



# Frost and drought: effects of extreme weather events on stem carbon dynamics

## in a Mediterranean beech forest

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### Abstract

The effects of short-term extreme events on tree functioning and physiology are still rather elusive. European beech is one of the most sensitive species to late frost and water shortage. We investigated the intra-annual C dynamics in stems under such conditions.

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Wood formation and stem CO<sub>2</sub> efflux were monitored in a Mediterranean beech forest for three years (2015–2017), including a late frost (2016) and a summer drought (2017).

The late frost reduced radial growth and, consequently, the amount of carbon fixed in the stem biomass by 80%. Stem carbon dioxide efflux in 2016 was reduced by 25%, which can be attributed to the reduction of effluxes due to growth respiration. Counter to our expectations, we found no effects of the 2017 summer drought on radial growth and stem carbon efflux.

The studied extreme weather events had various effects on tree growth. Even though late spring frost had a strong impact on beech radial growth in the current year, trees fully recovered in the following growing season, indicating high resilience of beech to this stressful event.

**Keywords:** drought, extreme weather events, *Fagus sylvatica* L. (beech), growth, late frost, resilience, stem carbon efflux, wood formation.

## Introduction

Tree stems play an important role in the carbon balance of forest ecosystems (Yang, He, Aubrey, Zhuang & Teskey 2016). Part of the carbon (C) fixed by photosynthesis is allocated to the stem, some is respired by stems and emitted into the atmosphere as CO<sub>2</sub>, and a minor part is released as volatile organic compounds (VOCs) (Rissanen, Vanhatalo, Salmon, Bäck & Hölttä 2020). Radial growth – an often used proxy for the overall allocation of C to the stem (Bascietto, Cherubini & Scarascia-Mugnozza 2004; Cuny *et al.* 2015; Chan, Berninger, Kolari, Nikinmaa & Hölttä 2018) – is largely related to the process of wood formation, which can be divided into five (main) developmental phases: *i*) cambial cell division; *ii*) cell enlargement; *iii*) secondary wall deposition and, *iv*) cell wall thickening (lignification); while *v*) in the case of vessels and fibres, also genetically-programmed cell death or *apoptosis* (Prislan, Čufar, De Luis & Gričar 2018). The whole process is known to be sensitive to many factors, such as leaf phenology (Michelot, Simard, Rathgeber, Dufrêne & Damesin 2012), temperature (Begum, Nakaba, Oribe, Kubo & Funada 2007), drought (Linares, Camarero & Carreira 2009), tree-size and social status (Rathgeber, Rossi & Bontemps 2011) and tree vigour (Gričar, Krže & Čufar 2009).

A recent global estimate (Yang *et al.* 2016) showed that the stem CO<sub>2</sub> efflux (ES) alone, from boreal to tropical forests combined, was 6.7 (± 1.1) Pg C yr<sup>-1</sup>, accounting for 11% and 20% of global forest ecosystem gross primary production (GPP) and net primary production (NPP), respectively. However, accurate field measurements of actual stem respiration (RS) are difficult if not impossible (Teskey, Saveyn, Steppe & McGuire 2008), and the most commonly measured proxy, stem CO<sub>2</sub> efflux (ES), is likely to underestimate local respiration (Trumbore, Angert, Kunert, Muhr & Chambers 2013). For example, ES was found to vary between 82–94% and 86–91% of RS in *Populus deltoides* W. Bartram ex Marshall (Saveyn, Steppe, Mc Guire, Lemeur & Teskey 2008) and *Dacrydium cupressinum* Lamb stems (Bowman *et al.* 2005), respectively. Lower values (up to 45%) were observed applying the mass balance approach (Teskey & McGuire 2007) and using the O<sub>2</sub> uptake technique, as an alternative proxy for actual respiration (up to 41%, Hilman *et al.* 2019). ES

and RS are different because part of the CO<sub>2</sub> produced by respiration is not released directly through the bark into the atmosphere, but is dissolved in xylem sap and is carried upward by the transpiration stream (Bloemen *et al.* 2014). In addition, ES is affected by CO<sub>2</sub> deriving from root respiration, which is also carried upward into the stem (Bloemen, McGuire, Aubrey, Teskey & Steppe 2013). Moreover, part of respired CO<sub>2</sub> can be fixed in xylem storage pools (De Roo, Salomón & Steppe 2020). ES is influenced by many factors, such as air temperature (Yang *et al.*, 2016), growth rate (Damesin, Ceschia, Le Goff, Ottorini & Dufrêne 2002), distribution and turnover of living cells (Collalti *et al.* 2020) and tree social status (Guidolotti, Rey, D'Andrea, Matteucci & De Angelis 2013). RS can be separated in growth respiration ( $R_G$ ), which provides the energy for synthesizing new tissues; and by maintenance respiration ( $R_M$ ), which maintains existing living cells (Thornley 1970; Amthor 2000). Separating RS into these components, which are partly included in ES, allows further investigation of stem carbon budgeting and tissue costs (Chan *et al.* 2018).

Even small changes in the mean or variation of a climate variable may cause disproportionately large changes in the frequency of extreme weather events, recognized as major drivers of current and future ecosystem dynamics (Frank *et al.* 2015). In the near future, the Mediterranean region is predicted to be the most vulnerable of the European regions to global change (Schröter *et al.* 2005). Changes in temperature and precipitation regimes may increase drought risk, which can negatively affect physiological performance (Rezaie *et al.* 2018), carbon allocation (D'Andrea, Guidolotti, Scartazza, De Angelis & Matteucci 2020), as well as the growth and competition strength (Peuke, Schraml, Hartung & Rennenberg 2002) of common beech, one of the most important and widespread broadleaved trees in Europe. Increasing spring temperatures can trigger earlier leaf unfolding (Gordo & Sanz 2010; Allevato *et al.* 2019), which in turn results in a higher risk that young leaves are exposed to late spring frost (Augspurger 2013), especially in Europe (Zohner *et al.* 2020) and at higher elevations (Vitasse, Schneider, Rixen, Christen & Rebetez 2018). Temperatures below  $-4^{\circ}\text{C}$  can kill the developing new shoots and leaves, thus reducing the photosynthetic area and ultimately the trees' growth. In the case of late frost, depending on the intensity of damage, the

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formation of new leaves requires a high amount of reserves (Dittmar, Fricke & Elling 2006; D'Andrea *et al.* 2019). Despite the crucial role of extreme events and increasing attention on their prospective increasing role in future climate scenarios, information on the effect of short-term extreme events on tree functioning is still fairly elusive (Carrer, Brunetti & Castagneri 2016; Gazol *et al.* 2019). In this respect, not much is known about the interaction between wood formation (i.e. xylogenesis) and ES. At the seasonal timescale, the capacity of stem micro-coring technique to identify phenological phases of wood formation allows to attribute metabolic costs to each one of them (Meir, Mencuccini & Coughlin 2019). A deeper investigation of this link is crucial, especially in the context of climate change, associated with increased frequency of extreme weather events (e.g., drought and late frost), which may greatly modify the contribution of these processes to the C cycle and their effect on tree performance. In this context, we monitored xylogenesis, together with ES and overall growth, in a mature Mediterranean beech forest (*Fagus sylvatica* L.) from 2015 to 2017 – a period characterized by a spring late frost (2016) and a summer drought (2017) – with the objective of unravelling the intra-annual C dynamics in stems under different climatic conditions and in response to extreme weather events. On the hypothesis that extreme weather events would alter the stem C dynamics at both tree and stand scales, we investigated tree scale physiological processes specifically, of growth (xylogenesis) and respiration (proxied by stem CO<sub>2</sub> efflux), and then up-scaled to stand-scale growth and C emission related to this process. We hypothesized that: 1) cambial activity and radial growth may have ceased soon after leaf death due to the 2016 spring late frost; 2) second leaf re-sprouting starts at the expense of stem growth; 3) the 2017 summer drought would have negatively impacted stem biomass production and effluxes; and that, 4) climatic variability and extreme weather events are important factors in C dynamics on tree and stand scales.

