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Homeostatic maintenance of nonstructural carbohydrates during the 2015–2016 El Niño drought across a tropical forest precipitation gradient

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Abstract

Nonstructural carbohydrates (NSCs) are essential for maintenance of plant metabolism and may be sensitive to short- and long-term climatic variation. NSC variation in moist tropical forests has rarely been studied, so regulation of NSCs in these systems is poorly understood. We measured foliar and

branch NSC content in 23 tree species at three sites located across a large precipitation gradient in Panama during the 2015–2016 El Niño to examine how short- and long-term climatic variation impact carbohydrate dynamics. There was no significant difference in total NSCs as the drought progressed (leaf $P=0.32$, branch $P=0.30$) nor across the rainfall gradient (leaf $P=0.91$, branch $P=0.96$). Foliar soluble sugars decreased while starch increased over the duration of the dry period, suggesting greater partitioning of NSCs to storage than metabolism or transport as drought progressed. There was a large variation across species at all sites, but total foliar NSCs were positively correlated with leaf mass per area, whereas branch sugars were positively related to leaf temperature and negatively correlated with daily photosynthesis and wood density. The NSC homeostasis across a wide range of conditions suggests that NSCs are an allocation priority in moist tropical forests.

KEYWORDS: climate, ENSO, NSC, Panama, storage, sugars, tropics, vegetation

1 INTRODUCTION

Tropical forests account for a large fraction of terrestrial live biomass (Pan, Birdsey, Phillips, & Jackson, 2013) and approximately half of terrestrial gross primary production (GPP; Beer et al., 2010). Drought is one of the largest threats to tropical forest structure and functioning (Davidson et al., 2012; McDowell et al., 2018) and can result in reduced carbon sequestration due to higher ecosystem respiration and lower GPP (Cavaleri et al., 2017), transitioning these ecosystems from carbon sinks to sources (Tian et al., 1998). The El Niño phase of the El Niño Southern Oscillation (ENSO) impacts some tropical forests through hotter, drier dry seasons and wet seasons with less solar insolation (Cavaleri et al., 2017). El Niños occur subdecadally (Allan, Lindsay, & Parker, 1996), and extreme events are expected to increase in frequency with climate change (Cai et al., 2014). Droughts with and without El Niños have been associated with increased mortality of canopy trees (Condit, Hubbell, & Foster, 1996; Laurance & Williamson, 2001), as well as shifts in allocation from leafing to fruiting (Detto, Wright, Calderón, & Muller-landau, 2018; Laurance & Williamson, 2001), and altered remote sensing signatures of the canopy surface (e.g., greenness and backscatter; Li, Xiao, & He, 2018; Nagai, Ichii, & Morimoto, 2007; Saatchi et al., 2013). Nonstructural carbohydrates (NSCs) provide the carbon skeletons for biosynthetic pathways associated with secondary metabolism (e.g., growth and defence) and for energy production (i.e., respiration through glycolysis and the tricarboxylic acid cycle; Chapin, Schulze, & Mooney, 1990; Heldt, 2005). Constraints on NSC storage and utilization have prompted widespread research on their role in autotrophic carbon cycling (e.g., Dietze

et al., 2014) and in the avoidance of carbon starvation (e.g., Adams et al., 2017). NSCs are stored, typically as starch, when supply of photosynthate exceeds demand (i.e., when carbon assimilation is greater than growth and metabolism; McDowell, 2011). These stored NSCs can then be utilized during periods when supply is unable to match demand (e.g., during periods when CO₂ assimilation may be reduced; Hoch, Richter, & Körner, 2003). Deep reserves of NSCs older than one decade may even be utilized in growth and respiration, thus providing some buffer to seasonal variation (Dietze et al., 2014; Martínez-Vilalta et al., 2016). In a global review of NSC dynamics across organs, Martínez-Vilalta et al. (2016) found that seasonal minimums remained relatively high and constant among functional types and biomes, supporting the idea that NSCs are maintained above some (undetermined) minimum threshold. They also found that, although depletion of starch was relatively common, depletion of soluble sugars or total NSCs was very rare, consistent with the role of starch as a storage reservoir and soluble sugars as substrate for immediate metabolic use. Relative to other biomes, tropical systems showed low seasonal variability in NSCs (Martínez-Vilalta et al., 2016). Data from the tropics, however, remains relatively limited. Less than 15% of studies in the global review by Martínez-Vilalta et al. (2016) were from the tropics, and several of these were performed on seedlings or understory shrubs. Several studies have shown a positive relationship between NSCs and tropical seedling survival during periods of stress (Myers & Kitajima, 2007; Newell, Mulkey, & Wright, 2002; O'Brien, Leuzinger, Philipson, Tay, & Hector, 2014; Poorter et al., 2010; Poorter & Kitajima, 2007), as well as higher seedling NSC concentrations in species from wetter tropical forests (Poorter & Kitajima, 2007). An accumulation of NSCs prior to or during seasonal drought is commonly observed (Körner, 2003; Latt, Nair, & Kang, 2001; Newell et al., 2002; Würth, Peláez-Riedl, Wright, & Körner, 2005; Würth, Winter, & Körner, 1998), particularly for deciduous trees (Newell et al., 2002), but this rise is small in comparison with the extra-tropical biomes (Martínez-Vilalta et al., 2016). This is consistent with the observed increase in NSCs associated with lower growth rates in stressed seedlings (Myers & Kitajima, 2007; Poorter et al., 2010; Poorter & Kitajima, 2007) and suggests storage accumulation as growth slows. Results from a long-term precipitation throughfall reduction experiment on mature trees showed no difference in NSCs between surviving droughted and control trees (Rowland et al., 2015), suggesting that at longer time periods during which mortality (thinning) occurs, NSCs may also be homeostatically regulated, in part by stand-scale processes (e.g., McDowell, Adams, Bailey, Hess, & Kolb, 2006). These studies, along with recent evidence from the temperate zone (Schönbeck et al., 2018), suggest that NSCs may increase, even if only slightly, in response to seasonal drought in tropical forests. However, across

