

## RESEARCH ARTICLE

# No risk—no fun: Penalty and recovery from spring frost damage in deciduous temperate trees

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## Abstract

1. Phenological shifts in response to changing climatic conditions is a key acclimation process for the persistence of perennial plants in temperate and boreal climates. The optimal time to leaf-out is the result of an evolutionary process determined by the trade-off between minimizing the risk of freezing damages and herbivory pressure while maximizing resource uptake to increase competitiveness against the other plants.
2. We quantified the penalty exerted by frost damage at the time of leaf emergence on plant development (reduction in leaf area, canopy duration and growth) over the potential gains without frost (increased biomass and non-structural carbohydrate reserves), depending on when leaf-out occurs. To this purpose, we exposed 960 saplings of four temperate deciduous tree species with contrasting cold hardiness to two frost intensities shortly after leaf emergence, which was artificially induced at four occasions to reflect the whole range of natural leaf-out dates.
3. One year above-ground biomass (AGB) increments following the frost revealed a clear ranking among the species depending on their strategy to cope with damaging frosts. *Prunus avium* (−41% of AGB-increment compared to control saplings) resprouted from the stem base, *Quercus robur* (−62%) rapidly produced new leaves from dormant reserve buds, *Fagus sylvatica* (−98%) showed highest chlorophyll content in autumn and delayed senescence together with *Carpinus betulus* (−105%), which overcompensated NSC reserves after the growing season but showed highest mortality (up to 32%). In all species, NSC reserves recovered rapidly to control levels at the expense of growth.
4. The timing of leaf-out (advanced and delayed artificially) significantly affected the performance and recovery (regreening and growth) of both frozen and non-frozen saplings, with the lowest performance found at the most delayed leaf-out date. We propose that the potential to recover from frost damages is an important component of a tree's performance, particularly at the juvenile stage. The ability to recover may become even more decisive in the future with the predicted increase of false springs in many extratropical regions.

## KEYWORDS

budburst, climate chamber experiments, climate warming, cold hardiness, forcing, growth, senescence, tree phenology

## 1 | INTRODUCTION

In temperate, boreal and alpine regions, the timing of leaf emergence in spring in relation to climatic fluctuations is one of the most influential events determining the persistence of plants in their environment (Chuine, 2010). This synchronization results from long-lasting evolutionary processes aimed at maximizing the acquisition of resources such as light, water and nutrients, which is essential at juvenile stage to out-compete neighbouring plants, while minimizing the risk of frost damage of the new sensitive leaves that would negate these effects (Kollas et al., 2014). To match this specific time window corresponding to optimal plant performance, temperate trees rely on temperature and photoperiod directly sensed at the bud level (Zohner & Renner, 2015). Thus, the onset of bud development in spring depends on a complex interplay between cool and warm temperatures, hereafter termed chilling and forcing, respectively, further fine-tuned through photoperiodic signals (Flynn & Wolkovich, 2018; Vitasse, Lenz, et al., 2014; Way & Montgomery, 2015; Zohner & Renner, 2015). Experiments have demonstrated that increasing exposure to chilling temperatures over the course of winter dormancy steadily increases the sensitivity of buds to forcing temperatures and thus reduces the amount of heat energy required for budburst (Baumgarten et al., 2021; Flynn & Wolkovich, 2018; Murray et al., 1989). Chilling requirement to progressively release winter dormancy has been adopted by most temperate trees to avoid a too early leaf-out during unpredictable warm spells in winter and is well reflected by the long-term probability to encounter frost (Lenz, Hoch, et al., 2016). Even more striking, the timing of spring phenology occurs at a precision to allow trees to maintain their 'safety margins against frost damages' along elevational gradients (Lenz et al., 2013).

However, with the extremely rapid ongoing rise in temperature due to anthropogenic greenhouse gas emissions, the optimal time window for balancing resource maximization and frost avoidance may have shifted outside the range of evolutionary experience. Indeed, while warmer springs caused by global warming have significantly advanced leaf emergence of temperate trees worldwide over the recent decades (Vitasse et al., 2022), the occurrence of the last spring frosts has advanced at a slower rate in most regions in Europe (Ma et al., 2019), North America (Augspurger, 2013) and worldwide (Zohner et al., 2020). Although dormant buds of deciduous tree species are well protected against minimum temperatures during winter, their freezing resistance substantially decreases before and during bud development in spring (Till, 1956; Vitra et al., 2017; Weiser, 1970). Thus, an earlier development of the vegetation in spring induced by warming may put plants at a higher risk of being damaged by frosts, particularly in Europe and Asia where tree species have lower freezing resistances (compared to North America) and are generally more responsive to temperature (Liu et al., 2018; Walde et al., 2022; Zohner et al., 2020). In fact, more damaging spring frosts have been observed in recent years, precisely because of the increased amount of heat energy that preceded leaf-out (Augspurger, 2009; Hufkens et al., 2012; Sangüesa-Barreda

et al., 2021; Vitasse et al., 2018). For instance in April 2017, after an extraordinary warm period in Western and Central Europe, the sudden arrival of cold air masses from the Arctic and the subsequent freezing events caused dramatic losses in fruit production in Central Europe and the amount of accumulated heat at the time of the frost was unprecedented in some stations since the beginning of meteorological records (Vitasse & Rebetez, 2018). For forest trees, most information on recovery from spring frost damages stems from European beech, as this species forms large dominant forests and canopy defoliations due to frost have been successfully detected by remote sensing in numerous areas across the southern European distribution of this species, particularly at higher elevations (Allevato et al., 2019; Bascietto et al., 2018; Olano et al., 2021). However, to reconstruct the frequency of such frost events beyond the period where satellite images with high spatial and temporal resolution became available, dendrochronological methods are currently the only method. Large-scale tree ring data across southern Europe indeed confirm an increasing frequency of late frost defoliations in European beech within a larger catchment area (Sangüesa-Barreda et al., 2021).

At the level of the individual tree, damaging frost effects are well reflected in the form of impaired annual rings. Since damaging spring frosts fall at the beginning of the growing season, the tree ring losses are usually as significant or even greater than the ones induced by extreme summer droughts (Rubio-Cuadrado, Camarero, et al., 2021; Vitasse et al., 2019). Although tree ring widths of beech are largely reduced during years with damaging spring frosts, no carry-over effects are generally observed in the following years (Príncipe et al., 2017). Once adult beech trees got hit by a frost during the critical stage of leaf emergence, new leaves appeared within 1 month and the canopy recovery was completed after roughly 2 months following the event (D'Andrea et al., 2019; Menzel et al., 2015). Similar recovery times have been found also for various deciduous tree species in North America with a large range in maximum canopy recovery depending on species (between 46% and 99% compared to 'normal' canopies; Augspurger, 2009). As a consequence of a delayed canopy greening due to frost damage, trees may extend their growing season by delaying leaf senescence as it has been reported for beech, thereby compensating for losses in productivity (Rubio-Cuadrado, Camarero, et al., 2021; Zohner et al., 2019).

The stage of leaf development in spring has been shown to largely determine the extent of frost damage, reflecting the frost tolerance of a given developmental stage (Allevato et al., 2019). While dormant buds are almost invulnerable to any freezing temperatures, the most critical stage appears to be around leaf-out (Vitra et al., 2017), before a slight increase in frost tolerance can be observed with leaf maturation (Taschler et al., 2004). Hence, the impact of the same frost can be less severe when leaf development has passed the point of maturation (Augspurger, 2011; Kreyling et al., 2012). Independent of the developmental stage, cold hardiness of buds and developing leaves is build up under cool preceding temperature conditions (Lenz, Hoch, et al., 2016; Neuner et al., 2020). While an earlier leaf-out increases the likelihood of frost damages, it has been reported

for European beech that the recovery time to rebuild the canopy is also shorter (Menzel et al., 2015).

The production of an additional leaf cohort requires an increased use of carbon from storage tissues (D'Andrea et al., 2019). However, it has been shown that non-structural carbohydrate concentrations were not critically reduced in storage tissues after frost-induced defoliation in adult beeches because storage levels were maintained at the expense of stem radial growth (Rubio-Cuadrado, Gómez, et al., 2021). The same study could demonstrate that nitrogen content in second cohort leaves emerging after frost were even higher compared to unfrozen control trees.

