

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

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UNCORRECTED MANUSCRIPT

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**Running head:**

Frost and drought effects on stemwood reserves

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## Abstract

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. NSCs 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.

**Key words:** *Fagus sylvatica* L., Late frost, Summer drought, Mediterranean region, Growth, Resilience

## 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, Stocker et al. 2014, Pederson 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  $-4^{\circ}\text{C}$  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, Guidolotti, et al. 2020), 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, Rezaie, et al. 2020) 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 synthesised 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, Ibrom, 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 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 ICP Forests monitoring network. 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, that 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 event on growth and carbohydrate dynamics (Dietrich et al. 2018, Li et al. 2018, Rubio-Cuadrado et al. 2020). 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 been so far 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; *ii*) evaluate the interplay and trade-off between C allocation to the canopy, stem growth and reserves.

## Material and methods

### Study site

Our 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) 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<sup>-1</sup>, a basal area of 45.8 m<sup>2</sup> ha<sup>-1</sup>, 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, Reyer et al. 2020). The experimental area is part of the LTER network (Long Term Ecological Research) since 1996.

### Climate and Phenology

Temperature and precipitation from 1989-2015, 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 (2016-2017), 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 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-day 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*, assumed to be the leaf maturity phase; (3) *senescence*, sharp decrease of photosynthetic activity and green leaf area; and (4) *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  $109 \pm 4$  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, 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 lyophilisation processes until NSC analyses.

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

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

where  $R$  is the radius of each  $i$  tree ( $\mu\text{m}$ ),  $t$  is the date of sampling, and  $\Delta t$  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:

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

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  $\mu\text{m}$  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**

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 (Fig.S1). 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 (Supporting Information Table S1) 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 xylogensis analysis and maintained in ethanol-formalin acetic acid solution (FAA). 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 (Fig. S2 - 4).

## 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 Fig S5.

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, SE), 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  $-6\text{ }^{\circ}\text{C}$  (Fig. 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  $2\text{ }^{\circ}\text{C}$ ) 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  $\sim 3\text{ }^{\circ}\text{C}$  (Fig 1a). Furthermore, from May to October 2017 a significant reduction of precipitation against the long-term average was observed (Fig. 1b), leading to total annual precipitation that was  $\sim 50\%$  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 for the site (Fig. 2a), while the second (re)green up, after the complete loss of leaves due to the late spring frost, started around June 28. In total, we estimated a leafless period of more than 60 days. In 2016, the beginning of the senescence phase occurred about one week earlier compared to the long-term average (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 the vegetative period assessed through remote sensing during from 2000 to 2015 was approximately 140 days, a value confirmed in 2017. In 2016 the same period was only 83 days.

The mean BAI in the 2000-2015 period was  $22.6 \pm 0.8 \text{ cm}^2 \text{ year}^{-1}$ , while it was  $3.7 \pm 1.1 \text{ cm}^2 \text{ year}^{-1}$  and  $18.6 \pm 3.8 \text{ cm}^2 \text{ year}^{-1}$  in 2016 and 2017, respectively (Fig. 2b inset panel). The late frost in spring 2016 reduced the stem radial growth by about 85% compared to 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 to 2017. In comparison, in 2017 the radial growth showed the usual seasonal pattern, reaching the highest increment ( $32.3 \pm 4.1 \text{ } \mu\text{m day}^{-1}$ ) in July (Fig. 2b).

## Intra-annual dynamics of NSCs

Dynamics of NSCs (Fig. 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 (Fig. 3a) was observed from bud break to the beginning of the green-up phase, due to increasing starch content (Fig. 3b). During the period between the onset and the middle of the maximum vegetative season, total NSC content decreased due to starch reduction, while the content of soluble sugars (Fig. 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  $8.15 \pm 0.58 \text{ mg g DW}^{-1}$  to  $5.25 \pm 0.43 \text{ mg g DW}^{-1}$ .

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, while 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  $11.57 \pm 1.51 \text{ mg g DW}^{-1}$  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 content was 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 accounted for 52, 43, and 80 %, while trees only explained 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 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, Collalti, Ibrom, et al. 2020). 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 remobilised 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 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 debated hypothesis of an active control of the accumulation and buffering role of NSCs in wood (Sala et al. 2012, Collalti, Tjoelker, et al. 2020).

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 hydrolysed and soluble non-structural carbon became increasingly older (D'Andrea et al. 2019). Soluble sugars were crucial for sustaining the second leaf re-sprouting and other demands, such as cambial activity (D'Andrea, Rezaie, et al. 2020). 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 production (D'Andrea, Rezaie, et al. 2020), 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 carbon, 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 carbon 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 carbon in biomass growth, while older trees prioritize the chemical defence of their biomass relative to acquisition of new biomass (Collalti, Ibrom, et al. 2020).

The strong reduction of sink activity in 2016 could have contributed to the earlier closure of the season compared to 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 (e.g. 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 till 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, Tjoelker, et al. 2020). 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 CO<sub>2</sub> 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 to 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 to 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 characterised by extreme weather events that strongly reduced C supply and increased C demand for sustaining stress-recovery (frost) and stress-tolerance (drought) processes further support the hypothesis that C reserves in plants can be actively managed by trees. In this view, wood NSC synthesis, cleavage, interconversion, mobilisation, 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, Merganičová et al. 2019, Collalti, Tjoelker, et al. 2020).

