



Differential response of oak and beech to late frost damage: an integrated analysis from organ to forest

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ABSTRACT

Intense spring freezing events can kill the recently produced cohort of leaves, forcing trees to expend additional carbon and nutrient stocks to produce a second cohort of leaves. The future trends in the frequency of late spring frosts will affect the adaptation of some tree species to their current habitats. Here we studied the effect of a late frost which occurred in 2017 on a mixed beech (*Fagus sylvatica*)-oak (*Quercus petraea*) forest located in central Spain, where these species reach their southernmost distribution limits. We followed a multi-scale approach from organ to forest levels. At the organ level, leaf and stem morphological and biochemical traits were compared between frost-damaged and non-damaged trees. At the tree level, we compared the 2017 radial growth between damaged and non-damaged trees. At the stand level, the 2017 Leaf Area Index (LAI) and daily variations of NDVI during 2017 were compared with those of average years in areas dominated by beech and oak. Finally, at the forest scale, daily NDVI dynamics during 2017 were compared with those of the three previous years. According to our results, beech trees damaged by late frost kept non-structural carbohydrate (NSC) concentrations stable by drastically reducing wood production. This growth reduction could compensate for the drop in carbon inputs due to the death of the first leaf cohort, the need to form a second leaf cohort, the one-month delay between both leaf flushes and the smaller photosynthetic surface of the second leaf cohort. In contrast, although the frost-damaged oaks lost the first cohort of leaves and formed a second one, no differences in leaf individual area and phenology, nor stem NSC concentrations and radial growth were found when comparing damaged and non-damaged oaks. The differential response between both tree species seems to provide oak with a competitive advantage over beech.

1. Introduction

The ongoing climate change is negatively affecting forest health worldwide (Allen et al., 2010), and will likely alter species distribution over this century (Benito Garzón et al., 2011; Hernández et al., 2017). Climate models predict a rising trend of climate warming at a planetary scale; this trend will be accompanied by an increase in climate variability, including an increase in the occurrence and severity of climate extremes such as frosts (Meehl et al., 2007). Winter warming has been linked to an advanced leaf-out in some deciduous tree species (Menzel, 2000; Menzel et al., 2003; Vitasse et al., 2018). This can extend the growing season of winter-deciduous species, but can also increase the

chances of spring freezing events (Gazol et al., 2019; Richardson et al., 2018). While adaptation of winter-deciduous tree species to freezing temperatures has coupled leaf-out to minimum temperatures (Lenz et al., 2013; Vitasse et al., 2018), it is relatively common that expanding leaves and flowers get damaged by spring freezing events (Augspurger, 2013, 2009; Hufkens et al., 2012). Since the pioneering study of Cannell (1985) analyzing the risks of tree frost damage in Britain, there has been no consensus on whether climate change will decrease (Dai et al., 2013; Hänninen, 2016; Menzel et al., 2003; Morin and Chuine, 2014; Scheffinger et al., 2003) or increase (Abbas et al., 2017; Augspurger, 2013; Bigler and Bugmann, 2018; Gu et al., 2008; Hänninen, 2016; Inouye, 2008; Kramer, 1994; Vitasse et al., 2014b) the risk of late frost damage.

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Leaf damage by spring freezing has a penalty to the tree. If the intensity and/or duration of the freezing event are moderate and do not kill the leaves, their morphology, physiology and chemical composition will be altered during most or all of their life span (Klosson and Krause, 1981a, 1981b; Sakai and Larcher, 1987). However, intense and/or long freezing events can kill the recently produced cohort of leaves, forcing the trees to flush a second cohort of leaves. Because the initial growth of spring shoots in winter-deciduous trees is sustained by carbon reserves stored in woody tissues (Hoch et al., 2003), trees have to re-mobilize a new supply of non-structural carbohydrates (NSC) and mineral nutrients to produce this new cohort. Frost impact on the NSC pools will depend on the amount of new leaves produced, their capacity to assimilate CO₂, and the extent to which carbon sinks (e.g., meristems) are down-regulated or carbon-limited after the freezing event.

Studies on temperate forests have reported abrupt declines in leaf area index (LAI) the year on which spring frost occurs, relative to a year with no leaf affection by freezing (Awaya et al., 2009). Because of this, the sapwood area potentially supplying water to a given leaf area (i.e. the Huber value; Huber, 1928) can increase relative to years with no freezing damage or relative to non-damaged trees. If the fewer new leaves benefit from having a delayed senescence (Zohner et al., 2018), higher nitrogen concentration, higher water supply, and thus higher rates of photosynthesis (Hoogesteger and Karlsson, 1992; Reich et al., 1999), the impact of reduced LAI on carbon gain at the tree or stand scales can be partly compensated. Wood production will also be likely affected by spring frosts (Hufkens et al., 2012). Altered hormonal signaling caused by reduced foliage and/or reduced carbon availability caused by the extra use of NSCs on resprouting can reduce stem growth (Buttò et al., 2020). Besides ring width, wood density can decrease after freezing damage to leaves, affecting the water storage capacity of stem sapwood (Vannoppen et al., 2018). There is therefore a coupling between carbon storage (e.g. NSC pools), carbon sources (e.g. leaf area), hydraulic conductivity (e.g. sapwood water conducting area) and carbon sinks (e.g. radial growth) that can differently extend to tree, stand and forest scales depending on species frost resilience.

The impact of leaf freezing on tree physiology scales-up to ecosystem processes, sometimes leading to a reduction in gross and net primary production (Awaya et al., 2009; Hufkens et al., 2012), due mainly to canopy recovery processes that can take several months (Nolè et al., 2018). Spectral indices derived from satellite images provide useful information on the consequences of spring freezing events on ecosystem functional traits, such as the reduction of canopy leaf area and primary productivity, or the duration of the freezing stress effects (Bascietto et al., 2018; Greco et al., 2018; Nolè et al., 2018). There is a need to combine information obtained at different spatial scales to study the impact of frost and the post-frost resilience at the organ, tree, stand, and forest scales. Despite its potential to understand the impact of freezing on forest stands, few studies have integrated information at different scales.

European beech (*Fagus sylvatica* L.) and sessile oak (*Quercus petraea* [Matt.] Liebl.) are co-occurring and widespread temperate tree species in central Europe. Since the last glaciation, European beech has expanded over Spain, often replacing sessile oak forests, a process that continues today (Gómez et al., 2019; Perea et al., 2020; Sánchez de Dios et al., 2016). Both species reach their southernmost limits of distribution at the studied forest (“El Hayedo de Montejo”, central Spain) (Pardo et al., 2004). This forest has a continental Mediterranean climate, with relatively frequent years in which leaves of a considerable number of trees are visibly damaged by late frosts (hereafter spring frosts; four in the last 25 years). If spring frost frequency and, thus, tree damage increases due to climate change, the vigor of these beech-oak populations may be compromised. An extraordinarily warm winter to early-spring period in 2017 caused the premature leaf out of these species in the “El Hayedo de Montejo” forest. A spring frost occurring over two consecutive nights in late April 2017, reaching -4.6°C, was enough to scorch the developing shoots of many beech and sessile oak trees (Figs. 1