sites, acclimation of leaf and plant traits such as height, leaf area, leaf mass per area, and stand density may allow homeostatic maintenance of NSC concentrations; that is, they maintain relatively stable values despite long-term environmental variation (see, e.g., homeostasis of NSCs, e.g., Rowland et al., 2015; leaf gas exchange, e.g., Ehleringer & Cerling, 1995, McDowell et al., 2006; or temperature, e.g., Michaletz et al., 2015, 2016, Blonder & Michaletz, 2018). The 2016 El Niño presented a unique opportunity to test the hypotheses that seasonal shifts will occur during drought within sites and that adaptation may allow homeostatic maintenance of NSCs across a long-term rainfall gradient. No prior study of NSCs in tropical forests has considered multiple sites and multiple dates throughout a seasonal drought, allowing investigation into short-term drought response (within sites) simultaneous with long-term acclimation response (across the precipitation gradient). We collected canopy tree NSCs across the Isthmus of Panama for the duration of the 2016 dry period, within the 2015–2016 El Niño period. We expected mild to moderate dry-season increases in NSCs (Körner, 2003; Latt et al., 2001; Würth et al., 1998; Würth et al., 2005) with homeostatic maintenance of NSCs across sites despite widely differing climatic regimes (Table 1). We hypothesized that NSCs would instead vary with intrinsic physiological traits (e.g., photosynthesis, leaf, and hydraulic traits) that can be mechanistically related to NSC dynamics.

2 MATERIALS AND METHODS

2.1 Site descriptions

We used three lowland tropical forest sites located across a precipitation gradient on the Isthmus of Panama for this study. Two of the sites have canopy-access cranes, maintained by the Smithsonian Tropical Research Institute, enabling sampling and measurement at the top of the forest canopy. The two canopy-access sites include a seasonally dry forest in the Parque Natural Metropolitano (PNM) near Panama City and a wet evergreen forest in the San Lorenzo Protected Area (SLZ), Colon Province. The third, and intermediate, site is located on Barro Colorado Island (BCI) in the Panama Canal. Historic (1998–2015) mean annual air temperature (\pm standard deviation) is 26.0°C (\pm 0.6), 25.9°C (\pm 0.7), and 25.3°C (\pm 0.6), and mean annual precipitation is 1,844, 2,352, and 3,282 mm for PNM, BCI, and SLZ, respectively, with ~85% of rainfall in the May–November wet season (Figure 1, Table 1; data provided by the Physical Monitoring Program of the Smithsonian Tropical Research Institute.). For more site information, refer to Basset, Horlyck, and Wright (2003).

TABLE 1 Site characteristics (1998–2015 mean)

	PNM	BCI	SLZ
Location	8°58'N, 79°34'W	9°10'N, 79°51'W	9°17'N, 79°58'W
Elevation (m)	50	70	70
Annual Precip. (mm)	1,844	2,352	3,282
Dry Season Precip. (mm)	210	308	655
Dry Season Solar Rad. ($\text{MJ}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$)	17	19	16
Wet Season Solar Rad. ($\text{MJ}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$)	13	14	13
Tmin ($^{\circ}\text{C}$)	23	24	24
Tmax ($^{\circ}\text{C}$)	31	30	28

Note. All three sites are characterized by a pronounced dry season (approximately from mid-December to the end of April) and a wet season (May to mid-December). Data provided by http://biogeodb.stri.si.edu/physical_monitoring/research/. PNM: Parque Natural Metropolitano; BCI: Barro Colorado Island; SLZ: San Lorenzo Protected Area.

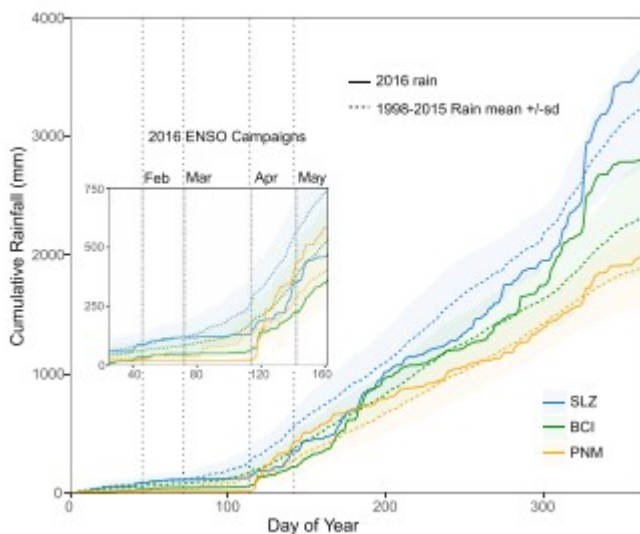


FIGURE 1 Cumulative annual rainfall by site. Monthly field campaigns (vertical grey dashed lines) were conducted at three sites across the Isthmus of Panama throughout the 2016 dry season (mid- Feb to mid-May; see inset). The Parque Natural Metropolitano crane site on the Pacific coast (PNM, yellow—dry) and the San Lorenzo crane site on the Caribbean Sea (SLZ, blue—wet) were sampled each month. The Barro Colorado Island site in the Panama Canal (BCI, green— intermediate) was only sampled in March. Each campaign included diurnal measurement of leaf traits. Cumulative annual rainfall (2016— solid lines; 1998–2015 mean—broken lines) was calculated from data provided by http://biogeodb.stri.si.edu/physical_monitoring/. Shaded regions indicate one standard deviation of the 1998–2015 mean

