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Homeostatic maintenance of non-structural carbohydrates during the 2015-2016 El Nino drought across a tropical forest precipitation gradient

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1 **Homeostatic maintenance of non-structural carbohydrates during the 2015-2016 El Niño**
2 **drought across a tropical forest precipitation gradient**

3

4 Running title: homeostasis of tropical forest carbohydrates

5

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32 **Abstract:** Non-structural carbohydrates (NSCs) are essential for maintenance of plant
33 metabolism, and may be sensitive to both short- and long-term climatic variation. NSC variation
34 in moist tropical forests has rarely been studied, so regulation of NSCs in these systems is poorly
35 understood. We measured foliar and branch NSC content in 23 tree species at three sites located
36 across a large precipitation gradient in Panama during the 2015-2016 El Niño to examine how
37 short- and long-term climatic variation impact carbohydrate dynamics. Across all sites, leaf
38 NSCs increased over diurnal time-periods. There was no significant difference in total NSCs as
39 the drought progressed (leaf $p=0.32$, branch $p=0.30$), nor across the rainfall gradient (leaf
40 $p=0.91$, branch $p=0.96$). Foliar soluble sugars decreased while starch increased over the duration
41 of the dry period, suggesting greater partitioning of NSCs to storage than metabolism or
42 transport as drought progressed. There was large variation across species at all sites, but total
43 foliar NSCs were positively correlated with leaf mass per area, while branch sugars were
44 positively related to leaf temperature and negatively correlated with daily photosynthesis and
45 wood density. The NSC homeostasis across a wide range of conditions suggests that NSCs are an
46 allocation priority in moist tropical forests.

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51 **Keyword index:** NSC; Panama; ENSO; tropics; climate; storage; sugars; vegetation

52 **Introduction**

53 Tropical forests account for a large fraction of terrestrial live biomass (Pan et al., 2013)
54 and approximately half of terrestrial gross primary production (GPP; Beer et al., 2010). Drought
55 is one of the largest threats to tropical forest structure and functioning (Davidson et al. 2012;
56 McDowell et al. 2018), and can result in reduced carbon sequestration due to higher ecosystem
57 respiration and lower GPP (Cavaleri et al. 2017), transitioning these ecosystems from carbon
58 sinks to sources (Tian et al. 1998). The El Niño phase of the El Niño Southern Oscillation
59 (ENSO) impacts some tropical forests through hotter, drier dry seasons and wet seasons with less
60 solar insolation (Cavaleri et al. 2017). El Niños occur sub-decadally (Allan et al. 1996), and
61 extreme events are expected to increase in frequency with climate change (Cai et al. 2014).
62 Droughts with and without El Niños have been associated with increased mortality of canopy
63 trees (Condit et al. 1996, Laurance & Williamson 2001), as well as shifts in allocation from
64 leafing to fruiting (Laurance & Williamson 2001, Detto et al. 2018), and altered remote sensing
65 signatures of the canopy surface (e.g. greenness, backscatter; Li et al. 2018, Nagai, Ichii, &
66 Morimoto. 2007; Saatchi et al. 2013).

67 Non-structural carbohydrates (NSCs) provide the carbon skeletons for biosynthetic
68 pathways associated with secondary metabolism (e.g. growth and defense), and for energy
69 production (i.e. respiration through glycolysis and the tricarboxylic acid cycle; Chapin et al.
70 1990, Heldt 2005). Constraints on NSC storage and utilization have prompted widespread
71 research on their role in autotrophic carbon cycling (e.g. Dietze et al. 2014) and in the avoidance
72 of carbon starvation (e.g. Adams et al. 2017). NSCs are stored, typically as starch, when supply
73 of photosynthate exceeds demand (i.e. when carbon assimilation is greater than growth and
74 metabolism; McDowell 2011). These stored NSCs can then be utilized during periods when

75 supply is unable to match demand (e.g. during periods when CO₂ assimilation may be reduced;
76 Hoch et al. 2003). Furthermore, deep reserves of NSCs older than one decade may even be
77 utilized in growth and respiration, thus providing some buffer to seasonal variation (Dietze et al.
78 2014, Martinez-Vilalta et al. 2016).

79 In a global review of NSC dynamics across organs, Martinez-Vilalta et al. (2016) found
80 that seasonal minimums remained relatively high and constant among functional types and
81 biomes, supporting the idea that NSCs are maintained above some (undetermined) minimum
82 threshold. They also found that, while depletion of starch was relatively common, depletion of
83 soluble sugars or total NSCs was very rare, consistent with the role of starch as a storage
84 reservoir and soluble sugars as substrate for immediate metabolic use. Relative to other biomes,
85 tropical systems showed low seasonal variability in NSCs (Martinez-Vilalta et al. 2016).

86 Data from the tropics, however, remains relatively limited. Less than 15% of studies in
87 the global review by Martinez-Vilalta et al. (2016) were from the tropics, and several of these
88 were performed on seedlings or understory shrubs. Several studies have shown a positive
89 relationship between NSCs and tropical seedling survival during periods of stress (Newell et al.
90 2002, Meyers & Kitajima 2007, Poorter & Kitajima 2007, Poorter et al. 2010, O'Brien et al.
91 2014), as well as higher seedling NSC concentrations in species from wetter tropical forests
92 (Poorter & Kitajima 2007). An accumulation of NSCs prior to or during seasonal drought is
93 commonly observed (Würth et al. 1998, Latt et al. 2001, Newell et al. 2002, Körner 2003, Würth
94 et al. 2005), particularly for deciduous trees (Newell et al. 2002), but this rise is small in
95 comparison to the extra-tropical biomes (Martinez-Vilalta et al. 2016). This is consistent with the
96 observed increase in NSCs associated with lower growth rates in stressed seedlings (Meyers &
97 Kitajima 2007, Poorter & Kitajima 2007, Poorter et al. 2010) and suggests storage accumulation

98 as growth slows. Results from a long-term precipitation throughfall reduction experiment on
99 mature trees showed no difference in NSCs between surviving droughted and control trees
100 (Rowland et al. 2015), suggesting that at longer time periods during which mortality (thinning)
101 occurs, NSCs may also be homeostatically regulated, in part by stand-scale processes (e.g.
102 McDowell et al. 2006).

103 These studies, along with recent evidence from the temperate zone (Schoenbeck et al.
104 2018), suggest that NSCs may increase, even if only slightly, in response to seasonal drought in
105 tropical forests. However, across sites, acclimation of leaf and plant traits such as height, leaf
106 area, leaf mass per area, and stand density, may allow homeostatic maintenance of NSC
107 concentrations; that is, they maintain relatively stable values despite long-term environmental
108 variation (see for example homeostasis of NSCs e.g. Rowland et al. 2015; leaf gas exchange e.g.
109 Ehleringer and Cerling 1995, McDowell et al. 2006; or temperature e.g. Michaletz et al. 2015,
110 2016, Blonder and Michaletz 2018). The 2016 El Niño presented a unique opportunity to test the
111 hypotheses that seasonal shifts will occur during drought within sites, and that adaptation may
112 allow homeostatic maintenance of NSCs across a long-term rainfall gradient. No prior study of
113 NSCs in tropical forests has considered multiple sites and multiple dates throughout a seasonal
114 drought, allowing investigation into short-term drought response (within sites) simultaneous with
115 long-term acclimation response (across the precipitation gradient). We collected canopy tree
116 NSCs across the Isthmus of Panama for the duration of the 2016 dry period, within the 2015-2016
117 El Niño period. We expected mild to moderate dry-season increases in NSCs (Würth et al. 1998,
118 Latt et al. 2001, Körner 2003, Würth et al. 2005) with homeostatic maintenance of NSCs across
119 sites despite widely differing climatic regimes (Table 1). We hypothesized that NSCs would

120 instead vary with intrinsic physiological traits (e.g. photosynthesis, leaf and hydraulic traits) that
121 can be mechanistically related to NSC dynamics.

122

123 **Materials and Methods**

124 *Site descriptions*

125 We used three lowland tropical forest sites located across a precipitation gradient on the
126 Isthmus of Panama for this study. Two of the sites have canopy-access cranes, maintained by the
127 Smithsonian Tropical Research Institute, enabling sampling and measurement at the top of the
128 forest canopy. The two canopy-access sites include a seasonally dry forest in the Parque Natural
129 Metropolitano (PNM) near Panama City and a wet evergreen forest in the San Lorenzo Protected
130 Area (SLZ), Colon Province. The third, and intermediate, site is located on Barro Colorado
131 Island (BCI) in the Panama Canal. Historic (1998-2015) mean annual air temperature (\pm standard
132 deviation) is $26.0 (\pm 0.6) ^\circ\text{C}$, $25.9 (\pm 0.7) ^\circ\text{C}$, and $25.3 (\pm 0.6) ^\circ\text{C}$, and mean annual precipitation
133 is 1844 mm, 2352 mm, and 3282 mm for PNM, BCI, and SLZ, respectively, with $\sim 85\%$ of
134 rainfall in the May-November wet season (Fig. 1, Table 1; data provided by the Physical
135 Monitoring Program of the Smithsonian Tropical Research Institute.). For more site information,
136 refer to Basset et al. (2003).

137 Twenty-three locally abundant canopy tree species (PNM $n=9$; BCI $n=5$; SLZ $n=9$) were
138 selected for intensive measurement of leaf NSCs and other traits (see Tables S1,S2, and S3 for
139 more information). Four monthly campaigns were conducted over the course of the 2016 dry
140 season from mid-February to mid-May (Fig. 1). During each campaign, two days were spent at
141 each location (except BCI; see below) conducting diurnal measurements of leaf gas exchange
142 and traits (see Table S1) on fully-expanded, upper canopy sunlit foliage of one target tree of each

143 species. Target species were selected to cover a wide range of wood densities. Individuals with
144 low or no liana infestation were chosen based on crane or tower access (see below). The same
145 individuals (“target trees”) were sampled during each campaign (see Table S3 for target tree
146 attributes). At PNM and SLZ leaves were measured and sampled via canopy-access cranes. At
147 BCI leaves were sampled from two telecommunication towers or by sling-shot, precluding
148 measurement of *in-situ* leaf gas exchange and branch sampling for NSC analysis and A-C_i
149 curves. BCI was only sampled in March due to the logistical difficulty of accessing the upper
150 canopy. Branches were sampled at PNM in March and at SLZ in March and April (see Table S4
151 for NSC sampling schematic) as described below.

