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

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24 **Summary**

25 • Extreme weather events are increasing in frequency and intensity due to global climate

26   change. We hypothesized that these have a strong impact on the stem radial growth and the

27   dynamic of non-structural carbohydrates (NSCs).

28 • In order to assess the effects on mature trees of a late frost occurred in spring 2016 and a

29   drought event characterizing the summer 2017, we monitored the phenology, the radial

30   growth and the dynamic of starch and soluble sugars in a Mediterranean beech forest.

31 • Growth was much more reduced by spring late frost than by summer drought, while NSCs

32   dynamic was deeply involved in counteracting the negative effects of both events,

33   supporting plant survival and buffering source-sink imbalances under such stressful

34   conditions, resulting in a strong trade-off between growth and NSCs dynamic in trees.

35 • Overall, our results highlight the key role of NSCs on trees resilience to extreme weather

36   events, confirming the relevant adaptability to stressful conditions. Such an insight is useful

37   to assess how forests may respond to the potential impacts of climate change on ecosystem

38   processes and to define how future management strategies can help adaptation of beech

39   forests in the Mediterranean area.

40

41 *Keywords: Carbon allocation, Carbon reserves, Drought, Fagus sylvatica L., Late frost,*

42 *Mediterranean, Phenology, Resilience*

Introduction

44   Global climate change is causing an increment in the frequency of extreme weather events

45 (Stocker *et al.*, 2014) that are recognized among the major drivers of current and future ecosystem

46 dynamics (Frank *et al.*, 2015). The Mediterranean basin is one of the two main hot-spots of climate

47 change (Giorgi, 2006; Noce *et al.*, 2017), showing increases in the inter annual variability and of

48 extreme environmental conditions (Flaounas *et al.*, 2013). In this region, the increasing risk of late

49 frost events represents one of the major threats associated with the future global change (Zohner *et*

50 *al.*, 2020). Indeed, increasing spring temperatures has been observed stimulating earlier leaf

51 unfolding (Gordo & Sanz, 2010; Allevato *et al.*, 2019), thus potentially exposing young leaves and

52 shoots to spring frost damage (Augspurger, 2013), especially at high elevation (Vitasse *et al.*,

53 2018). Depending on species, temperatures below −4°C can destroy the "fresh" leaves and shoots

54 reducing – up to even blocking – the photosynthetic capacity of trees for several weeks. In this

55 case, the resource requirements for new leaves formation, and tree life maintenance, must

56 necessarily rely on the remobilization of carbon (C) reserves (Dittmar *et al.*, 2006; D'Andrea *et al.*,

2019). Moreover, severity, duration, and frequency of drought events have all been increasing in the last decades (Spinoni *et al.*, 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 *et al.*, 2020a), reproductive capacity (Nussbaumer *et al.*, 2020), as well as the growth and competitive strength of the species (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) 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 *et al.*, 2012; Carbone *et al.*, 2013; Fatichi *et al.*, 2014; 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 *et al.*, 2013; D'Andrea *et al.*, 2019). Unfortunately, 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).

In this work, we studied the effects of spring late frost and summer drought in a long-term research plots established on a Mediterranean beech forest of Central-South Italy (Collelongo, Abruzzi Region, Italy). The site is located in the large area in 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 canopy. 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 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 inter-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

91 following the strong alteration of C activity; *iii*) evaluate the interplay and trade-off between carbon

92 allocation to canopy, stem and C reserves. The aim was to predict how these responses and

93 regulation processes could contribute to the resilience of beech to extreme weather events

94 associated with future global change.

Material and methods

Study site

97 The study was carried out during the years 2016 and 2017 in an even-aged, pure beech forest

98 (*Fagus sylvatica* L.) located at Selva Piana stand (41°50'58" N, 13°35'17" E, 1560 m elevation) in

99 the Central Apennine (Collelongo, Abruzzi Region, Italy). The experimental area is included in the

100 LTER network (Long Term Ecological Research) and more specific information about the site were

101 already reported in previous works (Guidolotti *et al.*, 2013; Collalti *et al.*, 2016; Rezaie *et al.*, 2018;

102 Reyer *et al.*, 2020) in which the soil, forest structure, and climate characteristics are described.

Climate and Phenology

104 The temperature and precipitation for the period 1989-2015, available on the Fluxnet2015

105 release, were used to characterize the, on average, climate conditions of the site. For the data gaps

106 occurred during the experimental trial (2016-2017), we used the ERA5 database produced by the

107 European Centre for Medium-Range Weather Forecasts (ECMWF)

108 (https://www.ecmwf.int/en/forecasts/datasets/archive-datasets/reanalysis-datasets/era5, data

109 accessed: [12/04/2018]), according to the Fluxnet2015 release formulations (Pastorello *et al.*, 2020).

110 To evaluate peculiarities of the climatic conditions in 2016 and 2017 we calculated monthly

111 differences with respect to the average values of precipitation and temperature observed in the site

112 in the historical time series 1989-2015.