Large-scale defoliations due to frost can have a significant impact on forest ecosystem functioning by affecting carbon uptake (Hufkens et al., 2012), water relations (Bréda & Granier, 1996) and by altering nutrient cycles (Estiarte & Peñuelas, 2015). In addition, populations of a vast variety of animal and fungal species, which depend on leaf tissue, fruits and seeds, may decline when frost damages occur (i.e. moth populations; Greco et al., 2018), which in turn likely affects other species at higher trophic levels (Augsburger, 2009).

While increasing effort has been made in recent years to predict potential harmful frost occurrences, little is known about species-specific responses to frost damages. Particularly, we lack information on the extent and velocity of recovery of forest tree species (others than European beech) in the year of the frost event and thereafter. If damaging frost events do increase in frequency, the cards may be reshuffled in the game of natural selection based on species responses (Augsburger, 2011).

Here, we aim to investigate how well temperate trees can recover from damaging frosts, depending on its intensity and on when leaf-out occurs. We tested saplings of four tree species with different levels of leaf cold hardiness at the time of leaf-out and different timing of flushing by monitoring their recovery in situ after inducing artificial frosts. Moreover, we investigated the recovery process of a natural, adult beech population in the Swiss Jura mountains that was damaged by a natural frost event in May 2020.

Growth increments, recovering leaf area and non-structural carbohydrates were monitored after inducing artificial frosts to estimate the trade-off between frost damage and potential growth. Specifically, we addressed the following research questions:

1. How fast and to what extent do trees recover after a damaging frost in terms of leaf area, growth increment and non-structural carbohydrates?
2. Does leaf-out timing influence the growth performance of undamaged control saplings and the recovery of frost-damaged ones?
3. Is recovery potential dependent on species life-history strategies (e.g. pioneer vs. late successional species)?

We expect early successional and early flushing species to recover faster from frost damages compared to more conservative, late successional and late flushing species. We further hypothesize that an earlier leaf-out timing leads to higher growth increments when

saplings avoid frost (control) and permits a better recovery after frost damage that translates into a higher biomass increment. Moreover, for the frost-damaged plants, we expect NSC reserves to recover quickly after producing the new cohort of leaves and to reach control levels (undamaged saplings) at least by the end of the growing season.

## 2 | MATERIALS AND METHODS

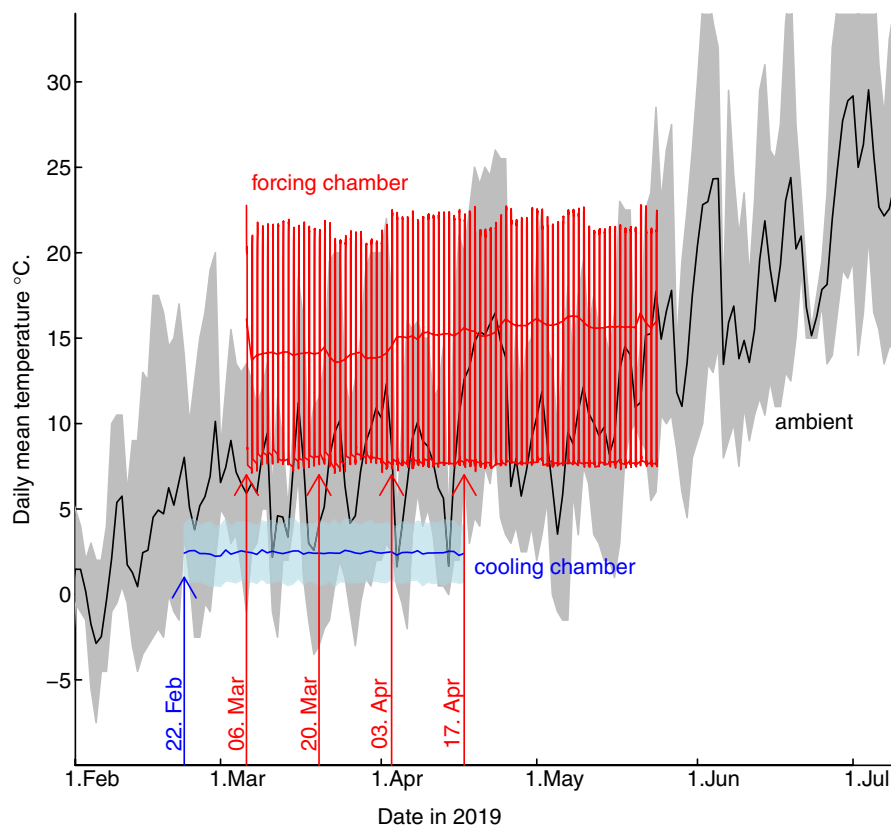
### 2.1 | Study species and study site

We selected four dominant deciduous tree species native to Europe with contrasting life strategies regarding spring phenology and freezing resistance: *Prunus avium* L. and *Carpinus betulus* L. which are early flushing species with very high and rather high-freezing resistance during leaf-out, respectively; *Fagus sylvatica* L. and *Quercus robur* L. which are both late flushing species at the study site with lower freezing resistance of emerging leaves; and *Fagus sylvatica* and *Carpinus betulus* which are both tolerant to shade in contrast to the other two species (see freezing resistance thresholds and shade tolerance index extracted from Till (1956) and Vitasse, Lenz, et al. (2014) and Niinemets and Valladares (2006), respectively summarized in Table S1). For clarity and brevity, hereafter, we refer to each species by its genus name.

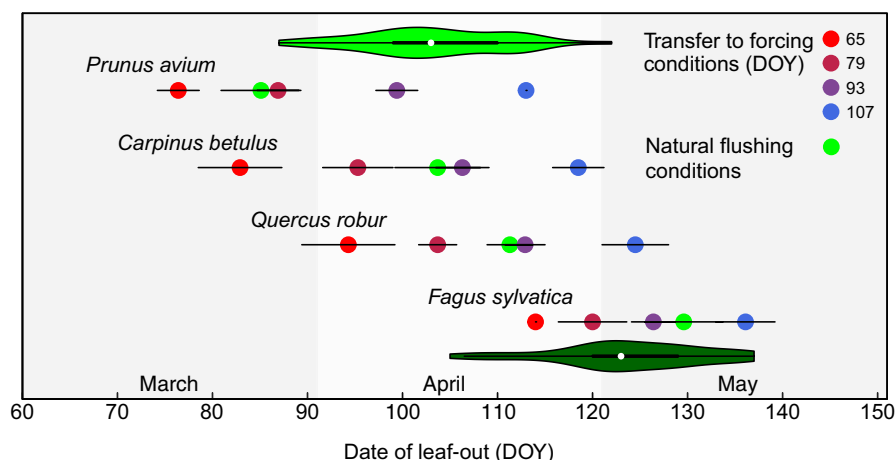
In autumn 2018, 2-year-old saplings were purchased from a local nursery (Wiler, Switzerland, 455 m a.s.l., 47°09'N, 7°33'E) and transferred to the WSL research institute situated in north-eastern Switzerland (Birmensdorf near Zurich; 47°21'38"N, 8°27'16"E; 550 m a.s.l.; mean annual temperature, 9.3°C; mean annual precipitation, 1134 mm; MeteoSwiss station Fluntern, 1981–2010), where they were exposed to ambient conditions until the start of the experiment (see Figure 1).

### 2.2 | Study design

The whole study design is depicted in Figure 1. On 22 February 2019, that is, when chilling requirements for dormancy release were likely to be satisfied, all saplings except the ones aiming for the natural leaf-out date, that is, 144 per species, were moved into a climate chamber at 3°C. On 6 March 2019 and every second week until 17 April 2019, a cohort of 36 saplings were transferred to another chamber simulating forcing conditions at 20°C to artificially advance or delay the date of leaf-out compared to natural leaf-out time (Figure 1). The photoperiod in both chambers was set to follow ambient conditions at Zurich (47°N latitude). For the natural leaf-out date, 36 saplings per species were left outside of the WSL research institute and were exposed to the ambient temperature conditions as shown in Figure 1. This resulted in five different leaf-out dates per species spanning the widest range (but the extent of the range being species specific) of possible leaf-out timings for the study site (four artificially induced, one natural, Figure 2). Thirty-two additional saplings per species were included to account for destructive NSC measurements (see Table S2).



**FIGURE 1** Daily mean (black line), minimum and maximum (shaded area) air temperature at the study site near Zurich, Switzerland from February to July 2019. The blue area corresponds to the daily minimum and maximum temperature in the cooling chamber with the mean as blue thick line in between. The red curve follows the hourly mean temperature of the forcing chamber with day and night fluctuations. Simulated daylength in the forcing chamber followed the increasing photoperiod at the study site, hence day-time temperature increasingly contributed to the mean (red line in between). Vertical lines indicate the transfer days from outside into the cooling chamber (blue) and from the cooling into the forcing chamber (red).



