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Research

Contrasting nonstructural carbohydrate dynamics of tropical tree seedlings under water deficit and variability

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Summary

• Drought regimes can be characterized by the variability in the quantity of rainfall and the duration of rainless periods. However, most research on plant response to drought has ignored the impacts of rainfall variation, especially with regard to the influence of nonstructural carbohydrates (NSCs) in promoting drought resistance.

• To test the hypothesis that these components of drought differentially affect NSC dynamics and seedling resistance, we tracked NSC in plant tissues of tropical tree seedlings in response to manipulations of the volume and frequency of water applied.

• NSC concentrations decreased in woody tissues under infrequent–high watering but increased under no watering. A faster decline of growth relative to stomatal conductance in the no watering treatment was consistent with NSC accumulation as a result of an uncoupling of growth and photosynthesis, while usage of stored NSCs in woody tissues to maintain function may account for the NSC decline under infrequent–high watering.

• NSCs, and specifically stem NSCs, contributed to drought resistance under severe water deficits, while NSCs had a less clear role in drought resistance to variability in water availability. The contrasting response of NSCs to water variability and deficit indicates that unique processes support seedling resistance to these components of drought.

Introduction

The importance of drought in shaping distributions of tree species in forests across the globe is increasing as climate change progressively alters the quantity (i.e. total rainfall), frequency (i.e. daily, weekly and monthly patterns) and intensity (i.e. rate of rainfall during events) of rainfall (Timmermann *et al.*, 1999; Huntington, 2006; Hartmann, 2011; Lewis *et al.*, 2011; Beier *et al.*, 2012). This increase in the role of drought in community dynamics and forest mortality has generated an increased interest in understanding traits that promote drought resistance. Nonstructural carbohydrate (NSC) concentration is one such trait that has been shown to be important for prolonging drought survival during severe water deficits (O'Brien *et al.*, 2014). Research on the role and response of NSCs to drought has commonly focused on severe deficits leading to mortality (Anderegg *et al.*, 2012; Adams *et al.*, 2013; Hartmann *et al.*, 2013; O'Brien *et al.*, 2014). However, the importance of NSC concentration for the response of trees to long-term minor deficits and variation in rainfall frequency has largely been ignored.

Plant response to drought is commonly categorized along a spectrum from isohydric (i.e. strong stomatal control with decreasing water availability) to anisohydric (i.e. weak stomatal control) (McDowell *et al.*, 2008; Mitchell *et al.*, 2013). An isohydric strategy can lead to mortality by carbon starvation if photosynthesis is inhibited and NSC reserves are depleted (McDowell *et al.*, 2008; Sala *et al.*, 2012). Anisohydric species that maintain growth and stomatal conductance during severe water deficits risk mortality from hydraulic failure and desiccation or carbon starvation as a result of water limitation of NSC transport (McDowell *et al.*, 2008; Bartlett *et al.*, 2012; Sala *et al.*, 2012; Hartmann *et al.*, 2013). The effect of fluctuating water supply on NSC dynamics is less well understood, as shifts from wet to dry soil conditions may induce a different response to that of water deficit alone (Parent *et al.*, 2008). We hypothesize three potential responses of NSC stores to variable water conditions: transient decreases in NSC concentrations as a result of use of stored NSCs for maintenance of metabolic function and growth during dry periods; transient decreases in NSC concentrations as a result of usage for rapid recovery of growth following dry periods; or transient increases in NSC concentrations as a result of an uncoupling of growth and photosynthesis during dry periods.

Furthermore, these different drought regimes may alter relative abundance among species across the isohydric–anisohydric spectrum. For example, species that maintain growth and risk hydraulic failure (i.e. anisohydric) may have an advantage under variable water conditions – which never reach the threshold of hydraulic failure before a rainfall event occurs – because

continued growth will provide them with a size advantage after continuous rainfall returns. Conversely, long-term water deficits may favour species that maintain high water potentials (i.e. isohydric) at the cost of slow growth under high water availability. However, the benefit of slower growth may be a lower risk of mortality if these species maintain hydraulic function when soil water potential drops below the wilting point during severe drought events (Bartlett *et al.*, 2012; Hartmann *et al.*, 2013). Therefore, these different aspects of drought may have differential effects on the relative abundance of species under variable rainfall versus severe drought events.

Aseasonal tropical forests provide an appropriate system to test the effects of rainfall variability and water deficits as both elements vary across a range of temporal scales. The rainfall regime of aseasonal tropical forest ecosystems is characterized by alternating short rainless periods and high or extreme rainfall, punctuated by irregular supra-annual droughts associated with El Niño southern oscillation events (Walsh & Newbery, 1999; Engelbrecht & Kursar, 2003; Engelbrecht *et al.*, 2007; Beier *et al.*, 2012). Previous work on the impacts of moisture variability in aseasonal forests has largely focused on landscape-level mortality rates as a result of severe drought (Nakagawa *et al.*, 2000; Gibbons & Newbery, 2002; Potts, 2003; Slik, 2004; Itoh *et al.*, 2012), but less has been done to examine the growth and physiological sensitivity of species to water deficits and variability and the traits that promote the ability of a species to survive and maintain function during drought (Burslem *et al.*, 1996; Tyree *et al.*, 1998; Cao, 2000; Baltzer *et al.*, 2008). Recent research has shown that higher NSC concentrations in tropical tree seedlings before drought prolong survival during drought (O'Brien *et al.*, 2014), but studying NSC concentration during drought remains necessary to elucidate the role of fluctuations of NSCs throughout various plant tissues in explaining seedling response.

We monitored NSC changes for seedlings of 10 Bornean tree species (Table 1, Supporting Information Table S1) during three different drought treatments relative to a frequently and well-watered control: minor water deficit (frequent–low volume: the frequency of application was every 2 d, the same rate as the control, but the volume of water was reduced to *c.* 20% of the control volume); variable water (infrequent–high volume: volume same as in the control but applied every 15 d); and severe deficit (no watering). We measured NSC concentrations in leaf, stem and root tissues through time for each drought treatment and examined relationships between changes in relative growth rates across species, xylem water potential responses and NSC storage or consumption in order to identify important NSC variables associated with resistance to the different drought treatments.

Materials and Methods

Study site

We conducted this experiment at the Malua Field Station (05°05′20″N, 117°38′32″E; 102 m above sea level, which is located *c.* 22 km north of Danum Valley Field Centre in the Malaysian state of Sabah (Hector *et al.*, 2011). The mean (SEM) annual rainfall recorded in Danum Valley from 1986 to 2010 was 2848.5 (94.0) mm, and over the last 100 yr, the variability of rainfall and the severity of water deficits have increased (Walsh & Newbery, 1999; Fig. S1). The experiment was conducted in 15 shadehouses under two layers of 70% shadecloth. Below the shadecloth, clear plastic polyethylene sheeting was used to exclude rainfall. The shadehouses provided the seedlings with a mean (SEM) of 3.9% (0.1%) of full daylight photosynthetically active radiation (PAR) and a mean (SEM) red : far-red ratio of 1.07 (0.01). Light was measured by simultaneous shadehouse and open sky PAR sensors (SKP 210 quantum sensor; Skye Instruments Ltd, Llandrindod Wells, UK). The temperature and humidity in the shadehouses were similar to those of measurements made at the weather station at the Malua Field Station (Methods S1).

