Supporting information
No risk – no fun: penalty and recovery from spring frost damages in deciduous temperate trees

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Tables

Table S1: Summary of **key variables** that characterise the strategy of the study species as well as height and diameter of the saplings prior to the experiment. LT_{50} and LT_{100} are the temperature thresholds in °C found in the literature at which 50% and 100% of the newly emerging leaves die, respectively. Applied values represent the measured mean minimum values during the artificial frost treatments.

Species	Literature values		Applied values		Shade tolerance index***	Initial stature Mean±SD	
	LT_{50}*	LT_{50}**	LT_{50}	LT_{100}		height (cm)	diameter (mm)
Prunus avium	-8.3±0.2	NA	-7.0	-10.0	3.33±0.33	83±10.5	7.2±1.0
Carpinus betulus	-7.2±0.1	-5	-7.0	-10.0	3.97±0.12	74±13.5	6.0±0.9
Fagus sylvatica	-6.8±0.2	-3	-4.0	-7.0	4.56±0.11	53±7.5	6.0±0.8
Quercus robur	-4.3±0.1	NA	-4.0	-7.0	2.45±0.28	25±6.5	4.5±0.7

* Values found by Vitasse *et al.* (2014)
**Values found by Till (1956)
***Shade tolerance scales range from 0 (no tolerance) to 5 (maximal tolerance), extracted from Niinemets and Valladares (2006).

Table S2: Number of replicates per species used. Indicated are only treatment combinations (frost and order of budburst; BB) for NSC, C/N and LSI measurements for which reduced sample sizes were used. For all other variables not indicated here 12 replicates were used (main experiment).

Treatment			NSC				C/N	LSI
Frost	BB order	main exp.	Feb*	BB*	June*	Nov	June	Sept-Nov
LT_{100}	1	12			4	4	-	4
	2	12			-	-	-	4
	3	12			-	-	-	4
	4	12			4	4	-	4
	natural	12			4	4	8	4
LT_{50}	1	12			-	-	-	-
	2	12			-	-	-	-
	3	12	4 (prior to treatments)	4 (prior to treatments)	-	-	-	-
	4	12			-	-	-	-
	natural	12			-	-	-	-
control	1	12			4	4	-	4
	2	12			-	-	-	4
	3	12			-	-	-	4
	4	12			4	4	-	4
	natural	12			4	4	8	4
Total		**180**	**-**	**-**	**24**	**24**	**16**	**40**

*additional saplings, not included in the main experiment

Table S3: Frost related **mortality** (%) after the second growing season for the 4 study species and the respective frost treatments.

species	Frost treatment		
	control	LT_{50}	LT_{100}
Prunus	0	5	8
Carpinus	0	13	32
Quercus	0	3	2
Fagus	0	12	8

Table S4: Effect of leaf-out timing (days relative to natural leaf-out) and frost intensity (LT_{50} vs. LT_{100}) on **maximum recovery** (% to control). Interactions are shown only when significant.

species	Coefficient	df	t-value	p
Prunus	Leaf-out Timing (LT)	88	1.59	0.115
	Frost LT100	88	0.44	0.663
	LT x Frost LT100	**88**	**-1.92**	**0.058**
Carpinus	Leaf-out Timing (LT)	76	-1.16	0.250
	Frost LT100	76	-1.18	0.240
Quercus	Leaf-out Timing (LT)	91	-1.40	0.165
	Frost LT100	**91**	**-3.03**	**0.003**
Fagus	Leaf-out Timing (LT)	87	-1.97	0.052
	Frost LT100	**87**	**-2.25**	**0.027**

Table S5: Results of a linear model testing the effect of frost (LT_{100}) on **C/N-ratio** of second cohort leaves for three species (no data for *Carpinus*). N = 8.

species	t value	p
Prunus	-2.60	0.021
Quercus	-3.40	0.0043
Fagus	-4.52	0.0005

Table S6: Effect of leaf-out timing (days relative to natural leaf-out) on calculated and standardized **above-ground biomass increment** (% to initial state) for unfrozen control saplings during 1. and 2. growing season, extracted from a linear mixed effect model.