## Material and methods

### *Study site*

The measurements were carried out between 2015 and 2017 in a long-term monitored beech stand (*Fagus sylvatica* L.) located at Selva Piana (41°50'58" N, 13°35'17" E, 1,560 m elevation), close to Collelongo (Abruzzi Region, Italy) in the Central Apennines. The site, established in 1991 and since 2006 part of the long-term ecological research (LTER) network, is located in a 3,000 ha forest included in the wider forest area of the external belt of Abruzzo-Lazio-Molise National Park. In 2017, the stand density was 725 trees ha<sup>-1</sup>, the basal area was 45.77 m<sup>2</sup> ha<sup>-1</sup> with a mean diameter at breast height (DBH) of 28.5 cm, and a mean tree height of 23 m. In 2013, mean tree age was estimated to be about 110 years. The site topography is gently sloping and the soil is humic alisol with a variable depth (40–100 cm), developed on calcareous bedrock. The climate is of Mediterranean mountain type, during the period 1989–2014 the mean annual temperature was 7.2°C, and the mean annual precipitation was 1178 mm, of which ~10% falls in summer (Guidolotti *et al.* 2013; Collalti *et al.* 2016; Reyer *et al.* 2020).

### *Tree selection, wood formation dynamics, xylem phenology and C fixation*

Sampling was performed on five trees, as done in other studies on wood formation and stem CO<sub>2</sub> efflux (e.g.: Ceschia *et al.*, 2002; Damesin *et al.*, 2002; Gruber *et al.*, 2009; Delpierre *et al.*, 2019), with a mean ( $\pm$  standard error) age of 109  $\pm$  4 years and mean DBH of 47.5  $\pm$  1.7 cm. Trees were selected for their similarity (*sensu* Gleichläufigkeit, GLK 70–85, which measures the percentage of common signs of year-to-year growth change between two series) with the site tree ring chronology (Expressed Population Signal, EPS = 0.89) as in Rezaie *et al.* (2018). We followed this approach because of the applied method for stem CO<sub>2</sub> efflux measurement, which needs big trees to allow fixing of collars. Hence, selected trees were larger than the average of stand trees, but still representative of the site growth pattern. Microcore collection and ES measurements were carried out from April 2015 – i.e., before leaf unfolding – until November 2017, when the trees had

completely lost their leaves. Microcores (2 mm diameter and 15 mm long each) were extracted from each tree at 1.1-1.7 m above ground using a Trephor tool (Rossi, Menardi, Fontanella & Anfodillo 2005). Microcores were collected 15, 12, and 14 times in 2015, 2016, and 2017, respectively. To avoid wound effects, cores were sampled at a distance of at least 5 cm from each other. The microcores, containing bark, cambium, newly developing xylem and 1-2 older xylem rings, were immediately stored in formaldehyde-ethanol-acetic acid solution (FEA) in the field. Cross-sections of the microcores were prepared following the standard methodology (Prislan, Gričar, De Luis, Smith & Cufar 2013) and were photographed in high definition under a Leica DM 4000 microscope (Leica Microsystems, Wetzlar, Germany) using transmission and polarized light. Histometrical analyses were performed on images taken with a Leica DFC 280 digital camera using the LAS (Leica Application Suite) image analysis system (Leica Microsystems, Germany). On each photographed cross-section, the number of cambium cells was counted and the widths of the developing xylem were measured along three parallel lines (Fig.1).

The dynamics of xylem formation were analysed by fitting the Gompertz function to xylem increments (Prislan *et al.* 2018; Rathgeber, Santenoise & Cuny 2018), corrected for the width of the previous tree ring to reduce the effect of the sampling position (Camarero, Guerrero-Campo & Gutierrez 1998; Oladi, Pourtahmasi, Eckstein & Bräuning 2011), as follows:

$$y = \alpha \exp[-e^{(\beta-kt)}] \quad (1)$$

where  $y$  is the cumulative ring width ( $\mu\text{m}$ ) at time  $t$  (day of the year),  $\alpha$  is the final asymptotic size representing the annual potential growth,  $\beta$  is the x-axis placement parameter and  $k$  is the rate of change parameter.

For each tree and monitoring year, the following phenological xylem formation phases were recorded: *i*) cambium *reactivation*, *ii*) beginning of cell *enlargement* (bE); *iii*) beginning of cell wall *thickening* (bW); *iv*) beginning of cell *maturation* (bM); *v*) *cessation* of cell enlargement (cE); and *vi*) *cessation* of cell wall thickening and *lignification* (cW). The date of cambium reactivation was

assessed as the average between dates when an increase of cambium cells was observed (i.e., from 3-4 to 6-7 cells in a radial row) (Čufar, Prislán, De Luis & Gričar 2008; Deslauriers, Rossi, Anfodillo & Saracino 2008). Phases of xylem growth and ring formation were computed using logistic regression, spanning from the 50% probability that phenophases have started or ended (Rathgeber *et al.* 2018). Based on phenological phases, the durations of key wood formation phases were calculated: *i*) the overall duration of the enlargement period ( $dE = cE - bE$ ); *ii*) the duration of the wall-thickening period ( $dW = cW - bW$ ); and *iii*) the total duration of wood formation (i.e., the duration of xylogenesis) ( $dX = cW - bE$ ). Data were analysed using the CAVIAR (v2.10-0) package (Rathgeber *et al.* 2018) built for R statistical software (R Development Core Team 2018). In April 2015, DBH of each sampled tree was measured using a dendrometric tape, and annual DBH increment in the measurement period was obtained through tree microcores analysis. The annual C fixed in the stem ( $SG_t$ ) was estimated for each of the sampled tree, as follows:

$$SG_t = \frac{0.46 \times (BS_t - BS_{t-1})}{\Delta t} \quad (2)$$

where  $SG_t$  is the amount of C fixed in the stem per year expressed in  $\text{Mg C yr}^{-1}$ , 0.46 is the carbon content of the woody tissue measured at the site (Scarascia-Mugnozza, Bauer, Persson, Matteucci & Masci 2000),  $BS_t$  and  $BS_{t-1}$  are the stem biomass in Mg Dry Matter (DW) at the beginning and at the end of each sampling year,  $\Delta t$  is the time variation (one year).

