Journal of
Plant Ecology

Highlight

The role of soluble sugars during drought in tropical tree seedlings with contrasting tolerances

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Handling Editor: Wen-Hao Zhang

Received: 25 March 2020, **Accepted:** 9 April 2020, **Advanced Access publication:** 13 April 2020

Citation: O'Brien MJ, Valtat A, Abiven S, *et al.* (2020) The role of soluble sugars during drought in tropical tree seedlings with contrasting tolerances. *J Plant Ecol* **13**:389–397. https://doi.org/10.1093/jpe/rtaa017

Abstract

Aims Non-structural carbohydrates (NSCs) are plant storage compounds used for metabolism, transport, osmoregulation and regrowth following the loss of plant tissue. Even in conditions suitable for optimal growth, plants continue to store NSCs. This storage may be due to passive accumulation from sink-inhibited growth or active reserves that come at the expense of growth. The former pathway implies that NSCs may be a by-product of sink limitation, while the latter suggests a functional role of NSCs for use during poor conditions.

Methods Using ^{13}C pulse labelling, we traced the source of soluble sugars in stem and root organs during drought and everwet conditions for seedlings of two tropical tree species that differ in drought tolerance to estimate the relative allocation of NSCs stored prior to drought versus NSCs assimilated during drought. We monitored growth, stomatal conductance, stem water potential and NSC storage to assess a broad carbon response to drought.

Important Findings We found that the drought-sensitive species had reduced growth, conserved NSC concentrations in leaf, stem and root organs and had a larger proportion of soluble sugars in stem and root organs that originated from pre-drought storage relative to seedlings in control conditions. In contrast, the drought-tolerant species maintained growth and stem and root NSC concentrations but had reduced leaf NSCs concentrations with a larger proportion of stem and root soluble sugars originated from freshly assimilated photosynthates relative to control seedlings. These results suggest the drought-sensitive species passively accumulated NSCs during water deficit due to growth inhibition, while the drought-tolerant species actively responded to water deficit by allocating NSCs to stem and root organs. These strategies seem correlated with baseline maximum growth rates, which supports previous research suggesting a trade-off between growth and drought tolerance while providing new evidence for the importance of plasticity in NSC allocation during drought.

Keywords: ^{13}C labelling, carbohydrate storage, drought tolerance, hydraulic function, *Shorea parvifolia*, *Shorea beccariana*, source–sink allocation

摘要：非结构碳水化合物(NSCs)是植物的贮藏化合物，用于代谢、运输、渗透调节和叶片脱落后的再生。即使在最适宜生长的条件下，植物也会继续储存NSCs。这种储存可能是由于生长受到抑制而产生的被动积累，也可能是由于以生长为代价而产生的主动储备。前者暗示NSCs可能是碳汇有限生长的副产物，而后者则表明NSCs在植物适应逆境中具有的功能作用。本研究中，利用^{13}C脉冲标记，我们追踪了具

有不同干旱耐受性的两种热带树种的幼苗在干旱和常湿条件下茎和根器官中可溶性糖的来源，以估计干旱前储存的NSCs与干旱期间同化的NSCs的相对分配。我们监测了生长、气孔导度、茎干水势和NSC储存以评估对干旱的全碳响应。结果表明，与对照幼苗相比，不耐旱树种生长速度减慢，在叶片、茎和根器官中储存NSCs，在茎和根器官中可溶性糖(源于干旱前的储存)的比例更大。相反，与对照幼苗相比，耐旱树种则能保持生长和茎根NSCs浓度，但叶片NSCs浓度降低，茎和根可溶性糖的比例更大，这些可溶性糖来自于新同化的光合产物。这些结果表明，不耐旱树种由于缺水导致生长受限而被动积累NSCs，而耐旱树种则通过分配NSCs到茎和根器官来积极响应缺水。这些策略似乎与基线最大生长速率相关，并且支持了以前的研究结果，表明在生长和耐旱性之间存在一种权衡关系，同时也为NSCs分配的可塑性在干旱中的重要性提供了新的证据。

关键词： ^{13}C标记, 耐旱性, 碳水化合物储存, 水力学功能, 娑罗双属植物, 源汇分配

INTRODUCTION

Non-structural carbohydrates (NSCs; e.g. soluble sugars, starch and lipids) mediate physiological responses of trees to water deficits (Chapin *et al.* 1990; O'Brien *et al.* 2014; Adams *et al.* 2017) by supporting metabolic function, hydraulic conductance and osmoregulation under drought conditions (Hartmann *et al.* 2013; O'Brien *et al.* 2014; Sevanto *et al.* 2014). However, NSC storage may occur at a cost to other functions—e.g. growth and reproduction (Chapin *et al.* 1990)—whereby trees allocate less assimilates to maximum growth and, instead, use them for the maintenance of NSC pools even under good growing conditions (Kitajima 1994; Sala *et al.* 2012; Wiley and Helliker 2012). Tracking storage and movement of NSCs during drought in trees with different growth rates and drought tolerance can elucidate strategic differences in NSC use in response to water deficit. This, in turn, would provide details on the importance of storage, movement and consumption of NSCs for drought tolerance, which has been highlighted as a research priority needed for predicting and modelling the impacts of drought on forests (Hartmann *et al.* 2015; O'Brien *et al.* 2017a; Hartmann *et al.* 2018).

Climate change is altering precipitation patterns across the world by increasing the severity and frequency of extreme water inundation and drought (Dai 2013; Chadwick *et al.* 2015; Donat *et al.* 2016). Functional traits allow plants to respond to novel drought conditions by reducing water loss—e.g. stomatal control—and by maintaining hydraulic function and osmoregulation during drought—e.g. wood density, vessel anatomy and NSCs—(McDowell 2011; McDowell *et al.* 2011; O'Brien *et al.* 2017a; Griffin-Nolan *et al.* 2018). The maintenance of hydraulic function is of particular importance for survival because many tree species grow in climates at the threshold of their hydraulic limits (Choat *et al.* 2012), and increased drought severity may push species beyond these thresholds (Choat *et al.* 2012). A recent synthesis by Adams *et al.* (2017) found hydraulic failure to be a persistent cause of drought-induced mortality across tree species, and other studies have found that turgor loss point (a proxy for hydraulic robustness) correlates with the sensitivity of trees to drought across ecosystems (Bartlett *et al.* 2012, 2014). NSCs have a functional role in the prevention of hydraulic failure as evidenced by an earlier study (O'Brien *et al.* 2014), which showed the importance of NSCs for maintaining stem water potentials and prolonging survival during drought. However, the temporal dynamics of NSCs throughout tree organs during drought are still not fully understood (see advances in Muller *et al.* 2011; Piper and Fajardo 2016) because multiple tree functions (e.g. respiration, growth and NSC assimilation and storage) are adjusting in concert in response to drought.

