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Research paper

Temporal controls on crown nonstructural carbohydrates in southwestern US tree species

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In trees, large uncertainties remain in how nonstructural carbohydrates (NSCs) respond to variation in water availability in natural, intact ecosystems. Variation in NSC pools reflects temporal fluctuations in supply and demand, as well as physiological coordination across tree organs in ways that differ across species and NSC fractions (e.g., soluble sugars vs starch). Using landscape-scale crown (leaves and twigs) NSC concentration measurements in three foundation tree species (*Populus tremuloides*, *Pinus edulis*, *Juniperus osteosperma*), we evaluated in situ, seasonal variation in NSC responses to moisture stress on three timescales: short-term (via predawn water potential), seasonal (via leaf $\delta^{13}\text{C}$) and annual (via current year's ring width index). Crown NSC responses to moisture stress appeared to depend on hydraulic strategy, where *J. osteosperma* appears to regulate osmotic potentials (via higher sugar concentrations), *P. edulis* NSC responses suggest respiratory depletion and *P. tremuloides* responses were consistent with direct sink limitations. We also show that overly simplistic models can mask seasonal and tissue variation in NSC responses, as well as strong interactions among moisture stress at different timescales. In general, our results suggest large seasonal variation in crown NSC concentrations reflecting the multiple cofunctions of NSCs in plant tissues, including storage, growth and osmotic regulation of hydraulically vulnerable leaves. We emphasize that crown NSC pool size cannot be viewed as a simple physiological metric of stress; in situ NSC dynamics are complex, varying temporally, across species, among NSC fractions and among tissue types.

Keywords: Bayesian, carbon starvation, labile carbon, memory, NSC, osmoregulation, sink-limited, tree growth, water stress.

Introduction

Non-structural carbohydrates (NSCs, soluble sugars and starches) link past conditions to current function, serving multiple roles within plant tissues related to growth and metabolism (Chapin et al. 1990, Dietze et al. 2014, Martínez-Vilalta et al. 2016), but also signaling, osmoregulation, and

defense (Kozłowski 1992, Dietze et al. 2014). As long-lived organisms, tree responses to climate integrate climate conditions across months and years (Becker 1989, Mazza and Manetti 2013, Peltier et al. 2018), driven in part by NSCs (Trugman et al. 2018). Nonstructural carbohydrate dynamics may be associated with or mediate physiological stress during

drought-related tree mortality (Adams et al. 2017). At subannual timescales, NSC pools in trees exhibit pronounced seasonal variation, where timing of seasonal maxima differs across species (Furze et al. 2018) and functional groups (Guo et al. 2020, Martínez-Vilalta et al. 2016), partly driven by endogenous processes such as leaf emergence and reproduction (Newell et al. 2002). Moreover, different NSC fractions may vary seasonally in response to different drivers; starch concentrations may be highest late in the growing season, reflecting accumulation and storage, whereas soluble sugar concentrations may peak in winter to confer freezing tolerance (Furze et al. 2018). In semiarid forests, seasonal variation in NSC concentrations may also be driven by seasonal moisture stress.

Forest communities in the semiarid southwestern USA (hereafter 'Southwest') are projected to experience increased drought stress (Williams et al. 2013). However, impacts on NSC dynamics and how these impacts may vary across species are poorly understood, likely due in part to efforts to oversimplify complex NSC dynamics. In general, NSC concentrations (particularly starch) often decline under moisture stress in semiarid forests characteristic of the Southwest (greenhouse: Adams et al. 2013, Sevanto et al. 2014; in situ: Dickman et al. 2015), where strong seasonal variation in moisture stress is an important component of climatic variability (Szejner et al. 2016). Nonstructural carbohydrates dynamics inferred from observations, however, are quite variable within and across species, where drought severity, drought duration, temperature stress, timing of extreme events and hydraulic or other physiological traits may also influence observed responses. *Populus tremuloides* (aspen) is a diffuse-porous, early successional angiosperm with high NSC storage capacity (Galvez et al. 2013, Landhäusser and Lieffers 2003), and its NSC concentrations may be only mildly affected by regional drought (Anderegg et al. 2012), although variation in starch concentrations in this species may be sensitive to temperature (Wiley et al. 2017). Aspen frequently co-occurs with *Pinus ponderosa* (ponderosa), a widespread, important species in the western USA, but the magnitude of seasonal cycles of NSCs in these two species likely strongly differs according to differing leaf phenology (Anderegg and Callaway 2012, Furze et al. 2018). *Pinus edulis* (piñon) and *Juniperus osteosperma* (juniper) are two foundation gymnosperms (Ellison et al. 2005) growing at lower elevations and subject to relatively greater seasonal moisture stress, but with classically different hydraulic behaviors (piñon, isohydric; juniper, anisohydric; West et al. 2008, Breshears et al. 2009, Resco et al. 2009). These two species also exhibited differing NSC dynamics under drought stress in a field manipulation (Dickman et al. 2015), but inconsistent results have been reported for potted trees (e.g., Adams et al. 2013, Anderegg and Anderegg 2013), potentially arising due to the complex nature of NSC storage and use in mature trees. Furthermore, gymnosperms (piñon and juniper) have generally wider hydraulic safety margins than angiosperms

(aspen) (Johnson et al. 2012), which may influence how NSC pools respond to moisture stress.

Nonstructural carbohydrates are stored in living cells throughout trees, and this decentralized distribution complicates efforts to scale NSC measurements from single organs to whole-tree NSC dynamics. For example, rapid changes in leaf NSC concentrations have been reported in response to varying environmental conditions (Adams et al. 2013, 2017, Dickman et al. 2015). Others, however, have shown constant crown NSC concentrations under drought stress in concert with depletion in longer-term storage organs such as roots (Hartmann et al. 2013), suggesting preferential allocation to certain organs under drought stress and recovery (Hagedorn et al. 2016) or sink limitations in others (Galvez et al. 2011). Differences in carbon allocation or organ-specific responses may also limit generalization across different species. Notably, many conifers (including piñon) may retain needles for many years, even under stressful conditions. Mobilization and translocation of NSCs from older needles could occur during spring flushing of new needles, but it is unknown whether moisture stress differentially affects NSC storage in different needle cohorts (Balster and Marshall 2000). Such translocation among organs may complicate interpretations of NSC responses to moisture stress.

To address the notion that NSC pools should respond in uniform, predictable ways to the environment across seasons, sites and species, we synthesize a network of NSC data from multiple organs, sites and species to quantify (i) seasonal variability in NSC concentrations, (ii) relationships of NSCs with indices of moisture stress and (iii) how NSC responses among different organs, such as leaves, twigs and needle cohorts, may be interrelated (Table 1). Specifically, we addressed the following questions: (Q1) how do NSC concentrations vary seasonally before and after the arrival of summer precipitation (monsoon) in leaves and twigs of three foundation tree species (aspen, piñon and juniper)? (Q2) How do NSC responses to water stress at different timescales—short-term (predawn water potential), seasonal (leaf $\delta^{13}\text{C}$) and annual (current year's ring width)—differ across tissues (leaves, twigs) in these same species? (Q3) Do responses to moisture stress differ across needle cohorts in piñon?

In addressing Q2, we propose simplified hypotheses couched within two frameworks for understanding the response of NSC pools to moisture stress. First, if direct control of carbon sinks by environmental variation is the dominant control, (H1) NSC concentrations would generally increase in response to moisture stress. Second, if NSC concentrations are driven by respiratory depletion and source limitation, (H2) moisture stress would generally reduce NSC concentrations. Throughout the paper, we strive to understand NSC dynamics by considering organismal and environmental context—when (season), where (organ) and what (species traits)—as well as the multiple competing functions of NSCs.