152

153 *Leaf gas exchange and ecophysiological traits*

154 Leaf gas exchange and temperature were measured with 5-6 portable gas exchange
155 systems (LI-6400XT, LI-COR Inc., Lincoln, NE, USA) equipped with 2x3 cm² leaf chambers
156 and red-blue light sources, and zeroed with a common nitrogen standard prior to each campaign.
157 Diurnal leaf gas exchange measurements (dataset available online, Rogers et al. 2017a) were
158 made on two to three leaves of each target tree using the canopy-access cranes at PNM and SLZ
159 as described previously (Rogers et al. 2004). Measurements were conducted approximately every
160 two to three hours from 6:00 to 19:00 local time for a total of five to seven measurements per
161 day. Chamber conditions mimicked outdoor conditions of humidity, temperature, and
162 photosynthetically active radiation. Leaf temperature was measured by a thermocouple in the
163 chamber during gas exchange measurement. Following *in-situ* gas exchange measurements,
164 leaves were immediately harvested for trait measurements. Leaves were sealed in humidified
165 plastic bags and stored in the dark, on ice for a maximum of 2 hours before further processing.

166 Leaf water potential (Ψ_l , MPa; dataset available online, Wolfe et al. 2017) was measured using a
167 pressure chamber (PMS, Albany, OR, USA). Following measurement, a known leaf area was
168 sampled with cork borers, weighed with a precision balance (Fisher Science Education, Model
169 SLF303, Hanover Park, IL), then dried to constant mass at 70°C for determination of dry mass
170 and calculation of leaf mass per area (LMA, g m^{-2} ; dataset available online, Ely et al. 2017).

171 Additional leaf punches were collected for NSC analysis at early (first after pre-dawn, ~6:00-
172 9:00), mid-day (between ~11:30-14:30), and late (last before sun-down, ~16:00-19:00) diurnal
173 time points and treated as described below (see *Non-structural Carbohydrate Analysis*). Leaf
174 samples were also collected before dawn to measure pre-dawn leaf water potential (Ψ_{PD} , MPa).

175 First-order branches at PNM and SLZ were collected at pre-dawn and kept in the shade
176 for measurement of A-C_i curves (dataset available online, Rogers et al. 2017b). Immediately
177 after harvesting, branches were re-cut under water, >1m from the initial cut, to remove
178 embolized xylem conduits. The cut segment was subsampled for NSC analysis. A-C_i curves were
179 measured using the same portable gas exchange systems used for diurnal gas exchange
180 measurement as described previously (Rogers et al. 2017c).

181 The same target tree species were measured for various hydraulic traits during the 2016
182 dry season, but independent of the diurnal measurement campaigns. Maximum stem area-
183 specific hydraulic conductivity ($K_{S_{max}}$, $\text{Kg s}^{-1} \text{MPa}^{-1} \text{m}^{-1}$) and the water potential at 50% loss of
184 stem hydraulic conductivity (P_{50} , MPa) were derived from measurements of terminal branches
185 collected from canopy trees of each species following the bench-top dehydration method of
186 Wolfe et al. (2016). P_{50} was calculated by plotting native stem-area specific hydraulic
187 conductivity as a function of stem water potential and fitting a Weibull curve through the 90%
188 percentile of with quantile regression. $K_{S_{max}}$ was calculated as the intercept of the equation

189 described for P₅₀. Leaf turgor loss point (Ψ_{TLP} , MPa) was calculated from two to six pressure
190 volume curves per species following Koide et al. (1989), except that leaf water potential was
191 measured on leaf discs with a leaf cutter psychrometer (J.R.D. Merrill Specialty Equipment,
192 Logan, Utah, USA). Maximum and minimum branch water potentials (Ψ_{bmin} , Ψ_{bmax} , MPa) were
193 measured with a pressure chamber at pre-dawn and midday, respectively, on leaves that were
194 bagged since predawn (at least 1 h before measurement) and covered with reflective foam
195 insulation to prevent overheating.

196 Additional measurements included ratio of leaf area to xylem area ($A_{\text{L}}:A_{\text{X}}$, $\text{m}^2 \text{m}^{-2}$), and
197 densities of bark (WD_{b} , g cm^{-3}), xylem (WD_{x} , g cm^{-3}), and whole stem (WD_{ws} , g cm^{-3} including
198 pith, xylem, and bark). We measured $A_{\text{L}}:A_{\text{X}}$ on three to five branches of each species that were
199 ~2m long and 19.4 ± 4.9 mm diameter at their base. Xylem area was measured with calipers
200 (excluding bark and pith) and leaf area was measured with an area meter (LI-3100C, LI-COR,
201 Lincoln, NE, USA). WD was measured on samples of the same terminal branches. After
202 removing pith and bark, the fresh volume of wood samples was measured with water
203 displacement on a digital balance and dry mass was measured after drying samples for >72 hours
204 at 65 °C.

205

206 *Non-structural Carbohydrate Analysis*

207 Nonstructural carbohydrates are defined here as free, low molecular weight sugars
208 (glucose, fructose, and sucrose) plus starch. Within two hours of collection, samples were
209 microwaved at 600 watts for 90 s to stop enzymatic activity before drying at 60 °C for 48hrs.
210 Leaf tissues were ball-milled to a fine powder (High Throughput Homogenizer, VWR
211 International, Radnor, PA, USA). Woody tissues were ground with a Wiley Mini Mill (Thomas

212 Scientific, Inc., Swedesboro, NJ, USA) prior to ball-milling. Samples were analyzed following
213 the protocol described by Hoch et al. (2002) modified for use with ethanol extraction
214 (Landhüsser et al. 2018), and are not subject to inter-lab comparison errors (Quentin et al.
215 2015).

216 Fine ground plant material was extracted three times with 80% ethanol for 10 min. in a
217 90 °C water bath (Isotemp 105, Fisher Scientific International, Inc., Hampton, NH, USA).
218 Ethanol was evaporated from the supernatant in a 50 °C oven overnight then reconstituted with
219 DI water in a 90 °C water bath and centrifuged (Allegra X-15R, Beckman Coulter, Inc., Brea,
220 CA, USA) for sugar quantification via enzymatic assay. The ethanol-insoluble pellet was dried at
221 50 °C overnight to remove residual ethanol and subsequently used for starch digestion and
222 quantification.

223 Soluble sugars (glucose, fructose, sucrose) and starch were quantified by enzymatic assay
224 (dataset available online, Dickman et al. 2018). For soluble sugar determination, sucrose in the
225 reconstituted extract was first hydrolysed to glucose and fructose by incubation with invertase
226 (Grade VII, from Baker's yeast, Sigma-Aldrich Co., St. Louis, MO, USA) for 40mins on a
227 microplate shaker. The invertase-treated sample was then incubated on a microplate shaker
228 (BioShaker M.BR-022UP, TAITEC) for 45 min. with phosphoglucose isomerase (from Baker's
229 yeast – Type III, Sigma-Aldrich Co., St. Louis, MO, USA), glucose hexokinase and glucose-6-P
230 dehydrogenase (Glucose Assay Reagent, Sigma-Aldrich Co., St. Louis, MO, USA), to convert
231 fructose to glucose and glucose to gluconate-6-phosphate. The concentration of free glucose in a
232 sample was determined photometrically in a 96-well microplate spectrophotometer (ELx800UV,
233 BioTek Instruments, Inc., Winooski, VT, USA), relative to glucose standards of known

234 concentration, by the increase in optical density at 340nm resulting from the reduction of NAD⁺
235 to NADH as glucose-6-P is oxidized.

236 Starch was converted into soluble oligosaccharides and then to glucose using a two-step
237 enzymatic digestion to avoid non-specific hydrolysis of non-starch polysaccharides (Denison et
238 al. 1990). In the first step, starch in the ethanol-insoluble pellet was hydrolyzed to water soluble
239 glucans using α -amylase from *Bacillus licheniformis*, (Sigma-Aldrich cat. no. A4551) at 85°C
240 for two hours. After centrifugation, the glucans contained in the supernatant were converted to
241 glucose using amyloglucosidase from *Aspergillus niger* (Sigma-Aldrich cat. no. 10115-5G-F) at
242 55°C for two hours. Following incubation, an aliquot of supernatant was used for photometric
243 quantification of glucose hydrolysate as described above.

244

245 *Statistical analyses*

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

257 compare random structures, maximum likelihood was used to compare fixed structures (ML),
258 and REML was used to evaluate the final model.

259 Leaf NSCs were found to increase over the course of the day (Fig. S1; Tables S5, S6;
260 consistent with Würth et al. 1998), so the late time point (late-afternoon) – as an integration of
261 daily leaf NSC assimilation – was used for all subsequent analyses (major conclusions did not
262 change when the early timepoint or daily average were used instead). We next tested changes in
263 leaf and branch NSCs across months with site and month as fixed effects, sample ID nested
264 within species as a random effect, and a corAR1 temporal autocorrelation structure for month
265 using the same model selection approach. When significant according to the likelihood ratio test,
266 month was included as a random effect in subsequent analyses (i.e. for tests of species
267 differences and trait relationships). Testing site and month effects on branch NSCs individually
268 (i.e. site differences with March only, and month differences with SLZ only) had no significant
269 impact on results (Tables S7, S8, S9). We next tested differences in leaf and branch NSCs
270 between species with species as a fixed effect and site as a random effect, or month and sample
271 ID nested within site as random effects with a corAR1 temporal autocorrelation structure for
272 month when month was found to be significant in the previous analysis. We finally tested the
273 relationships between leaf and branch NSCs and various leaf and hydraulic traits with traits as
274 fixed effects and species as a random effect, or month and species as a random effects with a
275 corAR1 temporal autocorrelation structure for month when month was previously found to be
276 significant. For post-hoc analysis of significant fixed effects, we used a general linear hypothesis
277 test with Tukey’s honest significant difference. We used R (R Core Team, 2017) with *nlme*
278 (Pinheiro et al., 2017) and *multcomp* (Hothorn T, F. Bretz & P. Westfall, 2008) to perform all
279 analyses.

280

281 **Results**

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

294 The high variability in NSCs observed across species (Fig. 4, Table S12) could be
295 partially predicted by structural and leaf traits (Figs. 6, 7, S1, Tables S1, S13). Leaf total NSCs
296 were positively related to LMA (Fig. 5, Table S13), while branch soluble sugars were negatively
297 related to leaf level photosynthesis (using the mean daily photosynthetic rate) and xylem wood
298 density (Fig. 6a, b, Table S13), and positively related to leaf temperature (Fig. 6c, Table S13).
299 Branch starch increased exponentially with turgor loss point (Fig. S4, Table S13). The other
300 traits tested, including parameters such as stomatal conductance (g_s), leaf water potential (Ψ_{leaf}),
301 maximum carboxylation rate (V_{cmax}), and stem water potential at 50% loss of hydraulic
302 conductivity (P_{50}) (Table S1), showed no significant relationships with NSCs. We also included

303 drought sensitivity metrics (Table S2), such as the difference between pre-dawn and mid-day leaf
304 water potential ($\Delta\Psi$) and the slope of the relationship between pre-dawn and mid-day leaf water
305 potential (slope Ψ_{PD} : Ψ_{MD} ; Figs. S5, S6, Tables S2, S13). Despite wide species variation in these
306 parameters we found poor fits to the NSC observations.