The site-specific allometric equation for beech used for BS was that proposed by Masci (2002):

$$BS = \frac{283.734 \times DBH^{2.134}}{10^6} \quad (3)$$

where BS is in Mg DW, and DBH (in cm) is the stem diameter at breast height (1.30 m) ( $R^2 = 0.96$ ,  $p$ -value < 0.01).

Up-scaling to stand scale was performed according to the following equation:

$$SG = SG_t \times \frac{BA}{CS_{st}} \quad (4)$$

with SG expressed in Mg C ha<sup>-1</sup> yr<sup>-1</sup>, BA is the stand Basal Area (m<sup>2</sup> ha<sup>-1</sup>), and CS<sub>st</sub> (m<sup>2</sup>) is the cross-sectional area of individual trees at 1.30 m.

### *Stem CO<sub>2</sub> efflux (ES)*

On the same trees monitored for wood formation, two PVC collars (10 cm diameter and 5 cm high, one facing north and one south) were fixed with flexible plastic ties and sealed leak tight with Terostat, a CO<sub>2</sub> neutral paste (Henkel KgaA, Germany). When present, bark mosses and lichens were removed. Stem CO<sub>2</sub> efflux was measured with a portable IRGA (EGM 4, PP-System, Hitchin, UK), equipped with a closed-dynamic chamber (SRC-1, PP-System, Hitchin, UK), which was tightened (Fig.S1). On a singular collar, each measurement consisted of a 120-second loop, in which the CO<sub>2</sub> concentration inside the chamber was measured every 5 seconds. During measurements, the CO<sub>2</sub> concentration typically increased by 10 to 50 μmol mol<sup>-1</sup>. Stem CO<sub>2</sub> efflux (ES) was calculated as:

$$ES = K_{CO_2} \div V_{mol} \times \frac{V_{cuv}}{A} \quad (5)$$

where ES is the stem CO<sub>2</sub> efflux per unit surface area (μmol m<sup>-2</sup> s<sup>-1</sup>),  $K_{CO_2}$  (μmol mol<sup>-1</sup> s<sup>-1</sup>) is the slope of the regression between CO<sub>2</sub> concentration and time during measurements, while  $V_{mol}$ , the molar volume, is the volume occupied by one mole of CO<sub>2</sub> (m<sup>3</sup> mol<sup>-1</sup>), at the air pressure (measured by the sensor built-in the EGM-4 soil respiration analyser) and air temperature ( $T_{air}$  in °C) at the measurement time,  $A$  is the exposed lateral surface area of the stem (m<sup>2</sup>), and  $V_{cuv}$  is the sum of the SRC-1 cuvette (Fig. S1) and collar volumes (m<sup>3</sup>).

Stem CO<sub>2</sub> efflux was measured 15, 12, and 11 times in 2015, 2016, and 2017, respectively. Measurements were always performed in the late morning starting between 10:30 and 12:00, and the overall sampling time was around 40 minutes.

An exponential function was used to assess the seasonal relationship between ES and  $T_{air}$

$$ES = a \times e^{T_{air} \times b} \quad (6)$$

and ES overall temperature sensitivity for a 10 °C increase ( $Q_{10}$ ) was calculated as:

$$Q_{10} = e^{10 \times b} \quad (7)$$

where  $b$  is derived from equation 6.

Considering the wood formation duration ( $dX = cW - bE$ ) as the difference between the end of the wall thickening phase ( $cW$ ) and the beginning of the enlargement phase ( $bE$ ) (see paragraph “*Tree selection, wood formation dynamics, xylem phenology and C fixation*”), we identified for each tree the wood formation period, when both maintenance and growth respirations occur ( $mg$ ), and non-wood formation periods, when only the maintenance respiration is active ( $m$ ), making it possible to divide the measured ES into two groups. According to Eq. 5, we calculated for each group the specific CO<sub>2</sub> efflux at a base air temperature of 15°C ( $ES_{15mg}$  and  $ES_{15m}$ ) and the specific  $Q_{10}$  ( $Q_{10mg}$  and  $Q_{10g}$ ).

According to the mature tissue method (Thornley 1970; Amthor 2000), during the non-wood formation period,  $ES_m$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) was constituted only by the effluxes derived by maintenance respiration, which was calculated as:

$$ES_m = ES_{15m} \times Q_{10m}^{\frac{(T_{air}-15)}{10}} \quad (8)$$

During the wood formation period,  $ES_{mg}$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), which is affected by both maintenance and growth respiration, was calculated as:

$$ES_{mg} = ES_{15mg} \times Q_{10mg}^{\frac{(T_{air}-15)}{10}} \quad (9)$$

According to Thornley (1970) and Rodríguez-Calcerrada *et al.* (2019),  $ES_m$  and its relationship with air temperature was assumed to be also valid during the wood formation period, although this

approach does not account for the acclimation of maintenance respiration to temperature during warmer periods (Collalti *et al.* 2018, and references therein). However, there are contrasting hypotheses on the magnitude of acclimation (Carey *et al.*, 1997; Stockfors & Linder, 1998). Under the above-mentioned assumptions, we calculated the stem CO<sub>2</sub> efflux due to growth respiration,  $ES_g$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), as:

$$ES_g = ES_{mg} - ES_m \quad (10)$$

The daily CO<sub>2</sub> effluxes of the whole stem were obtained by integrating, over the entire stem area, the effluxes through equation 7, 8, 9, using half-hourly  $T_{\text{air}}$  values measured at the site.

The stem area was calculated as follow:

$$LS = 0.464 \times DBH - 2.083 \quad (11)$$

where LS is the stem lateral surface ( $\text{m}^2$ ) ( $R^2 = 0.828$ ,  $p\text{-value} < 0.01$ , for more details on the equation see additional material Methods S1, Fig. S2-S3). Using DBH, we considered the measurement at 1.30 m to be representative of the whole stem, even though contrasting effects of height on stem CO<sub>2</sub> effluxes have been reported (e.g.: Damesin *et al.*, 2002 and Katayama *et al.*, 2019).

Daily stem CO<sub>2</sub> effluxes ( $\mu\text{mol CO}_2 \text{ tree}^{-1} \text{ day}^{-1}$ ) were converted in daily C effluxes ( $\text{g C tree}^{-1} \text{ day}^{-1}$ ) multiplying the moles of CO<sub>2</sub> for C molar mass ( $12 \text{ g mol}^{-1}$ ).

Annual values of the C effluxes of the five sampled trees ( $TES_{mg}$ ,  $TES_m$ ,  $TES_g$ ,  $\text{g C tree}^{-1} \text{ yr}^{-1}$ , see Table 1 for definitions) were obtained by summing up the daily values.

On the assumption that the selected trees were representative of the stand, annual values of each of the fluxes at stand scale ( $AES_{mg}$ ,  $AES_g$ ,  $AES_m$ , all in  $\text{Mg C ha}^{-1} \text{ yr}^{-1}$ , see Table 1 for definitions) were calculated as:

$$AES_x = 10^{-6} \times TES_x \times \frac{BA}{CS_{st}} \quad (12)$$

where  $TES_x$  was the effluxes at tree level ( $TES_{mg}$ ,  $TES_g$ ,  $TES_m$ ), BA is the stand Basal Area ( $m^2 ha^{-1}$ ), and  $CS_{st}$  ( $m^2$ ) is the cross-sectional area of individual trees at 1.30 m..

