



Sacrificing growth and maintaining a dynamic carbohydrate storage are key processes for promoting beech survival under prolonged drought conditions

Pierre-Antoine Chuste^{1,2} · Pascale Maillard^{1,2} · Nathalie Bréda^{1,2} · Joseph Levillain^{1,2} · Erwin Thirion^{1,2} · Rémi Wortemann^{1,2} · Catherine Massonnet^{1,2} 

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Abstract

Key message In case of a prolonged drought, the stored carbohydrates in trees were remobilized to fuel survival functions until their nearly depletion at death stage.

Abstract Dynamic global vegetation models project forest tree mortality in response to the recurrent severe droughts likely in the future. However, these models should better take into account the physiological processes involved in tree mortality. Faced with severe drought, the *Fagus sylvatica* L. tree strongly limits its cambial growth. This suggests that readjustments in carbon (C) allocation among sink functions are taking place in response to the lack of water and this could allow tree's survival. For 3 years, we induced a water shortage on 8-year-old beech trees in a rain exclusion system. During this period, we analysed the consequences of severe drought on survival rate, growth, and non-structural carbohydrate (NSC) dynamics in the aboveground and belowground compartments of control, water-stressed living, and dead trees. The survival rate after 3 years of drought was 87%, while primary and secondary growth was strongly reduced. The first 2 years, NSC concentrations increased in all tree compartments (stem, branches, and roots) in response to drought. However, during the third year, starch dropped markedly in water-stressed trees, while soluble sugar concentrations remained similar to control trees. All the compartments in dead trees were virtually empty of starch and soluble sugars. Maintaining an active C storage function at the expense of growth was certainly key to *F. sylvatica* survival under prolonged extreme drought conditions. Process-based models predicting mortality should better take into account C storage and remobilization processes in forest trees.

Keywords Carbon allocation · Carbon starvation · *Fagus sylvatica* L. · Mortality · Non-structural carbohydrates (NSC) · Storage

Introduction

Throughout their life, trees must face multiple biotic attacks and abiotic constraints, which disrupt their carbon (C) assimilation. To cope with these events, trees have developed strategies to manage their C resources and allocate them to growth, respiration, storage, reproduction, and defense (Chapin et al. 1990; Hoch et al. 2003). Carbon storage in trees consists in accumulating photosynthesized carbohydrates in specialized organs and tissues. These carbohydrates are mainly in the form of soluble sugars and starch, which make up remobilizable non-structural carbohydrates (NSC) (Dietze et al. 2014). The remobilization of stored C compounds is of primordial importance, especially for broad-leaved tree species, for maintaining tree functions and metabolism during periods of limited photosynthesis

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✉ Catherine Massonnet
catherine.massonnet@inra.fr

¹ Université de Lorraine, AgroParisTech, INRA, UMR Silva, 54000 Nancy, France

² INRA, UMR 1434 Silva, 54280 Champenoux, France

or a lack thereof (Chapin et al. 1990). This remobilization can occur at diurnal or seasonal scales (Li et al. 2002; Hoch et al. 2003, Hartmann and Trumbore 2016) or in response to biotic attacks or climatic hazards like drought (McDowell et al. 2008; Hoch 2014). Remobilization of *C* compounds may also help trees to prevent tissue dehydration to maintain cell elongation and differentiation shortly after the onset of a drought event (Boyer 1970). However, the degree of importance of *C* storage and remobilization processes involved in the responses of forest trees to long-term drought remains unclear.

The mechanisms that control *C* storage in trees are still under debate. For many years, *C* storage was thought to involve a buffer *C* reservoir, which was either passively filled or emptied in case of *C* surplus or *C* shortage, respectively. Recently, various authors have suggested an active control of *C* storage, competing with other sinks for *C* (Silpi et al. 2007; Wiley and Helliker 2012). These authors suggest that growth reduction, which is often observed in response to stress, actually would result from a decrease in the availability of photosynthetic *C*, which is preferentially allocated to storage at the expense of growth. Other recent studies have suggested that a sink limitation of growth, i.e., a meristematic activity limitation, may also be at play (Palacio et al. 2014; Hinman and Fridley 2018), and depends of tree species (Guillemot et al. 2015). They suggest that growth could only really be constrained by a lack of *C* in case of drastic events such as recurrent defoliation or severe drought (Palacio et al. 2014). The response of tree *C* allocation to stress is complex and undoubtedly depends on many factors such as species and the type, intensity, length, and period of constraint. It is questionable to what extent changes in the *C* allocation scheme can help trees to survive more frequent and extreme climatic events, as predicted by various climate change models (IPCC 2014). In recent years, extensive tree mortality and forest decline worldwide have been attributed to frequent extreme drought events (Breshears et al. 2005; McDowell et al. 2008; Anderegg et al. 2013). Many studies have focused on the causes, consequences, and climatic thresholds of drought-induced mortality (Anderegg and Anderegg 2013; McDowell et al. 2013; Gustafson 2014). Several hypotheses have been proposed concerning the mechanisms involved in tree mortality such as hydraulic failure, carbon starvation, phloem dysfunction, nitrogen starvation, and reduced defenses leading to successful opportunistic biotic attacks (McDowell et al. 2008; Sala et al. 2010; Gessler et al. 2017; Dannoura et al. 2019). Nevertheless, the respective importance of these physiological mechanisms is still under debate (Sevanto et al. 2014; Meir et al. 2015). Whereas the role of hydraulic failure has been demonstrated in juvenile tree mortality (Barigah et al. 2013) or hypothesized in mature trees (Choat et al. 2012, 2018), the role of *C* starvation is still understudied. Carbon starvation may

happen under prolonged soil water deficit due to consumption of *C* reserves, while stomatal closure leads to restricted *C* uptake, assimilation and translocation (Pangle et al. 2015). Considering the high quantity of *C* stored in adult forest trees (Genet et al. 2010; El Zein et al. 2011), *C* starvation may occur quite late, even after several years of drought (McDowell and Sevanto 2010; McDowell et al. 2011; Klein et al. 2014), making it very challenging to observe in experimental conditions. In response to prolonged severe drought events, it would be of interest to know what extent *C* stored can be remobilized to satisfy tree maintenance functions and tree survival. However, the role of *C* reserve dynamics in maintaining the vital functions of a tree during prolonged drought remains obscure.

To test the *C* starvation hypothesis and its involvement in drought-induced tree mortality, it is important to understand how tree *C* reserves change over time. Drought may modify NSC concentrations more at the organ level than at the whole-tree level (McDowell and Sevanto 2010; Galvez et al. 2011; Gruber et al. 2012). Furthermore, each perennial organ (roots, branches, twigs, and trunk) may have specific NSC dynamics (Körner 2003; Gaudinski et al. 2009; Dai et al. 2018). A better understanding of tree mortality also needs to consider tree ontogeny, since physiology and *C* allocation of seedlings and adult trees differ considerably within the same species (Baber et al. 2014, Genet et al. 2010). Observed mortality in very young (< 3 years) trees has been related to their limited rooting depth, which makes them more susceptible than adult trees to hydraulic failure (Hanson and Weltzin 2000). In older trees, the combination of greater belowground exploration (Lloret et al. 2004), higher carbohydrate storage, higher water storage, and lower transpiration per leaf area unit (Tyree 2003) should improve their resistance to prolonged drought compared to younger trees. Unfortunately, ecophysiological studies on adult forest trees remain scarce (Gérard and Bréda 2014; Gaylord et al. 2013; Cailleret et al. 2014). As underlined by Hartmann et al. (2018) and Bussotti et al. (2018), a focus on the physiological responses of mature trees to environmental stresses is urgent to better anticipate how forest trees will cope with extreme disturbances in the future.

Fagus sylvatica L. is a drought-sensitive species, especially when soil water shortage is severe and prolonged (Backes and Leuschner 2000; Dittmar et al. 2003; Lebourgeois et al. 2005; Gessler et al. 2007; Granier et al. 2007; Fotelli et al. 2009; Liu et al. 2017). These studies demonstrated that soil water deficit negatively affected beech cambial growth as well as annual twig growth. The ICP Forests network (International Co-operative Program on Assessment and Monitoring of Air Pollution Effects on Forests) that annually monitors forest health in Europe also reported a worsening of beech tree crown transparency in 2 years following the extreme drought event in 2003 (Lorenz

et al. 2005). However, very low concomitant mortality was reported in this species after this event (Eichhorn et al. 2005). Therefore, beech trees seem to mobilize physiological mechanisms that make it highly resistant to drought-induced tree mortality. In particular, since *C* assimilation is depressed under prolonged drought (Granier et al. 2008), how *C* storage and remobilisation processes in response to drought may intervene in the tree survival of this species?

In our study, we submitted 8-year-old beech trees to an extreme soil water deficit lasting 30 months; we expected these conditions to be severe enough to induce a tree *C* metabolic response. Our aim was to assess how two important *C* sinks, growth and storage, were modified during the water stress. Some of trees submitted to this prolonged drought died, so we analysed the amount of NSC remaining in dead organs in comparison to the amount of NSC in surviving drought and control trees. We made the following hypotheses concerning beech tree response to prolonged drought: (1) some trees will survive using their *C* reserves, to the detriment of *C* requirements for growth; (2) some trees will not withstand 3 years of severe drought; and (3) death will occur when NSC reaches a minimum threshold, or when the remaining stored NSC cannot be remobilized.

