Developmental constraints mediate the summer solstice reversal of climate effects on European beech bud set

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Abstract

Predicting the growing season of temperate zone trees under climate change requires factoring in developmental constraints that determine tree resource allocation. Recent work has identified the summer solstice (21 June) as a phenological "switch point," with pre-solstice warming advancing autumn bud set and post-solstice warming delaying it. However, the flexibility of this switch point remains poorly understood. We performed trans-solstice climate manipulation experiments on potted European beech (*Fagus sylvatica*) saplings to test (i) how spring leaf-out timing and June-August temperatures influence growth cessation (bud set), and (ii) whether daytime and nighttime temperatures before and after the solstice have different effects, given that trees primarily grow at night. Each day delay in spring leaf-out delayed bud set by 0.24 ± 0.06 days on average. July cooling delayed bud set by 4.9 ± 2.6 days in late-leafing individuals but had no effect in early-leafing ones. Conversely, August cooling advanced bud set by 4.4-4.5 ± 2.6 days in all trees. Daytime cooling before the solstice had no effect, while after the solstice it advanced bud set. Nighttime cooling always delayed bud set. Our findings support the Solstice-as-Phenology-Switch model and highlight the central role of developmental progression in constraining the growing seasons of

temperate trees. Faster early-season development–especially under nighttime warming–advances the point at which trees become sensitive to late-season cooling, thereby triggering senescence earlier. To improve growing season length projections, phenological models must account for these developmentally-mediated and diel-specific responses to warming.

Keywords: Carbon cycle | Climate change | Global warming | Plant-climate interactions | Plant development| Plant growth | Phenology

Introduction

Climate shifts are leading to rapid, species-specific changes in phenology and ecosystem productivity (Boisvenue & Running, 2006; Menzel *et al.*, 2006; Yang & Rudolf, 2010; Thackeray *et al.*, 2016). In temperate forests, changes in the timing of spring leaf-out, autumn leaf-senescence and bud set are modifying water, energy and carbon cycles (Peñuelas *et al.*, 2009; Richardson *et al.*, 2013), with extended growing seasons increasing net ecosystem carbon uptake by up to 9.8 gC m^{-2} day^{-1} (Keenan *et al.*, 2014). Therefore, understanding the interaction between climate change and temperate forest phenology is pivotal to improving forecasts of community dynamics and carbon sequestration.

The past few decades have seen delays in the onset of temperate autumn phenology, but these changes are much smaller in magnitude compared to the advances in spring leaf-out observed during the same period (Gill *et al.*, 2015; Piao *et al.*, 2019). This is unexpected, given that experiments have demonstrated a high sensitivity of leaf senescence to autumn warming, with phenological responses even surpassing the temperature sensitivity (days per

°C) of spring leaf-out (Fu *et al.*, 2018). One possible explanation for this discrepancy is that other factors may counterbalance the effects of autumn warming, with some studies finding that earlier leaf-out leads to earlier leaf senescence (Fu *et al.*, 2014; Keenan & Richardson, 2015; Zani *et al.*, 2020). This connection between spring and autumn phenophases could be due to developmental and nutrient constraints that affect carbon source-sink dynamics in temperate trees (Paul & Foyer, 2001; Zani *et al.*, 2020; Zohner *et al.*, 2023; Gessler & Zweifel, 2024).

The Solstice-as-Phenology-Switch hypothesis, supported by experiments and monitoring data, posits that warmer air temperatures before the summer solstice accelerate tree growth and development, leading to an earlier onset of leaf senescence, whereas warmer temperatures after the solstice slow the progression of leaf senescence (Zohner *et al.,* 2023). This reversal in temperature effects provides an explanation for the less pronounced shifts in autumn phenology compared to spring (Piao *et al.*, 2019). The effect reversal after the solstice points to the role of photoperiod in regulating plant physiology (Bauerle *et al.*, 2012), with trees becoming increasingly responsive to cooling temperatures as days continue to shorten. The precise timing of this reversal appears to be flexible and has been advancing in recent decades, potentially due to faster development driven by climate warming (Zohner *et al.*, 2023). For temperate trees, the key outcomes of development are production of viable seeds, cessation of primary and secondary growth, and maturation of tissues including leaf or flower buds (bud set) ready for overwintering before the onset of frost (Rohde & Bhalerao, 2007; Tanino *et al.*, 2010; Cooke *et al.*, 2012). Because opportunities for development will vary among years, the timing of the temperature effect

reversal should be flexible. However, this flexibility has yet to be demonstrated in processes directly related to development such as autumn bud set.

In this study, we investigate how changes in air temperature around the summer solstice affect primary growth cessation, using bud set as a key physiological marker for the end of primary growth and the beginning of autumn phenology (Zohner & Renner, 2019). Specifically, we explore the effects of pre- and post-solstice cooling, and of daytime versus nighttime cooling, on bud set timing. Diel temperature variations are relevant because trees mainly grow at night when temperatures and water deficit are lower than during the day (Zweifel *et al.*, 2021). Between 1950 and 2004, nighttime temperatures rose faster than daytime temperatures (Vose et al., 2005), but from 1991 to 2020 this appears to have reversed (Zhong et al., 2023). We also experimentally created early-leafing vs. late-leafing trees to assess whether slowed early-season development postpones the reversal date of temperature effects on autumn phenology (Fig. 1). As depicted in our conceptual model (Fig. 1), tree individuals are dynamic systems that continuously integrate and adapt their physiological responses to environmental conditions over time. Their responses to temperature between June and August and between day and night are therefore expected to differ, an expectation tested in this study.

Results

Experiment 1

Linear mixed-effects modelling showed that across all treatments, late-leafing trees cooled during spring experienced a delay to their bud set of 4.6 ± 1.3 days (mean ± 2 SE). Across all treatment pairings, the mean bud set date for the late-leafing group always occurred later

than for the early-leafing group (Fig. S2). On average, each day delay in spring leaf-out was associated with a delay of 0.24 ± 0.06 days in bud set timing.

The effect of July cooling differed strongly between the early- and late-leafing trees: Within the early-leafing group, moderate cooling in July lead to a small, non-significant delay in bud set compared to the ambient July treatment (1.4 ± 2.6 days). July cooling had a much greater impact on late-leafing trees, leading to a 4.9 ± 2.6-day delay in bud set compared to the ambient July treatment in the late-leafing group.

In contrast to July cooling, the effects of August cooling did not differ between early- and late-leafing trees, advancing bud set in all treatment groups (Fig. 2b). Cooling in August lead to a 4.5 ± 2.6-day and 4.4 ± 2.6-day advancement in bud set for early- and late-leafing trees, respectively. The extreme cooling treatments showed similar patterns, although slightly less clearly (Fig. S3). Effects of absolute and relative growth on bud set date can be found in the supporting information (Figs. S4-S5). Bud set timing had no effect on final bud length (Fig. S6).

Experiment 2

Linear mixed-effects modelling showed that pre-solstice full-day (day and night) cooling delayed autumn bud set by 4.1 ± 3.6 days (mean ± 2SE) (Fig. 3). Pre-solstice nighttime cooling had a similar effect, delaying bud set by 4.2 ± 3.6 days. By contrast, pre-solstice daytime cooling had no significant effect on bud set (0.2 ± 3.7 days). Post-solstice full-day cooling advanced autumn bud set by 5.2 ± 3.6 days. Similarly, post-solstice daytime cooling

advanced bud set by 5.3 ± 4.3 days. Conversely, post-solstice nighttime cooling delayed bud set by 3.8 ± 3.5 days.

Discussion

In this study, we conducted experiments on European beech to test how primary growth cessation and bud set respond to monthly and diel temperature changes around the summer solstice. We found large differences in the responses between trees subjected to daytime versus nighttime cooling before and after the solstice (Fig. 3) and between early- and late-leafing trees (Fig. 2). These differences suggest that early-season developmental timing not only influences the timing of autumn bud set *per se*, but also plays a critical role in determining the timing of the summer solstice effect reversal. Therefore, our findings support our conceptual model (Fig. 1), highlighting development as a key factor underpinning the Solstice-as-Phenology-Switch hypothesis. In the following sections, we discuss the effects of pre-solstice and post-solstice air temperature on development, along with the distinct impacts of daytime versus nighttime temperature variations.

