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**EFFECTS OF PRECIPITATION MANIPULATION ON
CARBOHYDRATE DYNAMICS AND MORTALITY
IN A PIÑON-JUNIPER WOODLAND**

by

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ABSTRACT

Drought-induced forest mortality is an increasing global problem with far-reaching consequences, yet mortality mechanisms remain poorly understood. Depletion of non-structural carbohydrate (NSC) stores has been implicated as a major factor in drought-induced mortality, but experimental field tests are rare. We conducted an ecosystem-scale precipitation manipulation experiment and evaluated leaf and twig NSC dynamics of two co-occurring conifers with different water regulation strategies; the relatively drought-averse piñon pine (*Pinus edulis*) and relatively drought-tolerant one-seed juniper (*Juniperus monosperma*). Experimental drought caused decreased leaf starch in dying trees of both species and increased allocation to glucose and fructose in juniper, consistent with osmoregulation requirements. For both species, average leaf starch content between drought treatment initiation and mortality was a good predictor ($R^2 = 0.77$) of the survival duration. These results, along with observations of drought-induced reductions to photosynthesis and growth, implicate carbon starvation as an important process during mortality of these two conifer species.

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Introduction:

Drought-associated tree mortality appears to be increasing across the globe (van Mantgem *et al.*, 2009; Allen *et al.*, 2010; Peng *et al.*, 2011), and has the potential to negatively influence CO₂ storage within forests (Kurz *et al.*, 2008; Hicke *et al.*, 2012). Despite decades of research, mechanisms of drought-induced tree mortality remain poorly understood (McDowell & Sevanto, 2010; Sala *et al.*, 2010; Sala *et al.*, 2012), limiting the ability of vegetation dynamics models to accurately capture and predict mortality events (Peng, 2000; Fisher *et al.*, 2010; McDowell *et al.*, 2013; Powell *et al.*, 2013; Xu *et al.*, 2013).

Two mutually-inclusive hypotheses of mortality mechanisms currently under debate are based on hydraulic and metabolic theory (McDowell *et al.*, 2008). Hydraulic failure is thought to occur when plant water potential reaches a critical value, either by equilibration with extremely dry soil or through water loss from transpiration, causing xylem cavitation and desiccation (Sperry *et al.*, 1998). Alternatively, prolonged stomatal closure to minimize water loss and the probability of hydraulic failure can promote a carbon starvation process, as respiratory carbon use exceeds assimilation and carbon stores diminish (Smith & Stitt, 2007; McDowell, 2011). Recent evidence also suggests these mechanisms may interact, causing phloem failure as carbon transport becomes constrained through osmotic or hydraulic mechanisms (McDowell *et al.*, 2013; Sevanto *et al.*, 2013).

These hypotheses remain largely untested because carbohydrate dynamics, particularly in natural settings, are poorly understood (Sala *et al.*, 2012). Currently there are limited data regarding effects of changing climate on carbohydrates, and how these dynamics may be related to mortality, though evidence supporting carbon starvation as a

critical process has been shown in mature Scots pine (*Pinus sylvestris*) and holm oak (*Quercus ilex* L.) forests in Spain (Martinez-Vilalta *et al.*, 2002; Galiano *et al.*, 2011; Galiano *et al.*, 2012; Poyatos *et al.*, 2013). Further, an interaction between carbon starvation and hydraulic failure has been supported in several species (Galvez *et al.*, 2011; Anderegg & Callaway, 2012; Adams *et al.*, 2013; Mitchell *et al.*, 2013; Quirk *et al.*, 2013; Sevanto *et al.*, 2013). Despite these advances, our understanding of the specific factors that control hydraulic failure, carbon starvation, and the progression of these processes toward mortality remains a critical limitation to our understanding of survival under climate change (Sevanto *et al.*, 2013).

Species with alternative strategies of stomatal control in response to drought, such as *Pinus edulis* (piñon pine) and *Juniperus monosperma* (one-seed juniper), may be differentially susceptible to drought-associated mortality (Tardieu & Simonneau, 1998; Williams & Ehleringer, 2000; Franks *et al.*, 2007; McDowell *et al.*, 2008; Breshears *et al.*, 2009; Mitchell *et al.*, 2013). Piñon-juniper woodlands are widespread across the Southwest US, a region that has experienced extensive drought-associated forest mortality in recent years (Breshears *et al.*, 2005; Allen *et al.*, 2010; Williams *et al.* 2013). Piñon pines are relatively isohydric, with complete stomatal closure at relatively low xylem water tensions, and are thus expected to be particularly vulnerable to carbon starvation. In contrast, junipers are relatively anisohydric, allowing their water potentials to decline by continuing to conduct water even as soils become very dry (Tardieu & Simonneau, 1998), and are predicted to be relatively less vulnerable to carbon starvation (McDowell, 2011).

We conducted a four-year ecosystem-scale experiment (Pangle *et al.*, 2012) to evaluate mortality mechanisms of these two co-occurring species under conditions of

elevated, ambient, and reduced precipitation leading to mortality. Our specific goal was to test whether the response of leaf and twig carbohydrates to precipitation manipulation was related to mortality and carbon starvation in mature piñon pine and juniper trees. We tested three hypotheses: 1) in both species, NSC content would vary with treatment such that average NSC content would be highest in irrigated and lowest in droughted trees, with higher average NSC content in surviving than dying drought trees; 2) the relatively isohydric piñon pine would experience greater declines in NSC content under drought than the relatively anisohydric juniper; and, 3) independent of species differences, trees with higher carbohydrate contents would survive longest.

Methods:

Site Description and Experimental Design

The study site is part of the US Long-Term Ecological Research network and Sevilleta National Wildlife Refuge, located in the Los Pinos Mountains of Socorro County, New Mexico (N 34° 23' 11", W 106° 31' 46", elevation 1911 m). Mean annual temperature is 12.7 °C, ranging from 2.2 °C in December to 23.3 °C in July. Mean annual precipitation is 363 mm, with mean monthly precipitation ranging from 13.0 to 67.3 mm, and approximately half of the annual total falling during the North American Monsoon (July-September). The site is dominated by piñon pine (*Pinus edulis*) and juniper (*Juniperus monosperma*), with total woody canopy coverage across the site averaging 36.7% (Pangle *et al.*, 2012).

A large precipitation manipulation experiment was established at the site in summer 2007 (Pangle *et al.*, 2012). The experimental treatments included 1) irrigation, 2) drought, 3) cover control, and 4) ambient control. Each treatment was replicated in three

blocks, or independent areas within the study site. Treatments were applied to 40 x 40 m plots (1600 m²) within each block, each including five target trees of piñon pine and five of juniper. In the irrigated treatment, precipitation was supplemented with 3 to 6 irrigations of 19 mm each growing season (April–October) using 16 equally spaced sprinklers mounted on 6.1m posts. In the drought treatment, precipitation was reduced by approximately 45% (relative to ambient) using troughs made of polycarbonate sheets fixed to rails approximately 1 m high and covering 45% of the plot area. In the cover control, the troughs were inverted to replicate the microenvironment under the water removal troughs without removing ambient precipitation (Pangle *et al.*, 2012). An important artifact of the cover control troughs was a 1 to 4 °C increase in maximum soil and ground-level air temperature during the growing season compared to ambient plots (Pangle *et al.*, 2012). Installation was completed on 22 August 2007 and irrigation began in 2008. See Pangle *et al.* (2012) for additional site descriptions.

Environmental Data

The site micrometeorological station included an air temperature and relative humidity sensor (Vaisala HMP45C, Louisville, CO, USA), a barometric pressure sensor (Vaisala PTB101B), a tipping bucket rain gauge equipped with a snowfall adapter (Texas Electronics, Dallas, TX, USA), a wind monitor (velocity and direction; R.M. Young 05103), a net radiometer (model NR-LITE, Kipp & Zonen, Delft, The Netherlands), and a PPFD/quantum sensor (LI190SB, Li-Cor, Lincoln, NE, USA). Continuous data were summed (precipitation) or averaged over 30 min intervals for 2007-2008, and over 15 min intervals for 2009-2010.