Twenty-three locally abundant canopy tree species (PNM $n = 9$; BCI $n = 5$; SLZ $n = 9$) were selected for intensive measurement of leaf NSCs and other traits (see Tables S1, S2, and S3 for more information). Four monthly campaigns were conducted over the course of the 2016 dry season from mid-February to mid-May (Figure 1). During each campaign, 2 days were spent at each location (except BCI; see below) conducting diurnal measurements of leaf gas exchange and traits (see Table S1) on fully expanded, upper canopy sunlit foliage of one target tree of each species. Target species were selected to cover a wide range of wood densities. Individuals with low or no liana infestation were chosen on the basis of crane or tower access (see below). The same individuals (“target trees”) were sampled during each campaign (see Table S3 for target tree attributes). At PNM and SLZ, leaves were measured and sampled via canopy-access cranes. At BCI, leaves were sampled from two telecommunication towers or by slingshot, precluding measurement of in situ leaf gas exchange and branch sampling for NSC analysis and A-C_i curves. BCI was only sampled in March due to the logistical difficulty of accessing the upper canopy. Branches were sampled at PNM in March and at SLZ in March and April (see Table S4 for NSC sampling schematic) as described below.

2.2 Leaf gas exchange and ecophysiological traits Leaf gas exchange and temperature were measured with 5–6 portable gas exchange systems (LI-6400XT, LI-COR Inc., Lincoln, NE, USA) equipped with 2 × 3 cm² leaf chambers and red-blue light sources and zeroed with a common nitrogen standard prior to each campaign. Diurnal leaf gas exchange measurements (dataset available online; Rogers et al., 2017a) were made on two to three leaves of each target tree using the canopy-access cranes at PNM and SLZ as described previously (Rogers et al., 2004). Measurements were conducted approximately every 2 to 3 hr from 6:00 to 19:00 local time for a total of five to seven measurements per day. Chamber conditions mimicked outdoor conditions of humidity, temperature, and photosynthetically active radiation. Leaf temperature was measured by a thermocouple in the chamber during gas exchange measurement. Following in situ gas exchange measurements, leaves were immediately harvested for trait measurements. Leaves were sealed in humidified plastic bags and stored in the dark, on ice for a maximum of 2 hr before further processing. Leaf water potential (Ψ_i , MPa; dataset available online; Wolfe et al., 2017) was measured using a pressure chamber (PMS, Albany, OR, USA). Following measurement, a known leaf area was sampled with cork borers, weighed with a precision balance (Fisher Science Education, Model SLF303, Hanover Park, IL), then dried to constant mass at 70°C for determination of dry mass and calculation of leaf mass per area (LMA, g m⁻²; dataset available online; Ely et al., 2018). Additional leaf punches were collected for NSC analysis at early (first after predawn, ~6:00–

9:00), midday (between ~11:30–14:30), and late (last before sundown, ~16:00–19:00) diurnal time points and treated as described below (see Section 2.3). Leaf samples were also collected before dawn to measure predawn leaf water potential (Ψ_{PD} , MPa).

First-order branches at PNM and SLZ were collected at predawn and kept in the shade for measurement of A-C_i curves (dataset available online; Rogers et al., 2017b). Immediately after harvesting, branches were recut under water, >1 m from the initial cut, to remove embolized xylem conduits. The cut segment was subsampled for NSC analysis. A-C_i curves were measured using the same portable gas exchange systems used for diurnal gas exchange measurement as described previously (Rogers, Serbin, Ely, Sloan, & Wullschleger, 2017).

The same target tree species were measured for various hydraulic traits during the 2016 dry season but independent of the diurnal measurement campaigns. Maximum stem area-specific hydraulic conductivity (Ks_{max} , Kg s⁻¹ MPa⁻¹ m⁻¹) and the water potential at 50% loss of stem hydraulic conductivity (P_{50} , MPa) were derived from measurements of terminal branches collected from canopy trees of each species following the bench-top dehydration method of Wolfe, Sperry, and Kursar (2016). P_{50} was calculated by plotting native stem-area specific hydraulic conductivity as a function of stem water potential and fitting a Weibull curve through the 90% percentile of with quantile regression. Ks_{max} was calculated as the intercept of the equation described for P_{50} . Leaf turgor loss point (Ψ_{TLP} , MPa) was calculated from two to six pressure volume curves per species following Koide, Robichaux, Morse, and Smith (1989), except that leaf water potential was measured on leaf discs with a leaf cutter psychrometer (J.R.D. Merrill Specialty Equipment, Logan, Utah, USA). Maximum and minimum branch water potentials (Ψ_{bmin} , Ψ_{bmax} , MPa) were measured with a pressure chamber at predawn and midday, respectively, on leaves that were bagged since predawn (at least 1 hr before measurement) and covered with reflective foam insulation to prevent overheating.

Additional measurements included ratio of leaf area to xylem area ($A_l:A_x$, m² m⁻²), and densities of bark (WD_b , g cm⁻³), xylem (WD_x , g cm⁻³), and whole stem (WD_{ws} , g cm⁻³ including pith, xylem, and bark). We measured $A_l:A_x$ on three to five branches of each species that were ~2 m long and 19.4 ± 4.9 mm diameter at their base. Xylem area was measured with callipers (excluding bark and pith), and leaf area was measured with an area metre (LI-3100C, LI-COR, Lincoln, NE, USA). WD was measured on samples of the same terminal branches. After removing pith and bark, the fresh volume of wood samples was measured with water displacement on a digital balance and dry mass was measured after drying samples for >72 hr at 65°C.

