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Research paper

Carbon dynamics of *Acer pseudoplatanus* seedlings under drought and complete darkness

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Carbon (C) storage is considered a key component to plant survival under drought and shade, although the combined effects of these factors on survival remain poorly understood. We investigated how drought and shade alter the C dynamics and survival of tree seedlings, and whether drought limits the access to or usage of stored C. We experimentally applied two levels of soil humidity (well-watered versus drought, the latter induced by dry-down) and light availability (light versus complete darkness) on 1-year-old seedlings of *Acer pseudoplatanus* L. for 3 months. We quantified the survival, biomass, growth rate and non-structural carbohydrates (NSC) of seedlings at their time of death or at the end of the experiment for those that survived. We found that the soil dried out faster when drought was combined with light than when it was combined with complete darkness. Seedlings subjected to both drought and light showed reduced growth and reached 100% mortality earlier than any other treatment, with the highest NSC concentrations at the time of death. Seedlings exposed to both drought and complete darkness died significantly earlier than seedlings exposed to complete darkness only, but had similar NSC concentrations at time of their death, suggesting that drought accelerated the use of stored C under complete darkness. Complete darkness significantly reduced seedling growth and whole-plant NSC concentrations regardless of soil humidity, while root NSC concentrations were significantly more reduced when complete darkness was combined with drought conditions. Thus, the C dynamics in *A. pseudoplatanus* seedlings under complete darkness was not hindered by drought, i.e., the access and use of stored C was not limited by drought. The contrasting growth and C storage responses driven by drought under light versus complete darkness are consistent with a key role of the drought progression in the C dynamics of trees.

Keywords: climate warming, C starvation, Patagonia, shade tolerance, sycamore, understory.

Introduction

In many temperate regions of the world, drought induced by climate change is causing tree mortality and forest decline (McDowell et al. 2008, Allen et al. 2010, Galiano et al. 2011, Anderegg et al. 2012). This pattern is expected to become more common, given that climatic projections predict more frequent and severe droughts for the next years (IPCC 2007). A better understanding of the physiological mechanisms that cause tree mortality is crucial to anticipate forests' responses to drought (McDowell and Sevanto 2010, Sala et al. 2010, McDowell et al. 2011, Anderegg et al. 2012, Klein 2015). Two of the principal, non-exclusive mechanistic explanations of

drought-induced tree mortality are hydraulic failure and carbon (C) starvation. Hydraulic failure is defined as the collapse of whole-tree hydraulic conductance due to massive, irreversible embolization (Tyree and Sperry 1989, McDowell et al. 2008); this type of failure is predicted to be particularly common under sudden and severe water stress (McDowell et al. 2008, Anderegg and Anderegg 2013, Mitchell et al. 2013). The C starvation hypothesis (CSH) proposes that plant death is caused by insufficient C availability due to a prolonged negative C balance under drought conditions (McDowell et al. 2008, McDowell 2011). Since not all stored C can be mobilized (Chapin et al. 1990, Millard et al. 2007), C starvation does not

necessarily imply complete C storage exhaustion (McDowell and Sevanto 2010, McDowell 2011, McDowell et al. 2011, Sevanto et al. 2014). In contrast to the hydraulic failure mechanism, the CSH is considered more likely to occur when drought severity increases gradually (McDowell et al. 2008, Hoch 2015), as this would warrant the minimal tissue hydration required to access and utilize stored C for a longer period of time (Hartmann et al. 2013a, 2013b, Sevanto et al. 2014). There is currently a consensus that the two mechanisms are interrelated and ultimately depend upon the C availability of plants (Sala et al. 2010, McDowell 2011, McDowell et al. 2011, Hartmann 2015); this has caused an increasing interest in the understanding of the C dynamics of plants subjected to drought (Hoch 2015, Maguire and Kobe 2015, Zhang et al. 2015).

The C dynamics of plants under drought has been modeled according to trajectories of C assimilation, C storage mobilization and C demands (McDowell 2011, McDowell et al. 2011, Hoch 2015). In the short term, C storage concentrations—commonly represented by non-structural carbohydrates (NSC)—increase due to higher dehydration limitations on C sink activity (i.e., cell elongation and differentiation) than on photosynthesis (Boyer 1970, Muller et al. 2011), although an alternative hypothesis to this effect is that increased C storage occurs at the expense of growth (Wiley and Helliker 2012). If drought persists throughout time and low C assimilation is prolonged, NSC concentrations will eventually decline as a result of C storage mobilization to meet C demands (e.g., maintenance respiration, osmoregulation and refilling) (McDowell et al. 2008, 2011, Hoch 2015). A critical question is whether drought restricts the access and utilization of stored C, which could increase the minimum *survivable* C storage level (sensu McDowell 2011). An increased demand of sugars for osmotic regulation and repair processes (e.g., prevention and repair of oxidative stress and destabilization of macromolecular integrity) may limit the C available for respiration, increasing NSC concentrations throughout the mortality process (McDowell 2011 and references therein). In addition, recent studies have shown that drought restricts NSC access, translocation and utilization in sites where carbohydrates are needed (Hartmann et al. 2013a, Sevanto et al. 2014, Savage et al. 2016), thus increasing NSC concentrations at the time of death, at least in some organs. For instance, Sevanto et al. (2014) found that slow-dying trees of *Pinus edulis* L. under drought survived longer and accounted for lower NSC concentrations at the time of death than fast-dying trees. In the same study, completely shaded and well-watered trees survived longer than fast-dying trees (and shorter than slow-dying trees), but completely exhausted their C storage. Similarly, Hartman et al. (2013a) found that drought accelerated mortality and limited the NSC utilization and translocation in CO₂-deprived *Picea abies* (L.) H. Karst. seedlings. The generality of these results is still uncertain, which limits the capacity to predict C levels at death. In particular, it is still unclear whether other taxa (e.g., angiosperms) would exhibit similar constraints.

