

Drought survival of tropical tree seedlings enhanced by non-structural carbohydrate levels

Michael J. O'Brien^{1*}, Sebastian Leuzinger², Christopher D. Philipson^{1,3}, John Tay⁴ and Andy Hector^{1,5}

Plants in most biomes are thought to be living at their hydraulic limits, and alterations to precipitation patterns consistent with climate change trends are causing die-back in forests across the globe^{1–4}. However, within- and among-species variation in plant traits that promote persistence and adaptation under these new rainfall regimes may reduce mortality in these changing climates^{5,6}. Storage of non-structural carbohydrates (NSCs) is posited as an important trait for resistance and resilience of forests to climate-change-induced drought, but the underlying mechanisms remain unclear^{7–10}. Here we demonstrate a positive relationship between NSCs and drought survival by manipulating NSC concentrations within seedlings of ten tropical tree species. Seedlings experimentally enriched in NSCs showed higher stem water potentials and sustained NSCs during drought. NSC use for maintenance of osmoregulation and hydraulic function therefore seems to underlie improved drought resistance. That drought mortality is delayed by higher NSC concentrations has implications for predicting the impacts of climate change on forest die-back^{2,4} and may help focus restoration efforts on species that increase the resistance and resilience of forests to climate change.

Precipitation patterns are changing across the globe causing drought-induced forest die-back and altering ecosystem function^{1–3,11}, and recent evidence shows that plants in nearly every forest biome are living at the edge of their functional hydraulic limits¹. However, within- and among-species variation of traits contributing to drought resistance may improve survival of species and adaptation to a changing climate⁵. In this way, biodiversity can be seen to have an insurance value⁶ by maintaining the presence of traits that support ecosystem resilience—in this case of forest ecosystems against drought. Identifying traits that promote plant resistance to drought is therefore important for predicting the effect of climate change on the persistence of species and communities^{11,12}. For poorly understood tropical forest communities, which sustain extremely high biodiversity and provide essential carbon sinks, the task of defining the functional traits influencing plant response to global change is a particularly important and pressing goal.

Inter-specific differences in non-structural carbohydrate (NSC) stores are assumed to be an important trait for plant survival under stress because they reflect, in part, the balance between photosynthesis and respiration and as such could influence carbon availability for growth depending on species-specific life-history strategies^{10,13}. NSC concentrations correlate with resistance to herbivory and with survival under low-light conditions^{10,14}. Although NSCs are also suspected to play a role in drought resistance and have

been manipulated during drought¹⁵, a direct relationship between drought resistance and NSC stores has not been demonstrated unambiguously^{3,7–10,16} in part owing to the difficulty of experimentally manipulating NSC concentrations without altering potential confounding factors such as plant size, hydraulics and morphology.

Although plant mortality from drought is a complex process dependent on multiple interrelated mechanisms^{16,17}, recent work has proposed three pathways to drought mortality: hydraulic failure, carbon starvation due to depletion of stored NSCs and the interaction between the two inhibiting transport and use of stored NSCs (refs 7,15,18). Hydraulic failure occurs when insufficient control of water loss during severe drought leads to the formation of embolisms, xylem damage and desiccation. Alternatively, when plants maintain water potentials through stomatal closure, photosynthesis is inhibited, which may lead to mortality from carbon starvation^{7,19}. Although depletions in plant NSC concentrations under drought have been observed in some systems^{19,20}, accumulation or maintenance of NSC stores is commonly documented^{3,7,21}. This accumulation is probably due to a decoupling of growth and photosynthesis as cell expansion and division are more sensitive to water deficit than photosynthesis^{21,22}. NSC concentrations also play a functional role in non-growth mechanisms such as plant metabolism, maintaining cell turgor, osmoregulation and embolism repair^{7,21,23–25}, and it has been proposed that active storage of NSCs by plants, which is in direct competition with growth, may also maintain basic metabolic functions to optimize long-term growth and survival^{7,10,13}. However, a direct link between stored NSC and drought resistance and its importance relative to other variables remains unclear^{7,26}. Regardless of the process, we hypothesize that increased NSC concentrations support and prolong basic plant functions, thereby improving tolerance of water deficit.