Early-season development rates alter responses to late-season temperature

Our trans-solstice climate manipulation experiments showed that trees delayed their aboveground primary growth cessation (bud set) in response to slow/late early-season development (delayed spring leaf-out). Regardless of the summer cooling treatments, late-leafing trees consistently set buds after their early-leafing counterparts (Fig. S2), with each day delay in spring leaf-out delaying bud set by an average of 0.24 days. This is in line with previous studies demonstrating a tight linkage between within-year variations in spring and autumn phenology (Fu *et al.*, 2014; Keenan & Richardson, 2015; Signarbieux *et al.*, 2017),

149 likely governed by developmental constraints (Zohner *et al.*, 2023), buildup of water and
150 nutrient stress (Paul & Foyer, 2001; Buermann *et al.*, 2018; Bigler & Vitasse, 2021), or leaf
151 aging (Lim *et al.*, 2007).
152
153 July cooling induced a delay in bud set dates 3.5 times greater in late-leafing trees compared
154 to early-leafing ones (4.9 versus 1.4 days delay), which agrees with the expectations derived
155 from our conceptual model (Fig. 1). As we had expected, the natural interannual variation in
156 early-season development causes year-to-year differences in the point at which cool
157 temperatures switch from delaying bud set to advancing it. This offers a physiological
158 explanation for the advancement in the effect reversal timing observed between 1966 and
159 2015 in *Fagus sylvatica, Aesculus hippocastanum, Quercus robur, and Betula pendula*
160 (Zohner *et al.*, 2023).
161
162 August cooling induced comparable advances in bud set timing in both early- and late-
163 leafing trees (4.4-4.5 days). As trees become increasingly responsive to cooling with
164 shortening days (Delpierre *et al.*, 2009; Körner *et al.*, 2016), they exhibit increasingly similar
165 responses to late-season (August) cooling, regardless of their prior developmental speeds
166 (Fig. 1). That the differences in the responses of early-leafing and late-leafing trees to cooling
167 diminishes between July and August suggests tree individuals are dynamic systems that
168 continuously integrate and adapt their physiological responses to environmental conditions
169 over time.
170
171 Effects of daytime vs. nighttime temperature

172 Our experiments showed that daytime and nighttime temperatures had different effects on
173 primary growth cessation before and after the summer solstice: Before the solstice, daytime
174 cooling had no impact on the timing of bud set, while nighttime cooling and full-day cooling
175 delayed bud set by 4.2 and 4.1 days, respectively (Fig. 3). Given that experimental cooling
176 reduced photosynthesis by 69-83% across all treatments (Fig. S9), these findings underscore
177 the role of developmental processes—such as cell division and expansion—which mostly
178 occur at night (Zweifel et al., 2021; Fig. 4a). The developmental arrest induced by night
179 cooling—and therefore also by full-day cooling—likely slowed bud growth and maturation,
180 effectively extending the growing season.
181
182 After the solstice, daytime and nighttime cooling of 8°C elicited opposite responses. Trees
183 subjected to post-solstice daytime cooling and full-day cooling set their buds earliest, more
184 than five days earlier on average than the control group. As days shorten, the temperature
185 threshold inducing this growth cessation must be lower at night, otherwise trees would risk
186 prematurely ending their growing season due to the daily minimum temperatures occurring
187 at night (Fig. 4b). This may explain why post-solstice nighttime cooling delayed, rather than
188 advanced, bud set: Cooling of 8°C was sufficient to trigger overwintering responses when
189 applied during the day but not during the night (see Fig. S10 for a conceptual schematic). It
190 is also possible that daytime cooling indirectly triggered bud set by reducing photosynthesis
191 (Fig 4a). Such a mechanism is based on the assumption that trees will prioritise dormancy
192 induction over growth once photosynthesis is not possible anymore (Körner *et al.*, 2016).
193 Indeed, downregulation of photosynthesis has previously been reported to accelerate
194 senescence (Krieger-Liszkay *et al.*, 2019), although this may simply follow a reduction in
195 growth activity (Paul & Foyer, 2001). However, reduced photosynthesis has also been linked

196 to delayed senescence in both observational (Meng *et al.*, 2023) and experimental studies
197 (Vitasse *et al.*, 2021; Zohner *et al.*, 2023). Therefore, the direct effects of post-solstice
198 photosynthesis on autumn phenology still need to be clarified.
199
200 Nighttime cooling always led to a delay in bud set of approximately four days (Fig. 3). As
201 trees mainly grow at night (Zweifel *et al.*, 2021), colder nighttime temperatures slow down
202 key developmental processes such as meristematic activity, tissue expansion and
203 maturation, which in turn likely caused the delays in primary growth cessation (Fig. 4). Cold
204 autumn nights also delay growth cessation and slow bud development in *Populus, Pinus* and
205 *Picea* species (Kramer, 1956; Malcolm & Pymar, 1975; Kalcsits *et al.*, 2009). Given the
206 consistency of the effect reversal across Northern Hemisphere temperate forests (Zohner *et*
207 *al.*, 2023), and the patterns seen in previous experiments (Kramer, 1956; Malcolm & Pymar,
208 1975; Kalcsits *et al.*, 2009; Zohner *et al.*, 2023), our results likely are generalisable across
209 temperate tree species.
210
211 In conclusion, earlier primary growth cessation in temperate trees under climate change can
212 be explained by higher developmental rates under warmer temperatures, an effect
213 predominantly driven by nighttime temperatures (since trees primarily grow at night).
214 Ultimately, to better predict future growing season lengths, models must begin to account
215 for the differential impacts of the annual and daily temperature cycles that temperate zone
216 trees are physiologically adapted to.
217

218 Materials and Methods

219 Experiment 1

To test the antagonistic effects of early-season development and late-season air temperature on autumn primary growth phenology, we set up an experimental population (n = 267) of 40-60 cm tall European beech (*Fagus sylvatica* L.) trees in Zurich, Switzerland in 2023. The trees were sourced from a local nursery, and each tree was placed individually in a 20 L plastic pot containing a 1:1:1 sand / peat / organic soil mixture with a Nitrogen (N) concentration of ~65 g m^{-3}, a Phosphate (P_2O_5) concentration of ~140 g m^{-3}, and a Potassium (K_2O) concentration of ~400 g m^{-3}. Individual trees were assigned randomly to one of 10 treatment groups ($26 \leq n \leq 27$). Treatment groups were cooled at different times of the year, using different cooling levels (Table 1). To arrest spring development and thereby generate early-leafing and late-leafing individuals, some trees were placed outside under ambient conditions, while others were cooled in climate chambers from 4 April to 24 May. The chambers were set to a low of 2°C at night and a high of 7°C during the day, following a simulated day-night cycle of temperature and light availability (13 h photoperiod at ~4,300 lux). Temperatures around 5°C are associated with low rates of growth and development processes such as cell division, expansion and maturation (Körner, 2003; Tumajer *et al.*, 2021). From 24 May to 21 June, all trees were kept outside under ambient conditions in a randomised block design. All trees were monitored to observe their individual leaf-out dates, which was defined as the date when >50% of their leaves had unfolded, corresponding to BBCH15 (Capdevielle-Vargas *et al.*, 2015).

During the summer treatments, control trees were placed outside under natural ambient conditions. We did not use climate chambers for the control groups because warm conditions in these chambers can introduce unique physiological stressors—such as aphid infestations—not present in cold chambers (Bezemer *et al.*, 1998). We noticed increased

244 aphid abundance in control chambers during experiment 2, however these stressors did not
245 appear to affect autumn phenology (see Experiment 2). The July treatments took place
246 directly after the summer solstice from 22 June to 23 July. Treated trees were placed in
247 climate chambers set to a low of 2°C at night and a high of 7°C during the day or a low of 8°C
248 at night and a high of 13°C during the day depending on their cooling level (extreme and
249 moderate, respectively). Trees in the chambers experienced a photoperiod of 16 h at ~7,300
250 lux. The extreme cooling was designed to severely impair cell division, expansion, and
251 maturation as described above. Additionally, under this temperature regime, photosynthesis
252 should be reduced by >40% (Körner, 2006). The moderate cooling regime should still impair
253 growth and development in temperate deciduous trees as well as reducing photosynthesis
254 by at least 30% (Körner, 2003, 2006; Lenz *et al.*, 2014). The August treatments took place
255 from 24 July to 25 August under the same conditions as the July treatments except with a 15
256 h photoperiod. For the remainder of the experiment, all trees were kept outside in a
257 randomised block design. Throughout the experiment, all trees were watered frequently to
258 ensure constant water supply (Fig. S1).
259
260 To observe the effects of our treatments on the development of overwintering buds, we
261 monitored bud growth to derive bud set dates, our marker for the cessation of primary
262 aboveground development and the beginning of autumn phenology, for all trees. On each
263 tree, the terminal bud of the primary shoot and the terminal bud on a random lateral stem
264 were selected and tagged for measurement. Each selected bud was measured to 0.01 mm
265 precision using a digital calliper (CD-P8”M, Mitutoyo Corp, Japan). We measured all buds on
266 a regular basis from 4 July to 2 November. Bud set was defined as the date when each bud
267 reached 90% of its own maximum length, which is considered to be an indicative stage of