**FIGURE 2** Leaf-out occurrences (day of year; DOY) of the four study species in response to artificial (blue to red points) and ambient (green points) spring conditions. Advanced and delayed leaf-out timings were induced by transferring saplings from a cool to a forcing chamber (indicated are the respective dates of transfer). Naturally flushing saplings experienced ambient condition at the research facility near Zurich, Switzerland. Each dot represents the mean  $\pm$  1 SD of 12 replicates. The violin diagrams represent the leaf-out time series of an adult cherry (light green, provenance from Bovenden, Germany, 38 record years) and beech (dark green, provenance from Denmark, 43 record years) tree that were recorded at the research facility. Shaded areas indicate the month for better readability.

## 2.3 | Monitoring of leaf-out dates

Bud development of every sapling was monitored by the same observer twice a week in spring 2019 using a four-stage categorical scale (Vitasse et al., 2013): at stage 0 (dormant bud), no bud development was visible; at stage 1 (bud swelling), buds were swollen and/or elongating; at stage 2 (bud burst), bud scales were open and

leaves were partially visible; at stage 3 (leaf-out), leaves had fully emerged from the buds but were still folded, crinkled or pendant, depending on species; and at stage 4 (leaf unfolded), at least one leaf was fully unfolded. For each sapling, the day of year of each stage was recorded as soon as 50% of all buds reached a new stage. Missing stages between two monitoring dates were estimated by linear interpolation.

## 2.4 | Freezing treatment

At the stage of leaf unfolding (>50% of the buds had at least one leaf fully unfolded), which is the most vulnerable stage to freezing temperatures (Lenz et al., 2013), both warming-induced and naturally flushing saplings were transferred into a freezing chamber to experience artificially induced freezing damages. Of 36 saplings per species, 12 were exposed to an artificial frost event that killed all leaves ( $LT_{100}$ , i.e. lethal temperature killing 100% of the leaves), and 12 others to an artificial frost event that partially damaged them ( $LT_{50}$  i.e. temperature aiming to damage around 50% of the leaf tissue). The  $LT_{50}$  and  $LT_{100}$  temperature thresholds have been adapted to each species according to their respective freezing resistance found in the literature (Till (1956); Vitasse, Lenz, et al. (2014); see Table S1). To apply the frost treatments, 24 saplings were first acclimatized at 4° for 1 h. With a freezing rate of 5°C per hour, temperature gradually decreased until reaching the  $LT_{50}$  target value, which was then maintained for 2 h before to move 12 saplings back into the 4°C chamber (' $LT_{50}$ -treatment'). The remaining 12 saplings per species were then further cooled down until reaching the  $LT_{100}$  target temperature that was again maintained for 2 h before transferring them back to 4°C conditions (' $LT_{100}$ -treatment'). To prevent any freezing damages to the roots, the sapling containers were put on top of a warm water bath with surrounding insulation (Figure S1) that prevented temperature to drop below 5°C throughout the freezing treatment (Figure S2). Finally, the remaining 12 individuals per species served as a control and were not subjected to a frost treatment (see Table S2 for number of replicates for all treatments).

Shortly after the frost treatment (i.e. within 24 h), all saplings including the control were planted outside on a former fertile grassland soil at the WSL research facility organized in three blocks (four replicates per block, 30 cm distance between saplings) under a shading net that intercepted  $\sim 65 \pm 4\%$  PAR (mean  $\pm$  SE; measured on 3 days in June and August with an LI-COR Li189 quantum PAR light sensor) simulating below canopy light conditions (Figure S3). This procedure was repeated five times for all four artificial as well as the naturally occurring leaf-out date.

After the first growing season, the shading net was removed to eliminate light limitation. Temperatures in all climate chambers were monitored throughout the experiment using temperature loggers (HOBO MX2203, TidbiT) at a minute interval to track freezing/thawing cycles in a high-temporal resolution. At the experimental site, air temperature at a height of 2 m was recorded under an aluminium shelter every 30 min (EL-USB-2+ sensor, Lascar Electronics). Saplings were watered at planting and at regular intervals depending on natural precipitation throughout the experiment to avoid any water limitations.

## 2.5 | Monitoring of recovering leaf area and mortality

Recovering and newly emerging leaf tissue after the freezing treatment was visually estimated by the same observer for each sapling

as the percentage of green leaf area compared to the control saplings (e.g. 100% equals full recovery) every second week in June and July and once in August and September respectively. Saplings were considered dead if they did not produce any new leaves or buds over the course of two growing seasons following the frost treatment (0% of recovery). Maximum recovery (% to control) was calculated as the mean for the last three monitoring dates to improve reliability. For better comparison between different leaf-out timings, results are displayed in growing degree days (GDD, threshold = 5°C) after frost exposure.

## 2.6 | C/N analysis

To evaluate the capacity of photosynthesis in recovering tissue after the frost treatment, we sampled leaf material from saplings that were subjected to the intense frost ( $LT_{100}$ ) and from the undamaged control saplings (eight replicates each), both from the natural leaf-out timing saplings batch. Leaves were harvested in June, when the second cohort leaves from the frost treatment reached full maturity (i.e. 2 weeks after leaf-out), dried and ground to fine powder.  $4.0 \pm 0.1$  mg of leaf material was weighted into  $4 \times 8$  mm tin capsules and processed for Carbon and Nitrogen content using a CN analyser (NC-2500, CE Instruments).

## 2.7 | Growth assessment

We measured diameter (2 cm above plant collar) and height of every sapling before budburst (prior to the treatments) as well as after the first and second growing season (after leaf fall) using a digital calliper (accuracy  $\pm 0.1$  mm) and a graduated pole (accuracy  $\pm 1$  mm). Above-ground biomass (g, AGB) was estimated following the allometric equation provided by Annighöfer et al. (2016):

$$AGB = \beta_1 \times (RCD^2 \times H)^{\beta_2}$$

with RCD = root collar diameter (cm);  $H$  = height (cm) and  $\beta_1$  and  $\beta_2$  = species-specific fitted coefficients provided in table 4 by Annighöfer et al. (2016). The increments in AGB in the 2019 and 2020 growing seasons were calculated by subtracting the estimated previous year's AGB.

Due to the loss of height as a result of the frost treatment in some saplings, increments were allowed to have negative values. Hence, AGB increments capture both dieback and new growth. In addition, height as an indicator for light competition was analysed separately.

After the second growing season, all saplings were dug out, washed and separated in roots and shoots for final below- and above-ground biomass assessment (drying for 3 days at 80°C). Calculated and measured AGB revealed a very high correlation ( $r = 0.97$  over all species,  $p < 0.0001$ ; see Figure S4).



## 2.8 | Monitoring of senescence

The progress of leaf senescence in autumn 2019 was assessed every second week between 12 September and 15 November (and weekly during accelerated senescence) using a leaf spectral index (LSI) as a proxy for the remaining chlorophyll content (MC-100, apogee instruments, mean of three representative leaves per replicate). In addition, the percentage of fallen leaves was monitored at the same occasions and we refined the total LSI of a sapling by assigning an LSI of 0 to already dropped leaves, following Zohner et al. (2019). For example, a sapling which already dropped 50% of its leaves and a measured LSI of 20 (remaining leaves) was rated with a total LSI of 10 ( $0.5 \times 20$ ). Individual saplings were considered senescent on the day when the LSI had decreased by 50% relative to the sapling's maximum LSI in summer.

## 2.9 | NSC measurements

We sampled pencil-thick pieces of the stem (with bark) from the main axis and root (with cortex) on four occasions: during dormancy (February), during first budburst before freezing, during emergence of second cohort leaves (June) and at the end of the growing season (November). Samples were taken for the earliest and latest artificial leaf-out timings as well as for the natural one for both LT<sub>100</sub> and control saplings (only occasions in June and November after treatments have started; see Table S2 for number of replicates). After harvesting, stem and root materials were placed directly in an oven at 80°C to prevent any further enzymatic activity and dried for at least 72 h. Tissue samples were then ground to fine powder with a ball mill (MM 400, Retsch GmbH).