In this study, we investigated the C dynamics of *Acer pseudo-platanus* L. (sycamore) seedlings in relation to reduced hydration (drought) and impeded C assimilation (complete darkness). We hypothesized that drought hinders C storage utilization and increases the minimum *survivable* C storage level. We thus expected that, under complete darkness, dehydrated seedlings would show higher NSC concentrations, lower growth rates, and higher and faster mortality rates than well-hydrated seedlings. As in previous studies (Piper et al. 2009, Sevanto et al. 2014, Fischer et al. 2015), we used prolonged darkness to impede C assimilation and induce reductions in C storage until reaching the minimum *survivable* level (McDowell 2011). To investigate whether this minimum *survivable* C storage is affected by drought, we combined complete darkness conditions with drought. Since the rate of soil desiccation tends to be lower under complete darkness than under high light conditions (Sánchez-Gómez et al. 2006, Martínez-Tillería et al. 2012), we also hypothesized that the drought-related impediments on growth and C storage utilization would be lesser under complete darkness than under light conditions, leading to higher growth, delayed mortality and lower NSC concentrations at the time of death in the former. Finally, we were also interested in examining soluble sugar (SS) and starch concentrations, as these compounds are the main form of storage in most angiosperms. Since starch is a typical storage compound with no other known metabolic function (Chapin et al. 1990), impediments in the use or access of stored C should mainly be evidenced by increases in the starch proportion. By contrast, an increased demand for osmotic regulation and maintenance of cell integrity might involve a higher proportion of sugar (McDowell 2011, McDowell et al. 2011, Dietze et al. 2014). Since the effect of drought on C storage may vary among organs (e.g., C depletion may occur locally) (Galvez et al. 2013, Hartmann et al. 2013a, Mitchell et al. 2013, Hoch 2015), we examined NSC concentrations at both the whole-plant and organ level.

Materials and methods

We conducted a pot experiment under outdoor conditions with *A. pseudoplatanus* seedlings near the city of Coyhaique (45° 57'S and 72° 03'W, 380 m above sea level), in the Aysén Region of the Chilean Patagonia. *Acer pseudoplatanus* is a tree species that was introduced to the region decades ago as an ornamental tree. It has become invasive in sites with diverse light availability, including both forest understories and open sites (Fuentes et al. 2014). We collected seeds from mature trees in a private location and sowed them in April 2009 (i.e., autumn in the Southern Hemisphere) at a depth of 1 cm in >100 small 0.25 l pots completely filled with homogenized mineral soil (a sand-clay mixture with 12.2 mg kg⁻¹ Olsen-P, 5.1% organic matter content, 0.20% total N and a pH of 6.4) collected from nearby locations. Each pot's soil was compacted to the same

level to assure similar soil bulk density. The pots were placed outdoors under intermediate-light conditions (50% canopy openness) provided by the shade of a few mature *A. pseudoplatanus* and *Populus deltoides* Bartr. ex Marsh. ssp. *deltoides* trees. Germination occurred in October 2009. Seedlings were regularly watered during the pre-experimental period with tap water (ion concentrations in mg l^{-1} : $\text{NO}_3^{-1} = 0.05$, $\text{Na}^{+1} = 3.20$, $\text{K}^{+1} = 0.43$, $\text{SO}_4^{-2} = 2.00$, $\text{Mg}^{+2} = 1.65$, $\text{PO}_4^{-3} = 0.03$, $\text{Ca}^{+2} = 2.69$; $\text{pH} = 6.9$) and exposed to natural rainfall (390 mm from 1 October 2009 to 31 January 2010) and temperatures (Figure 1). By the end of the pre-experimental period, seedlings had a mean initial height of 13.28 ± 3.88 cm (SD) (range: 4–22 cm) and a mean initial number of leaves of 4.60 ± 1.63 . They had also achieved complete bud break, as indicated by the similar number of leaves on control plants at the beginning and end of the experiment.