Annual ambient precipitation in all four years was lower than the 20-year (1991-2010) average of 362 mm (341, 331, 310, and 319 mm in 2007-2010 respectively, Fig. 1b). The drought plots received estimated reductions in ambient precipitation of 17% in 2007 and 45% in subsequent years (Pangle *et al.*, 2012). In the irrigated plots, annual total precipitation was 343, 388, 380, and 431 mm in 2007-2010 respectively, hardly exceeding the 20-year average until 2010, with supplemental irrigation of <1%, 17%, 22%, and 35% above ambient. Mean daily temperatures were similar for all four years, and ranged from -9.3 °C to 28.4 °C (Fig. 1b).

Non-Structural Carbohydrates (NSC)

Non-structural carbohydrates (NSC) are defined here as starch plus soluble sugars, including sucrose, glucose, and fructose. Leaf samples were collected on replicate block 3 starting in 2007, and collections began on the other two replicate blocks in 2009. Twig samples were also added to the collection protocol in 2009. We acknowledge that more frequent and representative sampling across tissues is necessary to make inferences regarding seasonality and whole tree carbon balance (Ryan, 2011), however more extensive and destructive sampling was not feasible at this site due to the numerous other studies being simultaneously conducted on the target trees. Based on biomass estimates for piñon and juniper (Grier *et al.*, 1992), our results for leaf and twig NSC content represent approximately 14% of biomass for piñon and juniper. Scaling from tissue to whole plant level is a critical next step that will require destructive harvesting to develop accurate allometrics at the individual level.

All samples were covered in dry ice immediately after collection and stored at -70 °C after transport to the lab. Samples were microwaved at 800 watts for 5 minutes to stop

enzymatic activity, then dried at 65 °C for 48 hours. Leaf tissues were ball-milled to a fine powder (High Throughput Homogenizer, VWR). Woody tissues were milled to 40 mesh prior to ball-milling (Wiley Mini Mill, Thomas Scientific). To reduce the number of samples for analysis, samples from non-drought plots were pooled by plot, species, and date, and thoroughly homogenized after milling.

Samples were analyzed following the protocol described by Hoch *et al.* (2002), with minor modifications. Approximately 12 mg of fine ground plant material was extracted in a 2mL deep-well plate with 1.6 mL distilled water for 60 minutes in a 100 °C water bath (Isotemp 105, Fisher Scientific). Following extraction, an NAD-linked enzymatic assay was used to evaluate NSC content. All sugars were hydrolysed to glucose, linked to the reduction of NAD⁺ to NADH, and monitored at 340 nm with a spectrophotometer (Cary 50 UV-Vis). All NSC values are expressed as percent of dry matter.

NSC content did not differ significantly between the cover control and ambient control (Table S1), so data from the cover control were excluded from further analysis. Analysis of pre-treatment NSCs also indicated there were no significant differences in NSC content within species prior to treatment initiation, though juniper had significantly higher leaf total NSC content than piñon ($p < 0.01$).

Leaf Water Potential

Pre-dawn and midday leaf water potentials (Ψ_{pd} and Ψ_{md} , respectively) of each target tree were measured at least once per month during the study. Two south-facing twigs with healthy foliage were collected from each tree before sunrise, immediately placed in a humidified plastic bag, and stored in a cooler until measurement with

Scholander-type pressure chambers (PMS Instruments, Corvallis, OR, USA). Midday samples were collected in batches of ten and measured sequentially to minimize time since cutting (always less than 20 min).

Sapflow and Hydraulic Conductance

Sap flux density (J_s) was measured using Granier heat dissipation probes (Granier, 1987). Two probes, each consisting of four 10 mm needles with internal thermocouples spaced 10 cm apart vertically and 5 cm apart horizontally, were inserted radially into the xylem of each target tree. The leftmost needles comprise the standard Granier configuration, with the downstream heated and the upstream unheated for reference, while the horizontally adjacent thermocouples correct for ambient axial temperature gradients (Goulden & Field, 1994; Pangle *et al.*, 2012; Plaut *et al.*, 2012). Sap flux density (J_s) was calculated according to the methods outlined in Granier (1987) and Goulden and Field (1994). Plant hydraulic conductance (K_s , mol m⁻² s⁻¹ MPa⁻¹) was calculated as:

$$K_s = (J_s)/\Delta\Psi$$

using mid-day J_s , where $\Delta\Psi$ is the difference between pre-dawn and mid-day leaf water potential and is a proxy for the soil to leaf water potential gradient assuming equilibrium in leaf and soil water potentials at pre-dawn. Values of $\Delta\Psi$ less than 0.5 MPa were excluded from calculation since the assumption of equilibrium in leaf and soil water potentials at pre-dawn may not be valid in isohydric piñon pine during extreme drought due to stomatal closure and hydraulic isolation from the soil (Plaut *et al.*, 2012).

Statistical Analyses

Absence of significant differences in carbohydrate NSC content between cover and ambient controls was confirmed using two-sample t-tests. Differences in mean NSC content between treatments and drought survival status, within species and tissue, were tested using one-way analyses of variance (ANOVAs). Since samples from non-drought plots were pooled by plot, species and date, ANOVAs were calculated using drought survival class means by plot, species, and date to homogenize variance. Pair-wise comparisons with Tukey's Honest Significant Difference were used to discriminate differences among treatments when ANOVAs revealed significant effects. Differences in treatment and species responses of NSC content to meteorologic and hydraulic variables were tested using analyses of covariance (ANCOVAs). For tree-specific hydraulic variables (e.g. K_s), ANCOVAs were calculated using plot means by species and date. All statistical analyses were performed using R software (version 2.15.2, R Core Team 2012).

Results:

Climate and Carbohydrate Dynamics

Leaf total NSC content (Fig. 1a) showed substantial seasonal variability in both species, increasing during the pre-monsoon seasonal drought and declining during the 20-year average monsoon period (July-Sept.). The pre-monsoon increase in NSC content was suppressed in years when the pre-monsoon period (May-June, Fig. 1b) was drier (2008 and 2010) as compared to wetter (2007 and 2009). Similarly, the major effect of precipitation manipulation was a decline in the seasonal amplitude of NSC content under drought (Fig. 1a).

In both species, leaf glucose and fructose were the only NSCs correlated with changes in climate. Climate drivers, however, differed by species (Fig. 2, Table 1). Juniper leaf glucose and fructose content was correlated only with changes in precipitation, with the log of leaf glucose and fructose content decreasing by 0.01 %dry weight (%dw) per mm increase in monthly precipitation ($p < 0.001$; all treatments) (Fig. 2a, Table 1). By contrast, piñon leaf glucose and fructose content was correlated with changes in temperature and vapor pressure deficit (VPD, Fig. 2b,c, Table 1). Piñon leaf glucose and fructose content decreased by 0.14 %dw per °C increase in mean daily temperature ($p < 0.001$, Fig. 2b, Table 1), and, for a given mean daily temperature, leaf glucose and fructose content was 0.6 %dw lower in droughted than ambient trees ($p = 0.03$, Fig. 2b, Table 1). The log of piñon leaf glucose and fructose content also decreased by 0.24 %dw per kPa increase in daily mean VPD ($p < 0.001$, all treatments; Fig. 2c, Table 1).