Summarizing, 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, mobilisation, 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 role of NSCs and the trade-off between growth *vs.* 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 expected increasing frequency of extreme weather events under future global changes are forecasted.

UNCORRECTED MANUSCRIPT

## Supplementary data

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

**Fig. S1** Collelongo-Selva Piana beech forest after the late frost of 2016.

**Fig. S2** 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. S3** 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. S4** Graphical validation of the assumptions of linear model describing the seasonal total NSC dynamic (homoscedasticity, normality of the error distribution, statistical independence of the errors, absence of influent points).

**Fig. S5** Multiple comparison of total NSCs, 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 reported (DOY\_Year). Point represent significant difference (P-value < 0.05).

## Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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### **References**

- Adams HD, Guardiola-Claramonte M, Barron-Gafford GA, Villegas JC, Breshears DD, Zou CB, Troch PA, Huxman TE (2009) Temperature sensitivity of drought-induced tree mortality portends increased regional die-off under global-change-type drought. *Proc Natl Acad Sci* 106:7063–7066. <https://www.pnas.org/content/106/17/7063>
- Aho K, Derryberry D, Peterson T (2014) Model selection for ecologists: The worldviews of AIC and BIC. *Ecology* 95:631–636.
- Akaike H (1974) A new look at the statistical model identification. *IEEE Trans Automat Contr* 19:716–723. <http://ieeexplore.ieee.org/document/1100705/>
- Allevato E, Saulino L, Cesarano G, Chirico GB, D’Urso G, Falanga Bolognesi S, Rita A, Rossi S, Saracino A, Bonanomi G (2019) Canopy damage by spring frost in European beech along the Apennines: effect of latitude, altitude and aspect. *Remote Sens Environ* 225:431–440. <https://doi.org/10.1016/j.rse.2019.03.023>
- Anderegg WRL (2012) Complex aspen forest carbon and root dynamics during drought A letter. *Clim Change* 111:983–991.

- Augspurger CK (2013) Reconstructing patterns of temperature, phenology, and frost damage over 124 years: Spring damage risk is increasing. *Ecology* 94:41–50.  
<http://doi.wiley.com/10.1890/12-0200.1>
- 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* 22:1201–10. <http://www.ncbi.nlm.nih.gov/pubmed/12464573>
- 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.
- Bascietto M, Bajocco S, Mazzenga F, Matteucci G (2018) Assessing spring frost effects on beech forests in Central Apennines from remotely-sensed data. *Agric For Meteorol* 248:240–250.
- Bolte A, Czajkowski T, Coccozza C, Tognetti R, de Miguel M, Pšidová E, Ditmarová L, Dinca L, Delzon S, Cochard H, Ræbild A, de Luis M, Cvjetkovic B, Heiri C, Müller J (2016) Desiccation and Mortality Dynamics in Seedlings of Different European Beech (*Fagus sylvatica* L.) Populations under Extreme Drought Conditions. *Front Plant Sci* 7:751.  
<http://journal.frontiersin.org/article/10.3389/fpls.2016.00751>
- Bonhomme M, Rageau R, Lacoïnte A, Gendraud M (2005) Influences of cold deprivation during dormancy on carbohydrate contents of vegetative and floral primordia and nearby structures of peach buds (*Prunus persica* L. Batch). *Sci Hortic (Amsterdam)* 105:223–240.  
<http://www.sciencedirect.com/science/article/pii/S0304423805000294>
- Carbone MS, Czimczik CI, Keenan TF, Murakami PF, Pederson N, Schaberg PG, Xu X, Richardson AD (2013) Age, allocation and availability of nonstructural carbon in mature red maple trees. *New Phytol* 200:1145–1155.
- Chamberlain CJ, Cook BI, Morales-Castilla I, Wolkovich EM (2021) Climate change reshapes the drivers of false spring risk across European trees. *New Phytol* 229:323–334.
- Chaves MM, Maroco J, Pereira JS (2003) Understanding plant responses to drought - From genes to

the whole plant. *Funct Plant Biol* · 30:239–264.

Collalti A, Ibrom A, Stockmarr A, Cescatti A, Alkama R, Fernández-Martínez M, Matteucci G, Sitch S, Friedlingstein P, Ciais P, Goll DS, Nabel JEMS, Pongratz J, Arneeth A, Haverd V, Prentice IC (2020) Forest production efficiency increases with growth temperature. *Nat Commun* 11:5322. <http://dx.doi.org/10.1038/s41467-020-19187-w>

Collalti A, Marconi S, Ibrom A, Trotta C, Anav A, D'andrea E, Matteucci G, Montagnani L, Gielen B, Mammarella I, Grünwald T, Knohl A, Berninger F, Zhao Y, Valentini R, Santini M (2016) Validation of 3D-CMCC Forest Ecosystem Model (v.5.1) against eddy covariance data for 10 European forest sites. *Geosci Model Dev*. <http://www.scopus.com/inward/record.url?eid=2-s2.0-84957990120&partnerID=MN8TOARS>

Collalti A, Tjoelker MG, Hoch G, Mäkelä A, Guidolotti G, Heskell M, Petit G, Ryan MG, Battipaglia G, Matteucci G, Prentice IC (2020) Plant respiration: Controlled by photosynthesis or biomass? *Glob Chang Biol* 26:1739–1753. <https://onlinelibrary.wiley.com/doi/abs/10.1111/gcb.14857>

Collalti A, Trotta C, Keenan TF, Ibrom A, Bond-lamberty B, Grote R, Vicca S, Reyer CPO (2018) Thinning Can Reduce Losses in Carbon Use Efficiency and Carbon Stocks in Managed Forests Under Warmer Climate. *J Adv Model Earth Syst*:2427–2452.