268 aboveground primary growth cessation (Signarbieux *et al.*, 2017; Zohner & Renner, 2019).
269 Bud lengths were linearly interpolated between measurement dates to derive the date at
270 which they reached the 90% threshold.
271
272 Experiment 2
273 To observe the effects of pre- and post-solstice daytime and nighttime temperature on bud
274 set phenology, we set up an experimental population (n = 180) of four-year-old *Fagus*
275 *sylvatica* trees in Zurich, Switzerland in 2022. The trees were sourced from a local nursery
276 and each tree was placed individually in a 20 L plastic pot containing a 1:1:1 sand / peat /
277 organic soil mixture with a Nitrogen (N) concentration of ~65 g m^{-3}, a Phosphate (P_2O_5)
278 concentration of ~140 g m^{-3}, and a Potassium (K_2O) concentration of ~400 g m^{-3}.
279
280 The ambient control treatment consisted of 36 trees exposed to natural ambient conditions.
281 The remaining eight treatments were each applied to 18 trees and included cooling in
282 climate chambers with simulated ambient day length (16 h) and light intensity (~6,900 lux).
283 The pre-solstice cooling treatments were applied between 22 May and 21 June. The post-
284 solstice cooling treatments were applied between 22 June and 21 July. The pre- and post-
285 solstice treatments included four levels each: Chamber control, where the trees were
286 continuously subjected to 20°C; Day cooling, where trees were subjected to 8°C in the
287 daytime and 20°C at night; Night cooling, where trees were subjected to 20°C in the day and
288 8°C at night; Full-day cooling, where trees were continuously subjected to 8°C (Table 2). Due
289 to warm temperatures, chamber control trees were subject to an increase in aphid
290 abundance, however this did not alter their bud set timing (see data analyses). After
291 treatment, all trees were placed in a randomised block design outside under ambient

conditions. Soil moisture content was regulated by frequent watering. We measured all buds on a weekly basis from 25 August to 3 November following the same methodology as in experiment 1. We also measured leaf-level CO_2 assimilation rates (see Zohner *et al.,* 2023 for methodology).

Data analyses

For experiment 2, we performed a one-way ANOVA that showed no significant differences between the bud set dates of the ambient, pre-chamber and post-chamber control groups ($F_{2,136}$ = 0.346, p =0.708, see Fig. S7). Therefore, for the following analyses we treated all three control treatments as one control treatment with 72 trees. For both experiments we ran linear mixed-effects models using treatment as a categorical fixed effect, bud type (apical vs. lateral) as a random effect and bud set day-of-year, absolute bud growth or relative bud growth as the response variable. Effect sizes were calculated in comparison to the corresponding control treatment, i.e., all early-leafing treatments were compared to the early-leafing control treatment and all late-leafing treatments were compared to the late-leafing control. Absolute and relative bud growth were calculated as:

$$Absolute = max\ length - min\ \ length$$

$$Relative = \left(1 - \frac{min\ length}{max\ length}\right) * 100$$

Where:

min length = the first measured bud length

max length = the measured bud length when the bud first surpassed 90% of its final length

To determine the sensitivity of autumn bud set timing to spring leaf-out timing, we ran a linear mixed-effects model using leaf-out day-of-year as the fixed predictor variable, bud type and summer temperature treatment as random effects, and bud set day-of-year as the response variable.

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Competing interests

The authors declare no competing interests.

Author contributions

D.R. conceived and developed the study, conducted the experiments and analyses, and wrote the manuscript. C.M.Z equally contributed to the conception and development of the study, contributed to the experiments and analyses, and the text. L.M., H.M., Z.W. and Y.Z. contributed to the conception and implementation of the first experiment. R.B. contributed to the conception and implementation of the second experiment. T.W.C. and S.S.R. contributed to the text. All authors provided comments and approved the final manuscript.

Data availability

The code and data for this study will be made available upon request or at Zenodo on publication of the manuscript.

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Figures

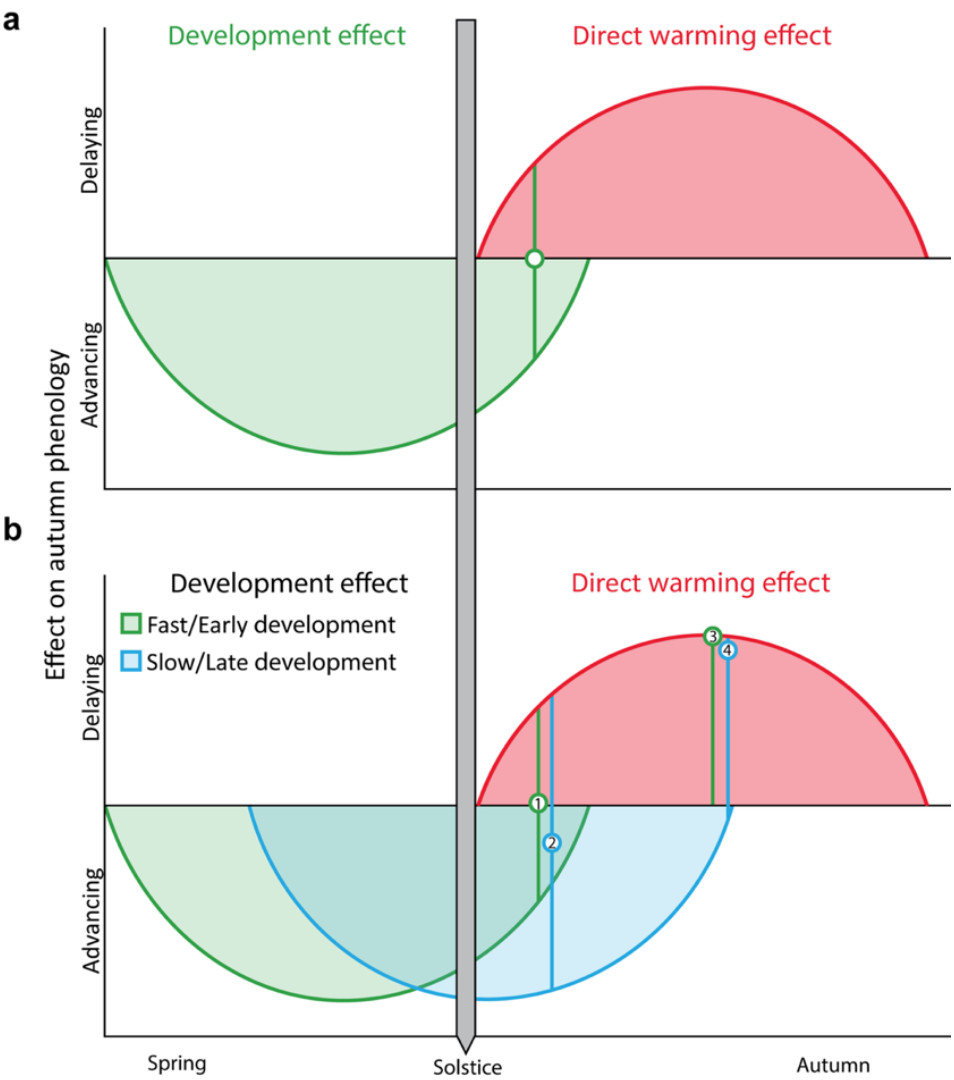

Figure 1. Conceptual model of autumn phenological responses of temperate trees to early-season development and late-season temperature. Autumn phenology, represented in this study by the timing of primary growth cessation (bud set), is influenced by two opposing factors: early-season development and late-season temperature. **a)** In our model, early-season development, which is driven by temperature, has an advancing effect on autumn phenology that lasts until shortly after the summer solstice (green curve). Higher temperatures cause trees to complete their annual life cycles faster, allowing them to set buds. After the summer solstice, as days shorten, trees become increasingly sensitive to cooling conditions, so late-season warming slows the progression of bud set and senescence, delaying autumn phenology (red curve). The compensatory point, where the advancing effect of early-season development is balanced by the delaying effect of late-season warming, is represented by the green circle. **b)** According to the model, the timing of this effect reversal is flexible and varies between years based on the speed of development. When development is slow or starts late (blue curve), the effect reversal occurs later than under fast or early development (green curve). Therefore, shortly after the solstice, the effect of temperature on autumn phenology differs between fast/early and slow/late developing individuals. For example, point 1 (green circle) shows no net temperature effect on phenology in fast/early trees. By contrast, point 2 (blue circle) shows that in slow/late developing trees, warmer temperatures shortly after the solstice (in July) still advance autumn phenology. However, as the growing season progresses and days shorten, trees become more responsive to cooling regardless of prior developmental speed. By August, both fast/early and slow/late trees should therefore exhibit similar phenological responses, with warming consistently delaying autumn phenology (points 3 and 4).