113 Leaf phenology was monitored using the MODIS Leaf Area Index product (LAI, MOD15A2H

114 product, https://modis.gsfc.nasa.gov/) with 8-day temporal resolution and 500-meter spatial

115 resolution (Myneni *et al.*, 2015). Critical dates, representing approximately linear transitions from

116 one phenological phase to another, were identified and defined according to Zhang *et al.* (2003) as:

117 (1) *green-up*, photosynthetic activity onset; (2) *maximum LAI*, supposed to be the leaf maturity

118 phase**;** (3) *senescence*, sharp decrease of photosynthetic activity and green leaf area; (4) *winter*

119 *dormancy*. In 2016, the leafless period after the late frost was identified from the day of the extreme

120 event and the second green up.

Selection, measurements and sampling of trees

122 Five trees were selected according to their similarity with site tree ring chronology, the trees

123 had diameters at breast height (DBH) ranging from 49 to 53 cm (for more details see D'Andrea *et*

4

124   *al.* 2020b). Trees were monitored from April 2016 to November 2017. Intra-annual radial growth of

125   each selected tree was measured using permanent girth bands with 0.1 mm accuracy (D1 Permanent

126   Tree Girth, UMS, Germany). Furthermore, stem diameter was recorded at the moment of each

127   sampling of xylem for biochemical analyses (20 sampling dates from April 2016 to November

128   2017).

129   From each tree, micro-cores (2 mm diameter, 15 mm long) of wood were collected after bark

130   removal, using the Trephor tool (Rossi *et al.*, 2005). All samples for biochemical analyses were

131   immediately placed in dry ice for transport to the laboratory, then stored at -20 °C and, finally,

132   stabilized through lyophilisation processes until NSCs analysis.

133   Daily radial increment (R_i, µm day^{-1}), was calculated as follow:

134

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

136

137   where *R* is the radius of each *i* tree (µm), *t* is the date of sampling, and *Δt* is the time interval

138   between the two sampling dates expressed in days.

139   In November 2017, at the end of the experimental trial, increment cores were collected at breast

140   height from each tree, using an increment borer. Tree ring width series were converted into tree

141   basal area increment (BAI, cm^2 year^{-1}), according to the following standard formula:

142

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

144

145   with *n* being the year of tree-ring formation.

146

147   **Starch and soluble sugar concentrations analysis**

148   The freeze-dried xylem samples were milled to a fine powder and used for all analytical

149   tests. For analysis of glucose, fructose, sucrose and starch, 10 mg of dry xylem powder were

150   extracted in 1 ml of 80% ethanol/water at 80 °C for 45 minutes. After centrifugation at 16,000 x g

151   for 5 minutes, soluble sugars were recovered in the supernatant while the pellet was resuspended in

152   1 ml of 40 mM acetate buffer (pH 4.5), then re-centrifuged 16,000 x g for 5 minutes. This procedure

153   was repeated 4-times. The final pellet was autoclaved for 45 minutes at 120 °C in the same wash

154   buffer. Enzymatic starch hydrolysis and the following glucose spectrophotometric assay were done

155   as described by Moscatello *et al.* (2017). The supernatant solution containing soluble sugars was

156   filtered on 0.2 µm nylon filters (GE-Whatman, Maidstone, UK), then analyzed by high-performance

157   anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Thermo

158   Scientific™ Dionex™ ICS-5000, Sunnyvale, CA U.S.A.)(Proietti *et al.*, 2017).

159

160   **Modelling of Intra-annual dynamics of non-structural carbohydrates**

161   To evaluate the effects of the spring late frost (2016) and the heat wave and drought stress

162   (2017) on the intra-annual NSCs dynamic, a representative benchmark of the typical intra-annual

163   carbohydrates dynamic of the study site was needed. With this aim, a dataset on NSCs dynamic

164   derived from other experimental trials at the site was used (Supporting Information Table S1).

165   Dataset was composed of data of different years (i.e.: 2001, 2002, 2013, 2014, 2015, and 2018).

166   This dataset included 39 observations of starch dynamic and 28 observations for both soluble sugars

167   (glucose, fructose and sucrose) and total NSCs dynamic. Observations for soluble sugars were

168   lower, because of the methodological sampling procedure used in 2015. During that year, woody

169   samples were collected for xylogenesis analysis and maintained in ethanol-formalin acetic acid

170   solution (FAA). Unfortunately, this methodology caused the loss of soluble sugars, while the starch

171   integrity was preserved, as verified by means of specific analytical tests on woody tissues.

172   Different models based on data of starch, soluble sugars and total NSCs were used looking

173   for possible patterns within the years and tested through the Akaike Information Criterion (AIC)

174   (Akaike, 1974; Aho *et al.*, 2014) to select the simplest model able in reproducing the *in situ*

175   observed pattern. The AIC quantifies the trade-off between parsimony and goodness-of-fit in a

176   simple and transparent manner, estimating the relative amount of information lost by a given model.

177   Hence, the model showing the lowest AIC is considered the model with the smallest information

178   loss and, potentially, the most representative one (Akaike, 1974). The four assumptions of linear

179   model (homoscedasticity, normality of the error distribution, statistical independence of the errors

180   and absence of influential points) were tested graphically (Fig. S1). Statistical analysis and figures

181   were made using R 3.5.0 (R Development Core Team, 2018).