### ***Meteorological and phenological data***

For the period 1989-2014, FLUXNET2015 data release was used for half-hourly air temperature and precipitation (Pastorello *et al.* 2020; Reyer *et al.* 2020). For the study period (2015-2017), measured data were gap filled using downloaded data by the ERA5 database of the European Centre for Medium-Range Weather Forecasts (ECMWF) (<https://www.ecmwf.int/en/forecasts/datasets/archive-datasets/reanalysis-datasets/era5>), according to FLUXNET 2015 release equations.

The Standardized Precipitation Evapotranspiration Index (SPEI), considered the most appropriate index for the Mediterranean climate (Vicente-Serrano *et al.* 2013), was used to assess the magnitude of the drought in 2017. This index is based on the difference between precipitation and potential evapotranspiration (PET), computed according to Hargreaves' equation. The 3-month SPEI was calculated for the site for the period 1989-2017, using the SPEI package in R.

Soil water content was measured at the site under the litter and at depths of 0.05 m, 0.20 m and 0.50 m in the soil by 4 permanent sensors using the Time Domain Reflectometry technique (CS-616, Campbell Scientific, Logan, UTAH, USA). Measurements were performed with 20 seconds frequency and averaged over half hour.

Leaf phenology was monitored using the MODIS Leaf Area Index product (LAI, MOD15A2H, <https://modis.gsfc.nasa.gov/>) with 8-day temporal resolution and 500-meter spatial resolution. The date of onset of photosynthetic activity (green up) and the date at which plant green leaf area peaked its annual maximum (maturity) were assessed through the rate of change in the curvature of the fitted logistic models (Zhang *et al.* 2003).

## *Statistical data analysis*

Descriptive parameters of growth and xylem phenology were tested using one-way repeated measures analysis of variance, with years as predictive factor, followed by the Holm–Sidak post-hoc method. Linear mixed models, considering “tree” and “sampling date” as crossed random effects, were used to account for the random variation of the ES inter-annual measurements. An exponential equation was used to evaluate the relationship between ES and  $T_{\text{air}}$ . Differences among ES parameters ( $Q_{10}$  and  $ES_{15}$ ) were tested using two-way repeated measures Anova (two factors repetition), using year and period (non-wood formation, wood formation) as predictive factors. Multiple comparisons were performed by the Holm-Sidak method. Linear regressions were used to assess the relationship between tree ring widths (TRW) and mean annual ES. We tested data normality and constant variance using the Shapiro-Wilk test and the Spearman rank correlation between the absolute values of the residuals and the observed value of the dependent variable.

## **Results**

### *Description of extreme weather events*

In the night between the 25<sup>th</sup> and 26<sup>th</sup> of April 2016 (Day of Year, DOY 115), a spring late frost occurred in Central and Southern Italy, causing leaf damage in many beech stands (Bascietto, Bajocco, Mazzenga & Matteucci 2018; Greco *et al.* 2018; Nolè, Rita, Ferrara & Borghetti 2018; Allevato *et al.* 2019). At the Selva Piana site, the air temperature reached  $-6^{\circ}\text{C}$  at canopy level, destroying the whole-stand canopy (Fig. 2) and leaving the trees without leaves for almost two months (Bascietto *et al.* 2018; D’Andrea *et al.* 2019).

In the summer of 2017, a positive temperature anomaly affected Central Italy and the Balkans, with a duration ranging from 20 to 35 days (Rita *et al.* 2019). In the same year, annual precipitation was 950 mm (while the 1989–2014 average was  $1136 \pm 40.8$  mm), with only 54 mm of precipitation throughout the entire summer (while the 1989–2014 average was  $183 \pm 13.9$  mm). In August 2017, the site experienced only 1 mm of rain (while the 1989–2014 average was  $50.7 \pm 8.6$  mm), with an

average maximum air temperature of 23.9 °C, about 3°C warmer than the long-term 1989–2014 average ( $20.8 \pm 0.3$  °C). Such particular weather conditions led to a summer SPEI  $< -1.5$  (Fig. 3) and to a soil water content (SWC, daily mean of the measurement at 5 and 20 cm depth) of  $0.22 \pm 0.01 \text{ m}^3 \text{ m}^{-3}$ . This value is lower ( $F=45.935$ ,  $p\text{-value} = <0.001$ ) than  $0.28 \pm 0.01$  and  $0.31 \pm 0.02 \text{ m}^3 \text{ m}^{-3}$  assessed in 2015 and 2016, respectively.

### ***Wood formation dynamics and leaf phenology***

The date of onset of photosynthetic activity, based on leaf area index (LAI) dynamics, differed among the study years, occurring at DOY 118, 95 and 127 in 2015, 2016 and 2017, respectively.

The second leaf unfolding, occurred after the 2016 late frost, did not lead to a second wood formation cycle. In all three years, cambium reactivation occurred after leaf unfolding at DOY  $123 \pm 4$ ,  $118 \pm 8$ ,  $138 \pm 6$  in 2015, 2016 and 2017, respectively (Fig. 4). In 2016, cambium cell production also continued after the late frost event, but at considerably lower rates. Different intra-annual growth patterns were observed during the three study years, especially in the year of the late frost (2016, Fig. 5, Table 2).

In 2016, the maximum growth rate (rx) ( $F = 8.469$ ,  $p\text{-value} = 0.014$ ) was lower and was reached 3 weeks earlier (tx) ( $F = 22.667$ ,  $p\text{-value} < 0.001$ ) than in the other two years. The different intra-annual growth patterns also resulted in significantly narrower tree rings in 2016 ( $230.12 \pm 1.54 \mu\text{m}$ ) ( $F = 13.272$ ,  $p\text{-value} < 0.01$ ) than in 2015 ( $1312.17 \pm 196.15 \mu\text{m}$ ) and 2017 ( $1234.80 \pm 269.32 \mu\text{m}$ ).

Differences were also observed for the beginning, cessation, and duration of wood formation phases (Fig. 5.b). The beginning of the enlargement phase occurred earliest in 2016 and latest in 2017 ( $F = 34.789$ ,  $p\text{-value} < 0.001$ ). The cessation of this phase was anticipated in 2016 ( $F = 17.155$ ,  $p\text{-value} < 0.01$ ). Consequently, the duration of the enlargement phase was longer in 2015 ( $110 \pm 22$  days) than in 2016 ( $82 \pm 4$  days) and 2017 ( $78 \pm 4$  days) ( $F = 8.025$ ,  $p\text{-value} = 0.01$ ).