To determine NSC concentrations, we applied the method of Wong (1990) modified by Hoch et al. (2002). For soluble sugar extraction, approx. 10 mg of the homogenized material was boiled in 2 ml distilled water for 30 min. After centrifugation, a 200- $\mu$ l aliquot of the extract was taken, and invertase and isomerase (Sigma-aldrich) were added to degrade sucrose and convert fructose into glucose. The amount of glucose was then determined photometrically with the glucose-hexokinase assay. In this assay, glucose is converted via hexokinase in the presence of ATP to glucose-6-P. In a second step, glucose-6-P and NAD are converted to gluconate-6-phosphate and NADPH by glucose-6-phosphate dehydrogenase. In the reaction, the concentration of NADPH (equating to the concentration of glucose) was determined photometrically at 340 nm. The total amount of NSC (soluble sugars plus starch) was determined by adding amylo-glucosidase from *Aspergillus niger* (Sigma-aldrich) to a 500- $\mu$ l aliquot of the plants extract and incubating it at 49°C for 15 h for starch digestion. Total glucose was determined as described above and the starch concentration was calculated as the difference between total NSC and soluble sugars. Pure starch, glucose, fructose, sucrose and standard plant powder (Orchard leaves; Leco) were included to control the reproducibility of the extraction process. Concentrations of sugars and starch are expressed on a dry matter basis in mg.g<sup>-1</sup>.

Samples were processed in a random manner to avoid any analytical bias related to analyses time.

## 2.10 | Natural frost damage in adult beech

In addition to the sapling experiment, we studied the impact of damaging spring frost on adult trees under natural conditions focusing on 10 adult beech trees growing at one of the highest mountain peaks in the Swiss Jura (Weissenstein, 47°25'79"N, 7°52'64"E; 1'385 m a.s.l.; mean temperature for July 14.8°C and -0.7°C for January; mean annual precipitation 1'635 mm, period of 1991–2021; CHELSA-W5E5 v.1.1; Karger et al., 2021). On 10 and 11 May 2020, a low-pressure system over Europe brought cold air from the north causing the temperature at this site to drop to a minimum of around -2.5°C (-1.1°C at the 9 km distant weather station Bettlachstock; 47°13'28"N 7°25'02"E; 1'100 m a.s.l. adjusted with a lapse rate of 0.5°C/100 m). As a consequence, the beech population at the highest elevation lost all newly emerged leaves, with damages visible down to ca. 1'150 m a.s.l (Figures S5 and S6). Ten additional adult beech individuals were selected on the same slope but at lower elevation (1'085 m a.s.l.), where no freezing damages have been observed. This latter site served as a control to compare the recovery of the damaged ones. Pencil-thick branches, 2 years old, were harvested (including the bark) for NSC analysis on five dates throughout the growing season 2020 (18 June, 30 June, 27 July, 3 September and 21 October). On the same dates, recovering leaf area was assessed visually in relation to the unfrozen control trees similar to the sapling experiment. A camera taking one photo per day (NatureView HD Essential, Bushnell Outdoor Products) was mounted on a tree at 4-metre height on 30 June in order to monitor the emergence of the new foliage.

## 2.11 | Data analysis and statistics

To account for initial differences in sapling stature and for better comparison among species biomass and height increments were standardized to the sapling's initial calculated AGB and height (prior to the experiment) and termed 'AGB and height increment (% to initial state)'.

All dependent variables were analysed within species using linear mixed effect models (lme function of the R package NLME v.3.1-148). The variable 'maximum recovery (% to control)' was log-transformed to obtain normality of residuals.

Fixed effects included leaf-out timing (days relative to control) as a numeric and frost treatment as a factorial explanatory variable. We included block as a random factor to account for the position of saplings within the experimental plot. Saplings flushing in natural conditions were excluded from this analysis as conditions prior to and during leaf-out differed from saplings manipulated in artificial climate chamber conditions. At first, this model was run for unfrozen control saplings only (without frost treatment as a fixed effect).

Leaf spectral index data were first analysed to test effects of frost and leaf-out timing for all measurement dates on absolute values using linear mixed effect models with individual saplings as random factor. In a second step, LSI values were standardized to maximum values to calculate the day of year at 10%, 50% and 90% of senescence for every replicate separately using a generalized linear model (glm function, package STATS v.4.0.2) with a quasibinomial distribution and a logit link function. Finally, a linear model was applied to test the effect of frost and leaf-out on the day of year at 10%, 50% and 90% senescence.

NSC concentrations (% dry weight) and C/N ratio were analysed using linear models adding leaf-out timing as a factor (for NSC), because this dataset consisted of the earliest and latest artificial flushing date only. Differences in NSC concentration and C/N ratio between frozen and unfrozen control groups were additionally analysed using Mann–Whitney *U* test.

For the additional experiment on adult beech trees at Weissenstein, Switzerland, NSC concentrations were analysed for each sampling date using linear mixed effect models with frost damaged trees/undamaged trees (population site) as a fixed and tree ID as a random effect to account for repeated measurements on the same individual.

All analyses were performed in R (R Development Core Team, 2020), v.4.0.2.

### 3 | RESULTS

#### 3.1 | Artificial and natural budburst timing

Temperature manipulation of saplings in climate chambers resulted in a wide range of budburst dates but with significant differences among species, spanning 37 days for *Prunus*, 36 days for *Carpinus*, 30 days for *Quercus* and only 22 days for *Fagus* (Figure 2). Two long-term series (>38 years) of leaf-out dates for adult cherry and beech (earliest and latest flushing species) show that this artificially induced range of leaf-out timing fairly reproduces the range of naturally occurring leaf-out timings observed in Zurich, Switzerland (Figure 2).

#### 3.2 | Induced freezing damages and mortality

Artificial freezing treatments of both temperature thresholds (LT<sub>50</sub> and LT<sub>100</sub>) caused severe damage to all leaves and led to a complete defoliation in all saplings and of all species (Figure S7). *Prunus* lost substantial parts of the main shoot, especially after the LT<sub>100</sub> treatment, and, as a consequence, reformed a new apical shoot from the stem basis. Frost-induced mortality was clearly highest in *Carpinus* for which 32% of the saplings died after the LT<sub>100</sub> and 13% after the LT<sub>50</sub> frost treatment respectively (Table S3). In *Fagus*, mortality was 8% and 12% (always LT<sub>100</sub> and LT<sub>50</sub>) followed by *Prunus* (8% and 5%) and *Quercus* (2% and 3%).

#### 3.3 | Short-term recovery (first growing season)

##### 3.3.1 | How fast do saplings recover their leaf-area from frost damages?

Naturally flushing saplings exposed to the LT<sub>100</sub> frost treatment reached close to maximum recovery values (leaf area compared to control) in all species already at the first monitoring campaign on 7 June (green dots in Figure S8A). While *Quercus* recovered fully with <280 GDD (Figure S8A; or less than 4 weeks, Figure S9A) using spare buds that were not deployed during the first flush, all other species showed two waves of recovery, the second being around the second flush of unfrozen control saplings (finished by end of July). Thereafter, leaf area compared to control remained stable until senescence.

Advanced leaf-out timing caused a lower first recovery wave even after similar GDD (Figure S8A).

##### 3.3.2 | How much do saplings recover their leaf area from frost damages?

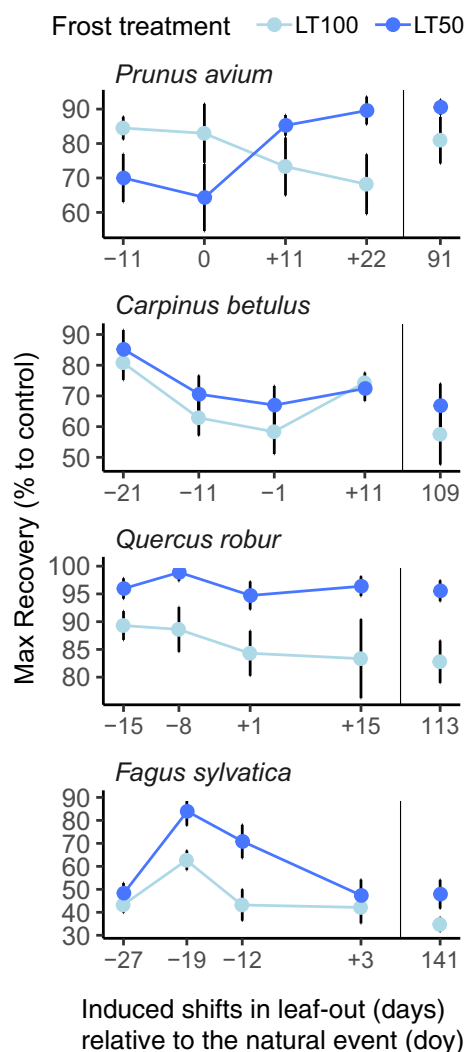
Following the LT<sub>100</sub> frost treatment, maximum recovery (mean leaf area in summer compared to control) of naturally flushing saplings was highest in *Quercus* (83±4%, Mean±SE; Figure 3) and *Prunus* (81±7%) and only reached 57±10% and 35±3% in *Carpinus* and *Fagus* respectively. Delayed leaf-out negatively affected maximum recovery, but it was significant in *Fagus* (LT<sub>50</sub> and LT<sub>100</sub>) and *Prunus* (LT<sub>100</sub>) only. While the most advanced leaf-out date did not fit in this pattern in *Fagus* and *Prunus*, the effect of leaf-out timing was clearly evident afterwards (Figure 3; maximum difference in state of recovery among artificial leaf-out dates: 24% in *Fagus* and *Carpinus*, 15% in *Prunus* and 6% in *Quercus* respectively).