182   **Results**

183   **Climate in the study period**

184   Monthly variations of temperature and precipitation in the Selva Piana beech forest are

185   reported in Figure 1a-b. In 2016 a severe late frost event occurred during the night between April 25

186   and 26, when the temperature at canopy level (~ 24 m) reached – 6 °C (Fig. 1a inset panel). The

187   extreme frost event followed an early spring season characterized by a temperature that during the

188   months of February and April was significantly higher (about 2°C) than the average value of the

189   site for the period 1989-2015 (Fig. 1a). In 2017, from May to August, the temperature was

190   significantly higher than the average value of the site, with an increase of ~3 °C (Fig 1a).

191 Furthermore, from May to October 2017 a significant reduction of precipitation against long term

192 average was observed (Fig. 1b), leading to an annual precipitation that was ~ 50% of the 1989-2015

193 average (Fig. 1b inset panel).

194

195 **Phenological parameters and radial growth**

196 The seasonal LAI trend, used to define the phenological phases of the stand, is reported in

197 Fig. 2a. The "first" green up in spring 2016 occurred between 20 and 30 days earlier than the

198 average of the site (Fig. 2a), while the "second" (re)green up, after the complete canopy destruction

199 due to the spring frost event, started around June 28, with a leafless period of more than 60 days. In

200 2016 the beginning of the senescence phase was anticipated of about one week compared to the

201 average of the long-term series (Fig. 2b). Maximum LAI was lower in 2016 (LAI = 4.79 $m^2\,m^{-2}$)

202 than in 2017 (LAI = 5.37 $m^2\,m^{-2}$), while the long-term average LAI of the site assessed with remote

203 sensing was ~5 $m^2\,m^{-2}$ (Fig. 2a). The average length of vegetative period assessed through remote

204 sensing during the 2000-2015 period was approximately 140 days, a value confirmed in 2017, while

205 it was 83 days in 2016.

206 The mean BAI in the 2000-2015 period was 22.64 ± 0.78 $cm^2\ year^{-1}$, while it was 3.69 ±

207 1.14 $cm^2\ year^{-1}$ and 18.75 ± 3.80 $cm^2\ year^{-1}$ in 2016 and 2017, respectively (Fig. 2b inset panel).

208 The late frost in spring 2016 reduced the stem radial growth of about 85% compared to the average

209 of the period 1989-2015. The late frost strongly affected the seasonal dynamic of stem diameter

210 growth during the year 2016, as shown by the lower and almost constant rate of stem growth

211 compared to 2017, when after the green up the radial growth followed the usual pattern, reaching

212 the highest increment (32.30 ± 4.14 $\mu m\ day^{-1}$) in July (Fig. 2b).

213

214 **Intra-annual dynamic of NSCs**

215 The values and the modelled intra-annual dynamics of NSCs (total sugars, starch and

216 soluble sugars content) measured in the beech stem wood are reported in Fig. 3 (panels a, b and c).

217 Dynamic of NSCs showed polynomial equation patterns at different grades, with R^2 ranging from

218 0.64 to 0.93 (Table 1). Comparing the modelled NSCs intra-annual dynamics and stand phenology,

219 an increase in total NSCs is observed from the bud break to the beginning of green-up phase, due to

220 the increasing starch content notwithstanding the decrease of soluble sugars. During the period

221 between the onset and the middle of the maximum vegetative season, total NSCs content decreased

222 due to starch reduction, while the amount of soluble sugar remained unchanged. In the late summer,

223 both starch and soluble sugars increased until the end of the vegetative season, determining an

224 increase of total sugars content (Fig. 3a). At the beginning of dormancy phase, a decrease of total

7

225 NSCs was recorded, driven by a severe decrease of starch although associated with a simultaneous

226 increase in soluble sugars.

227 In 2016, the lowest soluble sugars content (5.02 ± 0.46 mg g $_{DW}^{-1}$) was measured close to the

228 build-up of the new photosynthetic apparatus. In the same year, the maximum soluble sugars

229 content (15.29 ± 0.48 mg g$_{DW}^{-1}$) was measured at the end of the vegetative season, during the

230 dormancy phase, while two peaks of starch content were measured after the canopy destruction

231 (24.80 ± 0.20 mg g $_{DW}^{-1}$) and close to the beginning of the senescence phase (19.93 ± 2.82 mg g $_{DW}^{-1}$

232 $^{-1}$).

233 In 2017, the lowest content of soluble sugars (6.16 ± 1.36 mg g $_{DW}^{-1}$) was measured at Day of

234 the Year (DOY) 186, while the lowest starch contents were measured before the green up ($13.82 \pm$

235 1.51 mg g $_{DW}^{-1}$) and during the dormancy (9.37 ± 1.36 mg g $_{DW}^{-1}$).

