

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

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Abstract

Extreme weather events are increasing in frequency and intensity due to global climate change. We hypothesized that these have a strong impact on the stem radial growth and the dynamic of non-structural carbohydrates (NSCs). In order to assess the effects on mature trees of a late frost occurred in spring 2016 and a drought event characterizing the summer 2017, we monitored the phenology, the radial growth and the dynamic of starch and soluble sugars in a Mediterranean beech forest. Growth was much more reduced by spring late frost than by summer drought, while NSCs dynamic was deeply involved in counteracting the negative effects of both events, supporting plant survival and buffering source-sink imbalances under such stressful conditions, resulting in a strong trade-off between growth and NSCs dynamic in trees. Overall, our results highlight the key role of NSCs on trees resilience to extreme weather events, confirming the relevant adaptability to stressful conditions. Such an insight is useful to assess how forests may respond to the potential impacts of climate change on ecosystem processes and to define how future management strategies can help adaptation of beech forests in the Mediterranean area.

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Frost and drought effects on stemwood reserves

Summary

Extreme weather events are increasing in frequency and intensity due to global climate change. We hypothesized that these have a strong impact on the stem radial growth and the dynamic of non-structural carbohydrates (NSCs). In order to assess the effects on mature trees of a late frost occurred in spring 2016 and a drought event characterizing the summer 2017, we monitored the phenology, the radial growth and the dynamic of starch and soluble sugars in a Mediterranean beech forest. Growth was much more reduced by spring late frost than by summer drought, while NSCs dynamic was deeply involved in counteracting the negative effects of both events, supporting plant survival and buffering source-sink imbalances under such stressful conditions, resulting in a strong trade-off between growth and NSCs dynamic in trees. Overall, our results highlight the key role of NSCs on trees resilience to extreme weather events, confirming the relevant adaptability to stressful conditions. Such an insight is useful to assess how forests may respond to the potential impacts of climate change on ecosystem processes and to define how future management strategies can help adaptation of beech forests in the Mediterranean area.

Keywords: Fagus sylvatica L., Late frost, Mediterranean region, Resilience

Introduction

Global climate change is causing an increment in the frequency of extreme weather events (Stocker *et al.* 2014) that are recognized among the major drivers of current and future ecosystem dynamics (Frank *et al.* 2015). The Mediterranean basin is one of the two main hot-spots of climate change (Giorgi 2006; Noce, Collalti & Santini 2017), showing increases in the inter annual variability and of extreme environmental conditions (Flaounas *et al.* 2013). In this region, the increasing risk of late frost events represents one of the major threats associated with the future global change (Zohner *et al.* 2020). Indeed, increasing spring temperatures has been observed stimulating earlier leaf unfolding (Gordo & Sanz 2010; Allevato *et al.* 2019), thus potentially exposing young leaves and shoots to spring frost damage (Augspurger 2013), especially at high elevation (Vitasse, Schneider, Rixen, Christen & Rebetez 2018). Depending on species, temperatures below -4°C can destroy the “fresh” leaves and shoots reducing - up to even blocking - the photosynthetic capacity of trees for several weeks. In this case, the resource requirements for new leaves formation, and trees life maintenance, must necessarily rely on the remobilization of carbon (C) reserves (Dittmar, Fricke & Elling 2006; D’Andrea *et al.* 2019). Moreover, severity, duration, and frequency of drought events have all been increasing in the last decades (Spinoni, Naumann, Vogt & Barbosa 2015). European beech (*Fagus sylvatica* L.), one of the most diffused native tree species in Europe, is known to be drought sensitive (Bolte *et al.* 2016). Hence, drought events can negatively affect physiological performance (Rezaie *et al.* 2018), carbon allocation (D’Andrea, Guidolotti, Scartazza, De Angelis & Matteucci 2020a), reproductive capacity (Nussbaumer *et al.* 2020), as well as the growth and competitive strength of the species (Peuke, Schraml, Hartung & Rennenberg 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) dynamic are among the most strongly affected ecosystem processes by spring frost and summer drought (Li *et al.* 2018). An increasing body of evidence has shown that NSCs dynamic is not a pure passive deposit and removal of C compounds, but it represents a key process actively controlled by plants to finely regulate C source-sink balance and to buffer the difference between C supply and demand at different timescales (Scartazza *et al.* 2001; Sala, Woodruff & Meinzer 2012; Carbone *et al.* 2013; Fatichi, Leuzinger & Korner 2014; Martinez-Vilalta *et al.* 2016; Moscatello *et al.* 2017; Collalti *et al.* 2020). Therefore, NSCs could play a crucial role in counteracting the negative effects of extreme weather events on beech forests, contributing to their resilience and survival (Scartazza, Moscatello, Matteucci, Battistelli & Brugnoli 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-off with growth and reproduction in forest trees (Merganičová *et al.* 2019; Tixier *et al.* 2020). The stemwood plays a key role in the aforementioned mechanisms, because it, together with coarse roots, contains most of the NSCs of the tree plants (Barbaroux, Bréda & Dufrêne 2003; Richardson *et al.* 2015).