species	1. Growing season			2. Growing season		
	df	t-value	p	df	t-value	p
Prunus	44	-1.22	0.23	36	1.48	0.15
Carpinus	40	**-2.21**	**0.033**	31	-1.88	0.070
Quercus	43	-1.41	0.17	35	-1.33	0.19
Fagus	38	-0.54	0.59	**31**	**2.63**	**0.013**

Table S7: Effect of leaf-out timing (days relative to natural leaf-out) on standardized **height increment** (% to initial state) for unfrozen control saplings during 1. and 2. growing season, extracted from a linear mixed effect model.

species	df	t-value	p	df	t-value	p
		1. Growing season			**2. Growing season**	
Prunus	44	-0.973782	0.3355	36	1.480429	0.1475
Carpinus	40	**-3.010375**	**0.0045**	31.00	-1.141	0.2627
Quercus	43	**-3.4083**	**0.0014**	35	-0.224575	0.8236
Fagus	38	**-2.502058**	**0.0168**	31	1.948573	0.0604

Table S8: Fixed terms of the linear mixed effect model testing the effect of leaf-out timing (LO) and frost (LT_{100} and LT_{50}) on standardized **height increment** (% to initial state) during the 1. and 2. growing season (and both). Interactions are only shown if they significantly improved the model.

Spec	Treatments	df	t-value	p	df	t-value	p	df	t-value	p
			1. Growing season			**2. Growing season**			**Both Growing seasons**	
Prunus	Leaf-out Timing (LO)	132	-0.72	0.48	116	**2.32**	**0.022**	116	**2.24**	**0.027**
	Frost LT_{50}	132	**-7.39**	**<0.0001**	116	0.35	0.73	116	**-3.79**	**0.0002**
	Frost LT_{100}	132	**-5.08**	**<0.0001**	116	1.86	0.065	116	-1.47	0.14
	LO x Frost LT_{50}	132	1.81	0.073	-	-	-	-	-	-
	LO x Frost LT_{100}	132	-1.39	0.17	-	-	-	-	-	-
Carpinus	Leaf-out Timing (LO)	119	**-3.28**	**0.001**	101	**-3.19**	**0.0019**	101	**-4.22**	**0.0001**
	Frost LT_{50}	119	**-13.78**	**<0.0001**	101	-0.39	0.70	101	**-7.23**	**<0.0001**
	Frost LT_{100}	119	**-13.35**	**<0.0001**	101	0.79	0.43	101	**-6.28**	**<0.0001**
	LO x Frost LT_{50}	-	-	-	-	-	-	-	-	-
	LO x Frost LT_{100}	-	-	-	-	-	-	-	-	-
Quercus	Leaf-out Timing (LO)	133	**-4.23**	**<0.0001**	115	-0.20	0.84	119	**-3.22**	**0.002**
	Frost LT_{50}	133	-1.84	0.067	115	-0.15	0.88	119	-1.72	0.089
	Frost LT_{100}	133	**-4.37**	**<0.0001**	115	-0.64	0.52	119	**-3.69**	**0.0003**
	LO x Frost LT_{50}	-	-	-	-	-	-	-	-	-
	LO x Frost LT_{100}	-	-	-	-	-	-	-	-	-
Fagus	Leaf-out Timing (LO)	126	**-3.34**	**0.001**	109	**2.47**	**0.015**	109	1.80	0.074
	Frost LT_{50}	126	**-5.19**	**<0.0001**	109	**-3.96**	**0.0001**	109	**-4.64**	**<0.0001**
	Frost LT_{100}	126	**-7.20**	**<0.0001**	109	**-5.66**	**<0.0001**	109	**-6.63**	**<0.0001**
	LO x Frost LT_{50}	-	-	-	109	-0.84	0.40	109	-0.71	0.48
	LO x Frost LT_{100}	-	-	-	109	**-3.21**	**0.002**	109	**-3.05**	**0.003**