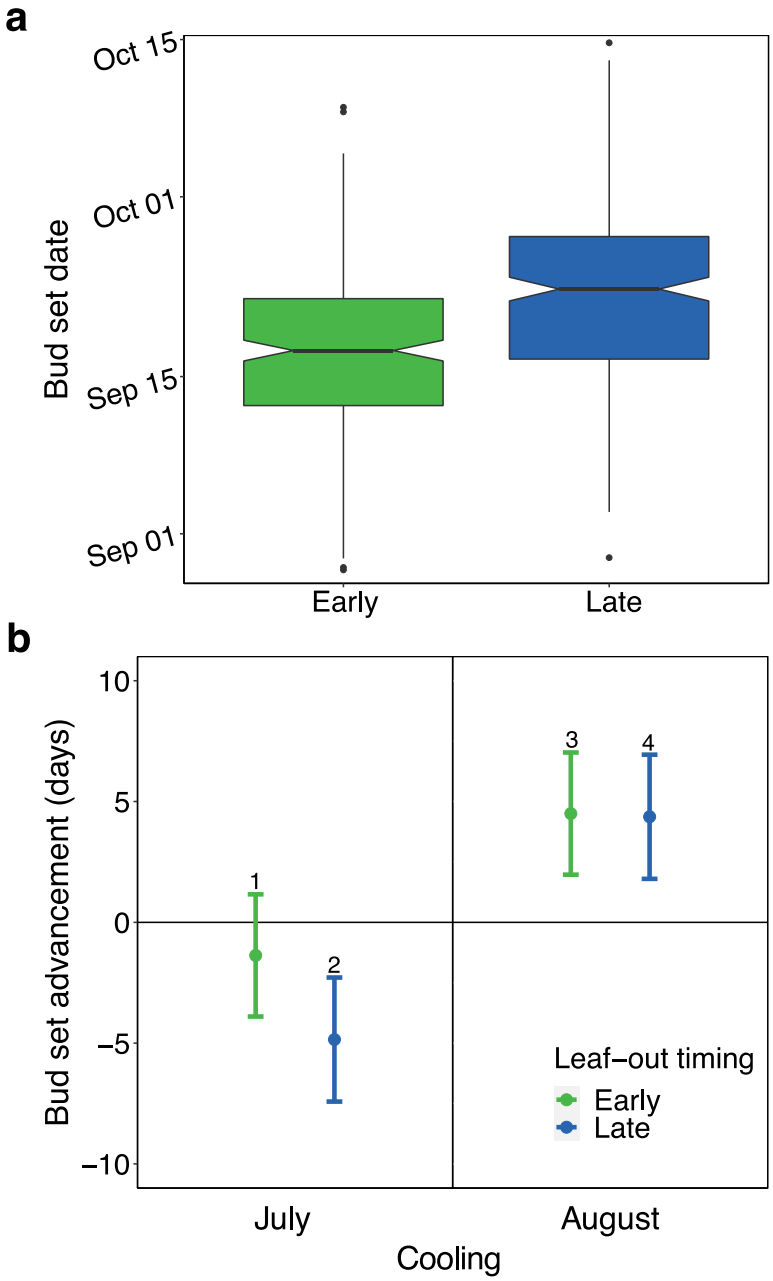

Figure 2. Effects of early-season development and late-season temperature on the timing of autumn bud set in *Fagus sylvatica* (experiment 1). a) Bud set dates for early- (green) and late- (blue) leafing trees including all treatments. Late-leafing trees were cooled (2-7°C) in climate chambers from 4 April to 24 May to arrest their development and delay leaf-out. **b)** Effects of July (22 June to 23 July) and August (24 July to 25 August) moderate cooling (8-13°C) on bud set date for early- (green) and late- (blue) leafing trees (see Fig. S3 for extreme cooling effects). Analyses show effect size means ± 95% confidence intervals from linear mixed models, including treatment as fixed effect and bud-type (apical vs lateral) as random effect. Early-leafing effects are calculated against the early-leafing control and late-leafing effects are calculated against the late-leafing control. Number labels (1-4) above each point are shown to aid comparison between points 1-4 in the conceptual model (Fig. 1b) and the observed effects. Positive values indicate advances in bud set and negative values indicate delays.

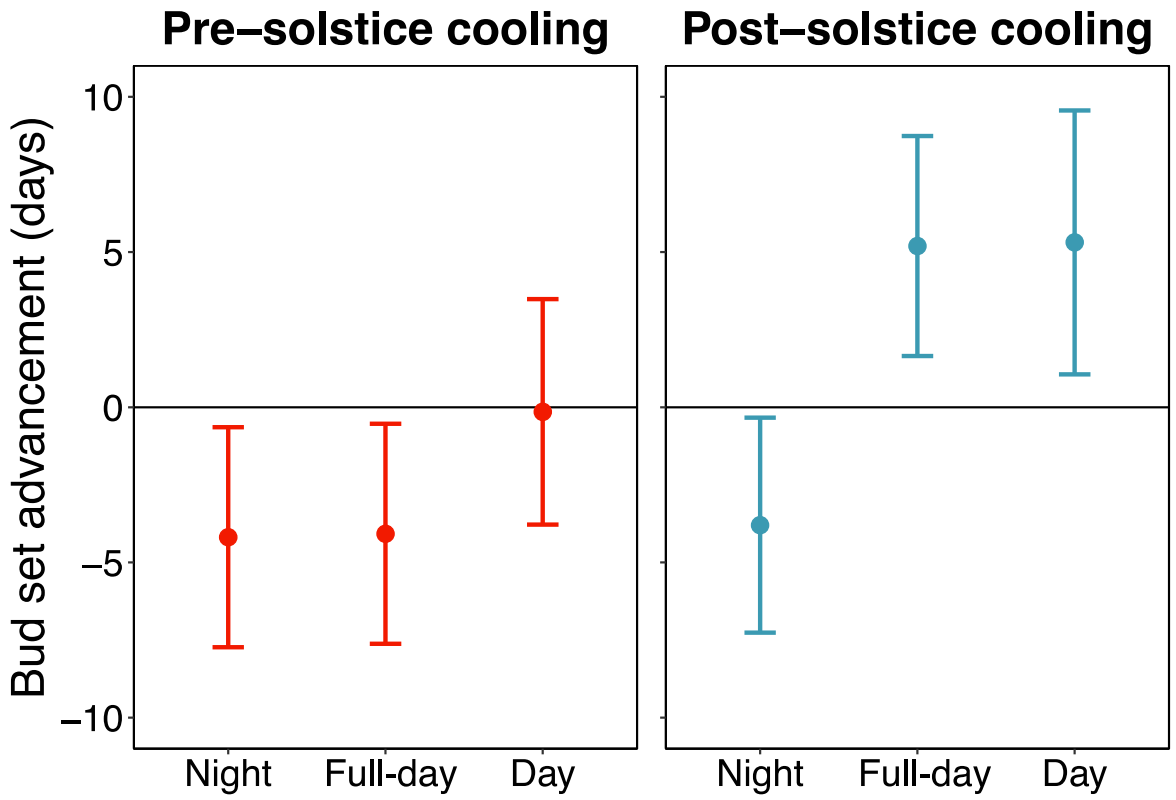

Figure 3. The pre- and post-solstice effects of night, full-day and day cooling on the timing of autumn primary growth cessation in *Fagus sylvatica* (experiment 2). Effects of pre-solstice (22 May to 21 June) and post-solstice (22 June to 21 July) cooling on bud set date. Full-day cooling trees were continuously cooled to 8°C, day cooling trees were cooled to 8°C in the day and kept at 20°C at night, night cooling trees were cooled to 8°C at night and kept at 20°C in the day. Analyses show effect size means ± 95% confidence intervals from linear mixed models, including treatment as fixed effect and bud-type (apical vs lateral) as random effect. Positive values indicate advances to bud set and negative values indicate delays.