Effects of Rainfall Manipulation on NSC content

The effects of rainfall manipulation on NSC content differed between species and tissues (Fig. 3, Table S2). In both species, leaf total NSC content declined significantly in droughted trees that died relative to ambient trees (juniper: $p = 0.04$, piñon: $p < 0.01$; Fig. 3a,b, full bar height, Table S2). This difference was driven primarily by declines in leaf starch content (Fig. 3a,b, lightest shade), with significantly lower leaf starch content in dying drought trees relative to ambient and irrigated trees (juniper ambient: $p = 0.02$, juniper irrigation: $p = 0.02$, piñon ambient: $p = 0.04$, piñon irrigation: $p = 0.05$). In juniper, leaf sucrose content (Fig. 3a, middle shade) was also lower in dying drought trees than irrigated ($p = 0.02$), ambient ($p = 0.04$), and surviving drought trees ($p = 0.02$). However, leaf

glucose and fructose (Fig. 3a, darkest shade) content actually increased in dying junipers relative to irrigated ($p < 0.01$) and ambient trees ($p = 0.05$). In piñon, there were no significant treatment differences in leaf soluble sugars (sucrose, glucose, and fructose, Fig. 3b, middle and darkest shades).

Twig total NSC content showed little variation with treatment in juniper (Fig. 3c, full bar height; Table S2), whereas the response of piñon twigs was similar to leaves (Fig. 3b,d, Table S2). Despite a lack of treatment differences in juniper twig total NSC content, juniper twig soluble sugars (sucrose, glucose, and fructose, Fig. 3c, middle and darkest shades) differed significantly between treatments. As in juniper leaves, twigs of dying droughted juniper had significantly higher glucose and fructose content (Fig. 3c, darkest shade) than irrigated ($p < 0.01$), ambient ($p < 0.01$), and surviving drought trees ($p < 0.01$). Twig sucrose content (Fig. 3c, middle shade) was also lower in irrigated relative to ambient junipers ($p = 0.05$), the only significant treatment difference between irrigated and ambient trees of either species. This absence of an irrigation treatment response suggests either that photosynthesis in ambient trees was not water limited, or, more likely, that any additional photosynthate was allocated to primary production rather than causing an increase in the NSC pool.

In contrast to the damped treatment response in juniper twigs, the drought treatment response in piñon twigs was actually greater than in piñon leaves. Piñon twig total NSC and starch content (Fig. 3d, full bar height and lightest shade) were significantly lower in dying drought trees than in ambient (total: $p < 0.01$, starch: $p = 0.03$) and irrigated trees (total: $p = 0.01$, starch: $p = 0.02$). Piñon twig sucrose content (Fig. 3d, middle shade) was also significantly lower in dying drought than in ambient ($p < 0.01$)

trees, and marginally lower than in irrigation ($p=0.08$) and surviving drought trees ($p=0.07$).

Piñon and juniper also differed greatly in overall NSC distribution between foliage and twigs (Fig. 4, Table 2). Juniper had a significantly lower ratio of twig to leaf total NSC content than piñon ($p<0.001$), with slopes of 0.3 and 1.0, respectively (Fig. 4, Table 2). Ratios in drought trees of both species were even more divergent, with a significantly lower ratio of twig to leaf NSC content in droughted than ambient junipers (0.26, $p<0.01$, Fig. 4, Table 2), and a significantly higher ratio of twig to leaf NSC content in droughted than ambient piñons (1.27, $p=0.07$, Fig. 4, Table 2).

Relationship between NSCs and Hydraulics

The correlation between leaf NSC content and changes in whole tree hydraulic conductance (K_s) differed significantly between species, but showed little variation with treatment (Fig. 5, Table 1). Juniper leaf total NSC content did not change significantly with K_s , while piñon leaf total NSC content decreased by 3.5 %dw ($p<0.01$) for each ($\text{mol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$) decrease in K_s (Fig. 5a, Table 1). Despite the difference in correlation between total NSC content and K_s , leaf starch content in both species decreased by 2.4 %dw ($p<0.001$) per unit ($\text{mol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$) decrease in K_s (Fig. 5b, Table 1). For a given K_s , however, juniper leaf starch content was 2 %dw higher than piñon ($p<0.01$, Fig. 5b, Table 1). The primary driver of species differences in the correlation between total NSC content and K_s was the change in leaf glucose and fructose content with changes in K_s . Juniper leaf glucose and fructose content increased by 2.5 %dw per unit ($\text{mol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$) decrease in K_s ($p<0.001$), with lower leaf glucose and fructose content in droughted than ambient junipers for a given K_s ($p=0.095$; Fig. 5c inset, Table 1). Piñon

leaf glucose and fructose content, by contrast, didn't change significantly with K_s (Fig. 5c, Table 1).

NSCs and Survival

Despite species differences (Figs. 1-5), average leaf starch content over the treatment period was the best predictor of survival time after drought initiation for both species (Fig. 6, Table 2). Survival time after drought initiation increased by 7.6 months for each 1.0 %dw increase in average leaf starch content ($p < 0.001$, $R^2 = 0.77$, Fig. 6, Table 2). Though sample sizes are limited, species-specific regressions were $y = 10.6 + 8.1x$ and $y = 11.1 + 4.1x$ for piñon and juniper, respectively (Fig. 6, Table 2).

Discussion:

Our findings support a role for carbohydrates in drought-induced mortality of conifers across a broad range of hydraulic regulation, and are consistent with the hypothesis that anisohydric species are less susceptible to the carbon starvation process. Although droughted piñons and junipers that died had significantly lower average leaf total NSC and starch content than trees under ambient conditions (Figs. 1, 3a,b, Table S2), the two species regulated NSC response to climate and drought manipulation differently (Figs. 2, 4, 5, Table 1). The net effect of this differential regulation was evident in the NSC response to changes in K_s . Severe, drought-induced reductions in K_s were related to a decline of up to 40% in piñon leaf total NSC content, while juniper leaf total NSC content showed a homeostatic response to equally severe K_s reductions (Fig 5a, Table 1). The decrease in leaf starch content with decreasing K_s in both species, however, suggests both were depleting utilizable storage (Fig. 5b, Table 1). Ultimately,

trees with lower leaf starch content died faster than those that maintained higher starch pools (Fig. 6; dry weight basis), implicating carbohydrates in mortality of these two conifer species. Juniper both maintained higher leaf starch content and survived longer than piñon (Fig. 5b, Table 1), suggesting anisohydry may confer a survival advantage under drought. These results have significant implications for our fundamental understanding of how plants die, and of carbohydrate dynamics in forests. If borne out through further tests in other ecosystems, these results will also be of general use to the community of modelers that are beginning to employ carbon starvation in part to drive vegetation dynamics (Fisher *et al.*, 2010; McDowell *et al.*, 2013).

Drought Impacts on Seasonal NSC Accumulation

Our chronic water availability manipulation was superimposed upon strong seasonal climate variation (Fig. 1), resulting in observed patterns of NSC content consistent with decades of theory regarding plant responses to water stress and resultant carbohydrate dynamics (Hsiao, 1973; Chapin *et al.*, 1990; Korner, 2003; Wurth *et al.*, 2005; McDowell, 2011; Muller *et al.*, 2011; Sala *et al.*, 2012; Wiley & Helliker 2012). NSC accumulated during the seasonally dry pre-monsoon drought period (Fig. 1), when photosynthesis was minimal but growth was zero (Limousin *et al.*, 2013; McDowell *et al.*, 2013), consistent with the greater sensitivity of growth than photosynthesis to environmental stress (Hsiao, 1973; Chapin *et al.*, 1990; Korner, 2003; Wurth *et al.*, 2005; McDowell, 2011; Muller *et al.*, 2011; Sala *et al.*, 2012). This pre-monsoon accumulation was reduced under drought manipulation and, to a lesser degree, in years when the pre-monsoon period was drier (2008 and 2010; Fig. 1), consistent with greater limitations on photosynthesis during extreme water limitation. NSC content subsequently declined

across all treatments during each monsoon period (Fig. 1) when photosynthetic rates are highest and growth occurs (Anderson-Teixeira *et al.*, 2011; Limousin *et al.*, 2013; McDowell *et al.*, 2013). Recurring reductions in dry season NSC accumulation under extreme drought may contribute to the C-starvation process. Such reductions in seasonal NSC storage diminish the safety margins needed for survival during severe stress (Sala *et al.*, 2012), reducing pools available to support subsequent growth (Chapin *et al.*, 1990), defense (Guerard *et al.*, 2007), and osmotic, maintenance, and transport demands (McDowell, 2011; Sevanto *et al.*, 2013). Consistent with these observations, dying trees with low NSC content also had the lowest allocation to xylem resin ducts and wood growth (Gaylord *et al.*, 2013; McDowell *et al.*, 2013).