Seedlings

Seeds from 10 species of shade-tolerant forest trees were collected during a landscape-scale masting event in August 2010 (Tables 1, S1). The species selected include eight species from the family Dipterocarpaceae, one from the Fabaceae and one from the Bombacaceae. We placed seeds under wet burlap sacks to germinate. After germination, each seed was planted into a large pot (20 cm diameter × 36 cm height; 11 l volume) filled with homogenized

Table 1 Species descriptions

Species	RLA (mm cm^{-2})	SLA (m^2 kg^{-1})	SRR (g g^{-1})	RMF (g g^{-1})	LMF (g g^{-1})	LAR (cm^2 g^{-1})
Dryobalanops lanceolata Burck	0.9 (0.2)	20.2 (0.5)	4.2 (0.2)	0.21 (0.01)	0.44 (0.02)	88.8 (4.3)
Durio oxleyanus Griffith	0.5 (0.1)	26.0 (0.5)	3.9 (0.2)	0.22 (0.01)	0.36 (0.01)	93.9 (2.2)
Hopea nervosa King	1.4 (0.1)	22.4 (0.5)	3.5 (0.3)	0.24 (0.01)	0.43 (0.02)	96.4 (5.6)
Koompassia excelsa Taub.	0.7 (0.1)	47.7 (1.1)	4.3 (0.2)	0.20 (0.01)	0.45 (0.01)	214.7 (6.1)
Parashorea malaanonan Merr.	1.6 (0.2)	25.6 (1.3)	3.3 (0.3)	0.26 (0.02)	0.44 (0.01)	114.5 (6.7)
Parashorea tomentella Meijer	0.9 (0.1)	23.7 (1.0)	3.3 (0.2)	0.24 (0.01)	0.45 (0.01)	104.9 (4.4)
Shorea argentifolia Symington	1.6 (0.2)	26.4 (0.5)	4.9 (0.3)	0.18 (0.01)	0.54 (0.01)	144.0 (4.1)
Shorea beccariana Burck	1.2 (0.1)	24.8 (0.7)	4.7 (0.3)	0.19 (0.01)	0.56 (0.01)	137.8 (4.5)
Shorea macrophylla Ashton	0.5 (0.1)	15.7 (0.6)	4.6 (0.2)	0.19 (0.01)	0.42 (0.01)	66.3 (3.7)
Shorea parvifolia Dyer	1.5 (0.2)	25.9 (0.7)	4.9 (0.3)	0.18 (0.01)	0.53 (0.01)	136.8 (4.4)

Mean (SE) allocation metrics from the frequent watering treatment are given. RLA, rooting depth : leaf area; SLA, specific leaf area; SRR, shoot : root ratio; RMF, root mass fraction; LMF, leaf mass fraction; LAR, leaf area ratio.

forest soil within the shadehouses. Seedlings were watered every 2 d for 3 months until all seedlings had abscised their cotyledons. We replaced individuals that died during the first 2 months from nursery-grown seedlings that had been collected during the same masting event.

Experimental design

On 19 November 2010, we assigned at random a selection of 22 seedlings of each species to each of four treatments (Fig. S2): 240 mm of water per month distributed in equal amounts every 2 d (frequent–high volume; control); 50 mm of water per month distributed in equal amounts every 2 d (frequent–low volume); 240 mm of water per month distributed in equal amounts every 15 d (infrequent–high volume); and complete dry down (no water).

Seedling growth, biomass and leaf area

We measured the height, diameter and leaf area of every seedling before the start of the treatments and every month thereafter. Leaf area was estimated from measurements of the length and width of every leaf on each seedling. These measures were used to fit linear models of leaf area from destructive harvests of seedlings (mean R^2 for all species = 0.93, range = 0.86–0.97; Fig. S3). Loss of leaf area from herbivory or browning was estimated visually as the percentage of the whole leaf lost in 5% increments.

We destructively harvested a sample of seedlings before the start of the treatments and then *c.* 30, 60 and 120 d after the treatments began. A final harvest of all living seedlings occurred 150 d after the treatments began. We harvested one seedling of each species in each treatment during the predawn (03:00–06:00 h), morning (08:00–10:00 h), midday (12:30–15:00 h) and late-afternoon (17:00–19:00 h) periods and made a variety of trait measurements on each individual. In order to assess the water status of each seedling, we measured leaf water potential on a single leaf (or a cluster of leaves for small seedlings when petioles were too small to fit into the chamber) and stem water potential on a single segment of the stem (*c.* 8 cm in length) using a Scholander pressure chamber (model 670; PMS Instrument Co., Corvallis, OR, USA). We took photographs of all the leaves and analysed their leaf area using ImageJ software (Rasband, 2013). These measured leaf areas were used to generate the linear models for estimating leaf area from the length and width measurements (Fig. S3). We removed all soil and measured the longest root length. Seedlings were dried at 64°C to a constant weight, and we weighed leaves, stem and roots separately to assess biomass allocation.

Seedling monitoring

We monitored the seedlings every 2 d for changes in leaf morphology. We recorded the date of leaf browning, decline in leaf angle, initial leaf loss and total leaf loss. Approximately every 10 d, we made measures of stomatal conductance (g_s) from 08:00 h until 18:00 h with a steady-state diffusion porometer (model SC-1; Decagon Devices Inc., Pullman, WA, USA). During each daily course, we performed measurements on 120 seedlings (three individuals of each species in each treatment) every 3 h. Each of the 120 seedlings received three stomatal conductance measurements per daily course (morning, midday and afternoon). Midday stomatal conductance through time was used to assess the effect of treatments on stomatal closure.

Environmental conditions

In order to measure temperature differences among treatments, we placed Thermocron Ibuttons (model DS1921G-F5#; Maxim Integrated Products Inc., Sunnyvale, CA, USA) in all four treatments in each shadehouse. In order to capture air and soil temperature differences, Ibuttons were buried 5 cm in the soil and suspended on a stick 5 cm above the soil and set to a 30-min measurement interval. Volumetric soil moisture content at the top and bottom of the pot was measured on one to two seedlings of each species in each treatment every week with an ML2x Theta Probe and HH2 moisture meter (Delta-T Devices; Burwell, Cambridge, UK). The relationship between soil water potential and volumetric soil moisture content was determined using the filter paper method (Deka *et al.*, 1995; O'Brien *et al.*, 2013). We measured relative humidity near the shadehouses every 30 min throughout the course of the experiment with a digital humidity probe (SKH 2000 probe; Skye Instruments Ltd).

