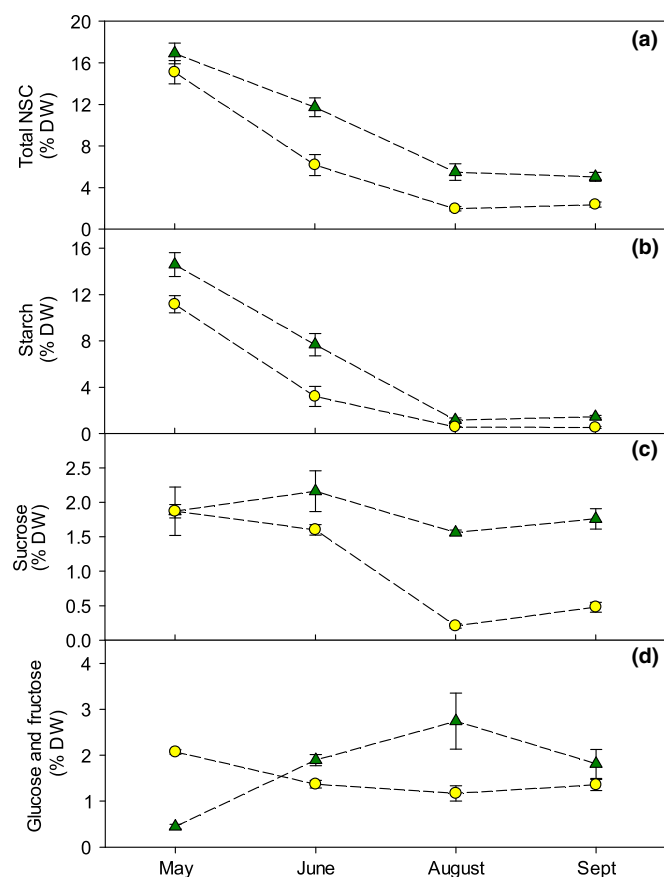


Fig. 5 Percent content of total nonstructural carbohydrate (NSC) components from foliage (a), starch (b), sucrose (c), and glucose and fructose (d) for *Juniperus monosperma* (green triangles) and *Pinus edulis* (yellow circles) during May, June, August and September. $n =$ two trees per species in May 2012, four trees per species in June 2011 and August 2012, and six trees per species in September 2012. Error bars, $\pm$ SE.

was greatest when water stress was most intense and least correlated when water stress was minimal. During May, there was no correlation between midday Ψ_1 and total NSC (Fig. 7a). During August, however, when water stress was most severe, mean total NSC was strongly negatively correlated with midday Ψ_1 ($r^2 = 0.95$, $P < 0.0001$; Fig. 7b). In September, by which time

summer rains had partially diminished soil water stress, the correlation between Ψ_1 and total NSC was still significant ($r^2 = 0.72$, $P = 0.0005$; Fig. 7c), although less pronounced than during August.

Discussion

The observed trends in Ψ_1 , K_{leaf} , A , g_s and NSC in these two species suggest that their contrasting strategies for the regulation of stomatal conductance and transpiration lead to significantly different consequences for their ability to maintain carbohydrate reserves during extended periods of soil water stress. *Juniperus monosperma* and *P. edulis* occupy very different positions on the isohydric–aniso-hydric continuum of stomatal regulation (West, 2006; Limousin *et al.*, 2013). The coordination of stomatal conductance with leaf hydraulics, such as the association of stomatal closure with loss of K_{leaf} and leaf water potential thresholds (Lo Gullo *et al.*, 2003; Woodruff *et al.*, 2007; Johnson *et al.*, 2009), demonstrates the direct relationship between K_{leaf} and assimilation (Sack & Holbrook, 2006) and thus a link between leaf hydraulics and C reserves. Our results support the hypothesized greater leaf hydraulic vulnerability in *P. edulis* than in *J. monosperma* (Fig. 1). The diurnal behavior of K_{leaf} in both species appeared to be highly consistent with their leaf hydraulic vulnerability curves (Figs 1, 2) and with their contrasting modes of osmotic and turgor regulation (Meinzer *et al.*, 2014). The ranking and relative difference between P_{50} for loss of K_{leaf} in *P. edulis* (c. -1.5 MPa) and *J. monosperma* (c. -3.25 MPa) were consistent with their most negative values of shoot Ψ at turgor loss (c. -3 and -6 MPa, respectively) observed during the study period (Meinzer *et al.*, 2014). These traits led to prolonged periods during which *P. edulis* was largely constrained hydraulically, presumably leading to increasing reliance on stored carbohydrates for metabolic function. By contrast, it was only during the most intense period of soil water stress during August when *J. monosperma* lost leaf hydraulic function because of excessively low Ψ_1 (Fig. 2). Thus, our second hypothesis, that the greater hydraulic vulnerability in *P. edulis*, relative to *J. monosperma*, would lead to greater constraints on K_{leaf} as leaf water potential declined, was supported. The leaf hydraulic behavior observed in