307

308 **Discussion**

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

317 Leaf and branch total NSCs were maintained both over the course of the 2016 dry period
318 when we made our measurements (Fig. 2a, S2a; Tables S7, S10), and across the precipitation
319 gradient (Fig. 3, S3a; Tables S7, S11). This homeostasis suggests that NSCs serve an important
320 function and are preserved as a priority carbon sink (Chapin et al. 1990, Dietze et al. 2014,
321 Martinez-Vilalta et al. 2016). This result is consistent with previous studies in which NSC
322 concentrations have been found to be relatively resistant to change under all but the most
323 extreme conditions. Variation in temperature and moisture have been shown to have modest
324 impacts on NSC concentrations over seasonal (Martinez-Vilalta et al. 2016) and decadal time-

325 scales (Rowland et al. 2015; Schoenbeck et al. 2018), with only the most severe conditions that
326 result in plant death causing larger NSC declines (Adams et al. 2017). The mechanisms driving
327 such homeostatic balance of NSCs against large changes in short- and long-term precipitation are
328 unknown, but include shifts in NSC consumption, e.g. to growth, defense, and energy
329 production, that match any shifts in photosynthesis during drought (McDowell 2011). The
330 homeostatic maintenance of leaf total NSCs throughout the seasonal drought in this study was
331 associated with a shift from soluble sugars to starch (Fig. 2b-d; Tables S7, S10), suggesting
332 either drought-constrained limitations on foliar metabolism and growth (Würth et al. 2005)
333 resulting in increased storage as drought progressed (McDowell 2011), or prioritization of foliar
334 storage over other processes during drought (Hartmann et al. 2015). The latter process is
335 consistent with the idea that these plants have experienced worse droughts (e.g. Condit et al.
336 1996), and may have adapted to maintain relatively high NSCs in case of an extremely prolonged
337 or severe drought (Wright 2005). Such adaptation could come in the form of shifts in uptake or
338 allocation of carbon that induce such homeostatic patterns. In contrast to leaves, branch soluble
339 sugars were higher and starch trended lower at the dry than the wet site (Fig. S3) which may be
340 related to a greater need for soluble sugars for embolism repair under drier conditions (Secchi et
341 al. 2011). The longer term response (across site) in branches and shorter term response (with
342 ENSO drought progression) in leaves is consistent with the transitory nature of carbohydrate
343 pools in leaves relative to branches.

344 Our observed relationships between NSCs and traits may serve to simplify both modeling
345 of carbon storage and collection of benchmark data, and emphasizes the importance of trait-
346 based modeling (van Bodegom et al. 2014) to capture species-level differences in NSCs. Our
347 observation of a positive relationship between leaf mass per area and foliar NSCs (Fig. 5, Table

348 S13) is consistent with an increase in leaf dry mass as sugars accumulate (Poorter et al. 2009).
349 Interestingly, branch soluble sugars were correlated with several metrics, including average
350 photosynthetic rate on the day of NSC sampling, xylem wood density, and leaf temperature (Fig.
351 6, Table S13). The negative relationship between branch soluble sugars and photosynthesis (Fig.
352 6a, Table S13) may be the result of feedback inhibition, whereby reduced sink strength and
353 phloem transport resulting from higher branch soluble sugars promotes a reduction in
354 photosynthetic rate (Paul & Foyer 2001, Thompson & Holbrook 2003, McCormick et al. 2009).
355 The decrease in branch soluble sugars with increasing wood density (Fig. 6b, Table S13) has
356 several possible explanations. Recent research has shown that increased sugar concentrations in
357 woody tissues reduce xylem vulnerability to cavitation (De Baerdemaeker et al. 2017), and
358 species with higher wood densities and therefore lower vulnerability (Hacke et al. 2001,
359 Jacobsen et al. 2005) would require less soluble sugar for repair. There is also evidence that
360 xylem parenchyma, the sugar transport and storage fraction in woody tissue that links the
361 heartwood and phloem, is related to embolism recovery (Secchi et al. 2017). Alternatively, wood
362 density is negatively correlated with phloem proportion in some species (Santini et al. 2012), so
363 there may simply be less soluble sugar transport tissue associated with denser wood. There may
364 also be a basic physical limitation whereby higher structural density reduces the space available
365 for sugar storage. Branch soluble sugars were also positively related to leaf temperature (Fig. 6c,
366 Table S13). This finding is contrary to research showing inhibition of assimilate export with
367 increased leaf temperature (Jiao & Grodzinski 1996). However Jiao & Grodzinski also show
368 declines in photosynthesis with leaf temperature (1996) which is consistent with the observed
369 negative relationship between branch soluble sugars and photosynthesis (Fig. 6a, Table S13)
370 discussed above. In our case, there was no observed correlation between photosynthesis and

371 leaf temperature across species ($r^2 = 0.14$). However, leaf temperature was consistently higher at
372 the driest site compared to the wettest site (Fig. S7), which may explain the site differences in
373 branch soluble sugars (PNM>SLZ; Fig. S3d).

374 Despite these few correlations, we did not find expected relationships between NSCs and
375 drought sensitivity or hydraulic metrics (Figs. S5, S6; Tables S1, S2), including leaf water
376 potential (Fig. S8), hydraulic conductivity, and P_{50} . Though increased leaf soluble sugars are
377 often associated with more negative water potentials (e.g. Dickman et al. 2014), these trees may
378 not have experienced dry enough conditions to necessitate osmotic regulation. We also explored
379 relationships with relative degree of isohydry (Fig. S5, Martinez-Villalta et al. 2014), yet there
380 was no significant correlation to NSCs (Fig. S6a) despite target species ranging from extreme
381 isohydry to extreme anisohydry (Fig. S6b, Table S2). Similarly, we found no correlation between
382 NSCs and $\Delta\Psi$, or difference between pre-dawn and mid-day leaf water potential (Table S2). This
383 absence of relationships across many functional traits, particularly hydraulic traits, further
384 emphasizes the homeostatic nature of NSCs in this system, that is, NSCs are relatively invariant
385 across a wide spectrum of hydraulic traits across the Isthmus of Panama; at least for the canopy
386 tree species explored here. To the extent that the natural rainfall gradient is a proxy for
387 adaptation to long term precipitation changes (specifically decreasing mean annual
388 precipitation), this suggests that NSCs will also be held homeostatic under future potentially
389 drier conditions, though manipulative studies (e.g. Rowland et al. 2015) are best utilized to test
390 this hypothesis. We note that many hydraulic metrics exist that we did not test (e.g.
391 vulnerability to embolism, Choat et al. 2012) that may provide more insight into regional
392 patterns of NSC regulation.

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

401

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406 Laboratory.

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600 **Tables**

601 **Table 1:** Site characteristics (1998-2015 mean). All three sites are characterized by a pronounced
 602 dry season (approximately from mid-December to the end of April), and a wet season (May to
 603 mid-December). Data provided by http://biogeodb.stri.si.edu/physical_monitoring/research/.

	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)	1844	2352	3282
Dry Season Precip. (mm)	210	308	655
Dry Season Solar Rad. (MJ m⁻² d⁻¹)	17	19	16
Wet Season Solar Rad. (MJ m⁻² d⁻¹)	13	14	13
Tmin (°C)	23	24	24
Tmax (°C)	31	30	28

604

605 **Figure legends**

606

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

616

617 **Figure 2:** Leaf total NSCs (a) don't change with drought duration, but ratio of leaf soluble
618 sugars to starch (b) declines due to increased starch (c) and decreased soluble sugars (d). Data
619 are from the late time point across all sites (PNM n = 18; BCI n = 15; SLZ n = 18). Error bars are
620 standard errors. Letters indicate significant differences in log transformed NSC for soluble
621 sugars:starch and starch, and square-root transformed NSC for soluble sugars.

622

623 **Figure 3:** No difference in leaf total NSCs across the precipitation gradient. Data are from the
624 late time point. Error bars are standard errors.

625

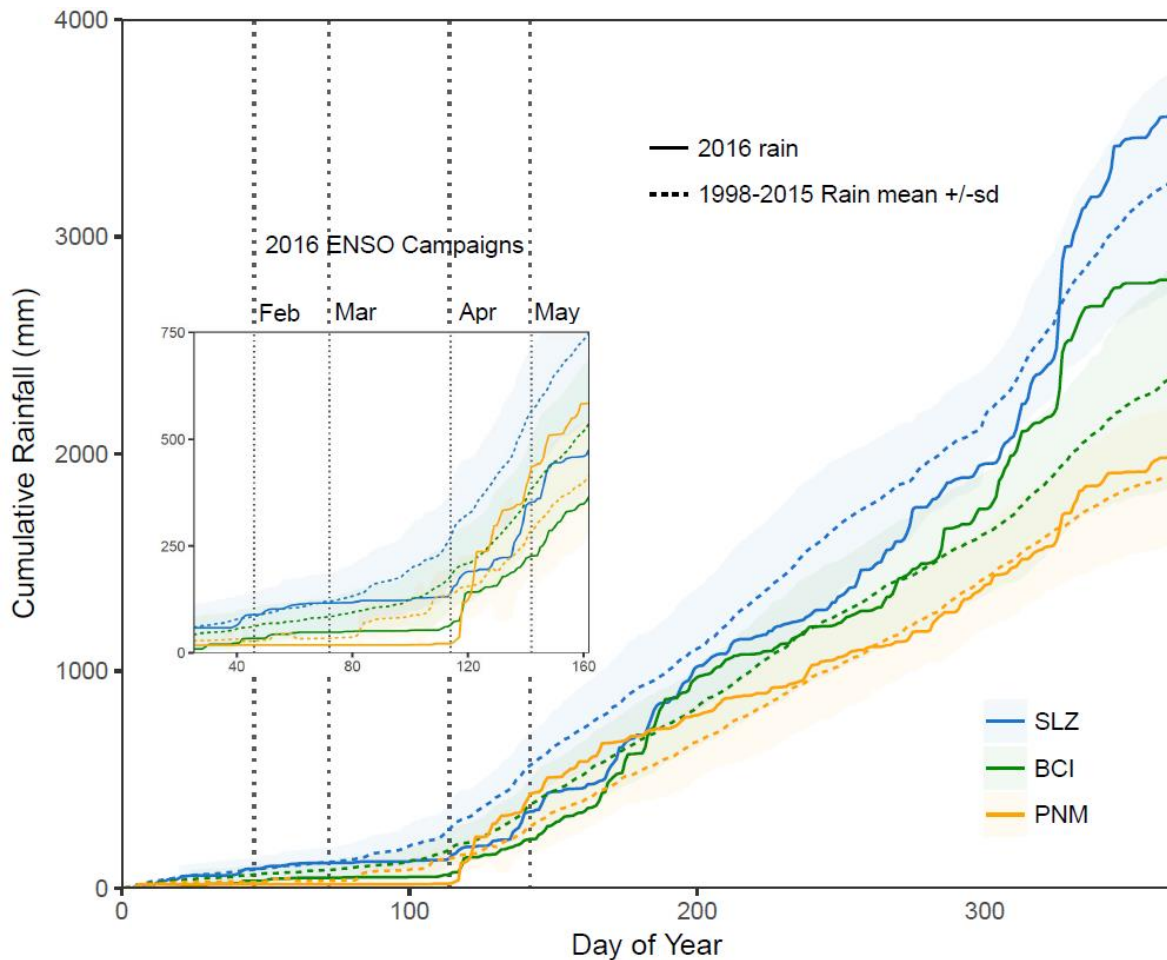
626 **Figure 4:** Large variation in NSCs across species. Leaf data are from the late time point. Branch
627 samples were collected at PNM in March and at SLZ in March and April. Branch samples were
628 not collected at BCI due to lack of canopy access. Species are arranged by site from left to right: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

631

632 **Figure 5:** Leaf mass per area explains some variation in leaf total NSCs. NSC data are from the
633 late time point. Each point represents one species.