Table 1. Approximate locations, elevation, descriptive climate information and sampled species associated with each of the main 22 study sites.

State	Site	Coordinates (dec. deg.)	Elevation (m)	MAP (mm)	MAT (°C)	Species
CO	AS1	37.7, −108.2	2791	681.51	4.20	POTR
CO	AS2	37.5, −106.2	2714	501.38	3.57	POTR
CO	AS3	37.8, −107.9	3147	932.10	1.90	POTR
CO	AS4	38.3, −108.1	2915	645.67	4.39	POTR
NM	AS5	36.6, −106.2	2825	552.12	5.25	POTR
UT	AS6	38.0, −111.8	2944	641.54	3.93	POTR
UT	AS7	38.3, −112.4	2941	838.98	4.08	POTR
AZ	AS8	35.2, −112.2	2157	606.58	9.69	POTR
AZ	AS9	36.4, −112.2	2781	821.65	6.10	POTR
AZ	AS10	34.0, −109.3	2845	660.81	5.95	POTR
NM	AS11	33.7, −108.6	2853	372.05	8.07	POTR
AZ	PJ1	36.5, −112.7	1680	320.66	12.7	PIED–JUOS
AZ	PJ2	35.9, −111.8	1937	347.84	9.60	PIED–JUOS
AZ	PJ3	35.2, −112.2	1896	538.72	11.1	PIED–JUOS
AZ	PJ4	34.6, −110.8	2001	400.84	10.46	PIED–JUOS
CO	PJ5	38.2, −108.4	1962	351.03	9.36	PIED–JUOS
NM	PJ6	37.0, −107.8	2069	391.15	9.85	PIED–JUOS
NM	PJ7	33.9, −108.5	2,140	360.11	9.13	PIED–JUOS
NM	PJ8	36.4, −107.1	2,246	372.05	7.85	PIED–JUOS
UT	PJ9	38.1, −109.5	2019	314.26	10.56	PIED–JUOS
UT	PJ10	37.2, −112.8	1827	390.25	11.72	PIED–JUOS
UT	PJ11	38.3, −110.1	1949	237.40	11.49	PIED–JUOS

MAT = mean annual temperature; MAP = mean annual precipitation; PIED = *P. edulis* (piñon); JUOS = *J. osteosperma* (juniper); POTR = *P. tremuloides* (aspen).

Materials and methods

Study sites

During the summers of 2016 and 2017, we sampled 24 sites experiencing varying degrees of moisture stress following high variability in 2015–16 winter precipitation (Figure 1, Table 1). We conducted three field sampling campaigns; the first occurred in June 2016 (day of year [DOY] 158–180), prior to monsoon onset (hereafter 'premonsoon'), to capture NSC concentrations during the premonsoon arid period. The second took place from August to September 2016 (DOY 229–261), after monsoon onset (hereafter 'postmonsoon'), to evaluate NSC changes after onset of monsoon precipitation. We collected tree cores in a third campaign across the summer of 2017 (see Measures of plant water stress section).

We sampled *P. tremuloides* (hereafter, aspen) at 11 sites (hereafter, sites AS1–AS11), ranging in elevation from 2160 to 3150 m above sea level (a.s.l.); we sampled co-occurring *P. edulis* (hereafter, piñon) and *J. osteosperma* (hereafter, juniper) at 11 sites (hereafter, sites PJ1–PJ11), spanning elevations of 1680–2250 m a.s.l. Tree dimensions (Table S1 available as Supplementary Data at *Tree Physiology* Online) were assessed by measuring diameter at breast height with diameter tapes (Forestry Suppliers Inc., Jackson, MS, USA) and estimating crown diameter (mean of N–S and E–W radii) and tree height with a laser range finder (TruPulse 200, Laser Technology Inc., CO, USA).

Nonstructural carbohydrates sample collection, preparation and measurement

Because NSC concentrations fluctuate diurnally in leaves (e.g., Baber et al. 2014, Werner and Gessler 2011), we standardized collections of crown tissues for NSC measurement in the afternoon (between 13:00 and 18:00 h). For each tree (5 trees/site for 22 sites, resulting in 33-species-site combinations; Table 1), leaves and twigs were sampled from three sides of the crown and pooled into a single sample for each tissue (leaves or twigs). Tissue samples for NSCs were immediately frozen on dry ice for transport to lab facilities in Flagstaff, Arizona. Transport times ranged from 1 to 6 days (most often a maximum of 2–3 days). After transport, samples were microwaved, oven dried for 72 h at 60 °C and subsequently frozen at −20 °C until analysis. Nonstructural carbohydrate concentrations in leaves and twigs were measured according to protocols described in Quentin et al. (2015) as revised in Landhäusser et al. (2018). Leaves and twigs from the most recent piñon cohort (2016) were analyzed separately (hereafter, 'young' needles or twigs), whereas the 2015 and 2014 cohorts were pooled across years (hereafter, 'old' needles or twigs). For a full description of the NSC assay method, see Methods S1 available as Supplementary Data at *Tree Physiology* Online.

Measures of plant water stress

We evaluated plant water stress at different timescales via multiple methods (Figure S1 available as Supplementary

