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Research paper

Unravelling resilience mechanisms in forests: role of non-structural carbohydrates in responding to extreme weather events

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Extreme weather events are increasing in frequency and intensity due to global climate change. We hypothesized that tree carbon reserves are crucial for resilience of beech, buffering the source–sink imbalance due to late frosts and summer droughts, and that different components of non-structural carbohydrates (NSCs) play specific roles in coping with stressful situations. To assess the compound effects on mature trees of two extreme weather events, first a late frost in spring 2016 and then a drought in summer 2017, we monitored the phenology, radial growth and the dynamics of starch and soluble sugars in a Mediterranean beech forest. A growth reduction of 85% was observed after the spring late frost, yet not after the drought event. We observed a strong impact of late frost on starch, which also affected its dynamic at the beginning of the subsequent vegetative season. In 2017, the increase of soluble sugars, associated with starch hydrolysis, played a crucial role in coping with the severe summer drought. Non-structural carbohydrates helped to counteract the negative effects of both events, supporting plant survival and buffering source–sink imbalances under stressful conditions. Our findings indicate a strong trade-off between growth and NSC storage in trees. Overall, our results highlight the key role of NSCs on beech trees, response to extreme weather events, confirming the resilience of this species to highly stressful events. These insights are useful for assessing how forests may respond to the potential impacts of climate change on ecosystem processes in the Mediterranean area.

Keywords: *Fagus sylvatica* L., growth, late frost, Mediterranean region, resilience, summer drought.

Introduction

Global climate change is causing an increase in the frequency of extreme weather events that are recognized among the major drivers of current and future ecosystem dynamics (Reichstein et al. 2013, Pederson et al. 2014, Stocker et al. 2014, Frank et al. 2015). The Mediterranean region is considered a primary climate change hotspot (Giorgi 2006, Pausas and Millán 2019) that shows an increase in the inter-annual climatic

variability (Flaounas et al. 2013). In this region, the increasing risk of late frost events represents one of the major threats for vegetation associated with future global change (Zohner et al. 2020). Indeed, increasing spring temperatures have been observed to stimulate earlier leaf unfolding in trees (Gordo and Sanz 2010), thus potentially exposing young leaves and shoots to spring frost damage (Augsburger 2013), especially at high elevation (Vitasse et al. 2018, Chamberlain et al.

2021). Depending on species, temperatures below -4°C can destroy the newly expanded leaves and shoots, reducing the photosynthetic leaf area and even blocking net photosynthesis of trees for several weeks, depending on the damage intensity. In this case, the resource requirements for leaf resprouting and tree metabolic processes must rely on the remobilization of carbon (C) reserves (Dittmar et al. 2006, D'Andrea et al. 2019). Moreover, the severity, duration and frequency of drought events have all been shown to increase in recent decades (Spinoni et al. 2015). European beech (*Fagus sylvatica* L.), one of the most widespread native tree species in Europe, is known to be drought sensitive (Bolte et al. 2016). Hence, drought events are expected to negatively affect its physiological performance (Rezaie et al. 2018), C allocation (D'Andrea et al. 2020a) and reproductive capacity (Nussbaumer et al. 2020), as well as its growth and competitiveness (Peuke et al. 2002), which may all impact its future distribution (Noce et al. 2017).

Growth and non-structural carbohydrates (NSCs; i.e., sucrose, fructose, glucose and starch) dynamics are among the physiological processes most strongly affected by spring frost (D'Andrea et al. 2020b) and summer drought (Li et al. 2018). An increasing body of evidence has shown that NSCs do not represent a purely passive accumulation and depletion of recently synthesized C compounds. Several studies have shown that reserves remobilization is an active physiological process, involving regulation of C source–sink balance in controlling and regulating the difference between C supply and demand at different timescales (Scartazza et al. 2001, Sala et al. 2012, Carbone et al. 2013, Fatichi et al. 2014, Martínez-Vilalta et al. 2016, Moscatello et al. 2017, Collalti et al. 2020a). Therefore, NSCs could play a crucial role in counteracting the negative effects of extreme weather events on beech forests, contributing to their resilience and future survival (Scartazza et al. 2013, D'Andrea et al. 2019). However, despite the recognized importance of NSCs for plant productivity and resilience, little is known regarding their seasonal regulation and trade-offs against growth and reproduction in forest trees (Merganičová et al. 2019, Tixier et al. 2020). Tree stemwood, together with coarse roots, contains most of the total NSC pools, hence playing a key role in the aforementioned mechanisms (Barbaroux et al. 2003, Richardson et al. 2015).

In this work, we studied the effects of spring late frost and summer drought in a site of the Italian Long-Term Ecological Research network (LTER-Italy), belonging also to the International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (ICP forests). Our study site is located in a Mediterranean beech forest of the Central-South Apennine (Collelongo-Selva Piana), where in spring 2016, due to unusually warm preceding weeks, leaf unfolding occurred up to 15–20 days earlier than the average, followed by a frost, which caused the complete loss of the newly grown leaves (Bascietto et al. 2018, Nolè et al. 2018, Allevato et al.