The experiment started the first week of February 2010 (mid-growing season in the Southern Hemisphere) by exposing the seedlings to four combinations of water and light availability set in outdoor conditions. First, in order to determine the influence of phenology on the seedlings' NSC concentrations during the experiment we used one set of 15 *initial* seedlings that were harvested at the onset of the experiment for NSC determination (see below); prior to harvesting, we measured their total height, and number of leaves. In the shade/drought experiment, seedlings were randomly assigned to watering (+W) or drought (-W), and to light (+L) or complete darkness (-L), resulting in the following four treatments: +W+L, +W-L, -W+L and -W-L. We assigned six seedlings to each treatment, and considered four blocks containing each of the four treatments (96 seedlings total). In the light treatments (+W+L, -W+L), we intended to imitate the typical light conditions where *A. pseudoplatanus* seedlings usually establish and grow in the region: seedlings were exposed to 40% canopy openness, receiving direct radiation for at least 3 h (09:00–12:00 am) per day, while they received diffuse radiation for the rest of the day. This was achieved by placing the pots under transparent acrylic (Plexiglas[®], 90% total light transmission) covers (1.8 m tall, 3 m² in area) located under

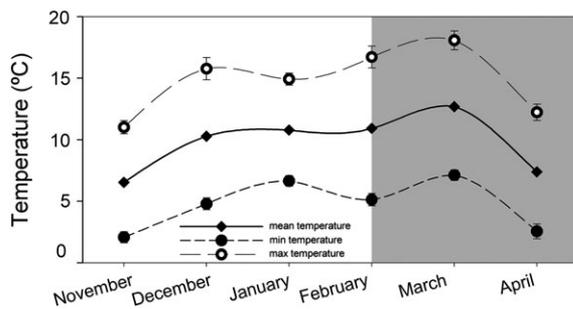


Figure 1. Mean, maximum and minimum temperatures for the growing season (November 2009–April 2010) at the experiment location (Coyhaique, Chile); the grey area indicates the experimental period (2 February 2009–23 April 2010).

natural shade and exposed to natural conditions of air temperature and humidity (Figure 1). To provide complete darkness, we constructed 2 m³ cages made of wood, with metallic roofs and one lateral window (50 × 70 cm) covered with black fabric. To ensure adequate air ventilation inside the cages and to minimize temperature differences among treatments, wood walls were set 2.5 cm above the ground, and the same space was left between the roof and the walls. Furthermore, the cages were located under natural shade and precluded from direct radiation. Watering (+W) consisted of the provision of 50 cc of tap water per pot (i.e., +W+L and +W-L) every other day, similar to the watering applied during the pre-experimental period. Drought was induced by a progressive dry-down of the pots' soil; thus, these pots received no watering throughout the experiment (Figure 2).

After the seedlings were assigned to different treatments, seedling survival and pot soil moisture were recorded on 23 February, 3 March, 10 March, 24 March, 4 April and 23 April. A seedling was considered dead when it showed complete tissue dehydration and a brownish colour. These two visual characteristics proved to be highly reliable indicators of mortality in a preliminary survey where five seedlings were exposed to severe drought. When these seedlings showed complete tissue dehydration and a brownish color they were dissected and indeed showed no green (i.e., alive) stem or root bark. The onset of

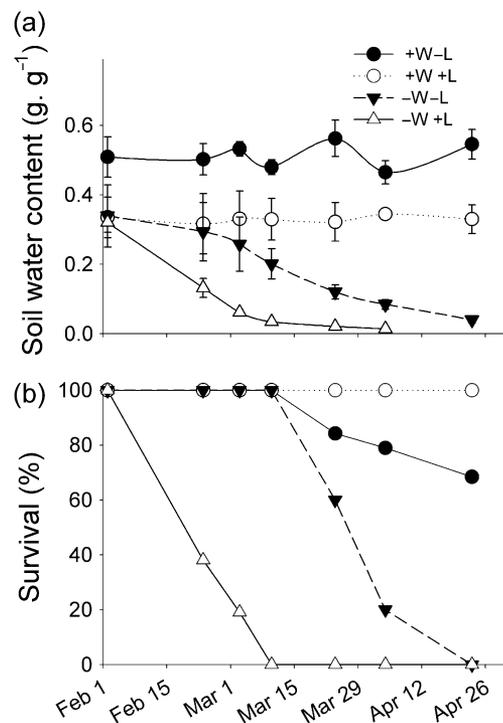


Figure 2. Time-course soil moisture (gravimetric soil water content, in grams of water per gram of dry soil) (a) and seedling survival (percentage of live seedlings in relation to the total number of seedlings per treatment) (b) of *A. pseudoplatanus* under drought and light (-W+L), drought and complete darkness (-W-L), watering and light (+W+L) and watering and complete darkness (+W-L), from 2 February to 23 April 2010 in Coyhaique, southern Chile.

mortality was defined as the first survival monitoring date when a seedling was found dead. Dead seedlings were immediately harvested, placed in a cooler and brought to the laboratory (Centro de Investigación en Ecosistemas de la Patagonia, Coyhaique, Chile) for biomass and chemical analyses. At the end of the experiment (late April 2010; the end of the growing season in the Southern Hemisphere), all of the surviving seedlings were harvested for biomass and chemical analyses.