In this work, we studied the effects of spring late frost and summer drought in a long-term research plot established in a Mediterranean beech forest (Collelongo, Abruzzi Region, Italy). The site is located in the large area of the Central-South Italy where in spring 2016, due to unusually warm preceding weeks, leaf unfolding occurred up to 15-20 days earlier than the normal average, followed by frost, that caused the complete loss of the newly grown leaves. Moreover, in 2017, a strong summer drought, due to a combination of drastic reduction of precipitation associated with high air temperature in late July and August, interested a huge area of the Mediterranean basin (Bascietto *et al.* , 2018; Nolè *et al.* , 2018; Allevato *et al.* , 2019; Rita *et al.* , 2019), including the Collelongo site. Notwithstanding these events were monitored through remote sensing techniques, *in situ* evaluations of their effects on ecosystem functionality are limited. Phenology, growth and stemwood NSCs dynamic in the Collelongo beech stand were investigated during 2016 (i.e. the year of the late frost event) and 2017 (i.e. the year of the summer drought event) and compared to the historical intra-annual data collected earlier at the site.

The objectives of the study were to: *i*) quantify the magnitude of the effects of such extreme events on ecosystem functioning; *ii*) verify the role of NSCs in mediating source-sink balance following the strong alteration of C activity; *iii*) evaluate the interplay and trade-off between carbon allocation to canopy, stem and C reserves. The aim was to predict how these responses and regulation processes could contribute to the resilience of beech to extreme weather events associated with future global change.

Material and methods

Study site

The study was carried out during the years 2016 and 2017 in an even-aged, pure beech stand (*Fagus sylvatica* L.) located at Selva Piana (Collelongo, Abruzzi Region, Italy 41°50'58" N, 13°35'17" E, 1560 m elevation) included in a 3000 ha forest within the external belt of Abruzzo-Lazio-Molise National Park (Central Apennine). The last dendrometric survey (2017) assessed a stand density of 725 trees ha⁻¹, a basal area of 45.77 m²ha⁻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 110 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 7.2degC, and the mean annual precipitation was 1178 mm, of which 10% concentrated during the summer (Guidolotti, Rey, D'Andrea, Matteucci & De Angelis 2013; Collalti *et al.* 2016; Rezaie *et al.* 2018; Reyer *et al.* 2020). The experimental area is part of the LTER network (Long Term Ecological Research) since 1996.

Climate and Phenology

The temperature and precipitation for the period 1989-2015, available on the Fluxnet2015 release, were used to characterize the, on average, climate conditions of the site. For the data gaps occurred dur-

ing the experimental trial (2016-2017), we used the ERA5 database produced by the European Centre for Medium-Range Weather Forecasts (ECMWF) (<https://www.ecmwf.int/en/forecasts/datasets/archive-datasets/reanalysis-datasets/era5>, data accessed: [12/04/2018]), according to the Fluxnet2015 release formulations (Pastorello *et al.* 2020). To evaluate peculiarities of the climatic conditions in 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 MODIS Leaf Area Index product (LAI, MOD15A2H product, <https://modis.gsfc.nasa.gov/>) with 8-days temporal resolution and 500-meters 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: (1) *green-up*, photosynthetic activity onset; (2) *maximum LAI*, supposed to be the leaf maturity phase; (3) *senescence*, sharp decrease of photosynthetic activity and green leaf area; (4) *winter dormancy*. In 2016, the leafless period after the late frost was identified from the day of the extreme event and the second green up.

Selection, measurements and sampling of trees

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

From each tree, micro-cores (2 mm diameter, 15 mm long) of wood were collected after bark removal, using the Trephor tool (Rossi, Menardi, Fontanella & Anfodillo 2005). All samples for biochemical analyses were immediately placed in dry ice for transport to the laboratory, then stored at -20 degC and, finally, stabilized through lyophilisation processes until NSCs analysis.

Daily radial increment (R_i , $\mu\text{m day}^{-1}$), was calculated as follow:

$$R_i = \frac{R_t - R_{t-1}}{\Delta t} \text{ eq.1}$$

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, increment cores were collected at breast height from each tree, using an increment borer. Tree ring width series were converted into tree basal area increment (BAI, $\text{cm}^2 \text{ year}^{-1}$), according to the following standard formula:

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

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 minutes. After centrifugation at 16,000 x g for 5 minutes, soluble sugars were recovered in the supernatant while the pellet was resuspended in 1 ml of 40 mM acetate buffer (pH 4.5), then re-centrifuged 16,000 x g for 5 minutes. This procedure was repeated 4-times. The final pellet was autoclaved for 45 minutes 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 analyzed by high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Thermo Scientific Dionex ICS-5000, Sunnyvale, CA U.S.A.)(Proietti *et al.* 2017).