2019). Moreover, in 2017, a strong summer drought, due to a combination of a drastic reduction in precipitation with high air temperature in late July and August, impacted our study site and a huge portion of the Mediterranean basin (Rita et al. 2019). Notwithstanding, in situ evaluations of their effects on ecosystem functions are still limited. Phenology, growth and stemwood NSC dynamics in the Collelongo-Selva Piana beech stand were investigated during 2016 and 2017. Moreover, using a historical dataset, we modelled the seasonal NSC dynamics, in order to derive a site-based reference for evaluating the impact of the late frost and summer drought. Previous works have addressed the effects of single stressful events on growth and carbohydrate dynamics (Dietrich et al. 2018, Li et al. 2018, Rubio-Cuadrado et al. 2021). To the best of our knowledge, in natural field conditions, the effects of consecutive extreme weather events of different types and intensities on physiological processes, which can likely trigger widespread tree mortality (Pederson et al. 2014, Druckenbrod et al. 2019), have not so far been analysed. Nevertheless, conditions similar to those investigated here are expected to occur more and more frequently in the future (Flaounas et al. 2013).

We hypothesized that the reserves of mature beech plants were able to buffer source–sink imbalance due to two consecutive and different weather extreme events, with starch and soluble sugars playing specific roles in coping with frost versus drought. The main aim of the study was to unravel the mechanistic role of stemwood NSCs in supporting tree resilience to climate stresses. Specifically, we aimed to (i) verify the role of NSCs in mediating source–sink balance following the strong alteration of C availability and (ii) evaluate the interplay and trade-off between C allocation to the canopy, stem growth and reserves.

Materials and methods

Study site

Our study was carried out during the years 2016 and 2017 in an even-aged, pure beech stand (*F. sylvatica* L.) located at Selva Piana (Collelongo, Abruzzi Region, Italy, $41^{\circ}50'58''$ N, $13^{\circ}35'17''$ E, 1560 m elevation) in a 3000 ha forest within the external belt of Abruzzo–Lazio–Molise National Park (Central Apennine). The last periodical dendrometric survey (2017) estimated a stand density of 725 trees ha^{-1} , a basal area of 45.8 $\text{m}^2 \text{ha}^{-1}$, a mean diameter at breast height (DBH) of 28.5 cm and a mean tree height of 23 m. In 2013, mean tree age was estimated to be about 115 years. The soil is humic alisol with a variable depth (40–100 cm), developed on calcareous bedrock. For the period 1989–2014, the mean annual temperature was 7.2°C and the mean annual precipitation was 1178 mm, of which 10% was concentrated during the summer (Guidolotti et al. 2013, Collalti et al. 2016, Rezaie et al. 2018, Reyher et al. 2020). The experimental area has been part of the LTER network since 1996.

Climate and phenology

Temperature and precipitation from 1989 to 2015, available on the Fluxnet2015 release, were used to characterize the average climate conditions of the site. For the limited data gaps during the experimental trial (2016–17), we used the ERA5 database produced by the European Centre for Medium-Range Weather Forecasts (<https://www.ecmwf.int/en/forecasts/datasets/archive-datasets/reanalysis-datasets/era5>, data accessed: 12 April 2018), according to the Fluxnet2015 release formulations (Pastorello et al. 2020). To evaluate peculiarities of 2016 and 2017, we calculated monthly differences with respect to the average values of precipitation and temperature observed in the site in the historical time series 1989–2015.

Leaf phenology was monitored using the Moderate Resolution Imaging Spectroradiometer leaf area index product (LAI, MOD15A2H product, <https://modis.gsfc.nasa.gov/>) with 8-day temporal resolution and 500 m spatial resolution (Myneni et al. 2015). Critical dates, representing approximately linear transitions from one phenological phase to another, were identified and defined according to Zhang et al. (2003) as (i) 'green-up', photosynthetic activity onset; (ii) 'maximum LAI', assumed to be the leaf maturity phase; (iii) 'senescence', sharp decrease of photosynthetic activity and green leaf area; and (iv) 'winter dormancy'. In 2016, the leafless period after the late frost was identified from the date of the event and that of the subsequent green-up.

Selection, measurements and sampling of trees

Five trees were selected according to their similarity with site mean tree ring chronology and were monitored from April 2016 to November 2017. These trees ranged from 49 to 53 cm DBH and had a mean age of 109 ± 4 years. Intra-annual radial growth of each selected tree was measured using permanent girth bands with 0.1 mm accuracy (D1 Permanent Tree Girth, UMS, Munich, Germany). Furthermore, stem diameter was recorded at the moment of each sampling of xylem for NSC analyses (20 sampling dates from April 2016 to November 2017).

After bark removal, micro-cores (2 mm diameter, 15 mm long) of wood were collected from each tree, using the Trephor tool (Rossi et al. 2005). All samples for NSC analyses were immediately placed in dry ice for transport to the laboratory, then stored at -20°C and, finally, stabilized through lyophilization processes until NSC analyses.