Because NSCs can be stored and mobilized again, they are assumed to help mediate plant function when resources are limited (Hsiao *et al.* 1976; Chapin *et al.* 1990; Martinez-Vilalta *et al.* 2016). If NSCs are actively stored, it may occur at the expense of maximum growth under good conditions (Chapin *et al.* 1990; Sala *et al.* 2012; Palacio *et al.* 2014). This active NSC storage under good conditions suggests that NCSs have a functional role, most likely to prepare the plant for more stressful conditions, such as drought (Chapin *et al.* 1990; O'Brien *et al.* 2014; Sevanto *et al.* 2014; Nardini *et al.* 2016). These NCSs stored during good conditions may then be translocated and used to prevent and repair damage during bad conditions *via* remobilization of starch or movement of soluble sugar (Myers and Kitajima 2007; Smith *et al.* 2018; Tomasella *et al.* 2020). Alternatively, NSCs may passively accumulate due to sink-induced growth inhibition (Eaton and Ergle 1948; Wiley and Helliker 2012). For example, drought uncouples growth and photosynthesis (Muller *et al.* 2011), which causes extra assimilates to accumulate as NSC surplus that may maintain plant functions under water shortage. In this scenario, photosynthates (mainly soluble sugars) produced during the stress may be preferentially allocated to non-photosynthetic tissues—i.e. stems and roots (O'Brien *et al.* 2015; Tomasella *et al.* 2020). To develop a complete picture of tree NSC storage, allocation and translocation under drought, experiments need to assess morphological and physiological responses while tracking NSC concentrations throughout organs while water availability decreases.

Stable isotopes are common in ecology to assess plant physiological responses to abiotic and biotic factors and to understand forest responses to global-change drivers at the ecosystem, community and individual level (Cernusak and English 2015; Pflug *et al.* 2015; Zhang *et al.* 2015). In natural settings, tracking isotopes at temporal, spatial and biological scales can elucidate differences in plant responses to environmental disturbance (Dawson *et al.* 2002; Moreno-Gutiérrez *et al.* 2012; Pflug *et al.* 2015). In addition, stable isotope labelling provides access to tracking the movement of elements through the soil–plant–atmosphere continuum (Studer *et al.* 2014), and *in situ* ^{13}C pulse labelling has been used to assess carbon allocation under different light conditions (Lacointe *et al.* 2004; Hartmann and Trumbore 2016). While a single pulse (a single injection of highly ^{13}C-enriched CO_2 in the atmosphere of the plants) offers information about C-transfer times, it usually fails at quantifying the allocation of C assimilated during photosynthesis over longer time spans (Studer *et al.* 2014). *In situ*, multiple-pulse labelling (i.e. the addition of enriched ^{13}C-CO_2 at regular intervals, inducing a partial but detectable and representative enrichment of the plant ^{13}C for the given labelling period) represents a practical alternative to continuous labelling, which is challenging to apply in the field. This technique in conjunction with growth and storage dynamics can detail source–sink transfer of NSCs during drought (Studer *et al.* 2014).

In this study, we labelled seedlings of a drought-tolerant and a drought-sensitive tropical tree species, in the genus *Shorea*, with ^{13}C prior to exposing them to drought (no watering) and control (ever-wet) conditions to assess the movement of NSCs from leaves to stem and root organs. We monitored growth, stomatal conductance and stem water potential to assess differences in growth, respiration and conductance responses between the two species. By comparing allocation to NSC pools, NSC concentration shifts and translocation

of labelled ^{13}C in stem and root soluble sugars between seedlings in control and drought conditions, we detailed distinct pathways in NSCs during water deficit. We posited that drought should result in greater allocation of NSC from leaf to stem and root organs relative to control seedlings and greater adjustments of NSC allocation in the drought-tolerant compared with the drought-sensitive species. In addition, we hypothesized that stem and root soluble sugars could originate from two sources. (i) Soluble sugars that were stored prior to the onset of drought (old sugars) could be preferentially allocated to stem and root organs to support hydraulic functions, allowing freshly assimilated photosynthates to maintain growth and leaf functions. This strategy would provide evidence for a functional role of the actively stored NSCs during good growing conditions. (ii) Freshly assimilated soluble sugars during drought (fresh sugars) could be allocated for the maintenance of hydraulic function, which would suggest that old sugars are likely not actively stored for stressful growing conditions but, instead, trees adjust allocation in response to water deficit to meet functional needs.

MATERIALS AND METHODS

Study site

The experiment was conducted at the Malua Field Station, located ~22 km north-east of Danum Valley Research Centre in Sabah, Malaysia (5°5′20″ N, 117°38′32″ E; 102 m a.s.l.). The site is aseasonal with an average monthly rainfall (standard error) of 240 mm (33 mm, within year) and an average yearly total of 2900 mm (90 mm, between years) as recorded at Danum Valley Field Centre from 1986 to 2014.

Experimental design

Seeds of two species with contrasting drought tolerance—*Shorea parvifolia* Dyer (drought-sensitive) and *Shorea beccariana* Burck (drought-tolerant)—were collected from the Malua Forest during a landscape-scale masting event in August 2010 (O'Brien *et al.* 2014, 2017). Seedlings were grown in nurseries under two layers of 70% shade cloth, reducing incoming light to about ~5% of direct sunlight for 3 years from 2010 to 2013. In November 2013, 96 seedlings (48 per species) were planted into 48 pots consisting of two compartments (20 cm × 20 cm area and 45 cm depth per compartment). The pots were filled with a 60:40 mixture of clayey soil collected nearby from the Malua Forest and sand. Each compartment per pot was planted with a single seedling with the same species planted in both compartments of the pot. The compartments were used to separate the watering treatments. Seedlings established in the pots for 5 months and any seedlings that died were replanted during this time.