Table S9: Effect of frost (LT_{100}) on leaf spectral index (LSI) at the beginning of senescence (early September) and towards the end of senescence (late October) for all leave-out dates pooled. Output of a linerar mixed effect model using individual tree as random factor.

species	df	t value	p	df	t value	p
Prunus	114	-0.66	0.51	**115**	**2.14**	**0.034**
Carpinus	102	**2.10**	**0.039**	**101**	**2.64**	**0.010**
Quercus	117	**-2.92**	**0.0042**	117	-0.95	0.34
Fagus	113	**7.24**	**<0.0001**	113	**1.94**	**0.054**

(header row: Begin September | End October)

Table S10: Results of a linear model testing the effect of frost (LT_{100}) on **total NSC concentration** (% dry matter) in June and end of summer for shoot and root tissue separately. Only saplings flushing in natural conditions were used. N = 4.

species	June shoot t value	p	June root t value	p	end of summer shoot t value	p	end of summer root t value	p
Prunus	**-6.52**	**0.0006**	**-3.01**	**0.024**	-1.74	0.13	-0.59	0.58
Carpinus	NA	NA	NA	NA	**2.46**	**0.049**	1.56	0.17
Quercus	**-3.57**	**0.012**	**-5.23**	**0.002**	-0.45	0.67	-0.89	0.41
Fagus	**-6.89**	**0.0005**	**-12.63**	**<0.0001**	0.67	0.53	-0.86	0.42

Table S11: Effect of a natural frost on **total NSC concentration** (% dry matter) for an adult beech population at **Weissenstein**, Switzerland at 5 sampling dates proceeding the frost event. Linear mixed effect models with tree individuum as a random factor to account for repeated measurements.

Date	df	t-value	p
18 June	4	**-4.59**	**0.01**
30 June	9	**-7.07**	**0.0001**
27 July	9	**-5.47**	**0.0010**
03 Sep	9	-1.70	0.12
21 Oct	9	-0.62	0.55

Figures

Fig. S1: A cohort of *Prunus* saplings that reached the stage of leaf unfolding are prepared for the freezing treatment in a climate chamber. Sapling container were put on top of a warm water bath, surrounded by insulation material.
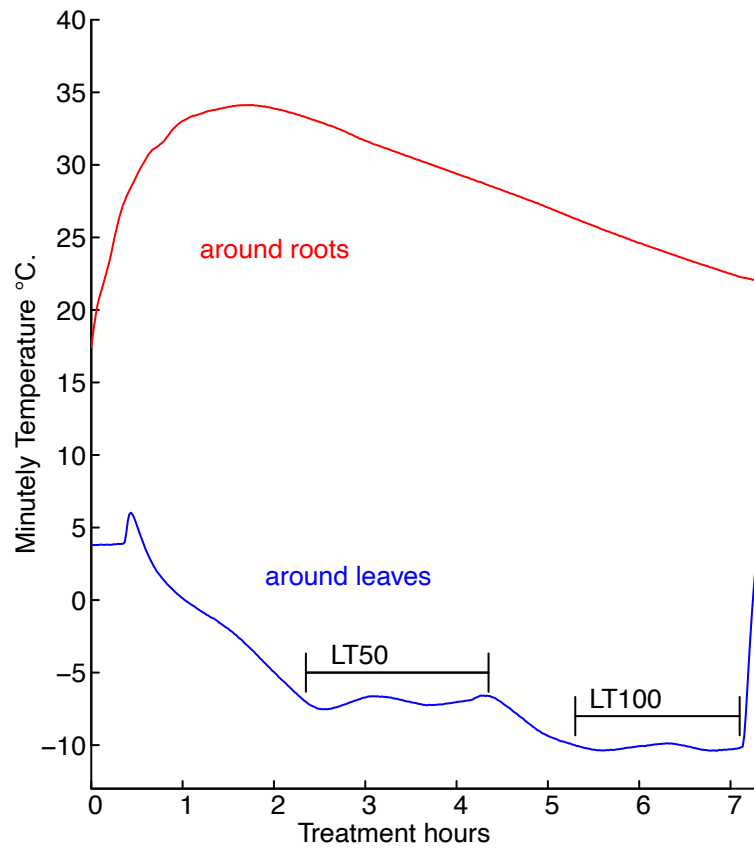
Fig. S2: Minutely temperature in the climate chamber during an LT_{100} freezing treatment (i.e. temperature killing all leaf tissue). Shoot and leaves experienced air temperature (blue line), while temperature underneath the sapling containers (red line) was buffered with the vapour of a warm water bath to prevent freezing damage. 12 saplings per flushing date and species were removed after the end of the LT_{50} target value (aiming to damage ~50 of the leaf tissue) and transferred to a cooling chamber around 3°C.