The beginning of the wall thickening phase did not differ among the years ( $F = 4.188$ ,  $p\text{-value} = 0.06$ ). The cessation of this phase occurred earlier in 2016 ( $F = 69.167$ ,  $p\text{-value} < 0.001$ ). The

duration of the wall thickening phase was thus shorter in 2016 ( $57 \pm 5$  days) than in 2015 ( $99 \pm 6$  days) and 2017 ( $75 \pm 4$  days) ( $F = 26.561$ ,  $p$ -value  $< 0.001$ ). We also observed a delay in the beginning of cell maturation in 2016 (at DOY  $200 \pm 4$ ) with respect to 2015 (at DOY  $178 \pm 4$ ) and 2017 (at DOY  $176 \pm 2$ ) ( $F = 11.650$ ,  $p$ -value  $< 0.01$ ). The overall duration of wood formation was longer in 2015 ( $128 \pm 5$  days) than in 2016 ( $98 \pm 8$  days) and 2017 ( $97 \pm 8$  days) ( $F = 12.561$ ,  $p$ -value  $< 0.001$ ).

### ***Stem CO<sub>2</sub> efflux***

During the monitoring period (April 2015 – November 2017), the measured ES ranged between  $0.16 \pm 0.03 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  (December 2015) and  $3.01 \pm 0.40 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  (August 2017) (Fig. 6). Mean ES measured in 2016 ( $0.68 \pm 0.19 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) was lower ( $F = 24.476$ ,  $p$ -value  $< 0.01$ ) than in 2015 ( $1.11 \pm 0.40 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) and 2017 ( $1.29 \pm 0.30 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ). Results from the linear mixed model showed that trees and sampling dates account for 11% and 75% of the total variance of ES, respectively.

In each year, ES was strongly related to air temperature through the standard exponential function (Fig. 7). The relation was confirmed at different aggregation levels of measurements (whole year, wood formation and non-wood formation periods; see also Table S1 in Supporting Information).

Considering the non-wood and wood formation period, average  $Q_{10}$  was  $2.75 \pm 0.15$ ,  $2.11 \pm 0.18$  and  $2.68 \pm 0.15$ , in 2015, 2016 and 2017, respectively (Table S1 in Supporting Information). The  $Q_{10}$  parameter was not strongly affected by the sampling year ( $p$ -value = 0.059), although the values in 2016 were 22% lower than in the other two years. Wood formation affected the  $Q_{10}$  parameter ( $F = 31.563$ ,  $p$ -value  $< 0.01$ ) with  $Q_{10\text{mg}}$  and  $Q_{10\text{m}}$  calculated to be  $3.06 \pm 0.15$  and  $1.93 \pm 0.14$  ( $t = 5.571$ ,  $p$ -value  $< 0.01$ ), respectively. This difference was confirmed for all the sampled years.

$ES_{15}$  was also affected by the different conditions of the monitoring years ( $F = 7.094$ ,  $p$ -value = 0.01) with mean values in 2016 ( $0.63 \pm 0.07 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) lower than in 2015 ( $0.93 \pm 0.12 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) and 2017 ( $0.82 \pm 0.12 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ). Similar to  $Q_{10}$ , the wood formation

period also affected  $ES_{15}$ , with  $ES_{15mg}$  ( $0.84 \pm 0.22 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) higher than  $ES_{15m}$  ( $0.73 \pm 0.02 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ,  $F = 7.094$ ,  $p\text{-value} = 0.01$ ). Furthermore, during the wood formation period,  $ES_{15w}$  was higher in 2015 ( $1.03 \pm 0.07 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) and 2017 ( $0.90 \pm 0.07 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) than in 2016 ( $0.60 \pm 0.09 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ). No differences among years were found for ES during the non-wood formation periods.

### ***Radial growth and stem CO<sub>2</sub> effluxes***

During the study period, annual average measured ES and tree ring widths were closely related (Fig. 8). Seasonal patterns of ES were similar in the three years, but with different amplitudes (Fig. 9). Moreover,  $ES_m$ , the stem CO<sub>2</sub> effluxes affected by maintenance respiration, showed a similar pattern among the three years. We observed a time-lag between the time of maximum growth rate (tx) and maximum ES values of  $23 \pm 2$  days,  $31 \pm 2$  days and  $29 \pm 1$  days in 2015, 2016 and 2017, respectively. Differences between years were not significant ( $F = 3.317$ ,  $p\text{-value} = 0.07$ ).

### ***From single tree to stand level***

Annual stand-level stem C emissions ( $AES_{mg}$ ) were lower in 2016 ( $0.94 \pm 0.11 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ ) than in 2015 ( $1.34 \pm 0.11 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ ) and 2017 ( $1.23 \pm 0.15 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ ) (Table 3). Annual stem C effluxes due to maintenance respiration ( $AES_m$ ) in 2016 ( $0.87 \pm 0.10 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ ) were lower than in 2015 ( $1.09 \pm 0.07 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ ) and 2017 ( $1.03 \pm 0.12 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ );  $AES$  due to growth respiration ( $AES_g$ ) was lower in 2016 ( $0.07 \pm 0.02 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ ) than in 2015 ( $0.24 \pm 0.05 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ ) and 2017 ( $0.20 \pm 0.04 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ ). The contribution of  $AES_g$  to the annual stem effluxes was  $19 \pm 0.02 \%$ ,  $7 \pm 0.02 \%$  and  $16 \pm 0.02 \%$  in 2015, 2016 and 2017, respectively.

The amount of carbon fixed in the stem biomass (SG) was lower in 2016 ( $0.28 \pm 0.07 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ ) than in 2015 ( $1.65 \pm 0.20 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ ) and 2017 ( $1.49 \pm 0.28 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ ). At the studied beech forest, the mean C construction cost of wood, defined as the slope of the relationship between  $AES_g$  and SG at tree level ( $R^2 = 0.842$ ,  $p\text{-value} < 0.01$ , see Supporting Information Fig. S4), was

0.23 g C emitted per g C fixed. On an annual scale, this parameter was  $0.15 \pm 0.01$  for 2015,  $0.24 \pm 0.05$  for 2016 and  $0.14 \pm 0.02$  for 2017 ( $F=2.797$ ,  $p$ -value = 0.104).

## Discussion

### *Cambial activity and radial growth are not entirely linked to leaf phenology*

In all three years, cambium reactivation and wood formation occurred within 1-3 weeks after leaf development, confirming the tight dependence of radial growth on leaf phenology and photosynthesis in diffuse-porous species (Čufar *et al.* 2008; Michelot *et al.* 2012). Nevertheless, in diffuse-porous trees, stem conductivity to water occurs in several outermost growth rings and is not limited to the youngest formed xylem, as found in ring-porous species (Schume, Grabner & Eckmüller 2004). Hence, in beech allocation to the current year wood, it is not as decisive as in ring-porous species, and newly formed photosynthates at the beginning of the season are preferably used for other crucial processes, such as foliage and fine root growth. At the same site, Matteucci (1998) analysed in parallel net ecosystem exchange (NEE) and carbon allocation to foliage and stem radial growth, finding that the latter started approximately 15 days after photosynthesis exceeded respiration. Until then, net absorbed carbon was allocated mostly to foliage growth. This can be related to the C allocation hierarchy across different C pools, which identifies newly developing leaves as the main C sink at the beginning of the growing season (Capioli *et al.* 2013; Collalti *et al.* 2016; Marconi, Chiti, Nolè, Valentini & Collalti 2017; Merganičová *et al.* 2019). Interestingly, and even counter to our expectations, in 2016, the cambium remained active at low rates even after complete canopy defoliation, probably fuelled by old C reserves.