2.3 NSC analysis

NSCs are defined here as free, low molecular weight sugars (glucose, fructose, and sucrose) plus starch. Within 2 hr of collection, samples were microwaved at 600 watts for 90 s to stop enzymatic activity before drying at 60°C for 48 hr. Leaf tissues were ball milled to a fine powder (High Throughput Homogenizer, VWR International, Radnor, PA, USA). Woody tissues were ground with a Wiley Mini Mill (Thomas Scientific, Inc., Swedesboro, NJ, USA) prior to ball milling. Samples were analysed following the protocol described by Hoch, Popp, and Körner (2002) modified for use with ethanol extraction (Landhäusser et al., 2018) and are not subject to interlab comparison errors (Quentin et al., 2015).

Fine ground plant material was extracted three times with 80% ethanol for 10 min in a 90°C water bath (Isotemp 105, Fisher Scientific International, Inc., Hampton, NH, USA). Ethanol was evaporated from the supernatant in a 50°C oven overnight then reconstituted with DI water in a 90°C water bath and centrifuged (Allegra X-15R, Beckman Coulter, Inc., Brea, CA, USA) for sugar quantification via enzymatic assay. The ethanol-insoluble pellet was dried at 50°C overnight to remove residual ethanol and subsequently used for starch digestion and quantification.

Soluble sugars (glucose, fructose, and sucrose) and starch were quantified by enzymatic assay (dataset available online; Dickman et al., 2018). For soluble sugar determination, sucrose in the reconstituted extract was first hydrolysed to glucose and fructose by incubation with invertase (Grade VII, from Baker's yeast, Sigma-Aldrich Co., St. Louis, MO, USA) for 40 min on a microplate shaker. The invertase-treated sample was then incubated on a microplate shaker (BioShaker M.BR-022UP, TAITEC) for 45 min with phosphoglucose isomerase (from Baker's yeast—Type III, Sigma-Aldrich Co., St. Louis, MO, USA), glucose hexokinase, and glucose-6-P dehydrogenase (Glucose Assay Reagent, Sigma-Aldrich Co., St. Louis, MO, USA), to convert fructose to glucose and glucose to gluconate-6-phosphate. The concentration of free glucose in a sample was determined photometrically in a 96-well microplate spectrophotometer (ELx800UV, BioTek Instruments, Inc., Winooski, VT, USA), relative to glucose standards of known concentration, by the increase in optical density at 340 nm resulting from the reduction of NAD⁺ to NADH as glucose-6-P is oxidized.

Starch was converted into soluble oligosaccharides and then to glucose using a two-step enzymatic digestion to avoid nonspecific hydrolysis of nonstarch polysaccharides (Denison, Fedders, & Tong, 1990). In the first step, starch in the ethanol-insoluble pellet was hydrolyzed to water soluble glucans using α -amylase from *Bacillus licheniformis*, (Sigma-Aldrich cat. no. A4551) at 85°C

for 2 hr. After centrifugation, the glucans contained in the supernatant were converted to glucose using amyloglucosidase from *Aspergillus niger* (Sigma-Aldrich cat. no. 10115-5G-F) at 55°C for 2 hr. Following incubation, an aliquot of supernatant was used for photometric quantification of glucose hydrolysate as described above.

2.4 Statistical analyses

For all tests detailed below, NSC data were log or square root transformed to meet assumptions of normality. NSC values of zero were excluded because we were interested in evaluating changes in NSC when present, as opposed to presence versus absence (note that statistical analyses were also performed with zeros included as values of 0.001, and no conclusions changed). We first tested diurnal change in leaf NSCs using data from March, which included all times of day (early, mid, and late) at all three sites. Data were analysed using linear mixed effects models with site and time of day as fixed effects, sample ID nested within species as a random effect, and a corAR1 temporal autocorrelation structure for time of day. The likelihood ratio test was used to compare models with and without the random effect and temporal autocorrelation, and selection of the most parsimonious model was confirmed using AICc model selection. As recommended by Zuur, Ieno, Walker, Saveliev, and Smith (2009), restricted maximum likelihood (REML) was used to compare random structures, maximum likelihood was used to compare fixed structures (ML), and REML was used to evaluate the final model.

Leaf NSCs were found to increase over the course of the day (Figure S1; Tables S5 and S6; consistent with Würth et al., 1998), so the late time point (late afternoon)—as an integration of daily leaf NSC assimilation—was used for all subsequent analyses (major conclusions did not change when the early timepoint or daily average were used instead). We next tested changes in leaf and branch NSCs across months with site and month as fixed effects, sample ID nested within species as a random effect, and a corAR1 temporal autocorrelation structure for month using the same model selection approach. When significant according to the likelihood ratio test, month was included as a random effect in subsequent analyses (i.e., for tests of species differences and trait relationships). Testing site and month effects on branch NSCs individually (i.e., site differences with March only, and month differences with SLZ only) had no significant impact on results (Tables S7, S8, and S9). We next tested differences in leaf and branch NSCs between species with species as a fixed effect and site as a random effect, or month and sample ID nested within site as random effects with a corAR1 temporal autocorrelation structure for month when month was found to be significant in the previous analysis. We finally tested the relationships between leaf and

branch NSCs and various leaf and hydraulic traits with traits as fixed effects and species as a random effect, or month and species as a random effects with a corAR1 temporal autocorrelation structure for month when month was previously found to be significant. For post hoc analysis of significant fixed effects, we used a general linear hypothesis test with Tukey's honest significant difference. We used R (R Core Team, 2017) with nlme (Pinheiro et al., 2017) and multcomp (Hothorn, Bretz, & Westfall, 2008) to perform all analyses.