The soil moisture content was determined using the gravimetric method, as the ratio of each soil sample's water mass to dry weight. For this, we added five additional pots without seedlings to each treatment; they had the same type of soil and received the same watering and light conditions as the corresponding treatments. These pots were weighed on the same days that the survival monitoring was carried out. A balance with a precision of ± 0.01 g (Boeco, Hamburg, Germany) was used. Finally, at the end of the experiment, they were dried at 100 °C until a constant weight was achieved using a forced-air stove (Mettler GmbH, Schwabach, Germany), after which they were weighed again. The water mass present in a pot at any given time was calculated as the difference between the pot's weight at that time (i.e., humid soil plus pot) and the pot's weight after drying (i.e., dried soil plus pot). Provided that the pots used for soil moisture measurements did not contain seedlings, we stress that our measurements are very conservative estimates of real soil moisture dynamics.

Biomass and chemical analyses

Seedlings' growth rates throughout the experiment were measured as the height increment relative to the initial height (RGR_h , in cm cm^{-1}). In addition, the seedlings' final biomass was computed for each treatment. In the laboratory, each seedling was separated into roots, stems (including branches and twigs), and leaves. The roots, leaves and shoots were thoroughly washed with tap water and gently brushed and put into labeled paper bags. Samples were then heated in a microwave for three 20-s cycles at 900 W in order to stop the enzymatic activity of the living tissues (Popp et al. 1996). After this, samples were placed to dry in a forced-air stove at 70 °C for 72 h; afterwards, dry tissues were weighed on a scale with a precision of 0.0001 g. Tissue samples were then ground into a fine powder with a ball mill (Retsch[®] MM200; Haan, Germany), and finally they were stored at 4 °C until the corresponding chemical analyses were carried out.

Our assessment of C storage was based on the determination of NSC, as the sum of low-molecular-weight SS and starch. This method is described in detail in Quentin et al. (2015) (laboratory 'W'). Soluble sugar and starch concentrations were determined using ~ 15 mg of the dried powder sample. Soluble sugar was extracted with a methanol:chloroform: water solution, which was separated from the pigments and lipids by adding water and chloroform (Rose et al. 1991). Soluble sugar was determined with the phenol sulphuric

method, using a 2% phenol reading at 490 nm (Chow and Landhäusser 2004). Sugar concentrations were determined using a sucrose standard. The residual pellet was dried overnight at 50 °C in a forced-air stove and the starch was then gelatinized (Rose et al. 1991) and hydrolyzed into glucose with amyloglucosidase (Sigma-Aldrich 10115, St. Louis, MO, USA) at 45 °C overnight. This glucose concentration was determined similarly to the SS concentrations (see above) but using glucose as a standard (Marquis et al. 1997, Poorter and Kitajima 2007). To account for the potential colorimetric interference driven by the reaction between amyloglucosidase and phenol (Chow and Landhäusser 2004), the absorbance of each sample was corrected by subtracting the absorbance of an enzyme blank. Soluble sugar and starch concentrations were expressed as milligram per gram of dry weight. Total NSC concentrations were estimated from the sum of SS and starch. According to our survival results, the biomass and NSC concentrations of the -W-L and -W+L treatments were assessed in dead seedlings, in the +W+L treatment they were evaluated in surviving seedlings, and in the +W-L treatment they were measured in both dead and surviving seedlings.

Statistical analyses

Seedling survival, biomass, relative growth rate and NSC concentration data were analyzed using mixed-effects models, with random effects for blocks and fixed effects for light (light versus complete darkness), drought (watering versus complete drought) and interactions. Thus, to estimate and test the differences in survival among the treatments, we used generalized linear mixed-effect models (GLMM) with a logistic link function, while seedling biomass, relative growth rate and NSC concentrations were modeled using linear mixed-effect models (LMM). All analyses were performed using R version 2.15.3 (R Development Core Team 2013), *lme4* (Bates et al. 2013) and *lmer* (Pinheiro et al. 2009) packages. To assess the significance of terms for GLMM, several challenges in calculating *P*-values must be overcome (Pinheiro and Bates 2001). Consequently, we used the recommended approach that uses the Akaike Information Criterion corrected for small sample size (AIC_c) and Likelihood Ratio Tests (LRT) (Bolker et al. 2009, Zuur et al. 2009). To determine whether drought hindered C use under complete darkness, we performed Student's *t*-tests to compare NSC, starch and sugar concentrations between dead seedlings from -W-L and +W-L treatments, and between dead and surviving seedlings from the +W-L treatment. The same analysis was performed to compare NSC, biomass and the growth rate of the seedlings before and after the experiment. Finally, we were also interested in estimating and testing differences in mortality time among treatments; in particular, we wanted to know whether drought affected the survival period under complete darkness. For this, we used a GLMM with a Poisson link function, where the response variable was the mean time for mortality onset across six time periods during the experiment. We conducted

post hoc multiple comparisons (Tukey's procedure) for particular treatment combinations using the *multcomp* package (Hothorn et al. 2008) in R.