In our system, damped seasonality under drought led to significantly lower average leaf total NSC content and starch content in droughted trees of both species that died than trees under ambient conditions (Figs. 1a, 3a,b, Table S2), consistent with our hypothesis that NSC content would be lowest in dying drought trees. In addition, leaf starch content decreased with declining K_s at the same rate in both species (Fig. 5b, Table 1), supporting the hypothesis that carbon metabolism and hydraulics are linked during mortality (McDowell, 2011; Sala *et al.*, 2012), and that two species with strongly contrasting hydraulic strategies appear to die of the same coupled processes (McDowell *et al.*, 2013). However, while piñon leaf total NSC content declined with decreasing K_s , consistent with the observed linear relationship between assimilation and leaf specific hydraulic conductance in ponderosa pine (Hubbard *et al.*, 2001), juniper leaf total NSC content remained unchanged at low K_s (Fig. 5a, Table 1) due to a shift from starch to glucose and fructose (Fig. 5c, Table 1). This species difference in the correlation between total NSC content and K_s supports our hypothesis of greater NSC content declines under

drought in the relatively isohydric piñon as compared to the relatively anisohydric juniper, consistent with the prediction of longer duration of negative carbon balance under drought in piñon than juniper due to stomatal closure at less negative water potentials (McDowell *et al.*, 2008).

Species Differences and Survival Implications

Juniper's homeostatic response of leaf total NSC content to changes in K_s may be a strategy to maximize and maintain a turgor difference from leaf to stem, allowing continued phloem transport and, hence, photosynthesis (Nikinmaa *et al.*, 2013). Consistent with their respective hydraulic strategies, conversion to leaf glucose and fructose was driven by soil water in anisohydric juniper (Figs. 2a, 3a, 5c, Table 1) and by atmospheric vapor demand in isohydric piñon (Fig. 2b,c, Table 1). Due to its greater stomatal sensitivity, piñon water potentials only rarely exceeded -3.0 MPa (Plaut *et al.*, 2012; Limousin *et al.*, 2013), making osmoregulation unnecessary (McDowell *et al.*, 2013). By contrast, juniper experienced extremely negative water potentials (up to -8.0 MPa, Plaut *et al.*, 2012), likely driving the observed accumulation of leaf glucose and fructose under water stress (Figs. 2a, 3a, 5c, Table 1), forcing large osmotic pressure to avoid turgor collapse (Fig. S1). Similar behavior has been directly associated with osmotic adjustment and turgor regulation under drought in species as diverse as black spruce (Tan *et al.*, 1992), oak and dogwood (Gebre & Tschaplinski, 2002), Indian plum (Clifford *et al.*, 1998), and beach strawberry (Zhang & Archbold, 1993). This increase in glucose and fructose content is facilitated by juniper's consistent maintenance of high leaf starch stores relative to piñon (Figs. 3a,b, 5b, Table 1) that can be hydrolysed for use in osmotic adjustment during drought stress (Hsiao *et al.*, 1976). In addition, juniper's

low ratio of twig to leaf total NSC content as compared to piñon's maintenance of equilibrium twig and leaf NSC content (slopes of 0.3 and 1.0, respectively, Fig. 4, Table 2) may reflect juniper's far greater requirement to maintain turgor and phloem function in order to maintain assimilation while frequently experiencing xylem water potentials far in excess of -4.0 MPa. The capacity to store large amounts of starch in the leaves may avoid both the hypothesized viscosity buildup (Holttta *et al.*, 2009) and the down-regulation of photosynthesis associated with leaf sucrose accumulation (Franck *et al.*, 2006). Juniper's strategy of maintaining high leaf starch stores may confer a survival advantage. Higher average leaf starch content over the treatment period was predictive of longer survival time after drought initiation in both species (Fig. 6, Table 2), consistent with the important role of leaf starch in maintaining normal rates of growth, photosynthesis, and respiration (Caspar *et al.*, 1985). A marginal increase in leaf starch also confers a greater survival advantage for juniper than piñon (Fig. 6, Table 2), conceivably because additional starch can be utilized to facilitate turgor maintenance as discussed above. This result suggests the relatively anisohydric juniper may be less vulnerable to the C-starvation process, though more susceptible to hydraulic failure under severe drought conditions (Hoffmann *et al.*, 2011), than the relatively isohydric piñon pine (McDowell *et al.*, 2008).

Conclusions:

Our results present a novel relationship between survival time and NSC content under drought that may prove useful in parameterization of mortality algorithms in dynamic vegetation models. Survival time of both species after drought initiation was highly correlated with average leaf starch content over the treatment period (Fig. 6, Table

2), supporting our hypothesis that trees with higher carbohydrate content would survive longest, and consistent with prior observations of a relationship between leaf starch and survival time in piñon pine (Sevanto *et al.*, 2013). Application of this trend to the relatively anisohydric juniper indicates that carbohydrates are important in mortality of conifers across a broad range of stomatal control (Figs. 5b, 6), and suggests that vegetation dynamics models are correct to include carbohydrate status as a predictive variable in determining vegetation survival (Fisher *et al.*, 2010). These results are particularly relevant for the US Southwest, where piñon and juniper are widespread, and predictions suggest conifers will be absent from the entire region by 2050 (Jiang *et al.*, 2013; Williams *et al.*, 2013). These results, representing conifers with widely disparate hydraulic strategies, are globally relevant because there is growing evidence that many forest types fall along the hydraulic continuum of predicted mortality (Mitchell *et al.*, 2013) represented by our focal species. Further research is necessary to elucidate the true extent to which the relationship between leaf starch and survival holds across a broad range of plant functional types and ecosystems.

Figures:

Figure 1. Seasonal leaf NSC dynamics are muted under drought. Timecourse of meteorological variables and leaf total NSC content, 2007-2011. Vertical dashed line indicates drought treatment initiation in August 2007. Shaded regions highlight the long-term average monsoon period (July-September). In panel A, colors indicate treatments (irrigation = blue, ambient = green, drought = red), line type indicates species (juniper = solid, piñon = dashed), and asterisks indicate mortality events. Four piñons died in August 2008, five in May 2009, and two in August 2009. One juniper died in March 2010, one in March 2011, and one in June 2011. Error bars are standard errors.