Materials and methods

Experimental design, growth conditions, and treatments

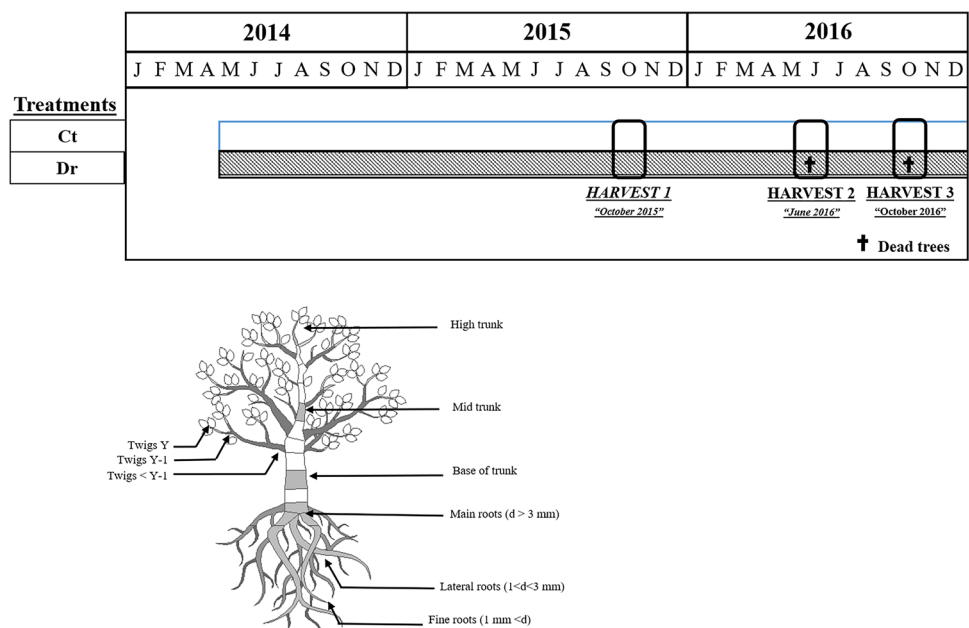
The study was conducted on 667 European beech (*Fagus sylvatica* L.) trees issued from seeds sown in 2006 and

transplanted in 2007 in open ground at the INRA Grand-Est nursery (Champenoux, France, 48°75'N, 6°34'E, 229 m asl). In 2014, a rain exclusion system was built 5 m above the 8-year-old trees: a semi-rigid structure supporting a transparent roof made of polycarbonate sheets. Nets were installed around the roof to intercept lateral rain. All trees were below the roof to guarantee similar radiation, air temperature, and relative humidity conditions between treatments. Two treatments were installed: a control (Ct, *n* = 334) and a drought (Dr, *n* = 333) treatment. In the Ct treatment, the soil was irrigated regularly throughout the experiment with an automatic drip watering system, which delivered between 2 and 4 L per tree two-to-three times a week. Irrigation was adjusted to avoid any water shortage, i.e., to maintain Relative Extractable Water (REW) > 40% (Granier et al. 1999).

Tree root system in the Dr treatment was isolated with a rigid waterproof plastic sheet (DELTA®-MS) of 1.80 m depth buried around the treatment. Trees were submitted to drought for three consecutive growing seasons (2014, 2015 and 2016, Fig. 1). Soil water deficit was kept low enough to induce long-lasting stomatal closure and reduced *C* assimilation, i.e., REW < 40% (Granier et al. 1999, 2008). REW dropped below 40% in July 2014 and stayed below this threshold throughout the experiment. Each autumn, trees in the Dr treatment were lightly irrigated (30–40 mm), though care was taken not to exceed the REW threshold of 40%.

We monitored the water status of a sub-set of trees in each treatment (*n* = 6 in 2014 and *n* = 30 in 2015 and 2016) by measuring pre-dawn water potential in twigs (ψ_{pd}) every month. We sampled one twig per tree before sunrise and performed the ψ_{pd} measurement with a pressure chamber (PMS Instruments, Albany, OR, USA).

Fig. 1 Schedule of the experiment (top) since the onset of the treatments (control, Ct and drought, Dr) in 2014. Dead trees were harvested in June and October 2016, while living trees were harvested in October 2015, June 2016, and October 2016. Diagram (bottom) of the organs sampled on each harvested tree. Twigs were taken in triplicate (*Y* year and *d* diameter)



To face woolly aphid attacks, phytosanitary treatments were carried out when necessary; this occurred once in 2015 and 2016.

Soil characteristics and soil water measurements

The common garden is characterized by homogeneous soil of 60 cm deep with a silty–clay loam texture (silt: $61 \pm 1.28\%$; clay: $27 \pm 0.98\%$; sand: $12 \pm 0.66\%$), a pH ranging from 7.5 to 8 and an organic matter content between 12.1 and $14.9 \text{ g} \cdot \text{kg}^{-1}$ (Silva 2010). Below 60 cm, the grey marl of the Jurassic inferior (Lotharingian) is characterized by a swelling heavy clay soil with a high bulk density.

We used a neutron probe (TROXLER TX 4301, Research Triangle Park, NC, USA) to measure the volumetric soil water content. Three neutron probe access tubes were installed in each treatment to quantify water content at different depths: two tubes measured from 0 to 1 m in depth and one other measured from 0 to 1.6 m. The Relative Extractable Water (REW, in %) was calculated according to Bréda et al. (1995).

Tree growth measurements and survival rate determination

In each treatment, at the end of each growing seasons (2014, 2015 and 2016) and the year before the installation of the treatments (2013), tree height (m) and trunk diameter (mm) of all trees were measured. Trunk diameter was taken at the collar with a caliper. Then annual mean height and mean diameter were calculated for each treatment.

Survival rate for each year was determined the following spring by counting the number of living trees (Lt), i.e., trees in each treatment, where budburst occurred. The survival rate (Sr) was determined for each treatment as follows:

$$Sr = 100 \times \frac{Lt}{Tt}, \quad (1)$$

where Tt is the total number of trees at the beginning of the experiment in each treatment.

Sampling and samples preparation

Trees were harvested in October 2015 ($n = 6$ per treatment), June 2016 ($n = 6$ per treatment + 6 dead trees in the Dr treatment) and October 2016 (4 per treatment + 4 dead trees in the Dr treatment). These seasonal dates were chosen to correspond to key phenological dates for NSC dynamics as detailed in the previous studies in beech mature trees (Barbaroux and Bréda 2002; El Zein et al. 2011). In June 2016, dead trees were sampled among trees, where no budburst had occurred in spring 2016 or budburst, but died just after. Dead trees harvested in October 2016 had crowns,

where all leaves had senesced and bark on the branches and trunk which had turned a reddish brown colour during the 2016 growing season. Then, trees were sampled by organ type, as presented in Fig. 1. Aboveground parts were separated into twigs and trunk and each organ was sampled for NSC analyses. More precisely, three branches were randomly chosen and cut into sections according to annual growth units: current year [Twigs Y], 1 year [Twigs Y-1], and older [Twigs < Y-1]. Slices of trunk were also sampled at three heights: top [High trunk], middle [Mid trunk], and base of trunk [Base of trunk]. For belowground parts, in June and October 2016, a sub-sampling of roots was gently cleaned with tap water and roots were separated according to their diameter: Fine roots ($d < 1 \text{ mm}$), Lateral roots ($1 < d < 3 \text{ mm}$), and Main roots ($d > 3 \text{ mm}$). In October 2015, only main roots were sampled. Fresh weight of all samples was determined; then, samples were frozen in liquid nitrogen and stored at $-80 \text{ }^\circ\text{C}$ before being freeze-dried [Dura-Top^(r), Dura-Dry^(r), FTS Systems^(r), Stone Ridge, NY, USA] for 5 days. Dried samples were weighed and then ground to a fine powder with a ball mill (CEPI SODEMI CB2200, Cergy, France). The rest of the trunk and branches was also dried at $80 \text{ }^\circ\text{C}$ during several days and then weighed to assess their total dry weight.

Soluble sugar and starch analyses

Soluble sugars (SS) were extracted from 10 mg of plant tissue powder mixed with 1 ml of 80% aqueous ethanol (v/v). This mixture was incubated in a water bath at $80 \text{ }^\circ\text{C}$ for 30 min and then centrifuged for 10 min at 10,000 g. The supernatant containing the soluble sugars was collected in a tube and SS were extracted twice again from the precipitate. The three supernatants were pooled and dried in a vacuum evaporator (Refrigerated CentriVap Vacuum Concentrators, Labconco) for 10 h, then resolubilized in 1 ml of ultrapure water. Starch contained in the precipitate was solubilized in 0.9 ml of NaOH and hydrolyzed in glucose with 100 μl of amyloglucosidase (SIGMA-A7420) solubilized in a citrate buffer at $\text{pH} = 4.6$ during 30 min at $50 \text{ }^\circ\text{C}$. SS and starch concentrations were measured following a modified anthrone method (Hansen and Moller 1975); they were determined spectrophotometrically at 620 nm (spectrophotometer UV–visible DU 640 B, Beckman Coulter, USA) in the presence of an anthrone–sulfuric acid reagent with glucose as a standard. The SS and starch concentrations of each organ were expressed in g of glucose per 100 g of dry matter (DM) and their sum is referred to as the non-structural carbohydrates (NSC). The NSC concentration ($\text{g} \cdot 100 \text{ g}^{-1} \text{ DM}$) for each organ is calculated as follows:

$$[NSC] = \frac{\left[\frac{C_{\text{spectro}} \times V_{\text{tot}} \times V_{\text{sam}} \times 10 \times 100}{DM} \right]}{1000}, \quad (2)$$

where C_{spectro} is the glucose concentration ($\mu\text{g} \cdot \text{ml}^{-1}$) read on spectrophotometer, and V_{tot} (ml) and V_{sam} (ml) are the total volume of the reaction mixture in the microcurve and the volume of the extract, respectively.