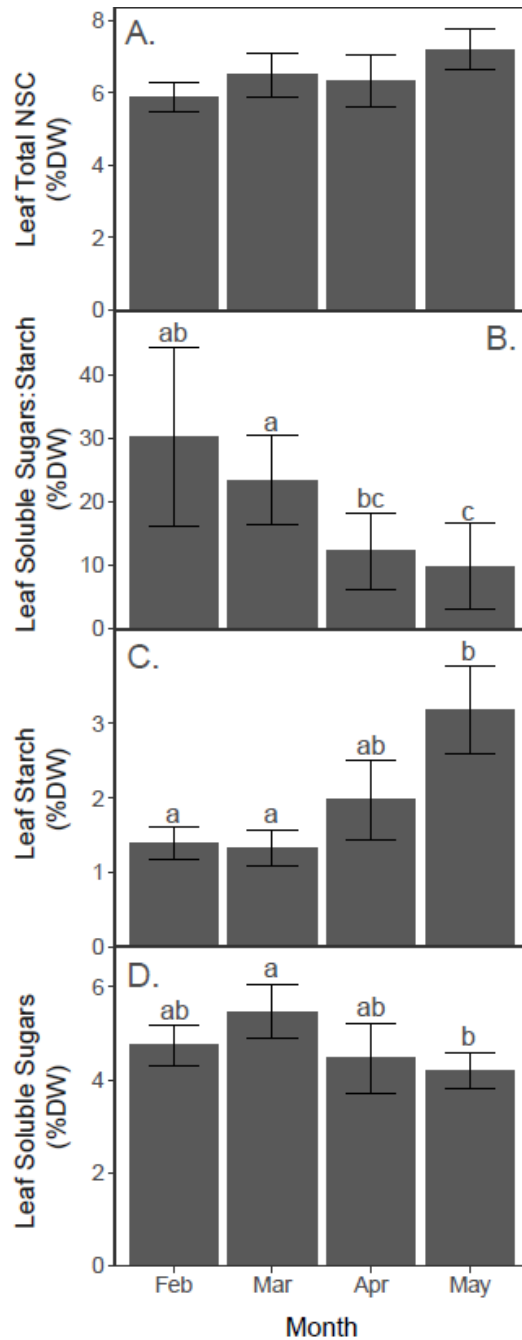
634

635 **Figure 6:** Branch soluble sugars are negatively related to photosynthesis and xylem wood
636 density, and positively related to leaf temperature. Each point represents one species.



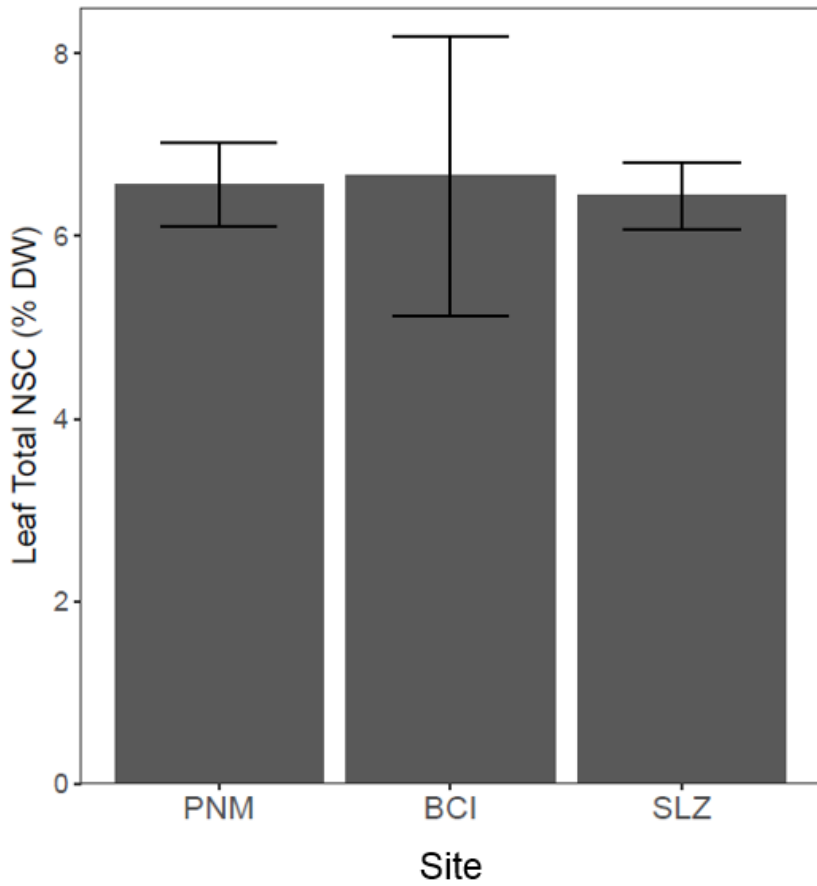
638

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



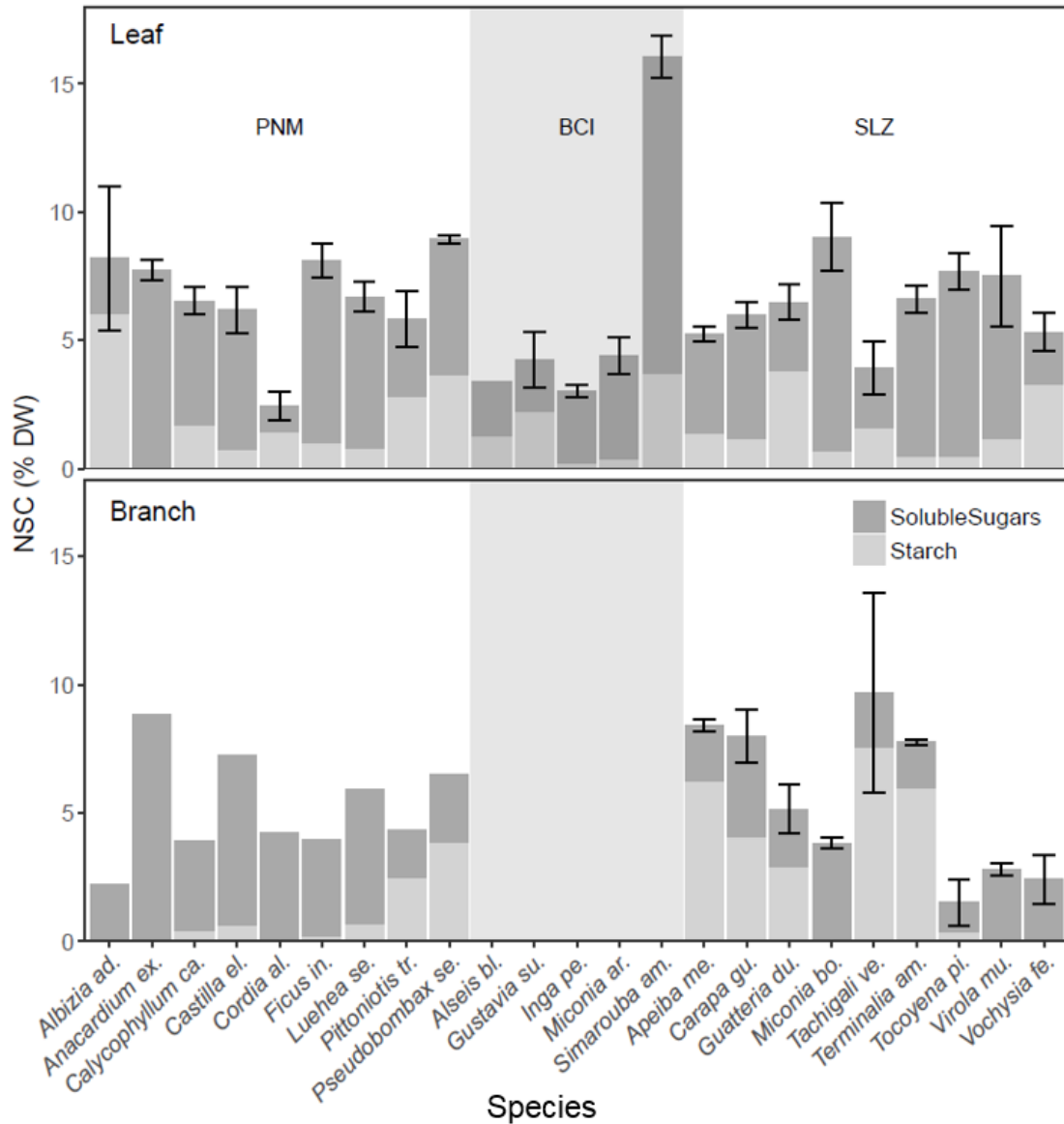
648

649 **Figure 2:** Leaf total NSCs (a) don't change throughout the drought, but ratio of leaf soluble
 650 sugars to starch (b) declines due to increased starch (c) and decreased soluble sugars (d). Data
 651 are from the late time point across all sites (PNM n = 18; BCI n = 15; SLZ n = 18). Error bars are
 652 standard errors. Letters indicate significant differences in log transformed NSC for soluble
 653 sugars:starch and starch, and square-root transformed NSC for soluble sugars .