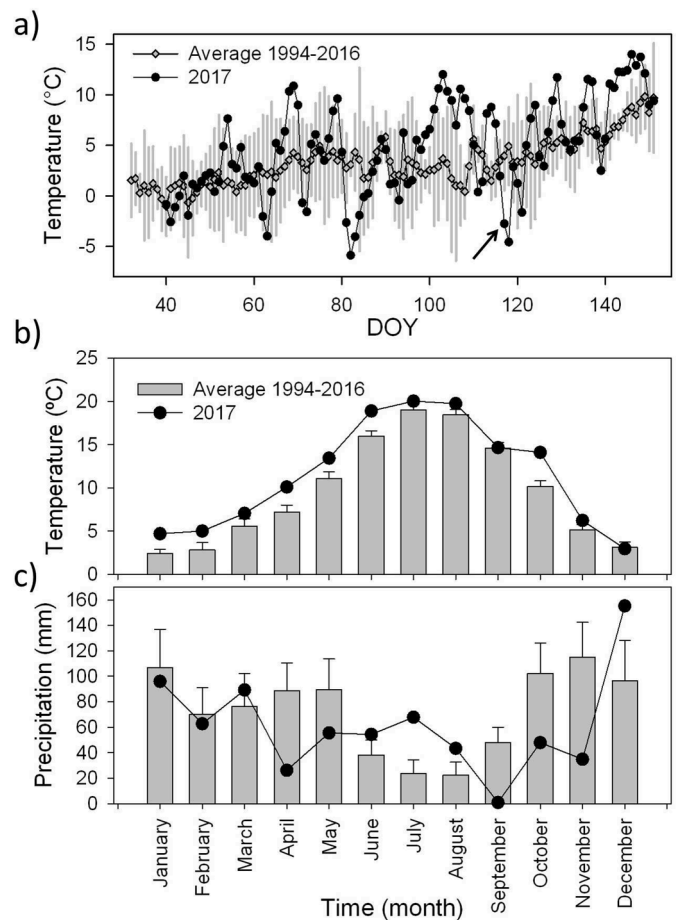


Fig. 1. Daily minimum air temperatures from February to May (a), average monthly temperatures (b) and total monthly precipitations (c) in 2017 compared with an average year for the 1994-2016 period. Vertical gray lines (a) and error bars (b and c) indicate 95% confidence interval. The arrow in the top panel points to the first day of the late frost. DOY stands for Julian day of the year.

and 2); in our research, we followed a multiscale approach to study its effects from organ to forest levels (Table 1). At the organ level, functional traits related with tree water transport and carbon balance were compared between trees damaged and non-damaged by the frost. At the tree level, we compared the 2017 radial growth between these two groups of trees. At the stand level, we analyzed the spring frost impact on LAI and Normalized Difference Vegetation Index (NDVI), distinguishing in both analyses between areas dominated by beech and oak. At the forest scale, the frost effect on NDVI was studied. We hypothesized that (i) the frost negative impact on carbon stocks, due to leaf scorching and the NSC expense to produce a second leaf cohort, would be buffered as a consequence of a decline in radial growth; (ii) LAI would be lower in 2017, due to a lower tree crown density and leaf size of the second leaf cohort and that, for these reasons, (iii) NDVI of 2017 would reach a lower and delayed maximum with respect to an average year.

2. Material and methods

2.1. Study site

The study site, “El Hayedo de Montejo”, is a mixed forest of 125 ha located at “Sistema Central” mountain range, central Spain (41° 07' N; 3° 30' W), between 1,250-1,500 m a.s.l. The dominant species are *Quercus pyrenaica* Willd., *Fagus sylvatica* L. (hereafter beech) and *Quercus petraea* (Matt.) Liebl. (hereafter oak), with relative basal areas of 50%,



Fig. 2. Damages produced by a late frost in beeches and oaks in the study area (“El Hayedo de Montejo”) located in central Spain. Inset shows scorched leaves in a beech tree.

27% and 23%, respectively. This study focused on beech and oak; it did not include *Quercus pyrenaica* because its late phenology prevented the occurrence of extensive damages in trees of this species. During centuries of firewood and cattle grazing use, the forest had an open woodland structure of dispersed old and large trees, and scarce tree recruitment (Pardo and Gil, 2005). However, cattle grazing was forbidden in 1961 and the last logging took place in 1962 (Gil et al., 2009; López Santalla et al., 2003), which has favored the increase of tree density in the last decades.

Climate data were obtained from a meteorological station located in the study forest and managed by the “Natural Systems and Forest History” research group of “Universidad Politécnica de Madrid”, Spain. The climate is continental subMediterranean, with 900 mm of mean annual rainfall and a prolonged dry period during July and August. The mean annual temperature is 9.5 °C and temperatures below 0° C can occur from November to February. In the last 25 years there were spring frosts damaging the trees in 1995, 2010, 2013 and 2017. The soil has been classified as humic cambisol (Pardo et al., 1997) and the horizon A reaches 50 cm of depth on average, enabling relatively high water storage during dry periods.

2.2. Frost event

In April 2017, few days after leaf-out in both species, there was an abrupt fall in temperature during two consecutive nights; between April 26 and 28, temperatures reached a negative peak of -4.6°C (Fig. 1a), killing the leaves of trees with advanced phenology (Fig. 2). Most of the trees were damaged by the spring frost (see more details in the section 2.5 Tree level). Only the trees or branches that had not sprouted yet, showed no frost damage.

2.3. Bud and leaf phenology

The overall leaf phenology of the forest was monitored weekly by the same observer during the spring of 2017, distinguishing between trees damaged and non-damaged by the frost. The number of trees on which phenology was measured after the spring frost was variable, with a minimum of 10 trees by measuring date (5 damaged and 5 non-damaged) for each species. The pre-frost phenology, i.e., the date when peaked the bud opening of the first cohort of leaves in 2017, was obtained from general observations of the beech stands on routine

weekly observations during the first months of 2017. It should be noted that those trees that had not sprouted when the frost occur delayed their bud opening as consequence of this frost. Four phenophases were considered: closed bud, bud opening and leaf elongation, extended leaves, and complete development of the leaves (expanded and hardened) following Millerón et al. (2012). A tree was included in a phenophase when more than 50% of its crown belonged to the same phase, and a given phenophase was assigned to the forest when more than 50% of the trees were in that phase. These data were compared with the phenology of a typical year characterized by a detailed inventory of data performed weekly in ten beech trees and eight oak individuals during six consecutive vegetative periods from 2006 to 2011 (Millerón et al., 2012).

2.4. Organ level

To estimate the frost effect on morphological and biochemical traits, we sampled the main-stem xylem, annual twigs and leaves in 10 adult beeches and 10 adult oaks (Table 1). Half of these trees (5 beeches and 5 oaks) had not been damaged by the spring frost (0% of the crown damaged) and the other half had been severely damaged (>90% of the crown damaged). The trees were selected from representative locations in the forest in terms of slope, orientation and basal area, which is 28.8 m² ha⁻¹ on average. The beech and oak trees sampled were located in homogeneous areas inside beech- and oak-dominated stands within a circular surface of 100 and 150 meters in diameter, respectively.

Stem xylem was sampled at the base of the trees using a Pressler increment borer at the following dates: May 8th, ten days after the frost event; June 1st, when non-damaged beech trees finished spreading the leaves; June 23rd, when damaged beech trees finished spreading the leaves of the new, second leaf cohort; July 24th, in the middle of the vegetative period; September 28th, near the end of the vegetative period but without trees showing yellowing leaves yet; December 22nd, during the dormant period. We extracted one 3-cm-long core per tree and date. After the extraction, bark and phloem were removed from the stem segments; the stem xylem was immediately placed in a Styrofoam box with ice to transport the samples to the laboratory, where they were stored at -80°C.