Fig. S3: Experimental setup at the WSL research facility near Zurich, Switzerland. The shading net was removed after the first growing season. To reduce edge effects, species position was changed for each of the 3 blocks (note the green cherry trees arranged from the front right to the back left).
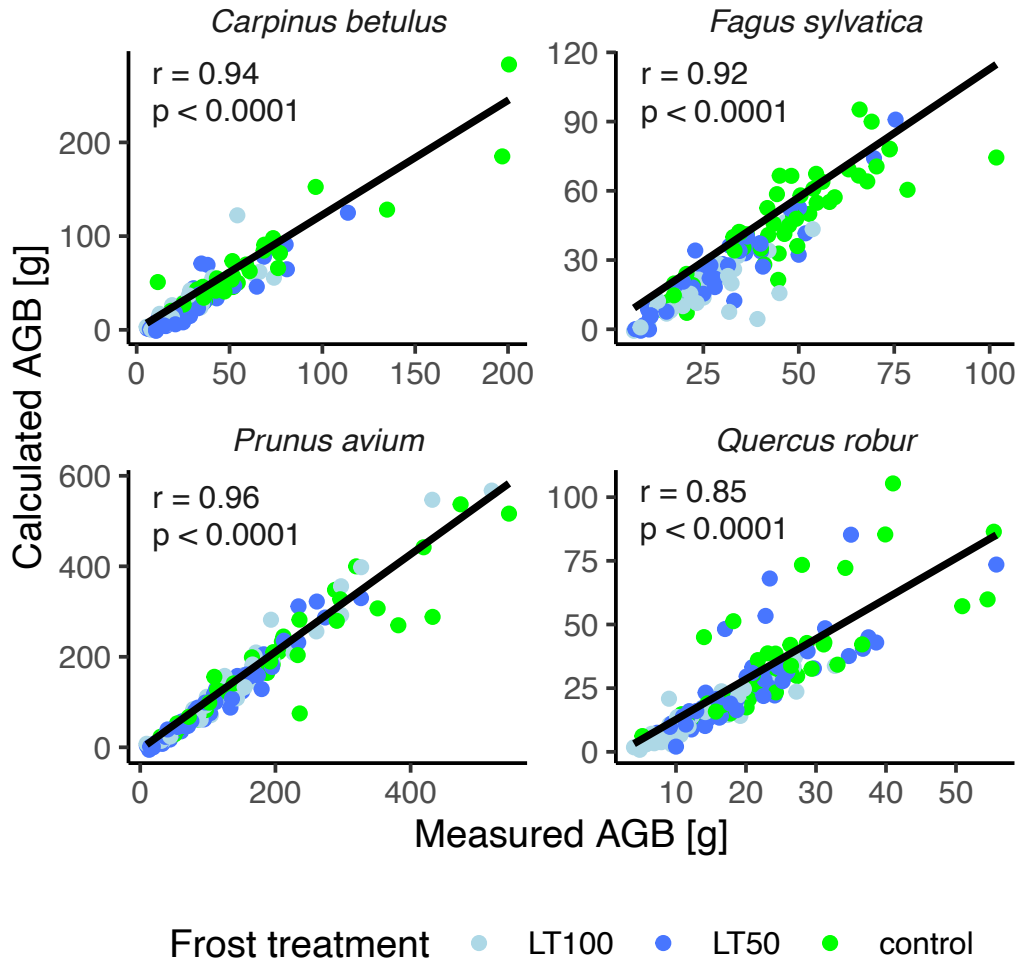
Fig. S4: Pearson correlation between calculated and measured above-ground biomass (AGB) after the second growing season. Calculations were made based on the allometric equation and species-specific parameters provided by Annighöfer *et al.* (2016) as well as the height and diameter of saplings.