236 Although the seasonal trends of carbohydrates accumulation in wood samples in 2016 and

237 2017, were similar to the modelled NSCs dynamic recorded in the reference period, some

238 substantial differences can be observed. In 2016, the higher starch content, balanced by a low

239 soluble sugars amount, was recorded soon before the green up. After the second leaf re-sprouting,

240 starch content decreased considerably, reaching a value lower than the modelled reference value at

241 the site. In August 2016, both starch and soluble sugars increased until leaves senescence, which

242 occurred earlier than the average of the site. After that, a reduction of the total carbohydrate

243 reserves was observed.

244 The lower amount of storage carbohydrates reached in 2016, directly affected the starch and

245 total NSCs amount recorded during the first part of the vegetative season in 2017, when a very low

246 content of starch and total carbohydrates was measured. At the end of July 2017, although the starch

247 content was lower than the modelled value of the site, a refilling of total carbohydrate reserves was

248 observed. The drought stress event of August 2017 strongly affected the composition of

249 carbohydrate reserves due to a severe starch hydrolysis, leading to a decrease of starch content of

250 about 35% and a parallel increase of soluble sugars. During the late phase of vegetative season of

251 2017, the carbohydrates pattern returned close to the modelled intra annual dynamic, although with

252 a limited reduction compared to the site average.

253 **Discussion**

254 **The buffering capacity of NSCs in response to the late frost**

255 As already observed at the site and as reported in the literature, the seasonal dynamics of

256 NSCs play a crucial role in regulating C source-sink balance through buffering the difference

257 between C supply and demand (Scartazza *et al.*, 2013; Fatichi *et al.*, 2014; Collalti *et al.*, 2018,

258 2020). The complete destruction of photosynthetic canopy and the strong reduction of stem radial
259 growth during springtime 2016 (May-June) following the frost event, were associated with an
260 increase of stemwood NSCs due to starch accumulation. An increase in total stemwood NSCs has
261 been previously observed from November to March in other temperate forests and it was attributed
262 to remobilization of sugars from storage compartments in coarse roots in advance of the C demands
263 associated with springtime growth (Hoch *et al.*, 2003; Hartmann & Trumbore, 2016). The NSCs
264 seasonal dynamic shows that starch accumulation in beech occurs during the formation of the new
265 crown, in the presence of the potentially dominating sink represented by new growing leaves and
266 shoots, while soluble sugars are decreasing. Furthermore, our results confirm that the accumulation
267 of starch in sapwood of beech trees during springtime is not necessarily supported by freshly
268 produced photosynthates. In 2016, it occurs, uniquely, as the result of the remobilization of already
269 existing soluble NSCs, including those remobilised from below-ground organs. The normal starch
270 rise in spring could be favoured by the destruction of the developing canopy leaves. This condition
271 leads to a high concentration of soluble sugars within the stemwood that, concurrently to the
272 springtime increased air temperatures, favour synthesis of starch over its degradation (Witt &
273 Sauter, 1994). Indeed, it was recently demonstrated in one-year old shoots of *Juglans regia* L. that
274 wood accumulation of starch can be increased when photosynthate export from the shoot is blocked
275 by girdling. In such case, the increase of starch accumulation is accompanied by an increase of the
276 total activity of ADPglucose pyrophosphorylase (Moscatello *et al.*, 2017), an enzyme well known
277 for its high control over starch synthesis. Thus, the spring programmed activation of starch
278 synthesis in wood can occur even when C resources are very limited by the absence of a
279 photosynthesizing crown. This strongly supports the hypothesis of an active control of the
280 accumulation and buffering role of NSCs in wood (Sala *et al.*, 2012; Collalti *et al.*, 2020).

281 The buffering role of NSCs to compensate the difference between C sink and C supply was
282 also particularly evident during the late spring and early summer 2016, when stemwood starch
283 reserves were partially hydrolysed for sustaining the second leaf re-sprouting, causing a slight
284 decrease of NSCs during July compared to the modelled values of the site. D'Andrea *et al*. (2019),
285 using the ^{14}C bomb technique, found that up to 5 years old reserves can be mobilized to sustain the
286 tree metabolic activities and leaf re-sprouting after the frost damage, further supporting the role of
287 NSCs in the resilience of beech to extreme weather events such as late spring frost. This
288 information is crucial in understanding the resilience capacity of Southern beech forest to late frost,
289 especially considering that extreme warm events may have particularly strong influences at the end
290 of winter when some species interrupt dormancy and the risk of freezing remains relatively high
291 (Ladwig *et al.*, 2019). After the complete canopy defoliation subsequent to the frost event, cambium

292 activity was maintained at low rates, representing an additional C sink during the leafless period
293 fuelled by the stemwood reserves (D'Andrea *et al.*, 2020b). During the second part of the season
294 (August-September), the new assimilates from the canopy are mainly used to sustain C sink
295 activities related to wall thickening and lignification phase (Prislan *et al.*, 2018) and to refill the
296 starch reserves within the stemwood. However, C allocation to cell wall thickening, was extremely
297 limited in 2016 due to strong reduction of xylem cells production (D'Andrea *et al.*, 2020b). This
298 possibly led to the increase of both starch and soluble sugars observed in stemwood of beech plants
299 in August 2016, notwithstanding the reduction of the net ecosystem exchange (NEE) compared to
300 the 2000-2015 values (Bascietto *et al.*, 2018). The NEE decrease in 2016 is related to canopy
301 destruction after the late frost and, overall, to the lower LAI, likely associated also with a reduction
302 of photosynthetic efficiency of defoliated beech trees (Gottardini *et al.*, 2020). In that year, the
303 strong reduction of sink activity, concomitantly with the seasonal decrease of air temperatures,
304 could contribute to the slightly anticipated closure of the season. After leaf shedding, starch was
305 partly hydrolysed and converted to soluble sugars to reduce cell osmotic potential and induce cold
306 tolerance (Bonhomme *et al.*, 2005; Tixier & Sperling, 2015).