Table 2 Percent content of nonstructural carbohydrate (NSC) constituents including total NSC, starch, sucrose and glucose plus fructose for *Juniperus monosperma* and *Pinus edulis* during May, June, August and September

	Species	Total NSC (%)	Starch (%)	Sucrose (%)	Glucose & fructose (%)
May	<i>J. monosperma</i>	16.91 (0.99)	14.59 (1.04)	1.87 (0.10)	0.45 (0.05)*
May	<i>P. edulis</i>	15.1 (1.13)	11.17 (0.73)	1.87 (0.35)	2.07 (0.05)*
June	<i>J. monosperma</i>	11.74 (0.92)*	7.69 (0.95)	2.16 (0.30)	1.89 (0.12)***
June	<i>P. edulis</i>	6.18 (1.01)*	3.21 (0.87)	1.60 (0.08)	1.37 (0.08)***
August	<i>J. monosperma</i>	5.49 (0.80)*	1.18 (0.16)*	1.56 (0.04)***	2.74 (0.61)*
August	<i>P. edulis</i>	1.96 (0.22)*	0.59 (0.03)*	0.21 (0.03)***	1.17 (0.17)*
September	<i>J. monosperma</i>	5.04 (0.43)**	1.47 (0.12)***	1.76 (0.15)***	1.81 (0.32)
September	<i>P. edulis</i>	2.37 (0.24)**	0.53 (0.08)***	0.48 (0.07)***	1.36 (0.12)

Asterisks indicate significant differences between species within a given month: *, $P \leq 0.05$; **, $P \leq 0.001$; ***, $P \leq 0.0001$. Values in parentheses are standard errors. NSC data represent samples from four trees per species in June 2011, two trees per species in May 2012 and seven trees per species in August and September 2012.