Čufar K, Prislan P, De Luis M, Gričar J (2008) Tree-ring variation, wood formation and phenology of beech (*Fagus sylvatica*) from a representative site in Slovenia, SE Central Europe. *Trees - Struct Funct* 22:749–758.

D'Andrea E, Guidolotti G, Scartazza A, De Angelis P, Matteucci G (2020) Small-Scale Forest Structure Influences Spatial Variability of Belowground Carbon Fluxes in a Mature Mediterranean Beech Forest. *Forests* 11:255. <https://doi.org/10.3390/f11030255>

D'Andrea E, Rezaie N, Battistelli A, Gavrichkova O, Kuhlmann I, Matteucci G, Moscatello S, Proietti S, Scartazza A, Trumbore S, Muhr J (2019) Winter's bite: beech trees survive complete defoliation due to spring late-frost damage by mobilizing old C reserves. *New Phytol*

224:625–631. <https://onlinelibrary.wiley.com/doi/abs/10.1111/nph.16047>

- D’Andrea E, Rezaie N, Prislan P, Gričar J, Collalti A, Muhr J, Matteucci G (2020) Frost and drought: effects of extreme weather events on stem carbon dynamics in a Mediterranean beech forest. *Plant Cell Environ.* <https://onlinelibrary.wiley.com/doi/abs/10.1111/pce.13858>
- Dietrich L, Delzon S, Hoch G, Kahmen A (2018) No role for xylem embolism or carbohydrate shortage in temperate trees during the severe 2015 drought. *J Ecol* 107:334–349.
- Dittmar C, Fricke W, Elling W (2006) Impact of late frost events on radial growth of common beech (*Fagus sylvatica* L.) in Southern Germany. *Eur J For Res* 125:249–259.
- Druckenbrod DL, Martin-Benito D, Orwig DA, Pederson N, Poulter B, Renwick KM, Shugart HH (2019) Redefining temperate forest responses to climate and disturbance in the eastern United States: New insights at the mesoscale. *Glob Ecol Biogeogr* 28:557–575.
- Fatichi S, Leuzinger S, Körner C (2014) Moving beyond photosynthesis: from carbon source to sink-driven vegetation modeling. *New Phytol* 201:1086–1095.  
<http://doi.wiley.com/10.1111/nph.12614>
- Flaounas E, Drobinski P, Vrac M, Bastin S, Lebeaupin-Brossier C, Stéfanon M, Borga M, Calvet JC (2013) Precipitation and temperature space-time variability and extremes in the Mediterranean region: Evaluation of dynamical and statistical downscaling methods. *Clim Dyn* 40:2687–2705.
- Fornier A, Valladares F, Bonal D, Granier A, Grossiord C (2018) Extreme droughts affecting Mediterranean tree species’ growth and water-use efficiency: the importance of timing. *Tree Physiol*
- Frank D, Reichstein M, Bahn M, Thonicke K, Frank D, Mahecha MD, Smith P, van der Velde M, Vicca S, Babst F, Beer C, Buchmann N, Canadell JG, Ciais P, Cramer W, Ibrom A, Miglietta F, Poulter B, Rammig A, Seneviratne SI, Walz A, Wattenbach M, Zavala MA, Zscheischler J (2015) Effects of climate extremes on the terrestrial carbon cycle: Concepts, processes and potential future impacts. *Glob Chang Biol* 21:2861–2880.

- Giorgi F (2006) Climate change hot-spots. *Geophys Res Lett* 33:1–4.
- Gordo O, Sanz JJ (2010) Impact of climate change on plant phenology in Mediterranean ecosystems. *Glob Chang Biol* 16:1082–1106. <http://doi.wiley.com/10.1111/j.1365-2486.2009.02084.x>
- Guidolotti G, Rey A, D'Andrea E, Matteucci G, De Angelis P (2013) Effect of environmental variables and stand structure on ecosystem respiration components in a Mediterranean beech forest. *Tree Physiol* 33:960–972.
- 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* 211:386–403. <https://onlinelibrary.wiley.com/doi/abs/10.1111/nph.13955>
- Hoch G, Richter A, Körner C (2003) Non-structural carbon compounds in temperate forest trees. *Plant, Cell Environ* 26:1067–1081.
- Lempereur M, Martin-stpaul NK, Damesin C, Joffre R, Ourcival J, Rocheteau A, Rambal S (2015) Growth duration is a better predictor of stem increment than carbon supply in a Mediterranean oak forest : implications for assessing forest productivity under climate change. :579–590.
- Li W, Hartmann H, Adams HD, Zhang H, Jin C, Zhao C, Guan D, Wang A, Yuan F, Wu J (2018) The sweet side of global change—dynamic responses of non-structural carbohydrates to drought, elevated CO<sub>2</sub> and nitrogen fertilization in tree species. *Tree Physiol* 38:1706–1723. <https://academic.oup.com/treephys/advance-article/doi/10.1093/treephys/tpy059/5035892>
- Martínez-Vilalta J, Sala A, Asensio D, Galiano L, Hoch G, Palacio S, Piper FI, Lloret F (2016) Dynamics of non-structural carbohydrates in terrestrial plants: a global synthesis. *Ecol Monogr* 86:495–516. <http://doi.wiley.com/10.1002/ecm.1231>
- McDowell NG (2011) Mechanisms Linking Drought, Hydraulics, Carbon Metabolism, and Vegetation Mortality. *Plant Physiol* 155:1051–1059.
- McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG, Yepez EA (2008) Mechanisms of plant survival and mortality during drought:

why do some plants survive while others succumb to drought? *New Phytol* 178:719–739.