Results

Drought and survival

Soil humidity decreased earlier in $-W+L$ than in $-W-L$, indicating that the soil dried out faster under light (+L) than under complete darkness (-L) (Figure 2a). At the end of the experiment, seedling survival was severely affected by darkness (-L) and drought (-W) (Figure 2b, Table 1). When single-factor models were compared with the only-random model (model 1 in Table 1), LRT clearly showed that drought was the factor that most affected seedling survival (Figure 2b, Table 1); drought reduced seedling survival to 0% regardless of the light conditions (i.e., $-W+L$ or $-W-L$). However, among well-watered seedlings the light condition was responsible for significant differences in final survival rates (100% versus 68% in $+W+L$ and $+W-L$, respectively). Thus, the model containing the interaction term between light and drought conditions was clearly the best model (relative to the second best model, light + drought, the ΔAIC_c was 2.12).

Treatments also strongly differed in their mean time for mortality onset, which was due to drought ($\chi^2 = 73.36$, $P < 0.001$) and darkness ($\chi^2 = 7.174$, $P = 0.008$). On average, the fastest onset of mortality was observed in $-W+L$ (mean time for mortality onset ≈ 3 weeks, 23 February) (Figure 2b). The mean time for mortality onset was significantly longer in $-W-L$ (c. 23 March; $z = 6.10$, $P < 0.001$), and even longer in $+W-L$ (c. 10 April; $z = 7.30$, $P < 0.001$). Thus, drought anticipated mortality under complete darkness.

Growth

Drought (but not light) significantly reduced seedling biomass and the RGR_h . The models including the drought \times light interaction were the best models with the lowest AIC_c (relative to the second best model, light + drought, the ΔAIC_c was 12.18 for biomass and 3.81 for the RGR_h), indicating that drought decreased the biomass and RGR_h of seedlings under light

Table 1. Summary of AIC_c , log-Likelihood ($\log Lik$), LRT estimates (χ^2) and their respective inference (P -value) for seedling survival after one growing season based on light (light versus complete darkness), drought (watering versus drought) and interactions among them for seedlings of *A. pseudoplatanus* grown near the city of Coyhaique, Patagonia, Chile. Data were analyzed using GLMM. ~1 refers to the random-only model.

Model	df	AIC_c	$\log Lik$	χ^2	P -value
~1	2	113.65	-54.83		
Light	3	113.73	-53.87	1.916	0.166
Drought	3	53.91	-23.95	61.745	<0.001
Light + Drought	4	49.47	-20.74		
Light \times Drought	5	47.35	-18.67	4.122	0.042

($-W+L$ versus $+W+L$), but not under complete darkness ($-W-L$ versus $+W-L$) (Table 2, Figure 3a and b). The highest biomass was found in $+W+L$, which was also the only treatment that significantly differed from the initial value ($t = -4.16$, $P < 0.001$) (Figure 3a). Regardless of the light condition, seedlings under drought conditions showed similar biomass values (Figure 3a) and did not significantly differ from the initial values ($-W-L$ versus initial: $t = -1.12$, $P = 0.27$; $-W+L$ versus initial: $t = -1.04$, $P = 0.30$). However, root biomass increased in all treatments when compared to pretreatment values (see Figure S1 available as Supplementary Data at *Tree Physiology* Online). Finally, the surviving seedlings of the $+W-L$ treatment had significantly higher biomass values ($t = -2.20$, $P = 0.04$) and similar RGR_h ($t = 0.38$, $P = 0.71$) when compared with their dead counterparts.

Non-structural carbohydrates

Seedlings under the $+W+L$ treatment had similar NSC concentrations (125.6 ± 10.4 mg g^{-1}) when compared with seedlings that were harvested at the beginning of the experiment (121.6 ± 4.3 mg g^{-1} , $t = 0.39$, $P = 0.70$, Figure 4). This ultimately shows that there was no phenological effect on C allocation to storage during the experiment.

Whole-plant NSC concentrations were significantly affected by light, but not by drought (Table 2, Figure 4). The same was true for SS and starch concentrations (see Table S1 available as Supplementary Data at *Tree Physiology* Online). Under light (+L), seedlings had on average, 137.43 (SE = 5.66) mg g^{-1} of

Table 2. Results of fixed effects in linear mixed models of seedlings' biomass, relative growth (RGR_h) and NSC concentrations, including AIC_c , log-Likelihood ($\log Lik$), LRT estimates (χ^2) and their respective inference (P -value). Likelihood ratio tests were used to test fixed effects of light (light versus complete darkness), drought (watering versus drought) and interactions among them for *A. pseudoplatanus* seedlings. ~1 refers to the random-only model.

	df	AIC_c	$\log Lik$	χ^2	P -value
<i>log</i> (biomass)					
~1	3	212.72	-103.36		
Light	4	211.53	-101.77	3.19	0.074
Drought	4	203.44	-97.72	11.28	<0.001
Light + Drought	5	201.76	-95.88		
Light \times Drought	6	189.58	-8.79	14.18	<0.001
<i>log</i> (RGR_h)					
~1	3	-53.45	29.73		
Light	4	-52.29	30.15	0.843	0.358
Drought	4	-67.76	37.88	16.307	<0.001
Light + Drought	5	-66.79	38.39		
Light \times Drought	6	-70.60	41.30	5.81	0.016
<i>log</i> (NSC)					
~1	3	118.48	-56.24		
Light	4	43.86	-17.93	76.617	<0.001
Drought	4	120.47	-56.23	0.008	0.928
Light + Drought	5	45.84	-17.92		
Light \times Drought	6	38.42	-13.21	9.417	0.002