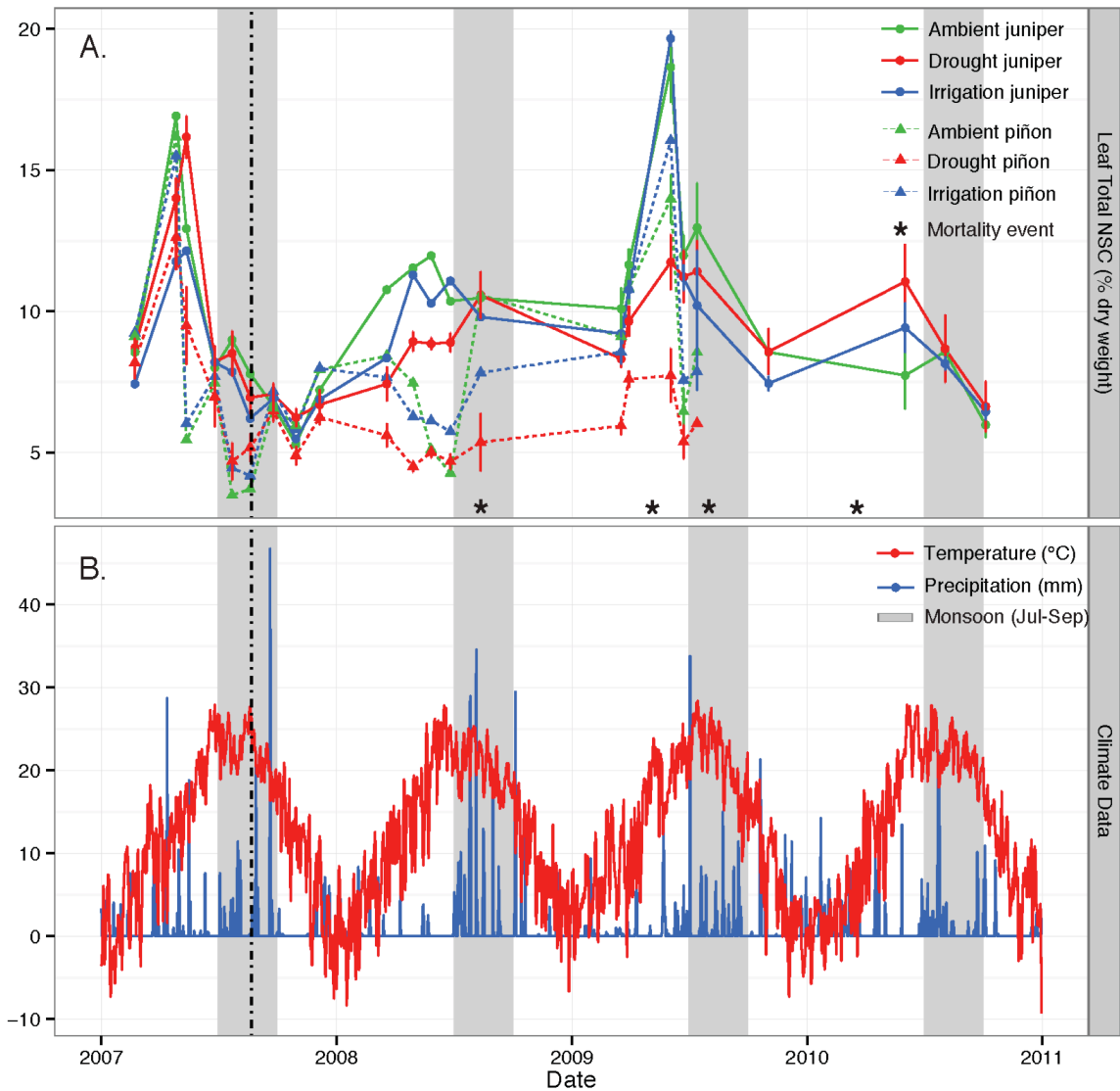


Figure 2. Leaf glucose and fructose content is responsive to changes in climate. Line type indicates species (juniper = solid, piñon = dashed), and the grey line is the species mean, provided as reference for species with non-significant relationships in respective panels. Where relationships are significant, irrigation = blue, ambient = green, and drought = red. Error bars are standard errors. See Table 1 for curve parameters and ANCOVA results.

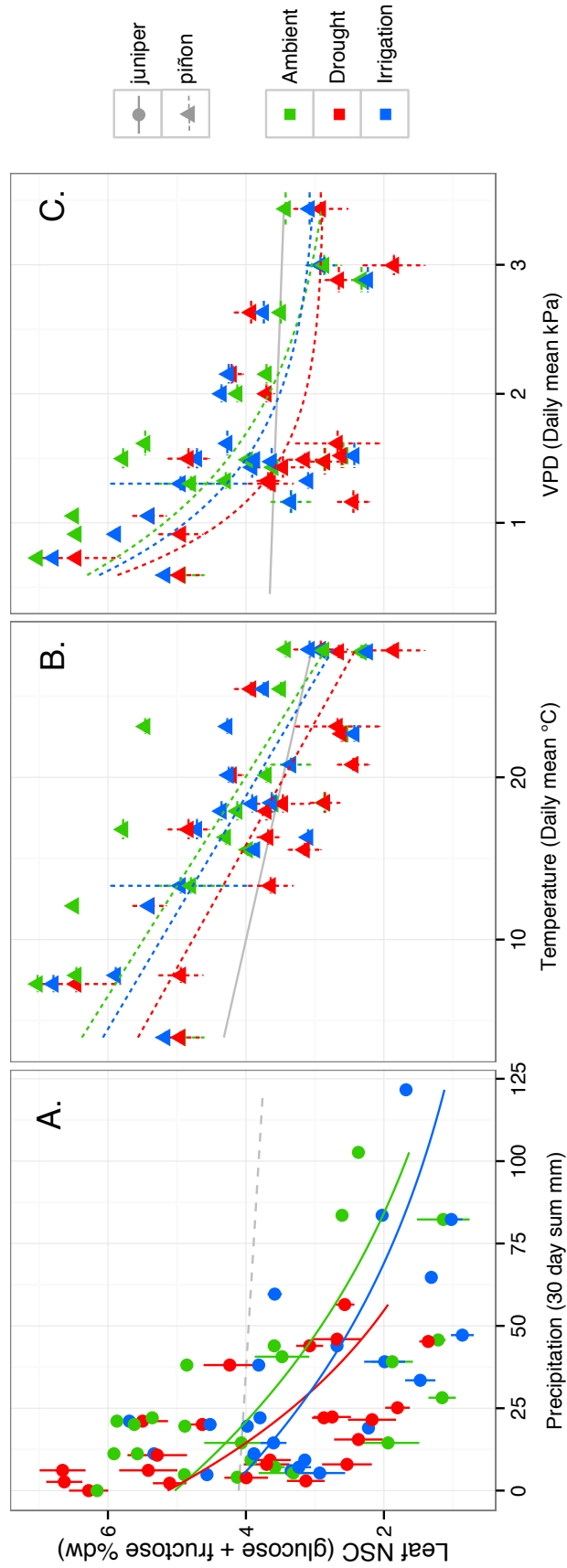


Figure 3. Average leaf total NSC and starch is lowest in droughted trees that die. Mean NSC content by treatment and survival status over the course of the study period. Colors indicate treatments (irrigation = blue, ambient = green, surviving drought = yellow, dead drought = red), and shading indicates NSC (glucose + fructose (GF) = darkest, sucrose (Suc) = middle, starch = lightest; full bar height = total NSC). Error bars are standard errors for total NSC content. Lower-case letters indicate significant differences in total NSC content (ANOVA $p < 0.05$). See Supplementary Table 2 for ANOVA results for all sugars. Trees considered “dead” for this analysis died by the end of the 2011 growing season.