For each study tree, daily radial increment (R_i , $\mu\text{m day}^{-1}$) was calculated as follows:

$$R_i = \frac{R_t - R_{t-1}}{\Delta t} \quad (1)$$

where R is the radius of each i tree (μm), t is the date of sampling and Δt is the time interval between the two sampling dates expressed in days.

In November 2017, at the end of the experimental trial, wood increment cores were collected at breast height from each study tree. Tree ring width series were converted into tree basal area increment (BAI, $\text{cm}^2 \text{ year}^{-1}$), according to the following standard formula:

$$\text{BAI} = \pi (R_n^2 - R_{n-1}^2) \quad (2)$$

with n being the year of tree ring formation.

Starch and soluble sugar concentrations analysis

The freeze-dried xylem samples were milled to a fine powder and used for all analytical tests. For analysis of glucose, fructose, sucrose and starch, 10 mg of dry xylem powder were extracted in 1 ml of 80% ethanol/water at 80°C for 45 min. After centrifugation at 16,000 g for 5 min, soluble sugars were recovered in the supernatant, whereas the pellet was resuspended in 1 ml of 40 mM acetate buffer (pH 4.5), then recentrifuged at 16,000 g for 5 min. This procedure was repeated four times. The final pellet was autoclaved for 45 min at 120°C in the same wash buffer. Enzymatic starch hydrolysis and the following glucose spectrophotometric assay were done as described by Moscatello et al. (2017). The supernatant solution containing soluble sugars was filtered on 0.2 μm nylon filters (GE-Whatman, Maidstone, UK), then analysed by high-performance anion exchange chromatography with pulsed amperometric detection (Thermo Scientific™ Dionex™ ICS-5000, Sunnyvale, CA, USA) (Proietti et al. 2017).

Modelling of intra-annual dynamics of NSCs

Considering that our study was performed in the context of a natural field experiment, the wide and strong impact of the late frost made it impossible to find trees without frost damage to use as a control (Figure S1 available as Supplementary data at *Tree Physiology Online*). However, previous studies on NSC seasonal dynamics have been performed in the study site, which gives us the potential to have of a control to our experiment. The historical dataset (Figure S1 available as Supplementary data at *Tree Physiology Online*) was used to develop models for describing the seasonal variation of starch, soluble sugars and total NSCs. The modelled sugar dynamics for the site were used as a reference to evaluate the effects of the stressful events studied in 2016 and 2017. The dataset was composed of data of different years (i.e., 2001, 2002, 2013, 2014, 2015 and 2018). This dataset included 39 observations of starch dynamics and 28 observations for both soluble sugars (glucose, fructose and sucrose) and total NSCs. Observations for soluble sugars were fewer, because of the methodological sampling procedure used in 2015. During that year, woody samples were collected for xylogenesis analysis and maintained in ethanol–formalin acetic acid solution. Unfortunately, this

methodology caused the loss of soluble sugars, but not of starch, as verified by means of specific analytical tests on woody tissues.

Different models based on data of starch, soluble sugars and total NSCs were used looking for possible patterns within the years and tested through the Akaike Information Criterion (AIC) (Akaike 1974, Aho et al. 2014) to select the simplest model able to reproduce the pattern observed in situ. The AIC quantifies the trade-off between parsimony and goodness-of-fit in a simple and transparent manner, estimating the relative amount of information lost by a given model. Hence, the model showing the lowest AIC is considered the model with the smallest information loss and, potentially, the most representative one (Akaike 1974). The four assumptions of linear model (homoscedasticity, normality of the error distribution, statistical independence of the errors and absence of influential points) were tested graphically (S2–S4 available as Supplementary data at *Tree Physiology* Online).

Statistical data analysis

Intra-annual differences among contents of starch and total sugars were tested using one-way repeated measures analysis of variance (one factor repetition), using sampling date as the predictive factor. The measured data of soluble sugars did not pass the normality test and were analysed by repeated measures analysis of variance on ranks. Multiple comparisons were performed by the Student–Newman–Keuls method and reported in Figure S5 available as Supplementary data at *Tree Physiology* Online.

Linear mixed models, considering ‘tree’ and ‘sampling date’ as crossed random effects, were used to account for the random variation of inter-annual starch, soluble sugars and total NSCs. Statistical analysis and figures were made using R 3.5.0 (R Development Core Team 2018).

Differences among modelled and measured data were identified using the interval of confidence (1.96 standard error), where the lack of overlap between the two intervals of confidence indicates the likelihood of statistically significant differences at the 95% level (P -value < 0.05).