NSC, a figure significantly higher ($P < 0.001$) than the 67.14 (SE = 3.76) mg g^{-1} of seedlings under complete darkness (-L). The model containing the interaction term between light and drought conditions was again the best model (relative to the second best model, light + drought, the ΔAIC_c was 7.42, Table 2), indicating that drought decreased the whole-plant NSC concentration under complete darkness and increased the NSC concentration under light (although it is important to note that the difference between +W-L and -W-L was not significant)

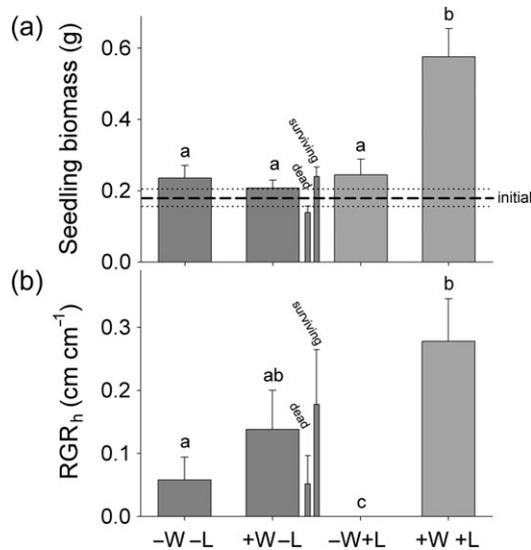


Figure 3. Mean (1SE) biomass (g) and relative growth rate in height (RGR_h , cm cm^{-1}) in seedlings of *A. pseudoplatanus* subjected to drought and light (-W+L), drought and complete darkness (-W-L), watering and light (+W+L) and watering and complete darkness (+W-L) conditions. Inset refers to surviving and dead seedlings for the +W-L treatment. Values for -W-L and -W+L correspond to dead seedlings; values for +W+L correspond to live seedlings. Different letters stand for significantly different ($P < 0.05$) mean values among treatments. Dashed and dotted lines represent the mean and SE, respectively, of pretreated seedlings.

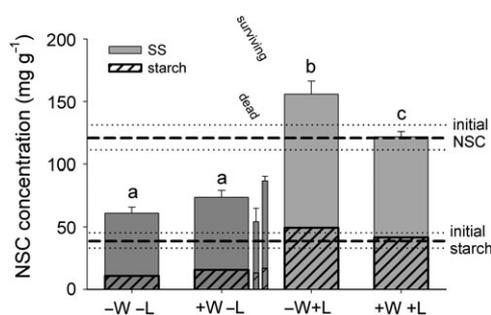


Figure 4. Mean (1SE) concentrations (mg g^{-1}) of whole-plant NSC (SS + starch), SS and starch in seedlings of *A. pseudoplatanus* subjected to drought and light (-W+L), drought and complete darkness (-W-L), watering and light (+W+L) and watering and complete darkness (+W-L) conditions. Inset refers to surviving and dead seedlings from +W-L. Values for -W-L and -W+L correspond to dead seedlings; values for +W+L correspond to live seedlings. Different letters stand for significantly different ($P < 0.05$) mean values among treatments. Dashed and dotted lines represent the mean and SE, respectively, of pretreated seedlings.

(Figure 4). These trends were mostly driven by sugars (i.e., the interaction between light and drought was highly significant for sugars, but marginally significant for starch) (see Table S1 available as Supplementary Data at *Tree Physiology* Online). Whole-plant NSC concentration trends were mainly driven by root and stem concentrations, although root NSC concentrations were significantly lower in -W-L than in +W-L (Table 3, Figures 4 and 5). Leaf NSC concentrations were similar among treatments (Figure 5).

Dead seedlings of -W-L and +W-L had similar NSC ($t = -0.98$, $P = 0.34$), starch ($t = 1.63$, $P = 0.12$) and sugar concentrations ($t = -1.67$, $P = 0.11$). However, surviving seedlings of +W-L showed significantly higher NSC ($t = 4.89$, $P < 0.001$), starch ($t = 2.90$, $P = 0.010$) and especially SS concentrations ($t = -4.98$, $P < 0.001$) than their dead counterparts (Figure 4).

Discussion

When comparing 1-year-old seedlings of *A. pseudoplatanus* that were subjected to both drought and complete darkness with those that were subjected only to complete darkness we found that the former died earlier, grew similarly and had similar NSC concentrations at their time of death. This result indicates that the minimum survivable C storage under complete darkness was not altered by drought. The fact that seedlings exposed to complete darkness showed similar NSC concentrations at the time of death regardless of the drought condition implies that

Table 3. Results of fixed effects in linear mixed models of seedlings' NSC concentrations in stems, roots and leaves of *A. pseudoplatanus* grown experimentally. These results include AIC_c , log-Likelihood ($\log\text{Lik}$), LRT estimates (χ^2) and their respective inference (P -value). Likelihood Ratio Tests were used to test fixed effects of light (light versus complete darkness), drought (watering versus drought) and interactions among them. ~1 refers to the random-only model.