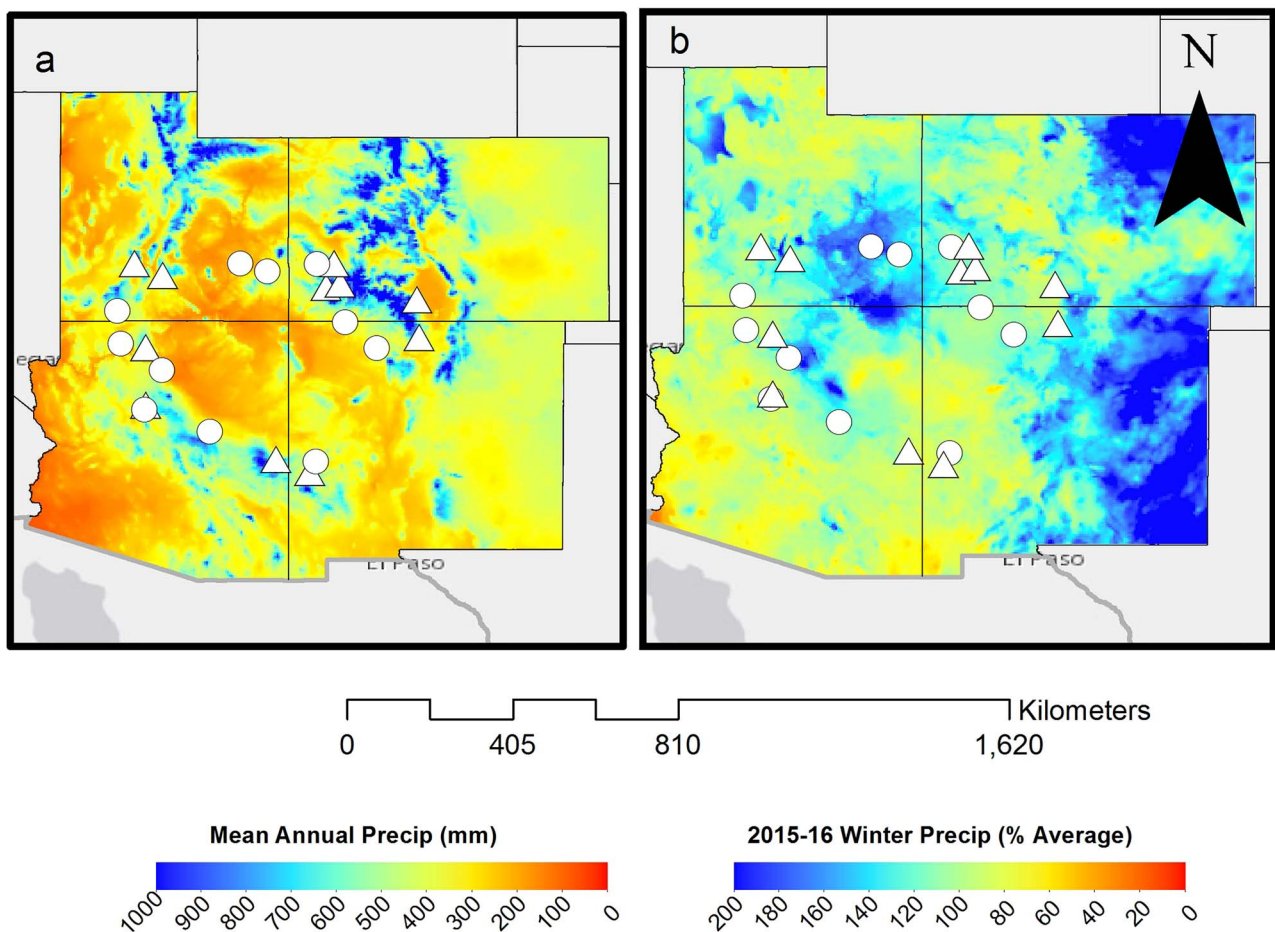


Figure 1. Locations of the sites used in this study overlaid on (a) mean annual precipitation and (b) 2015–16 winter precipitation anomalies (as % of average), where hotter colors represent lower (drier) values and cooler colors represent higher (wetter) values. Circles denote piñon–juniper sites, triangles denote aspen sites and squares denote ponderosa NMEG sites. Gray-filled symbols indicate sites where sapwood samples were collected for NSCs. Basemap sources: Esri, DeLorme, HERE, MapmyIndia, OpenStreetMap.

Data at *Tree Physiology* Online). To represent short-term water stress, predawn water potential (Ψ_{PD}) was measured on living twigs using a Scholander-type pressure chamber (PMS, Corvallis, OR, USA) on the same day as NSC sample collection in 2016. During each sampling campaign, twigs were excised before dawn and measured within 30 min of excision.

To assess seasonal moisture stress, following grinding of samples for NSC analysis (see NSC measurements, above), leaf material was subsampled to measure bulk-level carbon isotopes ($\delta^{13}C$), a measure of stomatal limitations to photosynthesis. We only used the most recent (2016) needle cohorts for piñon. Approximately 4 mg of dry material was weighed using a microbalance and encapsulated in a tin capsule. Samples were analyzed for $\delta^{13}C$ by gas isotope ratio mass spectroscopy by the Colorado Plateau Stable Isotope Lab at Northern Arizona University. $\delta^{13}C$ values are reported in per mil (‰) with reference to the Vienna Pee Dee Belemnite.

In summer 2017, tree cores were also collected with 5.15-mm increment borers (Haglof, Sweden) for ring width measurements to represent an annual index of drought stress (based on the ring width of the sampling year). Cores were obtained from multiple (two to three) sides of the tree, perpendicular to the slope when appropriate. Cores were subsequently mounted, air dried and sanded with increasingly finer sandpaper until cells and ring boundaries were clearly visible under a dissecting microscope. Cores were then visually cross dated and annual ring widths measured (Velmex, Bloomfield, NY, USA). Visual cross dating was verified with COFECHA (Grissino-Mayer 2001). Not all trees could be successfully cross dated at all sites (e.g., we were unable to cross date juniper trees at 3 of the 11 piñon–juniper sites). Tree ring widths were subsequently detrended for age effects following standard methodology (Fritts and Swetnam 1989), using the dplR (Bunn 2008) package in R (R Development Core Team 2019), as described in Peltier and Ogle (2019). We used each tree's detrended, unitless

ring width index for 2016 as a measure of annual moisture stress.

Estimating NSC seasonal patterns

To test for differences in concentrations of sugars and starch among species, tissues and seasons at the core sites (AS1–AS11 and PJ1–PJ11), we conducted a hierarchical, univariate three-way analysis of variance (ANOVA) for each fraction (sugars and starch), with species (aspen, juniper or piñon), tissue type (leaf or twig) and sampling date (premonsoon or postmonsoon) as factors. Model code is provided in [Supplementary Material A1](#) available as Supplementary Data at *Tree Physiology* Online.

Analysis of moisture stress effects on leaf and twig NSC concentrations

To understand variation in NSC concentrations ($n = 852$ each for sugars and starch) across the aspen (AS1–AS11) and piñon–juniper (PJ1–PJ11) sites, we evaluated leaf and twig NSC concentrations in relationship to three different moisture-related covariates, assuming these represent indices of moisture stress on varying timescales. Ψ_{PD} represents ‘instantaneous’ or short-term moisture stress, since Ψ_{PD} can vary from day to day (Guo and Ogle 2019, Hinckley et al. 1978), reflecting changes in soil water availability and the ability of the tree to equilibrate with soil water potentials during the night (Choat et al. 2015, Donovan et al. 2001, Martorell et al. 2014, Ritchie and Hinckley 1975). $\delta^{13}C$ represents an intermediate timescale index of water stress (Farquhar et al. 1982) that integrates over multiple weeks to a growing season (Lauteri et al. 1993). Annual ring width indices integrate effects of moisture availability over the entire growing season to multiple prior years, particularly in the Southwest (Peltier et al. 2018). Because they are normalized within a given tree core, use of ring width indices, rather than raw ring widths, removes effects of acclimation or adaptation to local site conditions, including local climate.

We transformed the three indices of moisture stress to a common scale, facilitating interpretation and comparison of effects and interactions. Critically, ring widths were detrended for age effects, yielding a unitless growth index with mean equal to 1, following standard methods (Fritts and Swetnam 1989). Thus, the RW index describes relative 2016 growth compared with the whole core, and differences in mean annual growth among sites are thus removed. Ψ_{PD} and RW indices were multiplied by -1 , and all three covariates were rescaled to each have mean 0 and standard deviation (SD) = 1. Hence, higher values (more positive) of the transformed Ψ_{PD} , $\delta^{13}C$ and RW indices correspond to greater moisture stress, whereas more negative values correspond to lower moisture stress.

Simple versus complex models for NSC pools

We further analyzed leaf and twig sugars and starch concentrations ($n = 852$ for each fraction) via two model-based

approaches. First, we implemented hierarchical linear models that related NSC concentrations to the three indices of moisture stress (potentially including all two-way interactions). The coefficients (parameters) in this model were allowed to vary by NSC fraction type, season (premonsoon or postmonsoon) and tissue (leaf or twig). Next, we implemented a simpler model to evaluate the importance of ignoring season, tissue type and various interaction effects. In this simple model, season and tissue type fixed effects were excluded, as well as all two-way interactions among the three moisture stress indices. While we primarily focus on the first approach, we include the simpler model as a strawman framework for understanding broad patterns in NSC responses and to also illustrate the pitfalls of treating NSC pool variation as a simple metric of physiological stress.

For each approach, six univariate models were applied, one for each combination of fraction (sugar and starch) and species (aspen, piñon and juniper). Thus, in the first approach, assuming logged NSC concentrations (for each observation, i) in tissue t and season m arise from a normal distribution with mean μ_i and SD σ^2 , then the full mean model is:

$$\mu_i = \beta_{0,t,m} + \sum_{j=1}^3 \beta_{j,t,m} X_{j,t,m} + \sum_{j=1}^3 \sum_{k=j+1}^3 \beta_{jk,t,m} X_{j,t,m} X_{k,t,m} \quad (1)$$

The intercept ($\beta_{0,t,m}$) describes the predicted, log-scale concentration of a given NSC fraction at ‘average’ water stress conditions (i.e., when the transformed moisture indices all equal 0); $\beta_{j,t,m}$ is the main effect of water stress index (covariate) j , and $\beta_{jk,t,m}$ is the interactive effect of covariates j and k ($j \neq k$). For piñon only, the coefficients in Eq. (1) are further indexed by cohort, c (e.g., in the first approach, $\beta_{0,t,m,c}$, $\beta_{j,t,m,c}$ and $\beta_{jk,t,m,c}$).