After the re-sprouting of leaves in 2016, cambium cell production decreased and became non-productive, although the environmental conditions were potentially still favourable for radial growth (Rossi *et al.* 2006; Camarero, Olano & Parras 2010). Stem radial growth of beech in Selva Piana was greatly affected by the extreme late spring frost in 2016 because of the premature

cessation of cambial cell production and the lower growth rate during the active period, which resulted in 82% narrower annual xylem increments compared to 2015 and 2017. Hence, after this stressful event, trees preferred to prioritize C resources allocation to newly developing leaves and to reserve C pools, the new main C sinks, rather than radial growth. In beech, previously reported growth reduction – as a consequence of late frost – ranged from 48 to 83%, with the maximum occurring at the northern fringe of the Alps (Dittmar *et al.* 2006). At the studied stand, radial growth rates had fully recovered in 2017, with no visible long-term effects of the late spring frost event in 2016, showing the high resilience of beech growth to late frost (Dittmar *et al.* 2006; Principe, Struwe, Wilmking & Kreyling 2017). However, as recently shown in D'Andrea *et al.* (2019), beech trees during 2016 were able to compensate the lost C reserve, completely refilling the pool to the same level as before the frost event. Hence, there was no need to prioritize reserve recharge over stem biomass production the subsequent year.

The 2017 summer drought became severe only in August (SPEI < -1.5), when the trees had already completed most of their radial growth, as already seen for other tree species growing in the Mediterranean adjusting the end xylem growth before potential stressful conditions may occur (e.g. Lempereur *et al.*, 2015; Forner *et al.*, 2018). Instead, the importance of favourable spring climatic conditions on beech growth has been reported for the Apennines and the Eastern Alps (Piovesan, Biondi, Di Filippo, Alessandrini & Maugeri 2008; Di Filippo, Biondi, Maugeri, Schirone & Piovesan 2012).

### ***Effluxes from stem are not entirely synchronised to radial growth***

Our results, being measured ES lower in 2016 despite similar air temperatures during the three monitoring years, demonstrated that stem CO<sub>2</sub> efflux cannot uniquely be estimated from temperature data. Mean annual values of “apparent” Q<sub>10</sub> ranged between 2.11 (2016) and 2.71 (2015) and were similar to the values estimated at the same site for co-dominant (2.59) and dominant (2.34) trees described in Guidolotti *et al.* 2013. Q<sub>10m</sub> and Q<sub>10mg</sub> estimated in this study are closely comparable with the dataset of various coniferous and broadleaf tree species as reported in

Damesin et al. (2002). Similar intra-annual variability of  $Q_{10}$  has also been observed in many other studies for different tree species, with higher  $Q_{10}$  during the growing period (Paembonan, Hagihara & Hozumi 1992; Carey, Callaway & DeLucia 1997; Stockfors & Linder 1998; Gruber *et al.* 2009). A higher  $Q_{10}$  during the wood formation period compared to the non-wood formation period can be explained by including the “apparent” growth respiration in the parameters’ estimates. This only “apparent” increase of  $Q_{10}$  is driven by the indirect effect of temperature on stem growth, which adds up to the maintenance respiration sensitivity to temperature (Salomón, De Schepper, Valbuena-Carabaña, Gil & Steppe 2018). However, other studies found (or assume) stable  $Q_{10}$  throughout the year (see for example Ceschia et al. 2002; Damesin et al. 2002). Similarly, the analysis of 136  $Q_{10}$  values from stem respiration showed a mean value of 2.18, with 42% of the values in the range from 1.5 to 2.0 and (Wang, Wang, Zu, Li & Takayoshi 2006).

As reported in other studies, the stem  $CO_2$  efflux at an air temperature of 15°C,  $ES_{15}$ , was sensitive to wood formation processes, showing an increase during the growing period (Ceschia *et al.* 2002; Damesin *et al.* 2002).

Maximum xylem production and maximum ES were not synchronized and an almost constant delay, of about one month, was observed, as similarly found in a young beech forest in which peak ES occurred *c.* 27 days after the maximum stem growth rate (Ceschia *et al.* 2002). Similarly, in an Australian stand dominated by *Eucalyptus tereticornis* Sm. a time-lag of 43 days was also observed (Salomón, Steppe, Crous, Noh & Ellsworth 2019). Furthermore, our results confirmed that the peak of ES occurred when xylem cells were still in the phase of wall thickening and lignification, as previously hypothesized (Ceschia *et al.* 2002). Moreover, when maximum ES was observed, it is very likely that trees were already refilling the stem C reserves pool (Scartazza, Moscatello, Matteucci, Battistelli & Brugnoli 2013).

### ***Only spring frost affects negatively stem C fluxes***

The amount of C fixed by stem biomass formation in 2015 and 2017 were 1.65 and 1.49 Mg C ha<sup>-1</sup> yr<sup>-1</sup>, lower than the values reported for a beech forest growing in plain in Germany that ranged between 1.69 to 2.41 Mg C ha<sup>-1</sup> yr<sup>-1</sup> (Mund *et al.* 2010). In 2016, however, we measured only 0.28 ± 0.07 Mg C ha<sup>-1</sup> yr<sup>-1</sup>, i.e., only about 20% of the fixation during the two other years, emphasizing how exceptionally negative this year was.

Annual stem CO<sub>2</sub> efflux (AES) is known to be highly variable in temperate forests (Yang *et al.* 2016). Our data ranges from 0.94 to 1.34 Mg C ha<sup>-1</sup> yr<sup>-1</sup> and thus is lower than 1.65 to 2.25 Mg C ha<sup>-1</sup> yr<sup>-1</sup> reported for a younger beech forest (Damesin *et al.* 2002). An earlier estimate of AES at the study site was 0.63 Mg C ha<sup>-1</sup> yr<sup>-1</sup> (Guidolotti *et al.* 2013) for 2007, year characterized by an extreme summer drought (SPEI < -2). The stem C efflux of the drought year presented in this study (2017, 1.23 Mg C ha<sup>-1</sup> yr<sup>-1</sup>) was about the double than 2007, which could be due to an increase in stem biomass (*c.* 15% lower in 2007 than in 2017), to different measurement tools and to the stronger drought. The contribution of AES<sub>g</sub> to annual stem effluxes, ranging from 7% to 19%, was lower than that measured in a young beech forest (Ceschia *et al.*, 2002), evidencing the importance of forest developmental stage in determining wood formation, growth respiration and their role in stand carbon balance.

The construction cost we found of 0.23 g C emitted per g C fixed is consistent with the lower range of values reported for beech from 0.2 to 0.4 g C fixed per g C emitted (Ceschia *et al.* 2002; Damesin *et al.* 2002). A large range of construction cost, from 0.16 to 0.87 g C emitted per g C fixed, were reported in Rodríguez-Calcerrada *et al.* (2019). The dataset includes different method for estimating construction costs, and their variability underlines how analytical methods affect the estimation of the respiratory component (Rodríguez-Calcerrada *et al.* 2019).