	df	AIC_c	$\log\text{Lik}$	χ^2	P -value
<i>log</i> (conc NSC) leaves					
~1	3	174.03	-84.02		
Light	4	162.06	-77.03	13.971	0.359
Drought	4	172.72	-82.36	3.314	0.069
Light + Drought	5	160.09	-75.05		
Light \times Drought	6	162.09	-75.04	0.001	0.972
<i>log</i> (conc NSC) stem					
~1	3	160.37	-77.18		
Light	4	109.87	-50.94	52.50	<0.001
Drought	4	162.21	-77.11	0.15	0.696
Light + Drought	5	111.57	-50.78		
Light \times Drought	6	107.40	-47.70	6.16	0.013
<i>log</i> (conc NSC) root					
~1	3	202.77	-98.38		
Light	4	142.51	-67.25	62.26	<0.001
Drought	4	203.69	-97.85	1.07	0.300
Light + Drought	5	142.08	-66.04		
Light \times Drought	6	139.13	-63.57	4.95	0.026

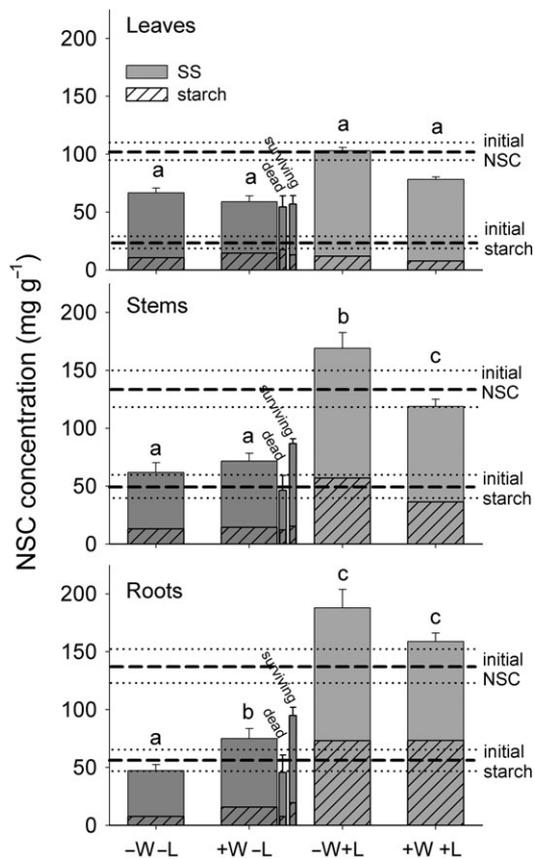


Figure 5. Mean (1SE) concentrations (mg g^{-1}) of leaves, stems and roots NSC (SS + starch), SS and starch in seedlings of *A. pseudoplatanus* subjected to drought and light (-W+L), drought and complete darkness (-W-L), watering and light (+W+L) and watering and complete darkness (+W-L). Inset refers to surviving and dead seedlings from +W-L. Values for -W-L and -W+L correspond to dead seedlings; values for +W+L correspond to live seedlings. Different letters stand for significantly different ($P < 0.05$) mean values among treatments. Dashed and dotted lines represent the mean and SE, respectively, of pretreated seedlings.

the access to and utilization of stored C under complete darkness was not significantly affected by drought. Rather, since such NSC concentrations were achieved earlier under drought (i.e., -W-L seedlings died earlier than +W-L seedlings) our results suggest that drought accelerated the consumption of the *potentially utilizable* stored C and thus shortened seedlings' survival time under conditions of zero C gain (i.e., complete darkness). This result contrasts with previous findings where drought increased seedlings' NSC concentrations at their time of death in seedlings subjected to reduced atmospheric CO_2 concentrations (Hartmann et al. 2013a). One possible explanation for this difference in results is the rate of soil desiccation. While Hartmann et al. (2013a) reported similar trends for seedlings exposed to drought, regardless of whether or not they were combined with reduced CO_2 concentrations, our seedlings experienced a much lower soil desiccation rate under complete darkness than under light, which could have led to slower tissue dehydration and hence a higher capacity of C utilization.

Another potential explanation is that winter deciduous species (*A. pseudoplatanus*) are better adapted than evergreens to remobilize, transport and utilize their C stores under stress, in general, and under drought conditions in particular. Winter deciduous species remobilize nutrients from woody tissues faster than evergreens (Grelet et al. 2001), and are more tolerant to defoliation thanks to their capacity to quickly use C and nitrogen stores (Piper and Fajardo 2014).

The faster NSC concentration decrease in seedlings exposed to both complete darkness and drought coincides with results from a previous study that compared the effects of shade and drought on the NSC levels of five broadleaved winter deciduous species of contrasting shade tolerance (Maguire and Kobe 2015). Although this study did not examine NSC at the time of death, it found that all of the studied species' NSC concentrations decreased most sharply in the shade + drought treatment. The authors suggested that carbon becomes more limited under both stresses than under shade or drought alone or that seedlings lose their ability to maintain carbon stores. Interestingly, the particular findings of Maguire and Kobe (2015) for *Acer rubrum* Marsch. (the most shade tolerant species examined) indicate a high degree of similarity to our results for *A. pseudoplatanus*: *A. rubrum* (along with *Betula papyrifera* Marsch.) did not decrease its NSC concentrations under drought and high light; it also exhibited the greatest reduction in SS under drought and shade conditions combined. *Acer rubrum* and *A. pseudoplatanus*' consistency in response to drought and shade suggests that shade tolerance may also be a determining factor in the access to and utilization of stored C under deep shade, as previously suggested (Myers and Kitajima 2007).