<http://doi.wiley.com/10.1111/j.1469-8137.2008.02436.x>

McDowell NG, Sevanto S (2010) The mechanisms of carbon starvation: how, when, or does it even occur at all? *New Phytol* 186:264–266. <https://doi.org/10.1111/j.1469-8137.2010.03232.x>

Merganičová K, Merganič J, Lehtonen A, Vacchiano G, Sever MZO, Augustynczyk ALD, Grote R, Kyselová I, Mäkelä A, Yousefpour R, Krejza J, Collalti A, Reyer CPO (2019) Forest carbon allocation modelling under climate change Polle A (ed). *Tree Physiol* 39:1937–1960.

<https://academic.oup.com/treephys/article/39/12/1937/5622724>

Michelot A, Simard S, Rathgeber C, Dufrêne E, Damesin C (2012) Comparing the intra-annual wood formation of three European species (*Fagus sylvatica*, *Quercus petraea* and *Pinus sylvestris*) as related to leaf phenology and non-structural carbohydrate dynamics. *Tree Physiol* 32:1033–1045.

Morgan JM (1984) Osmoregulation and Water Stress in Higher Plants. *Annu Rev Plant Physiol* 35:299–319. <https://doi.org/10.1146/annurev.pp.35.060184.001503>

Moscattello S, Proietti S, Augusti A, Scartazza A, Walker RP, Famiani F, Battistelli A (2017) Late summer photosynthesis and storage carbohydrates in walnut (*Juglans regia* L.): Feed-back and feed-forward effects. *Plant Physiol Biochem* 118:618–626.

<http://dx.doi.org/10.1016/j.plaphy.2017.07.025>

Noce S, Collalti A, Santini M (2017) Likelihood of changes in forest species suitability, distribution, and diversity under future climate: The case of Southern Europe. *Ecol Evol* 7:9358–9375. <http://doi.wiley.com/10.1002/ece3.3427>

Nolè A, Rita A, Ferrara AMS, Borghetti M (2018) Effects of a large-scale late spring frost on a beech (*Fagus sylvatica* L.) dominated Mediterranean mountain forest derived from the spatio-temporal variations of NDVI. *Ann For Sci* 75:83. <http://link.springer.com/10.1007/s13595-018-0763-1>

Nussbaumer A, Meusburger K, Schmitt M, Waldner P, Gehrig R, Haeni M, Rigling A, Brunner I,

Thimonier A (2020) Extreme summer heat and drought lead to early fruit abortion in European beech. *Sci Rep* 10:1–11. <http://dx.doi.org/10.1038/s41598-020-62073-0>

Pastorello G, Trotta C, Canfora E, Chu H, Christianson D, Cheah Y-W, Poindexter C, Chen J, Elbashandy A, Humphrey M, Isaac P, Polidori D, Ribeca A, van Ingen C, Zhang L, Amiro B, Ammann C, Arain MA, Ardö J, Arkebauer T, Arndt SK, Arriga N, Aubinet M, Aurela M, Baldocchi D, Barr A, Beamesderfer E, Marchesini LB, Bergeron O, Beringer J, Bernhofer C, Berveiller D, Billesbach D, Black TA, Blanken PD, Bohrer G, Boike J, Bolstad P V., Bonal D, Bonnefond J-M, Bowling DR, Bracho R, Brodeur J, Brümmer C, Buchmann N, Burban B, Burns SP, Buysse P, Cale P, Cavagna M, Cellier P, Chen S, Chini I, Christensen TR, Cleverly J, Collalti A, Consalvo C, Cook BD, Cook D, Coursolle C, Cremonese E, Curtis PS, D'Andrea E, da Rocha H, Dai X, Davis KJ, De Cinti B, de Grandcourt A, De Ligne A, De Oliveira RC, Delpierre N, Desai AR, Di Bella CM, di Tommasi P, Dolman H, Domingo F, Dong G, Dore S, Duce P, Dufrêne E, Dunn A, Dušek J, Eamus D, Eichelmann U, ElKhidir HAM, Eugster W, Ewenz CM, Ewers B, Famulari D, Fares S, Feigenwinter I, Feitz A, Fensholt R, Filippa G, Fischer M, Frank J, Galvagno M, Gharun M, Gianelle D, Gielen B, Gioli B, Gitelson A, Goded I, Goeckede M, Goldstein AH, Gough CM, Goulden ML, Graf A, Griebel A, Gruening C, Grünwald T, Hammerle A, Han S, Han X, Hansen BU, Hanson C, Hatakka J, He Y, Hehn M, Heinesch B, Hinko-Najera N, Hörtnagl L, Hutley L, Ibrom A, Ikawa H, Jackowicz-Korczynski M, Janouš D, Jans W, Jassal R, Jiang S, Kato T, Khomik M, Klatt J, Knohl A, Knox S, Kobayashi H, Koerber G, Kolle O, Kosugi Y, Kotani A, Kowalski A, Kruijt B, Kurbatova J, Kutsch WL, Kwon H, Launiainen S, Laurila T, Law B, Leuning R, Li Y, Liddell M, Limousin J-M, Lion M, Liska AJ, Lohila A, López-Ballesteros A, López-Blanco E, Loubet B, Loustau D, Lucas-Moffat A, Lüers J, Ma S, Macfarlane C, Magliulo V, Maier R, Mammarella I, Manca G, Marcolla B, Margolis HA, Marras S, Massman W, Mastepanov M, Matamala R, Matthes JH, Mazzenga F, McCaughey H, McHugh I, McMillan AMS, Merbold L, Meyer W, Meyers T, Miller SD, Minerbi S, Moderow U, Monson RK, Montagnani L,