We collected two annual twigs with leaves per tree with a 6-meter pole on September 28th. Due to the height of the trees, we could only sample twigs and leaves in 6 beech and 6 oak trees (4 damaged and 2

Table 1
Summary of the multiscale analysis performed.

Multiscale analysis				
Level	Organ	Tree	Stand	Forest
Aim	Study frost effect on morphological and biochemical traits	Study frost effect on tree growth and growth phenology over time	Study frost effect on tree canopy	Study frost effect on forest canopy
Sampling	Main-stem xylem, annual twigs and leaves in 10 beech and 10 oak trees	Radial growth in 63 beech trees and 8 oak trees	– Ground-based LAI in 17 beech-dominated points and 18 oak-dominated points – NDVI dynamics in beech- and oak-dominated stands	Forest NDVI dynamics
Time scale	– A single sampling of annual twigs and leaves in 2017 – 6 samplings of stem xylem over 2017 – 6 samplings of stem xylem over 2017	8 measurements per tree and year over 2017 and 2018	– A single sampling of LAI in 2015 and 2017 – NDVI was analyzed between 2014 and 2017 with daily resolution	NDVI was analyzed between 2014 and 2017 with daily resolution
Statistical analyses	Mann-Whitney <i>U</i> tests comparing damaged and non-damaged trees	– Growth modeling by fitting a sigmoid function to 2017 growth for each tree – Mann-Whitney <i>U</i> tests comparing damaged and non-damaged trees	– Mann-Whitney <i>U</i> tests comparing LAI values in 2015 and 2017 for each species – Kolmogorov-Smirnov test comparing average NDVI annual series for the 2014-2016 period and 2017 NDVI series for each species	Kolmogorov-Smirnov test comparing average NDVI annual series for the 2014-2016 period and 2017 NDVI series for the whole forest

non-damaged by the frost in both species). These samples were also preserved in the Styrofoam box with ice for transportation. In the laboratory, fresh leaves were scanned (Epson Expression 10000XL, Suwa, Japan) to measure leaf area, using the software WinFOLIA (Regent Instruments Inc., Canada). The base of the twig was debarked and measured with a precision electronic caliper to obtain the sapwood section and calculate the Huber value (i.e. ratio between sapwood area and distal leaf area; cf. Huber, 1928). The length of the apical bud was measured with an electronic caliper. Finally, the leaves were dried at 60°C during 72 h to obtain the dry weight, using a precision balance (OHAUS AR 2140, Pine Brook, New Jersey, USA), and the leaf mass per area (LMA; the ratio between dry weight and leaf area).

The chemical analyses of nitrogen (N) and non-structural carbohydrate (NSC) concentrations on stem sapwood and leaf samples were

performed following Palacio et al. (2012). First, all samples were dried at 60°C for 72 h and were milled to a fine powder in a ball mill (Retsch Mixer MM301, Leeds, UK). Total carbon (C) and N mass-based concentrations were analyzed in leaves with an elemental analyzer (Elementar VarioMAX N/CM, Hanau, Germany). Soluble sugars (SS) were extracted with 80% (v/v) ethanol and their concentration determined colorimetrically, using the phenol-sulphuric method of Dubois et al. (1956) as modified by Buysse and Merckx (1993). Starch and complex sugars remaining in the undissolved pellet after ethanol extractions were enzymatically reduced to glucose. NSCs measured after ethanol extraction are referred to as SS, whereas NSCs measured after enzymatic digestion are referred to as starch. Both are expressed in glucose equivalents. The sum of SS and starch is referred to as total NSCs.

The differences between groups (trees damaged vs. non-damaged by the frost) for organ-level variables were analyzed for each species with Mann-Whitney *U* tests (Table 1). In the twig variables the data were previously averaged per tree to avoid pseudo-replication error (Hurlbert, 1984). We calculated the relative differences between groups for twig and leaf traits (Eq. 1):

$$\text{Relative difference} = |(D - ND) / ((D + ND) / 2)| \quad (1)$$

where *D* and *ND* are the average values for damaged and non-damaged trees, respectively.

We also analyzed the temporal variation of the stem xylem traits, through the rate of change (Eq. 2):

$$RC_t = (S_t - S_{t-1}) / S_{t-1} \quad (2)$$

where *RC_t* is the rate of change at time *t* and *S_t* and *S_{t-1}* are a stem xylem variable at time *t* and *t-1*, respectively.

2.5. Tree level

Since 2015 there are dendrometer bands (DB 20, EMS Brno) placed on 71 beech trees and 8 oak trees in the study forest (Table 1). The dendrometers were placed at 1.3 m directly over bark on beech trees. In the oaks, the outermost dead bark was previously smoothed and removed to avoid stem deformities. These 79 trees were classified according to the frost damage suffered in 2017: <25%, 25-50% and >50% of the crown damaged. Measurements to the nearest 0.1 mm were acquired at variable time intervals during the year depending on the growth rate (average time interval during the growing season was 22 days). All measurements were acquired in the morning to avoid diurnal bias caused by stem shrinkage from transpiration (Sheil, 2003). Girth increment data were transformed to radial increments assuming a cylindrical tree shape. The basal area increments (BAI) and the basal area (BA, i.e. accumulated BAI) were calculated for each tree as:

$$BAI_t = \pi(r_t^2 - r_{t-1}^2) \quad (3)$$

$$BA_{yr} = \sum_{i=0}^n BAI_i \quad (4)$$

where *BAI_t* is the basal area increment at time *t*, *r_t* and *r_{t-1}* are the stem radius at times *t* and *t-1*, respectively, *BA_{yr}* is the basal area in the year *y* at time *t*, and *n* is the number of basal area increments measured in the year *y* until the time *t*.

For this study we used growth data of 63 beech trees, 12 of them having <25% of the crown damaged (hereafter non-damaged) and 51 having >50% of the crown damaged (hereafter damaged), leaving out those trees with intermediate crown damage (25-50%). Given the low number of monitored oaks (8), these were grouped in non-damaged (<50% of the crown damaged in this case; 2 trees) and damaged (>50% of the crown damaged; 6 trees). Based on these data, 72% and 80% of our sample of beech and oak, respectively, showed more than 50% of the crown damaged by the frost.

We modeled the growth of each tree over 2017 to estimate the day of year (DOY) when radial growth starts and ends (DOY_{beginning} and DOY_{end}, respectively), the length of the active growth period (DOY_{end} - DOY_{beginning}) and the time at which the maximum growth rate occurs. A sigmoid function was fitted to the 2017 growth of each tree by nonlinear least squares using the *nls* function in R statistical software (R Development Core Team, 2017):

$$BAS = a / (1 + e^{-b*(DOY-c)}) \tag{5}$$

where *BAS* is the standardized basal area according to the Eq. (6), with values from 0 to 1, and *a*, *b*, *c* are the model parameters, different for each tree, being *c* the DOY with maximum growth rate.

$$BAS_{it} = (BA_{it} - \min(BA_{it})) / \max(BA_{it} - \min(BA_{it})) \tag{6}$$

where *BAS_{it}* and *BA_{it}* are the standardized basal area and the basal area, respectively, for tree *i* at time *t*. Because the model estimated the start and end of growth asymptotically, we defined the start and the end of the active growth period as the day when *BAS* was 10% and 90% of the annual maximum (Moore et al., 2006).