Modelling of Intra-annual dynamics of non-structural carbohydrates

To evaluate the effects of the spring late frost (2016) and the heat wave and drought stress (2017) on the intra-annual NSCs dynamic, a representative benchmark of the typical intra-annual carbohydrates dynamic of the study site was needed. With this aim, a dataset on NSCs dynamic derived from other experimental trials at the site was used (Supporting Information Table S1). Dataset was composed of data of different years (i.e.: 2001, 2002, 2013, 2014, 2015, and 2018). This dataset included 39 observations of starch dynamic and 28 observations for both soluble sugars (glucose, fructose and sucrose) and total NSCs dynamic. Observations for soluble sugars were lower, 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 (FAA). Unfortunately, this methodology caused the loss of soluble sugars, while the starch integrity was preserved, 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, Derryberry & Peterson 2014) to select the simplest model able in reproducing the *in situ* observed pattern. 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 (Fig. S1 - 3).

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 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.

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 and total sugar contents. 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, SE), the lack of overlap between the two intervals of confidence indicates likely a statistically significant difference at the 95% level (P-value<0.05).

Results

Climate in the study period

Monthly variations of temperature and precipitation in the Selva Piana beech forest are reported in Figure 1a-b. 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 (Fig. 1a inset panel). The extreme frost event followed an early spring season characterized by a temperature that during the months of February and April was significantly higher (about 2°C) than the average value of the site for the period 1989-2015 (Fig. 1a). In 2017, from May to August, the temperature was significantly higher than the average value of the site, with a positive anomaly of ~3 °C (Fig 1a). Furthermore, from May to October 2017 a significant reduction of precipitation against long term average was observed (Fig. 1b), leading to an annual precipitation that was ~ 50% lower than the 1989-2015 average (Fig. 1b inset panel).

Phenological parameters and radial growth

The seasonal LAI trend, used to define the phenological phases of the stand, is reported in Fig. 2a. The “first” green up in spring 2016 occurred between 20 and 30 days earlier than the average of the site (Fig. 2a), while the “second” (re)green up, after the complete canopy destruction due to the spring frost event, started

around June 28, with a leafless period of more than 60 days. In 2016 the beginning of the senescence phase was anticipated of about one week compared to the average of the long-term series (Fig. 2b). Maximum LAI was lower in 2016 ($\text{LAI} = 4.79 \text{ m}^2 \text{ m}^{-2}$) than in 2017 ($\text{LAI} = 5.37 \text{ m}^2 \text{ m}^{-2}$), while the long-term average LAI of the site assessed with remote sensing was $\sim 5 \text{ m}^2 \text{ m}^{-2}$ (Fig. 2a). The average length of vegetative period assessed through remote sensing during the 2000-2015 period was approximately 140 days, a value confirmed in 2017, while it was 83 days in 2016.

The mean BAI in the 2000-2015 period was $22.64 \pm 0.78 \text{ cm}^2 \text{ year}^{-1}$, while it was $3.69 \pm 1.14 \text{ cm}^2 \text{ year}^{-1}$ and $18.75 \pm 3.80 \text{ cm}^2 \text{ year}^{-1}$ in 2016 and 2017, respectively (Fig. 2b inset panel). The late frost in spring 2016 reduced the stem radial growth of about 85% compared to the average of the period 1989-2015. The late frost strongly affected the seasonal dynamic of stem diameter growth during the year 2016, as shown by the lower and almost constant rate of stem growth compared to 2017, when after the green up the radial growth followed the usual pattern, reaching the highest increment ($32.30 \pm 4.14 \mu\text{m day}^{-1}$) in July (Fig. 2b).

Intra-annual dynamic of NSCs

The values and the modelled intra-annual dynamics of NSCs (total sugars, starch and soluble sugars content) measured in the beech stemwood are reported in Fig. 3 (panels a, b and c), while the intra-annual statistical differences and the multiple comparisons for 2016 and 2017 are shown in Fig. S4.

Dynamic of NSCs showed polynomial equation patterns at different grades, with R^2 ranging from 0.64 to 0.93 (Table 1). Comparing the modelled NSCs intra-annual dynamics and stand phenology, an increase in total NSCs is observed from the bud break to the beginning of green-up phase, due to the increasing starch content notwithstanding the decrease of soluble sugars. During the period between the onset and the middle of the maximum vegetative season, total NSCs content decreased due to starch reduction, while the amount of soluble sugars remained unchanged. In the late summer, both starch and soluble sugars increased until the end of the vegetative season, determining an increase of total sugars content (Fig. 3a). At the beginning of dormancy phase, a decrease of total NSCs was recorded, driven by a severe decrease of starch although associated with a simultaneous increase in soluble sugars.