Moore CE, Moors E, Moreaux V, Moureaux C, Munger JW, Nakai T, Neiryneck J, Nestic Z, Nicolini G, Noormets A, Northwood M, Noretto M, Nouvellon Y, Novick K, Oechel W, Olesen JE, Ourcival J-M, Papuga SA, Parmentier F-J, Paul-Limoges E, Pavelka M, Peichl M, Pendall E, Phillips RP, Pilegaard K, Pirk N, Posse G, Powell T, Prasse H, Prober SM, Rambal S, Rannik Ü, Raz-Yaseef N, Reed D, de Dios VR, Restrepo-Coupe N, Reverter BR, Roland M, Sabbatini S, Sachs T, Saleska SR, Sánchez-Cañete EP, Sanchez-Mejia ZM, Schmid HP, Schmidt M, Schneider K, Schrader F, Schroder I, Scott RL, Sedlák P, Serrano-Ortiz P, Shao C, Shi P, Shironya I, Siebicke L, Šigut L, Silberstein R, Sirca C, Spano D, Steinbrecher R, Stevens RM, Sturtevant C, Suyker A, Tagesson T, Takanashi S, Tang Y, Tapper N, Thom J, Tiedemann F, Tomassucci M, Tuovinen J-P, Urbanski S, Valentini R, van der Molen M, van Gorsel E, van Huissteden K, Varlagin A, Verfaillie J, Vesala T, Vincke C, Vitale D, Vygodskaya N, Walker JP, Walter-Shea E, Wang H, Weber R, Westermann S, Wille C, Wofsy S, Wohlfahrt G, Wolf S, Woodgate W, Li Y, Zampedri R, Zhang J, Zhou G, Zona D, Agarwal D, Biraud S, Torn M, Papale D (2020) The FLUXNET2015 dataset and the ONEFlux processing pipeline for eddy covariance data. *Sci Data* 7:225.

<http://www.nature.com/articles/s41597-020-0534-3>

Pausas JG, Millán MM (2019) Greening and Browning in a Climate Change Hotspot: The Mediterranean Basin. *Bioscience* 69:143–151.

Pederson N, Dyer JM, McEwan RW, Hessel AE, Mock CJ, Orwig DA, Rieder HE, Cook BI (2014) The legacy of episodic climatic events in shaping temperate, broadleaf forests. *Ecol Monogr* 84:599–620. <https://doi.org/10.1890/13-1025.1>

Peuke AD, Schraml C, Hartung W, Rennenberg H (2002) Identification of drought-sensitive beech ecotypes by physiological parameters. *New Phytol* 154:373–387.

Prislan P, Čufar K, De Luis M, Gričar J (2018) Precipitation is not limiting for xylem formation dynamics and vessel development in European beech from two temperate forest sites. *Tree Physiol* 38:186–197.

- R Development Core Team (2018) R: A Language and Environment for Statistical Computing.
- Reichstein M, Bahn M, Ciais P, Frank D, Mahecha MD, Seneviratne SI, Zscheischler J, Beer C, Buchmann N, Frank DC, Papale D, Rammig A, Smith P, Thonicke K, van der Velde M, Vicca S, Walz A, Wattenbach M (2013) Climate extremes and the carbon cycle. *Nature* 500:287–295. <https://doi.org/10.1038/nature12350>
- Reyer CPO, Silveyra Gonzalez R, Dolos K, Hartig F, Hauf Y, Noack M, Lasch-Born P, Rötzer T, Pretzsch H, Meesenburg H, Fleck S, Wagner M, Bolte A, Sanders TGM, Kolari P, Mäkelä A, Vesala T, Mammarella I, Pumpanen J, Collalti A, Trotta C, Matteucci G, D'Andrea E, Foltýnová L, Krejza J, Ibrom A, Pilegaard K, Loustau D, Bonnefond J-M, Berbigier P, Picart D, Lafont S, Dietze M, Cameron D, Vieno M, Tian H, Palacios-Orueta A, Cicuendez V, Recuero L, Wiese K, Büchner M, Lange S, Volkholz J, Kim H, Horemans JA, Bohn F, Steinkamp J, Chikalanov A, Weedon GP, Sheffield J, Babst F, Vega del Valle I, Suckow F, Martel S, Mahnken M, Gutsch M, Frieler K (2020) The PROFOUND Database for evaluating vegetation models and simulating climate impacts on European forests. *Earth Syst Sci Data* 12:1295–1320. <https://essd.copernicus.org/articles/12/1295/2020/> (13 June 2020, date last accessed ).
- Rezaie N, D'Andrea E, Bräuning A, Matteucci G, Bombi P, Lauteri M (2018) Do atmospheric CO<sub>2</sub> concentration increase, climate and forest management affect iWUE of common beech? Evidences from carbon isotope analyses in tree rings Epron D (ed). *Tree Physiol* 38:1110–1126. <https://academic.oup.com/treephys/advance-article/doi/10.1093/treephys/tpy025/4951454>
- Richardson AD, Carbone MS, Huggett BA, Furze ME, Czimczik CI, Walker JC, Xu X, Schaberg PG, Murakami P (2015) Distribution and mixing of old and new nonstructural carbon in two temperate trees. *New Phytol* 206:590–597.
- Rita A, Camarero JJ, Nolè A, Borghetti M, Brunetti M, Pergola N, Serio C, Vicente-Serrano SM, Tramutoli V, Ripullone F (2019) The impact of drought spells on forests depends on site