We tested the differences in BAI and growth-related phenological variables between damaged and non-damaged trees with Mann-Whitney *U* tests.

2.6. Stand and forest level

To study the frost effects at the stand level (Table 1), we compared Leaf Area Index (LAI) values measured in July 2015 (a year without freezing damage) and in July 2017, when the leaf development is maximum. LAI values in 2015 were retrieved from a field inventory with ForeStereo, a device that acquires stereoscopic hemispherical image pairs and enables estimation of basal area among other variables (Rodríguez-García et al., 2014; Sánchez-González et al., 2016). Ground-based LAI can be obtained from the hemispheric images through the Poisson gap frequency model with slope correction (Montes et al., 2007). In 2015, a total of 125 inventory points were measured on the nodes of a systematic 100 m × 100 m grid covering the entire forest area. For our current analysis, we selected points where one of the target tree species (beech and oak) dominates (>50% of the relative basal area): 17 beech-dominated points and 18 oak-dominated points. In 2017, the ForeStereo inventory was repeated in those points. Differences between LAI values in 2015 and 2017 were analyzed for each species with Mann-Whitney *U* tests.

Remotely sensed data acquired daily by the Moderate Resolution Imaging Spectroradiometer (MODIS) sensor and at 8-16 days frequency by Landsat sensors (Operational Land Imager—OLI, and Enhanced Thematic Mapper Plus—ETM+) were fused in accordance with Gao et al. (2015, 2006) (Fig. 3), obtaining synthetic daily data with 30 m of spatial resolution between 6th of March 2014 and 12th of January 2018 (see Appendix A of the Supplementary material). Areas dominated by beech or oak were defined as irregular polygons considering a recent

land cover cartography (Gómez et al., 2019) and assuring they encompassed beech or oak plots as mentioned above. Values of the entire forest NDVI series and those from the beech and oak polygons were extracted and analyzed with TIMESAT software (Eklundh and Jönsson, 2017). Savitsky-Golay, Gaussian, and double logistic functions (Eklundh and Jönsson, 2017) were fitted to NDVI series at the stand and entire forest scales. The start- and end-of-season parameters were determined based on seasonal amplitude, defined as the difference between the base and maximum NDVI values for each individual season. The start occurs when the left part of the fitted curve reaches 0.5 of the amplitude value, counted from the base level, and the end of season is defined similarly but for the right side of the curve. Parameters of the phenological season characterizing the start, end, and length of the vegetative period, as well as the annual maximum NDVI and time of maximum NDVI were obtained. We tested the differences between average NDVI annual series for the 2014-2016 period and 2017 NDVI series, both for each stand (dominated by beech or oak) and for the entire forest, using two-sample Kolmogorov-Smirnov test. We also tested the possible zonal affection of the frost, and analyzed the NDVI data by altitude, obtaining seasonality parameters for 8 altitudinal ranges.

3. Results

3.1. Bud and leaf phenology

In 2017, bud opening in part of the beech trees and oaks started approximately two weeks earlier than usual based on the 2006-2011 phenology (Fig. 4). Those trees were damaged by the freezing event, losing all open buds and leaves. The non-damaged beech trees sprouted three weeks later (during the second week of May) and the non-damaged oak trees five weeks later (during the last week of May) than damaged trees, considerably later than usual. The second cohort of leaves of the damaged beech and oak trees sprouted five weeks after the first cohort of leaves was damaged (during the last week of May). Thereafter, the phenology was similar in all oaks, damaged and non-damaged, whereas the damaged beech trees exhibited a delay in the phenophases when compared to those not damaged. For both species, the phenological phases occurred later than in an average year.

3.2. Organ level: leaves and stems

Due to the small number of twig and leaf samples collected, the confidence intervals of the statistical analyses were large (Table 2). Differences at *p* < 0.1 between damaged and non-damaged beech trees were detected in almost all leaf-related variables. Leaves of damaged beech trees were lighter, smaller and had lower LMA and NSC concentrations but higher N concentrations than leaves of non-damaged beech trees. Differences between damaged and non-damaged oaks were similar, but smaller and not significant. In this respect, the average relative difference between damaged and non-damaged oaks for leaf and twig traits was 0.15 compared to 0.30 for beeches (see Table S1 in

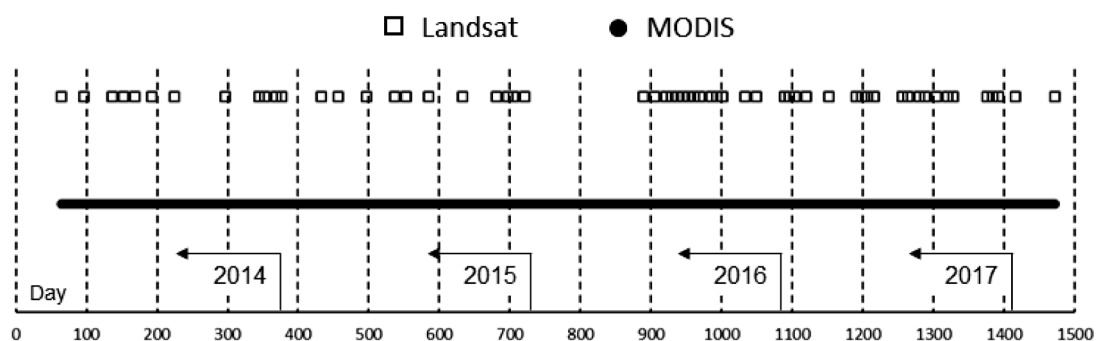


Fig. 3. Distribution of the time instants when the Landsat and MODIS images employed were acquired.

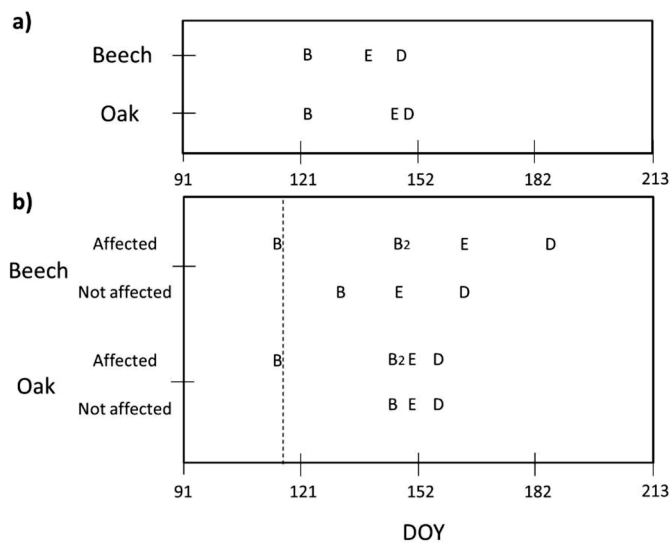


Fig. 4. Chronogram of average leaf emergence time in beech and oak (period 2006-2011) (a) compared with the late-frost year 2017 (b). Legend: B, bud opening and unfolding of the first leaf cohort; B₂, bud opening and unfolding of the second leaf cohort; E, extended leaves; D, complete leaf development. For 2017, data are given for damaged and non-damaged trees separately. The spring frost is highlighted with a dashed vertical line. The abbreviation “DOY” stands for Julian day of the year.