Fig. S5: The experimental site at Weissenstein, SO (Switzerland) in the Jura mountains were the natural frost damage occurred on 10/11 May 2020. Visible canopy defoliations of European beech on the studied slope were observed in a belt between 1'385m (top) and ~1'150m a.s.l. The photo was taken on 18 June 2020.

Fig. S6: Full canopy defoliations of European beech at the higher site at Weissenstein, 1'385m a.s.l. on 18 June 2020. Visible Green leaves stem from the more freezing resistant sycamore (*Acer pseudoplatanus*) and rowan (*Sorbus aucuparia)* trees.

Fig. S7: Example of an artificially frozen (left) and the unfrozen control (right) sapling of hornbeam (*Carpinus betulus*) after plantation under the shading net. Both LT_{50} and LT_{100} led to full canopy defoliation.
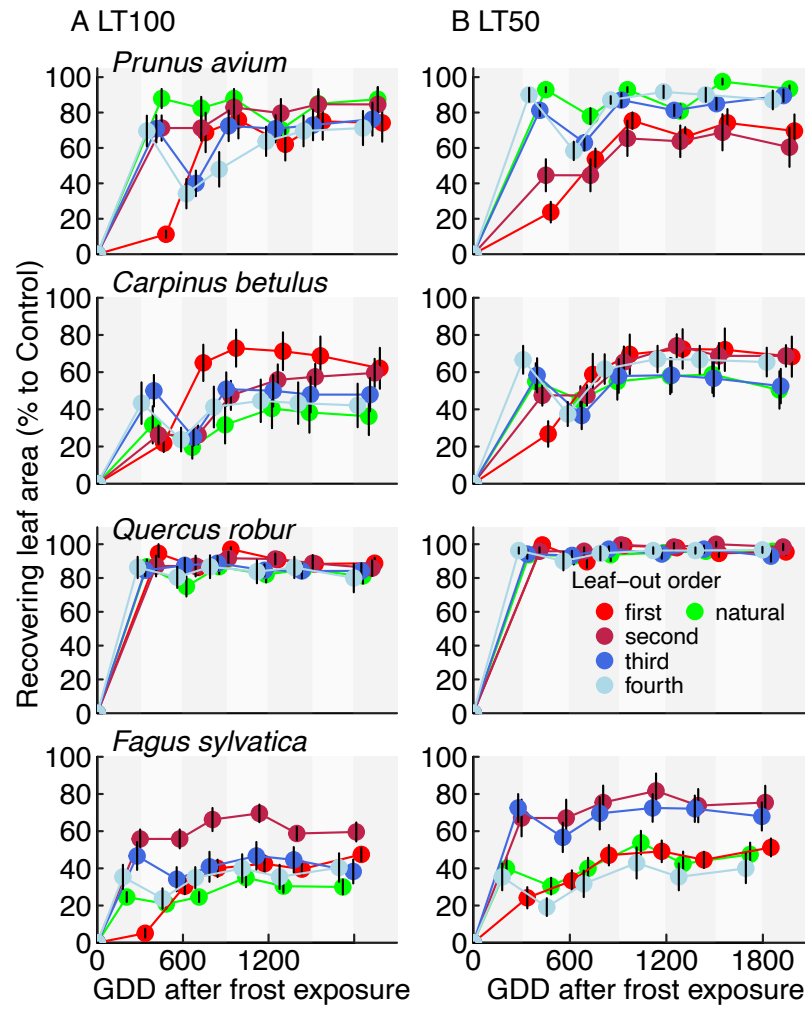