NSC analysis

We used three to four seedlings of each species from the first harvest, one seedling of each species in each treatment for each intermediate harvest and three to four seedlings of each species in each treatment from the last harvest to quantify NSC concentrations in the leaf, stem and root. Seedlings of three species did not survive until the final harvest and therefore the last seedlings to die were used for NSC analysis. At harvest, seedlings were immediately microwaved to stop enzymatic activity. We ground tissue samples with a ball mill and used 15–16 mg of sample for NSC analysis. We extracted soluble sugars with 80% ethanol at 27°C for one night followed by two additional 2-h periods (Marquis *et al.*, 1997; Myers & Kitajima, 2007). We digested the remaining starch with amyloglucosidase (A-7420; Sigma-Aldrich). The concentrations (mg mg^{-1}) of simple sugars and starch (measured as glucose equivalents) were measured at 487 nm by spectrophotometry after a 30-min phenol-sulphuric acid reaction (Dubois *et al.*, 1956; Ashwell, 1966). We used the weighted NSC concentration for total NSCs, starch and soluble sugars in a seedling to account for differences among seedlings in tissue biomass (i.e. each tissue concentration was multiplied by its dry biomass and their sum was divided by the whole seedling dry biomass).

Analysis

In order to assess the amount of soil drying and rewetting in the infrequent–high watering treatment, we modelled soil water potential in this treatment as a function of time (a continuous

variable, in days) interacting with a variable for soil water potential before and after watering (a fixed factor with two levels, before and after water addition) using a linear mixed-effects model with a random intercept for individual seedlings. The absolute value of soil water potential was log-transformed to meet assumptions of linearity, and the results are presented on the transformed scale; therefore, a steeper positive slope indicates faster soil drying. We used a linear mixed-effects model to assess declines in soil water potential in the no watering treatment as a function of time since the start of the treatment (a continuous variable, in days) and species identity (a fixed factor with 10 levels) with a random intercept for individual seedlings. Again, the absolute value of soil water potential was log-transformed to meet assumptions of linearity. We analysed soil temperature as a function of treatment (a fixed factor with four levels) using linear mixed-effects models with random intercepts for hour of the day, date and individual seedling.

We assessed seedling response to drought treatments by analysing living biomass (dead material was given a biomass of zero to account for the negative effect of dieback and mortality) as a function of time since the start of the experiment (a continuous variable), watering treatment (a fixed factor with four levels), species identity (a fixed factor with 10 levels) and all interactions (including the three-way interaction between treatment, species and time) with a generalized least squares model. We used initial leaf area for each individual seedling as a covariate to account for differences in initial seedling size, which was necessary to account for differences in water use (Fig. S4). The results of this analysis allowed us to calculate a relative growth rate of biomass (RGR) for each species in each treatment using an initial standardized seedling size (125 cm^2). In order to assess the effect of watering treatments on drought response among species, we calculated the difference in mean RGR between a treatment and the control treatment for each species as an assessment of drought resistance (ΔRGR). Therefore, a negative ΔRGR value for a species in a drought treatment indicates that the treatment reduced mean RGR relative to the control – as a result of slower growth, decreasing size or dieback/mortality (i.e. zero living biomass).

We analysed the response of NSC concentration as a function of time since the start of the experiment (a fixed factor with five levels, one for each harvest), watering treatment (a fixed factor with four levels) and their interaction. A random intercept for species identity was used in the model (a random factor with 10 levels). Furthermore, in order to examine the changes in NSCs within seedlings, we analysed the NSC concentration in leaf, stem and root tissues and for starch and soluble sugars separately using the same model as that used for NSC concentration (Fig. S5). Additionally, we calculated the difference in woody tissue (average allocation to stem and root; ΔWoody) and leaf tissue (ΔLeaf) between the initial and all other time-points for NSC, soluble sugar and starch concentrations. In order to determine the effects of watering treatments on changes in allocation, we analysed ΔWoody and ΔLeaf tissue concentrations as a function of treatment (a fixed factor with four levels). We plotted these metrics against each other for NSC, soluble sugar and starch concentrations and tested the difference in allocation to source (leaf tissue) and sink tissues (woody tissues) under each treatment relative to the initial allocation.

We used Pearson correlations between ΔRGR across species in each drought treatment and species traits in the control treatment to determine the traits that improved drought resistance. We used traits from the control treatment because the relationship between baseline traits and drought response was of interest and not the effect of drought on traits. The traits assessed in the correlation analysis were average initial biomass, RGR in the control treatment, total seedling and leaf, stem and root NSC concentrations and total seedling and leaf, stem and root starch and soluble sugar concentrations. A metric for xylem sensitivity to decreasing soil moisture potential – calculated as the mean difference in stem water potential and soil water potential in the no watering treatment at the last two harvests ($\Psi_x - \Psi_s$) – was used to assess the role of xylem tolerance to soil drying in drought resistance. We also used ΔWoody tissue soluble sugar concentration in order to assess the role of variation in osmotically important components for drought resistance. Significant correlations between ΔRGR and baseline traits were analysed with generalized least squares models to assess the effect of baseline traits on drought resistance. Standardized major axis regression (which accounts for variation in both variables) was used to visualize relationships between correlated traits (Warton *et al.*, 2006).

The effect of treatments on soil water potential and NSC concentration was analysed with the lme function in the nlme package (Pinheiro & Bates, 2000) of the R statistical software version 3.0.2 (R Development Core Team, 2013). Generalized least squares analysis on ΔRGR and traits was performed with the gls function in the nlme package (Pinheiro & Bates, 2000). Correlations were performed with the rcorr function in the Hmisc library. Standardized major axis regression between traits was performed with the sma function in the smatr package (Warton *et al.*, 2012). Analysis of soil temperature was performed with the lmer function in the lme4 library because of the complexity of the random effects (Bates *et al.*, 2013), and we calculated the 2.5% and 97.5% quantiles from 1000 resamples of the parameter estimates using the sim function in the arm library in order to obtain 95% confidence intervals (CIs) around temperature estimates because the lme4 library does not produce CIs (Gelman & Hill, 2007; Bagchi *et al.*, 2011).

Results

Soil drying

There were no differences in mean soil temperature between treatments (control, 25.5°C (95% CI 25.0–25.9°C); frequent–low, 25.4°C (95% CI 25.0–25.7°C); infrequent–high, 25.6°C (95% CI 25.2–26.0°C); and no water, 25.5°C (95% CI 25.1–25.9°C)). Soil water potential remained high in the control, frequent–low and infrequent–high watering treatments (Fig. 1a–c). However, pre-watering soil water potential in the infrequent–high watering treatment declined with days since the start of the treatment (slope of $\log_e$ MPa with days, 0.004; 95% CI 0.003–0.005), indicating that soil water was depleted more rapidly as