Table 2

Mean (and standard deviation) of leaf and twig functional traits for beech and oak measured at the end of the vegetative period of 2017. “•” indicates differences at $p < 0.1$ between trees damaged and non-damaged by the frost. LMA is leaf mass per area, SS is soluble sugars, NSC is non-structural carbohydrates, N is nitrogen and C is total carbon., expressed in % of dry mass.

Variable	Beech		Oak	
	Damaged	Non-damaged	Damaged	Non-damaged
Individual leaf dry weight (g)	0.05 (0.01) •	0.10 (0.01) •	0.33 (0.19)	0.50 (0.20)
Individual leaf area (cm ²)	13.22 (1.44) •	21.14 (0.95) •	45.40 (23.80)	71.10 (22.55)
LMA (g m ⁻²)	38.08 (4.09) •	45.86 (2.94) •	69.82 (7.99)	70.19 (6.19)
Dry leaf weight per twig (g)	0.27 (0.03) •	0.46 (0.02) •	2.04 (1.69)	2.64 (0.92)
Total leaf area per twig (cm ²)	70.52 (5.43) •	100.73 (2.28) •	289.22 (228.00)	372.25 (97.76)
Number of leaves per twig	5.35 (0.27)	4.77 (0.32)	5.95 (1.54)	5.28 (0.30)
Sapwood diameter of the annual stems (mm)	1.23 (0.19)	1.42 (0.05)	2.36 (0.75)	2.47 (1.01)
Huber value (10 ⁻⁴)	1.72 (0.47)	1.58 (0.09)	1.74 (0.51)	1.30 (0.71)
Average length of the apical buds (mm)	15.43 (1.69)	14.99 (0.28)	5.97 (0.72)	5.98 (0.25)
SS in leaves (%)	4.17 (0.55) •	6.06 (0.77) •	5.87 (1.11)	6.32 (0.30)
Starch in leaves (%)	2.88 (0.31) •	3.96 (0.54) •	5.76 (1.23)	6.35 (0.03)
NSC in leaves (%)	7.05 (0.84) •	10.02 (0.22) •	11.64 (2.22)	12.67 (0.27)
N in leaves (%)	2.40 (0.10) •	1.57 (0.02) •	1.95 (0.21)	2.07 (0.18)
C in leaves (%)	47.85 (0.69) •	48.70 (0.07) •	47.90 (0.34) •	46.84 (0.35) •
C/N ratio in leaves	19.95 (0.89) •	31.05 (0.36) •	24.83 (2.63)	22.74 (1.81)

Appendix B of the Supplementary material). Oak leaves only presented differences at $p < 0.1$ in C concentrations. With respect to stem xylem NSCs, only beech trees showed significant differences between damaged and non-damaged trees, and these were only found for SS concentrations at the beginning and at the end of the vegetative period (Fig. 5). On June 1st (DOY 152), when damaged beech trees were opening the buds and unfolding the leaves of the second leaf cohort and non-damaged beech trees were finishing spreading the leaves of the first leaf cohort, damaged trees exhibited higher SS concentration. This difference was caused by a decrease in SS concentration in non-damaged beeches, which was later compensated by a similar decrease in damaged trees (see rate of change values in Fig. 5). On the contrary, on September 28th (DOY 271), near the end of the vegetative period, damaged trees exhibited lower SS concentration than non-damaged trees. Differences in SS and NSC disappeared at the end of the year (on December 22nd, DOY 356), due to an increase in SS in damaged trees and a decrease in NSC in non-damaged trees.

3.3. Tree level: radial growth

Damaged and non-damaged beech trees showed highly significant differences in radial growth throughout the study period, except at the beginning of the vegetative period (Fig. 6). On the contrary, both groups of oaks only showed differences at $p < 0.1$, and these were found only at the beginning of the growing season. The 2017 frost also influenced the radial growth of beech in 2018 (Fig. S1 in Appendix C of the Supplementary material). In this year, the beech trees damaged by the 2017 frost had a significantly ($p < 0.001$) lower growth than those not damaged (9.0 vs. 14.9 cm² year⁻¹, respectively). On the contrary, oak growth in 2018 was not influenced by the 2017 frost ($p = 1$); the basal area increments of damaged and non-damaged oaks in 2018 were 5.6 and 6.2 cm² year⁻¹, respectively.

There were no significant differences between damaged and non-damaged trees in the estimated timing of maximum growth rate and growth end, nor in the length of the growth period of either species, but we found significant differences in the estimated growth start DOY for beech trees (Table 3). All phenological variables related with beech radial growth had higher variability in the group of trees suffering frost damage (Fig. S2 in Appendix C of the Supplementary material). In this regard, 15.7% of the damaged beech trees did not grow at all, 11.8% grew for a period equal to or less than one month, and 35% grew for a longer period than any of the beech trees not damaged by the frost. However, all non-damaged beech trees grew for more than one month. It must be noted that these estimates are potentially affected by the stem swelling and deflation associated to dendrometer data.

3.4. Stand and forest level

The LAI in beech-dominated areas was significantly ($p < 0.05$) lower in 2017 than in 2015 (3.67 with 0.93 of standard deviation vs. 4.52 ± 0.96; i.e. LAI decreased 18.7% by an effect of the late frost). In areas dominated by oak, LAI decreased 15.4%, although the p -value of the difference was 0.09 (3.29 ± 0.94 in 2017 and 3.83 ± 0.93 in 2015).

Gaussian curves were the best fitting function for the NDVI time series. When analysing areas dominated by beech or oak species, the start of the 2017 spectro-phenological season occurred later for both (DOY 152 for beech and DOY 154 for oak) as compared to previous years (DOY 131 and 125, respectively) (Fig. 7a and b). On the contrary, the end of the spectro-phenological season occurred later in 2017 (26 days later in areas dominated by beech and 23 days later in areas dominated by oak). Hence, the length of the spectro-phenological season (time period between sprouting and senescence of leaves as defined from NDVI values) was 171 days for areas dominated by beech and 182 days for areas dominated by oak. The NDVI peak occurred later in both species in 2017 than in previous years (DOY 234 and 238 compared with DOY 211 and 213 for beech and oak, respectively). Highly significant