conditions: The case of 2017 summer heat wave in southern Europe. *Glob Chang Biol*:1–13.

Rodríguez-Calcerrada J, Salomón RL, Gordaliza GG, Miranda JC, Miranda E, de la Riva EG, Gil L (2019) Respiratory costs of producing and maintaining stem biomass in eight co-occurring tree species. *Tree Physiol* 39:1838–1854.

Rubio-Cuadrado Á, Gómez C, Rodríguez-Calcerrada J, Perea R, Gordaliza GG, Camarero JJ, Montes F, Gil L (2020) Differential response of oak and beech to late frost damage: an integrated analysis from organ to forest. *Agric For Meteorol*

Sala A, Woodruff DR, Meinzer FC (2012) Carbon dynamics in trees: Feast or famine? *Tree Physiol* 32:764–775.

Scartazza A, Moscatello S, Matteucci G, Battistelli A, Brugnoli E (2013) Seasonal and inter-annual dynamics of growth, non-structural carbohydrates and C stable isotopes in a Mediterranean beech forest. *Tree Physiol* 33:730–42. <http://www.ncbi.nlm.nih.gov/pubmed/23933829> (6 November 2013, date last accessed ).

Scartazza A, Moscatello S, Matteucci G, Battistelli A, Brugnoli E (2015) Combining stable isotope and carbohydrate analyses in phloem sap and fine roots to study seasonal changes of source–sink relationships in a Mediterranean beech forest Cernusak L (ed). *Tree Physiol* 35:829–839. <http://treephys.oxfordjournals.org/cgi/doi/10.1093/treephys/tpv048>

Scartazza A, Proietti S, Moscatello S, Augusti A, Monteverdi MC, Brugnoli E, Battistelli A (2001) Effect of water shortage on photosynthesis, growth and storage carbohydrate accumulation in walnut (*Juglans regia* L.). *Acta Hort*:227–232. <https://doi.org/10.17660/ActaHortic.2001.544.30>

Spinoni J, Naumann G, Vogt J, Barbosa P (2015) European drought climatologies and trends based on a multi-indicator approach. *Glob Planet Change* 127:50–57. <https://www.sciencedirect.com/science/article/pii/S0921818115000284> (5 March 2020, date last accessed ).

Stocker TF, Qin D, Plattner GK, Tignor MMB, Allen SK, Boschung J, Nauels A, Xia Y, Bex V,

Midgley PM (2014) *Climate Change 2013 - The Physical Science Basis Intergovernmental Panel on Climate Change* (ed). Cambridge University Press, Cambridge.

<http://ebooks.cambridge.org/ref/id/CBO9781107415324>

Tixier A, Guzmán-Delgado P, Sperling O, Amico Roxas A, Laca E, Zwieniecki MA (2020)

Comparison of phenological traits, growth patterns, and seasonal dynamics of non-structural carbohydrate in Mediterranean tree crop species. *Sci Rep* 10:347.

<http://www.nature.com/articles/s41598-019-57016-3>

Tixier A, Sperling O (2015) Temperature-assisted redistribution of carbohydrates. *Am J Bot* 102:1216–1218.

Vitasse Y, Schneider L, Rixen C, Christen D, Rebetez M (2018) Increase in the risk of exposure of forest and fruit trees to spring frosts at higher elevations in Switzerland over the last four decades. *Agric For Meteorol* 248:60–69. <http://dx.doi.org/10.1016/j.agrformet.2017.09.005>