Total NSC quantity (g) for aerial parts (trunk and branches) is calculated as follows:

$$\text{NSC quantity} = \sum \frac{[\text{NSC}]_i \text{DM}_i}{100}, \quad (3)$$

where i are each organ of aboveground parts of trees for which [NSC] was determined (branches and trunk organs, Fig. 1).

Roots were excluded from the total NSC quantity calculation, because it was impossible to excavate the whole root system without damaging the surrounding trees.

Statistics

We used general linear mixed-effect models to assess the effect of treatment on seasonal pattern of pre-dawn water

potential, on growth (height, diameter, and biomass), and on NSC concentrations and quantity with individual trees set as random factor. If distribution was not normal, a logarithmic transformation was applied. Normality and homoscedasticity of standardized residuals were graphically checked on quantile-to-quantile and residual-vs-predicted plots. When a significant effect was revealed, Tuckey tests were performed. Data were analysed with the R software package (<http://www.r-project.org>, version 3.2.2, 2016-10-31). Values are presented as mean \pm standard error. Differences were considered significant when $p < 0.05$.

Results

Soil and branch water monitoring

In the irrigated control (Ct) treatment, the REW in the soil was held above 40% for the duration of the experiment (Fig. 2a) and the pre-dawn water potential (ψ_{pd}) always

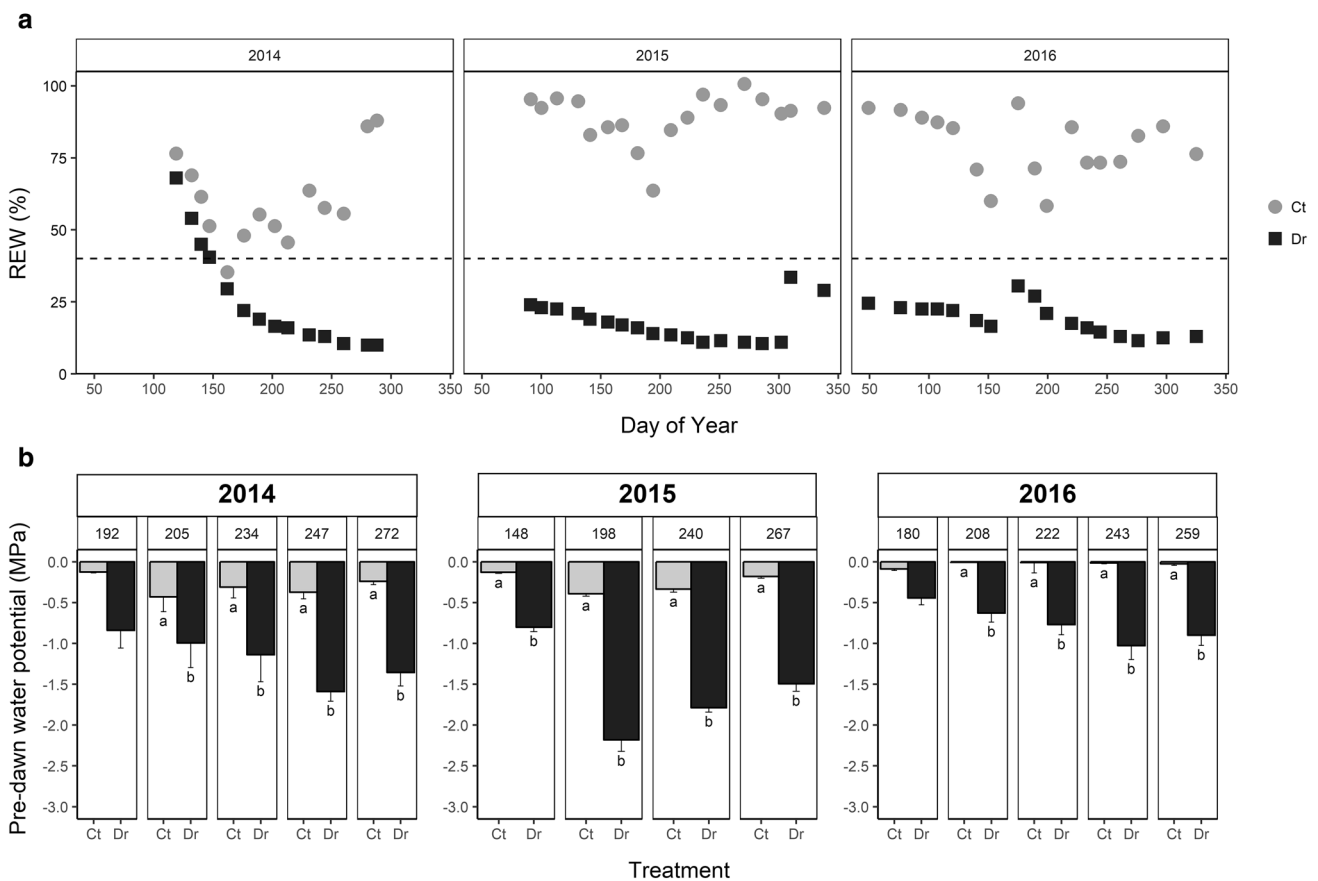


Fig. 2 a Seasonal dynamics of the relative extractable soil water (REW) content calculated at a depth of 1.6 m. Each point is the mean REW calculated for three access tubes. The dashed line indicates the REW threshold value (40%) below which stomatal conductance is affected, according to Granier et al. (1999). **b** Average pre-dawn

twig water potential in 10-year-old beech trees from year 2014 to year 2016 under soil water deficit (drought, Dr, in black) and with irrigation (control, Ct, in grey). Different letters indicate a significant difference ($p < 0.05$) between treatments for a given date

remained above -0.5 MPa (Fig. 2b). In the drought treatment (Dr), the soil water deficit was set up in 2014 with REW below 40% and this deficit lasted for the total duration of the experiment. A slight increase in REW was observed in November 2015 due to the light fall irrigation carried out in the Dr treatment and one other increase was observed in June 2016 when a heavy rain moistened the soil slightly. Each year, because of the progressively worsening soil water depletion, ψ_{pd} in Dr twigs decreased from -0.8 MPa (day of year (DOY) 192) to -1.6 MPa (DOY 247) in 2014, from -0.7 MPa (DOY 153) to -2.18 MPa (DOY 198) in 2015 and from -0.44 MPa (DOY 180) to -1.02 MPa (DOY 243)

in 2016 (Fig. 2b). Dr trees showed significantly lower ψ_{pd} than Ct trees for all dates, excepted in DOY 192 in 2014 and DOY 180 in 2016.

Impact of treatments on tree growth and survival

The Dr treatment had a strong impact on tree growth both in terms of height (Fig. 3a) and diameter (Fig. 3b). In 2013, before the beginning of the treatments, tree sizes were initially similar among treatments (Fig. 3a, b), while in 2016, after 3 years of experiment, Dr trees were significantly smaller [$p < 0.001$, mean height (-26%) and mean diameter