3 RESULTS

Leaf NSCs increased over the course of the day (Figure S1) driven by both starch and soluble sugar accumulation (Tables S5 and S6). Due to this diurnal trend, we used the late afternoon time point for all further analyses as it was the most representative of carbohydrate accumulation over the day, and it was the diurnal time point for which we had the most complete dataset. Both leaf and branch total NSCs were invariant over the course of the drought (Figures 2a and S2a, Tables S7 and S10), suggesting that NSC sources (photosynthates) and sinks (growth and metabolism) were balanced throughout the dry period. Leaf and branch total NSCs were also invariant across sites (Figures 3 and S3a, Tables S7 and S11) despite the substantial gradient in mean annual precipitation (Table 1). Despite these observed constancies, there were some changes associated with drought. Most notably, leaf NSC composition shifted from soluble sugars to starch throughout the dry period (Figure 2b-d, Tables S7 and S10), and branch soluble sugars were higher at the driest site (Figure S3d, Tables S7 and S11).

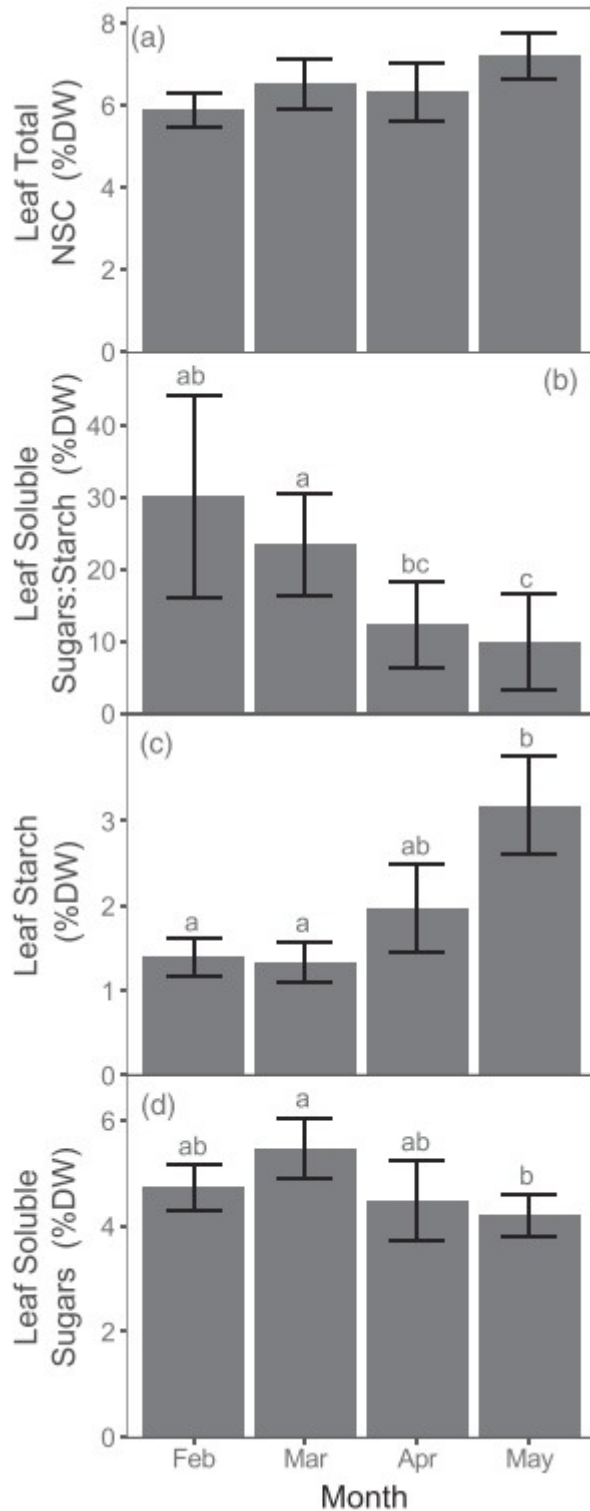


FIGURE 2 Leaf total nonstructural carbohydrates (NSCs) (a) do not change with drought duration, but ratio of leaf soluble sugars to starch (b) declines due to increased starch (c) and decreased soluble sugars (d). Data are from the late time point across all sites (PNM $n = 18$; BCI $n = 15$; SLZ $n = 18$).

Error bars are standard errors. Letters indicate significant differences in log transformed NSC for soluble sugars:starch and starch and square-root transformed NSC for soluble sugars.

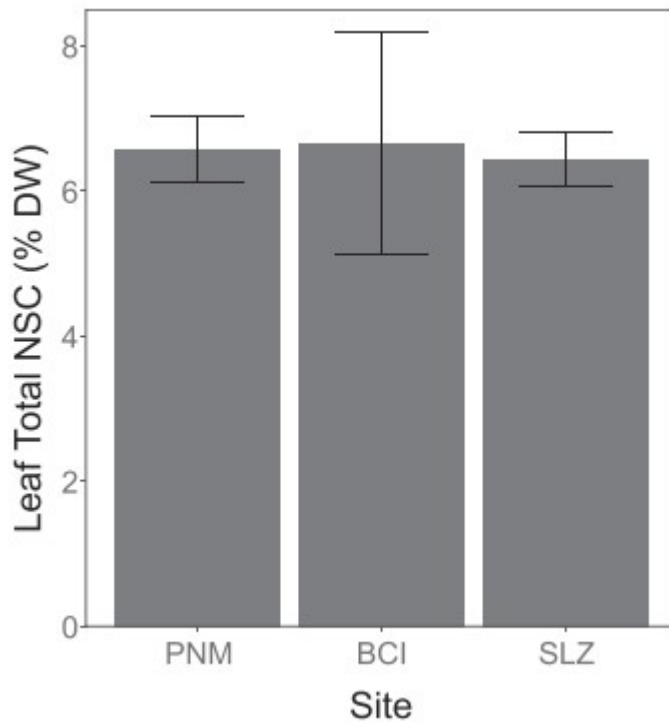


FIGURE 3 No difference in leaf total nonstructural carbohydrates (NSCs) across the precipitation gradient. Data are from the late time point. Error bars are standard errors.