307 **The role of NSCs to face the summer drought**

308 The slight reduction of C reserves at the end of the 2016 growing season impacted the
309 dynamic of the following year. Notwithstanding that the content of starch showed the typical
310 seasonal trend of the site, the starch content in woody tissue from bud break till the end of June
311 2017 was clearly lower than the modelled reference NSCs dynamic of the site, while there were not
312 relevant differences among modelled and measured content of soluble NSCs. This phase was
313 followed by a sharp rise in the stem radial growth as typical for the site (Scartazza *et al.*, 2013). In
314 summer 2017, the warm drought event had a strong effect on NSCs dynamic, leading to starch
315 hydrolysis and accumulation of soluble sugars in woody tissue. As drought induces a partial
316 stomatal closure that reduces C uptake, trees are forced to depend more on NSCs storage to sustain
317 metabolic activities, defence mechanisms against pathogens and osmoregulation processes
318 (McDowell, 2011; Hartmann & Trumbore, 2016). The negative effects of drought can be
319 exacerbated by the concomitant temperatures higher than average (i.e. the so-called "hot" drought),
320 strongly affecting water transport between roots and canopy (Hartmann & Trumbore, 2016).
321 Moreover, respiration increases with temperature leading to NSCs depletion (Guidolotti *et al.*,
322 2013; Collalti *et al.*, 2018), while, at the opposite, drought alone reduces whole-plant and root
323 respiration (Hartmann *et al.*, 2013). The observed increase of wood soluble sugars concentration
324 during July-August 2017 is in agreement with the key role of these C compounds as compatible
325 solutes for osmoregulation (Chaves *et al.*, 2003). Indeed, plants under drought conditions can

326 actively control the osmotic cell pressure to avoid tissue dehydration and maintain the physiological

327 functions by increasing the concentration of different kinds of compatible solutes such as betaines,

328 amino acids and sugars (Morgan, 1984). In our study, the increased concentration of stemwood

329 soluble sugars during drought was due to both hexoses (glucose and fructose) and sucrose (data not

330 shown), according to previous findings (Fu & Fry, 2010; Yang, 2013). In addition, NSCs have also

331 a relevant role to maintain xylem transport and embolism repair under drought conditions

332 (Scartazza *et al.*, 2015; Hartmann & Trumbore, 2016). The so called '*C starvation hypothesis*'

333 (Mcdowell *et al.*, 2008) speculates that the drought-induced stomatal closure minimizes hydraulic

334 failure but, at the same time, causes a decline of photosynthetic uptake, possibly leading to C

335 starvation as carbohydrates demand continues for the maintenance of metabolism and defence.

336 Moreover, the concomitance of elevated temperatures could accelerate the starch depletion leading

337 to tree mortality (Adams *et al.*, 2009), suggesting that trees, to avoid this risk, should be able to

338 maintain a minimum (safety) level of reserve under drought and warm conditions (McDowell &

339 Sevanto, 2010). Our results support this hypothesis, showing that, notwithstanding the partial starch

340 hydrolysis, the total NSCs contents were only slightly affected, indicating that beech trees were able

341 to counteract a relatively brief and intense "hot" drought event by the interconversion between

342 starch and soluble sugars without drastically affecting the total C storage reserves in woody tissue.

343 This extreme weather event delayed the starch accumulation in woody tissue during the late

344 summer-autumn period, when storage carbohydrates represent one of the major forest C sinks

345 (Scartazza *et al.*, 2013). However, at the end of the 2017 vegetative season, trees were able to store

346 similar amounts of starch and total NSCs compared to the modelled reference value of the site,

347 confirming that the studied forest showed an efficient internal regulation mechanism able to

348 respond to environmental factors with short- to medium-term homeostatic equilibrium (Scartazza *et*

349 *al.*, 2013; Dietrich *et al.*, 2018). The absence of a strong depletion of NSCs even during two

350 sequential years characterised by extreme weather events that strongly reduced C supply and, at the

351 same time, increased C demand for sustaining stress-recovery (frost) and stress-tolerance (drought)

352 processes, further support the hypothesis that C reserves in plants can be tightly actively managed.

353 In this view, wood NCS synthesis, cleavage, interconversion, mobilisation and allocation need to be

354 actively controlled at the physiological biochemical and molecular level, to optimize growth and

355 survival in the long-term (Sala *et al.* 2012; Collalti *et al.* 2018; Collalti *et al.* 2020).