We set out to test the extent to which higher NSC concentrations improve survival during drought in tropical forest species and the mechanism by which this resistance is achieved. We used a new approach to experimentally manipulate NSC concentrations in which seedlings experienced either high-then-low or low-then-high light conditions under the relatively constant aseasonal climate of our study system (Fig. 1). This manipulation produced seedlings either relatively enriched (low-to-high light) or depleted (high-to-low light) in NSC concentrations while maintaining similar seedling size and morphology, thus controlling for potentially confounding factors (Supplementary Figs 1–4). We used ten species of Bornean shade-tolerant seedlings (Supplementary Table 1) in this study because seedlings have limited NSC stores and a relatively small stature that allows a direct test of the role of

¹Institute of Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland, ²Institute for Applied Ecology New Zealand, School of Applied Sciences, Auckland University of Technology, Auckland 1142, New Zealand, ³Mountain Ecosystems WSL Institute for Snow and Avalanche Research, SLF, Flüelastrasse 11, CH-7260 Davos Dorf, Switzerland, ⁴School of International Tropical Forestry, University Malaysia Sabah, Jalan UMS, Kota Kinabalu, 88400 Sabah, Malaysia, ⁵Department of Plant Sciences, University of Oxford, OX1 3RB, UK.

*e-mail: mikey.j.obrien@gmail.com

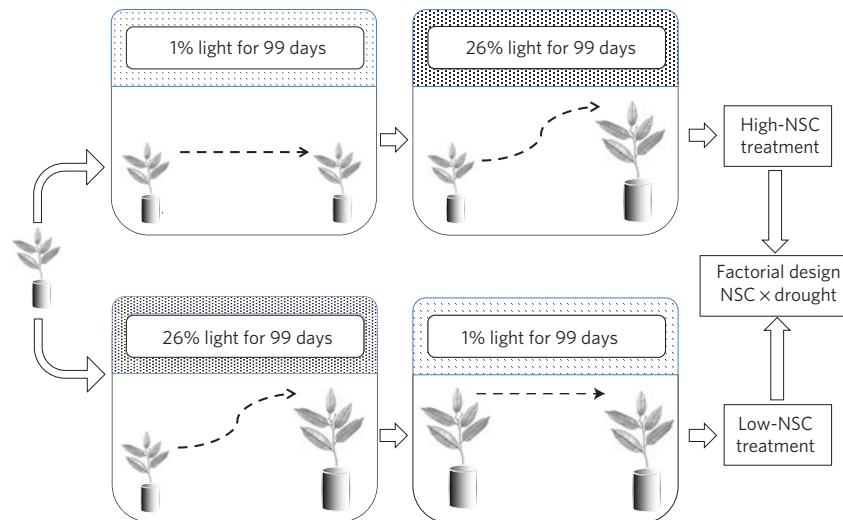


Figure 1 | Schematic of NSC manipulation. NSC concentrations in ten species of seedlings were manipulated while controlling for potentially confounding differences in size and morphology by growing randomly selected individuals under low-then-high (top row) or high-then-low light conditions (bottom row). There was little growth (dashed arrows) and NSC concentrations were depleted under the low light conditions. Growth and NSC concentrations increased under the high-light conditions. After light manipulation, seedlings grown under the different conditions had similar morphology but with NSC concentrations approximately 46% higher (11.8%) when grown under low-then-high light compared with when grown under the reverse order (8.1%).