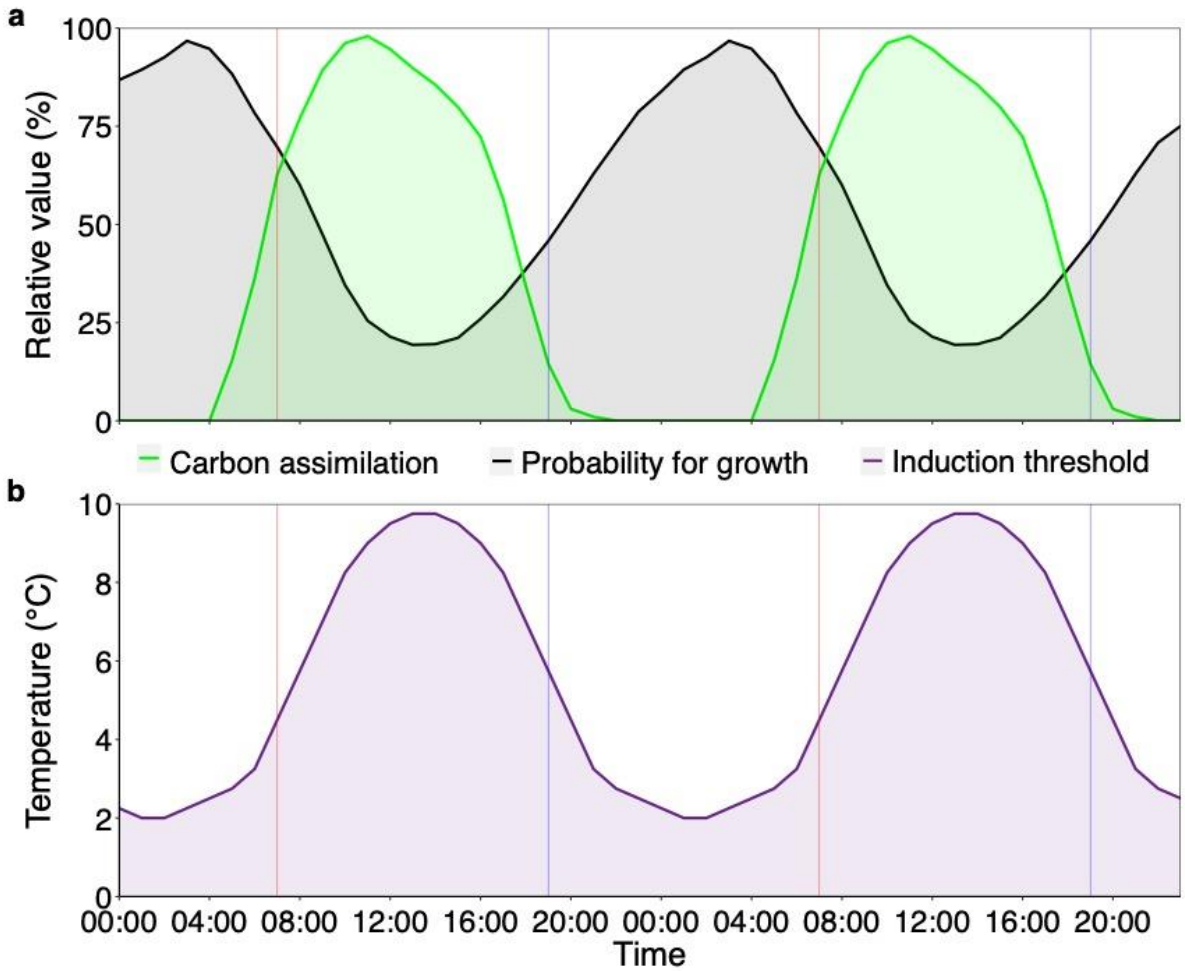

Figure 4. Diel patterns of relative growth, photosynthesis and theoretical thresholds for cold-induced bud set in *Fagus sylvatica*. Vertical red lines indicate the start of the day, and vertical blue lines indicate the start of the night, marking the boundaries between the 12-hour treatment windows used in experiment 2. **a)** The green curve shows relative carbon assimilation rate measured under controlled conditions (Urban *et al.*, 2014), with values linearly interpolated between measurements. The black curve shows the relative probability for growth (Zweifel *et al.*, 2021). Both curves were smoothed by taking the running mean of the target value, the previous value and the following value. At night, low temperatures slow the trees' developmental processes, possibly leading to delayed primary growth cessation, while low temperatures during the day reduce photosynthetic activity. **b)** As days shorten after the solstice, autumn bud set becomes increasingly responsive to cooling. Temperatures below a certain threshold induce overwintering responses, advancing autumn phenology. Our results indicate that daytime cooling of 8°C is below this threshold. However, because daily temperatures reach their minimum during the night, trees' induction thresholds should be lower at night than during the day. Post-solstice night-time cooling of 8°C may thus have delayed senescence by slowing development rather than inducing it.

Tables

Table 1. Description of the temperature treatments applied in experiment 1. July treatments were from 22 June to 23 July. August treatments were from 24 July to 25 August. Ambient means outside under natural conditions. Temperature ranges indicate the daily minimum and maximum temperatures experienced by trees inside climate chambers. The early-leafing and late-leafing trees were experimentally generated by placing potted trees in climate chambers from 4 April to 24 May and cooling them to 2°C at night and 7°C during the day to arrest spring development.

Treatment	1	2	3	4	5	6	7	8	9	10
Leaf-out timing	Early	Late	Early	Late	Early	Late	Early	Late	Early	Late
Treatment timing	-	-	July	July	July	July	August	August	August	August
Cooling level	Control (ambient)	Control (ambient)	Moderate (8-13°C)	Moderate (8-13°C)	Extreme (2-7°C)	Extreme (2-7°C)	Moderate (8-13°C)	Moderate (8-13°C)	Extreme (2-7°C)	Extreme (2-7°C)

Table 2. Description of the temperature treatments applied in experiment 2. June treatments were from 22 May to 21 June. July treatments were from 22 June to 21 July. Ambient means outside under natural conditions. The remaining temperature regimes are in the format day/night and refer to the temperatures applied to trees in climate chambers.

Treatment	Control	Pre-Chamber control	Pre-Day	Pre-Night	Pre-Full-day	Post-Chamber control	Post-Day	Post-Night	Post-Full-day
Cooling time of year	-	June	June	June	June	July	July	July	July
Cooling time of day	-	-	Day	Night	Full-day	-	Day	Night	Full-day
Temperature regime	Ambient	20/20°C	8/20°C	20/8°C	8/8°C	20/20°C	8/20°C	20/8°C	8/8°C

Supporting Information

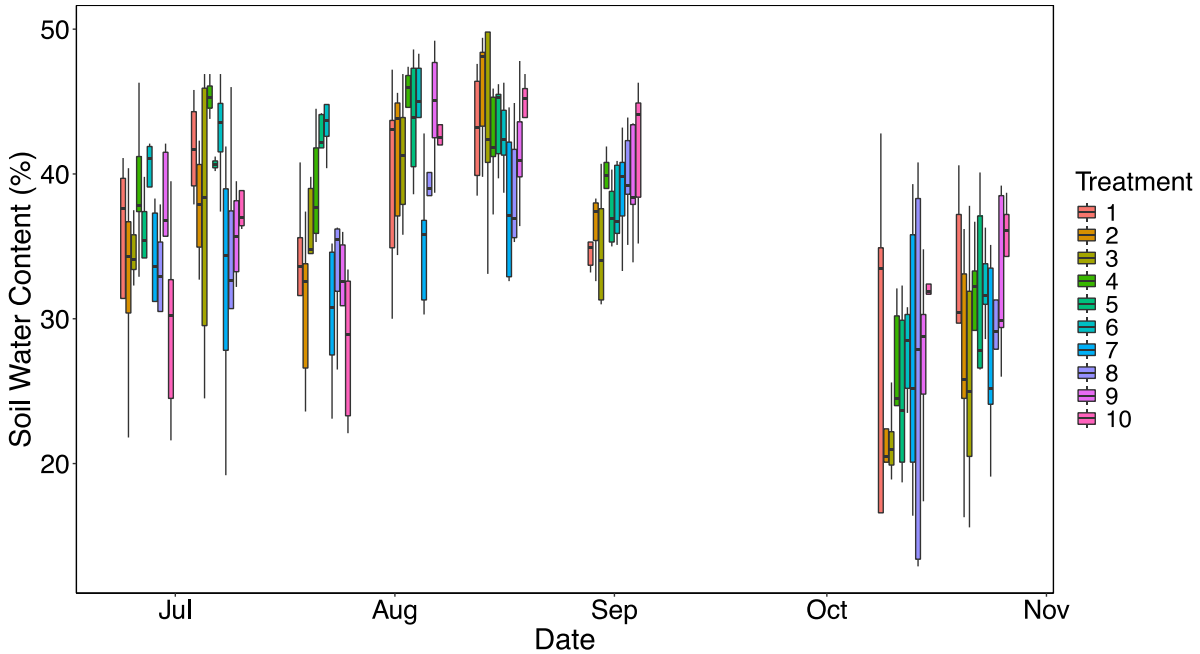

Figure S1. Seasonal soil water content (%) for each treatment (experiment 1). Five individuals per treatment were measured at each time step.