##### 3.3.3 | Does maximum recovery depend on frost intensity?

A lower frost intensity (LT<sub>50</sub> compared to LT<sub>100</sub>) resulted in significantly higher maximum recovery in naturally flushing saplings of *Quercus* (96±2% vs. 83±4%; Mean±SE) and *Fagus* (48±6% vs. 35±3%) only (Figure 3 and Table S4). Higher maximum recovery after exposure to reduced frost intensity was also evident in artificial leaf-out dates for all species, except for *Prunus* (Figure 3). In this species, we detected that maximum recovery increased with delayed leaf-out timing, contradictory to the response of saplings that were exposed to the LT<sub>100</sub> frost treatment (significant frost×leaf-out timing interaction, Table S4).

##### 3.3.4 | Do recovering leaves differ in C/N ratios?

Across all species tested (*Prunus*, *Quercus* and *Fagus*), C/N-ratios were significantly lower in the second cohort of leaves that have

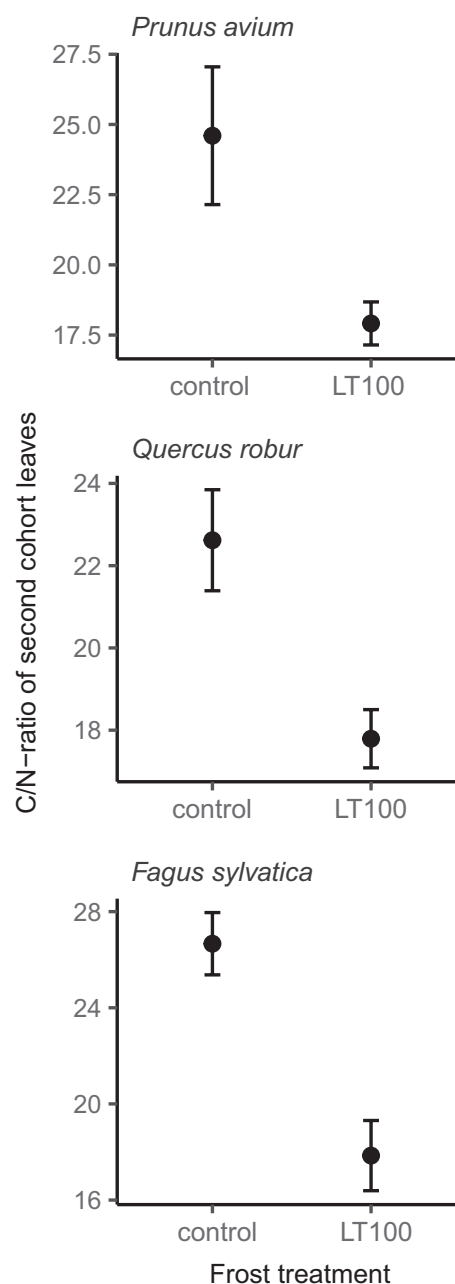


**FIGURE 3** Maximum recovery (mean leaf area in summer compared to control) after fully (LT100, A) and partly (LT50, B) frozen saplings compared to unfrozen control saplings in relation to leaf-out timing (days) relative to the natural event (day; day of the year). Dots represent the mean  $\pm 1$  SE of 12 replicates. Note the different scales on the y-axis.

emerged after the LT<sub>100</sub> frost treatment compared to the leaves from unfrozen control saplings (overall C/N ratio of  $18.8 \pm 0.9$  vs.  $27.3 \pm 1.4$ ; Figure 4 and Table S5). Thus, the new cohort of leaves which have emerged after the frost treatments had higher N concentrations per g dry weight.

### 3.3.5 | Is above-ground biomass (AGB) and height increment affected by leaf-out timing in unfrozen control saplings?

AGB increments during the first growing season following the frost treatment significantly decreased with delayed budburst in *Carpinus* only (green dots in Figure 5a and Table S6). However, lowest AGB increments were always found in most delayed leaf-out timings in

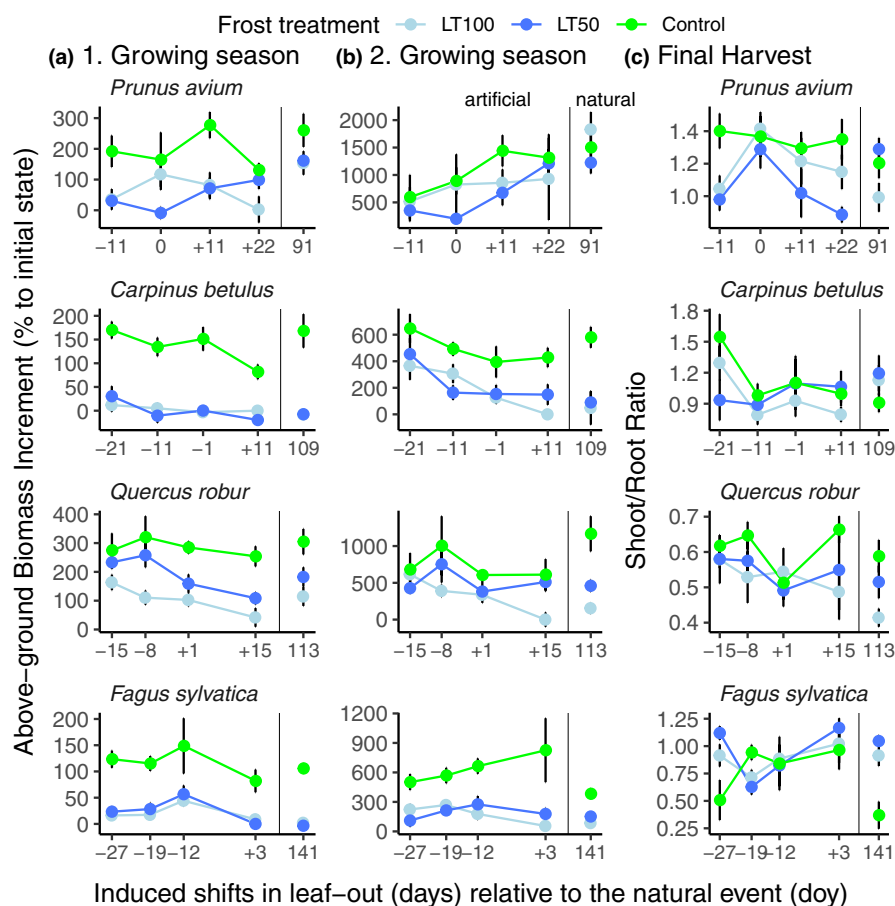


**FIGURE 4** C/N-ratio of second cohort leaves after the LT100 frost treatment compared to leaves from unfrozen control saplings. Mean  $\pm 1$  SE of 8 replicates. No data were collected for *Carpinus betulus* due to poor recovery (insufficient replicates).

all species. When focusing on height only, increments significantly decreased with delayed leaf-out timing by  $-28\%$  in *Fagus*,  $-21\%$  in *Carpinus* and  $-13\%$  in *Quercus* for 1 week of delay compared to the natural flushing date respectively (estimates from linear mixed effect models; no trend found for *Prunus*; Figure S10A and Table S7). During the second growing season following the frost treatment and leaf-out manipulation, *Carpinus* continued to show a trend in the same direction. *Fagus* compensated by significantly increasing AGB increments with delayed leaf-out timing (reverse lag effect; Figure 5b and Table S6).