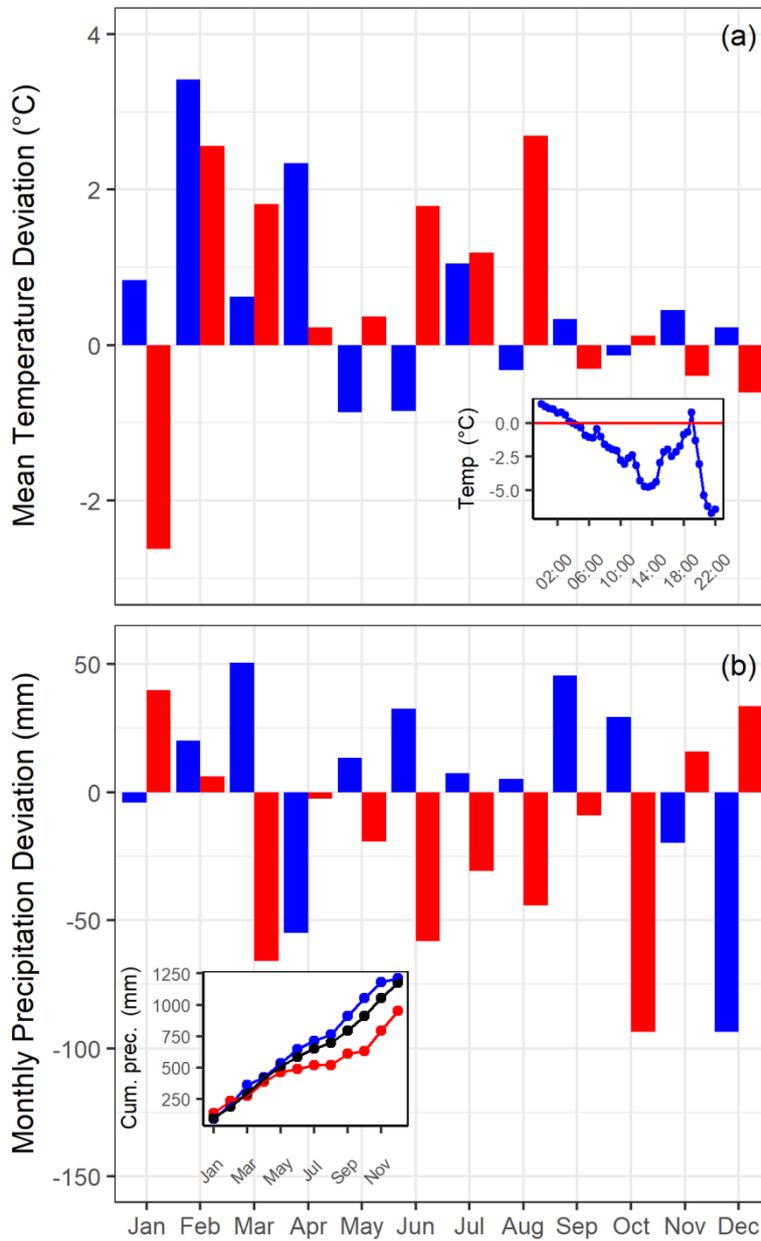
Witt W, Sauter JJ (1994) Starch metabolism in poplar wood ray cells during spring mobilization and summer deposition. *Physiol Plant* 92:9–16.

<https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1399-3054.1994.tb06648.x>

Zein R El, Maillard P, Bréda N, Marchand J, Montpied P, Gérant 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.

Zohner CM, Mo L, Renner SS, Svenning J-C, Vitasse Y, Benito BM, Ordonez A, Baumgarten F, Bastin J-F, Sebald V, Reich PB, Liang J, Nabuurs G-J, De-Miguel S, Alberti G, Antón-Fernández C, Balazy R, Brändli U-B, Chen HYH, Chisholm C, Cienciala E, Dayanandan S, Fayle TM, Frizzera L, Gianelle D, Jagodzinski AM, Jaroszewicz B, Jucker T, Kepfer-Rojas S, Khan ML, Kim HS, Korjus H, Johannsen VK, Laarmann D, Lang M, Zawila-Niedzwiecki T, Niklaus PA, Paquette A, Pretzsch H, Saikia P, Schall P, Šebeň V, Svoboda M, Tikhonova E, Viana H, Zhang C, Zhao X, Crowther TW (2020) Late-spring frost risk between 1959 and 2017 decreased in North America but increased in Europe and Asia. *Proc Natl Acad Sci*