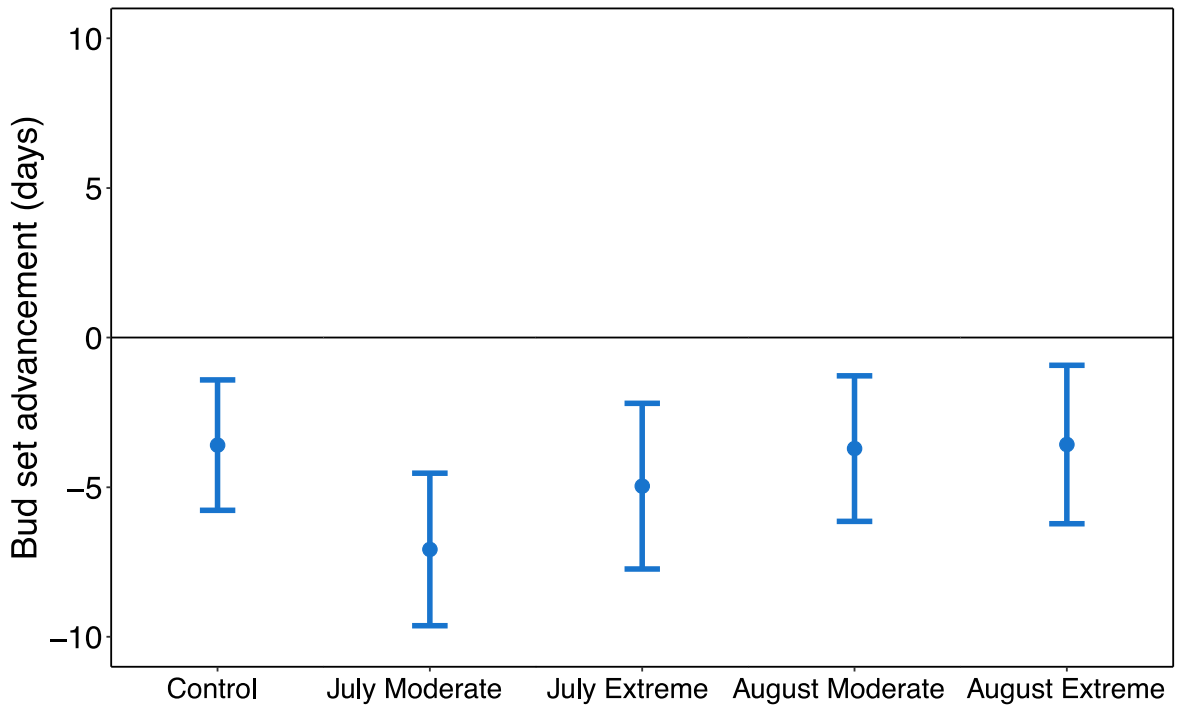

Figure S2. Effects of slow spring development on the timing of autumn bud set in *Fagus sylvatica* (experiment 1). Spring development was slowed by cooling the late-leafing trees from 4 April to 24 May in climate chambers. The late-leafing trees experienced a simulated day-night cycle of temperature and light availability, with minimum (2°C) and maximum (7°C) temperatures reached during the night- and daytime, respectively and otherwise simulated ambient conditions. Analyses show effect size means ± 95% confidence intervals from linear mixed models, including treatment as fixed effect and bud-type (apical vs lateral) as random effect. For each late-leafing treatment, the effect is calculated against the

equivalent early-leafing treatment. Positive values indicate advances to bud set and negative values indicate delays.

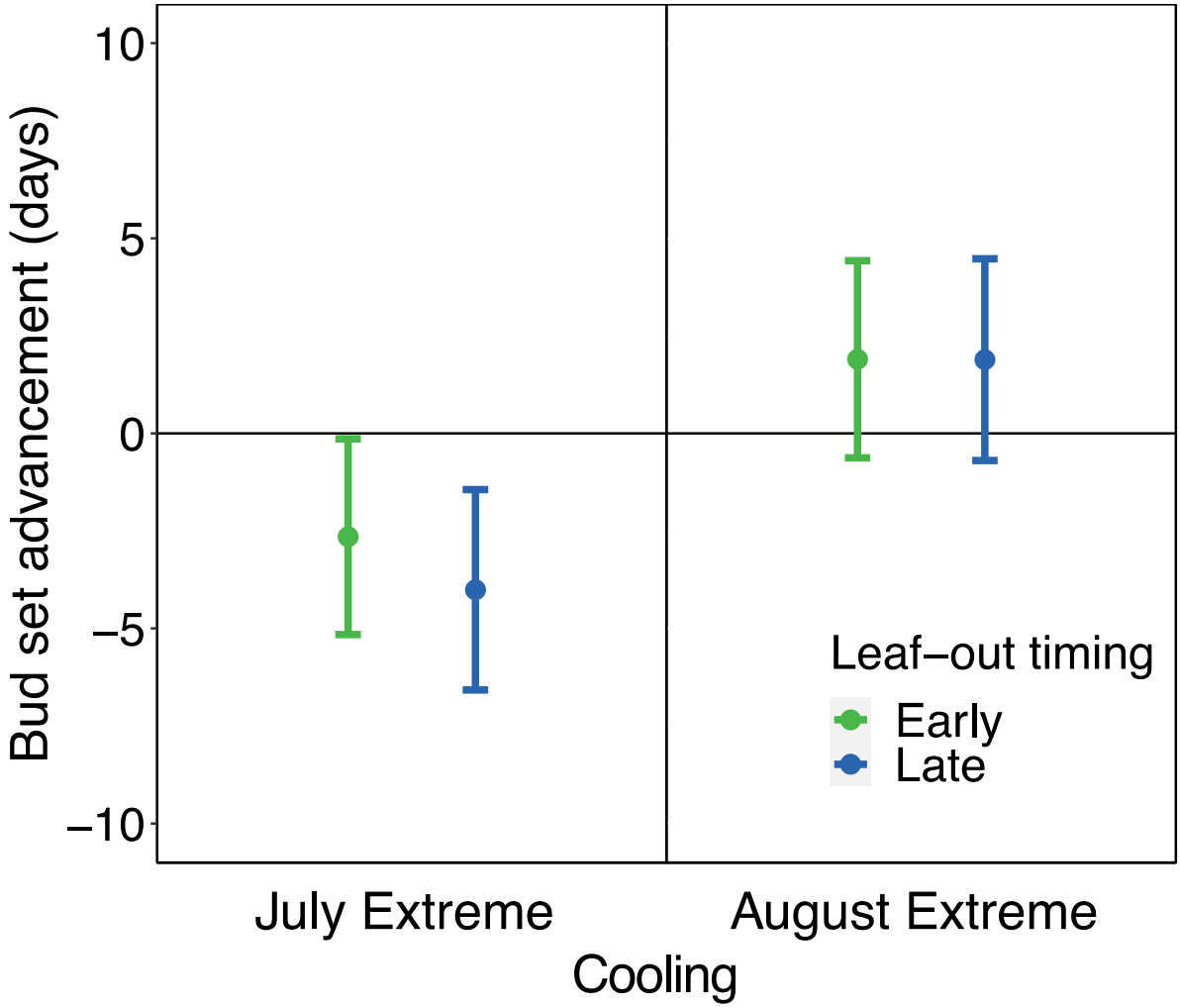

Figure S3. Effects of early-season development and extreme late-season cooling on the timing of autumn primary growth cessation in *Fagus sylvatica* (experiment 1). Effects of July (22 June to 23 July) and August (24 July to 25 August) extreme cooling (2-7°C) on bud set date for early- (grey) and late- (blue) leafing trees. Analyses show effect size means ± 95% confidence intervals from linear mixed models, including treatment as fixed effect and bud-type (apical vs lateral) as random effect. Early-leafing effects are calculated against the early-leafing control and late-leafing effects are calculated against the late-leafing control. Positive values indicate advances to bud set and negative values indicate delays.