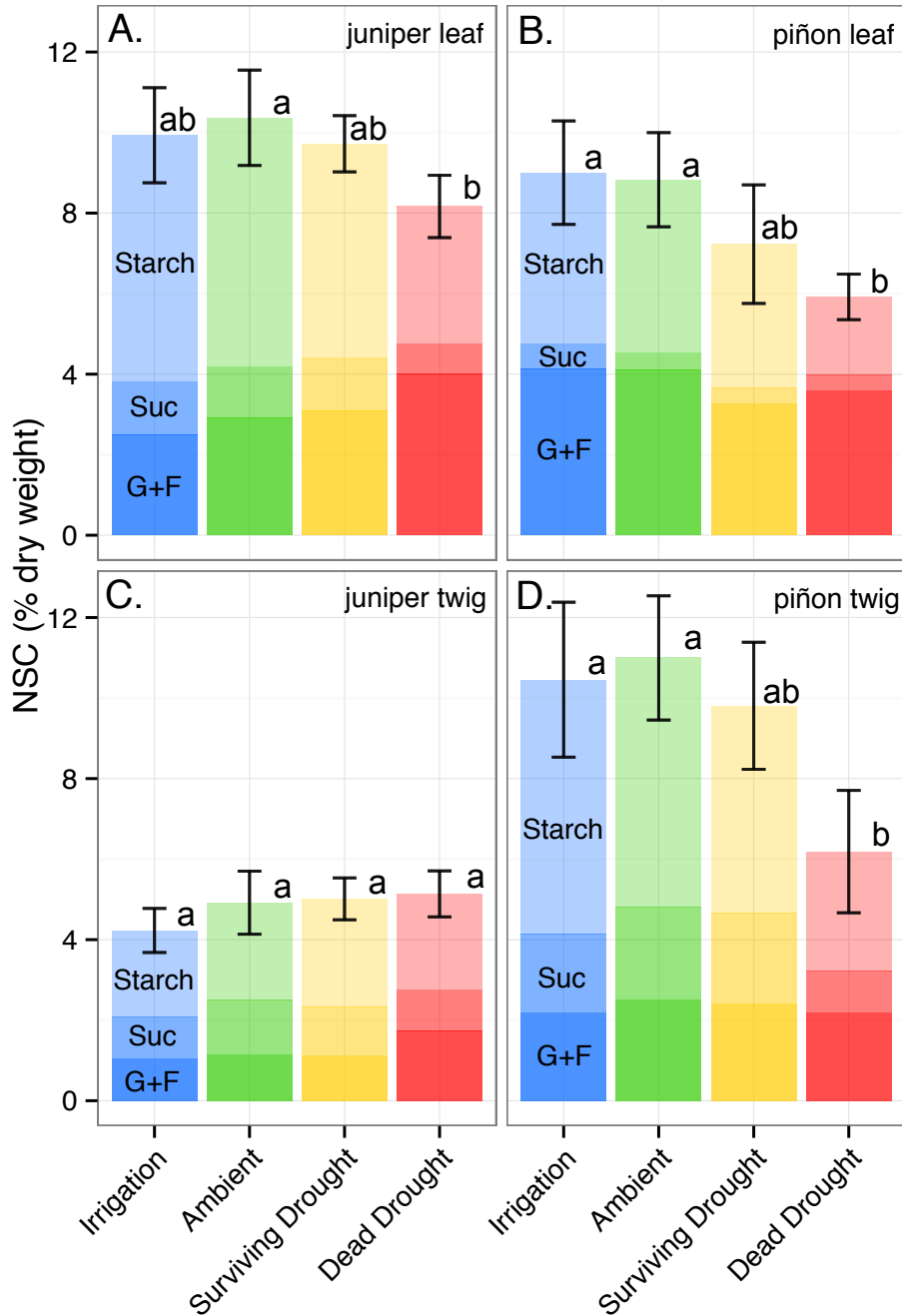


Figure 4. The ratio of twig to leaf total NSC content is a distinguishing feature of hydraulic strategy. Equations and R^2 presented represent species average curves (solid lines) for juniper (grey) and piñon (black). Dashed lines indicate treatments (irrigation = blue, ambient = green, drought = red). See Table 2 for treatment curve parameters and ANCOVA results.

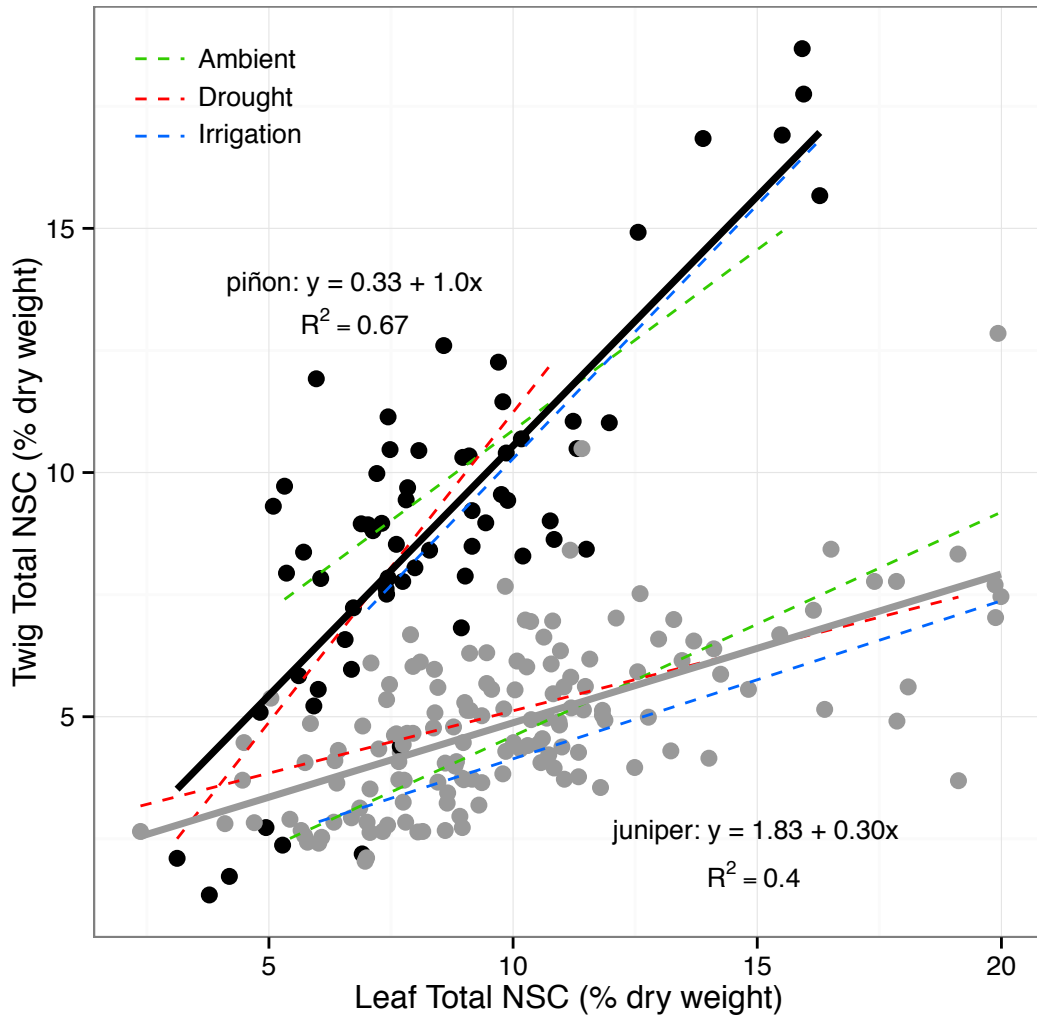


Figure 5. Leaf starch content declines with reduced hydraulic conductance. Hydraulic conductance (K_s) was calculated from sapflow and leaf water potential gradient. Solid lines are species average curves for juniper (grey) and piñon (black). Dashed lines shown in panel C highlight juniper treatment differences (irrigation = blue, ambient = green, drought = red). See Table 1 for curve parameters and ANCOVA results.