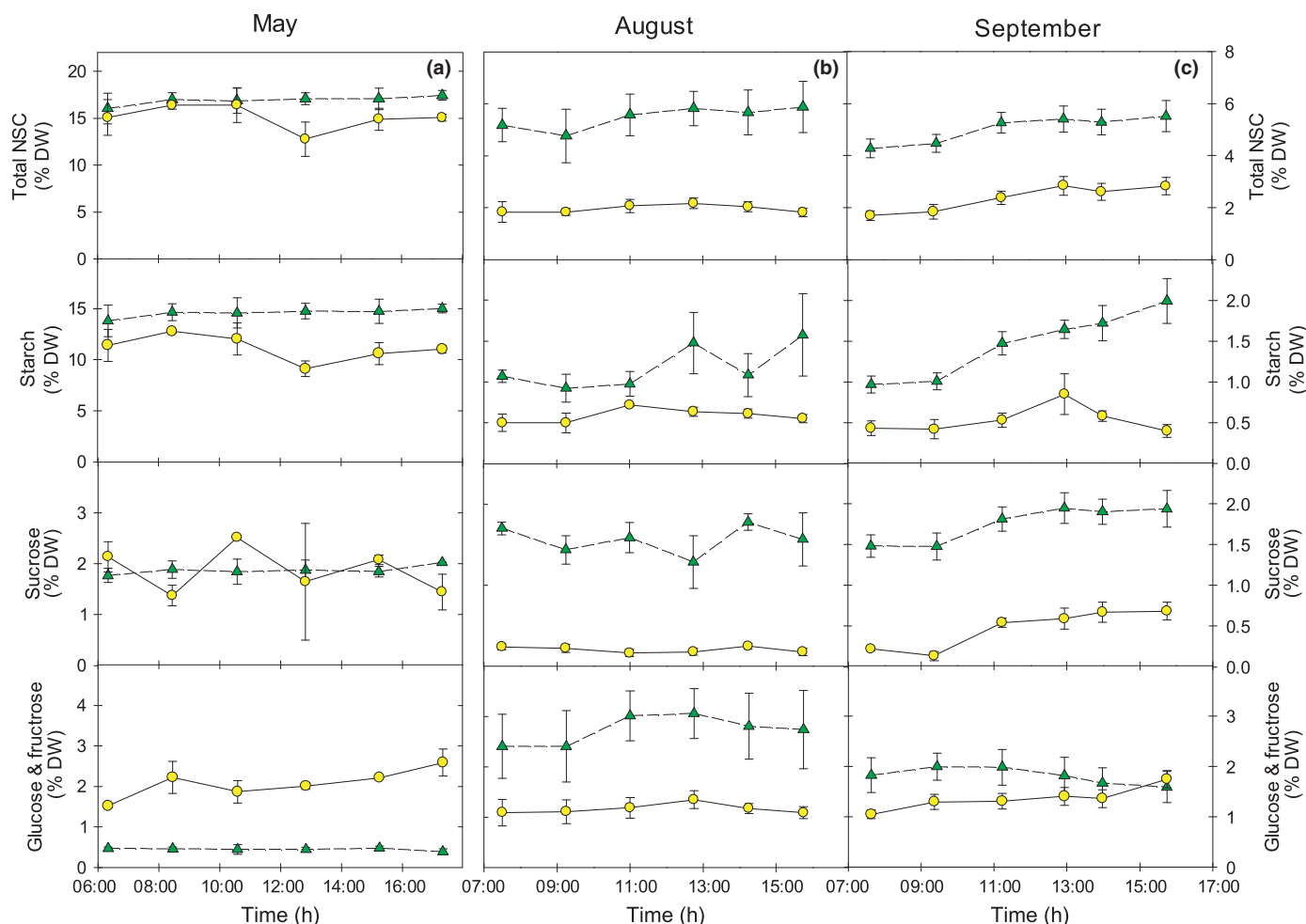


Fig. 6 Diurnal patterns of nonstructural carbohydrate (NSC) components from foliage for *Juniperus monosperma* (green triangles) and *Pinus edulis* (yellow circles). Panels show total NSC, starch, sucrose, and glucose plus fructose during May (a), August (b) and September (c). Note the different scales for the y-axes during May. n = two trees per species in May 2012, four trees per species in August and six trees per species in September 2012. Error bars, $\pm$ SE.

P. edulis and *J. monosperma* during this study was consistent with their respective isohydric and anisohydric modes of regulating stomatal conductance. The extended period of constrained hydraulic function in *P. edulis* and the relatively unaffected K_{leaf} in *J. monosperma* were consistent with the observed trends in A (Fig. 4), as well as with the substantially lower NSC levels in *P. edulis* relative to *J. monosperma*, particularly during August, when soil moisture stress was most severe (Figs 5, 6). Although NSC was only analyzed in foliar tissues in this study, in a recent study by Dickman *et al.* (2014), which involved the examination of NSC in both twig and leaf material of these same two species at the same site, *P. edulis* had a twig to leaf NSC content ratio that was approximately twice that of *J. monosperma*. This suggests that the differences in NSC observed in the current study are probably even greater when considering other tissues, such as xylem and bark. Diurnal patterns of A and g_s in the current study were also consistent with the observed patterns of NSC levels in both species. The respective hydraulic constraints, gas exchange patterns and NSC levels of *P. edulis* and *J. monosperma* during the growing season of 2012 were consistent with chronic stomatal