In situ ^{13}C multi-pulse labelling

A rectangular plastic chamber of 13.35 m^3 (7.9 m × 1.3 m area and 1.3 m height), constructed from transparent polyethylene sheeting under two layers of 70% shade cloth (~5% of direct sunlight), was built around all the seedlings to simultaneously label all plants with ^{13}C-enriched CO_2. Plastic was also used to cover the ground below the pots and the soil in the pots to reduce losses of ^{13}C gas and reduce the dilution of the label with CO_2 respired from the soil. Enrichment of $^{13}CO_2$ within the chamber was carried out by dissolving 30 g ^{13}C-enriched Na_2CO_3 (99 atom% ^{13}C, Cambridge Isotope, ReseaChem, Burgdorf, Switzerland) with 60 ml of acid. The reaction was done in six beakers to ensure equal distribution of CO_2 throughout the chamber, and the acid was applied at a drip to increase the CO_2 concentration in the chamber slowly (over ~30 min). The reaction increased the CO_2 concentration to a maximum of ~900 ppm, which resulted in an increase of 510 ppm above the assumed ambient concentration of 390 ppm. Battery-powered fans (KD1208PTS2 DC 12V, Sunon, Kaohsiung City, Taiwan) were placed in the chamber to ensure a good mixing of the CO_2 and to reduce the temperature of the chamber. We labelled for 4 days between 0800 and 1200 h (the period at which stomatal conductance reaches its daily maximums for these species) and allowed 1 day of recovery between each labelling treatment. The chamber was closed during the labelling and left open between labelling days to insure a good aeration. Following the multi-pulse labelling, seedlings were moved into four shade houses with a mean of 20% direct sunlight (95% confidence interval [CI]: 18–24%)—measured by simultaneous shade-house and open-sky sensors (SKP 210 quantum sensor, Skye instruments LTD, Llandrindod Wells, Powys, UK) every 30 min for a 24-h period. Seedlings were watered every 2 days for 30 days.

Drought treatment

On 31 May 2014 (30 days after labelling), watering was stopped in one compartment of each pot, while the other compartment was continually watered on a 3-day cycle (968 ml of water was applied each time, equivalent to 240 mm of rain per month). Volumetric soil moisture was measured every 3 days to assess soil drying relative to the watered compartment using an ML2x Theta Probe (Delta-T Devices, Burwell, Cambridge, UK). These measurements were converted to soil water potential following filter-paper methods (Deka *et al.* 1995; O'Brien *et al.* 2017). Soil drying was similar between species and reached a minimum of –2.5 MPa (95% CI: –3.4 to –2.0 MPa; see Supplementary Table S1 and Supplementary Fig. S1).

Seedling response

We measured mid-day stomatal conductance (1000–1300 h) weekly after the start of the drought treatment with a steady-state diffusion porometer (model SC-1, Decagon Devices Inc., Pullman, WA, USA) to assess stomatal closure. Seedling height (centimetres) was measured approximately every 7 days from the start of the drought treatment until the final harvest of seedlings on the 47th day after watering had stopped in the drought treatment. To assess physiological responses, NSC storage and ^{13}C movement throughout the experiment, we destructively harvested seedlings before dawn (0400 to 0600 h) at four different time points: (i) before labelling (four seedlings per species), (ii) after labelling (four seedlings per species), (iii) prior to drought (four seedlings per species) and (iv) after drought (10 seedlings of *S. beccariana* per treatment and 13 seedlings of *S. parvifolia* per treatment). At each harvest, we measured stem water potential using a Scholander pressure chamber (model 670; PMS Instrument Co., Corvallis, OR, USA). All roots were cleaned of soil. Seedlings were microwaved to stop enzymatic activity (Sala and Hoch 2009) and dried for 2–3 days at 64°C. Dry biomass was measured separately for leaves, stems and roots. Tissues were ground separately using a mortar and pestle and placed in 2-ml Eppendorf tubes (Eppendorf, Hamburg, Germany).

NSC analysis

In the lab, samples were further pulverized with a ball mill. We used 15–16 mg of each sample (leaf, stem and root for each seedling) to extract soluble sugars with 80% ethanol at 27°C for one night followed by two additional 2-h periods (Myers and Kitajima 2007; O'Brien *et al.* 2014). We digested the remaining starch supernatant with an amyloglucosidase enzyme (A-7420; Sigma-Aldrich, Co., St Louis, MO, USA) that converted the starch into glucose (O'Brien *et al.* 2014). The concentration of soluble sugars and starch (measured as glucose equivalents) were measured at 487 nm by spectrophotometry after a 30-min phenol-sulphuric acid reaction (O'Brien *et al.* 2014). We calculated the allocation of soluble sugar and starch to leaf, stem and roots by multiplying the concentration by organ biomass and dividing by the total soluble sugar and starch biomass in each organ biomass by the whole-plant soluble sugar and starch biomass.