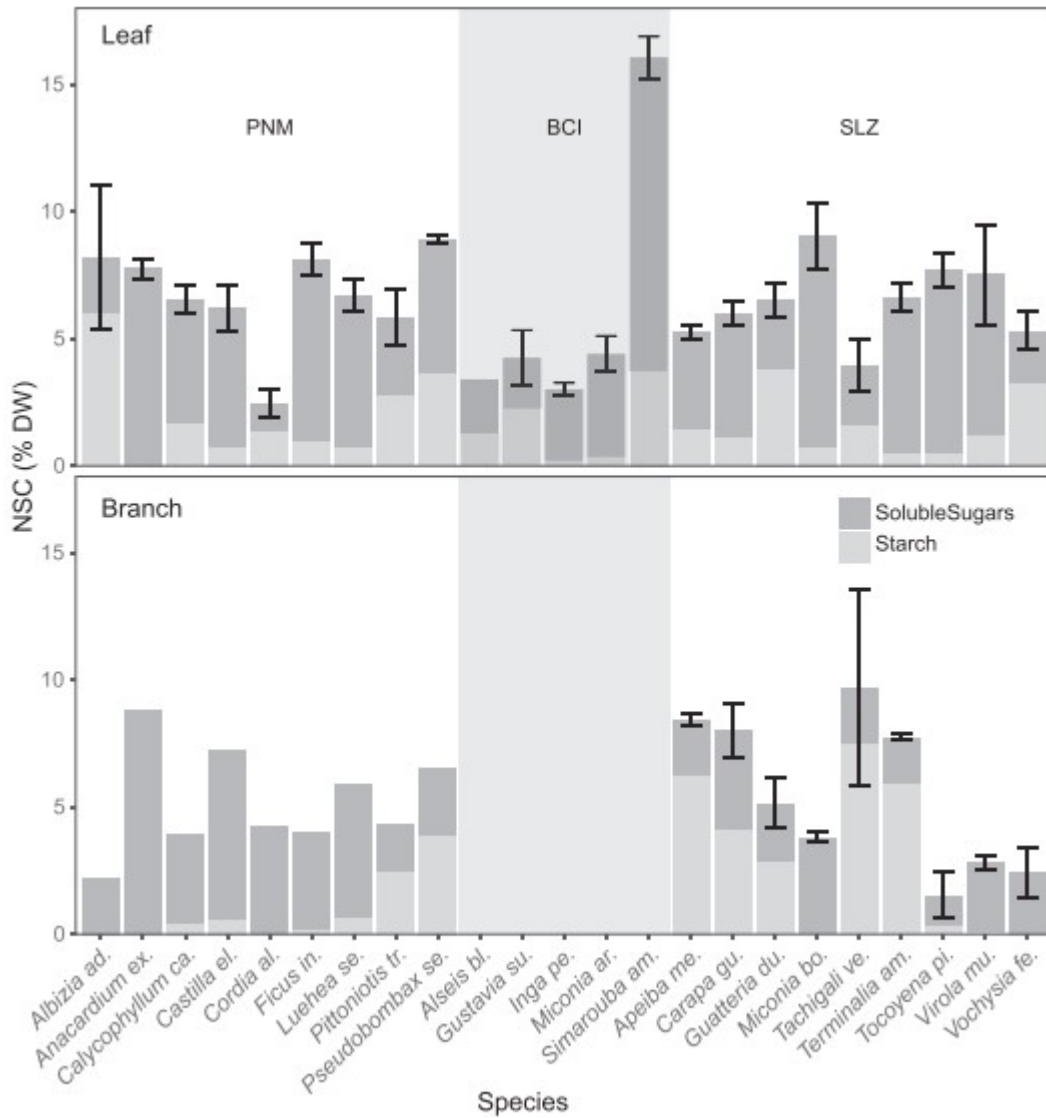


FIGURE 4 Large variation in nonstructural carbohydrates (NSCs) across species. Leaf data are from the late time point. Branch samples were collected at PNM in March and at SLZ in March and April. Branch samples were not collected at BCI due to lack of canopy access. Species are arranged by site from left to right: PNM, BCI, SLZ. Error bars are standard errors of total NSC. No error bars are shown for branches from PNM since there is only one sampling point

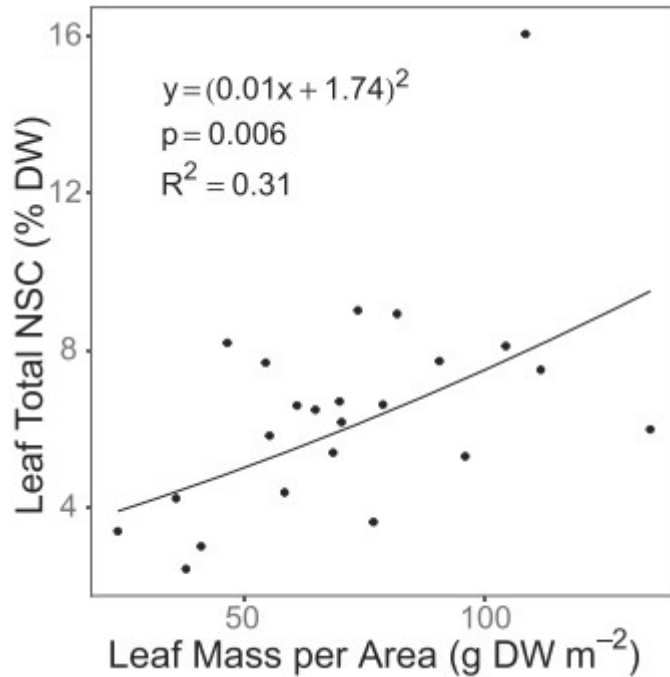


FIGURE 5 Leaf mass per area explains some variation in leaf total nonstructural carbohydrates (NSCs). NSC data are from the late time point. Each point represents one species.