closure to prevent excessive water loss, leading to long-term constraints on photosynthetic C gain in *P. edulis*. This was also consistent with our third hypothesis, that the constraints on K_{leaf} in *P. edulis* would, in turn, be associated with lower levels of assimilation and lower levels of C storage relative to *J. monosperma*, as well as the hypothesis that prolonged stomatal closure during the extended drought of 2002 led to widespread C starvation in *P. edulis* (McDowell *et al.*, 2008). The correlation of NSC with midday Ψ_1 was strongest when water stress was most intense (i.e. August, Fig. 7b) and weakest when water stress was minimal (i.e. May, Fig. 7a). The association of the strength of this correlation with water stress provides additional support for the third hypothesis because the association of more negative minimum midday water potentials with greater NSC is indicative of less rigorous control of transpiration, leading to lower constraints on C accumulation. The consistent grouping of the two species at disparate ends of this relationship between NSC and midday Ψ_1 reflects the contrasting strategies of these two species for the regulation of stomatal control of transpiration (i.e. isohydry vs anisohydry).

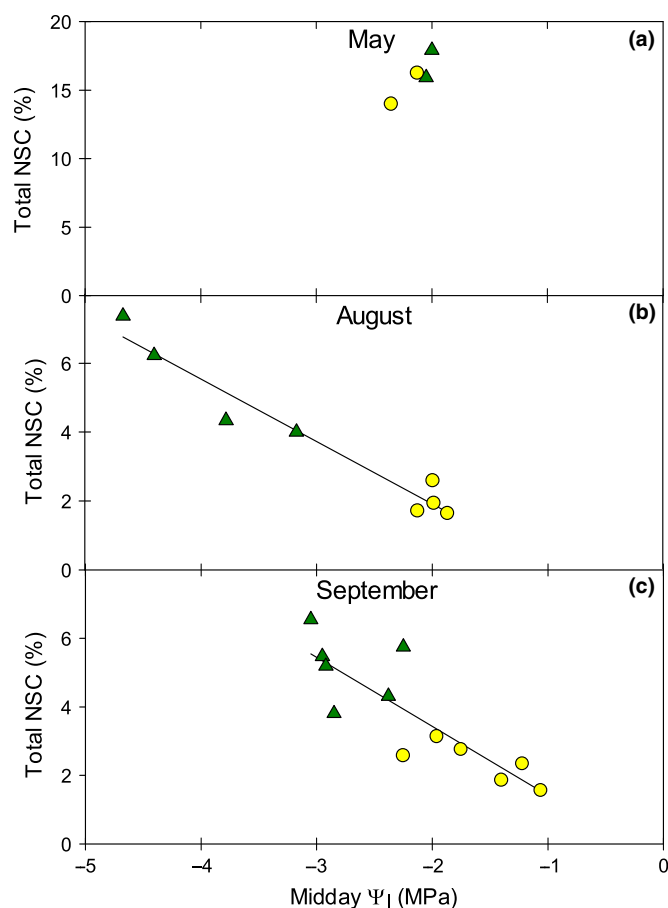


Fig. 7 Total nonstructural carbohydrate (NSC) from foliage for *Juniperus monosperma* (green triangles) and *Pinus edulis* (yellow circles) plotted against midday leaf water potential (Ψ_l) during May (a), August (b) and September (c). n = two trees per species in May 2012, four trees per species in August and six trees per species in September 2012.