The late frost event in 2016 reduced both wood growth and stem CO<sub>2</sub> efflux with respect to those measured in the other two years, but the percentage of growth reduction (80%) was much larger than the reduction of ES (25%). In this year, the strong reduction of fixed C, and the contemporary

lower reduction of stem CO<sub>2</sub> efflux, strongly affected the overall stem carbon balance. Results suggest that maintenance metabolism and respiration is mandatory for tree survival, even tapping on different C source (e.g. old C reserves). Moreover, the ability of modifying the sink hierarchy, reducing allocation to radial growth, allowed trees to face such stressful event. In contrast, the summer drought in 2017 did not have any effect on stem growth, and thus neither on CO<sub>2</sub> efflux due to growth respiration. Indeed, the summer drought became severe only in August when beech trees already completed the enlargement phase and most of the wall thickening/lignification, and ultimately, causing a negligible reduction of associated respiratory cost.

In conclusion, this study further highlights the sensitivity of European beech to leaf damage due to late spring frost. Since leaf development is expected to start earlier for beeches (and not only) due to global warming, the likelihood that spring frost may damage leaves will increase, as it is already occurring in Europe. We demonstrated that stem growth was significantly reduced due to the prolonged absence of photosynthesizing leaves after frost, since beech trees tapped their pool of old C reserves. However, the loss in growth was not completely compensated for after re-growth of leaves, but rather the cambium activity ceased shortly thereafter. Consequently, the trees fixed less C in the stem biomass, showing also a reduction of the stem carbon efflux related to growth respiration. Moreover, the summer drought occurred too late to affect wood formation and stem CO<sub>2</sub> effluxes. However, more investigations are needed to evaluate its effects on other physiological processes. This study also underlines the crucial role of spring weather conditions on the growth and physiology of beech trees. A better understanding of fine scale C dynamic will help in evaluating a medium- to long-term response to climate change under an increasing frequency of extreme events.

## Conflict of Interest statement

All co- authors declare no potential sources of conflict of interest.

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## Tables

**Table 1: List of terms used in the text**

Terms	Definition	Spatial Scale
ES	stem CO <sub>2</sub> efflux per surface area ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	Local
ES <sub>15w</sub>	specific CO <sub>2</sub> efflux at an air temperature of 15°C during the wood formation period ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	Local
ES <sub>15m</sub>	specific CO <sub>2</sub> efflux at an air temperature of 15°C during the non-wood formation period ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	Local
Q <sub>10mg</sub>	ES temperature sensitivity for a 10 °C increase during the wood formation period	Local
Q <sub>10m</sub>	ES temperature sensitivity for a 10 °C increase during the non-wood formation period	Local
ES <sub>mg</sub>	stem CO <sub>2</sub> efflux per surface area ( $\mu\text{mol m}^{-2}$ $\text{s}^{-1}$ ) during wood formation	Local
ES <sub>m</sub>	stem CO <sub>2</sub> efflux per surface area ( $\mu\text{mol m}^{-2}$ $\text{s}^{-1}$ ) due to maintenance respiration	Local
ES <sub>g</sub>	stem CO <sub>2</sub> efflux per surface area ( $\mu\text{mol m}^{-2}$ $\text{s}^{-1}$ ) due to growth respiration	Local

TES <sub>mg</sub>	annual stem C efflux (g C yr <sup>-1</sup> )	Tree
TES <sub>m</sub>	annual stem C efflux due to maintenance respiration (g C yr <sup>-1</sup> )	Tree
TES <sub>g</sub>	annual stem C efflux due to growth respiration (g C yr <sup>-1</sup> )	Tree
AES <sub>mg</sub>	annual stem C efflux (Mg C ha <sup>-1</sup> yr <sup>-1</sup> )	Stand
AES <sub>m</sub>	annual stem C efflux due to maintenance respiration (Mg C ha <sup>-1</sup> yr <sup>-1</sup> )	Stand
AES <sub>g</sub>	annual stem C efflux due to growth respiration (Mg C ha <sup>-1</sup> yr <sup>-1</sup> )	Stand
SG	annual C fixed in stem biomass (Mg C ha <sup>-1</sup> yr <sup>-1</sup> )	Stand

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**Table 2: Parameters describing the intra annual radial growth derived from the Gompertz function for the total xylem increment comprised of enlarging, wall thickening and mature cells.  $\alpha$  ( $\mu\text{m}$ ) is the upper asymptote, representing the final ring width at the end of the growing season; tx (DOY) is the day of the year at which the daily increment is maximum (Gompertz curve inflection point); rx is the maximum daily increment ( $\mu\text{m day}^{-1}$ ). Different letters represent significant differences among the monitored years.**

Total xylem increment				
Parameter	Year	Mean ( $\pm$ S.E.)	F	<i>p</i> -value
$\alpha$	2015	1312 ( $\pm$ 196) a		
$\alpha$	2016	230 ( $\pm$ 31) b	13.722	< 0.01
$\alpha$	2017	1235 ( $\pm$ 269) a		
rx	2015	26 ( $\pm$ 3) a		
rx	2016	6 ( $\pm$ 2) b	8.469	0.014
rx	2017	23 ( $\pm$ 4) a		
tx	2015	174 ( $\pm$ 2) a		
tx	2016	157 ( $\pm$ 6) b	22.667	< 0.001
tx	2017	175 ( $\pm$ 1) a		

**Table 3: Annual C stem fluxes. AESgm is the annual stem C efflux assessed using specific parameters for wood formation ( $Q_{10w}$  and  $ES_{15w}$ ) and non-wood formation phases ( $Q_{10nw}$  and  $ES_{15nw}$ ); AESm is the annual stem C efflux due to maintenance respiration; AESg is the annual stem C efflux due to growth respiration; SG is the annual amount of C fixed in the stem biomass; different letters represent significant differences  $p$ -value  $< 0.05$**

Year	Flux type	Mean $\pm$ S.E. Mg C ha <sup>-1</sup> y <sup>-1</sup>	F	$p$ -value
2015	AESgm	1.34 $\pm$ 0.12 a		
2016	AESgm	0.94 $\pm$ 0.11b	13.808	0.003
2017	AESgm	1.23 $\pm$ 0.15a		
2015	AESm	1.09 $\pm$ 0.07a		
2016	AESm	0.87 $\pm$ 0.10 b	8.015	0.012
2017	AESm	1.03 $\pm$ 0.12 a		
2015	AESg	0.24 $\pm$ 0.05 a		
2016	AESg	0.07 $\pm$ 0.05 b	7.634	0.014
2017	AESg	0.20 $\pm$ 0.04 a		
2015	SG	1.65 $\pm$ 0.20 a		
2016	SG	0.28 $\pm$ 0.07 b	18.639	0.001
2017	SG	1.49 $\pm$ 0.28 a		

## Figures

**Fig. 1:** Transverse section of a sample (15 July 2015) under polarized light microscopy: cambium (C), enlarging cells (E), wall thickening cells (W), mature cells (M).

**Fig. 2:** Selva Piana forest (Collelongo, Abruzzi region) photographed the 19 May 2016, 24 days after the late frost

**Fig. 3:** 3-month Standardized Precipitation Evapotranspiration Index (SPEI) at the experimental site of Selva Piana- Collelongo in 2015, 2016 and 2017.