356

357 **The interplay between carbon reserves and growth**

358 At the Collelongo-Selva Piana beech forest, after leaves development, stem radial growth

359 represents the main C sink during the first part of the vegetative season reaching the maximum rate

360   in July. Along the season in the late summer and early autumn period the photosynthates are mainly

361   allocated to the storage C compounds in woody tissues, as described by the models of carbohydrates

362   intra-annual dynamic and by previous studies (Michelot *et al.*, 2012; Scartazza *et al.*, 2013).

363   The complete canopy destruction in spring 2016 led to a drastic reduction of radial growth

364   without affecting stemwood C reserves dynamic during the first part of the vegetative season,

365   supporting the hypothesis that stem radial growth of diffuse-porous tree species starts soon after leaf

366   expansion and C needs are more likely to be supplied by the new assimilates rather than from the C

367   pool stored within the stem sapwood (Barbaroux & Bréda, 2002; Čufar *et al.*, 2008; Zein *et al.*,

368   2011; Michelot *et al.*, 2012). After the "second" green up in July 2016, the accumulation of C

369   reserves was prioritized over allocating recently fixed C to stem growth.

370   In 2017, at the beginning of vegetative season, the new assimilates produced by the canopy

371   photosynthesis were mainly used for sustaining the stem radial growth, which, differently from

372   2016, reached values of BAI similar to those observed for the reference period (1989-2015). In

373   2017, the extreme summer drought affected NSCs dynamic but had only very limited effects on

374   annual stem radial growth, as already observed for other tree species growing in the Mediterranean

375   area, which adopt a stress avoidance strategy, adjusting the end of xylem growth before potential

376   stressful conditions may occur (e.g. Lempereur *et al.* 2015; Forner, Valladares, Bonal, Granier &

377   Grossiord 2018). Ultimately, our results confirm that for Mediterranean beech, growth is more

378   negatively impacted by spring late frost than summer drought (Gazol *et al.*, 2019).

379   Moreover, our results suggest that in long-term adapted mature beech forests, summer

380   drought has limited effects on stem growth, being it mainly dependent on new photosynthates

381   produced in spring. At the opposite summer drought was reported to have important effects on stem

382   growth of young beech trees (Chuste *et al.*, 2020).

383   In beech trees, the duration of wood formation was found to be positively correlated with

384   increasing latitude, with warmer and drier conditions reducing the length of xylogenesis (Martinez

385   *et al.*, 2016). We speculate that the shorter period of wood formation in the more drought-prone

386   Mediterranean forests, could be also related to a local adaptation of beech to environmental

387   constraints (Jump & Peñuelas, 2007). The reduced sink activity (related to radial growth, wall

388   thickening and lignification) during extreme weather events could be functional to prevent NSCs

389   depletion (Anderegg, 2012; Dietrich *et al.*, 2018). The maintenance of high NSCs concentration and

390   control over NSCs metabolism (e.g. starch hydrolysis) during severe drought events contribute to

391   avoid xylem hydraulic failure and strong damages, as observed conversely in Central European

392   beech (Schuldt *et al.*, 2020). It should be noted that NSCs, including starch, can be rapidly

393   interconverted, ensuring a rapid hexose supply to the hexose phosphate pool. The hexose phosphate

394   pool then supports both metabolic and structural cell requirements for reduced carbon, ranging from

395   glycolysis and respiratory metabolism to cell wall polymer synthesis. On the contrary, assimilates

396   ending up in cell wall components cannot be reclaimed for metabolism, representing, in this respect,

397   almost a dead end, at least in the short period. Hence, under photosynthate famine conditions,

398   prioritization of photosynthate allocation to NSCs, might ensure the maintenance of a sufficient

399   amount of metabolically available reduced carbon. This acclimatory choice seems more

400   conservative than supporting end point-like allocation of photosynthates to cell wall components

401   and ensure a much higher plasticity to support plant response to environmental constraints.

402   Furthermore, allocation of photosynthates to NSCs is less energy costly than building new cell

403   walls polymers (Rodríguez-Calcerrada *et al.*, 2019) again making this choice more conservative in

404   case of reduced source activity. The ability of Mediterranean beech trees to store C reserves also

405   during stressful years could represent an active strategy for optimizing growth and survival and

406   coping with the increasing frequency of extreme weather events.

407   Summarizing, our study elucidated the mechanisms connected to the impact of late frost and

408   summer drought on sink processes (stem and foliage growth, allocation to reserve pool) in a

409   Mediterranean beech forest. Synthesis, cleavage, interconversion, mobilisation and allocation of

410   wood NSCs are all finely regulated processes and play a key role in counteracting the negative

411   effects of both late frost and summer drought, ensuring plant survival and buffering the difference

412   between C supply and demand under extreme weather event conditions. This information suggest

413   that C reserves could be crucial for resilience of beech because of the expected increasing frequency

414   of extreme weather events under the future global changes and may be useful for adaptive future

415   management strategies of beech forests in the Mediterranean area and Europe.