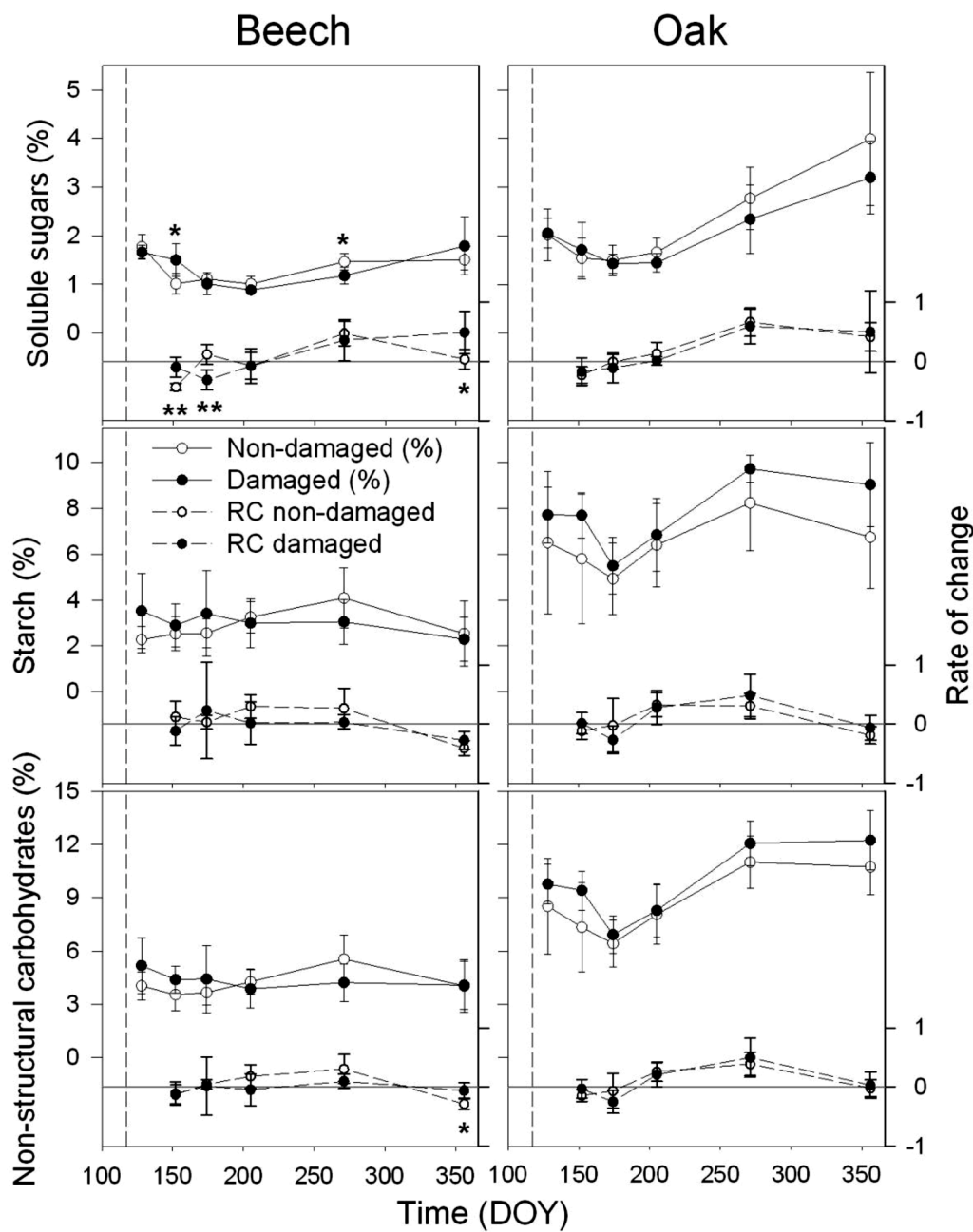


Fig. 5. Concentrations, as percentage of dry matter (solid lines; left y-axis), and rates of change (RC) of the concentrations (dashed lines; right y-axis) of soluble sugars, starch and non-structural carbohydrates in stem sapwood of beech and oak trees, either damaged or not damaged by a late frost, throughout 2017. Data are means (\pm standard deviation bars). Asterisks indicate significant differences (* $p < 0.05$, ** $p < 0.01$) for the same date. The spring frost is highlighted with dashed vertical lines. The abbreviation “DOY” stands for Julian day of the year. See Eq. (2) for definition of RC.

differences ($p < 0.001$) were found in both beech- and oak-dominated stands between the NDVI 2014-2016 average series and the NDVI 2017 series.

Highly significant differences ($p < 0.001$) were found between the NDVI 2014-2016 average series and the NDVI 2017 series for the entire forest. The length of the spectro-phenological season in 2017 was 154 days at the forest level (Fig. 7c), that is, considering jointly the areas dominated by beech and oak, as well as by the most abundant *Q. pyrenaica* (see section 2.1. Study site). At this level the average length of the spectro-phenological season in the period 2014-2016 was 172 days. In 2017, the season started on June 12th (DOY 163), 30 days later than the start averaged over the three previous years. The 2017 NDVI peak value, an indicative of maximum photosynthetic activity, occurred on August 20th (DOY 232), 23 days later than on an average year (DOY 209), and was lower than in previous years. The end of the spectro-phenological season was later in 2017 than in previous years (DOY 317, compared to DOY 305 for the period 2014-2016). The spring frost

impact on NDVI dynamics was similar for the whole forest, being negligible the effect of altitude (results not shown).

4. Discussion

4.1. Bud and leaf phenology: frost leaf scorching in early flushing trees delays senescence of new leaves

In beech, the sensitivity of bud bursting and leaf unfolding to temperature is mainly controlled by the photoperiod (Vitasse and Basler, 2013). This should make leaf phenology not particularly sensitive to warm winter conditions and prevent spring frost damage as compared with other deciduous tree species (Ma et al., 2019). Nevertheless, climate warming has advanced spring leaf unfolding of beech in Europe (Chen et al., 2018; Vitasse et al., 2018), particularly from the 1980s to 2000s; at different rates depending on site elevation (Kraus et al., 2016), local climate conditions or tree provenance (Chmura and Rozowski,

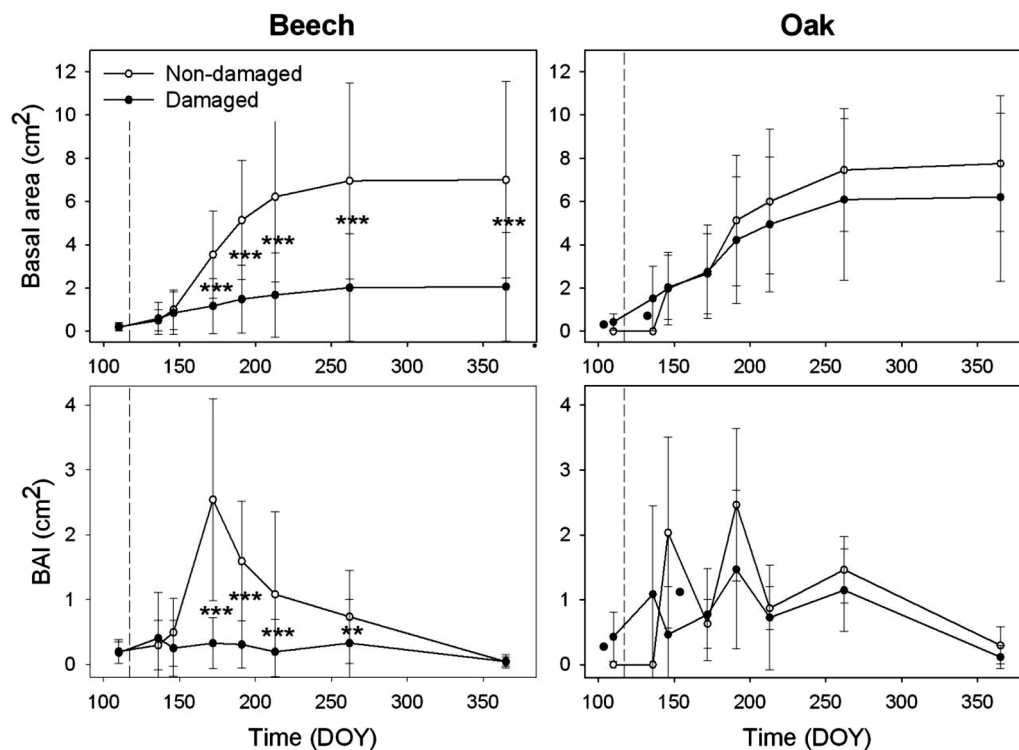


Fig. 6. Stem radial-growth dynamics in beech and oak trees, either damaged or not damaged by a spring frost, throughout 2017. Data are means (\pm standard deviation bars). Significance levels: \bullet $p < 0.1$, $**p < 0.01$ and $***p < 0.001$, respectively. The spring frost is highlighted with dashed vertical lines. BAI is basal area increment and DOY is Julian day of the year.