**FIGURE 5** Calculated and standardised above-ground biomass increments (% to initial state) after artificial frost events (LT100 and LT50 freezing target values) and control treatment during first (a) and second (b) growing season as well as the final shoot/root ratio (c) in response to leaf-out timing. The four artificial leaf-out dates are indicated as days relative to the natural leaf-out date (doy, day of year, right site on each panel), e.g. 0 corresponds to natural leaf-out timing. 91 = 1. Apr; 109 = 19. Apr; 113 = 23. Apr; 141 = 21. May.  $N = 12$  replicates. Dots represent the median  $\pm 1$  SE. Note the different scales on the y-axis.



### 3.3.6 | How much is AGB and height increment affected by frost?

Both LT<sub>100</sub> and LT<sub>50</sub> frost treatments led to a significant reduction in AGB increment in naturally flushing saplings of all species (Figure 5a and Table 1) during the first growing season. AGB increment following the LT<sub>100</sub> frost treatment was reduced by -105% in *Carpinus*, -98% in *Fagus*, -62% in *Quercus* and -41% in *Prunus* compared to unfrozen control saplings respectively (for naturally flushing saplings). The LT<sub>50</sub> frost treatment caused similar AGB reductions, except for *Quercus* which showed moderate reduction compared to the LT<sub>100</sub> treatment (-40%; see Table 1 for AGB and Table S8 for height increments). AGB increments after the LT<sub>100</sub> frost treatments significantly declined with delayed leaf-out timing in *Quercus* and *Carpinus* (Table 1 and Figure 5), with a reduction of -23.6% and -6% per week of delayed leaf-out respectively. Lowest increments were always found in the latest leaf-out date in all species. Similar to maximum recovery, *Prunus* showed a reverse trend after exposure to the LT<sub>50</sub> frost treatment, increasing AGB increment by +24.8% with delayed leaf-out timing (Table 1, interaction Frost\_LT<sub>50</sub> × Leaf-out timing). Reductions in height increments after LT<sub>100</sub> frost treatments followed the same pattern as for AGB increments (Figure S10, Table S8). Lag effects on AGB and height increments during the second growing season after the frost treatments are shown below (long-term recovery).

### 3.3.7 | Are chlorophyll content and senescence affected by leaf-out timing or frost?

In early September, at the beginning of senescence, chlorophyll content (leaf spectral index; LSI) was significantly higher in recovering leaves (after LT<sub>100</sub> frost treatment) of *Fagus* (+15%) and *Carpinus* (+3.1%) but lower in *Quercus* (-5.2%) compared to unfrozen control saplings (no difference detected in *Prunus*; Figure S11 and Table S9).

By the end of senescence in late October, chlorophyll content of frozen saplings was significantly higher in all species (+114% for *Carpinus*, +38% for *Fagus* and +29% for *Prunus*), except for *Quercus*, in which initial differences vanished (Table S9).

Delayed leaf-out timing significantly reduced chlorophyll content in frozen saplings of *Quercus* only, with a -55% reduction from earliest to latest leaf-out timing ( $df = 88$ ,  $t$ -value = -2.8,  $p = 0.006$ ; in September; Figure S11), that persisted throughout autumn senescence. Hence, in frozen saplings of *Quercus*, a delayed leaf-out led to a lower chlorophyll content.

### 3.3.8 | To what extent are NSC levels impacted by frost?

After an abrupt decrease in total NSC concentration (% dry matter) during the emergence of the first cohort leaves, NSC levels quickly

**TABLE 1** Fixed terms of the linear mixed effect model testing the effect of leaf-out timing (LT) and frost (LT<sub>100</sub> and LT<sub>50</sub>) on calculated and standardized above-ground biomass increment (% to initial state) during the 1. and 2. growing season (and both). Interactions are only shown if they significantly improved the model.

Species	Treatments	1. Growing season			2. Growing season			Both Growing seasons		
		df	t-value	p	df	t-value	p	df	t-value	p
<i>Prunus</i>	Leaf-out timing (LT)	136	-1.58	0.12	122	2.37	0.019	116	2.22	0.029
	Frost LT <sub>50</sub>	136	-5.89	<0.0001	122	-2.56	0.012	116	-3.00	0.003
	Frost LT <sub>100</sub>	136	-4.41	<0.0001	122	-0.75	0.45	116	-0.55	0.58
	LT × Frost LT <sub>50</sub>	136	2.52	0.013	—	—	—	—	—	—
	LT × Frost LT <sub>100</sub>	136	0.51	0.61	—	—	—	—	—	—
<i>Carpinus</i>	Leaf-out timing (LT)	133	-3.69	0.001	121	-4.41	<0.0001	101	-4.77	<0.0001
	Frost LT <sub>50</sub>	133	-12.33	<0.0001	121	-4.87	<0.0001	101	-5.70	<0.0001
	Frost LT <sub>100</sub>	133	-12.29	<0.0001	121	-5.69	<0.0001	101	-5.63	<0.0001
	LT × Frost LT <sub>50</sub>	—	—	—	—	—	—	—	—	—
	LT × Frost LT <sub>100</sub>	—	—	—	—	—	—	—	—	—
<i>Quercus</i>	Leaf-out timing (LT)	134	-3.76	0.001	122	-2.24	0.027	119	-2.46	0.015
	Frost LT <sub>50</sub>	134	-3.83	0.001	122	-1.68	0.096	119	-2.25	0.026
	Frost LT <sub>100</sub>	134	-6.76	<0.0001	122	-3.64	0.001	119	-4.39	<0.0001
	LT × Frost LT <sub>50</sub>	—	—	—	—	—	—	—	—	—
	LT × Frost LT <sub>100</sub>	—	—	—	—	—	—	—	—	—
<i>Fagus</i>	Leaf-out timing (LT)	132	-1.29	0.19	123	3.91	0.001	109	3.69	0.001
	Frost LT <sub>50</sub>	132	-6.85	<0.0001	123	-6.83	<0.0001	109	-6.05	<0.0001
	Frost LT <sub>100</sub>	132	-8.19	<0.0001	123	-8.08	<0.0001	109	-7.60	<0.0001
	LT × Frost LT <sub>50</sub>	—	—	—	123	-3.13	0.002	109	-2.27	0.025
	LT × Frost LT <sub>100</sub>	—	—	—	123	-3.91	0.001	109	-3.50	0.001

*d*-value shown in bold when *p* < 0.05.

recovered in unfrozen saplings (Figure 6). Shortly after the emergence of a new cohort of leaves for the frozen saplings (June), total NSC concentrations were significantly reduced in all species (no data for *Carpinus*) compared to unfrozen control saplings in shoot as well as in root tissue (Figure 6 and Table S10). Specifically, total NSC concentration was reduced by  $-78\%$  in *Fagus*,  $-69\%$  in *Quercus* and  $-55\%$  in *Prunus* respectively ( $LT_{100}$  compared to control). However, by the end of the growing season (November), total NSC concentrations of frozen saplings recovered to control levels in both root and shoot tissues with *Carpinus* even significantly overcompensating and hence exceeding control levels by  $+24\%$  (shoot tissue, Table S10). No difference in NSC concentration was found between earliest and latest leaf-out timing, irrespective of species (Figure S12)