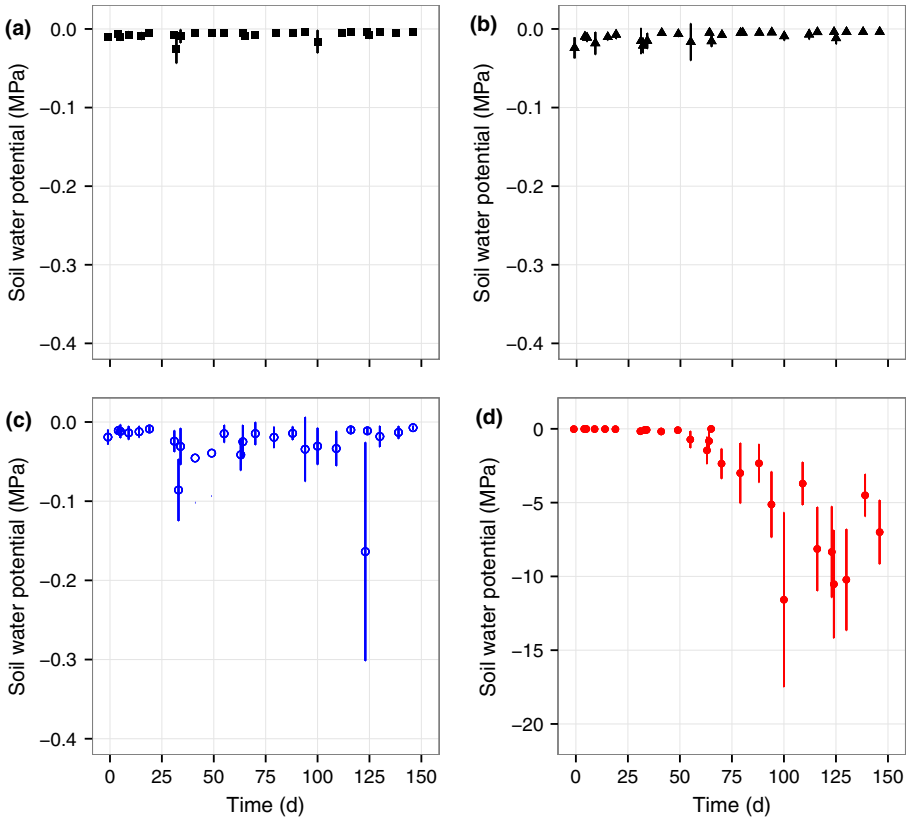

Fig. 1 The soil water potential (mean ± 95% confidence interval) of each treatment throughout the course of the experiment. (a) Control (black squares), (b) frequent–low watering (black triangles), (c) infrequent–high watering (blue circles) and (d) no watering (red circles). Note the change in scale in (d).

plants grew larger. Post-watering soil water potential in the infrequent–high watering treatment remained relatively constant through time (slope of $\log_e$ MPa with days, −0.0002; 95% CI −0.002 to 0.002), which implies that every watering event was recharging the soil water to an equivalent extent. Soil water potential began to decline significantly from zero in the no watering treatment after *c.* 45 d (Fig. 1d). This decline varied slightly among species, with *Durio oxleyanus* having the steepest change (slope of $\log_e$ MPa with days, 0.07; 95% CI 0.04–0.09) and *Hopea nervosa* having the slowest change (slope of $\log_e$ MPa with days, 0.04; 95% CI 0.03–0.05; Fig. 2a).

Seedling mortality, growth and stomatal response

Average mortality of species was not significantly different among the control, frequent–low and infrequent–high watering treatments (control: 2.3%, 95% CI 0.2–4.3%; frequent–low: 2.3%, 95% CI 0.5–5.1%; and infrequent–high: 1.4%, 95% CI 0.0–2.8%) but was significantly higher in the no watering treatment (29.5% (95% CI 18.8–40.3%)). All species exhibited higher mortality in the no watering than in the control, frequent–low and infrequent–high watering treatments (Table S2).

The timing and extent of leaf loss varied among species, with *Koompassia excelsa* dropping leaves first and *H. nervosa* last in response to no water. During the 150 d of no water, only three species had a positive RGR (*H. nervosa*, *K. excelsa* and *Shorea parvifolia*), and only *H. nervosa* had an RGR statistically indistinguishable from that of the control. *Shorea macrophylla* had the fastest decline in living biomass ($-0.15\ g\ g^{-1}\ d^{-1}$; 95% CI -0.23 to $-0.1\ g\ g^{-1}\ d^{-1}$; Fig. 2b). Because large seedlings declined faster as a result of earlier soil drying (i.e. higher water demands), we also calculated the decline in soil water potential with time for each species in the no watering treatment and modelled RGR as a function of soil water potential instead of days. The decline in RGR among species with decreasing soil water potential was significantly correlated with the decline in RGR with days (Pearson $r = 0.95$; 95% CI 0.8–0.99). As these two metrics were highly correlated, we retained the use of RGR as a function of days in order to maintain comparisons across treatments instead of using RGR as a function of soil water potential solely for no watering.

Dryobalanops lanceolata, *Shorea argentifolia* and *Shorea beccariana* had significantly lower RGR in the infrequent–high watering treatment relative to the control, while all other species had RGRs that were statistically indistinguishable from the control values (Fig. 2b). *Dryobalanops lanceolata* and *S. beccariana* were the only species that grew significantly less in the frequent–low watering treatment relative to the control (Fig. 2b).

Stomatal conductance was statistically indistinguishable among all treatments for 60 d (Fig. S6). After 80 d, stomatal conductance in the no watering treatment was statistically lower than in the other three treatments mean stomatal conductance difference between no watering and other treatments = –166 mmol $m^{-2}\ s^{-1}$, 95% CI –299 to –33). In this treatment, mean stomatal conductance was significantly below 100 mmol $m^{-2}\ s^{-1}$ after 100 d and reached a minimum after 150 d (*c.* 10 mmol $m^{-2}\ s^{-1}$). The other three treatments were never statistically distinguishable from each other for the entire experiment.