Fig. S8: Recovering leaf area of fully (LT$_{100}$, A) and partly (LT$_{50}$, B) frozen saplings compared to unfrozen control saplings in relation to growing degree days (GDD, threshold=5°C) experienced after frost exposure over the course of the first growing season. Dots represent the mean ± 1 SE of 12 replicates for the natural (green) and the order of artificial (red to blue) leaf-out dates. See method section for detailed leaf-out dates.
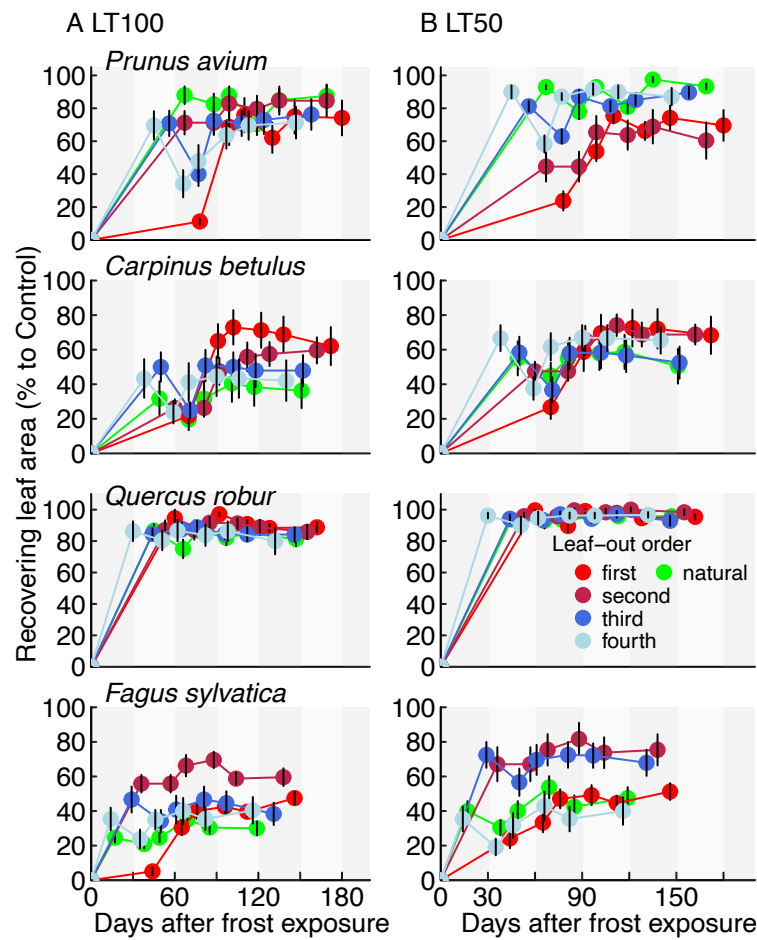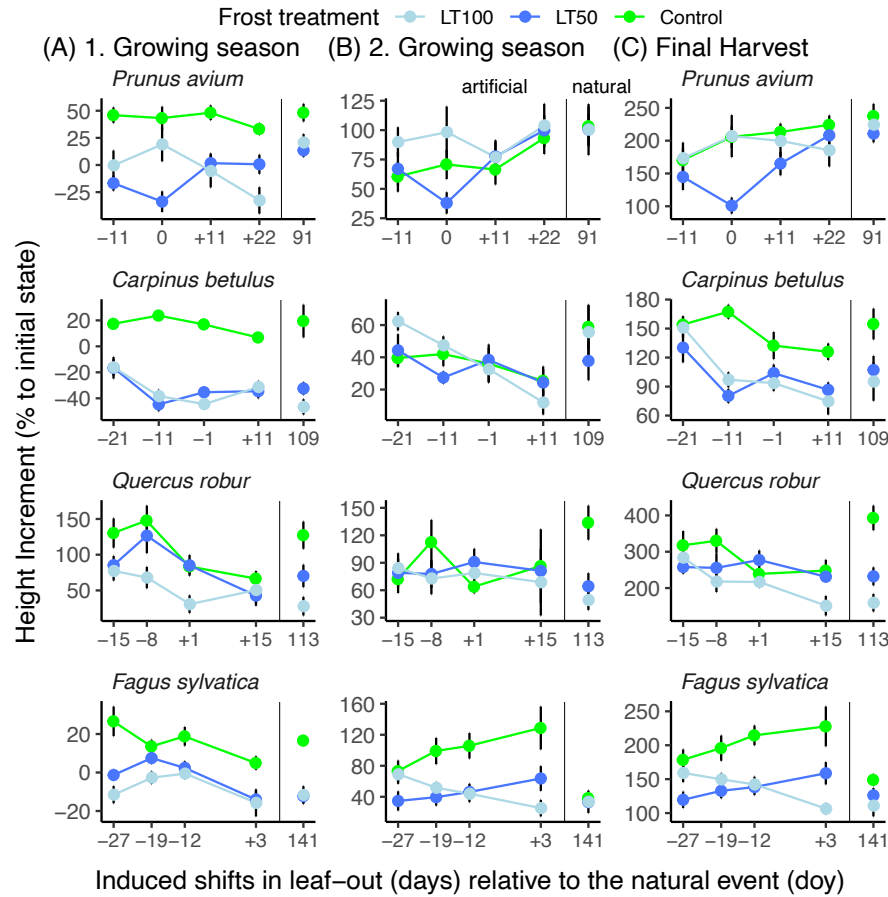
Fig. S9: Recovering leaf area of fully (A, LT_{100}) and partly (B, LT_{50}) frozen saplings compared to unfrozen control saplings in relation to the days after the frost exposure. Dots represent the mean ± 1 SE of 12 replicates for the natural (green) and the order of artificial (red to blue) leaf-out dates. See method section for detailed leaf-out dates.