In 2016, during the leafless period soluble sugars content significantly decreased from $8.15 \pm 0.58 \text{ mg g DW}^{-1}$ to the lowest value ($5.25 \pm 0.43 \text{ mg g DW}^{-1}$), which was measured close to the build-up of the new photosynthetic apparatus. Before the senescence, a peak of soluble sugars content was observed (241 vs 215 Day of the Year, DOY), while during the dormancy phase the maximum soluble sugars content ($16.75 \pm 0.50 \text{ mg g DW}^{-1}$) was assessed. 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 after the canopy destruction ($24.80 \pm 0.20 \text{ mg g DW}^{-1}$). Subsequently, a decline in the starch content was observed before a second seasonal peak (DOY 241), while the lowest starch content was recorded during the dormancy phase ($9.37 \pm 0.78 \text{ mg g DW}^{-1}$). In 2016, two seasonal peaks of stemwood total sugars content were observed, 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 (241 vs 215 DOY) a significant increment of total sugars was recorded.

In 2017, during spring and early summer a significant decrement of stemwood content of soluble sugars until the lowest seasonal value ($6.16 \pm 0.41 \text{ mg g DW}^{-1}$, DOY 186) was measured. A significant increment of soluble sugars content was observed between July and August (218 vs 241 DOY), before reaching the maximum value of $15.27 \pm 0.76 \text{ mg g DW}^{-1}$ during the senescence. In spring and early summer 2017, stemwood starch content significantly increased from $11.57 \pm 1.51 \text{ mg g DW}^{-1}$ to $20.26 \pm 1.92 \text{ mg g DW}^{-1}$. At 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 sugars were lower at the beginning of the vegetative period ($23.51 \pm 2.38 \text{ mg g DW}^{-1}$) than during the senescence, when the maximum value was reached ($31.94 \pm 3.03 \text{ mg g DW}^{-1}$).

Results from the linear mixed model showed that sampling dates account for 52, 43, and 80 % of the total variance of starch, total NSCs and soluble sugars, respectively. Meanwhile, trees explain 8, 11, 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 NSCs dynamic recorded in the reference period, some substantial differences can be observed. After the leaf re-sprouting 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 leaves senescence. After that, a reduction of starch and consequently of the total carbohydrate reserves was observed. The lower amount of storage carbohydrates reached in 2016, directly affected the starch amount recorded during the first part of the vegetative season in 2017. At the beginning of August 2017, although the starch content was lower than the modelled value of the site, a refilling of total carbohydrate reserves was observed. The drought stress event of August 2017 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 dynamic, although during the dormancy stemwood starch content was lower than the modelled value of 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, 2020). The complete destruction of photosynthetic apparatus and the consequent strong reduction of stem radial growth during springtime 2016 (May-June), which in diffuse-porous tree species is mainly supplied by the new assimilates (Barbaroux & Bréda 2002; Čufar, Prislan, De Luis & Gričar 2008; Zein *et al.* 2011; Michelot, Simard, Rathgeber, Dufrêne & Damesin 2012), were associated with an increase of stemwood NSCs due to starch accumulation. An increase in total stemwood NSCs has been previously observed from November to March in other temperate forests and it was attributed to remobilization of sugars from storage compartments in coarse roots in advance of the C demands associated with springtime growth (Hoch, Richter & Körner 2003; Hartmann & Trumbore 2016). The NSCs seasonal dynamic shows that starch accumulation in beech occurs during the formation of the new crown, in the presence of the potentially dominating sink represented by new growing leaves and shoots, while 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 occurs, uniquely, as the result of the remobilization of already existing soluble NSCs, including those remobilised from below-ground organs. The normal starch rise 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, concurrently to the springtime increased air temperatures, favour synthesis of starch over its degradation (Witt & Sauter 1994). Indeed, it was recently demonstrated in one-year old shoots of *Juglans regia* L. that wood accumulation of starch, accompanied by an increase of the total activity of ADPglucose 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, much debated, hypothesis of an active control of the accumulation and buffering role of NSCs in wood (Sala *et al.* 2012; Collalti *et al.* 2020).