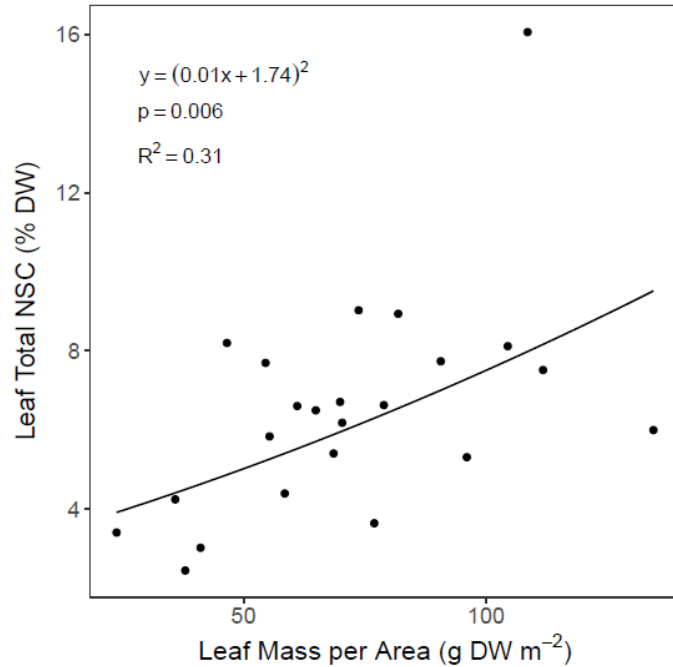
654

655 **Figure 3:** No difference in leaf total NSCs across the precipitation gradient. Data are from the
656 late time point (PNM n = 54; BCI n = 15; SLZ n = 72). Error bars are standard errors.



657

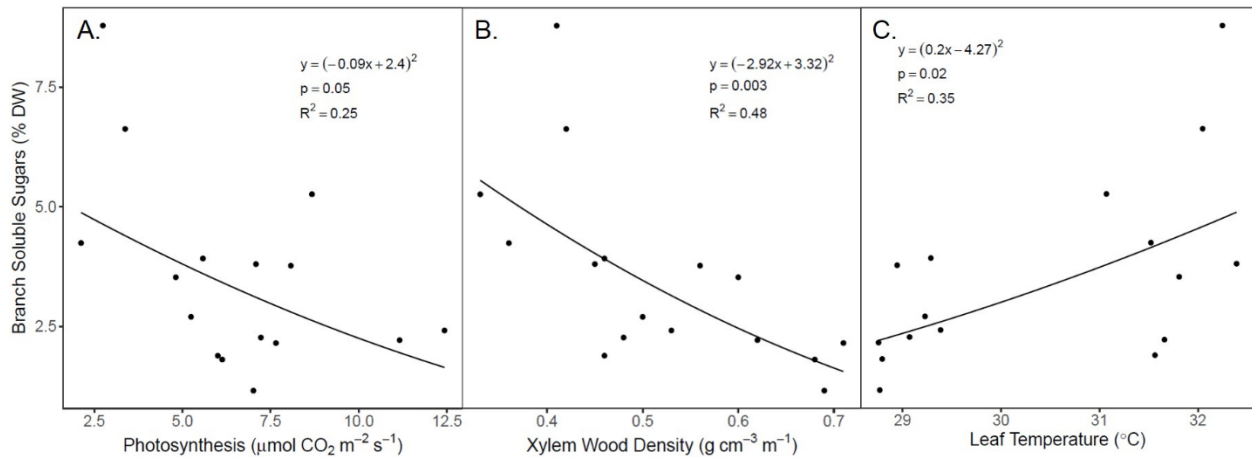
658 **Figure 4:** Large variation in NSCs across species. Leaf data are from the late afternoon time
 659 point (PNM n = 6; BCI n = 3; SLZ n = 8). Branch samples were collected at PNM in March (n =
 660 1) and at SLZ in March and April (n = 2). Branch samples were not collected at BCI due to lack
 661 of canopy access. Species are arranged by site from left to right: PNM, BCI, SLZ. Dark shading
 662 represents soluble sugars, light shading represents starch (see inset color key). Error bars are
 663 standard errors of total NSC. No error bars are shown for branches from PNM since there is only
 664 one sampling point.



665

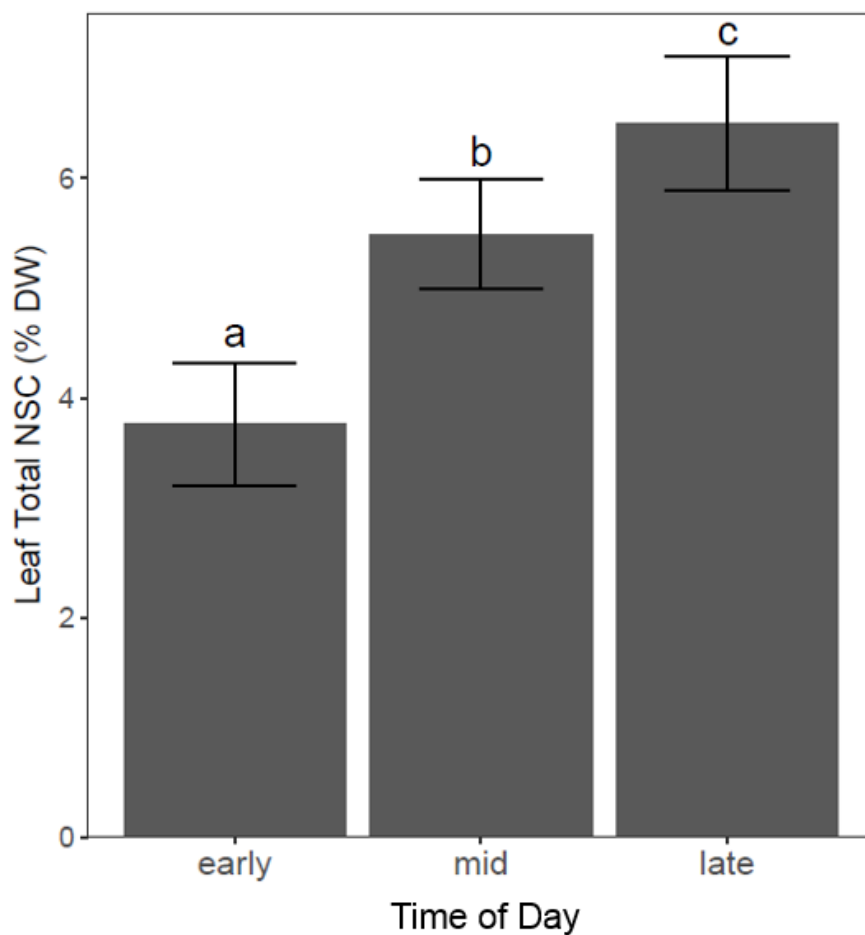
666 **Figure 5:** Leaf mass per area explains some variation in leaf total NSCs. NSC data are from the
 667 late time point. Each point represents one species. The regression without the highest NSC point
 668 remains significant but the correlation declines ($p=0.02$, $R^2=0.24$).

669



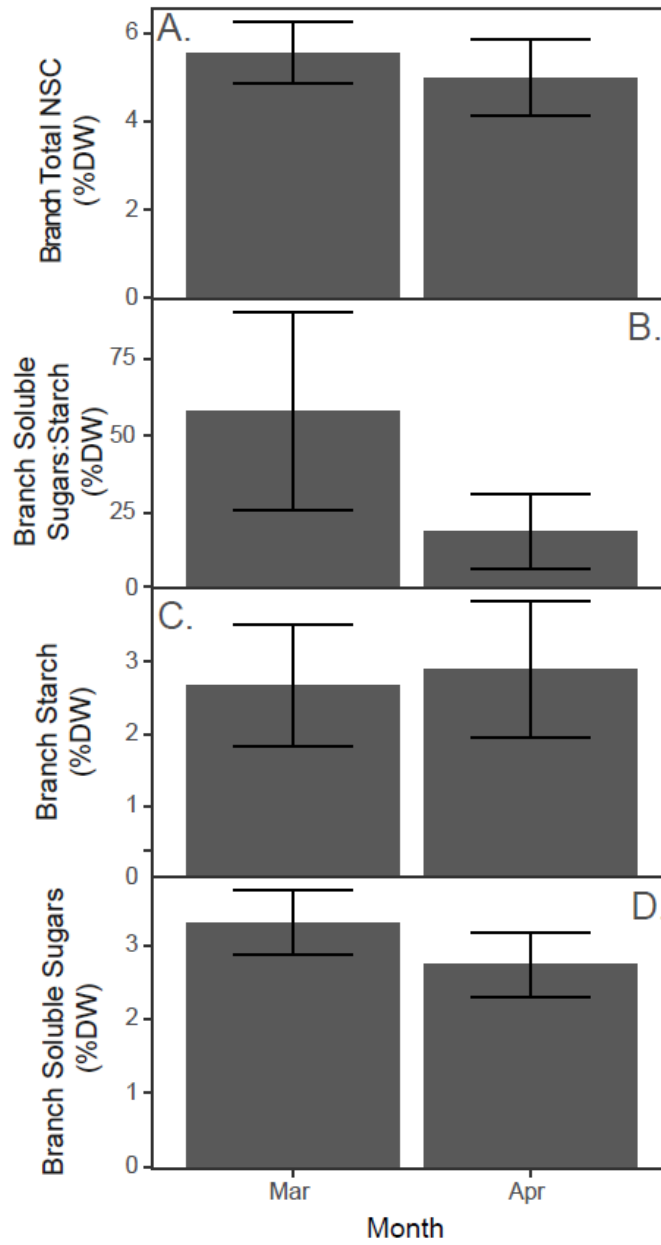
670

671 **Figure 6:** Branch soluble sugars are negatively related to mean daily photosynthesis and xylem
 672 wood density, and positively related to leaf temperature. Each point represents one species.



676 **Figure S1:** Leaf total NSCs increase over the course of the day. Samples were collected in the
677 morning (early), at mid-day (mid), and before sun-down (late) at all sites in March (n = 22).

678 Error bars are standard errors. Letters indicate significant differences in square root transformed
679 NSC.



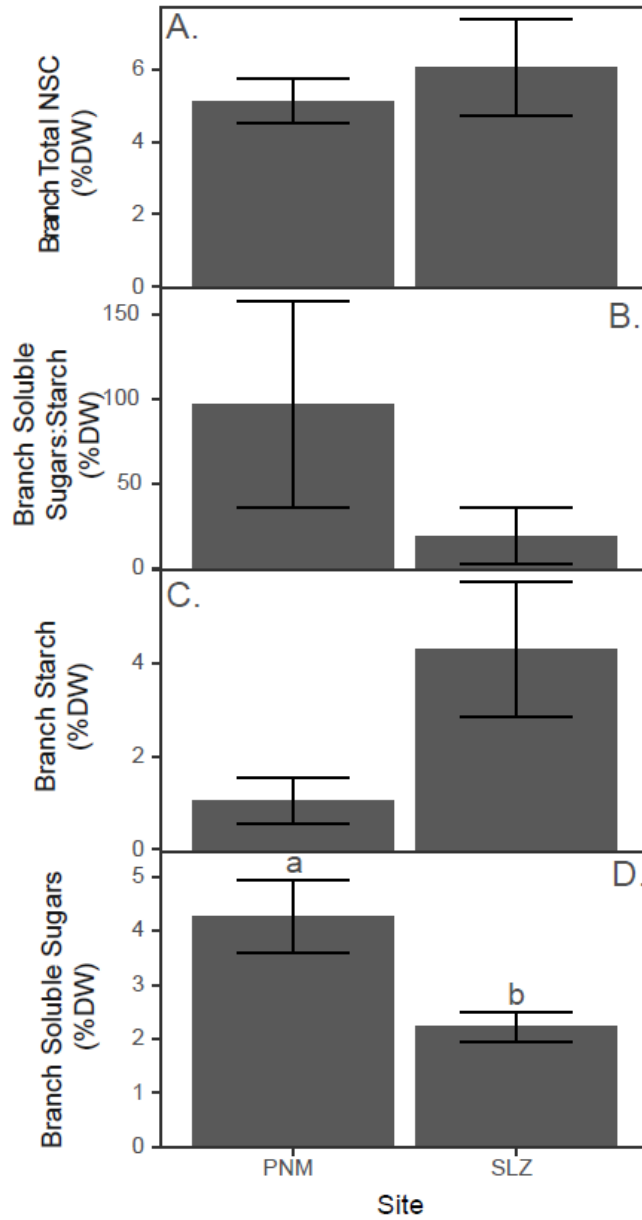
681

682 **Figure S2:** Branch NSCs don't change throughout the drought, though ratio of branch soluble

683 sugars to starch (b) trends lower as drought progresses ($p = 0.16$). Data are from the SLZ site (n

684 = 18). Error bars are standard errors.