The simple models (second approach) follow a similar structure for the mean model as in Eq. (1), but the intercept and main effects do not vary by tissue or season (e.g., β_0 , β_j), and all interaction terms are excluded (i.e., no $\beta_{jk,t,m}$ parameter).

For the first approach, we performed a simple backward stepwise model selection process where nonsignificant interactions (95% credible interval [CI] includes zero) in the full models described by Eq. (1) were excluded from the final models. This selection process was implemented separately for each of the six models, such that the structure of the final models differed among the six different species–fraction combinations. Some observations were missing covariate data such that 3%, 21% and 9% of the Ψ_{PD} , $\delta^{13}C$ and RW^{2016} observations were missing, respectively, and we imputed these missing values within the model. Relatively noninformative and fairly standard priors were specified for all model parameters. More details on the final models from the first approach are reported in [Table S2](#) available as Supplementary Data at *Tree Physiology* Online. All other model details and all model code are provided in [Supplementary Material A2](#) available as Supplementary Data at *Tree Physiology* Online. Raw data provided in [Table S3](#) available as Supplementary Data at *Tree Physiology* Online. We interpret results from the final models only.

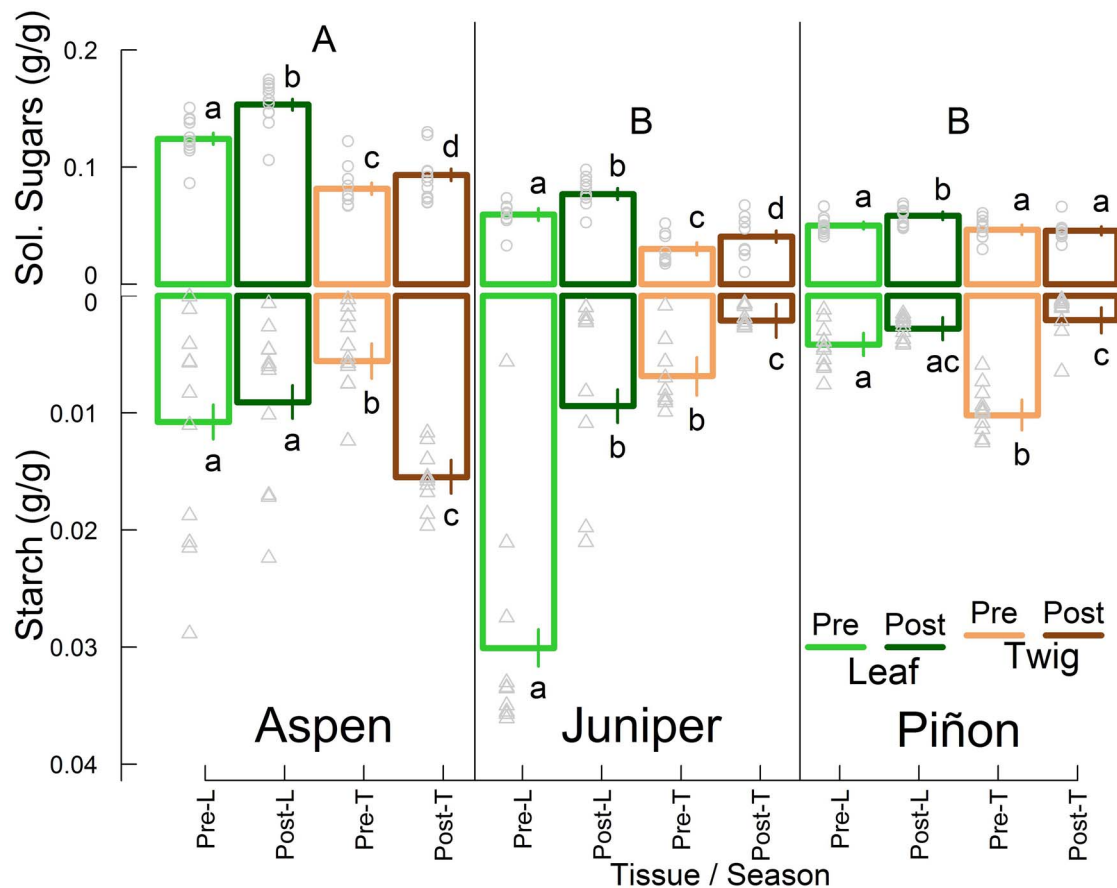


Figure 2. Posterior means (open bars) estimated from the hierarchical Bayesian three-way ANOVA for soluble sugars (positive axis, up from zero) and starch (negative axis, down from zero; note difference in scale), for all combinations of species (aspen, left; juniper, middle; piñon, right), tissue types (leaves or needles, greens; twigs, browns) and seasons (premonsoon, light green or brown; postmonsoon, dark green or brown) with associated 95% CIs (vertical lines). Labels along x-axis denote combinations of season (premonsoon, Pre; postmonsoon, Post) and tissue (leaves, L; twigs, T). Point symbols (gray) depict site-level data (sample means) for soluble sugars (open circles) and starch (open triangles) for the 11 sites sampled per species (AS1–AS11 for aspen and PJ1–PJ11 for juniper and piñon). For visual ease, lowercase letters denote significant within-species differences among tissue-sampling date for a given NSC fraction, based on 95% CIs that do not contain the posterior mean of another group. For soluble sugars, significant differences among species-level means are also denoted with uppercase letters (no significant differences among species were found for starch).

With respect to interactions among moisture stress covariates, we assume moisture stress indices operating at longer timescales influence the response of NSC pools to moisture stress at shorter timescales, and not vice versa. For example, we assume that Ψ_{PD} , representing short-term moisture stress, cannot influence the response of NSC pools to seasonal or annual moisture stress that accumulated prior to the Ψ_{PD} measurement. Rather, when moisture stress interactions are significant, we assume annual moisture stress, RW^{2016} , governs the response of NSC pools to both Ψ_{PD} and $\delta^{13}C$, and seasonal moisture stress, $\delta^{13}C$, influences the response of NSC pools to Ψ_{PD} .

Model implementation

The models—hierarchical three-way ANOVA and the two model-based approaches for NSC—were implemented in a hierarchical Bayesian framework in JAGS 3.4.0 (Plummer 2003) via R (R Development Core Team 2019), using the package rjags (Plummer 2013). For each model, three chains were initialized

with dispersed initial values, and posterior samples were simulated until convergence. After an initial burn-in period, models were updated for an additional 100,000 iterations, from which summary statistics (posterior means, SDs and 95% CIs) were computed.

Results

Seasonal differences in NSC concentrations (Q1)

Nonstructural carbohydrate concentrations in nearly all species and tissues were seasonally dynamic (Figure 2). Soluble sugar (i.e., ‘sugar’) concentrations increased from the pre- to postmonsoon periods in aspen and juniper, regardless of tissue type, whereas sugars in piñon only showed small increases in leaves (Figure 2). Conversely, starch concentrations declined from the pre- to postmonsoon period in juniper (both tissues) and piñon twigs, but increased in aspen twigs (Figure 2). Among species, sugar concentrations were higher in aspen tissues than

in juniper and piñon, but starch concentrations did not differ among species (Figure 2, uppercase letters). Nonstructural carbohydrate concentrations were generally highest in leaves, with two exceptions being postmonsoon starch in aspen and premonsoon starch in piñon (Figure 2). There was considerable site-to-site variation for any given tissue and season, particularly for starch.