The buffering key role of NSCs to compensate the difference between C sink and C supply was also particularly evident during the late spring and early summer 2016, when stemwood starch reserves were partially hydrolysed, and soluble non-structural carbon became increasingly older (D'Andrea *et al.* 2019), for sustaining the second leaf re-sprouting and other plants needs, as cambium activity (D'Andrea *et al.* 2020b). Usually, during the second part of the season (August-September), the new assimilates from the canopy are mainly used to sustain C sink activities related to wall thickening and lignification phase (Prislan, Čufar, De Luis & Gričar 2018) and to refill the starch reserves within the 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 cells

production (D’Andrea *et al.* 2020b) leading to the increase of both starch and soluble sugars in stemwood of beech plants at the end of August. The reduced sink activity (related to radial growth, wall thickening and lignification) during extreme weather events could be functional to prevent NSCs depletion (Anderegg 2012; Dietrich, Delzon, Hoch & Kahmen 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 carbon, ranging from glycolysis and respiratory metabolism to cell wall polymer synthesis. On the contrary, assimilates ending up in cell wall components cannot be used for metabolism, because compartmentalized and sequestered away from sites of phloem loading, potential dead alley for their usage. Hence, under photosynthate famine and starving conditions, prioritization of photosynthates allocation to NSCs over any other form of growth, might ensure the maintenance of a sufficient amount of metabolically available level of carbon reserves. This allocation choice 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 response to environmental constraints (Rodríguez-Calcerrada *et al.* 2019; Collalti *et al.* 2020).

In 2016 year, the strong reduction of sink activity, concomitantly with the seasonal decrease of air temperatures, could contribute to the slightly anticipated closure of the season. After leaf shedding, starch was partly hydrolysed and converted to soluble sugars to reduce cell osmotic potential and induce cold tolerance (Bonhomme, Rageau, Lacoite & Gendraud 2005; Tixier & Sperling 2015).

The summer drought effects on growth and NSCs

In 2017, at the beginning of vegetative season, the new assimilates produced by the canopy photosynthesis were mainly used for sustaining the stem radial growth, which, differently from 2016, reached similar values of BAI to those observed for the reference period (1989-2015). In 2017, the summer drought affected NSCs dynamic while had only very limited effects on annual stem radial growth. Such a behaviour has been already observed for other tree species growing in the Mediterranean area which adopt a stress avoidance strategy, adjusting the end of xylem growth before potential stressful conditions may occur (e.g. Lempereur *et al.* 2015; Forner, Valladares, Bonal, Granier & Grossiord 2018).

The slight reduction of C reserves at the end of the 2016 growing season impacted the dynamic of the following year. Notwithstanding that the content of starch showed the typical seasonal trend of the site, the starch and total sugars content in woody tissue from bud break till the end of June 2017 was clearly lower than the modelled reference NSCs dynamic of the site. In summer 2017, the warm drought event had a strong effect on NSCs dynamic, leading 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 NSCs storage to sustain metabolic activities, defence mechanisms against pathogens and osmoregulation processes (McDowell 2011; Hartmann & Trumbore 2016; Collalti *et al.* 2020). The observed increase of wood soluble sugars concentration during July-August 2017 is in agreement with the key role of these non-structural compounds as solutes for osmoregulation (Chaves, Maroco & Pereira 2003). Plants under drought conditions can actively control the osmotic cell pressure to avoid tissue dehydration and to maintain the physiological functions by increasing the concentration of different kinds of compatible solutes such as betaines, amino acids and sugars (Morgan 1984). In our study, the increased concentration of stemwood soluble sugars during drought was due to both hexoses (glucose and fructose) and sucrose (data not shown), according to previous findings (Fu & Fry 2010; Yang 2013). In addition, NSCs have also a relevant role to maintain xylem transport and embolism repair under drought conditions (Scartazza, Moscatello, Matteucci, Battistelli & Brugnoli 2015; Hartmann & Trumbore 2016). The so called ‘*C starvation hypothesis*’ (McDowell *et al.* 2008) speculates that the drought-induced stomatal closure minimizes hydraulic failure but, at the same time, causes a decline of photosynthetic uptake, possibly leading to C starvation as carbohydrates demand continues for the maintenance of metabolism and defence. In addition, the concomitance of elevated temperatures could accelerate metabolism and, thus, the starch depletion potentially leading to tree mortality (Adams *et al.* 2009), suggesting that trees, to avoid this risk, should be able to maintain a minimum (safety) level of reserve under drought and warm conditions (McDowell & Sevanto 2010). Our results support this

hypothesis, showing that, notwithstanding the partial starch hydrolysis, the total NSCs contents were only slightly affected, indicating that at least beech trees are 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 were able to store similar amounts of starch and total NSCs compared to the modelled reference value of 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 characterised by extreme weather events that strongly reduced C supply and, at the same time, increased C demand for sustaining stress-recovery (frost) and stress-tolerance (drought) processes, further support the hypothesis that C reserves in plants can be tightly actively managed. In this view, wood NCS synthesis, cleavage, interconversion, mobilisation and allocation need to be actively controlled at the physiological biochemical and molecular level, to optimize growth and survival in the long-term (Sala *et al.* 2012; Collalti *et al.* 2018; Merganikova *et al.* 2019; Collalti *et al.* 2020).