### 3.4 | Long-term recovery (lag effect)

#### 3.4.1 | Impact of damaging frosts on AGB and height increment during the second growing season

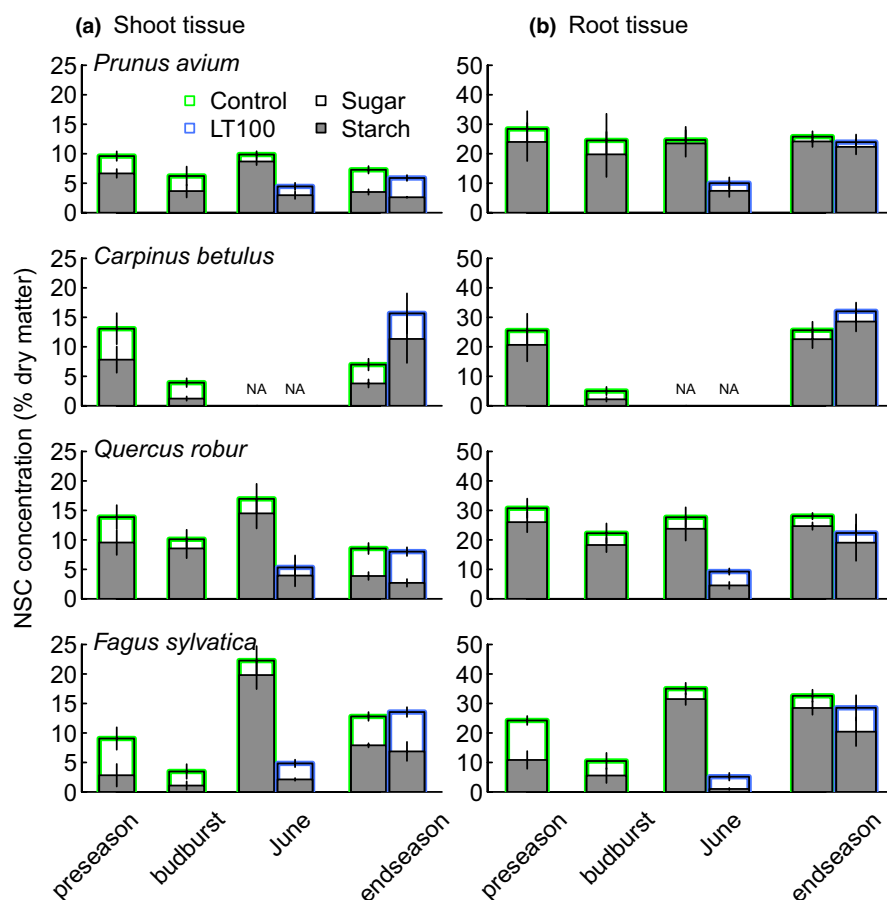
Growth reduction induced by the two frost treatments ( $LT_{100}$  and  $LT_{50}$ ) during the first growing season was also significant during the second growing season (Figure 5b and Table 1). In naturally flushing saplings, AGB increments were still reduced in  $LT_{100}$ -treated saplings

by  $-92\%$  in *Carpinus*,  $-87\%$  in *Quercus* and  $-78\%$  in *Fagus*, respectively, except for *Prunus* which increased AGB increment by  $+22\%$  compared to unfrozen control saplings. However, the  $LT_{50}$  frost treatment still caused a reduction of  $-18\%$  in this species. Delayed leaf-out timing during the first year continued to cause decreased AGB increments in *Carpinus* and *Fagus* during the second growing season, enhancing the effect found in the previous growing season (Table 1). The same pattern was found also for height increments (Figure S10b and Table S8, see therein also effect on both growing seasons). Shoot/root ratio was only found to be significantly lower in saplings of *Prunus* exposed to the  $LT_{50}$  frost treatment ( $-0.14$ ;  $t = -2.0$ ,  $p = 0.046$ ; Figure 5c).

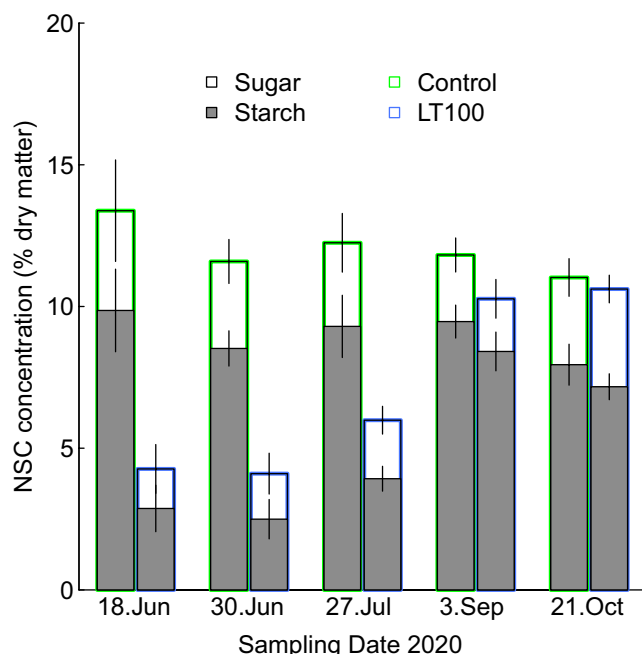
### 3.5 | Recovery after a natural frost damage in a mature beech stand

#### 3.5.1 | Recovery of foliage

First signs of emerging leaves appeared on 30 June 2020, that is, 51 days after the frost event (10 May). Then, within 2 weeks, the canopies of the selected trees reached a greenness of around 70% compared to unfrozen control trees located 300m lower in elevation and remained unchanged until the end of the growing season.



**FIGURE 6** NSC concentration over the season 2019 in shoot (a) and root tissue (b). In June and at the end of season also saplings from the  $LT_{100}$  freezing treatments (blue rectangles) were sampled in addition to unfrozen control saplings (green rectangles). No saplings were harvested in June for *Carpinus* due to limited number of replicates. Error bars indicate  $\pm 1$  SE around the mean of 4 replicates. Note the different scaling of the y-axis for shoots and roots.



**FIGURE 7** Shoot NSC concentration in adult trees at Weissenstein (SO, Switzerland) that were either exposed to a damaging natural spring frost event (10/11 May 2020; blue rectangles) or not (control located 300m below; green rectangles) over the course of the season 2020. Dates indicate the respective sampling occasion. On 30 June, second cohort leaves started to emerge after the canopy dieback. Error bars indicate  $\pm 1$  SE around the mean of 10 replicates.

### 3.6 | NSC

Trees that were exposed to the natural frost event showed a significant depletion in shoot NSC reserves which persisted at least until the end of July (Figure 7, Table S11). On 30 June, total NSC concentration of frozen trees was reduced by  $\sim 55\%$  compared to unfrozen control trees at lower elevation (Figure 7). However, both starch and sugar concentrations recovered to control levels by the end of the growing season (Figure 7).

## 4 | DISCUSSION

This experimental study allowed us to assess the combined effects of leaf-out timing (within a realistic range) and frost ( $LT_{100}$  and  $LT_{50}$  temperature) on recovering leaf area, growth, senescence and NSC concentration in saplings of four species with contrasting freezing tolerances and successional strategy (early to late flushing).

Overall, our results showed that recovering leaf tissue appeared within a month, had higher N-content and reached different target values of maximum leaf-area depending on species ( $>80\%$  for *Prunus* and *Quercus*,  $<40\%$  for *Carpinus* and *Fagus*; naturally flushing saplings compared to unfrozen control saplings). Chlorophyll content in recovering leaves was maintained at a higher level in autumn indicating delayed senescence in all species except *Quercus*. Above-ground

biomass (AGB) increment was significantly diminished after both frost intensities by c.  $\sim 100\%$  (*Fagus* and *Carpinus*), c.  $\sim 60\%$  (*Quercus*) and c.  $\sim 40\%$  (*Prunus*), with shoot growth (apical meristem) being more affected than radial (cambium) growth. Delayed leaf-out timing caused increments and/or maximum recovery to decrease with the latest leaf-out date generally showing poorest performance. NSC concentrations were clearly diminished by at least  $\sim 55\%$  in June (compared to unfrozen control saplings) but fully recovered to control levels by the end of the growing season. These NSC patterns on saplings were corroborated by the assessment of adult beech trees following a natural frost event. Moreover, we show that delayed leaf-out in unfrozen control saplings led to reduced height increments, with all species showing lowest increments in the latest leaf-out timing, for which only *Fagus* (over-) compensated in the second growing season following the leaf-out manipulation (lag effect).

### 4.1 | NSC restoration is prioritized at the expense of growth

As found in many studies manipulating disturbance (drought, shading, defoliation), NSC reserves were restored quickly after frost at the expense of growth activity (Schönbeck et al., 2018; Weber et al., 2018). Presumably, trees aim to maintain a large safety margin in terms of carbohydrate reserves in case of injuries due to extreme biotic or climatic events with frost being one among them (Klein et al., 2016). The substantial lower NSC concentrations found in frozen compared to unfrozen saplings (all species, experiment) and adult trees (beech, Weissenstein) in June are contradictory to the study by Rubio-Cuadrado, Gómez, et al. (2021), where no differences were found in stem tissue without bark of adult beech trees. We assume that changes in reserve consumption/allocation are more sensitive (1) in twig samples including the bark (which we measured as pooled samples), (2) in saplings presumably less capable of compensating and buffering large NSC demands and (3) in shaded compared to unshaded conditions (Weber et al., 2019). For *Carpinus*, in which total NSC levels of frozen saplings exceeded the control by the end of the growing season, it is unclear if NSC levels increased as a result of an active investment or passively increased with declining growth similar to what is observed with growth restrictions at the upper treeline (Hoch & Körner, 2012). Although we could not measure NSC levels for *Carpinus* in June, we assume that depleted NSC reserves contributed to the high mortality observed as was also reported by Weber et al. (2019) for this species in strongly shaded (6% of full sunlight) and defoliated saplings.