Nonstructural carbohydrates responses to moisture stress (Q2 and Q3)

To summarize results from the complex versus simple model of NSC dynamics, we present a graphical approach to visualizing the results (Figures 3 and 4). The simple model appears to incompletely describe NSC responses to moisture stress, where the more complex model, Eq. (1), explained an average of 10% more variation in NSC concentrations. Coefficients of determination (R^2) between predicted and observed sugar concentrations for the complex (vs. simple) model were 0.57 (vs 0.50) in aspen, 0.67 (vs 0.52) in juniper, but only 0.39 (vs 0.36) for piñon. For starch, R^2 was lower for aspen at 0.57 (vs 0.39), high for juniper at 0.81 (vs 0.72) and moderate for piñon at 0.69 (vs 0.60).

The two modeling approaches generally agreed with respect to piñon's NSC responses: short-term moisture stress negatively impacts NSC concentrations, seasonal moisture stress was not important and annual moisture stress positively impacted starch concentrations (Figure 3). While the simple model indicated that short-term moisture stress reduced NSC concentrations and annual moisture stress increased starch concentrations (Figure 3b and d), the complex model demonstrated that responses differed strongly across seasons. For example, greater short-term moisture stress (more negative Ψ_{PD}) negatively impacted NSC concentrations in the postmonsoon (Figure 3a and b). Additionally, premonsoon leaf starch and postmonsoon twig starch increased with greater annual moisture stress (relatively smaller 2016 ring-width indices; Figure 3a and b, Table S2 available as Supplementary Data at *Tree Physiology* Online). Cohort-specific responses emerged, whereby sugar concentrations decreased with greater short-term moisture stress for old twigs (premonsoon) and current-year leaves (postmonsoon) (Figure 3a and c).

Similarly, for juniper, both approaches generally agreed on the negative effects of short-term moisture stress and the positive effects of seasonal and annual moisture stress on NSC concentrations (Figure 4). However, the complex model indicates that responses again differed strongly across seasons: for example, under greater annual moisture stress, twig starch increased in the premonsoon but leaf starch concentrations increased during the postmonsoon. Similarly, twig sugar concentrations increased under both greater seasonal and annual moisture stress, but only in the postmonsoon (Figure 4a). Furthermore, moisture stress indices often interacted to amplify positive effects of

moisture stress, and these interactions also depended strongly upon seasonal or tissue context (Figure 4a). For example, positive interactions were apparent during the premonsoon, where leaf and twig sugar concentrations increased with greater seasonal moisture stress if annual moisture stress was also high (more positive; Figure 4a). However, under low (more negative) annual moisture stress, premonsoon sugar concentrations were unaffected by (leaves) or tended to decrease (twigs) with greater seasonal moisture stress (Figure 4a). Similarly, in the postmonsoon, twig sugars decreased with short-term moisture stress under average to below-average (more negative) seasonal moisture stress, but increased with short-term moisture stress under higher (more positive) seasonal moisture stress. In contrast, postmonsoon leaf starch decreased with greater short-term moisture stress, and this effect was amplified under greater (more positive) annual moisture stress (Figure 4a).

Unlike piñon or juniper, aspen premonsoon starch concentrations decreased with greater annual moisture stress, as indicated by both the simple (Figure 4d) and complex (Figure 4c) models. Conversely, short-term moisture stress tended to increase starch concentrations (Figure 4c and d). But, similar to the other two species, the complex model revealed important interactions among the different moisture stress indices and differences across seasons (Figure 4c). For example, leaf starch increased with increasing seasonal (pre- and postmonsoon) and short-term (premonsoon) moisture stress, but only under high (positive) longer-term moisture stress indices. Conversely, postmonsoon leaf starch increased with greater short-term stress, particularly under low (more negative) annual or seasonal stress. Twig starch decreased with greater short-term moisture stress, particularly when seasonal moisture stress was low or annual moisture stress was high (Figure 4c), but only during the premonsoon. Postmonsoon twig starch was insensitive to moisture stress. Finally, leaf sugars increased with greater seasonal (premonsoon) or short-term (postmonsoon) moisture stress (Figure 4c), whereas the simple model failed to detect responses of sugars to moisture stress (Figure 4d).

Discussion

Our results provide clear evidence that NSC concentrations cannot be used as a simple index for understanding tree physiological status. Across three species and 22 sites in the southwestern USA ('Southwest'), seasonal variation in NSC concentrations and NSC responses to moisture stress were not solely consistent with either respiratory depletion (H1) or sink limitation (H2; Figure 5). Interpretation of our results is not possible using either of these frameworks alone, pointing to the utility of integrating multiple theoretical frameworks in interpreting stress impacts on NSC dynamics in trees, and the need for a broad range of datasets to constrain unknowns in these frameworks. Our results similarly highlight the multiple