Table 3

Means (and standard deviation) of growth-related phenological variables in beech and oak trees, either damaged or non-damaged by a spring frost, throughout 2017. p -values indicating the significance of differences between damaged and non-damaged trees are shown. The abbreviation “DOY” stands for Julian day of the year.

	Variable	Damaged	Non-damaged	P
Beech	DOY beginning	127 (16)	143 (10)	< 0.001
	DOY end	214 (36)	205 (17)	0.217
	DOY of maximum growth rate	168 (19)	173 (7)	0.171
	Growth length (days)	66 (49)	62 (24)	0.685
Oak	DOY beginning	115 (30)	138 (7)	0.182
	DOY end	231 (18)	231 (15)	0.505
	DOY of maximum growth rate	174 (16)	184 (13)	0.317
	Growth length (days)	116 (41)	93 (8)	0.182

2002), reinforcing the controversy on the factors that govern leaf unfolding (Menzel et al., 2015; Zohner et al., 2016). In contrast, oak depends largely on temperature for the right timing of leaf-out (Vitasse et al., 2014a). The study oak is one of the European deciduous species with latest leaf unfolding (Vitasse et al., 2014a), which allows this species minimizing the risk of frost damage. In the study area, bud opening and leaf unfolding occurs on average at approximately the same time in both species, although leaves usually expand and develop more slowly in oak than in beech (Fig. 4a). In 2017, leaf phenology was advanced in part of the trees (Fig. 4b) due to the warm winter (Fig. 1b) and the high temperatures that were reached during the week previous to bud opening, with daily minima above 10°C (Fig. 1a). Trees less sensitive to warming, which took longer to break dormancy, avoided frost damage to their leaves. The non-damaged oak trees sprouted five weeks after the frost-damaged trees, while the non-damaged beech trees

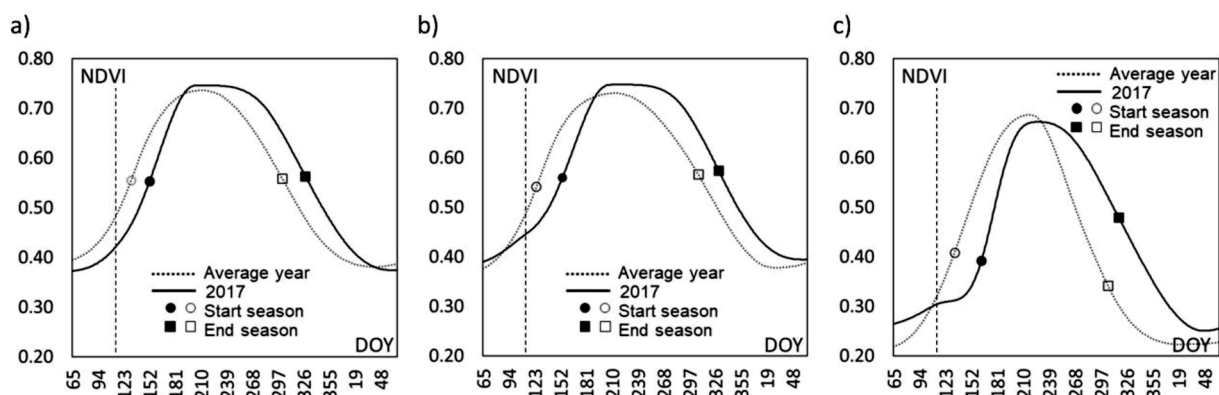


Fig. 7. Gaussian model of the NDVI time series of “El Hayedo de Montejo” in areas dominated by beech (a) and oak (b) and in the entire forest (c). Start and end of spectro-phenological season were determined as the DOY at which the fitted curves reach 0.5 of their amplitude value, counted from the base level of the left and right side of the curve, respectively. The spring frost is highlighted with dashed vertical lines. The abbreviation “DOY” stands for Julian day of the year.

sprouted three weeks later, possibly due to the greater importance of the photoperiod in beech trees.

The recovery of the crown in damaged trees differed between species. In an experimental study, Zohner et al. (2018) found that after a late frost event, oak leaves were developed more rapidly and reached their maximum development at the same time as the trees not subjected to the spring frost treatment, while beech trees damaged by frost had a slower leaf development compared to undamaged trees. Our results show that after the frost and subsequent sprout or re-sprout in non-damaged or damaged oak trees, respectively, leaf development (from bud opening to complete development) was completed faster than in an average year (Fig. 4). This rapid development partially compensated the delay in bud opening which affected oaks after the spring frost. On the contrary, in beech trees, the development of the different phenological phases was slower in 2017 than in an average year, something that is not observed in other studies (Awaya et al., 2009). In any case, NDVI data pointed out to a delay in leaf senescence in both species (Fig. 7), probably related to high autumn temperatures (Fig. 1b), which partially compensated for the reduction of the growing-season length caused by the spring frost. In addition to climatic factors, there may be other factors favoring delayed senescence. In this sense, recent studies suggest that autumn phenology is affected by the preceding spring development, with delays in leaf senescence compensating for late sprouts (Fu et al., 2014; Keenan and Richardson, 2015; Liu et al., 2016; Signarbieux et al., 2017; Zohner et al., 2018). Other studies, however, do not show a delay in leaf senescence in frost-damaged trees (Awaya et al., 2009).

The fact that early-sprouting trees were damaged by frost, while late sprouting trees were not, hinders to unequivocally attribute differences between trees to an effect of the spring frost or the tree phenology. However, since previous studies have found no relationship between leaf phenology and some of the traits studied here, such as radial tree growth (Čufar et al., 2015), we believe that the differences observed between damaged and non-damaged trees can be mainly attributed to the spring frost effects.

4.2. Organ level: frost did not induce a carbon limitation to stem growth

Second-cohort leaves produced by damaged beech trees had lower LMA, SS, starch and NSC concentrations than leaves of the non-damaged beech trees (Table 2), in line with other studies of broadleaf species damaged by a late frost (St. Clair et al., 2009). Thinner leaves would have lower rates of photosynthesis because they would have less photosynthetic machinery (pigment-protein complexes and enzymes) per leaf unit area (Rodríguez-Calcerrada et al., 2008). Lower photosynthetic carbon uptake by leaves of the second cohort could explain their lower NSC concentrations. On the other hand, the second cohort of leaves of beech trees had lower C/N ratio and higher N concentration than the leaves of non-damaged beech trees. Higher mass-based photosynthetic capacity, which is highly linked to N concentration (Hoogesteger and Karlsson, 1992; Reich et al., 1999), would allow re-sprouted leaves to compensate, at least partially, for their lower LMA and photosynthetic machinery per leaf area. This result would be in accordance with previous studies on similar species where the second cohort of leaves produced after insect defoliation was more resource acquisitive (Fuenzalida et al., 2019). In contrast, no significant differences (Table 2) and lower relative differences (Table S1 in Appendix B of the Supplementary material) in leaf traits were found between leaves of the damaged and non-damaged oaks, which would allow them to maintain similar rates of photosynthesis. In any case, our small sample size advocates for a cautious interpretation of these results.