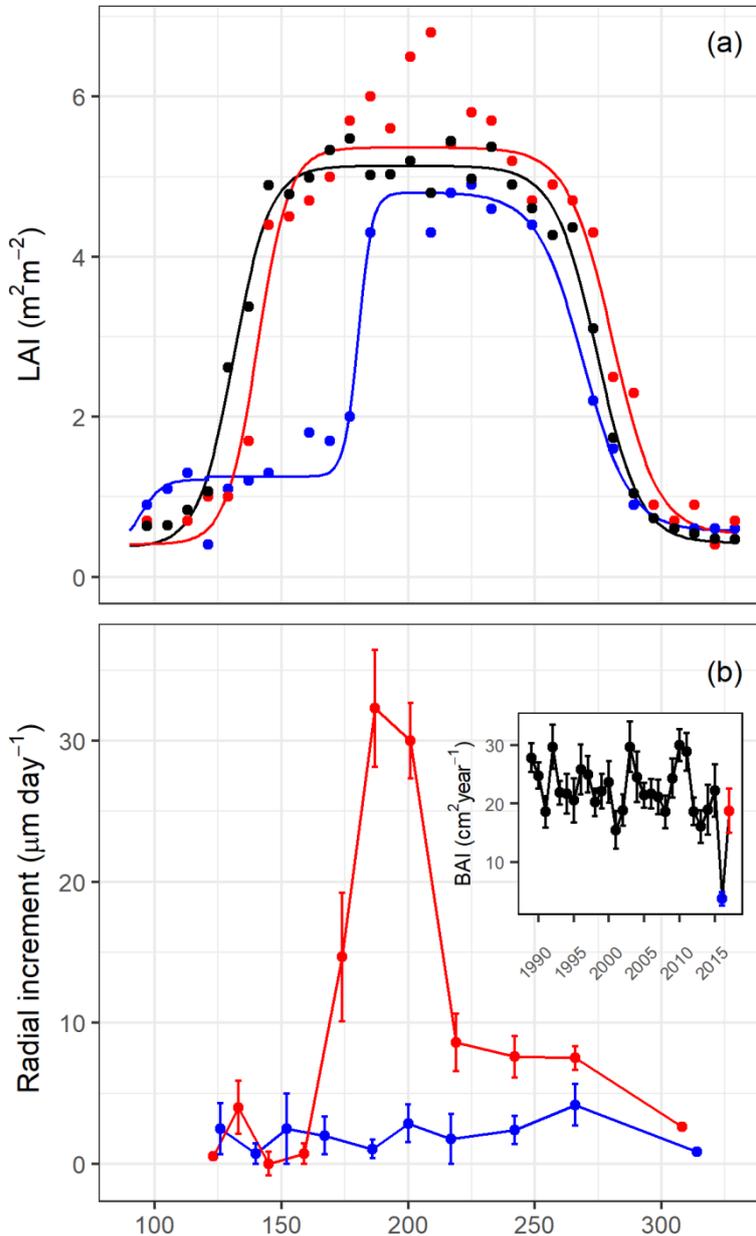
**Figures legends**



**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 2000-2015 average value at the site. Temperature of April 25<sup>th</sup>, 2016 measured at canopy level (24 m) is reported in inset graph of panel

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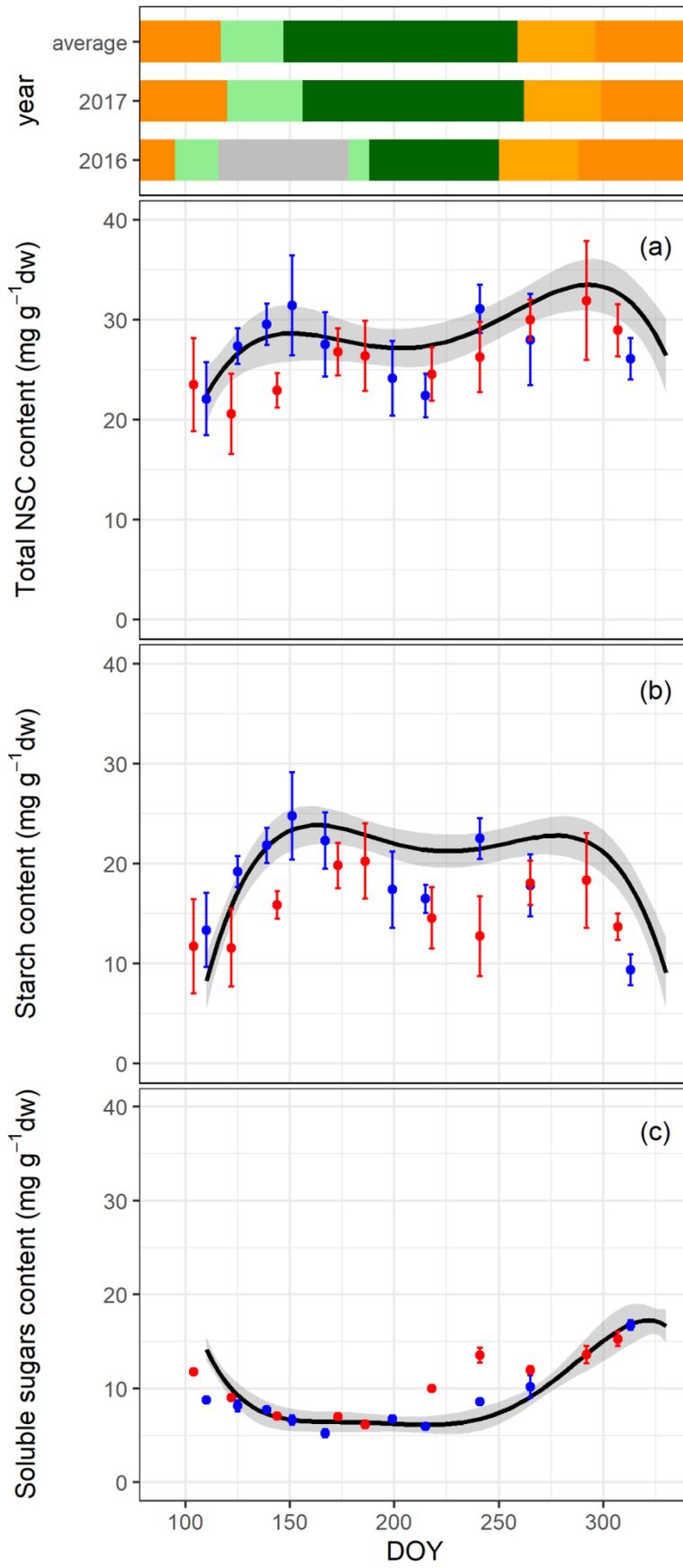
*a*, while the annual precipitation of the year 2016 (blue dots), 2017 (red dots) and the long-term average (black dots) is reported in the inset graph of panel *b*.



**Figure 2:** Seasonal dynamics of Leaf area index (LAI,  $\text{m}^2 \text{m}^{-2}$ , 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,  $\text{cm}^2 \text{ year}^{-1}$ ), where the last two dots represent the BAI value obtained in 2016 (blue dot) and 2017 (red dot), respectively.

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**Figure 3:** Phenological data for the experimental beech forest site (top panel) and seasonal dynamic of NSC 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,  $m^2 m^{-2}$ ) 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.

## Tables

**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     | yo    | a     | b      | c     | d      | e     | n  | R <sup>2</sup> | 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  |