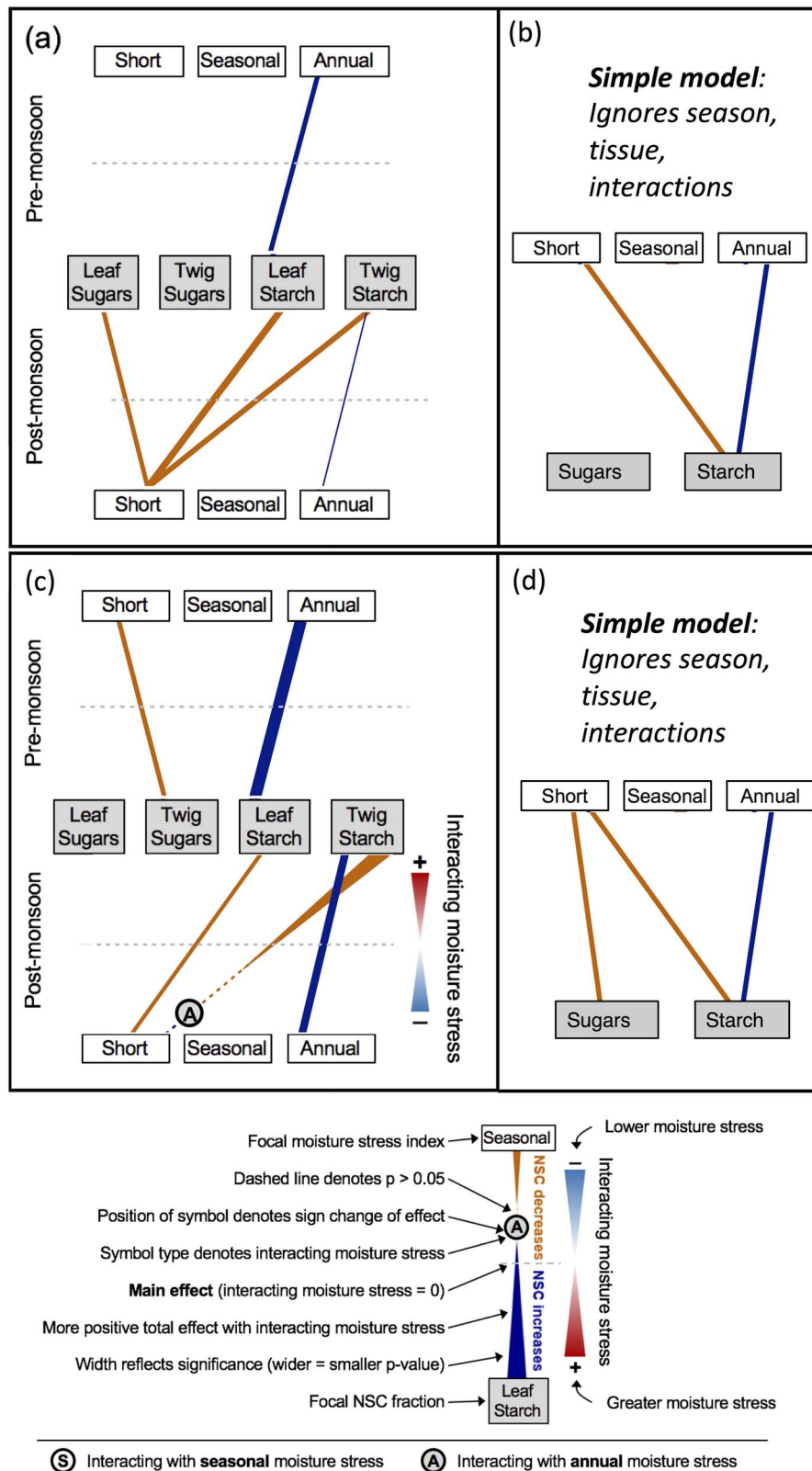


Figure 3. (a, c) Complex model (Eq. 1) total responses of NSC fractions (leaf or twig soluble sugars or leaf or twig starch) to moisture stress indices at different timescales in piñon (a) current-year needle cohorts and (c) old needle cohorts, during the premonsoon (upper half of panels) or postmonsoon (lower half of panels). (b, d) Equivalent responses based on the simple model (ignores seasons, tissues and interactions) for (b) current-year needle cohorts and (d) old needle cohorts. Lines connecting NSC fractions to water stress indices denote significant responses to that water stress index, with thicker lines reflecting smaller associated P -values (dashed line: $P > 0.05$; solid lines based on $P < 0.05$, 0.01 or 0.001); the connecting line color denotes the sign of the total effect or response (orange: NSC concentration decreases with increasing moisture stress;

functional roles of NSCs (poorly captured by the simple model), such as osmoregulation, storage and stress tolerance, and their variation across seasons. For example, moisture stress responses in one NSC fraction may be consistent with sink limitation (increased sugars in leaves with greater moisture stress, under direct limitation on growth activity, e.g., [Fatichi et al. 2014](#)), but contemporaneous responses in the other fraction are not (reduced starch under greater moisture stress, consistent with respiratory depletion). Responses to moisture stress were also clearly different across three tree species, potentially reflecting different hydraulic strategies and mean environmental conditions ([Figure 5](#)). Finally, particularly in juniper and aspen, important interactions among moisture stress at different timescales highlight the importance of considering physiological history ([Ogle et al. 2015](#)). That is, NSC pools respond differently to moisture stress at short timescales if they have experienced greater or reduced moisture stress in past seasons or, likely, years ([Figure 4](#)).

Species traits align with different functional roles of NSC

Nonstructural carbohydrates serve numerous roles within tree tissues, complicating interpretation of seasonal variability or responses of multiple NSC pools to moisture stress ([Dietze et al. 2014](#), [Martínez-Vilalta et al. 2016](#)). Furthermore, moisture stress can act directly on sink activity (e.g., can reduce growth), leading to accumulation of NSCs in source organs ([Sala and Hoch 2009](#), [Woodruff and Meinzer 2011](#), [Würth et al. 2005](#)). However, the functional role of NSCs in osmoregulation is increasingly being recognized as an important component of drought tolerance ([Kannenberg et al. 2018](#), [Martínez-Vilalta et al. 2019](#), [Sapes et al. 2019](#)). For example, coexisting piñon and juniper experienced similar climatic and drought conditions, but exhibited different NSC responses to moisture stress ([Figure 5](#)). Such species-specific responses of different NSC fractions to short-term (Ψ_{PD}), seasonal (bulk-leaf $\delta^{13}C$) and annual (ring-widths indices) moisture stress help to differentiate the multiple functional roles of NSCs in tree crown tissues and to highlight the disadvantages of simplistic treatments of NSC variation.

In juniper and aspen, NSC responses to short-term and seasonal moisture stress were not solely consistent with respiratory depletion or sink limitation, instead reflecting multiple contemporaneous NSC functions, likely driven by hydraulic traits ([Figure 5](#)). There is evidence for links between strategies of stomatal regulation (e.g., iso/anisohydry) and plasticity in leaf turgor loss point ([Meinzer et al. 2014](#)), where more anisohydric

species may actively regulate osmotic potential under increasing moisture stress, though it is unclear how NSCs are directly involved ([Nolan et al. 2017](#)). Suggestive patterns have been observed in piñon (isohydric, decreased leaf sugar concentrations under stress) and juniper (anisohydric, increased leaf sugar concentrations under stress), though previous results have been interpreted as growing season depletion in piñon due to its isohydric strategy ([Woodruff et al. 2015](#)). Consistent decreases in NSC concentrations with greater short-term moisture stress ([Figure 3](#)) suggest simple respiratory depletion in piñon, likely due to stomatal closure in response to lower needle water potentials ([Adams et al. 2013](#), [Dickman et al. 2015](#), [West et al. 2008](#)). Even if piñon does not close stomata earlier than co-occurring juniper, greater embolism may lead to reductions in water transport to the crown and associated reductions in photosynthetic capacity ([García-Forner et al. 2016](#)). Of course, different NSC responses could also occur if piñon and juniper trees occur in different microclimate conditions or support roots at very different depths ([West et al. 2007](#)).

In contrast to piñon, juniper has been characterized as an anisohydric species, and the large number of positive responses of NSC concentrations to seasonal and annual moisture stress—NSC concentrations often increase with greater moisture stress ([Figure 2](#), [Figure 4a and b](#), [Table S2](#) available as Supplementary Data at *Tree Physiology Online*) is consistent with osmoregulation ([Dickman et al. 2015](#)). Momentarily focusing on the main effects of the different moisture stress indices, only leaf starch, and to some extent twig sugars, decreased with greater short-term moisture stress, and only during the postmonsoon ([Figure 4a](#)). In contrast, leaf sugars were unaffected by moisture stress during the postmonsoon (after the onset of monsoonal precipitation). One interpretation for this suite of responses is transformation of leaf starch to sugars and translocation of twig sugars to leaves, both of which could help regulate osmotic potential under declining leaf water potentials ([West et al. 2008](#)). Assuming 50% water content of juniper leaves, which is realistic for piñon or juniper ([Stimson et al. 2005](#)), and assuming all sugars are glucose, the increase in average leaf soluble sugar concentrations across sites from pre- to postmonsoon ([Figure 2](#)) represents a change in sugar concentration of roughly 0.3–0.4 molar. This represents a physiologically important change in osmotic (solute) potential that acts to maintain turgor pressure, as periods between sporadic monsoon rainfall can be just as warm as the premonsoon ([Knowles et al. 2020](#)).

The relative insensitivity of leaf sugars to moisture stress in juniper—and premonsoon increases in leaf sugars under

blue: NSC concentration increases). In cases involving significant interactions, widening connecting lines depict dependence of moisture stress responses on interactions with moisture stress at longer timescales (e.g., short-term \times seasonal, short-term \times annual or seasonal \times annual). The longer timescale interacting moisture stress indices are indicated by the symbol type (A = annual; S = seasonal), where the position of the symbols indicates where the total moisture stress effect is zero relative to the interacting moisture stress index value. The vertical scale at the right indicates variation in the interacting moisture stress indices, from low to high ('-' to '+'; -3σ to $+3\sigma$; horizontal dotted gray line denotes average index value of 0).