Results

Climate in the study period

In 2016, a severe late frost event occurred during the night between April 25 and 26, when the temperature at canopy level (~24 m) reached -6°C (Figure 1a, inset panel). The extreme frost event followed an early spring season characterized by temperatures during the months of February and April that were significantly higher (about 2°C) than the average value of the site for the period 1989–2015 (Figure 1a). In 2017, from May to August, the temperature was significantly higher than the average value of the site, with a positive anomaly of

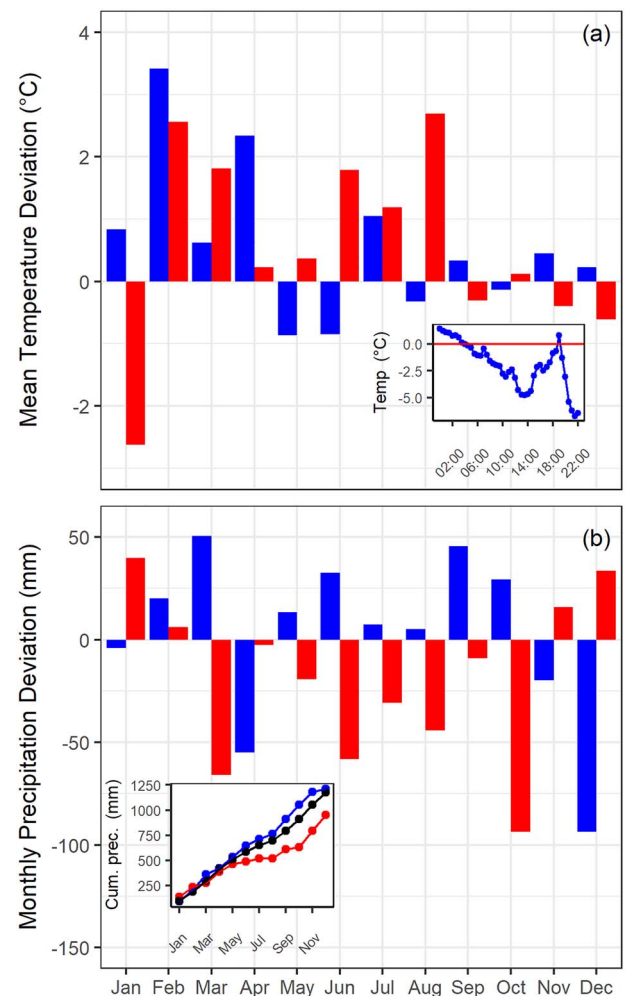


Figure 1. Deviations of monthly mean temperature (a) and precipitation (b) for 2016 (blue bars) and 2017 (red bars) calculated as the difference from the 2000 to 2015 average value at the site. Temperature of 25 April 2016 measured at canopy level (24 m) is reported in inset graph of (a), whereas the annual precipitation of the year 2016 (blue dots), 2017 (red dots) and the long-term average (black dots) is reported in the inset graph of (b).

$\sim 3^{\circ}\text{C}$ (Figure 1a). Furthermore, from May to October 2017, a significant reduction of precipitation against the long-term average was observed (Figure 1b), leading to total annual precipitation that was $\sim 50\%$ lower than the 1989–2015 average (Figure 1b, inset panel).

Phenological parameters and radial growth

The seasonal LAI trend, used to define the phenological phases of the stand, is reported in Figure 2a. The ‘first’ green-up in spring 2016 occurred between 20 and 30 days earlier than the average for the site (Figure 2a), whereas the second (re)green-up, after the complete loss of leaves due to the late spring frost, started around 28 June. In total, we estimated a leafless period of >60 days. In 2016, the beginning of the senescence phase occurred about 1 week earlier compared with the long-term average (Figure 2b). Maximum LAI was lower in 2016