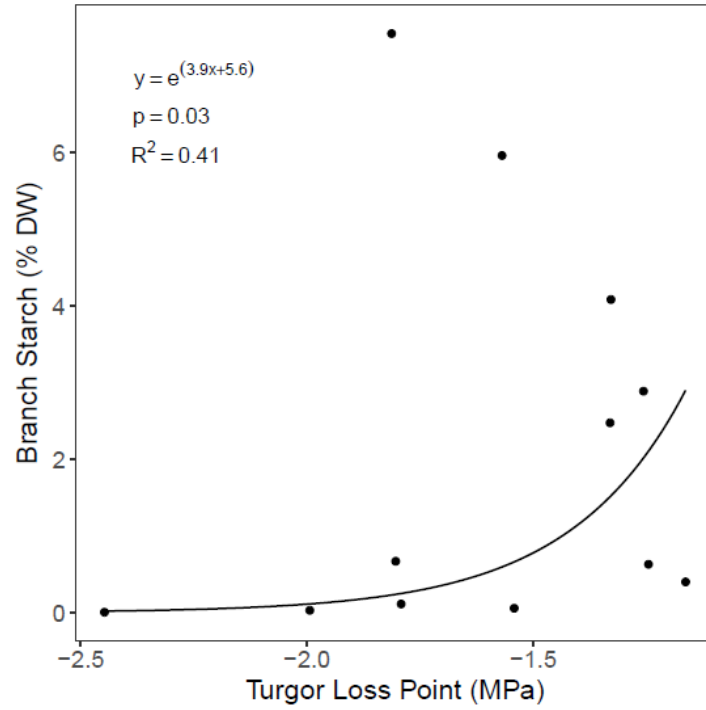
685



686

687 **Figure S3:** Branch total NSCs (a) don't differ across sites, but ratio of branch soluble sugars to
 688 starch (b) trends higher at the drier site (PNM; $p = 0.09$) due to decreasing starch (c; $p = 0.16$)
 689 and increased soluble sugars (d; $p = 0.01$). Data are from March (PNM $n = 10$; SLZ $n = 9$). Error
 690 bars are standard errors. Letters indicate significant differences ($p \leq 0.05$) in square-root
 691 transformed NSC for soluble sugars.

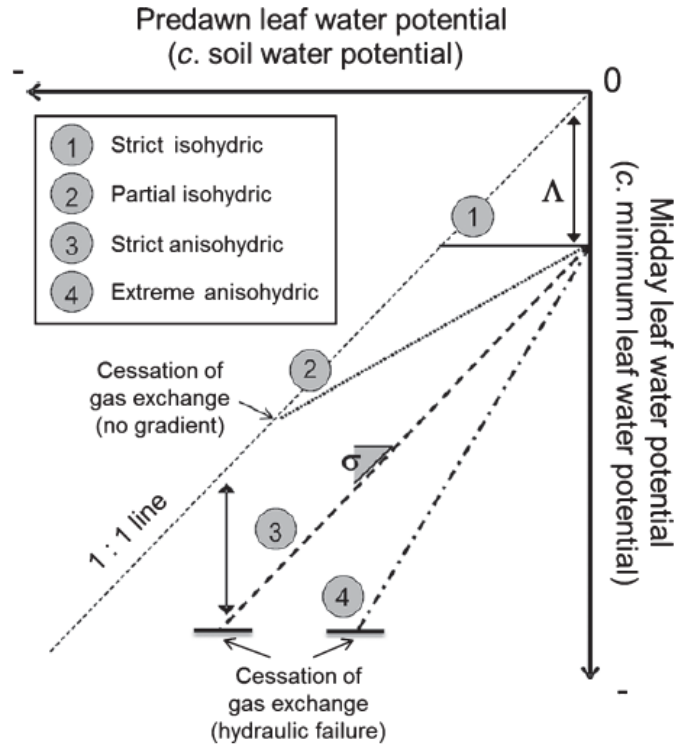
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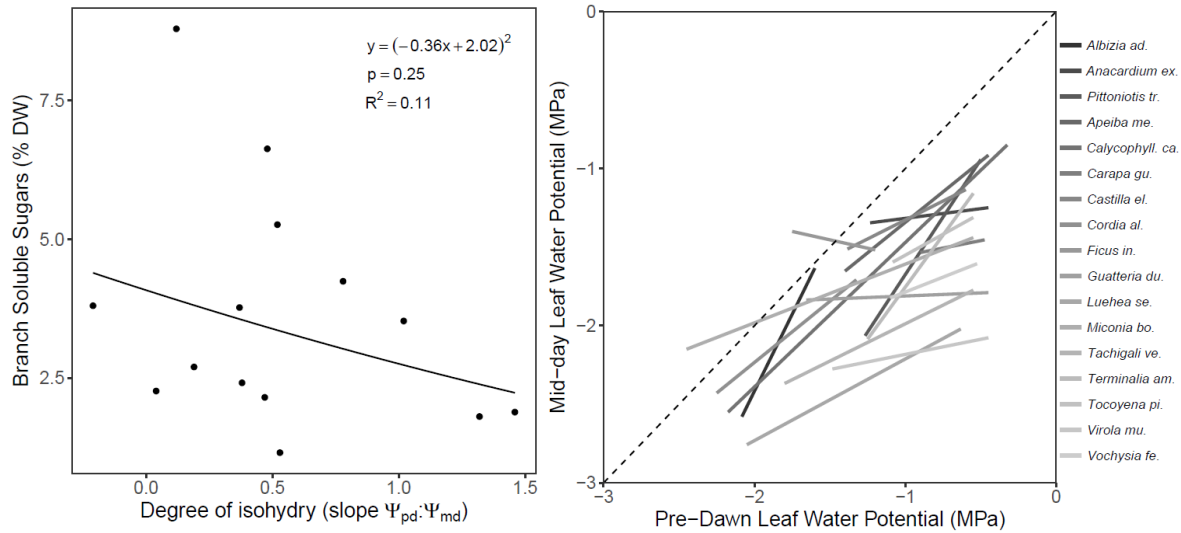
694 **Figure S4:** Branch starch increases exponentially with turgor loss point. Each point represents
695 one species.

696



697

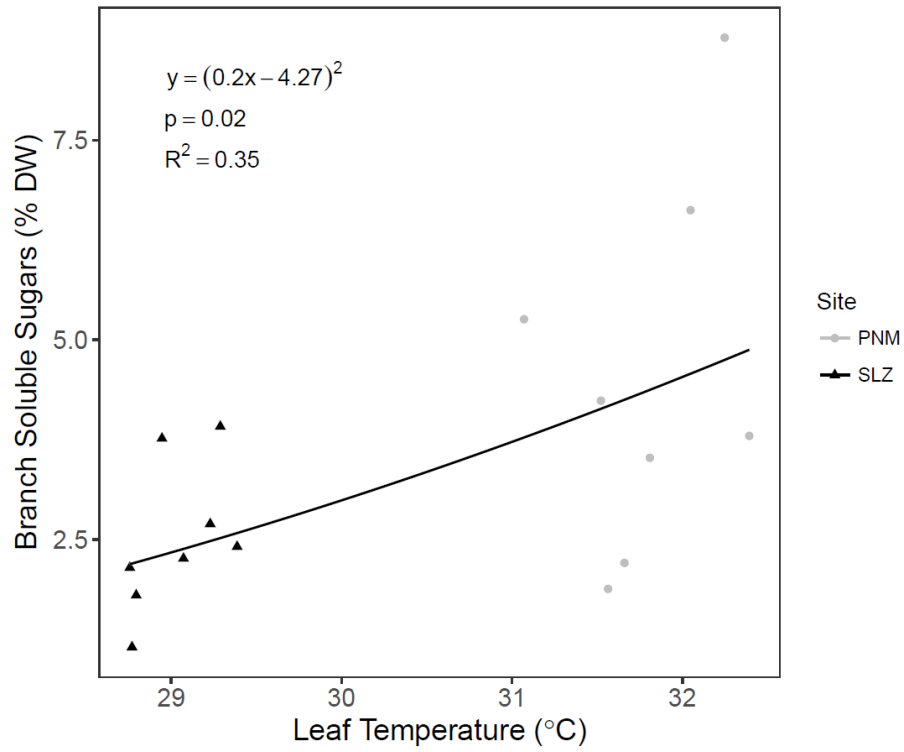
698 **Figure S5:** Relationship between predawn (Ψ_{PD}) and midday (Ψ_{MD}) leaf water potentials
 699 according to Martinez-Vilalta et al. 2014, Fig. 1. Four different behaviors are depicted, all
 700 sharing the same intercept (Λ) but different slopes (σ): strict isohydric ($\sigma = 0$), partial isohydric
 701 ($0 < \sigma < 1$), strict anisohydric ($\sigma = 1$) and extreme anisohydric ($\sigma > 1$). The point of cessation of
 702 gas exchange is also represented: for isohydric behaviors; it occurs when $\Psi_{PD} = \Psi_{MD}$; for
 703 anisohydric relationships, it occurs when Ψ_{MD} reaches the water potential inducing complete loss
 704 of plant hydraulic conductance. The 1:1 line is also depicted.



705

706 **Figure S6:** Branch soluble sugars are not related to drought response strategy. Degree of
 707 isohydry (a) is defined by the slope of pre-dawn vs. mid-day leaf water potential (b), per
 708 Martinez-Villalta et al. (2014) where strict isohydry: slope = 0; partial isohydry $0 < \text{slope} < 1$;
 709 strict anisohydry: slope = 1; extreme anisohydry: slope > 1 . Each point represents one species.
 710 Species for which range in pre-dawn water potential was less than 0.5MPa (Table S2) were
 711 excluded.