The high variability in NSCs observed across species (Figure 4, Table S12) could be partially predicted by structural and leaf traits (Figures 5, 6, and S1, Tables S1 and S13). Leaf total NSCs were positively related to LMA (Figure 5, Table S13), whereas branch soluble sugars were negatively related to leaf level photosynthesis (using the mean daily photosynthetic rate) and xylem wood density (Figure 6a,b, Table S13) and positively related to leaf temperature (Figure 6c, Table S13). Branch starch increased exponentially with turgor loss point (Figure S4, Table S13). The other traits tested, including parameters such as stomatal conductance (g_s), leaf water potential (Ψ_{leaf}), maximum carboxylation rate (V_{cmax}), and stem water potential at 50% loss of hydraulic conductivity (P_{50}) (Table S1), showed no significant relationships with NSCs. We also included drought sensitivity metrics (Table S2), such as the difference between predawn and midday leaf water potential ($\Delta\Psi$) and the slope of the relationship between predawn and midday leaf water potential (slope Ψ_{PD} : Ψ_{MD} ; Figures S5 and S6, Tables S2 and S13). Despite wide species variation in these parameters, we found poor fits to the NSC observations.

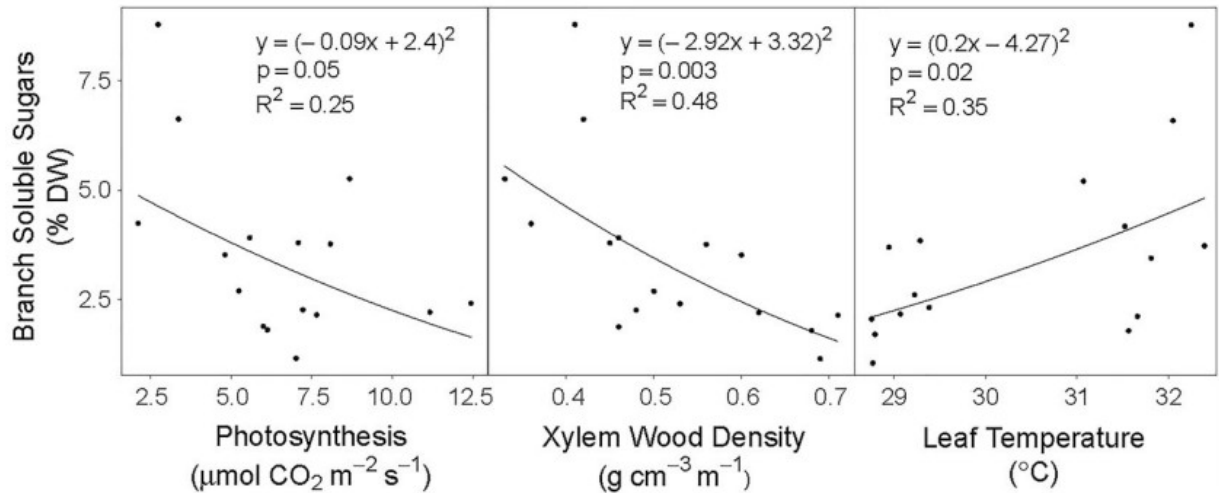


FIGURE 6 Branch soluble sugars are negatively related to photosynthesis and xylem wood density and positively related to leaf temperature. Each point represents one species.

4 DISCUSSION

We tested the hypothesis that NSCs would increase during the drought progression but be maintained at relatively constant, or homeostatic, levels under long-term (across site) variation in precipitation. These hypotheses were tested across communities located along a large precipitation gradient during the drought imposed by the 2015–2016 El Niño. Despite large variation in NSCs across species (Figure 4, Table S12), we found total NSC content of foliage and branches was held relatively homeostatic both across the drought period and across the precipitation gradient (Figures 2a, 3, S2a, and S3a; Tables S7, S10, and S11). Traits explained some, but not all, of the observed variation in NSCs across species (Figures 5, 6, and S1, Table S13).

Leaf and branch total NSCs were maintained both over the course of the 2016 dry period when we made our measurements (Figures 2a and S2a; Tables S7 and S10) and across the precipitation gradient (Figures 3 and S3a; Tables S7 and S11). This homeostasis suggests that NSCs serve an important function and are preserved as a priority carbon sink (Chapin et al., 1990; Dietze et al., 2014; Martínez-Vilalta et al., 2016). This result is consistent with previous studies in which NSC concentrations have been found to be relatively resistant to change under all but the most extreme conditions. Variation in temperature and moisture have been shown to have modest impacts on NSC concentrations over seasonal (Martínez-Vilalta et al., 2016) and decadal timescales (Rowland et al., 2015; Schönbeck et al., 2018), with only the most severe conditions that result in plant death causing larger

NSC declines (Adams et al., 2017). The mechanisms driving such homeostatic balance of NSCs against large changes in short- and long-term precipitation are unknown, but include shifts in NSC consumption, for example, to growth, defence, and energy production, that match any shifts in photosynthesis during drought (McDowell, 2011). The homeostatic maintenance of leaf total NSCs throughout the seasonal drought in this study was associated with a shift from soluble sugars to starch (Figure 2b-d; Tables S7 and S10), suggesting either drought-constrained limitations on foliar metabolism and growth (Würth et al., 2005) resulting in increased storage as drought progressed (McDowell, 2011), or prioritization of foliar storage over other processes during drought (Hartmann, McDowell, & Trumbore, 2015). The latter process is consistent with the idea that these plants have experienced worse droughts (e.g., Condit et al., 1996) and may have adapted to maintain relatively high NSCs in case of an extremely prolonged or severe drought (Wright, 2005). Such adaptation could come in the form of shifts in uptake or allocation of carbon that induce such homeostatic patterns. In contrast to leaves, branch soluble sugars were higher and starch trended lower at the dry than the wet site (Figure S3), which may be related to a greater need for soluble sugars for embolism repair under drier conditions (Secchi, Gilbert, & Zwieniecki, 2011). The longer term response (across site) in branches and shorter term response (with ENSO drought progression) in leaves is consistent with the transitory nature of carbohydrate pools in leaves relative to branches.