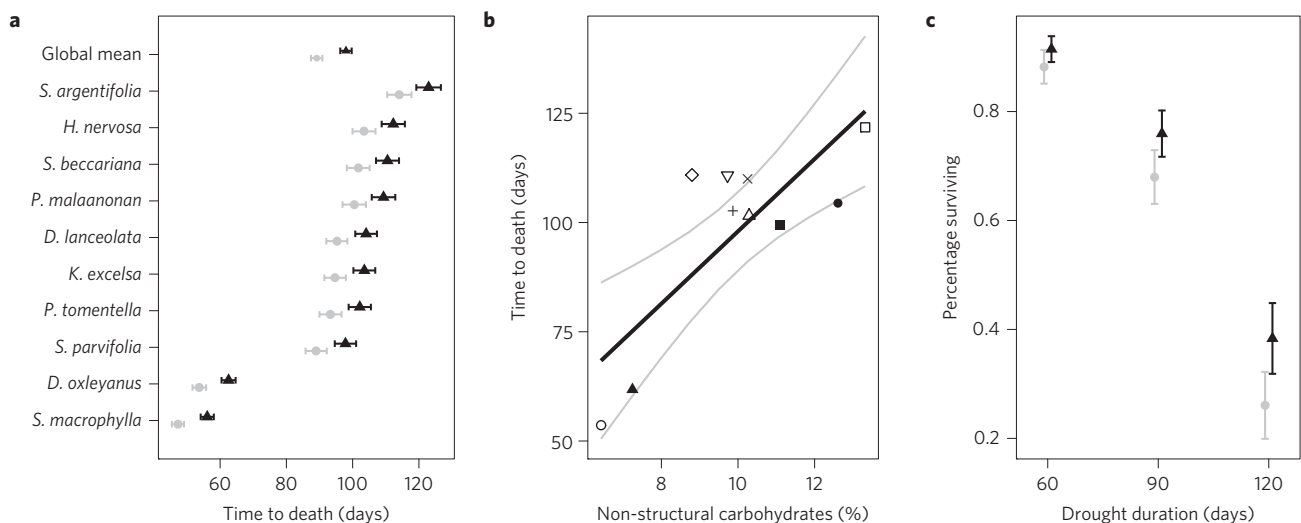


Figure 2 | Effect of NSC on drought mortality. **a**, Time to death (days) under drought for NSC-enriched (black triangles) and NSC-depleted (grey circles) seedlings for each species (s.e.m.) and the global mean (least significant difference intervals). **b**, Time to death of each species as a function of average weighted NSCs before drought (95% CI). (*Dryobalanops lanceolata*: plus symbol, *Durio oxleyanus*: filled triangle, *Hopea nervosa*: diamond, *Koompassia excelsa*: filled circle, *Parashorea malaanonan*: downtriangle, *P. tomentella*: open triangle, *Shorea argentifolia*: open square, *S. beccariana*: cross, *S. macrophylla*: open circle, *S. parvifolia*: filled square.) **c**, Percentage of enriched and depleted seedlings (95% CIs) surviving after drought from survival analysis (durations represent a mild, moderate and severe event).

NSC. Furthermore, Borneo provides a diverse, ecologically sensitive forest system, which is facing increased drought under climate change²⁷. We monitored seedling mortality, NSC levels, pre-dawn stem water potential and stomatal conductance under drought of NSC-enriched and NSC-depleted seedlings of all ten species to examine the extent to which NSC concentrations affect survival. We deliberately maintained drought conditions until all seedlings were dead with a hypothesis that greater NSC concentration would extend the time to death both within- and among-species either through prolonged hydraulic integrity or reduced carbon starvation.

When exposed to prolonged drought all seedlings of the ten species died within 168 days whereas there was complete survival of seedlings in the watered controls. Within the drought treatment, individuals enriched in NSC concentrations lived longer, a result

that was consistent across all ten species (Fig. 2a). Averaged across all species, seedlings with NSC-enriched concentrations survived 8.9 (95% CI: 5.5–12.2) days longer than NSC-depleted seedlings (Fig. 2a). This result was robust when a covariate for root mass to leaf area and shoot to root ratio were included in the analysis to account for potential differences in initial biomass allocation between enriched and depleted NSC seedlings (enriched lived 9 (5–14) days longer after controlling for root mass to leaf area ratio and 11 (5–17) days after controlling for shoot to root ratio; Supplementary Fig. 5a,b). Survival analysis using a Cox proportional hazard model supported this result as seedlings with experimentally depleted NSC stores had a 1.7 (1.4–2.1) times greater risk of dying than seedlings enriched in NSCs (Supplementary Fig. 6a). The results of our experimental manipulation were supported