^{13}C isotope analysis

Isotopic analysis was done for the extracted soluble sugars and starch solutions. These samples were prepared by pipetting the solution into tin capsules and evaporating off the liquid in a fume hood. In total 2.55 ml of sample solution were added to each capsule and the milligram equivalent of soluble sugar and starch (dry mass) was calculated from the measured concentration. Across all samples, there was on average 0.062 mg (95% CI: 0.058–0.065 mg) of soluble sugar or starch per capsule. The isotopic analyses of leaf, stem and root NSCs of four individuals of each species in each treatment at each harvest were done by isotope ratio mass spectrometry (Picarro G2131-i with Combustion module, Santa Clara, California, USA). To estimate the isotope ratios, the solid samples were combusted in an elemental analyser (EA 1110, Carlo Erba, Val de Reuil, France), and the resulting CO_2 was transferred in a helium stream via a variable open-split interface (ConFlo II, Thermo Finnigan, San Jose, CA, USA) to the mass spectrometer (Delta S, Thermo Finnigan, San Jose, CA, USA). The isotopic ratios measured were expressed in delta (δ) notation relative to the international standard Vienna Pee Dee Belemnite ($R_{V\text{-}PDB}$, $^{13}C/^{12}C = 0.0111796$; Coplen 2011). These delta values were converted to atom fraction—commonly denotated as $x^{13}C$ (see Coplen 2011 and Studer *et al.* 2014)—because atom fraction responds linearly with increasing ^{13}C label, while $\delta^{13}C$ increases exponentially with increasing ^{13}C label (Coplen 2011; Brand and Coplen 2012). The atom fraction within each plant organ was calculated following Coplen (2011):

$$x^{13}C = \frac{1}{1 + \frac{1}{((\delta^{13}C/1000)+1) \times R_{V-PDB}}}$$

These values were used to calculate the amount of isotope contributed by previously stored sugars using the mixing models described in Dawson *et al.* (2002), whereby the difference between ^{13}C of soluble sugars in the leaves prior to labelling and the observed ^{13}C in soluble sugars within stem and root organs was divided by the difference between ^{13}C of soluble sugars in the leaves prior to labelling and ^{13}C of soluble sugars in the leaves prior to drought.

$$\%\ Reallocation = 100 \times \frac{x^{13}C_{woody\text{-}postdrought} - x^{13}C_{leaves\text{-}prelabel}}{x^{13}C_{leaves\text{-}predrought} - x^{13}C_{leaves\text{-}prelabel}}$$

We detected significant increases in atom fraction in the NSC after labelling relative to before labelling, which indicates that the labelling of the seedlings with ^{13}C was successful (see Supplementary Table S2 and Supplementary Fig. S2).

Statistical analysis

We assessed the response of seedlings to drought by analyzing growth as a function of species (a fixed factor with two levels; *S. beccariana* and *S. parvifolia*), watering treatment (a fixed factor with two levels; control and no water), days since the start of no watering (a continuous variable), all possible two-way interactions and the three-way interaction. We used a linear mixed-effects model with random terms for individual seedling identity and its interaction with time (see Supplementary Table S3 for the Wald statistics). We further analyzed the biomass allocation of seedlings at the end of the experiment as a function of species (a fixed factor with two levels; *S. beccariana* and *S. parvifolia*), watering treatment (a fixed factor with two levels; control and no water), organ (a fixed factor with three levels; leaf, stem and root) and all possible two two-way interactions. We used a linear mixed-effects model with a random term for individual seedling identity (see Supplementary Table S4 for the Wald statistics).

We analysed stomatal conductance as the proportion of stomatal openness relative to the average stomatal conductance in the control as a function of species, day and the two-way interaction with random terms for individual seedling identity and its interaction with time (see Supplementary Table S5 for the Wald statistics). Stem water potential was analysed with the same function as growth but using a generalized least squares model with a separate variance structure for harvest time due to the increasing variance in response to no watering through time (see Supplementary Table S6 for the Wald statistics). To meet assumptions of linearity, the absolute value of this variable was log-transformed.

NSC response

Soluble sugar and starch allocation among organs were analyzed as a function of species (a fixed factor with two levels; *S. beccariana* and *S. parvifolia*), watering treatment (a fixed factor with two levels; control and no water), organ (a fixed factor with three levels; leaf, stem and root) and all possible two two-way interactions. We used linear models for this analysis (see Supplementary Tables S7 and S8 for the analysis of variance [ANOVA] tables).

The response of soluble sugar and starch concentrations to drought (>30 days of no water) were analysed separately as the ratio of the observed concentration in each organ after drought over the average concentration of each organ in the control. This response variable was log-transformed and analysed as a function of species (a fixed factor with two levels; *S. beccariana* and *S. parvifolia*), organ (a fixed factor with two levels; leaf and wood) and their interaction with a random term for individual seedling identity (see Supplementary Tables S9 and S10 for the Wald statistics).

Soluble sugar translocation

The percentage allocation of $x^{13}C$ to the stem and root organs from stored soluble sugars in the leaves (calculated from the mixing equation described above) was analyzed as a function of species, watering treatment and their interaction using a random term for individual seedling identity (see Supplementary Table S11 for the Wald statistics). The percentage allocation was log-transformed to meet assumptions of linearity. All analyses were performed with the asreml-R package (ASReml 4, VSN International, UK) in the R statistical software (version 3.6.1; http://r-project.org).

RESULTS

Seedling response

Height growth was decreased for *S. parvifolia* under drought resulting in significantly shorter seedlings relative to the control (difference = –4.2 cm, 95% CI: –7.2 to –1.2; Fig. 1a; Wald statistics in Supplementary Table S3), and biomass was significantly lower than in control seedlings for the three plant organs leaf (difference = –0.7 g, 95% CI: –1.2 to –0.3), stem (difference = –0.6 g, 95% CI: –1.0 to –0.1) and roots (difference = –0.5 g, 95% CI: –1.0 to –0.1) after >30 days of drought (Fig. 1b; Wald statistics in Supplementary Table S4). Seedling height of *S. beccariana* was similar in drought and control conditions (difference = –0.9 cm, 95% CI: –4.0 to 2.1; Fig. 1c), and biomass was maintained to levels similar to the control seedlings in the three plant organs leaf (difference = –0.1 g, 95% CI: –0.5 to 0.4), stem (difference = 0.1 g, 95% CI: –0.4 to 0.6) and roots (difference = 0.1 g, 95% CI: –0.3 to 0.6) organs (Fig. 1d).