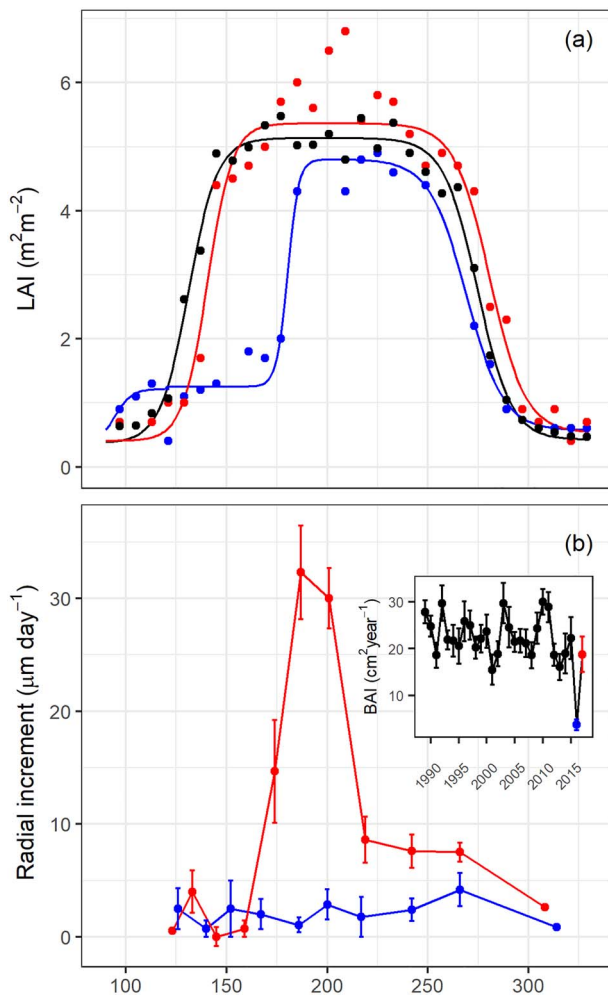


Figure 2. Seasonal dynamics of leaf area index (LAI, $\text{m}^2 \text{m}^{-2}$) (a) and daily stem radial increment (b) for the years 2016, 2017 and the 2000–15 reference period. The LAI was derived from Moderate Resolution Imaging Spectroradiometer (MODIS, see Materials and methods), for 2016 (blue line), 2017 (red line) and for the 2000–15 reference period (black line). Solid lines are the modelled LAI pattern, using two logistic functions for the increasing and decreasing phases. Dots are the raw MODIS–LAI values. In (b), the daily radial increment for 2016 (blue dots) and 2017 (red dots) are shown, whereas the inset graph reports the long-term series data of basal area chronology (BAI, $\text{cm}^2 \text{year}^{-1}$), where the last two dots represent the BAI value obtained in 2016 (blue dot) and 2017 (red dot), respectively.

(LAI = $4.79 \text{ m}^2 \text{m}^{-2}$) than in 2017 (LAI = $5.37 \text{ m}^2 \text{m}^{-2}$), whereas the long-term average LAI of the site assessed with remote sensing was $\sim 5 \text{ m}^2 \text{m}^{-2}$ (Figure 2a). The average length of the vegetative period assessed through remote sensing from 2000 to 2015 was ~ 140 days, a value confirmed in 2017. In 2016, the same period was only 83 days.

The mean BAI in the 2000–15 period was $22.6 \pm 0.8 \text{ cm}^2 \text{year}^{-1}$, whereas it was 3.7 ± 1.1 and $18.6 \pm 3.8 \text{ cm}^2 \text{year}^{-1}$ in 2016 and 2017, respectively (Figure 2b, inset panel). The late frost in spring 2016 reduced the stem radial growth by about 85% compared with the average of the period 1989–2015. The late frost strongly affected the seasonal dynamics

of stem diameter growth during the year 2016, as shown by the lower and almost flat rate of stem growth compared with 2017. In comparison, in 2017, the radial growth showed the usual seasonal pattern, reaching the highest increment ($32.3 \pm 4.1 \mu\text{m day}^{-1}$) in July (Figure 2b).

Intra-annual dynamics of NSCs

Dynamics of NSCs (Figure 3) showed different polynomial equation patterns, with R^2 ranging from 0.64 to 0.93 (Table 1). Comparing the modelled NSC intra-annual dynamics and stand phenology, an increase in total NSC content (Figure 3a) was observed from bud break to the beginning of the green-up phase, due to increasing starch content (Figure 3b). During the period between the onset and the middle of the maximum vegetative season, total NSC content decreased due to starch reduction, whereas the content of soluble sugars (Figure 3c) remained unchanged. In the late summer, both starch and soluble sugars increased until the end of the vegetative season, leading to an increase of total sugar content. At the beginning of the dormancy phase, a decrease of total NSCs was recorded. This trend was due to a severe decrease of starch content, even though associated with a simultaneous increase in soluble sugars.

During the leafless period in 2016, soluble sugar content significantly decreased from 8.15 ± 0.58 to $5.25 \pm 0.43 \text{ mg g dry weight (DW)}^{-1}$.

Before senescence, a peak of soluble sugar content was observed (241 vs 215 day of the year, DOY). During dormancy, the maximum soluble sugar content was observed to be $16.75 \pm 0.50 \text{ mg g DW}^{-1}$. A significant increment of starch content was observed from the beginning of the vegetative period ($13.35 \pm 1.89 \text{ mg g DW}^{-1}$) until the loss of leaves due to the late frost ($24.80 \pm 0.20 \text{ mg g DW}^{-1}$). Along with that event, a decline in the starch content was observed before a second seasonal peak (DOY 241). The lowest starch content was recorded during the dormancy phase ($9.37 \pm 0.78 \text{ mg g DW}^{-1}$). Therefore, two seasonal peaks of stemwood total NSC content were observed in 2016, with a significant increase (110 vs 151 DOY) and decrease (151 vs 215 DOY) in spring and summer, respectively. At the end of the vegetative season, a significant increase of total NSC content was recorded (241 vs 215 DOY).

In 2017, during spring and early summer, we measured a significant decrease of soluble sugar content in stemwood until DOY 186 ($6.16 \pm 0.41 \text{ mg g DW}^{-1}$). A significant increment of soluble sugar content was observed between July and August, whereas the maximum was reached during senescence ($15.27 \pm 0.76 \text{ mg g DW}^{-1}$). In spring and early summer 2017, stemwood starch content significantly increased from 11.57 ± 1.51 to $20.26 \pm 1.92 \text{ mg g DW}^{-1}$. By the end of August, a significant decrement of starch content was observed ($12.76 \pm 1.92 \text{ mg g DW}^{-1}$, DOY 241). Stemwood total NSC

Table 1. Parameters of the polynomial model describing the intra-annual variation of non-structural carbohydrates (total NSC, starch and soluble sugar content) in beech wood; n is the number of samplings used for the analysis and RMSE (mg g DW⁻¹) is the root mean square error.