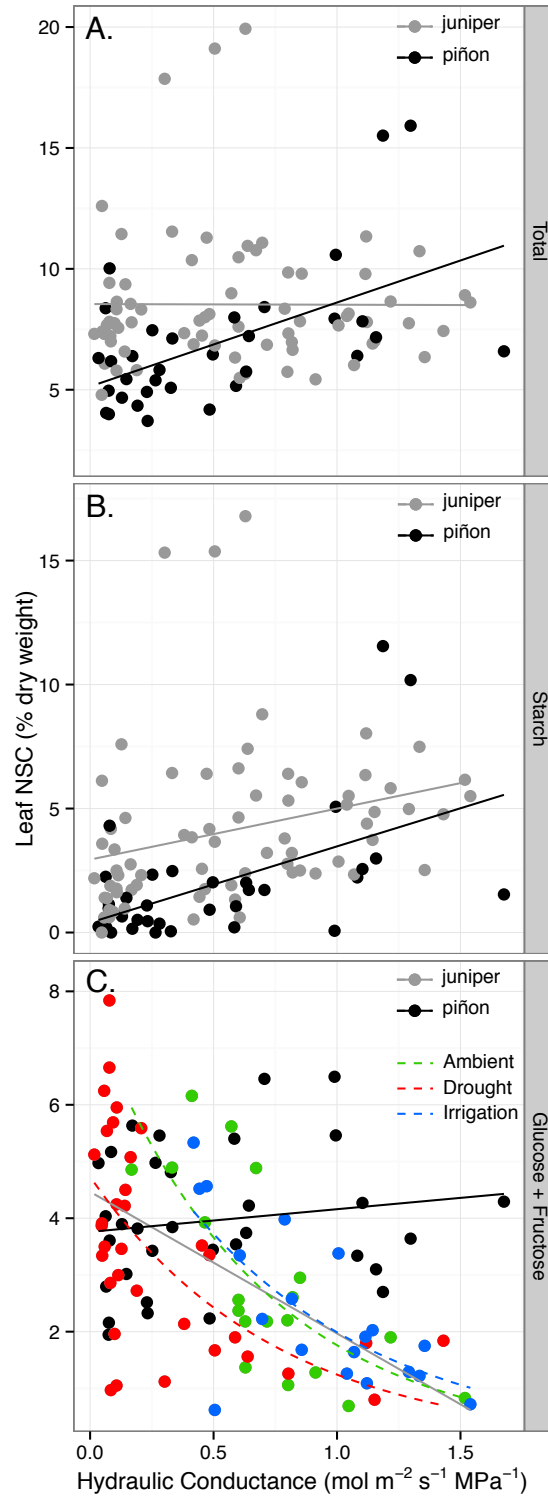
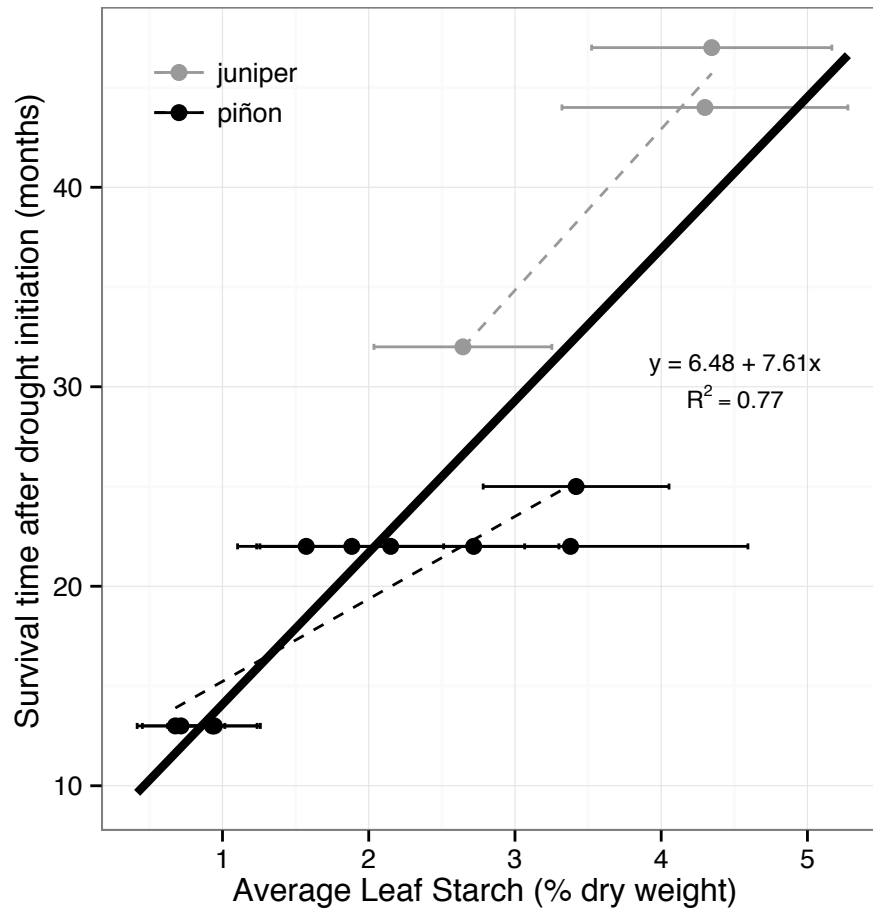


Figure 6. Average leaf starch content predicts survival time after drought initiation. Error bars are standard errors. Equation and R^2 presented represent the interspecific average curve (solid line). Dashed lines are species-specific curves for juniper (grey) and piñon (black). See Table 2 for species-specific curve parameters.



Tables:

Table 1. Regression coefficients and ANCOVA results for correlation between leaf NSC content and meteorologic and hydraulic variables (Figs. 2, 5). Equations are in the form: linear (lin): $y = a + bx$; exponential (exp): $\log(y) = a + bx$, where y = leaf NSC content. For tests of treatment differences (Figs. 2, 5c inset), the ambient treatment is the baseline, and p-values indicate significant differences from zero. For drought and irrigation treatments, p-values indicate significant differences from ambient. For tests of species differences (Fig. 5), juniper is the baseline, and p-values indicate significant differences from zero. For piñon, p-values indicate significant differences from juniper. Variable (var.): Precip, 30-day sum precipitation (mm); Temp, daily mean temperature (°C); VPD, daily mean vapor pressure deficit (kPa); K_s , hydraulic conductance calculated from sapflow ($\text{mol m}^{-2} \text{s}^{-1} \text{MPa}$). NSC: GF, glucose + fructose; Suc, Sucrose; Starch; Total, Total NSC. Significance codes, p: $0 \leq *** \leq 0.001 \leq ** \leq 0.01 \leq * \leq 0.05 \leq ' \leq 0.1 \leq ' ' \leq 1$. NS = not significant, NA = not applicable. Note: parameter estimates presented are from ANCOVA fits including species or treatment interaction, however R^2 values are from linear model fits for individual species or treatments, for which parameter estimates may differ slightly.

Table 1

Fig.	Var.	Panel	NSC	Species	Test	Treatment	Line	a (p-value)	b (p-value)	R ²
2	Precip	A	GF	juniper	Treatment	Ambient	Exp	1.48 (2e-16***)	-0.01 (8.7e-08***)	0.34
		A	GF	juniper		Drought	Exp	NS	NS	NA
		A	GF	juniper		Irrigation	Exp	NS	NS	NA
		A	GF	piñon	None	All	Lin	4.1 (<2e-16***)	-0.003 (0.66)	0
	Temp	B	GF	juniper	None	All	Lin	4.53 (3.3e-12***)	-0.05 (0.06.)	0.04
		B	GF	piñon	Treatment	Ambient	Lin	6.82 (2e-16***)	-0.14 (2.5e-12***)	0.52
		B	GF	piñon		Drought	Lin	6.23 (0.03*)	NS	0.59
		B	GF	piñon		Irrigation	Lin	6.63 (0.48)	NS	0.67
	VPD	C	GF	juniper	None	All	Lin	3.69 (3.2e-12 ***)	-0.07 (0.78)	0
		C	GF	piñon	Treatment	Ambient	Exp	1.75 (2e-16***)	-0.24 (10e-07***)	0.34
		C	GF	piñon		Drought	Exp	NS	NS	NA
		C	GF	piñon		Irrigation	Exp	NS	NS	NA
5	K _s	A	Total	juniper	Species	All	Lin	8.54 (< 2e-16***)	-0.03 (0.97)	0
		A	Total	piñon		All	Lin	5.13 (0.0002***)	3.47 (0.008**)	0.28
		B	Starch	juniper	Species	All	Lin	2.75 (3.5e-07***)	2.38 (0.0004***)	0.06
		B	Starch	piñon		All	Lin	0.75 (0.001**)	NS	0.25
		C	GF	juniper	Species	All	Lin	4.47 (< 2e-16***)	-2.50 (5.3e-10***)	0.38
		C	GF	piñon		All	Lin	3.76 (0.11)	0.40 (1.7e-05***)	0
		C	GF	juniper	Treatment	Ambient	Exp	1.69 (7.5e-15***)	-1.13 (1.3e-08***)	0.53
		C	GF	juniper		Drought	Exp	1.42 (0.095.)	NS	0.3
		C	GF	juniper		Irrigation	Exp	1.74 (0.76)	NS	0.32