Baseline stomatal conductance in control plants was higher for *S. parvifolia* (455 mmol m^{-2} s^{-1}, 95% CI: 414–498) than for *S. beccariana* (391 mmol m^{-2} s^{-1}, 95% CI: 337–444). Both species closed their stomata under drought (Fig. 1e; Wald statistics in Supplementary Table S5), although drought seedlings of *S. parvifolia* closed their stomata to a greater degree (29% of control, 95% CI: 15–43%) than drought seedlings of *S. beccariana* (44% of control, 95% CI: 30–57%). Therefore, *S. parvifolia* (132 mmol m^{-2} s^{-1}) had lower stomatal conductance under drought at the

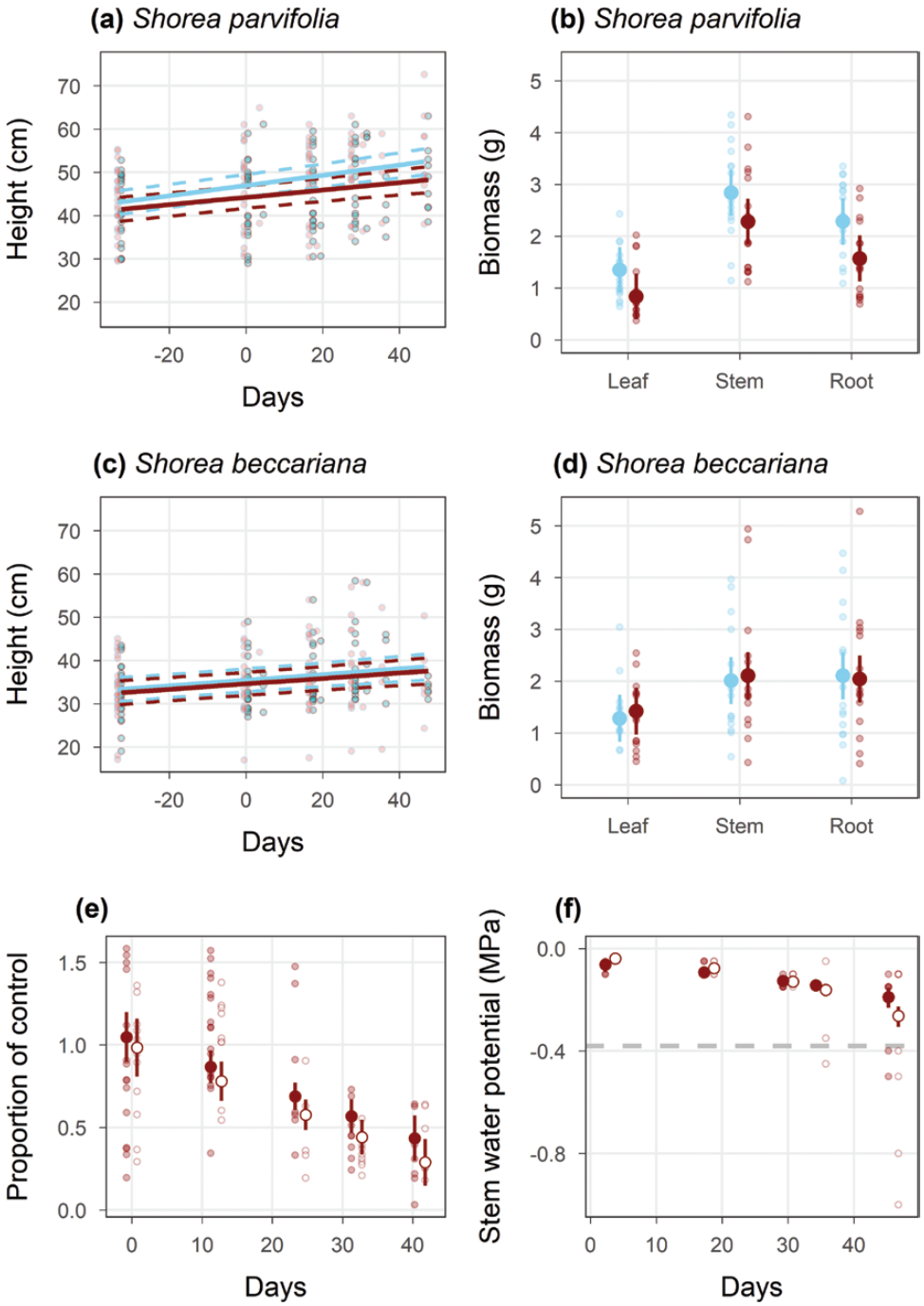

Figure 1: Seedling responses to drought. Height growth (95% CI indicated by dash lines) since the start of the drought for (**a**) drought-sensitive *S. parvifolia* and (**c**) drought-tolerant *S. beccariana* for drought (red line) and control (blue line) treatments. Biomass after >30 days of control (blue circle) and drought (red circle) conditions for (**b**) *S. parvifolia* and (**d**) *S. beccariana*. (**e**) Proportion of stomatal conductance (95% CI) relative to the control in the drought treatment since the start of the drought for *S. parvifolia* (open circle) and *S. beccariana* (closed circle). Average stomatal conductance in the control was lower for *S. beccariana* (386 mmol m^{-2} s^{-1}) than for *S. parvifolia* (465 mmol m^{-2} s^{-1}). (**f**) Stem water potential (95% CI) since the start of the drought (colours and symbols as in **e**). The grey dash line is the assumed threshold for 50% hydraulic failure for these species (Tyree *et al.* 1998). The absolute values of stem water potential were log-transformed for analysis but back-transformed for the figure.

end of the experiment than *S. becarriana* (172 mmol m^{-2} s^{-1}). Stem water potential significantly decreased below the average in control seedlings under drought (Fig. 1f; Wald statistics in Supplementary Table S6) and by a significantly greater extent in *S. parvifolia* (–0.27 MPa, 95% CI: –0.34 to –0.21) than in *S. beccariana* (–0.20 MPa, 95% CI: –0.26 to –0.15), although both species maintained stem water potentials above the 50% hydraulic failure threshold (Tyree *et al.* 1998).

NSC response

Allocation among organs of soluble sugar and starch was not significantly different between drought and control seedlings (ANOVA table in Supplementary Tables S7 and S8), but allocation of soluble sugar was significantly different between *S. parvifolia* and *S. beccariana* in the aboveground plant organs leaf (difference = 5.6%, 95% CI:

1.2–10) and stem (difference = 6.6%, 95% CI: 2.4–10.9) but not roots (Fig. 2a). The same allocation pattern was found for starch in all three plant organs leaf (difference = 10.3%, 95% CI: 6.3–14.2), stem (difference = 11.3%, 95% CI: 7.5–16.2) and roots (no difference) in both *S. parvifolia* and *S. beccariana* (Fig. 2b).