712

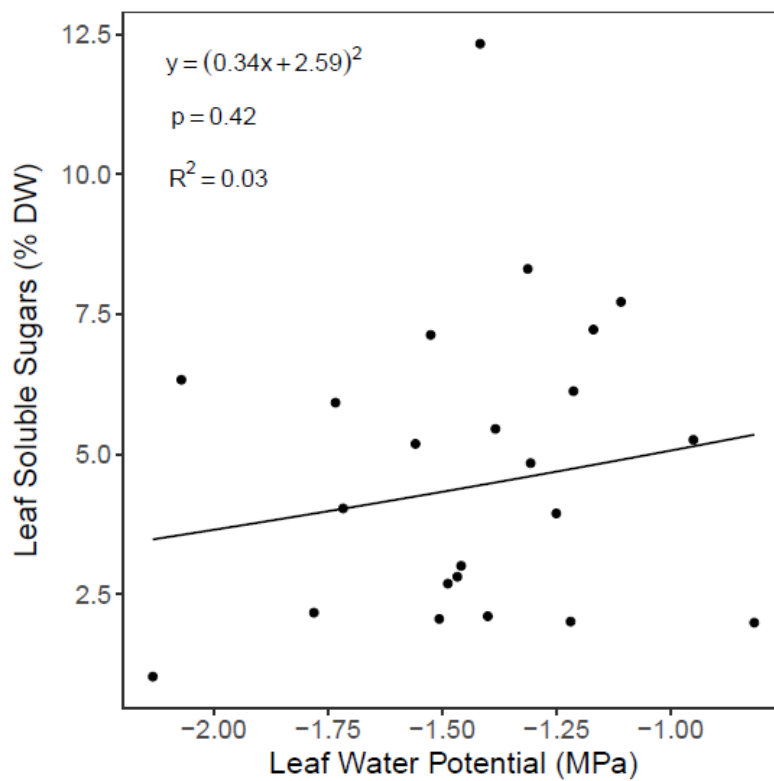


713

714 **Figure S7:** Leaf temperature is consistently higher at the driest site (PNM) compared to the
715 wettest site (SLZ), which may explain the site differences in branch soluble sugars (PNM>SLZ)

716

717



718

719 **Figure S8:** Leaf water potential doesn't explain variation in leaf soluble sugars. NSC data are
720 from the late time point. Each point represents one species.

721 **Table S1:** Traits tested for correlation with NSCs. Only significant correlations are shown (see
 722 Figure S9).

Trait	Units	Description	Methods detail
T_{leaf}	$^{\circ}\text{C}$	Sample specific leaf temperature measured with LiCor-6400XT	
A	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$	Sample specific photosynthetic assimilation measured with LiCor-6400XT	
C_i	$\mu\text{mol CO}_2 \text{ mol}^{-1}$	Sample specific intercellular CO_2 concentration measured with LiCor-6400XT	
g_s	$\text{mol H}_2\text{O} \text{ m}^{-2} \text{ s}^{-1}$	Sample specific stomatal conductance measured with LiCor-6400XT	
E	$\text{mol m}^{-2} \text{ s}^{-1}$	Sample specific transpiration measured with LiCor-6400XT	
Ψ_1	MPa	Sample specific leaf water potential	
LMA_{samp}	g m^{-2}	Sample specific leaf mass per area	
$\Delta\Psi$	MPa	Species specific difference between midday and predawn leaf water potential (from campaigns).	
$\Psi_{\text{PD}}:\Psi_{\text{MD}}$	unitless	Slope of relationship between midday and predawn leaf water potential (from campaigns). Metric of isohydry.	See Martinez-Villalta et al. 2014
V_{cmax}	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$	Maximum carboxylation rate extracted from A- C_i curves measured monthly during campaigns	
J_{max}	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$	Maximum electron transport rate extracted from A- C_i curves measured monthly during campaigns	
P_{50}	MPa	Target tree stem water potential at 50% loss of hydraulic conductivity	Stem P_{50} value. Stem water potential at 50% loss of hydraulic conductivity (MPa). 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.
K_{smax}	$\text{Kg s}^{-1} \text{ MPa}^{-1} \text{ m}^{-1}$	Target tree maximum stem-area-specific hydraulic conductivity (stem area includes bark and pith)	Maximum stem-area-specific hydraulic conductivity (stem area includes bark and pith). The intercept of the equation described for P_{50} above. $\text{Kg s}^{-1} \text{ MPa}^{-1} \text{ m}^{-1}$.
Ψ_{TLP}	MPa	Target tree leaf turgor loss point	Mean leaf turgor loss point (MPa). Calculated from 2-6 pressure volume curves per species. The pressure volume curves were produced with a leaf discs in a psychrometer rather than a whole leaf in a pressure chamber.

Ψ_{bmin}	MPa	Target tree minimum branch water potential measured in the field	Minimum branch water potential measured in the field. (MPa) measured with a pressure chamber at midday on leaves that were bagged and insulated since predawn.
Ψ_{bmax}	MPa	Target tree maximum branch water potential measured in the field	Maximum branch water potential measured in the field. (MPa) with a pressure chamber on leaves that were bagged and insulated at predawn at least 1 h before measurement.
LMA_{sp}	$g\ m^{-2}$	Target tree leaf mass per area	
$A_l:A_x$	$m^2\ m^{-2}$	Target tree leaf area per xylem area	
WD_b	$g\ cm^{-3}$	Target tree bark density	
WD_{ws}	$g\ cm^{-3}$	Target tree whole stem density	
WD_x	$g\ cm^{-3}$	Target tree xylem density	

724 **Table S2:** Study species by site and drought sensitivity metrics. Species specific differences
725 between midday and predawn leaf water potential ($\Delta\Psi$) measured during sampling campaigns
726 and their standard deviations (sd $\Delta\Psi$). Coefficients of the relationship between midday and
727 predawn leaf water potential (intercept and slope $\Psi_{PD}:\Psi_{MD}$) measured during sampling
728 campaigns. Slope $\Psi_{PD}:\Psi_{MD}$ provides a metric of isohydry (Martinez-Villalta et al. 2014). Species
729 for which the range in measured predawn leaf water potentials ($\Delta\Psi_{PD}$) was less than 0.5 MPa
730 were excluded.

Site	Species	$\Delta\Psi$	sd $\Delta\Psi$	intercept $\Psi_{PD}:\Psi_{MD}$	slope $\Psi_{PD}:\Psi_{MD}$	$\Delta\Psi_{PD}$
PNM	<i>Albizia adinocephala</i>	0.24	0.31	1.49	1.96	0.48
	<i>Anacardium excelsum</i>	0.50	0.31	-1.19	0.12	0.78
	<i>Pittoniotis trichantha</i>	0.66	0.22	-0.21	1.46	0.77
	<i>Calycophyllum candidissimum</i>	1.09	0.75	-1.05	1.02	1.85
	<i>Castilla elastica</i>	0.30	0.21	-0.85	0.48	0.88
	<i>Cordia alliodora</i>	0.28	0.19	-0.68	0.78	0.93
	<i>Ficus insipida</i>	0.08	0.37	-1.77	-0.21	0.55
	<i>Luehea seemanii</i>	1.02	0.33	-1.69	0.52	1.42
	<i>Pseudobombax septenatum</i>	0.26	NA	-0.89	NA	0
BCI	<i>Alseis blackiana</i>	0.61	NA	-1.86	NA	0
	<i>Gustavia superba</i>	0.50	NA	-1.62	NA	0
	<i>Inga pezizifera</i>	0.90	NA	-1.82	NA	0
	<i>Miconia argentea</i>	1.37	NA	-1.93	NA	0
	<i>Simarouba amara</i>	0.75	NA	-1.45	NA	0
SLZ	<i>Apeiba membranacea</i>	0.34	0.11	-0.57	0.78	0.95
	<i>Carapa guianensis</i>	0.77	0.31	-1.36	0.19	0.43
	<i>Guatteria dumetorum</i>	0.70	0.61	-1.77	0.04	1.21
	<i>Miconia borealis</i>	0.23	0.50	-1.23	0.37	1.90
	<i>Tachigali versicolor</i>	0.87	0.37	-1.51	0.47	1.25
	<i>Terminalia amazonia</i>	0.74	0.13	-0.43	1.32	0.70
	<i>Tocoyena pittieri</i>	0.61	0.51	-1.02	0.53	0.53
	<i>Virola multiflora</i>	1.29	0.39	-1.99	0.19	1.03
	<i>Vochysia ferruginea</i>	0.86	0.16	-1.41	0.38	0.58

732 **Table S3.** Target tree attributes by site.

Site	Species	Phenology	Height (m)	DBH (mm)
PNM	<i>Albizia adinocephala</i>	evergreen	29.4	295
	<i>Anacardium excelsum</i>	evergreen	39	1319
	<i>Pittoniotis trichantha</i>	brevideciduous	19	210
	<i>Calycophyllum candidissimum</i>	evergreen	20.1	395
	<i>Castilla elastica</i>	facultative deciduous	23.5	380
	<i>Cordia alliodora</i>	wet season deciduous	22	283
	<i>Ficus insipida</i>	evergreen	31.2	954
	<i>Luehea seemannii</i>	evergreen	26	632
	<i>Pseudobombax septenatum</i>	obligate deciduous	34	1234
BCI	<i>Alseis blackiana</i>	brevideciduous	19.1	275
	<i>Gustavia superba</i>	evergreen	15.3	241
	<i>Inga pezizifera</i>	evergreen	25.3	340
	<i>Miconia argentea</i>	evergreen	17.7	239
	<i>Simarouba amara</i>	evergreen	21.7	374
SLZ	<i>Apeiba membranacea</i>	brevideciduous	29	805
	<i>Carapa guianensis</i>	evergreen	33.9	620
	<i>Guatteria dumetorum</i>	evergreen	35	590
	<i>Miconia borealis</i>	evergreen	24.8	340
	<i>Tachigali versicolor</i>	evergreen	30.4	574
	<i>Terminalia amazonia</i>	evergreen	27	529
	<i>Tocoyena pittieri</i>	evergreen	26.6	533
	<i>Virola multiflora</i>	evergreen	22.7	351
	<i>Vochysia ferruginea</i>	evergreen	29.4	580

734 **Table S4.** NSC sample collection. Text indicates a sample was collected at a given site and date.
 735 Green indicates leaf, brown indicates branch. Leaf samples were collected at early, mid-day, or
 736 late diurnal time points.