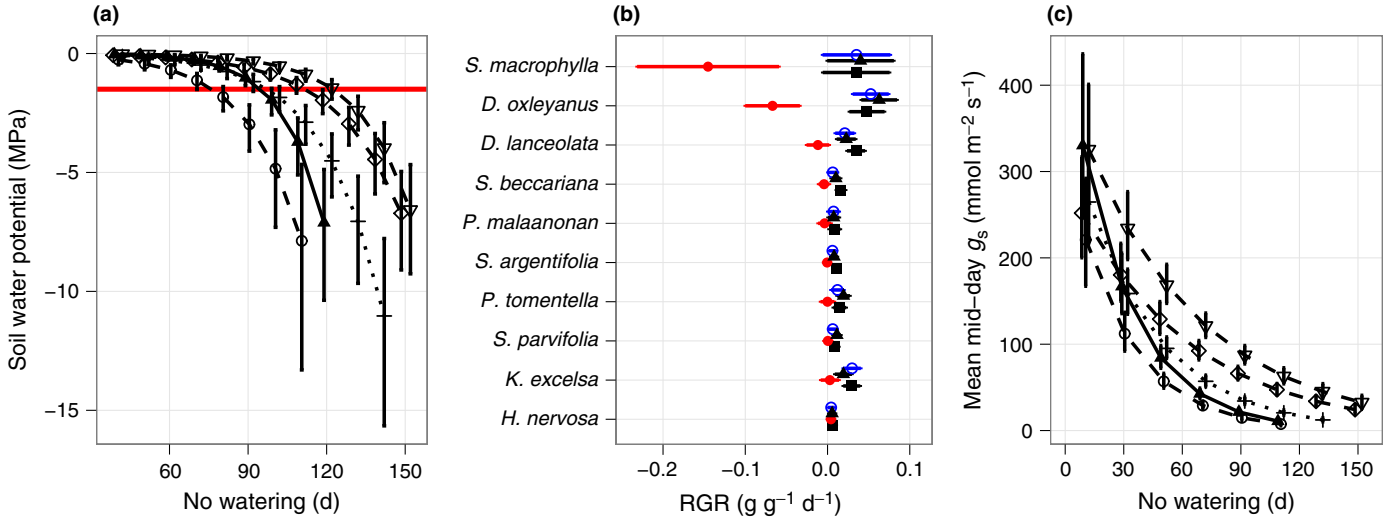

Fig. 2 Soil water potential, relative growth rates (RGRs) and stomatal conductance. (a) The lines represent the model estimates (mean ± SEM) of soil water potential as a function of days of no watering, species and their interaction. Estimates are back-transformed from log-transformed absolute values. The red line represents an assumed wilting point at −1.5 MPa (only the following species are presented for readability, and they represent the spectrum of initial leaf areas: crosses, *Dryobalanops lanceolata*; open circles, *Shorea macrophylla*; closed triangles, *Durio oxleyanus*; downward triangles, *Parashorea malaanonan*; open diamonds, *Hopea nervosa*). (b) The points represent the mean (95% confidence interval) for each species in each treatment (red circles, no water; blue circles, infrequent–high water; black triangles, frequent–low water; black squares, control). Species are ordered from the most negative RGR to the least negative RGR in the no watering treatment (top to bottom). All species except *H. nervosa* had significantly slower growth under no watering compared with the control. (c) Mean (SEM) midday stomatal conductance in the no watering treatment. The same species are presented as in (a). *K. excelsa*, *Koompassia excels*; g_s, stomatal conductance.

Species varied in their decline in stomatal conductance, with *Parashorea malaanonan* dropping significantly below 100 mmol m^{-2} s^{-1} after 100 d while *S. macrophylla* took only 44 d (Fig. 2c).

NSC response

NSC concentrations in seedlings varied with drought treatment: no watering caused a significant increase in NSCs above the control, while seedlings exposed to infrequent–high watering had significantly lower NSC concentrations than those in the control (Fig. 3a). In the no watering treatment, leaf NSC concentration increased above that of the control for 30 d (mean difference in leaf NSC between no watering and control, 3.2%; 95% CI 0.7–5.6%; Fig. 3b) and then decreased to values similar to those of the other treatments. Stem NSC concentration in the no watering treatment increased over the 150 d to concentrations significantly higher than those of the control (mean difference in stem NSCs between no watering and control, 3.8%; 95% CI 1.5–6.0%; Fig. 3c), while root NSCs remained higher in the no watering treatment but fluctuated more, reaching values significantly higher than the control values after 30 d (mean difference, 4.0%; 95% CI 1.4–6.7%) and 150 d (mean difference, 3.6%; 95% CI 1.1–6.1%; Fig. 3d). Soluble sugars remained relatively constant through time in the leaf, stem and root, while starch concentrations increased in the stem and root (Fig. S5g,k).

NSC concentration in the infrequent–high watering treatment contrasted with that of the no watering treatment, with a decline to minimum values at *c.* 60 d (mean difference in stem NSCs between infrequent–high watering and control, −2.0%; −3.7 to −0.4%) followed by a recovery to a concentration similar to that of the control (Fig. 3a). Stem NSC concentration was the main driver of this decline as it reached a minimum at 60 d (Fig. 3b). This decline was evident in both starch and soluble sugar concentrations, but the recovery was delayed in soluble sugars (Fig. S5e–l). NSC concentrations in the frequent–low watering treatment were statistically indistinguishable from those of the control throughout the experiment.

Under no watering, woody tissue significantly increased in total NSC concentration (2.3%; 95% CI 1–3.5%), which was driven by a significant increase in starch (2.4%; 95% CI 1.2–3.5%; Fig. 4a,b). Soluble sugars in woody tissue on average were not significantly different from values at the initial time-point under no watering (Fig. 4c). Under infrequent–high watering, woody tissue significantly decreased in total NSC (−1.0%; 95% CI −2.0 to −0.1%; Fig. 4a) and soluble sugar (−1.3%; 95% CI −1.8 to −0.7%; Fig. 4c) concentrations, while on average starch concentration remained similar to that at the initial time-point. Furthermore, soluble sugars in woody tissues were significantly lower under infrequent–high watering than in the controls (difference between infrequent–high and control watering, 0.5%; 0.1–0.8%). Allocation under control and frequent–low water was similar for all NSC components and only varied significantly from the initial time-point with a decrease in soluble sugars (−0.8%; −1.3 to −0.3%; Fig. 4c). Allocation to leaf tissue was statistically indistinguishable from that at the initial time-point for all treatments (Fig. 4).

Baseline traits and drought resistance

In the infrequent–high watering treatment, ΔRGR was positively correlated with ΔWoody soluble sugars ($r = 0.74$; 95% CI

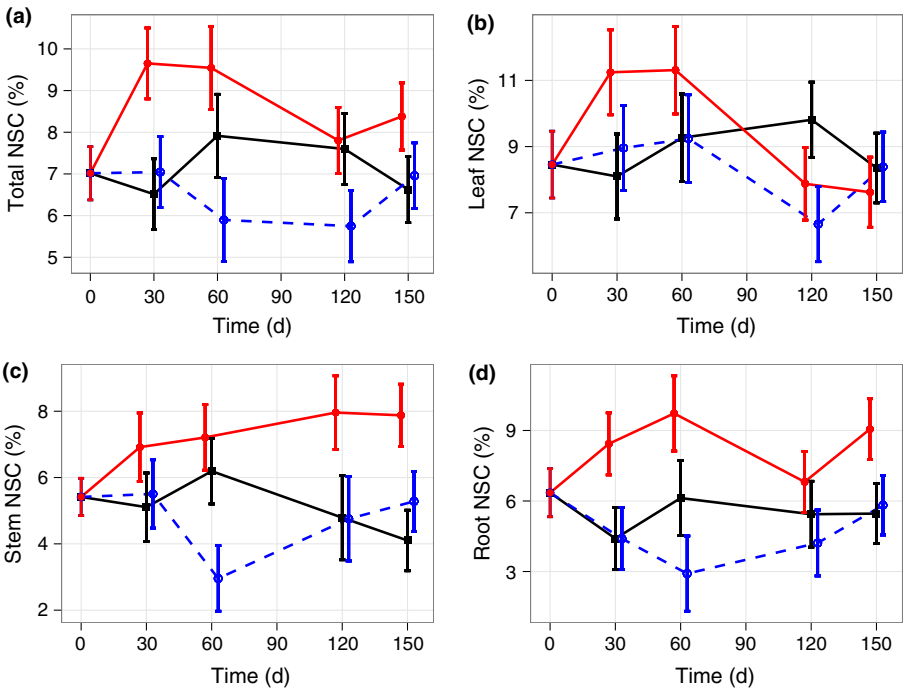

Fig. 3 Change in total, leaf, stem and root nonstructural carbohydrates (NSCs) for no water (red circles), infrequent–high water (blue circles) and the control (black squares). (a) Total NSC concentration was significantly lower at 60 and 120 d under infrequent–high watering and significantly higher at 30 and 150 d under no watering relative to the control. (b) Leaf NSC concentration was significantly higher at 30 d under no watering and significantly lower after 120 d under both infrequent–high and no watering. (c) Stem NSC concentration was significantly lower at 60 d under infrequent–high watering and significantly higher after 120 d under no watering relative to the control. (d) Root NSC concentration was significantly higher after 30 and 150 d under no watering relative to the control. NSC concentration in the controls was statistically indistinguishable from that at day zero in all tissues at all sampling times. The points represent the mean (± SEM) for all species at each time-point.