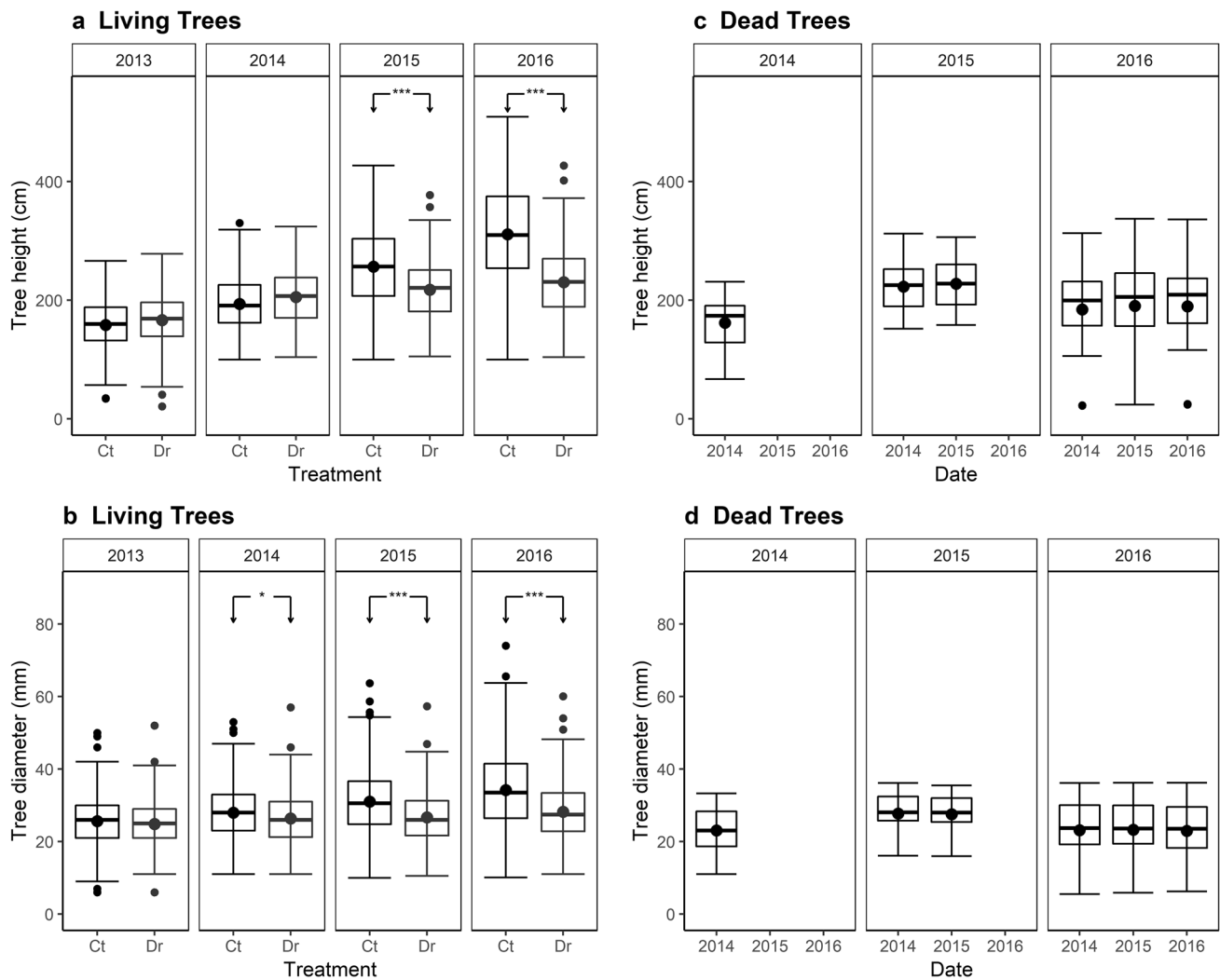


Fig. 3 Boxplot showing the height (cm, **a**) and diameter (mm, **b**) of beech trees under soil water deficit (drought, Dr, $n=333$ —number of dead trees) or irrigation (control, Ct, $n=334$) from 2013 to 2016 for living trees. In c and d, the panels 2014, 2015, and 2016 present the height (c) and diameter (d) of Dr trees dead in 2014, 2015, and 2016, respectively. For the trees dead in 2014 (panel 2014, $n=10$), height and diameter of these trees measured the year of the death (2014) are presented. For the trees dead in 2015 (panel 2015, $n=16$), height and

diameter of these trees measured the year of the death (2015) and the year before death (2014) are presented. For the trees dead in 2016 (panel 2016, $n=16$), height and diameter of these trees measured the year of the death (2016) and 2 years before death (2014 and 2015) are presented. Significant differences between dates for each treatment and between treatments for each date are presented with: * $p < 0.05$; ** $p < 0.01$, and *** $p < 0.001$. In each boxplot, the point represents the mean value

(−17%)] than Ct trees. Furthermore, differences in height and diameter between treatments increased throughout the experiment. Differences increased from −3 (*ns*) to −15% ($p < 0.001$) for height and from −5.8% ($p < 0.05$) to −15% ($p < 0.001$) for diameter from 2014 to 2015. The date effect (e.g., between 2013 and 2016) was significant in both Ct and Dr treatments ($p < 0.001$). Moreover, monthly relative increment in diameter slowed in Dr trees compared to Ct trees from DOY 180 in 2014 and through 2015 and 2016 growing seasons (Suppl. Figure S1). In 2015, relative increment in diameter in Dr trees even presented some negative values, indicating a shrinkage of trunks in response to drought. In 2016, very low growth was observed in Dr trees with a maximum value of 0.9% vs 3.4% in Ct trees.

At the beginning of each growing season, the survival rate of the previous year was calculated in the two treatments by noting trees, whose buds did not burst (Table 1). During the experiment, no mortality occurred in the Ct treatment, i.e., the survival rate stayed at 100%. In the Dr treatment, the survival rate decreased from year to year reaching 87.4% after 3 years of severe drought. The annual mortality rate was 3% the first year and 4.8% each of the following 2 years. Dead trees varied considerably in size and reflected different social status, from suppressed to dominant trees. Indeed, the height of dead trees ranged from 67 to 231 cm in 2014, from 158 to 306 cm in 2015 and from 24 to 336 cm in 2016 (Fig. 3c). The diameter of dead trees ranged from 11.04 to 33.27 mm in 2014, from 15.99 to 35.49 mm in 2015, and from 6.25 to 36.24 mm in 2016 (Fig. 3d). Mean trunk diameter of the trees died in 2015 and 2016 was slightly smaller than mean trunk diameter measured on these same trees the previous year, revealing a shrinkage of trunks in these trees (Fig. 3d).

Change over time in NSC concentrations in aboveground and belowground compartments in response to soil water deficit

In October 2015, 18 months after the onset of the drought treatment when seasonal stocks of *C* reserves are typically at their maximal level in trees, only a slight difference in NSC concentrations was observed between treatments

(Fig. 4, Suppl. Table S1). Dr trees had significantly higher total NSC concentrations than Ct trees in only two young compartments: the youngest twigs (Twigs Y, $p < 0.05$) and the higher part of the trunk (high trunk, $p < 0.05$) with NSC values of, respectively, 5.63% and 7.27% in Ct trees and of, respectively, 13.6% and 14.1% in Dr trees (Fig. 4, Suppl. Table S1). For these two compartments, NSC concentrations of Dr trees differed from Ct trees by 196% and 90%, respectively (Suppl. Figure S2). These two compartments (Twigs Y, $p < 0.05$, high trunk ($p < 0.05$) plus main root ($p < 0.05$)) presented significantly higher SS values in Dr trees than in Ct ones (from 4.91 to 5.1% in Dr trees and from 2.47 to 2.8% in Ct trees). Starch presented similar values between Dr and Ct treatments whatever the compartments.

Six months later, in June 2016, when stocks of *C* reserves were minimal, compartments of Dr trees presented similar (for Twigs Y, Mid trunk and Fine roots) or higher (other compartments) values of NSC concentration than Ct trees, but these differences were not significant. All compartments presented higher starch concentrations in Dr trees than in Ct trees, but these differences were significant only in lateral roots (2.17% for Ct and 7.02% for Dr, $p < 0.001$). SS concentrations were similar between treatments whatever the compartments. Contrariwise, dead trees presented very low NSC concentrations in all compartments (between 0.91 in Base of trunk and 2.16% in Twigs Y, Fig. 4, Suppl. Table S1). They presented significantly lower NSC concentrations than Ct trees and alive Dr trees in all compartments except for Twigs Y-1 and Fine roots. The difference ranged from −57% for Twigs Y-1 to −89% for the Base of trunk compared to Ct trees (Suppl. Figure S2). In dead trees, significantly lower starch concentrations than in Ct trees were measured in all compartments except for Twigs Y Twigs Y-1 and Fine roots (Fig. 4, Suppl. Table S1). Starch concentration values ranged from 1.9 to 6.8% in Ct trees, from 2.26 to 10.6% in Dr trees and from 0.39 to 1.47% in dead trees (Fig. 4, Suppl. Table S1). Dead trees had significantly lower SS concentrations than Ct trees only in Twigs Y and Base of trunk (3.67% and 1.27%, respectively).

Finally, in October 2016, after 3 years of drought, when stocks of *C* reserves were at maximal levels in trees, NSC concentrations were similar to the ones measured in October 2015 in Ct treatment (mean of 10.21% in 2015 and 8.04% in 2016). However, NSC concentrations had strongly decreased in Dr treatment (mean of 13.7% in 2015 and 4.2% in 2016) for all compartments (Fig. 4, Suppl. Table S1). Drought decreased NSC concentrations between −42% and −79%, depending on the compartment, compared to the Ct treatment (Suppl. Figure S2), but these differences were significant only in Twigs Y and Fine roots. Starch concentrations were significantly lower in Dr trees than in Ct trees for all compartments except for high trunk and base of trunk. For SS concentrations, significantly higher values were observed

Table 1 Changes over time in the number of living trees per treatment (control and drought) and annual mortality rate (%) in the drought treatment

	Years			
	2013	2014	2015	2016
Control	334	334	334	334
Drought	333	323	307	291
Annual mortality rate under drought (%)	0	3	4.8	4.8