Results from previous research on NSC content in response to drought have been inconsistent, as there are confounding factors involved in how NSC content is influenced by the timing, duration and severity of the drought experienced, as well as the temporal dynamics and magnitude of the natural patterns of increase and depletion of NSC in different species. In some studies, NSC content has been shown to increase with drought (Sala & Hoch, 2009; Woodruff & Meinzer, 2011; Gruber *et al.*, 2012) and decline in others (Köerner, 2003; Sayer & Haywood, 2006; Anderegg, 2012). In some cases, NSCs have been shown to remain relatively unaffected by drought (Anderegg & Anderegg, 2013). The occurrence of increasing NSC storage under conditions of drought is probably related to the timing and magnitude of dynamics between water stress and the supply and demand of NSCs. As a result of the greater sensitivity of turgor-driven cell expansion than photosynthesis to water stress (Hsiao, 1973), moderate water stress is generally associated with an increase in NSC and a 'surplus' of photosynthate which the plant is unable to use for the more drought-sensitive, turgor-driven cell growth. If drought persists and/or becomes more severe, however, the previously accumulated excess of NSCs may be depleted as a result of ongoing metabolism and other demands on carbohydrate

reserves when photosynthesis is constrained (McDowell, 2011). The rate at which soil moisture stress develops can also have an impact on NSC dynamics. The use of sample material that has developed either *in situ* or under conditions in which soil moisture is depleted in such a way that it mimics a natural system can be important when examining relationships between hydraulics and NSC dynamics. Although substantial, the levels of water stress experienced by the trees in the present study were reached relatively gradually and *in situ*. In some studies examining the effects of drought on NSC storage in potted plants in glasshouses, where soil water potential is reduced at a very rapid rate, severe effects of dehydration and even mortality can be observed with little impact on NSC storage (Anderegg & Anderegg, 2013; Hartmann *et al.*, 2013; Sevanto *et al.*, 2014). The lack of decline in NSC in these cases of very rapid soil drying may be caused in part by dehydration-induced failure of the phloem transport system (Sevanto, 2014; Sevanto *et al.*, 2014; Woodruff, 2014), resulting in NSC being quickly sequestered. In addition to limiting C availability in growing tissues and at sites of metabolism, reduced phloem transport could also exacerbate water stress because of constraints on solute-mediated regulation of cell turgor (Turner & Jones, 1980; Morgan, 1984) or if sugars cannot be translocated to where they are possibly needed for osmotic refilling of embolized vessels (Bucci *et al.*, 2003; Nardini *et al.*, 2011). Modeling phloem sieve cell conductivity (k_p) using measured anatomical and phloem sap compositional parameters obtained along a gradient of sustained water stress in Douglas-fir trees provided evidence that water stress leads to significant constraints on phloem transport capacity (Woodruff, 2014). These constraints appeared to be influenced to an extent by changes in phloem sap composition characteristics associated with increased viscosity, but more so by water stress-related changes in sieve cell structure.

Depending on a range of factors, such as photosynthetic activity, season and time of day, plants may either draw on or store NSC reserves for C demands associated with physiological processes, such as growth, reproduction, respiration and the production of defensive compounds. Fluctuations in these processes lead to variability in the demand for and availability of C. Phenological cycles, in particular, can lead to large seasonal fluctuations in NSC storage (Newell *et al.*, 2002; Woodruff & Meinzer, 2011). As such, isolated measurements of NSC can be potentially misleading, and it may be necessary to evaluate NSC storage repeatedly throughout the day and throughout the year in order to more accurately evaluate tree C status. For example, seasonal depletions of NSC reserves are typically attributed to the allocation of stored NSC to growth, independent of the sustained period of water stress that led to prolonged stomatal closure. However, by comparing seasonal patterns of Ψ_l , K_{leaf} and NSC storage and depletion during an extended period of declining soil water potential, we were able to examine relationships between the disparate hydraulic strategies represented by these two species and the depletion or relative maintenance of NSC storage. Although no tree mortality was observed in our study trees, the very low levels of NSC in *P. edulis*, particularly during August and September, are consistent with isohydric regulation of

stomatal conductance leading to pronounced reductions in C storage during extended periods of severe drought.

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