Components	xo	a	b	c	d	e	n	R ²	RMSE	P-value
Total NSC	28.29	11.21	-5.47	-3.68	-10.23	4.95	28	0.64	2.36	<0.001
Starch	19.55	4.98	-26.09	2.43	-14.76	—	39	0.78	2.57	<0.001
Soluble sugars	9.41	8.13	20.28	-3.31	1.72	-3.97	28	0.93	1.00	<0.001

content was lower at the beginning of the vegetative period (23.51 ± 2.38 mg g DW⁻¹) than during the senescence, when the maximum value was reached (31.94 ± 3.03 mg g DW⁻¹).

Results from the linear mixed model showed that sampling dates accounted for 52, 43 and 80%, whereas trees only explained 8, 11 and 1% of the total variance of concentration of starch, total NSCs and soluble sugars, respectively.

Although the seasonal trends of stemwood carbohydrates accumulation in 2016 and 2017 were similar to the modelled NSC dynamics recorded in the reference period, some substantial differences were observed. After leaf resprouting in 2016, starch content decreased considerably, reaching a value lower than the modelled reference value at the site. In August 2016, soluble sugars increased until leaf senescence. After senescence, we observed a reduction of starch and, consequently, in the total carbohydrate reserves. The lower amount of storage carbohydrates reached in 2016 directly affected the amount of starch during the first part of the vegetative season in 2017. Although the starch content was lower than the modelled value for the site by the beginning of August 2017, a refilling of total carbohydrate reserves was observed. The drought stress event of August 2017 also strongly affected the composition of carbohydrate reserves due to a severe starch hydrolysis, leading to a decrease of starch content of about 35% and a parallel increase of soluble sugars. During the late phase of the 2017 vegetative season, the carbohydrates pattern returned close to the modelled intra-annual dynamics. We do note that stemwood starch content during dormancy was lower than the modelled value for the site.

Discussion

The buffering capacity of NSCs in response to the late frost

The seasonal dynamics of NSCs play a crucial role in regulating C source–sink balance through buffering the difference between C supply and demand (Scartazza et al. 2013, Fatichi et al. 2014, Collalti et al. 2018, 2020a). In 2016, during springtime (May–June), we found that the complete frost-induced defoliation and, therefore, destruction of the photosynthetic apparatus was associated with an increase of total stemwood NSCs, due to starch accumulation, and with a strong reduction of stem radial growth, which in beech is strongly dependent on the new assimilates (Barbaroux and Bréda 2002, Čufar et al. 2008, Zein et al. 2011, Michelot et al. 2012). An increase in total stemwood

NSCs from November to March has been previously observed in other temperate forests, being attributed to remobilization of sugars from storage compartments in coarse roots in advance of the C demands associated with springtime growth (Hoch et al. 2003, Hartmann and Trumbore 2016). The seasonal dynamics of NSCs in our study show that starch accumulation in beech occurs during the formation of the new crown and in the presence of the potentially dominating sink represented by new growing leaves and shoots, even if soluble sugars are decreasing. Our results confirm that the accumulation of starch in stemwood of beech trees during springtime is not necessarily supported by freshly produced photosynthates. In 2016, it seems to have occurred as the result of the remobilization of already existing soluble sugars, including those remobilized from below-ground organs. In that year, the normal rise of starch in spring could be favoured by the destruction of the developing canopy leaves. This condition leads to a high concentration of soluble sugars within the stemwood that favours the synthesis of starch over its degradation, concurrently to rising springtime temperatures (Witt and Sauter 1994). Indeed, it was recently demonstrated in 1-year-old shoots of *Juglans regia* L. that wood accumulation of starch, accompanied by an increase of the total activity of ADP-glucose pyrophosphorylase, can be increased when photosynthate export from the shoot is blocked by girdling (Moscatello et al. 2017). Thus, the spring programmed activation of starch synthesis in wood can occur even when C resources are very limited by the absence of a photosynthesizing crown. This strongly supports the debated hypothesis of an active control of the accumulation and buffering role of NSCs in wood (Sala et al. 2012, Collalti et al. 2020b).