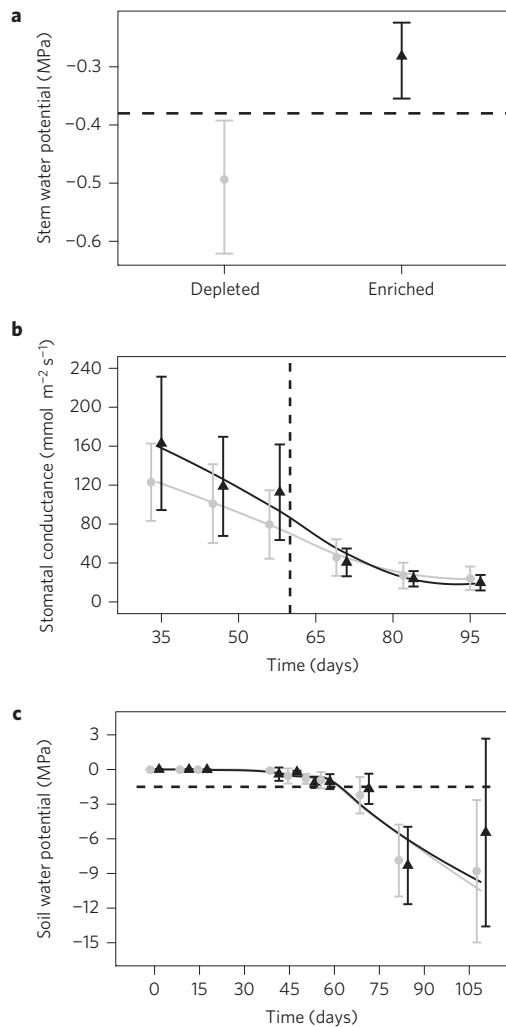


Figure 3 | Prolonged hydraulic function of NSC-enriched seedlings.

a, Mean stem water potential (least significant difference intervals) for seedlings with enriched (black triangles) and depleted NSCs (grey circles) after more than 80 days of drought. Estimates are back-transformed from log-transformed absolute values. The dashed line represents the average xylem potential at which 50% loss of conductance occurs for dipterocarps²⁸. **b**, Stomatal conductance (95% CIs) during drought between enriched and depleted NSC. The dashed line represents the approximate day the wilting point was achieved (-1.5 MPa). **c**, Soil water potential (95% CIs) during drought for enriched and depleted NSC seedlings (drying was not statistically different). The dashed line represents the wilting point at -1.5 MPa.

by a similar positive interspecific relationship between NSC and survival. Initial mean NSC concentration of species before drought was negatively correlated to Cox hazard risk (Spearman rank correlation coefficient = -0.79 , $p < 0.0001$; Supplementary Fig. 6b) and positively correlated with mean days to death (0.74 , $p < 0.001$; Supplementary Fig. 6c). Furthermore, average time to death among species increased by 8.3 days per 1% increase in NSC concentration (3.2–13.3; Fig. 2b). As with the within-species analysis, this result was robust when the same covariates were included in the statistical models to account for potential differences in initial biomass allocation among species (a 1% increase in NSC prolonged survival by 6.4 (-0.2 –13.1) days after controlling for differences in root mass to leaf area and 8.3 (2.6–14) days after controlling for shoot to root ratios; Supplementary Fig. 5d,e).

After the permanent wilting point (assumed as -1.5 MPa) was reached in the soil (~ 60 days), seedlings enriched in NSCs had