The response of soluble sugar concentrations to drought depended on species and plant organ (Fig. 2c; Wald statistics in Supplementary Table S9), whereby soluble sugars in leaf and stem and root organs were statistically similar between the drought and control seedlings of *S. parvifolia* (Fig. 2c). However, *S. beccariana* had significantly decreased leaf soluble sugars under drought relative to the control seedlings. Starch concentrations of *S. beccariana* and *S. parvifolia* of both species had significantly reduced leaf concentrations and statistically similar stem and root concentrations relative to the control (Fig. 2d; Wald statistics in Supplementary Table S10).

Soluble sugar translocation

The percentage allocation from old and fresh leaf soluble sugars to stem and root soluble sugars was statistically different between drought and control treatments and the direction of the difference was species dependent (Fig. 3; Wald statistics in Supplementary Table S10). *Shorea beccariana* allocated significantly less old soluble sugars to stem and roots in the drought than in the control treatment (difference = −9.9%, 95% CI: −26.2 to −1.2), while *S. parvifolia* allocated significantly more old soluble sugars to stem and roots in the drought than in the control treatment (difference = 13.4%, 95% CI: 3.2–19.1).

DISCUSSION

Our experiment using ^{13}C labelling to trace translocation of old and fresh soluble sugars in leaves to stem and root organs highlights two complex pathways for the maintenance of soluble sugars in stem and root organs during drought (Fig. 4). During soil water deficit, the drought-sensitive species (*S. parvifolia*) had reduced growth, conserved NSC pools and concentrations among organs and reallocated more old soluble sugars to stem and root organs than seedlings in the control conditions. In contrast, the drought-tolerant species (*S. beccariana*)

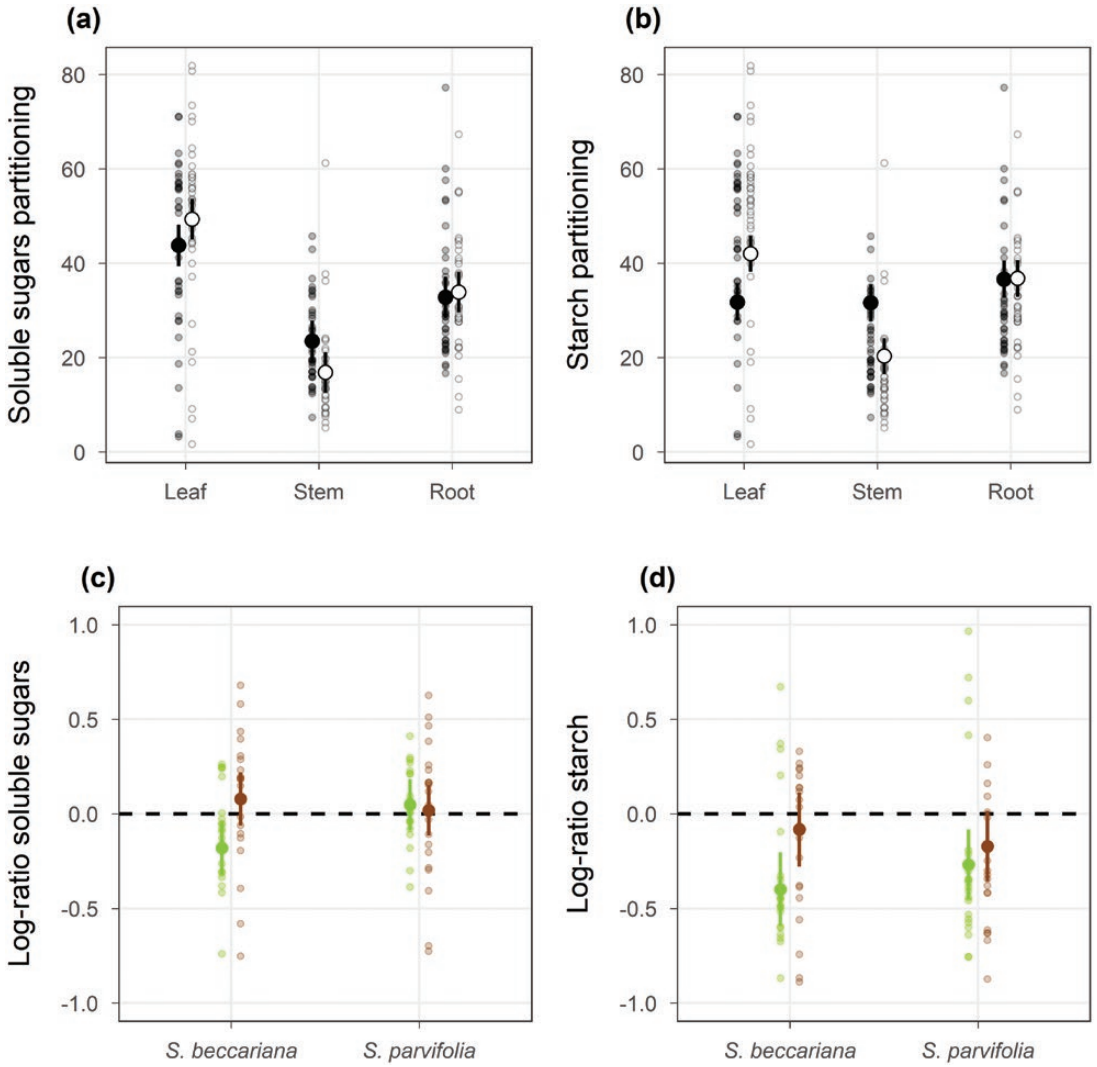

Figure 2: Response of soluble sugar and starch to drought. (**a**) Percentage of the total soluble sugar pool (95% CI) present in each of the seedling organs in *S. parvifolia* (open circle) and *S. beccariana* (closed circle). (**b**) Percentage of the total starch present in each of the seedling organs in *S. parvifolia* and *S. beccariana*. (**c**) Change in soluble sugar concentration (95% CI) relative to control in the leaf (green circle) and stem and root (brown circle) organs. (**d**) Change in starch concentration relative to control in the leaf and stem and root organs. CIs that do not cross the dash line at zero represent a significant change relative to control concentrations.

maintained growth and NSC pools relative to control seedlings but reduced leaf soluble sugar concentrations relative to control seedlings while reallocating less old soluble sugars to stem and root organs than seedlings in the control. These results indicate a more complex pattern of response in seedlings than expected (Fig. 4), which likely depended on growth and photosynthesis decoupling in the drought-sensitive species but concentration adjustments in the drought-tolerant species. The use of ^{13}C as a tracer allowed us to elucidate these different pathways in greater detail than was possible in previous studies (O'Brien *et al.* 2014, 2015) and suggests that plastic responses of soluble sugar concentrations is an important component of tolerance to decreasing water availability.