Summarizing, our study gives some new insights while 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, mobilisation and allocation of wood NSCs are all finely regulated processes and play a key role in counteracting the negative effects of both late frost and summer drought, ensuring plant survival and buffering the difference between C supply and demand under extreme weather event conditions. This information suggests that C reserves could be crucial for resilience of beech, but not only, because of the expected increasing frequency of extreme weather events under the future global changes and may be useful for adaptive future management strategies of forests in the Mediterranean area and Europe.

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Table 1 : Parameters of the polynomial model describing the intra-annual variation of non-structural carbohydrates (total sugars, starch and soluble sugars 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	yo	a	b	c	d	e	n	R ²	RMSE	p-value
Total sugars	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

Figure legends

Figure 1 : Deviations of monthly mean temperature and precipitation for 2016 (blue bars, panel A) and 2017 (red bars, panel B) calculated as the difference from the 2000-2015 average value at the site. Temperature of April 25th, 2016 measured at canopy level (24 m) is reported in inset graph of panel *a* , while 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 panel *b* .

Figure 2 : Seasonal dynamics of Leaf area index (LAI, m² m⁻², panel *a*) and daily stem radial increment (panel *b*) for the years 2016, 2017 and the 2000-2015 reference period. LAI was derived from Moderate Resolution Imaging Spectroradiometer (MODIS, see Materials and Methods), for 2016 (blue line), 2017 (red line) and for the 2000-2015 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 panel *b* the daily radial increment for 2016 (blue dots) and 2017 (red dots) are shown, while the inset graph reports the long-term series data of Basal Area chronology (BAI, cm² year⁻¹), where the last two dots represent the BAI value obtained in 2016 (blue dot) and 2017 (red dot), respectively.

Figure 3 : Phenological data for the experimental beech forest site (top panel) and seasonal dynamic of NSCs content as total NSCs (panel *a*), starch (panel *b*) and soluble sugars (panel *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, m² 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 the panels *a*, *b*, and *c*, blue and red dots represent carbohydrate concentrations of 2016 and 2017, respectively, while 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 Material and Methods). Modelled values are derived from 39 and 28 measurements of starch and soluble sugar content, respectively.