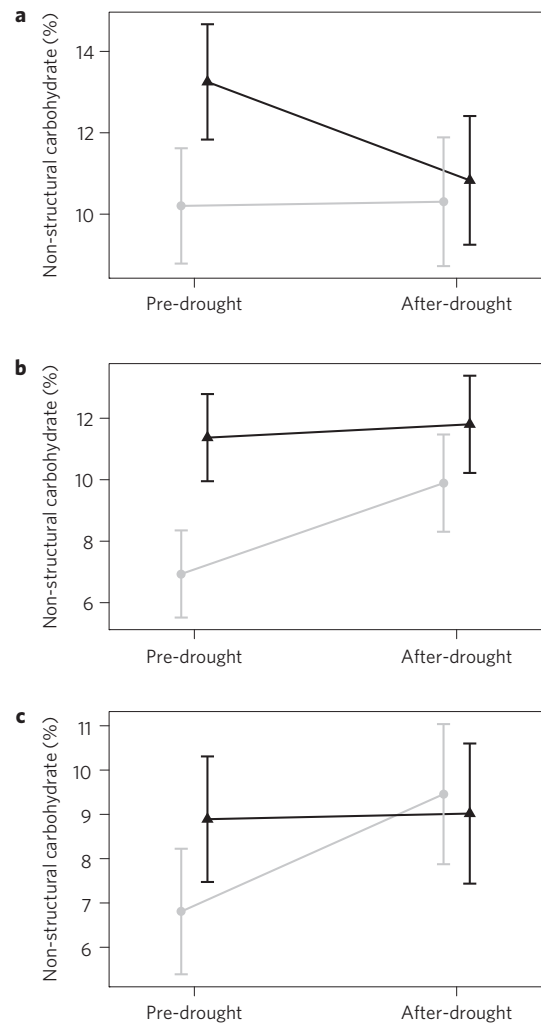


Figure 4 | Change in NSC concentrations of plant tissues during drought.

a–c, The change in average (95% CIs) NSC concentrations in leaves (**a**), stems (**b**) and roots (**c**) after prolonged drought for enriched (black triangles) and depleted NSC treatments (grey circles) from initial pre-drought NSCs. The after-drought measurements were measured from destructively harvested seedlings before they had died of drought on day 83, 102 and 123.

higher pre-dawn stem water potentials than individuals depleted in NSCs (-0.3 MPa versus -0.6 MPa; Fig. 3a) and maintained pre-dawn water potentials above -0.38 MPa—the point at which 50% loss of hydraulic conductance is reached in these species²⁸. In addition, seedlings from both treatments maintained similar stomatal conductance and soil drying throughout the experiment (Fig. 3b,c). Furthermore, seedlings initially depleted in NSCs maintained or even increased their NSC concentrations during drought to levels similar to the enriched seedlings in leaves, stems and roots (Fig. 4). The increase in NSCs during drought may indicate that whereas enriched seedlings were able to maintain hydraulic function and growth, NSC-depleted seedlings could not, with earlier slowing of growth associated with a greater accumulation of NSC (Supplementary Fig. 7).

Shade-tolerant tree species in the lowlands of Borneo are susceptible to embolism, cavitation and hydraulic dysfunction, and because embolism repair is a carbon-demanding process²⁴, we suggest that higher NSC concentrations could improve the ability of an individual to maintain functioning xylem. Furthermore, NSC concentrations are important for the maintenance of osmoregulation and cell turgor, functions that are probably

coupled with sustained hydraulic function^{15,16,18}. We can exclude the potentially confounding effects of differences in soil water potential, vapour pressure deficit, plant size and wood density (which suggests similar cavitation resistance) because these were experimentally controlled and similar between NSC treatments (Fig. 3c and Supplementary Figs 1–5). Carbon depletion can also be discounted as a mechanism for the rapid mortality of the NSC-depleted seedlings because their NSC concentrations increased during drought, suggesting that a deficit of NSCs was not responsible for the shorter time to death. However, recent work has implied the importance of carbon starvation due to transport limitation as a mechanism of drought mortality^{15,18}. Although we cannot definitively discount this process as the mechanism of mortality, our results provide no evidence in support of this cause of death. Our design exposes seedlings from both treatments equally to low- and high-light conditions, and our data provide no support of differences that could affect transport. On the contrary, the increase in NSC concentrations in stems and roots is consistent with ongoing NSC transport from source (leaves) to sink tissue during drought, and not with inhibited transport in NSC-depleted seedlings. Therefore, these physiological responses under a controlled climatic environment strongly suggest prolonged hydraulic function and maintenance of osmoregulation as important mechanisms by which NSCs improved the survival of the NSC-enriched seedlings^{21,23,24}.