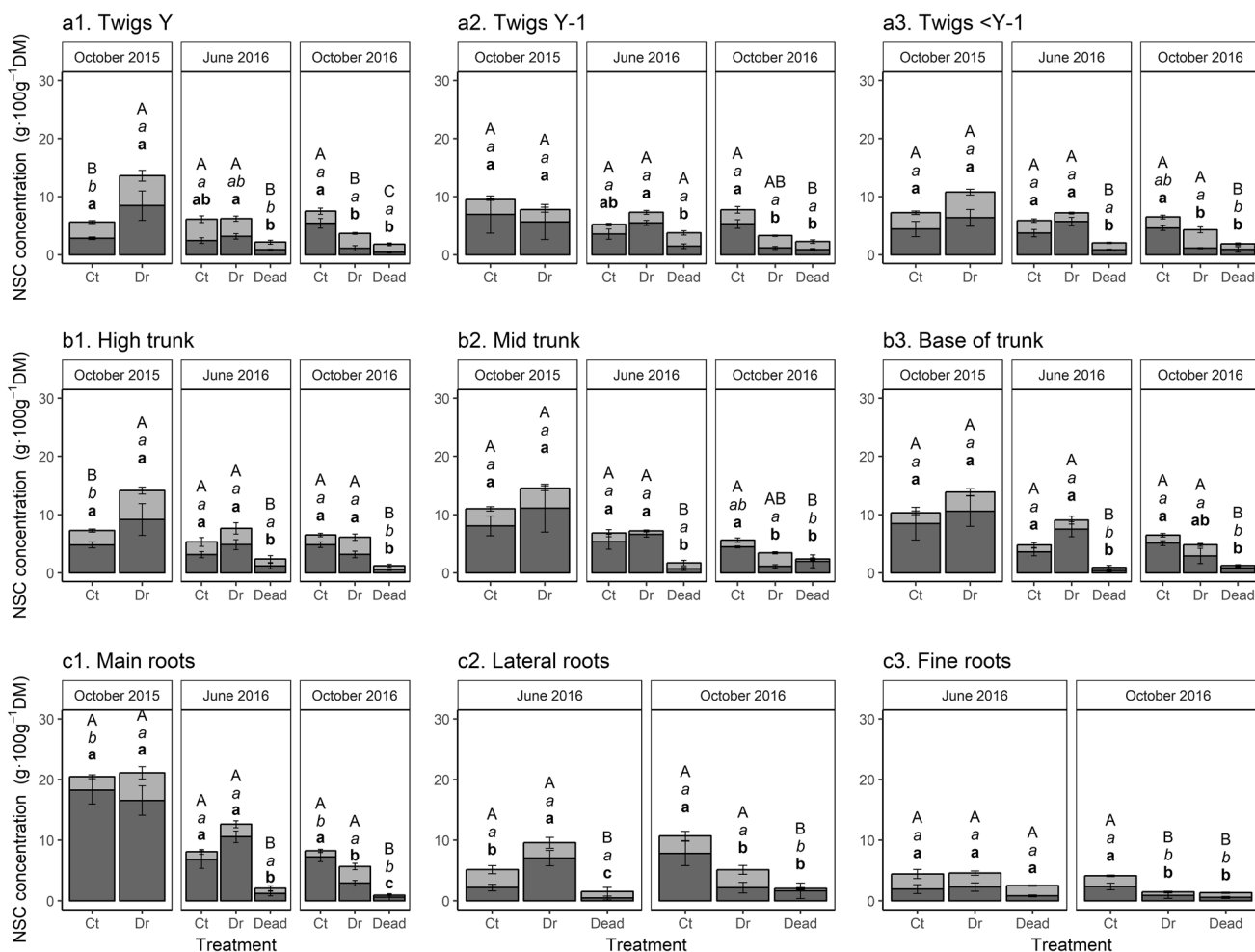


Fig. 4 Mean concentrations ($\text{g} \cdot 100 \text{g}^{-1}$ Dry Matter) of non-structural carbohydrates (NSC) in branches [twigs Y (a1), twigs Y-1 (a2), and twigs <Y-1 (a3)], trunk [high trunk (b1), mid trunk (b2), and base of trunk (b3)] and roots [main roots (c1), lateral roots (c2), and fine roots (c3)] in drought (Dr) and control (Ct) trees. The sampling protocol is presented in Fig. 1. Dead trees were also sampled in June and October 2016 when mortality was recorded. Starch concentrations are in dark grey and soluble sugar concentrations are in light grey. Different letters indicate a significant difference ($p < 0.05$) between treat-

ments for a given organ. Differences between starch concentrations are given in the lower line in bold lowercase letters, while differences in soluble sugar concentrations are in the middle line in italics. Differences in NSC between treatments are given in the upper line in uppercase letters. $n = 6$ each for Ct and Dr trees in October 2015 and June 2016 and $n = 4$ in October 2016. Six dead trees (trees which presented no budburst in spring 2016) were sampled in June 2016 and four trees in October 2016 (trees which died during the 2016 growing season). Complementary statistical analysis is in the Suppl. Table S1

in Dr trees compared to Ct trees in main roots, whereas lower values were observed in fine roots and similar values were found in all other compartments. Dead trees had significantly lower NSC concentrations than Ct trees for all compartments ($p < 0.001$). A deviation ranging from -55% to -92% according to the compartment was observed between dead and Ct trees (Suppl. Figure S2). A reduction in both starch and SS concentrations was observed in dead trees compared to Ct trees in all compartments for starch and excepted in main roots, mid trunk, and all twigs for SS. Dead trees presented also significantly lower NSC concentrations than alive Dr trees for all compartments except Twigs Y-1, mid trunk, and fine roots (Fig. 4, Suppl. Table S1). SS in dead

trees were lower than in alive Dr trees in all compartments except for Twigs Y, Twigs Y-1, and fine roots. Starch in dead trees was significantly lower than in alive Dr trees in main roots and high trunk and was similar in other compartments.

Impact of drought on growth and storage partitioning in aboveground organs

The biomass and NSC quantity in aboveground organs (trunk and branches) were compared between treatments at three sampling dates (Fig. 5a). In October 2015, branch, trunk, and total aboveground biomass and NSC quantities were similar in Ct and Dr trees (Fig. 5a, b). In June

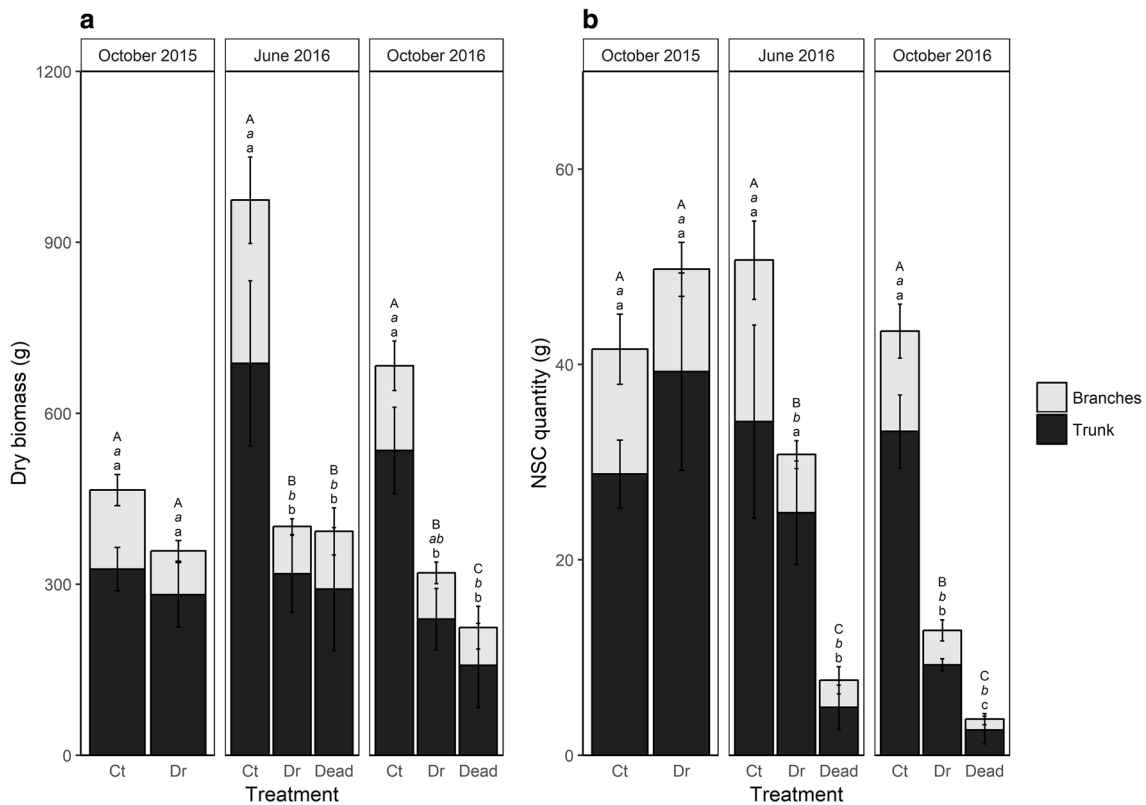


Fig. 5 Dry biomass (**a**, **g**) and non-structural carbohydrates (**b**, NSC, **g**) content in branches and trunks in drought (Dr) and control (Ct) trees. Dead trees were sampled in June and October 2016. Uppercase letters indicate differences between treatments for total NSC or biomass, middle lowercase letters indicate differences between values for branches, and lowercase letters at the bottom indicate differences for

trunk values only. $n=6$ each for Ct and Dr trees in October 2015 and June 2016; $n=4$ in October 2016. Six dead trees (trees which presented no budburst) were sampled in June 2016 and four (trees which died during the 2016 growing season) in October 2016. Differences are significant at $p < 0.05$

2016, Dr alive and dead trees had significantly less trunk, branch, and total aboveground biomass than Ct trees (Fig. 5a), which is coherent with the increasing impact on growth of the continuous drought (Fig. 3). No significant difference in NSC quantity was noticed between Dr and Ct trunks, while the NSC quantity in Dr branches was significantly lower (–65%) than in Ct branches (Fig. 5b). Dead trees had lower NSC quantities in all compartments (–85%) compared to Ct trees. Finally, by October 2016, soil water deficit had decreased both trunk (–55%) and whole aboveground biomass in Dr trees (–55% compared to Ct trees), but branch biomass was similar. Dead trees had lower branch (–55%), trunk (–70%) and total aboveground biomass (–68%) than Ct trees (Fig. 5a). Dr trees had lower NSC quantities in the branch (–65%), trunk (–73%), and total aboveground (–70%) compartments compared to Ct trees (Fig. 5b). Dead trees had significantly lower quantities of NSC in the trunk (–93% compared to Ct trees), branches (–90%), and total aboveground biomass (–91%) than Ct trees and Dr alive trees.