423

Author contribution

E. D'A., A.S, S.M., N.R., G.M. contributed to the design of the research. Fieldwork was carried out by E. D'A., N.R.. Soluble Sugars Content analysis were performed by S.M., A.B, S.P, and A.S.. Data analysis was done by E. D'A, A.S., S.M., data interpretation by all co-authors. The manuscript was written by E. D'A, A.S., S.M., A.C.. All authors read and commented the manuscript.

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642 **Table 1**: Parameters of the polynomial model describing the intra-annual variation of non-structural

643 carbohydrates (total sugars, starch and soluble sugars content) in beech wood, n is the number of

644 samplings used for the analysis and RMSE (mg g $_{DW}^{-1}$) is the root mean square error.

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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

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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, m^2 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, cm^2 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, 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 interval of confidence, respectively. Each point is the mean of five beech trees and bars are the standard errors (see Material and Methods). Modelled values are derived from 39 and 28 measurements of starch and soluble sugar content, respectively.
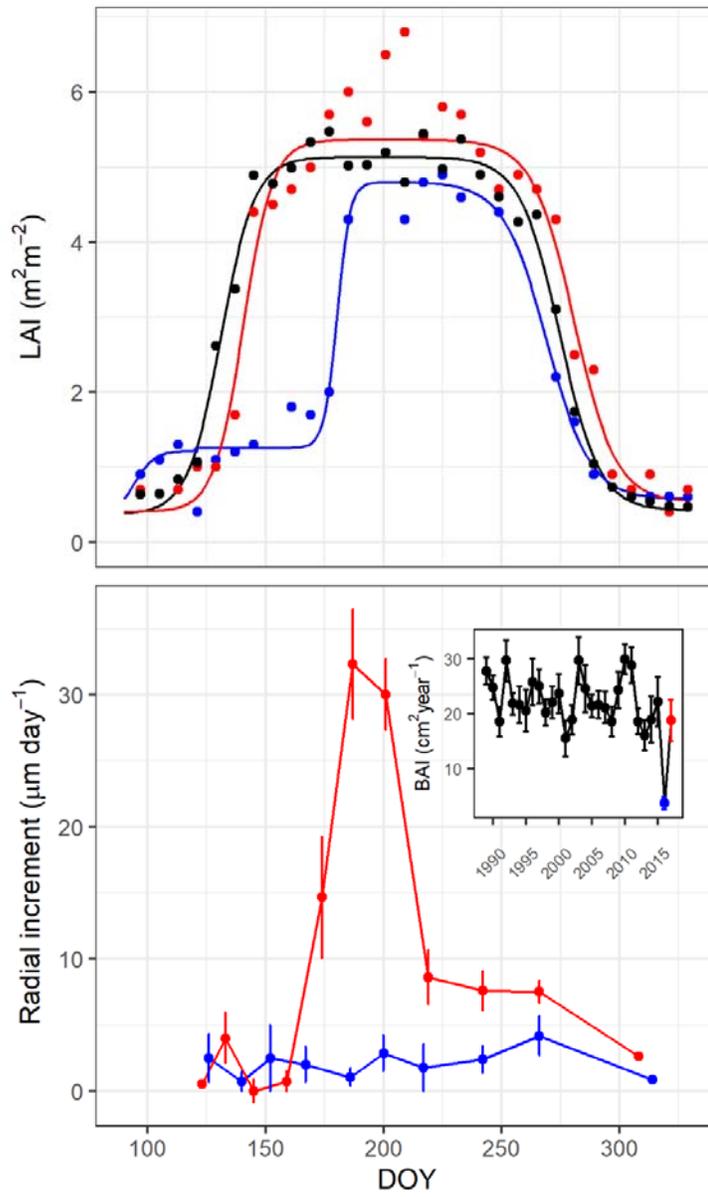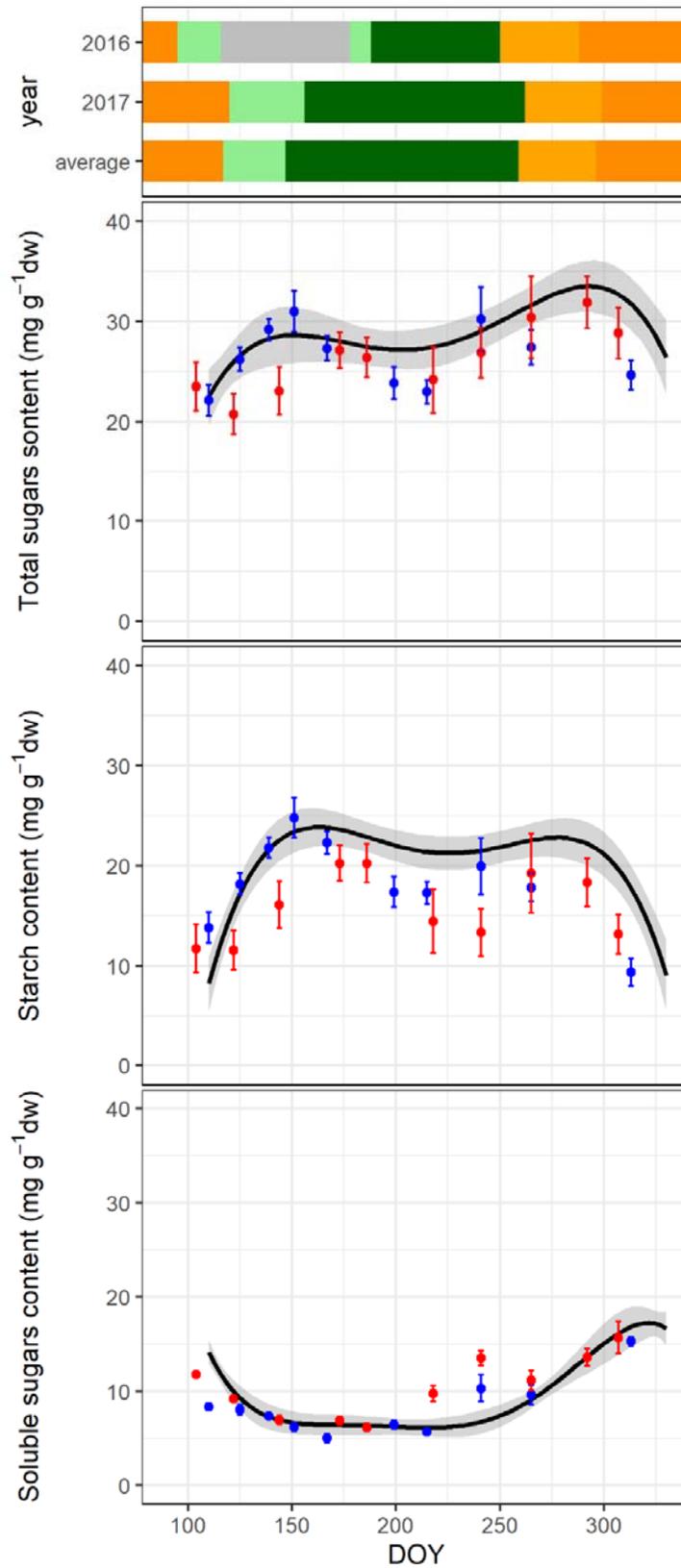
699 **Figure 1**