The interspecific relationship between NSC concentration and survival times is consistent with correlations found between NSC concentration and other stressors: both shade and herbivory correlate with among-species variability in NSC concentrations¹⁴. Here we demonstrate that higher NSC concentrations also provide a survival advantage to tropical tree seedlings under drought, a relationship consistent in both our among-species comparison and within-species experimental manipulation. However, a one percentage point difference in average weighted NSC concentration among species is associated with a larger change in drought survival than that resulting from our experimental manipulation within species, indicating that interspecific differences in drought resistance are also due to traits other than NSC concentration alone such as slower relative growth rates and greater allocation to below versus aboveground biomass²⁹. These results emphasize that a multi-dimensional suite of traits contribute to the drought response of a seedling.

In combination, our analyses show better drought survival of both species and individuals with higher NSC concentrations through the maintenance of tissue water potentials and hydraulic conductance and not by delayed carbon starvation. After 90 days, only 24% of NSC-enriched seedlings had died versus 33% of NSC-depleted seedlings (Fig. 2c), and removing the two most sensitive species (*Shorea macrophylla* and *Durio oxleyanus*) increased this relative difference in mortality (7% versus 14%). Events of the duration of our experiment or longer were recorded in this region of Borneo 12 times between 1968 and 1998²⁷. Furthermore, many parts of Borneo experienced nearly four rainless months during the severe El Niño/Southern Oscillation event in 1998^{1,27}. During these rainless periods, it is likely that drying occurs more rapidly than in our experiment owing to lower cloud cover, competition for soil water *in situ* and higher vapour pressure deficits, which would increase the rate of mortality. However, long-term studies of precipitation patterns and community composition will be needed to further establish the importance of drought in structuring these communities. Furthermore, drought induces flowering of mature trees in this system and a broader understanding of the overall importance of NSCs in the ecology of the forests will require an integrated understanding of their roles in both reducing mortality due to biotic and abiotic stressors and their functional contribution to drought-induced mast-fruiting events.

Not surprisingly, a large, highly replicated field experiment of this type cannot shed light on the more detailed mechanism of how NSC reserves alleviate drought stress, and the biological and physiological processes by which NSC concentrations maintain hydraulic function should be a key target for the next generation of experiments in this area¹⁰. Furthermore, it remains to be shown whether the same patterns exist for mature trees that have large NSC reserves and plants in other biomes. Species-specific physiology may be of particular importance in plant response to drought, especially in terms of NSC transport and storage. For example, recent experiments manipulating CO₂ uptake and drought on isohydric species reported declines in NSCs under drought, which contrasts our findings^{15,18}.

Our results are an experimental demonstration of an unambiguous positive relationship between higher NSC concentrations before drought and plant survival during drought. This relationship is supported by both a comparative analysis among species and an experimental manipulation of NSC concentrations within species that controls for potentially confounding effects of size and morphology. We are also able to demonstrate that the general mechanism underlying this relationship is the maintenance of hydraulic function rather than limitation of carbon for metabolic function. This relationship between NSCs and drought resistance will help to predict the dynamics of seedling regeneration and drought mortality under climate change as well as contribute to the selection of species for restoration schemes that can increase long-term resistance and resilience of forest ecosystems.

Methods

We conducted this experiment at the Sabah Biodiversity Experiment (N05° 05'20" E117° 38'32"; 102 m a.s.l.) located about 22 km north of Danum Valley Field Center in Malaysian Borneo. Seeds from ten species of shade-tolerant trees (Supplementary Table 1) were collected during a landscape-scale masting event in August 2010. The species encompass a range of relative-growth rates (indicating differences in carbon storage). We planted 140 seeds per species into circular pots (20 × 36 cm) within a nursery (Supplementary Methods).