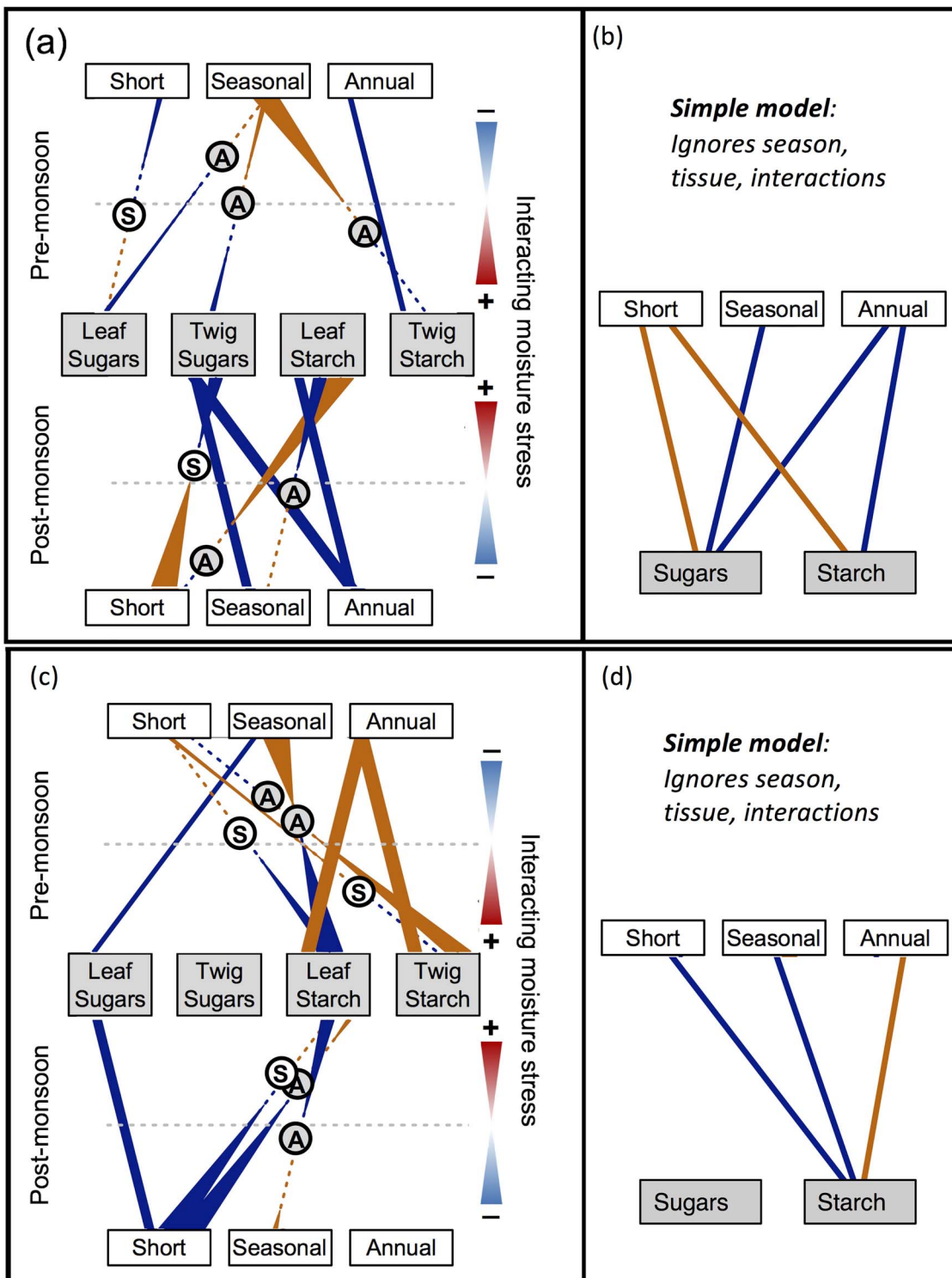


Figure 4. Total responses of NSC fractions (leaf or twig soluble sugars or leaf or twig starch) to moisture stress indices at different timescales in (a, b) juniper and (c, d) aspen. See Figure 3 for a detailed explanation and graphical legend.

seasonal moisture stress—is also consistent with maintenance of leaf sugar concentrations under moisture stress at the expense of storage as starch or storage in proximate tissues (Guo et al. 2020). Depletion of root, but not crown NSCs

under drought has been observed in other conifer species (Hartmann et al. 2013). Increased twig sugars and leaf starch with greater seasonal and annual moisture stress could thus represent acclimation of NSC storage prioritization in the face

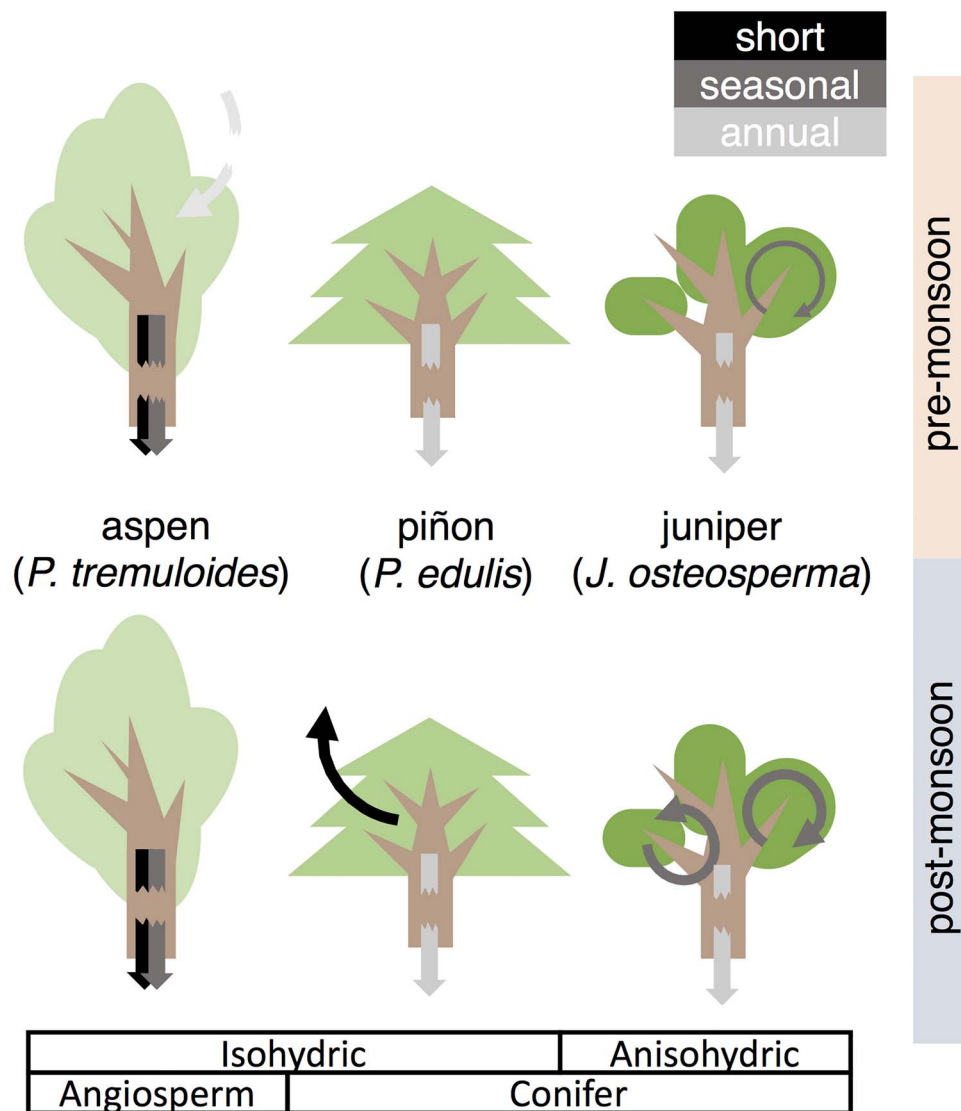


Figure 5. Responses to moisture stress broadly differ across three tree species (aspen, piñon and juniper) and timescales (short-term, black arrows; seasonal, dark gray arrows; annual, light gray arrows). Though effect strengths differ, sink-limitation (downward-pointing broken arrows) is universal and is driven by moisture stress at different timescales in different species (e.g., relatively fast timescales in aspen and longer timescales in the two conifers). Respiratory depletion of NSC pools (upward-pointing arrows) occurs in different seasons for different species. Osmotic regulation (cycling arrows) is likely a dominant driver of NSC pool variability only in juniper, which is typically classified as anisohydric.