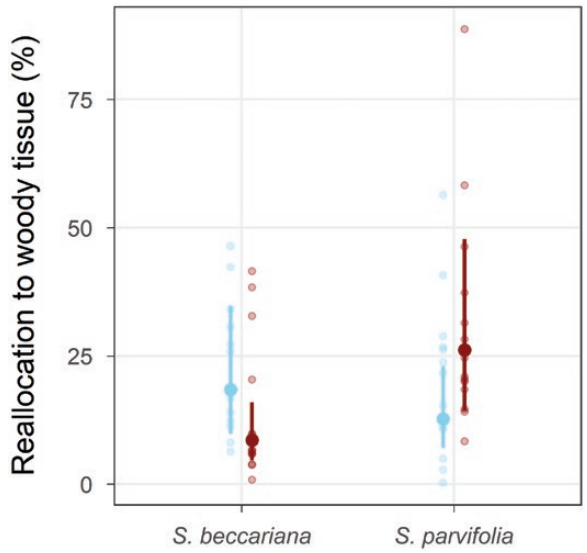

Figure 3: Reallocation of C from old leaf soluble sugars to stem and root organs. Percentage of soluble sugar C (95% CI) in stem and root organs that originated from leaf soluble sugars stored prior to the drought in the control (blue circle) and drought (red circle) treatments.

Seedling response

The faster growth in control conditions and greater growth and biomass reduction under drought of the drought-sensitive species relative to the drought-tolerant species agrees with previous work demonstrating that fast-growing species are less drought tolerant than slow-growing species (Piper 2011; Mitchell *et al.* 2013; Ouédraogo *et al.* 2013). The stomatal conductance of the drought-sensitive species also decreased by a greater proportion of the control relative to the drought-tolerant species, which suggests that it was showing stronger responses to reduced soil water availability than the drought-tolerant species (Fig. 1e). However, this proportion of stomatal closure was likely still not inhibiting photosynthesis as evidenced by the maintained NSC values in the drought-sensitive species. The similar pattern of stem water potentials during drought for both species suggests that hydraulic function was not yet inhibited by reduced soil water availability, although the drought-sensitive species had more individuals with stem water potentials below the threshold of 50% hydraulic failure than the drought-tolerant species (Fig. 1f). This maintained hydraulic function was likely due to the short duration of the drought (45 days), which is below typical drought survival times for these species (O'Brien *et al.* 2014). For example, *S. parvifolia* lives about 100 days and *S. beccariana* more than 110 days during complete dry down and both species can survive at soil water potentials below –1.5 MPa (O'Brien *et al.* 2014). This study only achieved that severity of soil water deficit at the end of the experiment (after more than 35 days). A more prolonged or severe drought might have caused larger differences in hydraulic function between these two species. Regardless, hydraulic conductance was

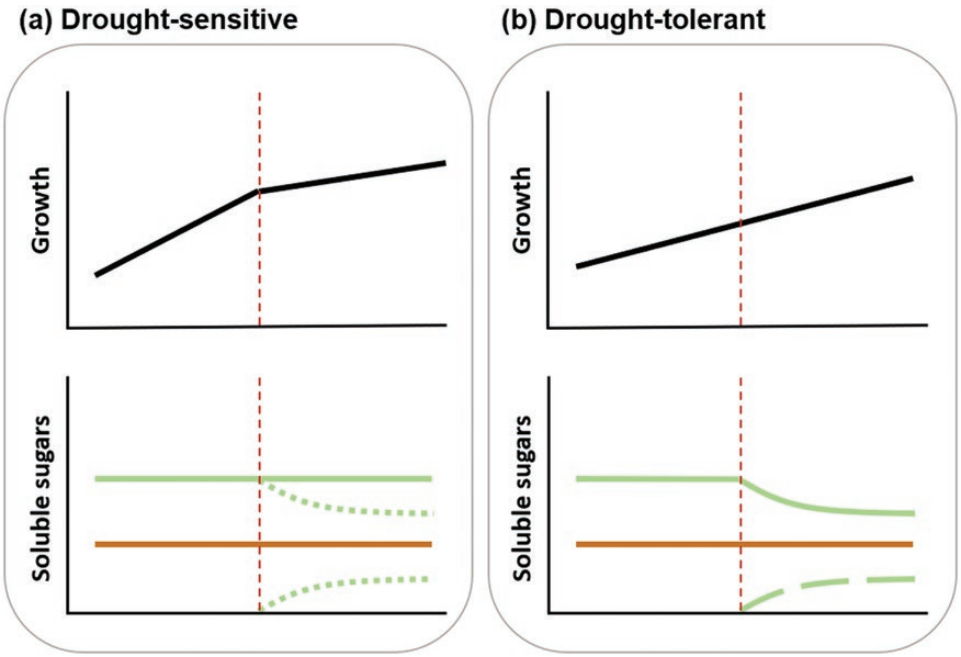

Figure 4: Conceptual description of contrasting responses. (**a**) The drought-sensitive species had reduced growth (black bent line) during drought (start indicated by red dashed line), which decouples growth and photosynthesis causing NSC accumulation. Both leaf (green solid line) and wood (brown solid line) soluble sugars were maintained at similar levels relative to control seedlings but the proportion of pre-drought stored soluble sugars (green dotted line) decreased in leaves—replaced by accumulated sugars from reduced growth—and increased in wood. (**b**) The drought-tolerant species maintained growth (black straight line) during drought but leaf soluble sugars (green curved line) declined relative to control seedlings. Wood soluble sugars were maintained *via* an increasing proportion of newly assimilated soluble sugars (green dash line).

uninhibited for most individuals, which implies that NSC movement was likely unrestricted.