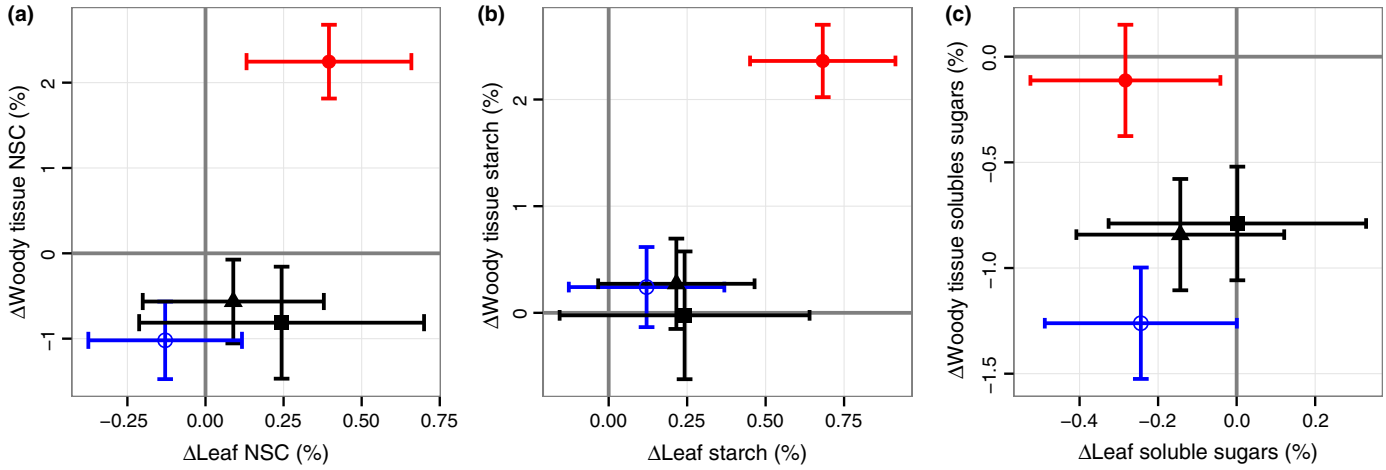

Fig. 4 Change in total nonstructural carbohydrates (NSC), starch and soluble sugar concentrations between the initial time-point and all other time-points for all treatments (mean ± SEM). (a) Woody tissue (ΔWoody) had a significantly lower NSC concentration under infrequent–high watering (blue circles) and a significantly higher NSC concentration under no watering (red circles), while NSC concentration in leaf tissue (ΔLeaf) was not statistically different from that at the initial time-point. Control (black squares) and frequent–low (black triangles) watering were statistically similar in both tissues through time. (b) Starch concentration in woody tissue was significantly higher through time under no watering but was similar through time for all other treatments. Starch in leaf tissue was statistically indistinguishable among treatments and through time. (c) Only no watering had a similar soluble sugar concentration through time in woody tissue, while all other treatments had significant decreases in woody tissue soluble sugars, with infrequent–high watering declining the most through time. Leaf tissue soluble sugar was statistically indistinguishable among treatments and through time.

0.2–0.9), which indicates that species with a greater negative biomass response to infrequent–high watering also had a larger reduction in soluble sugar concentrations in their woody tissues. In the frequent–low watering treatment, ΔRGR declined with increasing initial stem NSCs (slope of stem NSCs to ΔRGR for frequent–low, −0.005; 95% CI −0.01 to −0.002). Initial size negatively affected ΔRGR in the no watering treatment (slope of ΔRGR with increasing biomass, −0.03; 95% CI −0033 to −0.02; Fig. 5a), whereby larger seedlings had more negative ΔRGR. Initial size did not significantly affect the other two drought treatments (Table S3). Fast growth in the control negatively affected ΔRGR (slope of ΔRGR with increasing RGR in the control, −3.1; 95% CI −5.2 to −1.0; Fig. 5b). ΔRGR in the no watering treatment significantly increased with NSC concentration (slope of ΔRGR with increasing NSC concentration, 0.03; 95% CI 0.01–0.04; Fig. 5c) and stem NSC concentration (slope of ΔRGR with increasing stem NSC concentration, 0.03; 95% CI −0.00007 to 0.1; Fig. 5d). Initial biomass and RGR in