of higher probability and/or frequency of short-term moisture stress. In fact, aforementioned decreases in leaf starch with short-term moisture stress were largest when annual moisture stress was also high (Figure 4a), consistent with acclimation. Acclimation of other traits (e.g., gas exchange) in *Juniper* spp. have been documented, but not clearly in NSC concentrations (Grossiord et al. 2017, 2018, Limousin et al. 2013), though again, NSC concentrations in juniper have been shown to be less reduced by drought stress than co-occurring piñon (e.g., Dickman et al. 2015). Sugar accumulation under greater short-term and seasonal moisture stress could reflect sink limitation (direct climate limitations on growth or respiration) or limitations on the export of sugars from the leaves under weak turgor (Hsiao 1973). However, starch concentrations did not increase

with greater short-term or seasonal water stress, and in certain cases, starch actually decreased (Figure 4a, Table S2 available as Supplementary Data at *Tree Physiology* Online). This is more consistent with transformation of starch to osmotically active sugars under heightened short-term and seasonal moisture stress (e.g., Wang and Stutte 1992).

In contrast to the two gymnosperm species, aspen responses to short-term and seasonal moisture stress were consistent with sink limitations, whereas responses to long-term stress suggested depletion (Figures 4c, d, and 5). Aspen is sometimes characterized as relatively isohydric (Dang et al. 1997, Hogg et al. 2000, Landhäusser and Loeffers 2012), though widespread cavitation has been observed in this species (Anderegg et al. 2012). Aspen occurs at higher elevations

in the Southwest (Table 1), where populations have been experiencing significant dieback during recent regional droughts (Worrall et al. 2010). Our study primarily found increases in crown NSC concentrations (except twig starch) under short-term and seasonal moisture stress. In aspen stands in this region experiencing drought mortality, no clear declines in NSC concentrations in this species were observed (Anderegg et al. 2012); rather, NSC concentrations may initially increase in tandem with greater drought stress in this species (Anderegg 2012). Here, higher leaf sugars with greater short-term moisture stress during the premonsoon could indicate osmoregulation, but as starch also accumulated with short-term moisture stress, this is more consistent with direct limitations on sinks (Fatichi et al. 2014). Decreased starch concentrations in twigs during short-term moisture stress is not clearly consistent with this interpretation, but could reflect respiratory depletion in twigs receiving reduced NSCs from adjacent leaves under short-term transport limitations (Hsiao 1973). We argue growth sinks were more strongly inhibited than photosynthesis or stomatal conductance by short-term moisture stress in aspen, consistent with the moderate water potentials observed for this species (Figure S1 available as Supplementary Data at *Tree Physiology* Online).

In contrast, starch concentrations in aspen decreased with greater annual moisture stress, suggesting respiratory depletion or carbon limitation. This response could reflect that trees with relatively narrow ring widths in 2016 also tended to have narrower ring widths in 2015. While ring widths were detrended for age effects (thus removing differences in mean ring width across trees), we did not apply any procedures to reduce autocorrelation in the tree-ring series. Thus, a negative response of starch to annual moisture stress could reflect an effect of past growth status (i.e., past ring widths), or even legacies from the 2012 drought. This was also the case for piñon and juniper tree rings, but other work with this dataset suggests aspen trees have comparatively longer memory (sensu Ogle et al. 2015) of antecedent precipitation conditions, and thus may be influenced by climate over longer timescales. Respiratory depletion under annual, but not short-term, moisture stress is somewhat contradictory, but may suggest sink limitations under short-term moisture stress are transient (or ultimately released), whereas moisture stress over annual timescales captures the reduction in annual photosynthetic assimilation under persistent stomatal limitation in this species in hotter or drier years. That starch concentrations in both twigs and leaves were reduced when annual moisture stress was high also supports a physiological limitation on carbon assimilation at the leaf level (Figure 4a).

Implications of cohort-specific responses

Needle cohorts are rarely individually considered in studies of NSC dynamics (Palacio et al. 2012). Damage to previous-year needle cohorts has been suggested as a possible mechanism underlying observed drought legacies in conifers

(Peltier et al. 2016), and physiological function, such as photosynthetic capacity, declines with needle age (Ethier et al. 2006, Warren 2006). In piñon, NSC dynamics in old tissue cohorts were sensitive to short-term moisture stress, particularly sugar concentrations in twigs (Table S2 available as Supplementary Data at *Tree Physiology* Online), but there was little other evidence that older cohorts were more sensitive to moisture stress than younger cohorts (Figure 3), though it is possible they are more hydraulically vulnerable to embolism. Experimental drought treatments have shown moisture stress to differentially affect emergence and development of young foliage in piñon, particularly along secondary branches (Adams et al. 2015). Isotope-labeling studies (Epron et al. 2012) could explicitly test whether NSC translocation occurs among needle and twig cohorts in response to moisture stress.

Conclusions

Notable effort has been invested in measuring NSC concentrations in diverse species and ecosystems, and recent efforts have attempted to make measurements consistent across research groups (Landhäusser et al. 2018). However, failure to recognize the multiple functions of NSC can lead to overly reductionist arguments about the function of NSCs. That is, if only the dominant phases of variability (Figure 4b and d) in NSC concentrations are detected, we are left with an incomplete view of the function and role of this carbon pool, challenging our ability to predict or simulate NSC dynamics under novel climates or conditions. Integrating landscape scale measurements of NSCs and multiple indices of water stress for multiple trees and species, we show that the responses of NSC concentrations to moisture stress at three timescales vary across species (Figure 5), as well as organs, seasons and perhaps needle cohorts (Figures 3 and 4). These differing responses illustrate the breadth of functional roles of NSCs in tree physiological processes, including respiratory depletion and sink limitation, and also osmoregulation (Figure 5). We also know tree NSC pools are built up and drawn down over timescales much longer than those over which we typically measure (Carbone et al. 2013). Interactive effects of moisture stress at different timescales on NSC pools reflect this and highlight the limitations on inference drawn from 'snapshot' measurements of NSC concentrations. 'Memory' or 'legacies' of prior moisture stress on tree growth is another clear example of this dynamic (Kannenberg et al. 2020). For the field to move forward, comprehensive (across organs and time) measurements are needed, along with a full consideration of the drivers of variability of different NSC fractions and their interrelationships. Most likely, certain NSC pools, such as leaf sugars, exhibit fast temporal fluctuations in comparison with even high-frequency NSC measurements (Guo et al. 2020). Thus, such empirical and experimental efforts should be coupled with improved physiological models

of NSC pools. We hope these types of approaches can improve our understanding of the physiological functioning of trees, and the movement and drivers of NSCs within these complex organisms.

Supplementary Data

Supplementary Data for this article are available at *Tree Physiology* online.

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Conflict of interest

None declared.

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