NSC allocation

Allocation to NSC pools across organs did not show differences between treatments in either species despite small decreases in the biomass of the drought-sensitive species. However, species showed distinct allocation differences, whereby the drought-sensitive species maintained greater NSCs in leaves and the drought-tolerant species greater NSCs in stems (Fig. 2). Greater allocation to NSC pools in stems has been reported in shade-tolerant species relative to shade-intolerant species (Myers and Kitajima 2007), and these higher concentrations in stem and root organs promoted recovery from herbivore damage (Myers and Kitajima 2007). Prolonged drought inhibits growth and leaf dieback in these species (O'Brien *et al.* 2015), and greater storage in the stem would allow rapid recovery upon re-wetting similar to recovery of aboveground biomass in species that re-sprout (Smith *et al.* 2018). In addition, consumption of stem soluble sugars has been found during hydraulic recovery after drought in tree species (Tomasella *et al.* 2020), suggesting that greater allocation of NSCs in stems is a beneficial strategy for faster post-drought recovery.

The drought-tolerant species also adjusted NSC concentrations by reducing leaf soluble sugars and starches while maintaining stem and root sugars and starches relative to control seedlings. In contrast, the drought-sensitive species showed minimal adjustments in leaf and wood NSC concentrations (Fig. 2). Similarly, decreased soluble sugar concentrations in leaves and maintained concentrations in stem and root organs have been observed in other species as synthesized in Adams *et al.* (2017). This reduced leaf NSC concentration and sustained wood NSC concentration seems to suggest an active adjustment of NSCs in response to drought (Tomasella *et al.* 2020).

Soluble sugar translocation

Interestingly, the species showed different movement of soluble sugars in the ^{13}C tracer (Fig. 3), which suggests unique strategies in response to soil water deficits between the two species. The stem water potentials above 50% hydraulic failure indicate that drought had not yet inhibited movement of soluble sugars from the leaf to other organs. The drought-sensitive species with reduced growth had a higher proportion of old sugars in stem and root organs. The mechanism behind this is not clear, but we argue that photosynthesis decoupled growth and photosynthesis causing NSC accumulation in leaves (Muller *et al.* 2011; Palacio *et al.* 2014), which then promoted movement of older sugars to stems and roots. Therefore, the concentration of leaf and stem and root soluble sugars did not decrease (Fig. 2) but the proportion of old and fresh assimilates changed relative to the control seedlings (Fig. 4). The drought-tolerant species allocated more photosynthates to storage in stem and root organs causing a dilution of the ^{13}C label in the stem and root organs (Fig. 2 and Supplementary Fig. S2). This process was evidenced by the decrease of soluble sugars in leaves but maintained soluble sugars in stem and root organs. We have previously suggested that the movement of soluble sugars from leaf to stem and root organs could be a mechanism for the maintenance of hydraulic connectivity in these species (O'Brien *et al.* 2015). These contrasting allocation patterns suggest a passive response by the drought-sensitive species and active adjustments by the drought-tolerant species.

CONCLUSIONS

The use of multi-pulse labelling revealed a detailed pattern of NSC storage, allocation and movement underlying the differential drought responses of the two species. The most important result indicates an active movement of NSCs from leaves to stems and roots in the drought-tolerant species, and this plasticity in NSC allocation is a novel mechanism for the resistance and potentially post-drought recovery in drought-tolerant species. Here, we could only investigate one species for each strategy type. NSC allocation and concentration measurements of more species along the spectrum of drought tolerance will be required to test whether our results can be generalized and to identify potential trade-offs in this response with other traits. Our initial assessment suggests that maximum growth rate is likely a simple metric to identify active versus passive NSC responses under drought.

Supplementary Material

Supplementary material is available at *Journal of Plant Ecology* online.
Table S1: Wald-statistics of soil water potential.
Table S2: Wald-statistics of atom fraction.
Table S3: Wald-statistics of height growth.
Table S4: Wald-statistics of height biomass.
Table S5: Wald-statistics of proportion of stomatal conductance.
Table S6: Wald-statistics of stem water potential.
Table S7: ANOVA table of soluble sugar allocation.
Table S8: ANOVA table of starch allocation.
Table S9: Wald-statistics of the log ratio of soluble sugars.
Table S10: Wald-statistics of the log ratio of starch.
Table S11: Wald-statistics of 13C allocation.
Figure S1: Soil water potential for no watering and control treatments.
Figure S2: Atom fraction for both species.
Figure S3: Observed NSC concentrations in leaf and stem and root organs.

Funding

This work was supported by the Universität Zürich MSc Ecology Program with additional support by the Universität Zürich Research Priority Program on Global Change and Biodiversity (URPP-GCB). M.O.B. was supported by the Atracción de Talento Investigador Modalidad I Fellowship from the Comunidad de Madrid (grant number 2018-T1/AMB-11095) during the preparation of the manuscript.

Acknowledgements

This project was carried out with the help of the research assistants supported by the Southeast Asia Rainforest Research Partnership with special thanks for project oversight by Musa bin Boy. We appreciate the generosity of ReseaChem GmbH in Burgdorf, Switzerland, for providing affordable ^{13}C for the *in situ* labelling. This publication is number 22 from the research team associated with the Sabah Biodiversity Experiment.

Author contributions

M.O.B. and A.V. setup and designed the experiment with input from B.S. A.V. carried out the experiment and wrote an initial version of the manuscript. M.O.B. analysed the data and wrote the final manuscript. S.A. and M.S.S. provided technical guidance on the ^{13}C labelling and performed the isotope analysis. R.O. provided logistical and technical support to carry out the work in Sabah, Malaysia. M.O.B. and B.S. led the revisions of the manuscript.

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