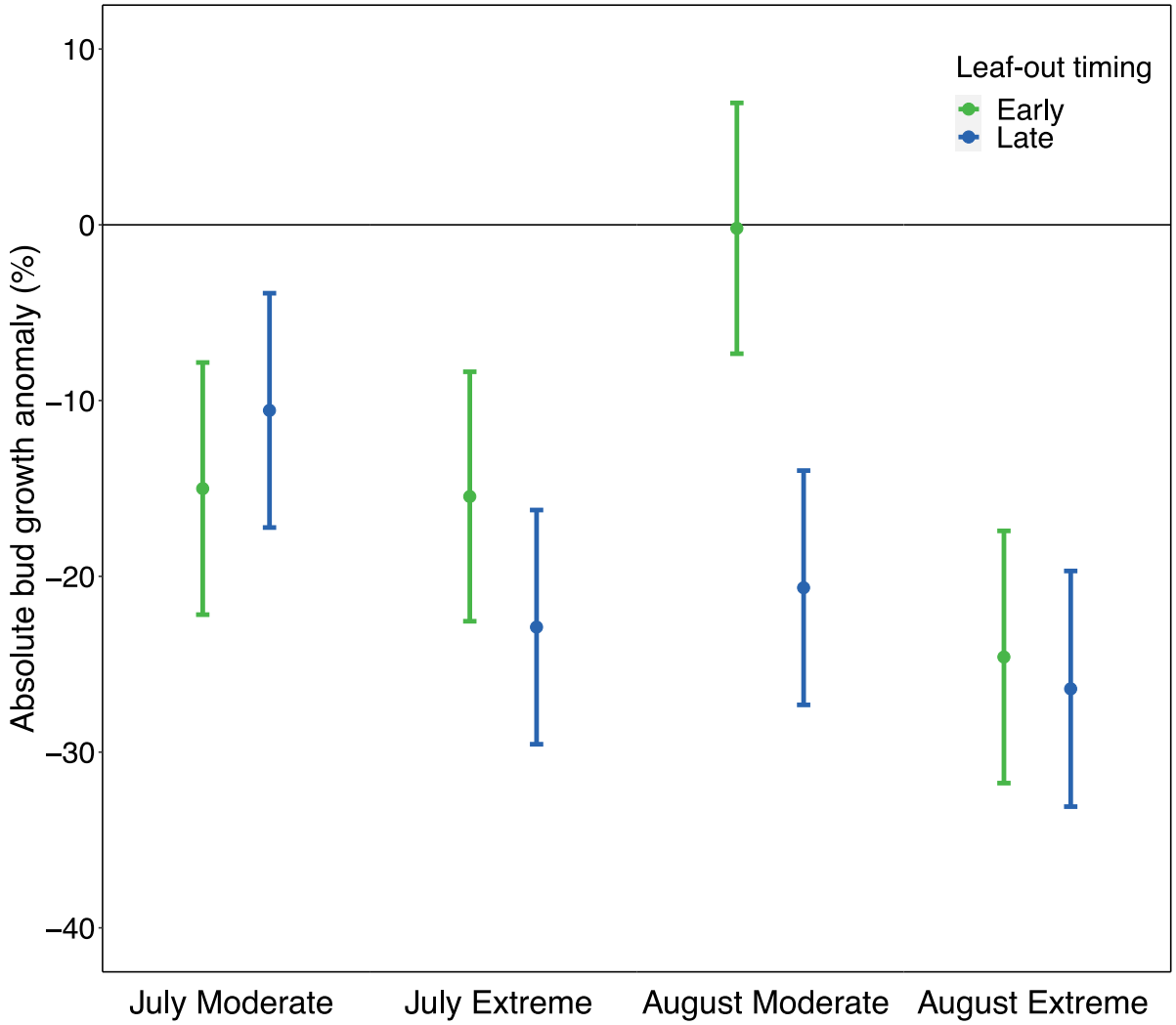

Figure S4. Effects of early-season development and late-season cooling on total bud growth in *Fagus sylvatica* (experiment 1). Effects of July (22 June to 23 July) and August (24 July to 25 August) moderate and extreme cooling (8-13°C and 2-7°C, respectively) on total seasonal (4 July to bud set date) bud growth for early- (grey) and late- (blue) leafing trees. Analyses show effect size means ± 95% confidence intervals from linear mixed models, including treatment as fixed effect and bud-type (apical vs lateral) as random effect. Early-leafing effects are calculated against the early-leafing control and late-leafing effects are calculated against the late-leafing control.

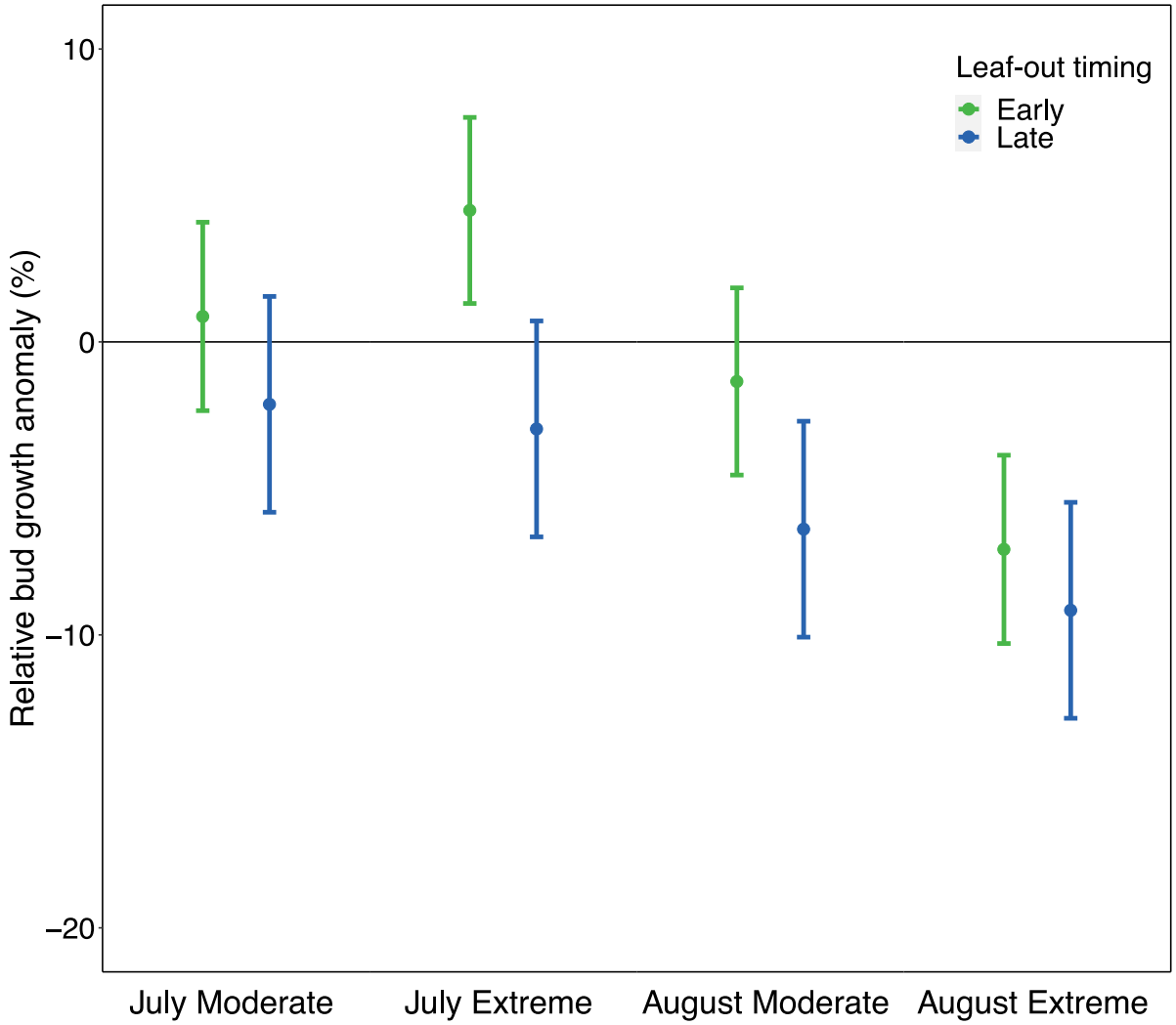

Figure S5. Effects of early-season development and late-season cooling on relative bud growth in *Fagus sylvatica* (experiment 1). Effects of July (22 June to 23 July) and August (24 July to 25 August) moderate and extreme cooling (8-13°C and 2-7°C, respectively) on relative seasonal (4 July to bud set date) bud growth for early- (grey) and late- (blue) leafing trees. Analyses show effect size means ± 95% confidence intervals from linear mixed models, including treatment as fixed effect and bud-type (apical vs lateral) as random effect. Early-leafing effects are calculated against the early-leafing control and late-leafing effects are calculated against the late-leafing control.