The temporal dynamics of the concentrations of soluble sugars, starch and NSCs in stem sapwood for beech and oak throughout 2017 were very similar between damaged and non-damaged trees, and concurs with previous studies (Hoch et al., 2003). We found only significant differences of soluble sugars in two measurements in beech (Fig. 5). On

June 1st, stem xylem soluble sugar concentrations were higher in damaged trees, possibly due to the mobilization of soluble sugars to form the second cohort of leaves (Klein et al., 2016). This is consistent with the drop in the soluble sugars concentration (see rate of change in Fig. 5), which occurred after the formation of the second cohort of leaves was completed by the damaged beech trees (Fig. 4). An alternative explanation to this result is the lower consumption of soluble sugars in stem growth in damaged trees, which was reduced by the late frost already by June 1st (Fig. 6) (Deslauriers et al., 2009). On the contrary, on September 28th, non-damaged beech trees had higher soluble sugar concentrations than damaged trees. The lower leaf (Table 2) and stem xylem soluble sugar concentrations on this date in the frost-damaged beech trees could be related to a lower resource acquisition capacity of the new leaves. Despite these punctual, small differences in NSCs between beech trees, NSC reserves were rather similar along the year in both species (being practically identical at the end of the year), indicating no limitation on carbon availability for growth (Hoch et al., 2003; Körner, 2003). The decrease in NSC concentrations in non-damaged beech trees observed after the end of the vegetative period (see rate of change in Fig. 5) could be related to the increased respiration rates in those trees showing more wood formation (Rodríguez-Calcerrada et al., 2019). Thus the higher resource acquisition capacity of non-damaged beeches would be partly compensated by increased respiration rates.

4.3. Tree level: frost reduced radial growth

Oak begins to produce early wood prior to or at the same time that budburst using NSC reserves whereas beech growth starts forming wood later, just after budburst, and use mostly carbon assimilated by the leaves of the current year, with a maximal growth rate when the leaves are mature (Michelot et al., 2012; Puchařka et al., 2017). These different growth strategies could explain that NSC concentrations were more variable in oak than beech over time (Fig. 5), and the notable impact of frost on radial growth of beech (Fig. 6). The beginning of the radial growth in oak resulted in a drop in NSC concentrations, which was similar in damaged and non-damaged trees and showed a recovery during the growing season. The differences at $p < 0.1$ in radial growth between damaged and non-damaged oaks at the beginning of the growth period could be due to the earlier start of radial growth in damaged oaks, consistently with leaf phenology (Fig. 4), but this should be further checked using xylogenesis data. These differences, however, were later compensated when the growth of non-damaged oaks began. The clear reduction in radial growth in damaged beech trees suggest that carbon storage is an actively competing sink in beech trees and that, in case of defoliation, primary growth, crown recovery and carbon storage are favored over secondary carbon sinks as radial growth (Dietze et al., 2014; Miranda et al., 2020; Wiley et al., 2017).

Dendrometer data have lower precision as radial-growth measures than xylogenesis data derived from repeated microcore sampling. However, the results are similar when comparing both methods (Michelot et al., 2012). In addition, our results (Table 3) are consistent with those obtained in previous xylogenesis studies on beech (Martínez del Castillo et al., 2016). In all phenological variables related to radial growth, the variability in damaged beech trees was greater than in non-damaged trees (Table 3), probably due to differences in the vigor of the trees before the frost and/or the different impact of the frost among trees (showing between 50% and 100% of dead leaves on the trees classified as damaged). Accordingly, the most damaged beech trees and/or those with less vigor would reduce the length of their growing season while those that exhibited a moderate damage and/or were more vigorous would lengthen it. In fact, the largest BAI in damaged beech trees occurred between August 1st (DOY 213) and September 19th (DOY 262) while in non-damaged trees it occurred between May 26th (DOY 146) and June 21st (DOY 172) (Fig. 6).

4.4. Stand and forest level: frost reduces LAI and delays the NDVI annual peak

Our results showed a greater reduction of LAI in beech-dominated areas than in oak-dominated areas after the frost. This is in agreement with the reduction of total leaf area per twig, which was 29% in beech and 22% in oak (although only in beech the *p*-value of the difference was lower than 0.1) (Table 2). LAI reduction in our study was lower as compared to others (Awaya et al., 2009), possibly due to differences in the amount of damaged leaves and trees in the studied areas, and the existence of other non-damaged tree species in beech- and oak-dominated areas.

Fusing Landsat and MODIS satellite data provides synthetic data with high spatial and temporal resolutions that enable a detailed analysis of NDVI variations over time and precise determination of leaf emergence and senescence times. The NDVI peak value and the length of the 2017 spectro-phenological season were similar to previous years in areas dominated by beech and oak (Figs. 7a and b), suggesting that, although the vegetative period was delayed in 21 and 29 days, respectively, by incidence of the late frost, the total photosynthetic activity at stand level in 2017 was similar to previous years, partly due to the high autumn temperatures (Fig. 1b). As the study scale is enlarged, the detail is reduced. In this respect, it was possible to differentiate between frost-damaged and non-damaged trees at organ and tree levels, and to obtain the LAI *in situ* in areas where one or the other species dominate. However, when using satellite images there is a mixture of species at each of the areas considered, with intermingled damaged and non-damaged trees, which produces a blurring of the frost effects. For example, a LAI reduction in damaged trees could favor competing trees, and the overall NDVI at the stand level would not change.

The impact of the frost on the spectro-phenological season length was higher at the forest level (Fig. 7c) than in the areas dominated by beech or oak, with an 18-day reduction in 2017 compared to previous years. This is likely because the late-sprouting *Quercus pyrenaica*, with a much later phenology than beech and oak, is the most abundant species outside the areas dominated by beech and oak in the studied forest. Thus, the shorter 2017 growing season length would reflect the high contribution of *Quercus pyrenaica* to the overall forest NDVI trajectory. Although *Quercus pyrenaica* was barely affected by the late frost (occurring two weeks before its average budburst time; in any case, during field surveys, it was the species that showed the least number of frost-damaged leaves), the drop of temperature at this time could have imposed a further delay in this species leaf emergence.

5. Conclusions

Early leaf-out in beech and oak trees caused by an unusually warm winter resulted in massive leaf scorching after a spring frost. Trees remobilized carbon and nutrient reserves to produce a second cohort of leaves. For beech, however, crown recovery was incomplete: leaves were smaller and thinner, and twigs had smaller leaf area than those of trees not affected by the frost. This did not have a significant impact on stem sapwood NSC concentrations, suggesting that beech trees prioritize maintaining carbon storage to secondary growth after leaf frost damage. Frost effects diluted as analyses scaled up from organ to forest levels, although some frost effects at stand level reflected the different organ- and tree-level sensitivity between species (e.g. the higher frost-induced reduction of LAI in beech stands). Delayed leaf senescence compensated for the frost-induced delay in carbon uptake, caused by leaf scorching and the time needed to form a second leaf cohort, alleviating the frost impact on the length of the vegetative period. Nonetheless, the differential response between the two study species may give oak a competitive advantage over beech, and may limit the current expansion of beech if the frequency of spring late frosts increases.

Declaration of Competing Interest

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

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.agrformet.2020.108243.

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