The key buffering role of NSCs to compensate for the difference between C sink and C supply was also particularly evident during the late spring and early summer of 2016, when stemwood starch reserves were partially hydrolyzed and soluble non-structural C became increasingly older (D'Andrea et al. 2019). Soluble sugars were crucial for sustaining the second leaf resprouting and other demands, such as cambial activity (D'Andrea et al. 2020a). Usually, during the second part of the season (August–September), new assimilates from the canopy are mainly used to sustain C sink activities related to wall thickening and lignification (Prislan et al. 2018) as well as refilling starch reserves in stemwood. However, after the second green-up in July 2016, C allocation to cell wall thickening was extremely limited due to strong reduction of xylem cell

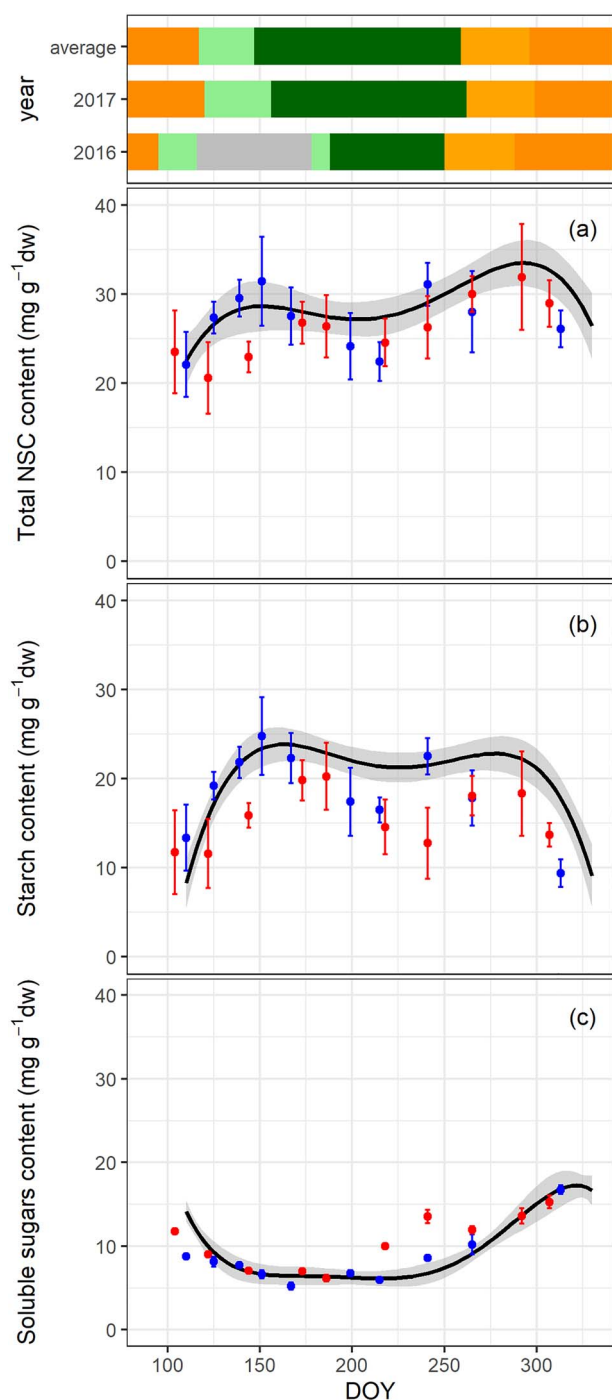


Figure 3. Phenological data for the experimental beech forest site (top panel) and seasonal dynamic of NSC content as total NSCs (a), starch (b) and soluble sugars (c). In the top panel, the different colours represent dormancy (dark orange), the period between the green-up and the maximum leaf area index (LAI, $\text{m}^2 \text{m}^{-2}$) value (light green), the maximum LAI (dark green), the senescence phase (light orange) and, finally, the leafless period after the late frost in 2016 (grey). In (a–c), blue and red dots represent carbohydrate concentrations of 2016 and 2017, respectively, whereas the black lines and grey area show modelled intra-annual dynamic of carbohydrates and 95% interval of confidence (1.96 SE), respectively. Each point is the mean of five beech trees and bars are the intervals of confidence (1.96 SE) (see Materials and methods). Modelled values are derived from 39 and 28 measurements of starch and soluble sugar content, respectively.

production (D'Andrea et al. 2020b), leading to the increase of both starch and soluble sugars in stemwood of beech trees at the end of August. The reduced sink activity (related to radial growth, wall thickening and lignification) during extreme weather events could be enough to prevent NSC depletion (Anderegg 2012, Dietrich et al. 2018). It should be noted that NSCs, including starch, can be rapidly transformed, ensuring a rapid hexose supply to the hexose phosphate pool. The hexose phosphate pool then supports both metabolic and structural cell requirements for reduced C, ranging from glycolysis and respiratory metabolism to cell wall polymer synthesis. In contrast, assimilates ending up in cell wall components cannot be used for metabolism because they are compartmentalized and sequestered away from sites of phloem loading, which can lead to a potential dead end for their usage. Hence, under photosynthate famine and starving conditions, prioritization of photosynthate allocation to NSCs over any other form of growth might ensure the maintenance of a sufficient amount of metabolically available C reserves. This allocation pattern reflects a more conservative and less costly strategy than supporting end point-like allocation of photosynthates to cell wall components and ensures a much higher plasticity to sustain plant responses to environmental constraints (Rodríguez-Calcerrada et al. 2019). Moreover, young trees that must compete for light and nutrients have to invest more C in biomass growth, whereas older trees prioritize the chemical defence of their biomass relative to acquisition of new biomass (Collalti et al. 2020a).