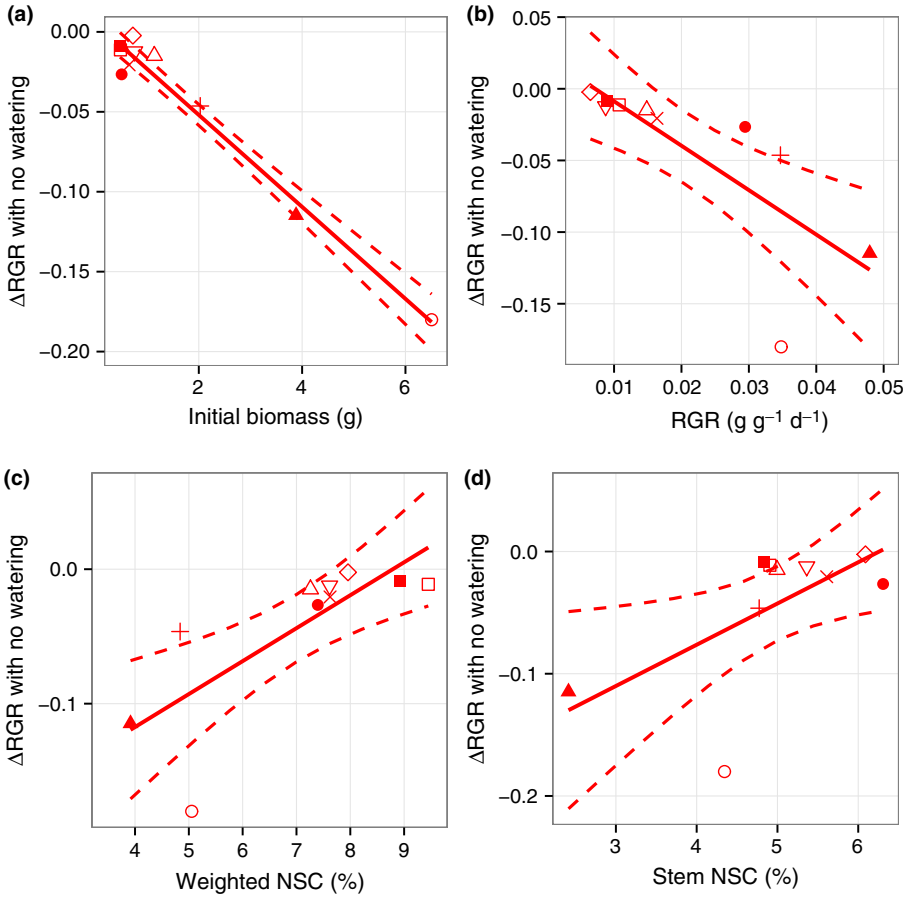

Fig. 5 The difference in relative growth rates (RGRs) between a treatment and the control watering treatment (ΔRGR) as a function of baseline traits. (a) ΔRGR declined significantly with increasing initial seedling biomass. (b) ΔRGR declined significantly with increasing RGR in the control treatment. (c) ΔRGR increased significantly with increasing baseline total nonstructural carbohydrates (NSCs). (d) ΔRGR increased significantly with increasing baseline stem NSCs. Lines are generalized least squares model fits, and dashed lines represent 95% confidence intervals. Points represent mean values for each species (cross, *Dryobalanops lanceolata*; closed triangle, *Durio oxleyanus*; open diamond, *Hopea nervosa*; closed circle, *Koompassia excels*; downward open triangle, *Parashorea malaanonan*; upward open triangle, *Parashorea tomentella*; open square, *Shorea argentifolia* × *Shorea beccariana*; open circle, *Shorea macrophylla*; closed square, *Shorea parvifolia*).

the control were highly correlated ($r = 0.7$; 95% CI 0.1 to −0.9; Fig. 6a). However, these variables traded off with weighted NSC concentration (biomass–NSC concentration, $r = -0.8$ (95% CI −0.9 to −0.3) and RGR–NSC concentration, $r = -0.9$ (95% CI −0.97 to −0.6), respectively; Fig. 6b,c). RGR in the control treatment also traded off with stem NSC concentration ($r = -0.7$ (95% CI −0.9 to −0.03); Fig. 6d; Table S4).

Discussion

We examined the effect of multiple components of the drought regime on NSC storage and depletion, and the importance of NSC storage in mediating the drought response of tropical tree seedlings. Interestingly, infrequent–high watering (alternation between sufficient water and deficit) and no watering (sustained and increasing water deficit) were associated with contrasting patterns of NSC concentration in woody tissues, resulting in accumulation of NSCs under sustained water deficit and consumption of NSCs under a variable water regime. These NSC responses were coupled with contrasting growth responses to drought, which suggests that accumulation occurred in response to inhibited growth under no water and depletion occurred as a result of the maintenance of growth, especially in the first 120 d, under infrequent–high water. NSC traits were not strong predictors of seedling growth under frequent–low and infrequent–high watering. However, under prolonged severe drought with no water, species with greater total and stem NSC concentrations, slower growth and smaller initial size were less susceptible to reduction in growth during drought. These results suggest that NSC storage trades off with growth rate when water availability is nonlimiting and that baseline NSC storage is indicative of the relative drought resistance among species.

Differential NSC response to infrequent–high water and no water

The contrasting effects of infrequent–high and no water on NSC concentration indicate differential seedling responses to the two treatments: NSC consumption and continued growth under infrequent–high water and NSC accumulation and inhibited growth under no water. Usage of NSC stores in the stem and roots to maintain metabolic function and sustain growth may account for the decrease in NSC concentrations during the first 120 d in the infrequent–high watering treatment. Alternatively, NSC use for growth following watering events may also cause the decrease in NSC concentration in woody tissues, but measurements of NSC concentrations pre- and post-watering would be necessary to distinguish between these two mechanisms. Not surprisingly, the strongest declines occurred in osmotically active soluble sugars, which may support the hypothesis that NSC is being used during short dry periods to maintain function. However, there was no evidence for translocation of NSCs from

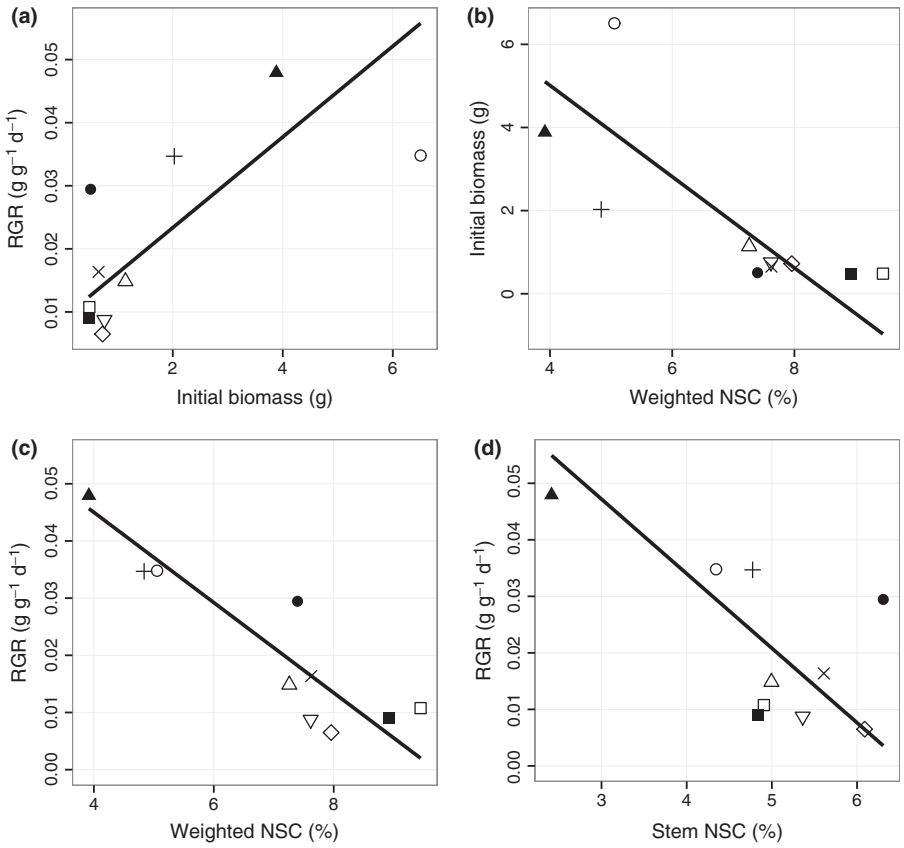

Fig. 6 Relationships between mean baseline trait values among species. (a) Relative growth rate (RGR) was significantly positively correlated with initial biomass, but both (b) biomass and (c) RGR traded off with baseline nonstructural carbohydrate (NSC) concentration. (d) RGR also traded off with baseline stem NSC concentration. Standardized major-axis lines are used for visualization of the relationship, and points represent mean values for each species (cross, *Dryobalanops lanceolata*; closed triangle, *Durio oxleyanus*; open diamond, *Hopea nervosa*; closed circle, *Koompassia excels*; downward triangle, *Parashorea malaanonan*; upward triangle, *Parashorea tomentella*; open square, *Shorea argentifolia*; cross, *Shorea beccariana*; open circle, *Shorea macrophylla*; closed square, *Shorea parvifolia*).

source to sink tissues, as leaf tissue concentration never deviated significantly from initial concentrations.