	PNM			BCI			SLZ		
Feb	early		late				early	mid	late
Mar	early	mid	late	early	mid	late	early	mid	late
	branch						branch		
Apr	early						early	mid	late
							branch		
May	early		late				early		late

737

738 **Table S5.** Summary of linear mixed models of leaf NSCs by site (PNM, BCI, SLZ) and time of
 739 day (early, mid, late) for each sugar. The response variable is transformed leaf NSC data from
 740 March. Significant fixed effects at $p < 0.05$ are bolded.

NSC	Fixed Effects	Random	Correlation	numDF	denDF	F-value	p-value
sqrt(Total NSC)	Intercept	yes	no	1	82	223.03	<.0001
	Site			2	19	0.7142	0.5023
	Timeofday			2	82	27.644	<.0001
log(Starch)	Intercept	yes	no	1	38	11.484	0.0016
	Site			2	16	0.1137	0.8932
	Timeofday			2	38	7.7839	0.0015
sqrt (Soluble Sugars)	Intercept	yes	no	1	80	171.27	<.0001
	Site			2	19	0.4172	0.6648
	Timeofday			2	80	17.784	<.0001
log (SS:Starch)	Intercept	yes	no	1	36	39.985	<.0001
	Site			2	16	0.1340	0.8756
	Timeofday			2	36	1.3621	0.2690

Note: Full model: lme(NSC ~ Site + Timeofday, random=~1|Species/Sample_ID, na.action=na.exclude, method="REML", correlation = corAR1(form=~Timeofday|Species/Sample_ID))

741
 742
 743 **Table S6:** Parameter estimates from linear mixed models of leaf NSCs by site and time of day
 744 for each sugar. The response variable is transformed leaf NSC data from March. Letters indicate
 745 significant differences at $p < 0.05$.

	early	mid	late
sqrt(leaf TNSC)	1.77 a	2.22 b	2.43 c
log(leaf Starch)	-1.97 a	-0.83 b	-0.53 b
sqrt(leaf SS)	1.70 a	2.08 b	2.19 b
log(leaf SS:Starch)	2.47	2.2	1.85

746

747 **Table S7.** Summary of linear mixed models of NSCs by site (PNM, BCI, SLZ) and month (Feb,
748 Mar, Apr, May) for each tissue and sugar. The response variable is transformed NSC data. For
749 leaves, data are from the late time point. Significant fixed effects at $p < 0.05$ are bolded.

NSC	Fixed Effects	Random	Correlation	numDF	denDF	F-value	p-value
sqrt(leaf TNSC)	Intercept	Yes	no	1	79	575.38	<.0001
	Site			2	20	0.0979	0.9072
	Month			3	79	1.1843	0.3211
log(leaf Starch)	Intercept	yes	no	1	79	5.9238	0.0172
	Site			2	20	0.3537	0.7064
	Month			3	79	8.4823	0.0001
sqrt(leaf SS)	Intercept	yes	no	1	79	245.03	<.0001
	Site			2	20	0.0150	0.9851
	Month			3	79	3.3796	0.0223
log(leaf SS:Starch)	Intercept	yes	no	1	62	22.957	<.0001
	Site			2	20	0.0653	0.9370
	Month			3	62	10.909	<.0001
sqrt(branch TNSC)	Intercept	no	yes	1	25	265.31	<.0001
	Site			1	-	0.0025	0.9603
	Month			1	-	1.1196	0.3001
log(branch Starch)	Intercept	no	yes	1	20	0.7137	0.4082
	Site			1	-	1.7567	0.2000
	Month			1	-	1.4835	0.2374
sqrt(branch SS)	Intercept	no	no	1	25	492.62	<.0001
	Site			1	-	8.7087	0.0068
	Month			1	-	0.4851	0.4925
log(branch SS:Starch)	Intercept	no	yes	1	14	7.4502	0.0163
	Site			1	14	2.7115	0.1219
	Month			1	6	2.2392	0.1852

Note: Full model: lme(NSC ~ Site + Month, random=~1|Species/Sample_ID, na.action=na.exclude, method="REML", correlation = corAR1(form=~Month|Species/Sample_ID))

751 **Table S8.** Summary of linear mixed models of branch NSCs from the SLZ site by month (Mar,
752 Apr) for each sugar. The response variable is transformed branch NSC data. Significant fixed
753 effects at $p < 0.05$ are bolded.

NSC	Fixed Effects	Random	Correlation	numDF	denDF	F-value	p-value
sqrt(TNSC)	Intercept	no	yes	1	16	95.283	<.0001
	Month			1	-	1.0094	0.33
log(Starch)	Intercept	no	yes	1	13	0.1101	0.7453
	Month			1	-	1.4870	0.2443
sqrt(SS)	Intercept	no	no	1	16	348.91	<.0001
	Month			1	-	0.6586	0.429
log(SS:Starch)	Intercept	no	yes	1	13	0.6948	0.4196
	Month			1	-	2.2480	0.1577

Note: Full model: lme(NSC ~ Month, random=~1|Species/Sample_ID, na.action=na.exclude, method="REML", correlation = corAR1(form=~Month|Species/Sample_ID))

754

755 **Table S9.** Summary of linear mixed models of branch NSCs from March by site (PNM, SLZ) for
 756 each sugar. The response variable is transformed branch NSC data. Significant fixed effects at p
 757 < 0.05 are bolded.

NSC	Fixed Effects	Random	numDF	denDF	F-value	p-value
sqrt(TNSC)	Intercept	no	1	17	238.97	<.0001
	Site		1	-	0.1389	0.714
log(Starch)	Intercept	no	1	14	0.4479	0.5142
	Site		1	-	2.2224	0.1582
sqrt(SS)	Intercept	no	1	17	364.15	<.0001
	Site		1	-	8.6057	0.0093
log(SS:Starch)	Intercept	no	1	14	5.9514	0.0286
	Site		1	-	3.3879	0.0870

Note: Full model: lme(NSC ~ Site, random=~1|Species/Sample_ID, na.action=na.exclude, method="REML")

758

759 **Table S10:** Parameter estimates from linear mixed models of NSCs by month for each tissue and
 760 sugar. The response variable is transformed NSC data. For leaves, data are from the late time
 761 point. Letters indicate significant differences at $p < 0.05$.

	Feb	Mar	Apr	May
sqrt(leaf TNSC)	2.38	2.44	2.43	2.61
log(leaf Starch)	-0.27 a	-0.58 a	0.18ab	0.67b
sqrt(leaf SS)	2.08 ab	2.21 a	1.94 ab	1.91 b
log(leaf SS:Starch)	1.75 ab	2.02a	1.05bc	0.5c
sqrt(branch TNSC)	-	2.28	2.11	-
log(branch Starch)	-	-0.36	0.02	-
sqrt(branch SS)	-	1.76	1.61	-
log(branch SS:Starch)	-	1.43	2.29	-

762

763

764 **Table S11:** Parameter estimates from linear mixed models of NSCs by site for each tissue and
 765 sugar. The response variable is transformed NSC data. For leaves, data are from the late time
 766 point. Letters indicate significant differences at $p < 0.05$.

	PNM	BCI	SLZ
sqrt(leaf TNSC)	2.51	2.38	2.46
log(leaf Starch)	0.09	-0.27	-0.06
sqrt(leaf SS)	2.08	2.03	2.08
log(leaf SS:Starch)	1.26	1.59	1.42
sqrt(branch TNSC)	2.22	-	2.23
log(branch Starch)	-1.16	-	0.24
sqrt(branch SS)	2.01 a	-	1.54 b
log(branch SS:Starch)	2.51	-	0.64

767

768 **Table S12.** Summary of linear mixed models of NSCs by species (leaves n = 23; branches n =
769 18) for each tissue and sugar. The response variable is transformed NSC data. For leaves, data
770 are from the late time point. Significant fixed effects at $p < 0.05$ are bolded.

NSC	Fixed Effects	Random	Correlation	numDF	denDF	F-value	p-value
sqrt(leaf TNSC)	Intercept	no	no	1	109	2892.5	<.0001
	Species			22	-	3.9182	<.0001
log(leaf Starch)	Intercept	no	no	1	90	0.0037	0.9515
	Species			22	-	2.7070	0.0005
sqrt(leaf SS)	Intercept	no	no	1	109	3034.5	<.0001
	Species			22	-	10.472	<.0001
log(leaf SS:Starch)	Intercept	no	no	1	90	109.49	<.0001
	Species			22	-	5.1682	<.0001
sqrt(branch TNSC)	Intercept	no	no	1	10	914.55	<.0001
	Species			17	-	3.5639	0.0229
log(branch Starch)	Intercept	no	no	1	7	2.1559	0.1855
	Species			15	-	11.783	0.0015
sqrt(branch SS)	Intercept	no	no	1	10	1041.7	<.0001
	Species			17	-	3.6649	0.0208
log(branch SS:Starch)	Intercept	no	no	1	7	46.927	0.0002
	Species			15	-	10.090	0.0024

Note: Full model: lme(NSC ~ Species, random=~1|Site or ~Month|Site/Sample_ID, na.action=na.exclude, method="REML", correlation = corAR1(form=~Month|Site/Sample_ID))

771

772 **Table S13.** Summary of linear mixed models of NSCs by traits for select tissues and sugars. The
773 response variable is transformed NSC data. For leaves, data are from the late time point. Traits
774 with significant relationships (bolded, $p < 0.05$) to NSCs include LMA (leaf mass per area, g dw
775 m^{-2}), Ψ_{TLP} (turgor loss point, MPa), A (photosynthetic assimilation, $\mu mol m^{-2} s^{-1}$), WD_x (xylem
776 wood density, $g cm^{-3} m^{-1}$), and T_{leaf} (leaf temperature, $^{\circ}C$). $\Psi_{PD}:\Psi_{MD}$ (slope of pre-dawn to mid-
777 day leaf water potential, unitless) was also included as a metric for relative isohydry, however
778 there was no significant relationship to NSCs. Other non-significant relationships are not shown.
779 Traits are species averages.

NSC	Fixed Effects	Random	Correlation	numDF	denDF	F-value	p-value
sqrt(leaf TNSC)	Intercept	no	no	1	21	732.17	<.0001
	LMA			1	-	9.2092	<.0063
log(branch Starch)	Intercept	no	no	1	10	1.6751	0.2247
	Ψ_{TLP}			1	-	6.8477	0.0257
sqrt(branch SS)	Intercept	no	no	1	14	270.14	<.0001
	A			1	-	4.5958	0.0501
sqrt(branch SS)	Intercept	no	no	1	14	392.97	<.0001
	WD_x			1	-	13.052	0.0028
sqrt(branch SS)	Intercept	no	no	1	14	315.21	<.0001
	T_{leaf}			1	-	7.6984	0.0149
sqrt(branch SS)	Intercept	no	no	1	14	235.89	<.0001
	$\Psi_{PD}:\Psi_{MD}$			1	-	2.2380	0.1569

Note: Full model: lme(NSC ~ Trait, random=~1|Species or ~Month|Species, na.action=na.exclude, method="REML", correlation = corAR1(form=~Month|Species))