Fig. S10: Standardized Median height increment (% to initial height) after artificial frost events (LT_{100} and LT_{50} freezing target values) and control treatment during first (A) and second (B) growing season as well as at the final harvest (C) in response to manipulated leaf-out timing in the first year. The four artificial leaf-out dates are indicated as days relative to the natural leaf-out date (doy, day of year, right dots 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 ± 1 SE. Note the different scales on the y-axis.
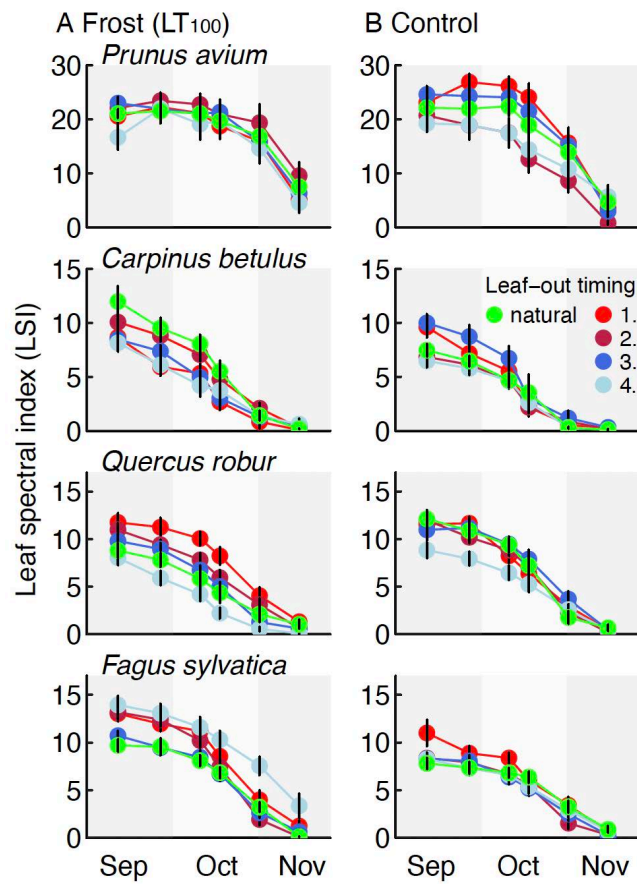
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Fig. S11: Leaf spectral index (LSI) as a proxy for chlorophyll content and senescence over autumn 2019 after artificial frost (LT$_{100}$ freezing target value; A) and control treatment for the four artificial (red to blue) as well as the natural (green) leaf-out timing. N=4 replicates for each measurement date. Note the different scales on the y-axis.
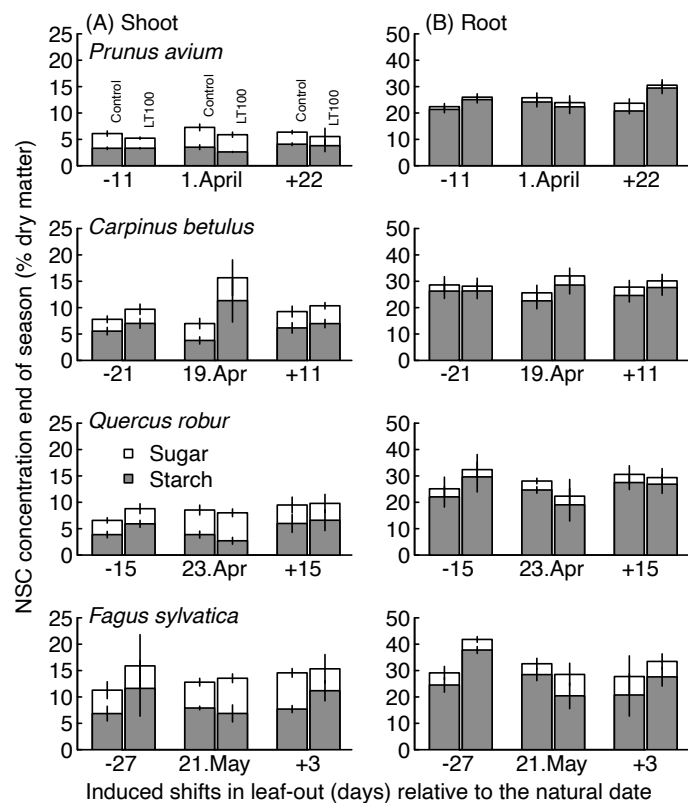
Fig. S12: NSC concentration in frozen (LT_{100}) and control saplings with most advanced and delayed, as well as natural leaf-out dates at the end of the season 2019 in shoot (A) and root tissue (B). Dates indicate the mean date of leaf-out (stage 3). Error bars indicate ±1SE around the mean of 4 replicates. Note the different scaling of the y-axis for shoots and roots.

Fig. S13: Stem recovery of *Prunus avium*. Initial main shoot (labelled and dead; right) died following the LT$_{100}$ frost treatment. After resprouting from the stem basis the new shoot (thick; left) reached again the height of unfrozen control saplings by the second growing season.

References

Annighöfer P, Ameztegui A, Ammer C, Balandier P, Bartsch N, Bolte A, Coll L, Collet C, Ewald J, Frischbier N. 2016. Species-specific and generic biomass equations for seedlings and saplings of European tree species. *European Journal of Forest Research* **135**(2): 313-329.

Niinemets U, Valladares F. 2006. Tolerance to shade, drought, and waterlogging of temperate Northern Hemisphere trees and shrubs. *Ecological Monographs* **76**(4): 521-547.

Till O. 1956. Über die frosthärte von pflanzen sommergrüner laubwälder. *Flora oder Allgemeine Botanische Zeitung* **143**(4): 499-542.

Vitasse Y, Lenz A, Hoch G, Körner C. 2014. Earlier leaf-out rather than difference in freezing resistance puts juvenile trees at greater risk of damage than adult trees. *Journal of Ecology* **102**(4): 981-988.