In the infrequent–high watering treatment, NSC concentrations recovered to values that were similar to those of the control after 150 d. We hypothesize this was attributable to an increase in water deficit between watering events as seedlings grew larger and water demands increased causing growth to slow or stop and NSCs to accumulate. This hypothesis is supported by the significant decrease in pre-watering soil moisture availability through time in the infrequent–high watering treatment. Alternatively, an adaptation of seedlings to infrequent–high watering with time by increased allocation to root growth would reduce the effect of the dry periods and may explain the NSC recovery. Therefore, species could avoid the dry period and recover their NSC concentrations (Poorter & Markesteijn, 2008; Markesteijn & Poorter, 2009). Our results cannot directly elucidate the cause of this recovery in NSC concentrations, but there is no support for adaptation of morphology because we did not find significant changes to the root mass fraction or the root mass to leaf area ratio in the infrequent–high watering treatment (Fig. S6). Furthermore, the trajectory of change in the stem and root NSC concentrations indicates a steady increase from 60 to 150 d (Fig. 3c, d), and if NSC concentrations continued to increase, then initial NSC accumulation from uncoupled growth and photosynthesis can be assumed.

The increase in NSC concentrations in the no watering treatment is consistent with an uncoupling of growth and photosynthesis (Muller *et al.*, 2011). Growth began to decline significantly at *c.* 30 d of no water (Fig. S4), while stomatal closure did not decline significantly until 45 d of no watering for a few species and more than 70 d for most species (Fig. 2c), while sustained low conductance (< 100 mmol m^{-2} s^{-1}) was not achieved until 80 d of no watering (Fig. S6). Therefore, this decline in growth probably occurred before photosynthesis was inhibited, resulting in an accumulation of NSCs (Muller *et al.*, 2011). Our results support other studies that have found an accumulation of stored NSCs in fast-growing species (Mitchell *et al.*, 2013). However, a dry-down experiment in pots has its limitations as it represents a simplified drought scenario. It is possible that soil drying could occur more rapidly during a natural drought than in our experiment as a result of reduced cloud cover, more competition for soil water with overstorey trees and higher vapour pressure deficits. Research examining plant response during severe El Niño-driven droughts is the next step to validate our results in a natural setting.

Interestingly, leaf NSC concentrations behaved similarly in all treatments (remaining statistically indistinguishable among treatments and across time), although an initial increase in the no watering treatment for 30 d was observed. Furthermore, starch increased more than soluble sugars under no watering (Fig. S5g, k), while under infrequent–high watering only soluble sugars decreased. This result may indicate that soluble sugars are important for short-term stress tolerance, while accumulation under severe water shortage promotes starch storage.

Traits promoting drought resistance

Resistance to severe water deficits from no watering was associated with the multiple interrelated variables of initial size, baseline RGR, total weighted NSC concentration and stem NSC concentration. The relationship between ΔRGR and initial biomass was driven by the three largest species (Fig. 5c). Although these three species experienced dieback earlier than the other species because of more depletion of soil water, mortality of the two species with the largest seedlings (*S. macrophylla* and *D. oxleyanus*) also occurred at higher soil water potentials than mortality of other species (Fig. 2a). Therefore, these larger seedlings depleted the soil water faster but were also less tolerant of low soil water potentials than seedlings of other species. Size artifacts alone did not account for differences in ΔRGR in the no watering treatment. Initial leaf area displayed a trade-off with total weighted NSC concentration, which indicates that the larger individuals stored less NSCs (Fig. 6a). Furthermore, seed size correlated with larger initial leaf area (0.98, 95% CI 0.90–0.99), and seedlings of large-seeded species grow faster in the first few months of development (O'Brien *et al.*, 2013). Therefore, species with a faster pre-drought growth rate had lower NSC stores relative to their size before the start of the drought, which may have caused the rapid subsequent declines in ΔRGR and higher mortality in response to drought (O'Brien *et al.*, 2014).

Species with a greater baseline NSC concentration before drought were less affected by the no water treatment. Stem NSC concentration was particularly important for drought resistance and specifically stem soluble sugars, which were marginally correlated with ΔRGR, while starch was not correlated (Table S3). The importance of stem NSC, and specifically soluble sugars, for drought tolerance suggests that NSCs play a role in maintaining basic functions during prolonged drought. Our data support earlier results (O'Brien *et al.*, 2014), which show that greater NSC concentrations prolong time to death and xylem water potentials both within and among the same species of Bornean trees. We suggest that greater stores of NSCs could indicate a larger budget for the dissolution of embolisms, osmoregulation and/or maintenance of cell turgor (Hartmann *et al.*, 2013; Sevanto *et al.*, 2014), and therefore support the importance of stem NSCs in particular for drought resistance.

Few NSC variables were significantly correlated with ΔRGR under infrequent–high watering and frequent–low watering. However, a positive correlation between ΔWoody tissue soluble sugar concentrations and ΔRGR for both treatments was found, which indicates either that more resistant species maintained higher soluble–sugar concentrations by allocating more to woody tissue or that drought-resistant species had less demand for soluble sugars in response to these watering regimes. We hypothesize that adjustments to leaf morphology and below- versus aboveground allocation are more important for maintenance of growth rate in response to mild soil water deficits (Poorter & Markesteijn, 2008; Markesteijn & Poorter, 2009) and that species with greater capacity to express plasticity in these traits place reduced demands on stores of soluble sugars for drought resistance. Interestingly, ΔWoody soluble sugar concentration was negatively correlated with baseline total stem NSC concentration, which suggests that low baseline stem storage is related to an inability to move NSCs to the stem or high demands for NSCs in the stem.

Conclusions

We found a differential response of NSC concentrations in tropical tree seedlings to variability in water availability and severe deficit, which was characterized by an initial decrease in NSC concentration in the early stages of the variable watering regime and an increase in NSC concentration in response to greater water deficit. Furthermore, under severe water deficit, high baseline stem NSC concentration expressed a trade-off with fast growth under high water availability and contributed to drought resistance of seedlings. Variability among species in NSC storage under ambient high rainfall conditions may play a mediating role in differential responses of species to the increase in frequency and severity of El Niño-driven droughts that are predicted by some climate change scenarios.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Histogram of rainfall variability over the last 20 yr.

Fig. S2 Watering patterns of the four treatments.

Fig. S3 Model fits for leaf area estimates from leaf length and width.

Fig. S4 Biomass through time for each species in each treatment.

Fig. S5 Concentration of total NSCs, starch and soluble sugars in leaf, stem and root tissues.

Fig. S6 Morphological and physiological changes in each treatment through time.

Table S1 Compiled information on species distributions

Table S2 Total mortality counts by species and treatment

Table S3 Correlations between RGR response to treatments and baseline traits

Table S4 Correlations between traits

Methods S1 Details of experimental conditions and trait measurements.

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