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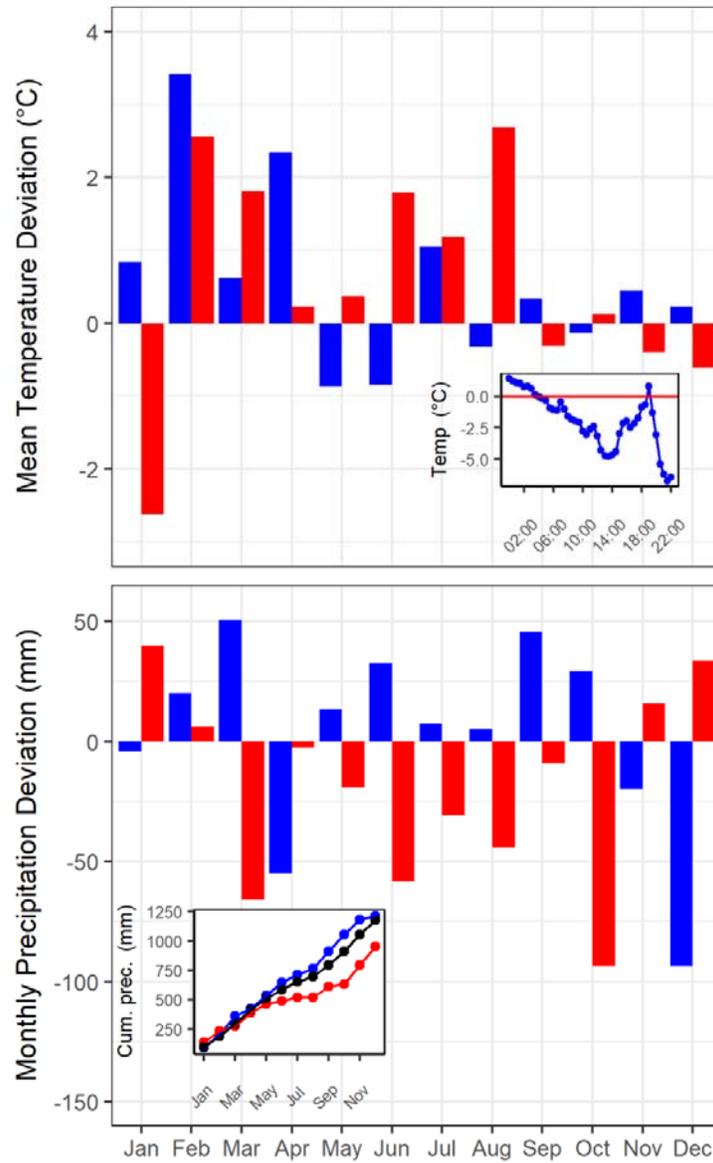
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703 **Figure 2**

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706   **Figure 3**

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708 **Supporting Information**

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

711 carbohydrates.