As expected, drought had contrasting effects on C dynamics depending on light availability. Under light conditions, the soil dried out quickly, which most likely reduced plant hydration, rapidly inhibiting growth before photosynthesis and thus causing NSC to accumulate (Boyer 1970, McDowell 2011, Muller et al. 2011, Hoch 2015). The early mortality of seedlings exposed to both drought and light conditions and their corresponding high NSC concentrations at the time of death appear to be compatible with impeded C storage access and utilization and a massive hydraulic failure, as found in other studies that also applied severe drought (Anderegg and Anderegg 2013, Mitchell et al. 2013, Sevanto et al. 2014). Under complete darkness, by contrast, the slower progression of soil desiccation probably implied a delay in tissue dehydration, allowing sustained growth along with a higher NSC remobilization and utilization to meet growth and respiration demands that would otherwise be met by C assimilation. Eventually, the capacity to access, transport and utilize NSC under slow soil desiccation was most likely responsible for the delayed mortality of -W-L seedlings when compared with their -W+L counterparts. On the other hand, the fact that under complete darkness drought did not promote increases in NSC concentrations or decreases in growth rates

(as it did under light) casts some doubt on the hypothesis that NSC accumulation under drought could be driven by a drought-induced change in C allocation priorities from growth to storage (Wiley and Helliker 2012). If drought induced more C to be allocated to storage rather than growth, then drought should have promoted a decrease in growth along with increased NSC concentrations in seedlings under light, but also under complete darkness, which was not the case.

Our study suggests that C sinks under complete darkness were altered by drought. Well-watered seedlings under complete darkness (+W–L) and seedlings exposed to both drought and complete darkness (–W–L) invested a similar portion of their C stores into increasing their height and biomass. Since both the NSC concentrations at the time of death and the growth rates were similar for these two treatments, the anticipated mortality observed in –W–L seedlings could have been driven by an accelerated C use for growth. However, this explanation is unlikely, provided that growth is highly sensitive to drought (Boyer 1970, Muller et al. 2011). These seedlings most likely had an increased C demand from sinks other than growth. One of these sinks could be root growth and metabolism, which can be stimulated in response to drought (Hartmann 2015). In accordance with this, several studies have also found significant reductions in root NSC concentrations in response to drought (Galvez et al. 2013, Hartmann et al. 2013a, 2013b, Mitchell et al. 2013), a response that we also found in *A. pseudoplatanus*. In addition, maintenance respiration could increase under drought and thus lead to a faster C depletion, earlier attainment of the minimum *survivable* NSC concentration, and eventually, anticipated mortality (McDowell 2011).

Under conditions of complete darkness, we found that starch but especially SS concentrations were lower in dead seedlings of both –W–L and +W–L treatments than in the surviving seedlings of the +W–L treatment. Our results thus support the notion that SS (low molecular-weight sugars) play a critical role in seedling survival under drought (Dietze et al. 2014), and are consistent with the idea that increased osmotic demand may explain seedlings' anticipated mortality under drought (McDowell 2011, McDowell et al. 2011, Galvez et al. 2013). Nevertheless, the lower SS concentrations in dead than in surviving seedlings of the +W–L treatment might suggest that osmotic disruption is the ultimate cause of mortality in plants with impeded C assimilation, even if they are well hydrated. Although the sugar concentrations of dead seedlings of the +W–L treatment seem high enough to argue in favor of osmotic limitations, the absolute NSC concentrations may be very difficult to precisely determine (e.g., they could be overestimated) and therefore should be interpreted with caution (Quentin et al. 2015).

Conclusions

Carbon availability is critical to plant survival under drought conditions, as it mediates the hydraulic functioning of plants and

stored C can meet the plant's C demands when C assimilation is limited for a long period. However, previous studies have found that NSC access, translocation and utilization are impaired by drought (Hartmann et al. 2013a, Sevanto et al. 2014). In this study, we found no evidence of drought-induced limitations to the access and utilization of stored C in *A. pseudoplatanus* seedlings under complete darkness. On the contrary, drought appeared to accelerate the use of C reserves under complete darkness. Similar results have been found in seedlings of other shade tolerant, winter deciduous species (Maguire and Kobe 2015), suggesting that this could be a more generalized response in this plant functional group. Our study is also in line with previous studies that have found significant C storage reductions in seedlings subjected to slow desiccation (Galvez et al. 2013, Mitchell et al. 2013), highlighting that the progression of drought severity is a key aspect for C dynamics. It remains to be investigated whether and how drought severity progression also determines the C dynamics of adult trees, for which criteria on drought severity can be more difficult to define.

Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

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Conflict of interest

None declared.

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