Discussion

Jeopardizing growth and maintaining dynamic whole-tree carbohydrate storage enabled beech survival during the two first years of severe drought

In our experiment, beech trees exhibited a drastic reduction in growth in both secondary and primary stem tissues in response to soil water deficit. This is a common response for *Fagus sylvatica* L. and other isohydric species (Bigler and Veblen 2009; Voltas et al. 2013; Hentschel et al. 2016). This decrease in growth could result from water deprivation for cell expansion (sink limitation) and/or a C limitation (source limitation). However, Galiano et al. (2011) showed that limiting C availability alters tree growth only in some extreme cases. Our results provide further arguments rather in favour of a sink limitation, at least during the two first years of drought. Indeed, shrinkage of the trunk was observed under drought, particularly

in 2015 (Suppl. Figure S1). This likely indicates a modification in the within-tree water content, as confirmed by the lower water content in trunks of Dr trees compared to Ct trees in October 2015 and June 2016 (Suppl. Figure S3). In our Dr trees, *C* assimilation was also markedly reduced (D. Epron, personal communication), but this *C* limitation had been able to be compensated by stored *C* compounds. Stored *C* compounds are a fundamental source of *C* for active *C* sinks, which are essential for a tree's survival. This role seemed to be still functioning perfectly in our beech trees even after 2 growing seasons of soil water deficit. Indeed, in October 2015, after 18 months of drought, NSC concentrations and stocks were similar between Dr and Ct trees. The perennial organs near leaves (Twig Y and High trunk) and the root compartment even tended to have increased NSC concentrations due to an increase in SS in response to drought. This result would suggest that after two growing seasons under severe drought, our 9-year-old beech trees were able to restrict their *C* consumption enough to avoid resorting to their *C* reserves. Though, despite the reduction in growth, other important *C* sinks were still active (e.g., transport, respiration, and defense) or may have even been up-regulated (e.g., SS increase for osmoregulation or hydraulic integrity). This indicates that to maintain NSC under drought conditions, the dynamic storage function was likely preserved, through active or passive processes, this is still unclear (Dietze et al. 2014; Piper et al. 2017). A combination of both a semi-passive control of *C* storage (decreasing *C* allocated to growth) and a semi-active control (accumulating SS for osmoregulation, i.e., protection against cell dehydration) could be occurring, as suggested by Piper et al. (2017) on Douglas fir. In our study, we also observed these two processes (reduction in growth and accumulation of SS) in Dr trees. The increase in SS that we observed did not result from starch–sugar interconversion, since there was no variation in starch concentrations in response to drought in October 2015. This increase probably reflects the tree's crucial need for SS recently assimilated to maintain metabolic functions under drought (osmoregulation and transport), particularly near the meristematic zones. The increasing SS concentrations that we found in the root compartment, while starch concentrations remained constant is also the sign that the transport of sugars did not stop in Dr trees even if it slowed compared to Ct trees (Dannoura et al., 2019).

Three years of drought strongly decreased NSC and threatened tree survival

Time is one of the important factors to take into account when interpreting *C* dynamics in trees under stress (Sala et al. 2012). In our 10-year-old beech trees, a changeover

began to occur in NSC dynamics during the third year of drought. The storage function seemed to become impaired by the prolonged soil water deficit. The very detailed sampling method used in our study allowed us to map the complete within-tree variability in NSC, as shown schematically in Fig. 6. In June, whereas NSC concentrations usually decrease following their mobilization for spring growth, the NSC concentrations remained high in the drought treatment, particularly in the root system. Dr trees hardly consumed their *C* reserves, contrary to Ct trees. However, during the third consecutive season of drought, Dr trees markedly consume their *C* reserves instead of stocking them. Indeed, in October 2016, total NSC concentrations in Dr trees were lower than the ones in Ct trees in all organs, including roots and trunk, the two main long-term storage organs. The strongest differences concerned the roots. This shows that the storage organs had begun with remobilising their *C* reserves to help the trees survival under persistent drought. The decrease in NSC concentrations was mostly due to a

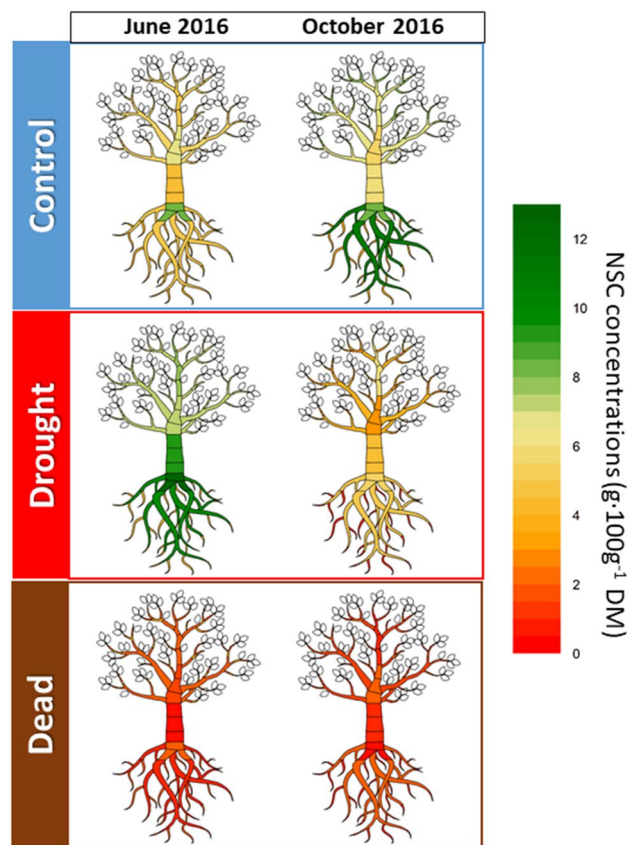


Fig. 6 Schema of the within-tree distribution in non-structural carbohydrates (NSC) concentrations ($\text{g} \cdot 100 \text{g}^{-1}$ dry matter (DM)) during the third year of treatment in drought (Dr), control (Ct) and dead trees. Two dates: June 2016 (on the left) and October 2016 (on the right) are presented. The colour range (see legend) from red (the lowest concentrations) to green (the highest concentrations) represents the concentration level of NSC

strong decrease in starch concentrations in all compartments of Dr trees. A concomitant increase in SS concentrations was also observed in some compartments of Dr trees, suggesting that a starch–sugar inter-conversion had occurred. We only monitored the dynamics of NSC compounds in our study, but it is possible that the strong decrease in starch concentrations was the precursor of a more generalized senescence process in tissues at the whole-tree scale. Although the role of sugars in the senescence process is not clear (Wojciechowska et al. 2018), a long-term source–sink imbalance could induce whole-tree senescence before death. Trees could also remobilise other compounds such as hemicellulose, proteins, or lipids, which can be a source of C for plants enduring extreme conditions (Bathelier et al. 2009). As Munné-Bosh and Alegre (2004) described for leaf senescence, a prolonged drought could lead to a programmed cell death in various organs of the tree. The programmed cell death process is known to take part in the defense response of plants to environmental stress (Locato and De Gara 2018). With this process, plants limit respiratory C sink through the partial death of tissues and at the same time remobilise sugars from the catabolism of membrane lipids via the neoglucogenesis process.

In the third year of drought, we sampled a few dead beech trees. Both starch and soluble sugars had been strongly reduced in the perennial organs of these trees, suggesting that trees had either used the soluble sugars locally or transported them from perennial organs to photosynthetic tissue before dying. These trees had considerably reduced their carbohydrate stocks by the moment of death, but had died before depleting them completely. Total depletion (NSC = 0) seems highly unlikely (McDowell et al. 2011; Hartmann et al. 2013). NSC use was likely no longer possible with persistent, harsh soil desiccation, but determining if dead trees had reached a lethal NSC threshold would require further investigation. We cannot exclude possible contributions of internal nutrient deficiency or hydraulic failure in the physiological mechanisms leading to the death of trees. Some hydraulic conductance measurements done in 2016 on trees of our experiment showed a mean embolism rate of 54% with data ranging from 21% to 74% in branches of alive Dr trees (R. Wortemann, Personal communication). This possible disruption of hydraulic network under drought may explain why soluble sugars and starch had not yet been totally depleted at death. In fact, the enzymatic hydrolysis of starch requires water (Stitt and Zeeman 2012) and this process may be seriously impaired under severe soil water deficit due to local hydraulic dysfunction (Sevanto et al. 2014; Aguadé et al. 2015; García de la Serrana et al. 2015). Moreover, SS play a key role in osmotic adjustment, possible embolism refilling and root system growth. Therefore, if the conversion of starch to glucose or sucrose to glucose/fructose is no longer possible, C depletion can exacerbate

hydraulic dysfunction (Sala et al. 2012). Inversely, starch could be blocked in its reservoir due to embolism and be unavailable for tree metabolism (Sala et al. 2012; Sevanto 2014). The lack of water and carbon can be particularly harmful when droughts become longer and harsher.

Implications for predicting beech tree mortality in response to hazards

In our study, the total mortality rate was 12.6% after 3 years of drought (3% the first year and 4.8% each of the two following years). According to technical reports published annually by the European monitoring network, ICP Forests, the natural annual mortality for beech trees is less than 0.3% on average (0.1% in France, IGN, 2016), and only reached 0.6% in 2005, as a consequence of the 2003 drought and a Europe-wide summer heat wave. In other words, our experiment induced excess mortality, with 16 times more dead trees than the natural level and 8 times more than the extreme level induced by the 2003 drought and heat-wave event. It should also be noted that our experiment induced a lower pre-dawn potential in twigs (−2.5 MPa) in August 2015 compared to the one recorded in 2003 (−2.0 MPa) in a nearby beech forest (Peiffer et al. 2014), thus highlighting the extreme stress suffered by our trees. However, although we obtained excess mortality compared to natural mortality rate, we also found that the mortality rate was stable from year to year and did not increase dramatically after 3 years of severe drought as expected. Contrary to the numerous mortality studies that work on very young potted trees, our conditions were closer to field conditions for adult trees with a well-developed root system and strong storage functions. In our case, *Fagus sylvatica* L. confirmed its high resistant status to drought-induced tree mortality events. *Fagus sylvatica* L. is a diffuse-porous species, whose phenology (wood growth starts after spring leaf burst) and wood properties (with small vessels) make it few vulnerable to winter embolism and few dependant on C reserves in spring (Gasson 1987; Hacke and Sauter 1996; Barbaroux et al. 2003). These hydraulic properties and the strategy to preserve C storage pools to the detrimental of growth could explain the small number of trees which died after 3 years of severe soil water deficit. However, this small number could also be explained, because we have made sure to eliminate one of the main hazards threatening forest ecosystems: pathogens. Indeed, we were careful to prevent any pathogen attacks and used phytosanitary treatments when necessary (once in 2015 and 2016). Under natural conditions, pests and diseases (*Taphrorychus bicolor*, *Cryptococcus fagi*, and *Agilus* sp.) may either make trees more susceptible to mortality by weakening defense metabolism before a drought or may accelerate an already ongoing decline process that eventually kills weakened trees (Manion 1981; Zweifel et al.