Table 2. Regression coefficients and ANCOVA results for carbohydrate distribution ratios (Fig. 4), and NSC content vs. survival (Fig. 6). Equations are in the form: linear (lin): $y = a + bx$. For Fig. 4, y = twig NSC content and x = leaf NSC content. For Fig. 6, y = months survival after drought initiation and x = average leaf starch content (%dw) over the treatment period. For tests of species differences, juniper is the baseline, and p-values indicate significant differences from zero. For piñon, p-values indicate significant differences from juniper. For tests of treatment differences, the ambient treatment is the baseline, and p-values indicate significant differences from zero. For drought and irrigation treatments, p-values indicate significant differences from ambient. Significance codes, p : $0 \leq *** \leq 0.001 \leq ** \leq 0.01 \leq * \leq 0.05 \leq ' \leq 0.1 \leq ' ' \leq 1$. NS = not significant, NA = not applicable. Note: parameter estimates presented are from ANCOVA fits including species or treatment interaction, however R^2 values are from linear model fits for individual species or treatments, for which parameter estimates may differ slightly.

Fig.	Test	Species	Treatment	a (p-value)	b (p-value)	R²
4	Species	juniper	All	1.83 (7.4e-06***)	0.30 (4e-14***)	0.40
		piñon		0.33 (0.04*)	1.02 (<2e-16***)	0.67
	Treatment	juniper	Ambient	0.02 (0.98)	0.46 (3.2e-11***)	0.70
			Drought	2.57 (0.002**)	0.26 (0.007**)	0.27
			Irrigation	0.90 (0.38)	0.33 (0.13)	0.73
	Treatment	piñon	Ambient	3.47 (0.07.)	0.74 (0.0002***)	0.44
			Drought	-1.46 (0.05*)	1.27 (0.07.)	0.49
			Irrigation	-0.10 (0.18)	0.99 (0.24)	0.80
6	Species	juniper	All	10.61 (0.16)	8.08 (0.001**)	0.95
		piñon		11.1 (0.95)	4.14 (0.08.)	0.74

Appendices:

APPENDIX A: Supporting Information

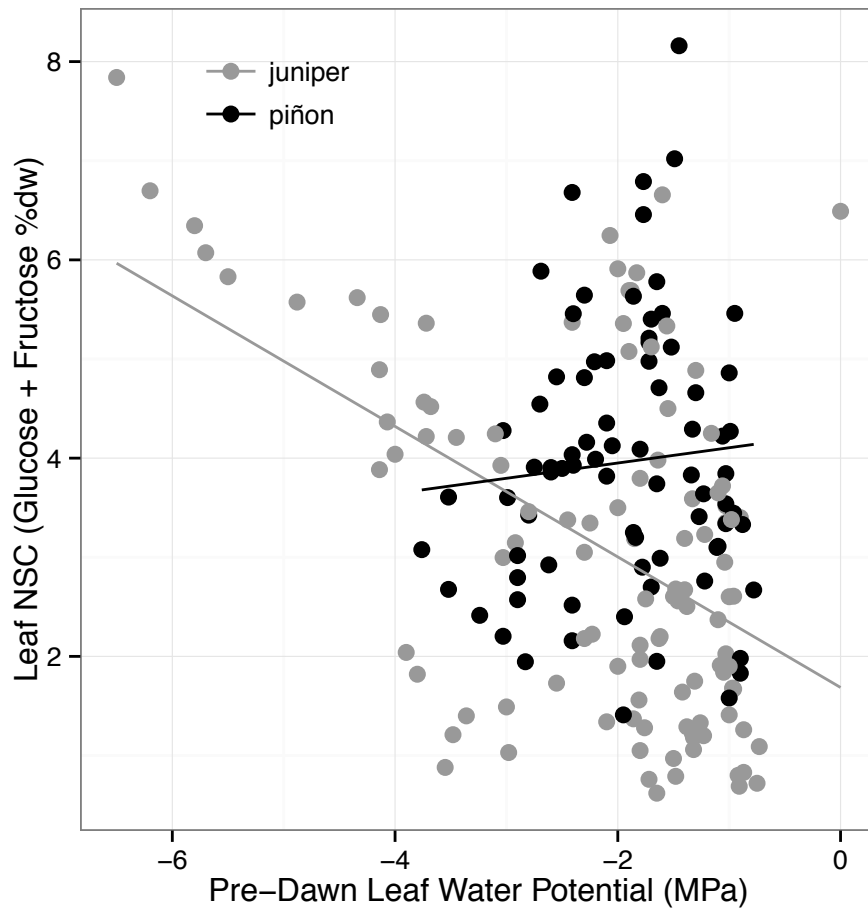
Table S1. Test for significant differences in NSC content of cover control and ambient control trees. Results are p-values from two-sample t-tests of sample means from four dates in 2009. NSC: GF, glucose + fructose; Suc, Sucrose; Starch; Total, Total NSC.

Species	Tissue	NSC			
		GF	Suc	Starch	Total
juniper	leaf	0.844	0.634	0.825	0.655
juniper	twig	0.614	0.100	0.993	0.599
piñon	leaf	0.843	0.396	0.603	0.445
piñon	twig	0.689	0.602	0.873	0.699

Table S2. Multiple comparisons of mean NSC content by treatment and drought survival status. ANOVA results for Figure 3. Significance values are from Tukey's Honest Significant Difference test. P-values ≤ 0.1 are italicized, ≤ 0.05 are bolded and italicized, and ≤ 0.01 are bolded. ANOVAs were calculated using treatment and survival class means by date to homogenize variance. NSC: GF, glucose + fructose; Suc, Sucrose; St, Starch; Tot, Total NSC.

Comparison	A. juniper leaf				B. piñon leaf				C. juniper twig				D. piñon twig			
	GF	Suc	St	Tot	GF	Suc	St	Tot	GF	Suc	St	Tot	GF	Suc	St	Tot
Surviving Drought-Ambient	0.96	1.00	0.72	0.80	0.46	1.00	0.95	0.598	1.00	0.60	0.91	1.00	0.99	1.00	0.87	0.88
Dead Drought-Ambient	<i>0.05</i>	<i>0.04</i>	<i>0.02</i>	<i>0.04</i>	0.50	1.00	<i>0.04</i>	<i><0.01</i>	<i><0.01</i>	<i>0.11</i>	1.00	0.97	0.63	<i><0.01</i>	<i>0.03</i>	<i><0.01</i>
Irrigation-Ambient	0.67	1.00	1.00	0.93	1.00	0.64	1.00	1.00	0.93	<i>0.05</i>	0.91	0.39	0.53	0.69	1.00	0.96
Surviving Drought-Dead Drought	0.14	<i>0.02</i>	0.19	0.22	0.95	1.00	0.63	0.76	<i><0.01</i>	0.61	0.92	1.00	0.92	0.07	0.46	0.18
Irrigation-Surviving Drought	0.38	1.00	0.75	0.99	0.44	0.82	0.96	0.51	0.97	0.51	0.54	0.28	0.90	0.90	0.82	0.99
Irrigation-Dead Drought	<i><0.01</i>	<i>0.02</i>	<i>0.02</i>	0.13	0.47	0.55	<i>0.05</i>	<i><0.01</i>	<i><0.01</i>	1.00	0.96	0.30	1.00	0.08	<i>0.02</i>	<i>0.01</i>

Figure S1. Piñon isohydry obviates osmoregulation. Relationship between pre-dawn leaf water potential (MPa) and leaf glucose and fructose content (%dry weight) for juniper (grey) and piñon (black).



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