The strong reduction of sink activity in 2016 could have contributed to the earlier closure of the season compared with the long-term average. The decrease of starch after leaf shedding was related to a partial hydrolysis and conversion to soluble sugars to reduce cell osmotic potential and induce cold tolerance (Bonhomme et al. 2005, Tixier and Sperling 2015).

The summer drought effects on growth and NSCs

At the beginning of the 2017 vegetative season, the new assimilates produced by canopy photosynthesis were mainly used for sustaining stem radial growth, which, in contrast to 2016, reached similar values of BAI to those observed for the reference period (1989–2015). The impact of the 2017 summer drought on NSC dynamics had very limited effects on annual stem radial growth. Such behaviour has been observed for other tree species growing in the Mediterranean area. Trees there frequently adopt a stress avoidance strategy by adjusting the end of xylem growth before potential stressful conditions may occur (Lempereur et al. 2015, Forner et al. 2018).

The slight reduction of C reserves at the end of the 2016 growing season impacted the dynamics of the following year. Notwithstanding that the content of starch showed the typical seasonal trend of the site, the starch and total sugar content in woody tissue from bud break until the end of June 2017 was clearly lower than the modelled reference NSC dynamics within

the same site. The warm drought of 2017 had a strong effect on NSC dynamics and led to starch hydrolysis and accumulation of soluble sugars in woody tissue. As drought induces a partial stomatal closure that reduces C uptake, trees depend more on NSC storage to sustain metabolic activities, defence mechanisms against pathogens and osmoregulation processes (McDowell 2011, Hartmann and Trumbore 2016, Collalti et al. 2020b). The observed increase of wood soluble sugar concentration during July–August 2017 agrees with the key role of these non-structural compounds as solutes for osmoregulation (Chaves et al. 2003). Plants under drought conditions can actively control cell osmotic pressure to avoid tissue dehydration and maintain physiological functions by increasing the concentration of different kinds of compatible solutes such as betaines, amino acids and sugars (Morgan 1984). The increased concentration of stemwood soluble sugars during drought in our study was due to both hexoses (glucose and fructose) and sucrose (data not shown), in agreement with previous works (Fu and Fry 2010, Yang 2013). In addition, NSCs have also a relevant role to maintain xylem transport and embolism repair under drought conditions (Scartazza et al. 2015, Hartmann and Trumbore 2016). The so-called 'C starvation hypothesis' (McDowell et al. 2008) speculates that drought-induced stomatal closure minimizes hydraulic failure and the resulting decline of photosynthetic CO₂ uptake can possibly lead to C starvation as carbohydrates demand continues for the maintenance of metabolism and defence. In addition, elevated temperatures frequently co-occur with drought, a condition that could accelerate metabolism, bringing about the depletion of too much starch, potentially leading to tree mortality (Adams et al. 2009). This suggests that trees should be able to maintain a minimum (safety) level of reserve under drought and warm conditions to avoid this risk (McDowell and Sevanto 2010). Notwithstanding partial starch hydrolysis, our results support this hypothesis, showing that the total NSC contents were only slightly affected. This indicates that beech trees might be able to counteract a relatively brief and intense hot drought event by the interconversion between starch and soluble sugars without drastically affecting the total C storage reserves in woody tissue. However, at the end of the 2017 vegetative season, trees stored similar amounts of starch and total NSCs compared with the modelled reference values for the site, confirming that the studied forest showed an efficient internal regulation mechanism able to respond resiliently to environmental factors with short- to medium-term homeostatic equilibrium (Scartazza et al. 2013, Dietrich et al. 2018). The absence of a strong depletion of NSCs at the end of two sequential years characterized by extreme weather events that strongly reduced C supply and increased C demand for sustaining stress-recovery (frost) and stress-tolerance (drought) processes further supports the hypothesis that C reserves in plants can be actively managed by trees. In this view, wood NSC synthesis, cleavage, interconversion,

mobilization and allocation are likely tightly controlled at the physiological, biochemical and molecular level to optimize long-term growth and survival (Sala et al. 2012, Collalti et al. 2018, 2020b, Merganičová et al. 2019).

To summarize, our study adds new insights for elucidating the mechanisms connected to the impact of late frost and summer drought on sink processes (stem and foliage growth, allocation to reserve pool) in a Mediterranean beech forest. Synthesis, cleavage, interconversion, mobilization and allocation of wood NSCs seem to be finely regulated processes and play a key role in counteracting the negative effects of both late frost and summer drought. These processes seem likely to improve plant survival by buffering the difference between C supply and demand under extreme weather events. Hence, our data support the hypothesis of mechanistic connections between changes in NSCs, tree growth and resilience. Moving forward, more experimental studies are necessary to verify this hypothesis. Although both the key roles of NSCs and the trade-off between growth versus protection are not novel, this work likely represents the first evidence in the field under two consecutive extreme weather events. This information suggests that C reserves could be crucial for resilience of beech in the Mediterranean area and Europe, where an increasing frequency of extreme weather events under future global changes are forecasted.

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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