### 4.2 | Non-linear effects of leaf-out timing

Recovery indicators (leaf-area, height and AGB increments) were clearly affected by leaf-out timing with the latest timing generally causing lowest performance. However, only for *Carpinus* and *Quercus*, we could find a negative linear trend with delaying leaf-out

timing. It appears that this relationship is true for all species within a certain range of leaf-out timing only. *Prunus* and *Fagus* could not profit or even performed less under most advancing leaf-out scenarios (indicating a non-linear relationship). We assume that other trade-offs such as an increasing herbivory pressure and/or temperature near the freezing tolerance may have led to this lower performance in early spring. Indeed, an increased infestation of aphids (*Phyllaphis fagi* L. and *Myzus cerasi* F.) was found in saplings with most advanced leaf-out timing, for *Fagus* and *Prunus* respectively (personal observation). In addition, the strong photoperiodic control known for *Fagus* (Vitasse & Basler, 2013) may have slow down further leaf development and hence recovery. Menzel et al. (2015) also reported a shorter recovery time of earlier flushing adult beech trees, although this effect could be the result of enhanced freezing resistance after leaf maturation (Allevato et al., 2019).

### 4.3 | Presumably higher photosynthetic capacity in second cohort leaves

The higher N-content per g dry weight in second cohort leaves emerging after the frost treatment indicates that these saplings had sufficient access to critical nutrients and the new investment might compensate for their reduced canopy foliage by higher rates of photosynthesis (Reich et al., 1995). This is in line with results from Rubio-Cuadrado, Gómez, et al. (2021) who found increased N concentrations in recovering leaves of frozen compared to unfrozen adult trees of *Fagus sylvatica*. Hence, even in natural conditions, where trees are likely strongly competing for nutrients, adult trees seem to allocate increased amounts of nitrogen to the recovering leaf tissue. There are strong indications that beech uses stored N early in the season to support growth emerging leaves (when low soil temperature prevents substantial N uptake by roots) whereas later in the season newly taken up N strongly contributes to the nitrogen pool allocated in the tree and supporting the demand of above-ground organs (Gessler et al., 1998). We therefore conclude that saplings as well as adult trees are neither carbon nor nitrogen limited and dispose of enough reserves to build up an entire canopy. Consistently, substantially higher chlorophyll content in frozen *Fagus* saplings compared to unfrozen ones was found at the beginning of autumn, in agreement with the higher N-content in recovering leaves.

### 4.4 | Species-specific strategies to cope with damaging frost events

We observed a clear ranking of recovery performance among the species investigated that was not related to successional stage. Both pairs of early and late flushing species showed contrasting responses with rather species-specific responses:

Fully frozen saplings (LT<sub>100</sub>) of *Prunus* lost their main shoot down to the stem basis and resprouted from there, forming a new apical

shoot (Figure S13), that, surprisingly, reached the final height of control saplings after the second growing season. Partly frozen (LT<sub>50</sub>) saplings recovered less but still considerably reflecting the high growing potential of this species.

*Quercus* showed minor losses and a fast recovery after frost treatments. Second cohort leaves emerged quickly from dormant buds which amount to c. 60% of total buds (personal observation also on adult trees), barely showing any visible impact of the frost treatments comparable to findings of other studies (Rubio-Cuadrado, Gómez, et al., 2021; Vander Mijnsbrugge et al., 2021).

*Fagus* resprouted from adventive buds and produced smaller leaves (personal observations) and a visibly reduced canopy found also by Menzel et al. (2015) and Rubio-Cuadrado, Camarero, et al. (2021). Higher chlorophyll content of frozen leaves which remained longer in autumn presumably compensated for assimilation losses to some extent (Vander Mijnsbrugge et al., 2021; Zohner et al., 2019). Such stress-related delay in senescence and compensation of assimilation losses has also been observed in beech recovering from drought (Hagedorn et al., 2016) and other studies on frost events (Zohner et al., 2019).

Frost-related dieback of the apical meristem may lead to a more shrub-like stature ('stem forking'; Rosique-Esplugas et al., 2022) with increasing frequency of frost damages. Beech trees at the high elevation site in the Jura mountains appeared to have indeed several main axes with more frequent branching (personal observation). It seems that the strong photoperiodic control over leaf-out in *Fagus sylvatica* triggers earlier leaf-out at higher elevations than could be expected from temperature alone (Vitasse & Basler, 2013). This results in a higher risk of frost damage during late spring often observed at higher elevations for this species (Bascietto et al., 2018). In some cases, frost-damaged trees occur within an elevational belt (Allevato et al., 2019) depending on frost intensity, leaf-out timing and state of leaf maturation (Menzel et al., 2015).

*Carpinus* suffered from high mortality after the LT<sub>100</sub> frost treatments. Saplings with a very low recovering leaf area appeared to die mainly in the following growing season. Such a cascading mortality effect was also observed in saplings of *Aesculus glabra* after a natural frost event (Augspurger, 2011) and highlights the importance to assess frost effects beyond the first growing season. Otherwise, *Carpinus* overcompensated NSC reserves presumably as a result of diminished growth and delayed senescence.

### 4.5 | Limitations of the study

To assess the isolated effect of leaf-out timing on the speed and the extent of recovery after frost, one needs to control for environmental conditions, which will otherwise largely determine the growth responses after leaf-out. Hence, part of the effects of leaf-out timing reported in this study reflect the weather conditions after plantation in the field. Nevertheless, by doing so, we simulated a variety of realistic 'starting scenarios' that introduced



variability to capture more reliable plant responses within a single year. While saplings do not necessarily reflect responses of adult trees (e.g. advanced leaf-out timing; Vitasse, 2013), they are often more sensitive to stress conditions and are therefore the bottleneck of long-term persistence of a species through the critical phase of recruitment and establishment (Bolte & Ibisch, 2007). Bianchi et al. (2019) found indeed very high mortality rates in seedlings of deciduous trees most likely related to spring frost events because of their opportunistic behaviour. The pronounced lag effect of frost on growth increments (during the second growing season) reflects mainly the difference in saplings' stature caused by the frost treatments during the first growing season. It is likely, that such a carry-over effect diminishes with increasing tree size and maturation as indicated by the high resilience to frost found in tree ring studies (Príncipe et al., 2017; Vitasse et al., 2019), though this carry-over effect has also been found in adult beech population in central Spain (Rubio-Cuadrado, Camarero, et al., 2021).

Earliest flushing sapling benefit from more light as the canopy is still open under natural conditions during this time of year. Thus, by planting saplings directly under a shading net (simulating canopy closure), we may have underestimated the advantage of earlier flushing, especially in shade-intolerant species like cherry.

Frost as well as drought events are expected to increase in frequency with an increasing possibility that both events occur in the same year, which may cause a tipping point for tree resilience. Future experiments should therefore aim at assessing the effect of multiple frosts in several subsequent years as well as combined effects of other stressors like drought events within the same growing season. Besides, it remains unknown to what extent trees in natural stands could profit from lower competition, when neighbouring trees are damaged by a spring frost.

## 5 | CONCLUSION

Our study demonstrates that recovery from frost events involves a relatively fast (saplings) but slower (adult trees) regreening with leaves of presumably higher photosynthetic efficiency, that are maintained longer in autumn. NSC reserves recover always at the expense of growth and overall recovery performance declines with delayed leaf-out timing. However, we observed large differences in recovery performance among the tested species that were not related to their life-history strategy (pioneer vs. late successional species). Species' strategies to cope with frost are various and included the deployment of reserve buds (oak), the ability to resprout from the stem base (cherry), higher N-content in recovering leaves (all species), overcompensation of NSC reserves (hornbeam) and delayed senescence (hornbeam and beech).

A common explanation to optimize leaf-out timing is the trade-off between freezing damage and competition through a shorter growing season. Here, we propose another key trait, the recovery potential, that enables a species to take greater risks even at the cost of losing the whole leaf tissue of first cohort buds in some

years. In an evolutionary perspective, this means that fast and well-recovering trees are selected to leaf-out earlier thereby consistently taking more risk than could be expected from the performance of avoiding frost damages alone. Such competitive advantages may reshuffle the cards of evolution with increasing frequency of damaging frost events and (combined with) other stressors.

## AUTHOR CONTRIBUTIONS

Frederik Baumgarten and Yann Vitasse planned and designed the experiment. Frederik Baumgarten conducted the experiment, analysed the data and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.69p8cz95f> (Baumgarten et al., 2022).

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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