Our observed relationships between NSCs and traits may serve to simplify both modelling of carbon storage and collection of benchmark data and emphasizes the importance of trait-based modelling (van Bodegom, Douma, & Verheijen, 2014) to capture species-level differences in NSCs. Our observation of a positive relationship between leaf mass per area and foliar NSCs (Figure 5, Table S13) is consistent with an increase in leaf dry mass as sugars accumulate (Poorter, Niinemets, Poorter, Wright, & Villar, 2009). Interestingly, branch soluble sugars were correlated with several metrics, including average photosynthetic rate on the day of NSC sampling, xylem wood density, and leaf temperature (Figure 6, Table S13). The negative relationship between branch soluble sugars and photosynthesis (Figure 6a, Table S13) may be the result of feedback inhibition, whereby reduced sink strength and phloem transport resulting from higher branch soluble sugars promotes a reduction in photosynthetic rate (McCormick, Watt, & Cramer, 2009; Paul & Foyer, 2001; Thompson & Holbrook, 2003). The decrease in branch soluble sugars with increasing wood density (Figure 6b, Table S13) has several possible explanations. Recent research has shown that increased sugar concentrations in woody tissues reduce xylem vulnerability to cavitation (De Baerdemaeker, Salomón, De Roo, & Steppe, 2017), and

species with higher wood densities and therefore lower vulnerability (Hacke, Sperry, Pockman, Davis, & McCulloh, 2001; Jacobsen, Ewers, Pratt, Paddock, & Davis, 2005) would require less soluble sugar for repair. There is also evidence that xylem parenchyma, the sugar transport and storage fraction in woody tissue that links the heartwood and phloem, is related to embolism recovery (Secchi, Pagliarani, & Zwieniecki, 2017). Alternatively, wood density is negatively correlated with phloem proportion in some species (Santini, Schmitz, & Lovelock, 2012), so there may simply be less soluble sugar transport tissue associated with denser wood. There may also be a basic physical limitation whereby higher structural density reduces the space available for sugar storage. Branch soluble sugars were also positively related to leaf temperature (Figure 6c, Table S13). This finding is contrary to research showing inhibition of assimilate export with increased leaf temperature (Jiao & Grodzinski, 1996). However, Jiao and Grodzinski (1996) also show declines in photosynthesis with leaf temperature, which is consistent with the observed negative relationship between branch soluble sugars and photosynthesis (Figure 6a, Table S13) discussed above. In our case, there was no observed correlation between photosynthesis and leaf temperature across species ($r^2 = 0.14$). However, leaf temperature was consistently higher at the driest site compared with the wettest site (Figure S7), which may explain the site differences in branch soluble sugars (PNM > SLZ; Figure S3d).

Despite these few correlations, we did not find expected relationships between NSCs and drought sensitivity or hydraulic metrics (Figures S5 and S6; Tables S1 and S2), including leaf water potential (Figure S8), hydraulic conductivity, and P_{50} . Though increased leaf soluble sugars are often associated with more negative water potentials (e.g., Dickman, McDowell, Sevanto, Pangle, & Pockman, 2014), these trees may not have experienced dry enough conditions to necessitate osmotic regulation. We also explored relationships with relative degree of isohydry (Figure S5, Martínez-Vilalta, Poyatos, Aguadé, Retana, & Mencuccini, 2014), yet there was no significant correlation to NSCs (Figure S6a) despite target species ranging from extreme isohydry to extreme anisohydry (Figure S6b, Table S2). Similarly, we found no correlation between NSCs and $\Delta\Psi$, or difference between predawn and midday leaf water potential (Table S2). This absence of relationships across many functional traits, particularly hydraulic traits, further emphasizes the homeostatic nature of NSCs in this system; that is, NSCs are relatively invariant across a wide spectrum of hydraulic traits across the Isthmus of Panama, at least for the canopy tree species explored here. To the extent that the natural rainfall gradient is a proxy for adaptation to long-term precipitation changes (specifically decreasing mean annual precipitation), this suggests that NSCs will also be held homeostatic under future

potentially drier conditions, though manipulative studies (e.g., Rowland et al., 2015) are best utilized to test this hypothesis. We note that many hydraulic metrics exist that we did not test (e.g., vulnerability to embolism; Choat et al., 2012) that may provide more insight into regional patterns of NSC regulation.

Our results suggest that, despite broad species diversity, NSCs in tropical canopy trees are maintained homeostatically at the community level through a seasonal and ENSO-influenced drought, across a long-term climatic gradient and across a wide variety of functional traits. We cannot exclude the possibility that more exceptional droughts, particularly with increased dry down of soil moisture, could cause depletions of NSC in these tropical forests. However, these observations indicate that maintenance of NSCs is prioritized and may simplify our ability to represent NSC dynamics in next-generation Earth Systems Models, which currently use carbon storage as a proxy to simulate tree mortality (e.g., Fisher et al., 2010; McDowell et al., 2013).

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