**Fig. 4:** Annual trends of the number of cambium cells (N. cells, left Y axis) and leaf area index (LAI,  $\text{m}^2\text{m}^{-2}$ , right y-axis). Grey circles and dashed lines are the MODIS-LAI values and the modelled intra-annual dynamic at Selva Piana beech forest, respectively. Green and blue vertical lines represent the green-up and maturity phases of leaf phenology, respectively. The red vertical line represents the late frost of 25<sup>th</sup> April 2016. Black triangles are the average number of cambial cells of the five sampled beech trees. Bars represent  $\pm 1$  standard error.

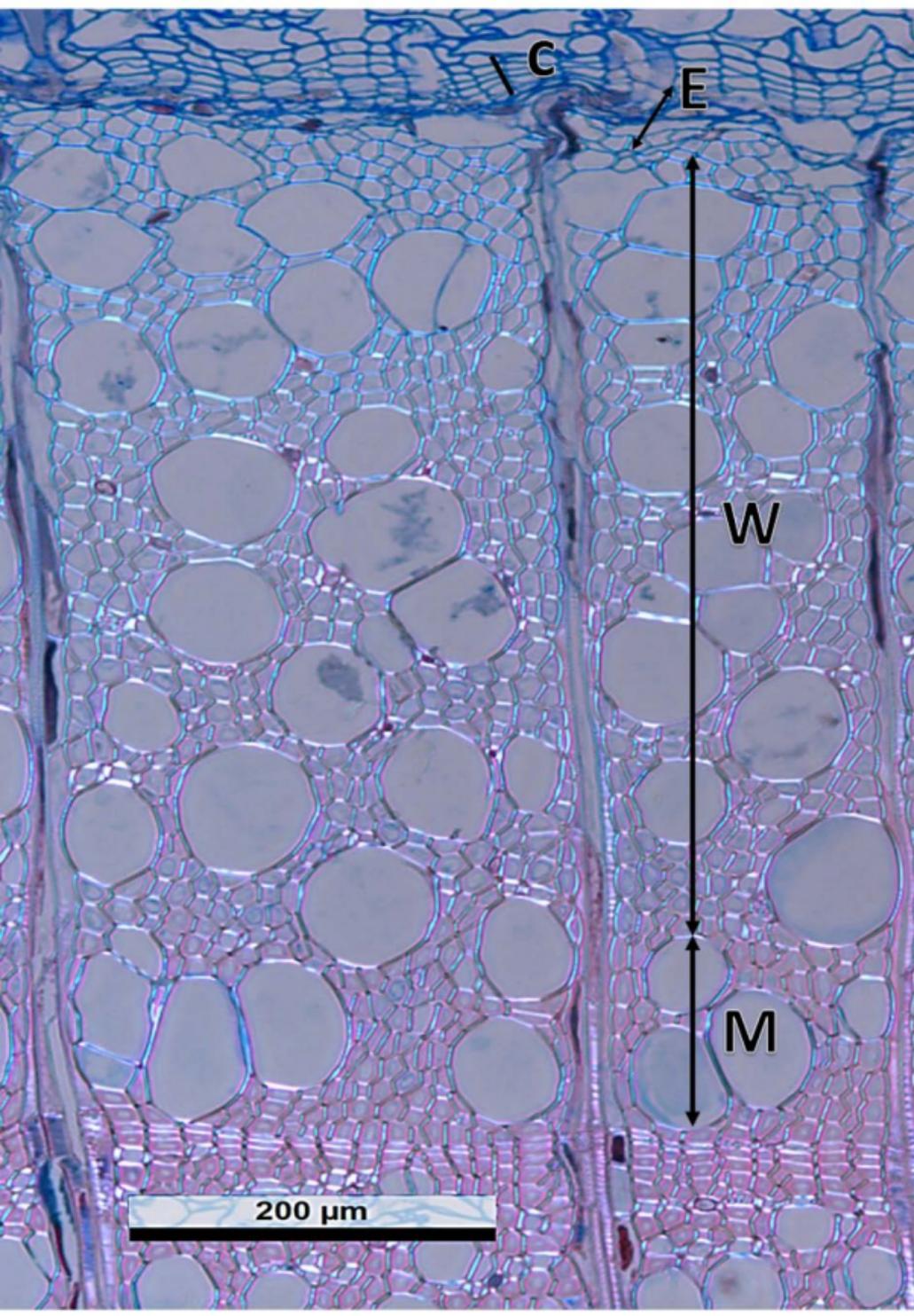
**Fig. 5:** Formation and phenology of the xylem. (a) Intra-annual dynamics of xylem formation ( $\mu\text{m}$ ) in 2015 (green dots and solid line), 2016 (blue dots and solid line) and 2017 (red dots and solid line). Gompertz functions were fitted to the total xylem increment comprised of enlarging, wall thickening and mature cells. Each point is the mean of 5 sampled *Fagus sylvatica* trees and bars are  $\pm 1$  standard error, \*\*\*  $p$ -value  $< 0.001$ . (b) Critical dates and duration of wood formation phases. Different letters represent significant differences among the beginning (dots) of the enlargement and the wall thickening phases, cessation (triangle) of the enlargement and the wall thickening phases ( $p$ -value  $< 0.05$ ). Solid and dashed rectangles represent the overall duration of the enlargement and wall thickening phases. Each value is the mean of 5 sampled trees per year and the bars error represent  $\pm 1$  standard error.

**Fig. 6: (a)  $T_{\text{air}}$  ( $^{\circ}\text{C}$ ) at the day of sampling in 2015 (green), 2016 (blue) and 2017 (red). (b) Measured stem  $\text{CO}_2$  effluxes ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) in 2015 (green), 2016 (blue) and 2017 (red). Each point is the mean of 5 *Fagus sylvatica* trees. Bars are  $\pm 1$  standard error.**

**Fig. 7: Relationship between ES ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) and air temperature ( $T_{\text{air}}$ ,  $^{\circ}\text{C}$ ). Annual, considering the whole measurements for each year, each point is the mean of five sampled trees. Each point represents the mean of those trees, which are in the non-wood and wood formation periods, at a given sampling date. Bars are  $\pm 1$  standard error. \*\*\*  $p$ -value  $< 0.001$ , \*\*  $p$ -value  $< 0.01$ , \*  $p$ -value  $< 0.05$ .**

**Fig. 8: Relationships between ring widths (mm) and the mean annual ES ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) measured in 2015 (green), 2016 (blue), and 2017 (red). Each point represents a sampled *Fagus sylvatica* tree per year. Bars represent  $\pm 1$  standard error. \*\*\*  $p$ -value  $< 0.001$ .**

**Fig. 9: Daily stem increment and stem Carbon effluxes of *Fagus sylvatica* at stand level. Top panel: the black line is the daily increment during 2015, 2016 and 2017, respectively. The red line represents ES, the daily C effluxes. Lower panel: contribution of maintenance respiration to daily stem  $\text{CO}_2$  efflux calculated as  $\text{ES}_m/\text{ES}_{\text{mg}}$ .**





SPEI