2012; Gaylord et al. 2013; Oliva et al. 2014), thus resulting in higher mortality rates. We stopped the experiment after 3 years of water stress but we would probably have observed higher annual mortality rates in the following years, considering the low levels of carbohydrate reserves measured in the organs of Dr trees at the end of the third year.

Finally, survival time since the onset of the drought treatment varied among individual trees, as it does in natural conditions. Predicting tree mortality in response to hazards at the individual scale is very challenging, because each tree's sensitivity to drought depends on its long-term history of growth and exposure to stress (Berdanier and Clark 2016). Vulnerability factors and robust mortality predictors still need to be identified to improve our ability to accurately predict tree mortality (Martinez-Vilalta et al. 2019).

Conclusion

Our study showed that 10-year-old beech trees could survive a 3-year drought suggesting an optimistic message concerning the resistance of beech trees faced with future drought-induced-mortality events. However, in natural conditions, it is likely that biotic attacks would weaken trees more rapidly or may induce more mortality. Though we cannot exclude mechanisms other than C starvation from contributing to beech tree mortality in our study, we are sure that NSC availability is an important driver to take into account. More than quantity, the availability of stored NSC and the tree's ability to mobilize stored C are likely to be fundamental in ensuring beech tree survival. Consequently, C storage and remobilization processes should be better taken into account in process-based models predicting mortality and resilience.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

References

- Aguadé D, Poyatos R, Gómez M, Oliva J, Martínez-Vilalta J (2015) The role of defoliation and root rot pathogen infection in driving the mode of drought-related physiological decline in Scots pine (*Pinus sylvestris* L.). *Tree Physiol* 35:229–242
- Anderegg WRL, Anderegg LDL (2013) Hydraulic and carbohydrate changes in experimental drought-induced mortality of saplings in two conifer species. *Tree Physiol* 33:252–260
- Anderegg WRL, Kane JM, Anderegg LDL (2013) Consequences of widespread tree mortality triggered by drought and temperature stress. *Nat Clim Change* 3:30–36
- Baber O, Slot M, Celis G, Kitajima K (2014) Diel patterns of leaf carbohydrate concentrations differ between seedlings and mature trees of two sympatric oak species. *Botany* 92:535–540
- Backes K, Leuschner C (2000) Leaf water relations of competitive *Fagus sylvatica* and *Quercus petraea* trees during 4 years differing in soil drought. *Can J For Res* 30:335–346
- Barbaroux C, Bréda N (2002) Contrasting distribution and seasonal dynamics of carbohydrate reserves in stem wood of adult ring-porous sessile oak and diffuse-porous beech trees. *Tree Physiol* 22:1201–1210
- Barbaroux C, Bréda N, Dufrêne E (2003) Distribution of above-ground and below-ground carbohydrate reserves in adult trees of two contrasting broad-leaved species (*Quercus petraea* and *Fagus sylvatica*). *New Phytol* 157:605–615
- Barigah TS, Charrier O, Douris M, Bonhomme M, Herbet S, Ameglio T, Fichot R, Brignolas F, Cochard H (2013) Water stress-induced xylem hydraulic failure is a causal factor of tree mortality in beech and poplar. *Ann Bot* 112(7):1431–1437
- Bathelier C, Tcherkez G, Bigny R, Gout E, Cornic G, Ghashghaie J (2009) Metabolic origin of the $\delta^{13}\text{C}$ of respired CO_2 in roots of *Phaseolus vulgaris*. *New Phytol* 181:387–399
- Berdanier AB, Clark JS (2016) Multiyear drought-induced morbidity preceding tree death in south-eastern US forests. *Ecol Appl* 26(1):17–23
- Bigler C, Veblen TT (2009) Increased early growth rates decrease longevities of conifers in subalpine forests. *Oikos* 118:1130–1138
- Boyer JS (1970) Leaf enlargement and metabolic rates in corn, soybean, and sunflower at various leaf water potentials. *Plant Physiol* 46:233–235
- Bréda N, Granier A, Aussenac G (1995) Effects of thinning on soil and tree water relations, transpiration and growth in an oak forest (*Quercus petraea* (Matt.) Liebl.). *Tree Physiol* 15:295–306
- Breshears DD, Cobb NS, Rich PM, Price KP, Allen CD, Balice RG, Romme WH, Kastens JH, Floyd ML, Belnap J, Anderson JJ, Myers OB, Meyer CW (2005) Regional vegetation die-off in response to global-change-type drought. *PNAS* 102:15144–15148
- Bussotti F, Pollastrini M, Gessler A, Luo Z-B (2018) Experiments with trees: from seedlings to ecosystems. *Environ Exp Bot* 152:1–6
- Cailleret M, Nourtier M, Amm A, Durand-Gillmann M, Davi H (2014) Drought-induced decline and mortality of silver fir differ among three sites in Southern France. *Ann For Sci* 71:643–657
- Chapin FS, Schulze ED, Mooney HA (1990) The ecology and economics of storage in plants. *Ann Rev Ecol Syst* 21:423–447
- Choat B, Jansen S, Brodribb TJ et al (2012) Global convergence in the vulnerability of forests to drought. *Nature* 491:752–756