To prepare seedlings with enriched or depleted NSC concentrations, we used two contrasting light environments to alter early plant development in which individuals were exposed to either high-then-low or low-then-high light conditions (Supplementary Methods). This manipulation produced seedlings either enriched (low-to-high light) or depleted (high-to-low light) in NSC concentrations while maintaining similar seedling size and morphology (Fig. 1). This pre-treatment phase began on 14 December 2010 with seedlings of ten species (70 individuals × 10 species = 700 individuals of both enriched and depleted NSC levels; N = 1,400). After 99 days, all seedlings were moved to the alternative light environment. After a second 99 day period, all seedlings were measured for height, diameter and leaf number. Following this 198 days of pre-treatment, seedlings that received a low-to-high light treatment had enriched NSC concentrations (11.8% NSC ± 0.6 s.e.m.) that were increased by approximately 46% relative to depleted seedlings that received a high-to-low light treatment (8.1% ± 0.3; Supplementary Fig. 1). Our light-swapping treatment produced substantial differences in NSC concentrations while minimizing differences in stem diameter (Enriched: 5.5 mm ± 0.1 versus Depleted: 5.5 mm ± 0.1), stem height (Enriched: 40.4 cm ± 0.7 versus Depleted: 43.0 cm ± 0.8) and leaf counts (Enriched: 11.2 leaves ± 0.2 versus Depleted: 13.3 leaves ± 0.3; Supplementary Fig. 2). Furthermore, leaf formation under high- and low-light environments was similar for NSC-enriched and NSC-depleted seedlings indicating similar average leaf vasculature (Supplementary Fig. 3).

Sixty seedlings of each species were assigned to a drought treatment with no water (30 NSC-depleted and 30 NSC-enriched), and 60 seedlings were given an average rainfall treatment with 240 mm of water per month on two-day intervals (30 NSC-depleted and 30 NSC-enriched) producing a full factorial manipulation of drought and NSC concentration (seven species had individuals die during light swapping and five individuals were harvested for NSC measures; therefore, only 60 seedlings from each pre-treatment were used to keep a balanced design). We measured height, diameter and leaf number before the start of the drought treatment and approximately every month thereafter. We monitored the seedlings every two days for mortality (that is, the point when no green tissue was observable under the bark on the stem; some seedlings were re-watered to ensure mortality had occurred). We destructively harvested a subset of seedlings before the start of the drought and again at 35, 72, 83, 102 and 123 days of drought (2–5 seedlings per species per treatment at each time point). Destructively harvested

seedlings were used for analysis of NSCs in leaf, stem and roots, leaf and stem water potential and dry biomass. We calculated NSC concentration weighted by the relative contribution of each organs biomass to total biomass (these weighted NSC concentrations are used for all analyses except in Fig. 4). Stomatal conductance was measured on a subset of seedlings in each NSC treatment in each watering treatment every ten days (Supplementary Methods).

To examine the global response of all species, we assessed time to death as a function of NSC treatment with a random effect for species using mixed-effects models. This relationship was robust to the inclusion of root mass-to-leaf area and shoot-to-root ratio to account for potential differences in seedling size and allocation (Supplementary Methods). We tested differences in enriched and depleted NSC individuals at the species level using generalized least-squares models with time to death as a function of NSC treatment, species and their interaction. However, the analysis strongly favoured the model without the interaction ($\Delta\text{BIC} = 45.2$; BIC is the Bayesian Information Criterion), and it was removed. We used a Cox proportional hazard model to assess differences in seedling survival between depleted and enriched NSC treatments. We tested the effect of NSC concentrations on among-species mortality by examining the number of days to death as a function of mean NSC concentration of each species (mean across both NSC-enriched and NSC-depleted seedlings).

To test the water status of seedlings after severe water deficit, we analysed the difference between pre-dawn stem water potentials for the NSC-enriched and NSC-depleted seedling using mixed-effects models with day and species within day as random effects. We tested the effect of drought on NSCs (pre- versus post-drought concentrations) of enriched and depleted seedlings using a mixed-effects model with species as a random effect (Supplementary Methods).

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Author contributions

M.J.O'B. conceived, designed and carried out the experiment, extracted NSC, analysed the data and wrote the manuscript. S.L. contributed in designing the physiological method, analysis and writing. C.D.P. contributed to the study design, analysis and writing. J.T. was the local collaborator helping with the logistics of research in Sabah. A.H. contributed to the study conception, analysis and writing. Revisions were led by M.J.O'B. with input from all authors.

Additional information

Supplementary information is available in the [online version of the paper](#). Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to M.J.O'B.

Competing financial interests

The authors declare no competing financial interests.