Figure 1

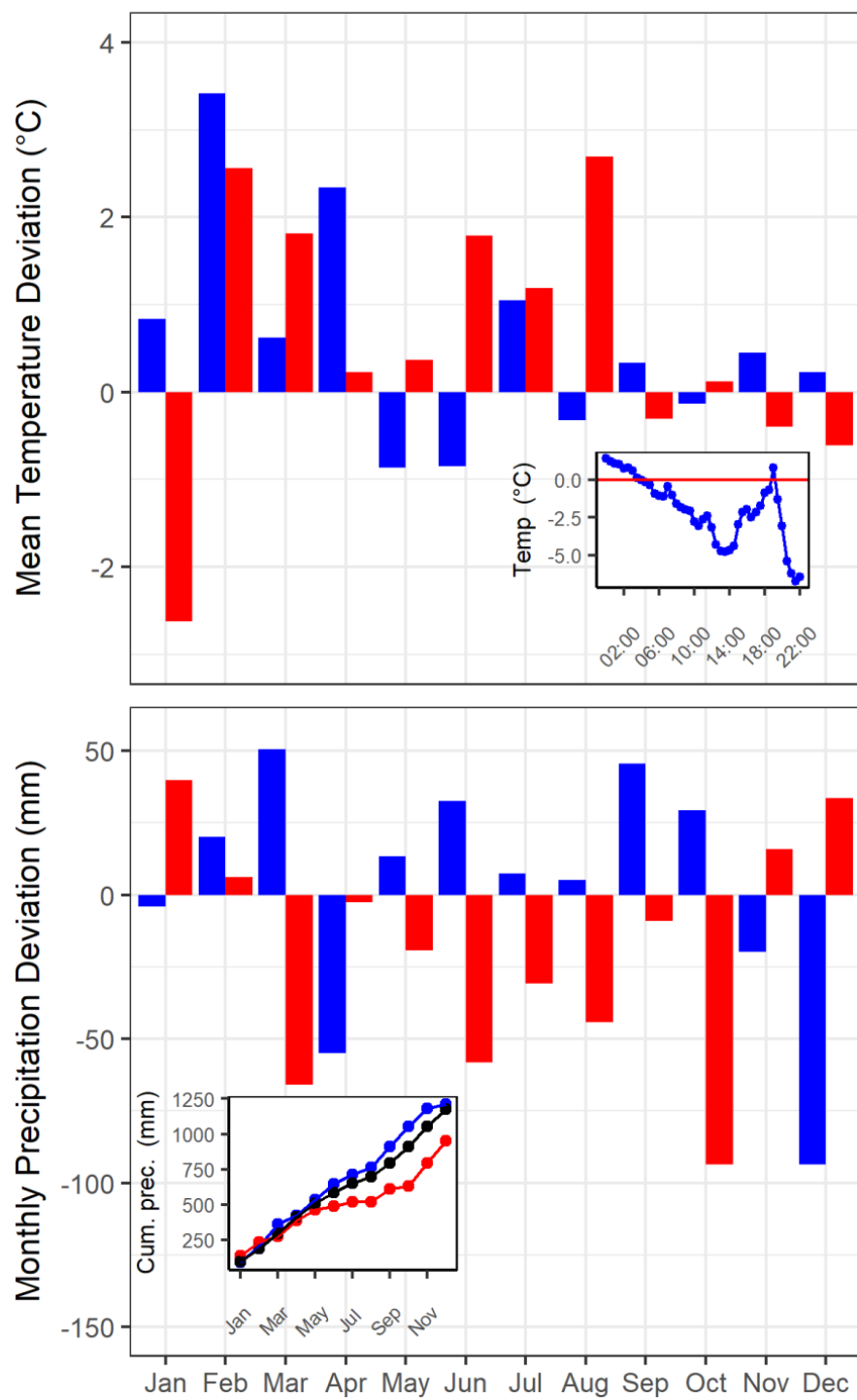


Figure 2

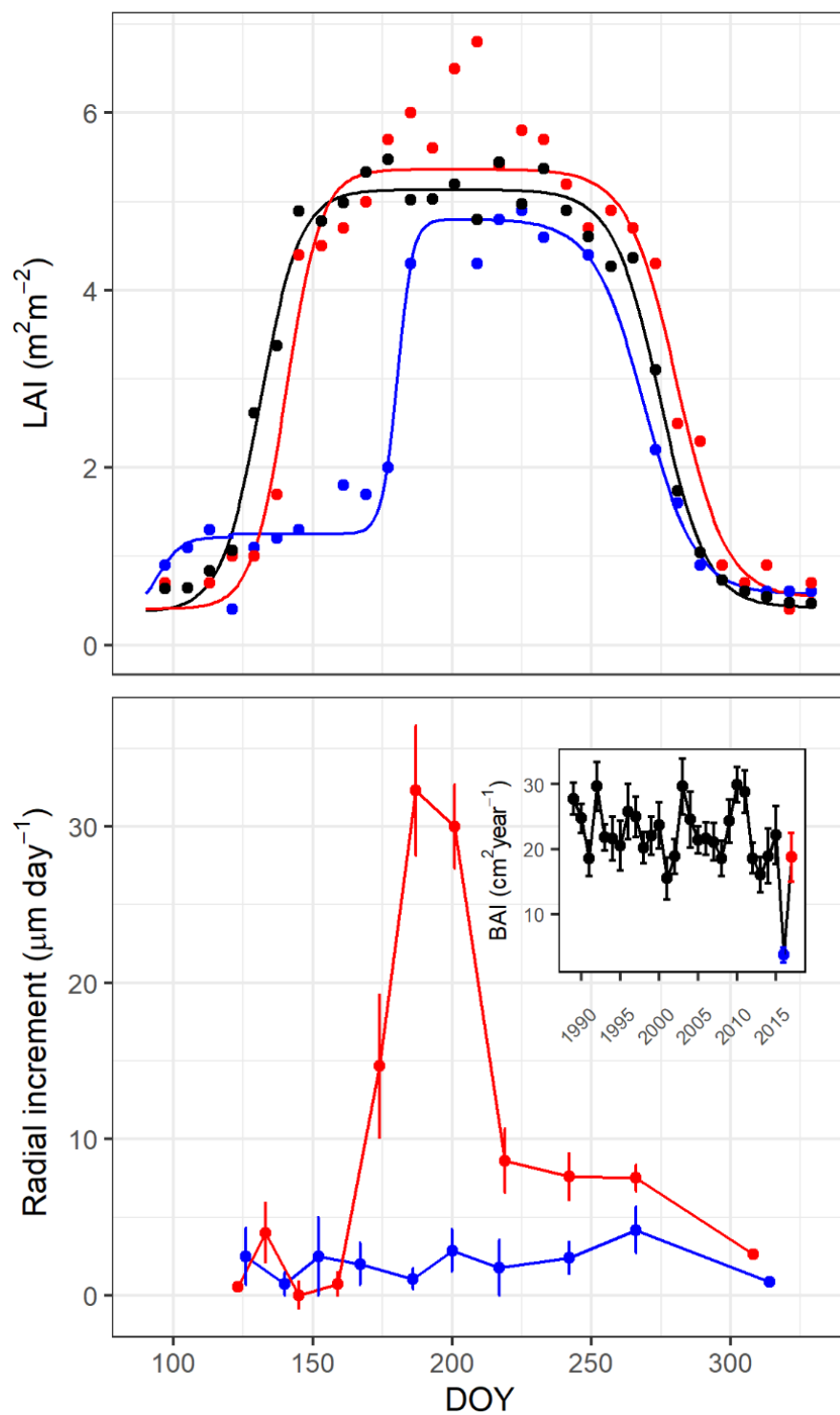
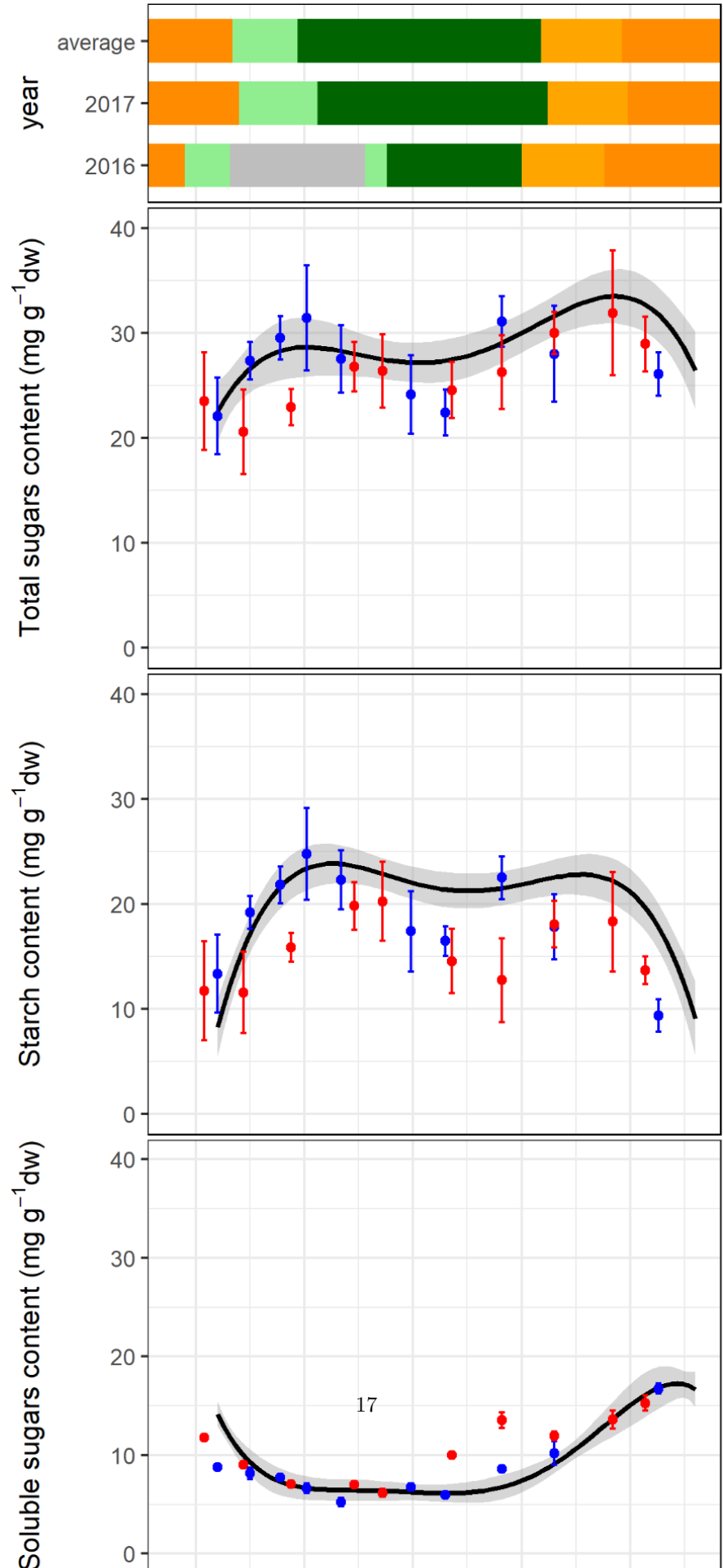


Figure 3



Supporting Information

Table S1 : Dataset of soluble sugar (glucose, fructose and sucrose), starch and total non-structural carbohydrates.

Fig. S1 Graphical validation of the assumptions of linear model describing seasonal dynamic of soluble sugars (homoscedasticity, normality of the error distribution, statistical independence of the errors, absence of influent points).

Fig. S2 Graphical validation of the assumptions of linear model describing seasonal starch dynamic (homoscedasticity, normality of the error distribution, statistical independence of the errors, absence of influent points).

Fig. S3 Graphical validation of the assumptions of linear model describing the seasonal total carbohydrates dynamic (homoscedasticity, normality of the error distribution, statistical independence of the errors, absence of influent points).

Fig. S4 Multiple comparison of total sugars, starch, and soluble sugars performed by the Student-Newman-Keuls Method. On the axis the day of the year (DOY) and the year of the samplings were reported (DOY_Year). Point represent significant difference (P-value < 0.05).