- Choat B, Brodribb TJ, Brodersen CR, Duursma R, Lopez R, Medlyn B (2018) Triggers of tree mortality under drought. *Nature* 558:531–539
- Dai Y, Wang L, Wan X (2018) Relative contributions of hydraulic dysfunction and carbohydrate depletion during tree mortality caused by drought. *AoB Plants* 10:plx069. <https://doi.org/10.1093/aobpla/plx069>
- Dannoura M, Epron D, Desalme D, Massonnet C, Tsuji S, Plain C, Priault P, Gérard D (2019) The impact of prolonged drought on phloem anatomy and phloem transport in young beech trees. *Tree Physiol* 39:201–210. <https://doi.org/10.1093/treephys/tpy070>
- Dietze MC, Sala A, Carbone MS, Czimczik CI, Mantooth JA, Richardson AD, Vargas R (2014) Non-structural carbon in woody plants. *Annu Rev Plant Biol* 65:667–687
- Dittmar C, Zech W, Elling W (2003) Growth variations of Common beech (*Fagus sylvatica* L.) under different climatic and environmental conditions in Europe—a dendroecological study. *For Ecol Manag* 173:63–78
- Eichhorn J, Icke R, Isenberg A, Paar U, Schönfelder E (2005) Temporal development of crown condition of beech and oak as a response variable for integrated evaluations. *Eur J Forest Res* 124:335–347
- El Zein R, Maillard P, Bréda N, Marchand J, Montpied P, Gérard D (2011) Seasonal changes of C and N non-structural compounds in the stem sapwood of adult sessile oak and beech trees. *Tree Physiol* 31:843–854
- Fotelli MN, Nahm M, Radoglou K (2009) Seasonal and interannual ecophysiological responses of beech (*Fagus sylvatica*) at its south-eastern distribution limit in Europe. *Forest Ecol Manag* 257:1157–1164
- Galiano L, Martínez-vivalta J, Lloret F (2011) Carbon reserves and canopy defoliation determine the recovery of Scots pine 4 yr after a drought episode. *New Phytol* 190:750–759
- Galvez DA, Landhäuser SM, Tyree MT (2011) Root carbon reserve dynamics in aspen seedlings: does simulated drought reserve limitation? *Tree Physiol* 31:250–257
- García de la Serrana R, Vilagrosa A, Alloza JA (2015) Pine mortality in southeast Spain after an extreme dry and warm year: interactions among drought stress, carbohydrates and bark beetle attack. *Trees Struct Funct* 29:1791–1804
- Gasson P (1987) Some implications of anatomical variations in the wood of pedunculate oak (*Quercus robur* L.), including comparison with common beech (*Fagus sylvatica* L.). *IAWA J* 8:149–166
- Gaudinski JB, Torn MS, Riley WJ, Swanson C, Trumbore SE, Joslin JD, Majdi H, Dawson TE, Hanson PJ (2009) Use of stored carbon reserves in growth of temperate tree roots and leaf buds: analyses using radiocarbon measurements and modeling. *Glob Change Biol* 15:992–1014
- Gaylor ML, Kolb TE, Pockman WT, Plaut JA, Yezzer EA, Macalady AK, Pangle RE, McDowell NG (2013) Drought predisposes piñon-juniper woodlands to insect attacks and mortality. *New Phytol* 198:567–578
- Genet H, Bréda N, Dufrière E (2010) Age-related variation in carbon allocation at tree and stand scales in beech (*Fagus sylvatica* L.) and sessile oak (*Quercus petraea* (Matt. Liebl.) using a chronosequence approach. *Tree Physiol* 30:177–192
- Gérard B, Bréda N (2014) Radial distribution of carbohydrate reserves in the trunk of declining European beech trees (*Fagus sylvatica* L.). *Ann Forest Sci* 71:675–682
- Gessler A, Keitel C, Kreuzwieser J, Matyssek R, Seiler W, Rennenberg H (2007) Potential risks for European beech (*Fagus sylvatica* L.) in a changing climate. *Trees Struct Funct* 21:1–11
- Gessler A, Schaub M, McDowell NG (2017) The role of nutrients in drought-induced tree mortality and recovery. *New Phytol* 214:513–520
- Granier A, Bréda N, Biron P, Villette S (1999) A lumped water balance model to evaluate duration and intensity of drought constraints in forest stands. *Ecol Model* 116:269–283
- Granier A, Reichstein M, Bréda N et al (2007) Evidence for soil water control on carbon and water dynamics in European forests during the extremely dry year: 2003. *Agric For Meteorol* 143:123–145
- Granier A, Bréda N, Longdoz B, Gross P, Ngao J (2008) Ten years of fluxes and stand growth in a young beech forest at Hesse, North-eastern France. *Ann Forest Sci* 65:704
- Gruber A, Pirkebner D, Florian C, Oberhuber W (2012) No evidence for depletion of carbohydrate pools in Scots pine (*Pinus sylvestris* L.) under drought stress. *Plant Biol* 14:142–148
- Guillemot J, Martin-StPaul NK, Dufrière E, Francois C, Soudani K, Ourcival JM, Delpierre N (2015) The dynamic of the annual carbon allocation to wood in European tree species is consistent with a combined source–sink limitation of growth: implications for modelling. *Biogeosciences* 12:2773–2790
- Gustafson EJ (2014) Applicability of predictive models of drought-induced tree mortality between the mid-west and northeast United States. *Forest Sci* 60:327–334
- Hacke U, Sauter JJ (1996) Xylem dysfunction during winter and recovery of hydraulic conductivity in diffuse-porous and ring-porous trees. *Oecologia* 105:425–439
- Hansen J, Moller I (1975) Percolation of starch and soluble carbohydrates from plant tissue for quantitative determination with anthrone. *Anal Biochem* 68:87–94
- Hanson PJ, Weltzin JF (2000) Drought disturbance from climate change: response of United States forests. *Sci Total Environ* 262:205–220
- Hartmann H, Trumbore S (2016) Understanding the roles of nonstructural carbohydrates in forest trees from what we can measure to what we want to know. *New Phytol* 171:46–59
- Hartmann H, Ziegler W, Trumbore S (2013) Lethal drought leads to reduction in nonstructural carbohydrates in Norway spruce tree roots but not in the canopy. *Funct Ecol* 27:413–427
- Hartmann H, Moura C, Anderegg WRL et al (2018) Research frontiers for improving our understanding of drought-induced tree and forest mortality. *New Phytol* 51:15–28
- Hentschel R, Hommel R, Poschenrieder W, Grote R, Holst J, Biernath C, Gessler A, Priesack E (2016) Stomatal conductance and intrinsic water use efficiency in the drought year 2003—a case study of a well-established forest stand of European beech. *Trees Struct Funct* 30:153–174
- Hinman ED, Fridley JD (2018) To spend or to save? Assessing energetic growth-storage tradeoff in native and invasive woody plants. *Oecologia* 188:659–669
- Hoch G (2014) Carbon reserves as indicators for carbon limitation in trees. *Prog Bot* 76:321–346
- Hoch G, Richter A, Körner C (2003) Non-structural carbon compounds in temperate forest trees. *Plant Cell Environ* 26:1067–1081
- Klein T, Hoch G, Yakir D, Körner C (2014) Drought stress, growth and nonstructural carbohydrate dynamics of pine trees in a semi-arid forest. *Tree Physiol* 34:981–992
- Körner C (2003) Carbon limitation in trees. *J Ecol* 91:4–17
- Lebourgeois F, Bréda N, Ulrich E, Granier A (2005) Climate-tree-growth relationships of European beech (*Fagus sylvatica* L.) in the french permanent plot network (RENECOFOR). *Trees Struct Funct* 19:385–401
- Li MH, Hoch G, Körner C (2002) Source/sink removal affects mobile carbohydrates in *Pinus cembra* at the Swiss treeline. *Trees Struct Funct* 16:331–337
- Liu JF, Arend M, Yang WJ, Schaub M, Ni YY, Gessler A, Jiang ZP, Rigling A, Li MH (2017) Effects of drought on leaf carbon source and growth of European beech are modulated by soil type. *Sci Rep* 7:42462–42471

- Lloret F, Peñuelas J, Estiarte M (2004) Experimental evidence of reduced diversity of seedlings due to climate modification in a Mediterranean-type community. *Glob Change Biol* 10:248–258
- Locato V, De Gara L (2018) Programmed cell death in plants: an overview. *Methods Mol Biol* 1743:1–8
- Lorenz M, Fischer R, Becher G, Mues V, Seidling W, Roskams P, Kraft P, Nagel HD (2006) Forest condition in Europe. 2005 Technical Report of ICP Forests. BFH, Hamburg, pp 58
- Manion PD (1981) Tree disease concepts. Prentice Hall, Englewood Cliffs, p 324
- Martínez-Vilalta J, WAnderegg WRL, Sapes F, Sala A (2019) Greater focus on water pools may improve our ability to understand and anticipate drought-induced mortality in plants. *New Phytol* 223(1):22–32
- McDowell NG, Sevanto S (2010) The mechanisms of carbon starvation: How, when, or does it even occur at all? *New Phytol* 186:264–266
- McDowell N, Pockman WT, Allen CD et al (2008) Mechanisms of plant survival and mortality during drought: Why do some plants survive while others succumb to drought? *New Phytol* 178:719–739
- McDowell NG, Beerling DJ, Breshears DD, Fisher RA, Raffa KF, Stitt M (2011) The interdependence of mechanisms underlying climate-driven vegetation mortality. *Trends Ecol Evol* 26:523–532
- McDowell NG, Fisher RA, Xu C et al (2013) Evaluating theories of drought-induced vegetation mortality using a multimodel-experiment framework. *New Phytol* 200:304–321
- Meir P, Mencuccini M, Dewar RC (2015) Drought-related tree mortality: addressing the gaps in understanding and prediction. *New Phytol* 207:28–33
- Munné-Bosh S, Alegre L (2004) Die and let live: leaf senescence contributes to plant survival under drought stress. *Funct Plant Biol* 31:203–216
- Oliva J, Stenlid J, Martínez-Vilalta J (2014) The effect of fungal pathogens on the water and carbon economy of trees: implications for drought-induced mortality included in theoretical models for drought induced mortality. *New Phytol* 203:1028–1035
- Palacio S, Hoch G, Sala A, Körner C, Millard P (2014) Does carbon storage limit tree growth? *New Phytol* 201:1096–1100
- Pangle RE, Limousin JM, Plaut JA, Yopez EA, Hudson PJ, Boutz AL, Gehres N, Pockman WT, McDowell NG (2015) Prolonged experimental drought reduces plant hydraulic conductance and transpiration and increases mortality in a piñon–juniper woodland. *Ecol Evol* 5:1618–1623
- Peiffer M, Bréda N, Badeau V, Granier A (2014) Disturbances in European beech water relation during an extreme drought. *Ann Forest Sci* 71:821–829
- Piper F, Fajardo A, Hoch G (2017) Single-provenance mature conifers show higher non-structural carbohydrate storage and reduced growth in a drier location. *Tree Physiol* 37:1001–1010
- Sala A, Piper F, Hoch G (2010) Physiological mechanisms of drought-induced tree mortality are far from being resolved. *New Phytol* 186:274–281
- Sala A, Woodruff DR, Meinzer FC (2012) Carbon dynamics in trees: feast or famine? *Tree Physiol* 32:764–775
- Sevanto S (2014) Phloem transport and drought. *J Exp Bot* 65:1751–1759
- Sevanto S, McDowell NG, Dickmann LT, Pangle R, Pockman WT (2014) How do trees die? A test of the hydraulic failure and carbon starvation hypotheses. *Plant Cell Environ* 37:153–161
- Silpi U, Lacoite A, Kasempap P, Thanysawanyangkura S, Chantuma P, Gohet E, Musigamart N, Clément A, Ameglio T, Thaler P (2007) Carbohydrate reserves as a competing sink: evidence from tapping rubber trees. *Tree Physiol* 27:881–889
- Silva DE (2010) Ecologie du hêtre (*Fagus sylvatica L.*) en marge sud-ouest de son aire de distribution. Lorraine University. Thèse, Université de Lorraine, Nancy, France
- Stitt M, Zeeman SC (2012) Starch turnover: pathways, regulation and role in growth. *Curr Opin Plant Biol* 15:282–292
- Tyree MT (2003) Desiccation tolerance of five tropical seedlings in Panama. relationship to a field assessment of drought performance. *Plant Physiol* 132:1439–1447
- Voltas J, Camarero JJ, Carulla D, Aguilera M, Ortiz A, Ferrio JP (2013) A retrospective, dual-isotope approach reveals individual predispositions to winter-drought induced tree dieback in the southernmost distribution limit of Scots pine. *Plant Cell Environ* 36:1435–1448
- Wiley E, Helliker B (2012) A re-evaluation of carbon storage in trees lends greater support for carbon limitation to growth. *New Phytol* 195:285–289
- Wojciechowska N, Sobieszczuk-Nowicka E, Bagniewska-Zadwornal A (2018) Plant organ senescence—regulation by manifold pathways. *Plant Biol* 20:167–181
- Zweifel R, Bangerter S, Rigling A, Sterck FJ (2012) Pine and mistletoes: how to live with a leak in the water flow and storage system? *J Exp Bot* 63:2565–2578

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