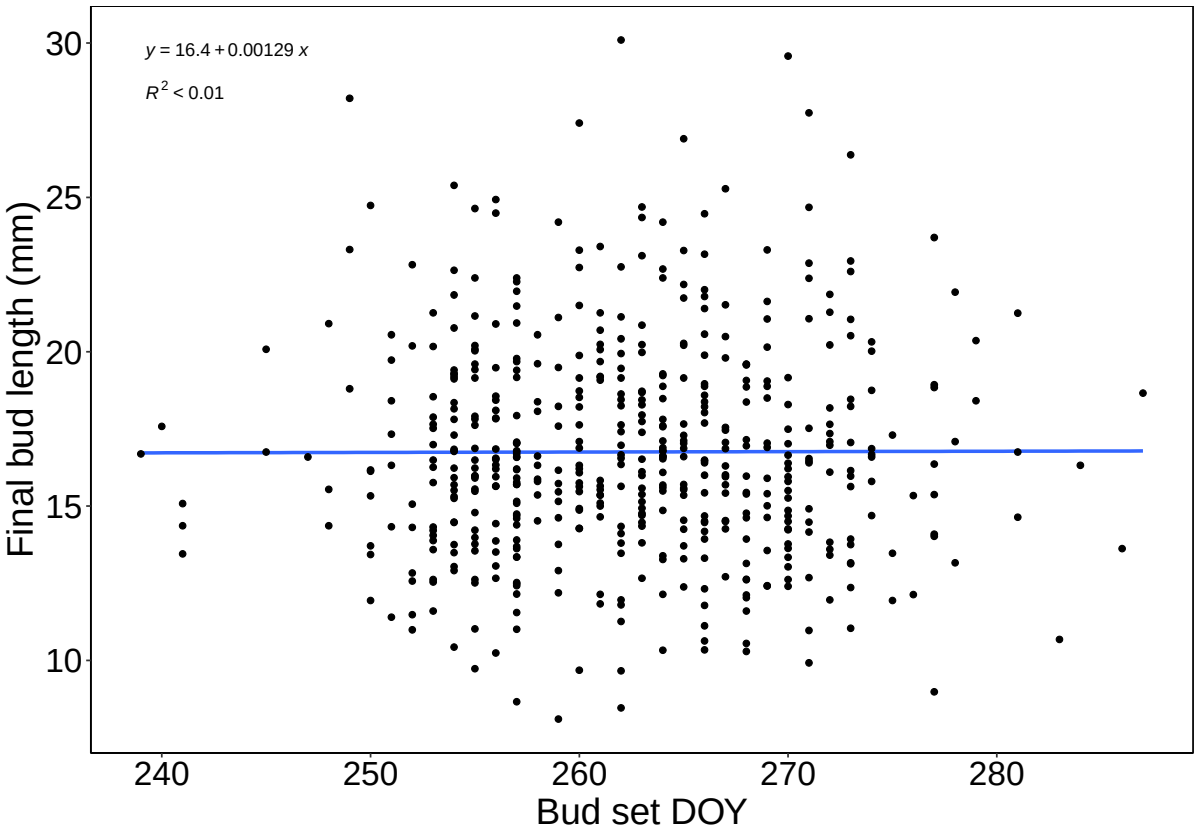

Figure S6. Relationship between end of season bud length and the timing of primary growth cessation in *Fagus sylvatica* (experiment 1). Effect of bud set DOY (day-of-year) on the final bud length at the end of the growing season from a linear model with 95% confidence interval.

Table S1. Mean and 95% confidence intervals of 50% leaf-out (day-of-year), bud set (day-of-year) and absolute bud growth (cm) for each treatment in experiment 1.

TREATMENT	LEAF-OUT DOY (95% CI)	BUD SET DOY (95% CI)	ABSOLUTE BUD GROWTH CM (95% CI)
1	**131.6 (130.2-133.)**	**260.5** (259.1-261.9)	**12.88** (11.96-13.80)
2	**148.1 (147.8-148.4)**	**264.1** (262.4-265.8)	**14.21** (13.53-14.89)
3	**131.2 (129.7-132.7)**	**261.9** (260.0-263.8)	**10.95** (10.29-11.61)
4	**148.6 (148.2-149.0)**	**268.9** (267.2-270.6)	**12.72** (11.99-13.45)
5	**130.1 (128.9-131.3)**	**263.1** (261.1-265.1)	**10.89** (10.41-11.37)
6	**148.7 (148.4-149.0)**	**268.1** (266.2-270.0)	**10.97** (10.35-11.59)
7	**130.2 (128.6-131.8)**	**256.0** (254.6-257.4)	**12.85** (12.17-13.53)
8	**148.9 (148.6-149.2)**	**259.7** (257.7-261.7)	**11.28** (10.63-11.93)
9	**132.3 (130.8-133.8)**	**258.6** (256.5-260.7)	**9.71** (9.22-10.20)
10	**148.0 (147.6-148.4)**	**262.2** (260.5-263.9)	**10.46** (9.81-11.11)

Table S2. Mean and 95% confidence intervals of bud set (day-of-year) and absolute bud growth (cm) for each treatment in experiment 2.

TREATMENT	BUD SET DOY (95% CI)	ABSOLUTE BUD GROWTH CM (95% CI)
AMBIENT CONTROL	**257.2** (254.7-259.7)	**4.47** (3.80-5.14)
PRE-CHAMBER-CONTROL	**257.1 (254.1-260.1)**	**4.73 (3.82-5.54)**
PRE-FULL-DAY	**260.6** (257.3-263.9)	**5.18** (4.26-6.10)
PRE-DAY	**257.2** (252.7-261.7)	**4.95** (3.96-5.94)
PRE-NIGHT	**261.0** (257.9-264.1)	**5.01** (4.34-5.68)
POST-CHAMBER-CONTROL	**255.4 (251.8-259.0)**	**4.24 (3.45-5.03)**
POST-FULL-DAY	**251.6** (248.3-254.9)	**2.58** (2.08-3.08)
POST-DAY	**251.5** (248.0-255.0)	**2.92** (2.32-3.52)
POST-NIGHT	**260.6** (256.7-264.5)	**5.58** (4.70-6.46)

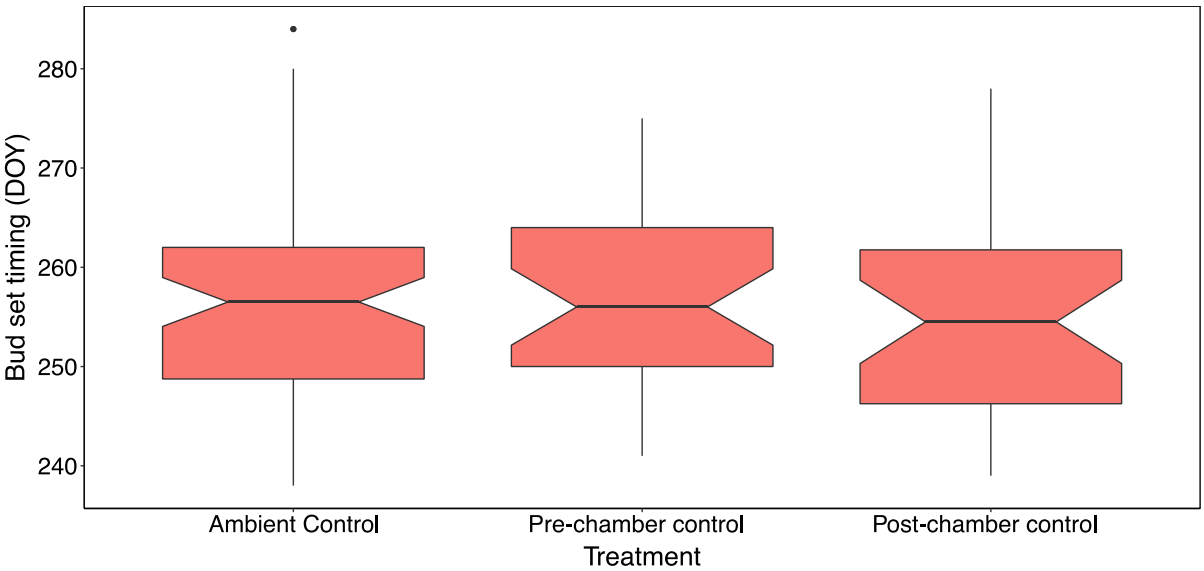

Figure S7. Bud set timing (day-of-year) for each control treatment in experiment 2. One-way ANOVA revealed no significant differences in bud set timing between the three control groups ($F_{2,136}$ = 0.346, p =0.708). Ambient control trees were placed outside throughout the experiment, Pre-Chamber control trees were in a climate chamber under simulated ambient conditions between 22 May and 21 June, Post-Chamber control trees were placed in a climate chamber under simulated ambient conditions between 22 June and 21 July.

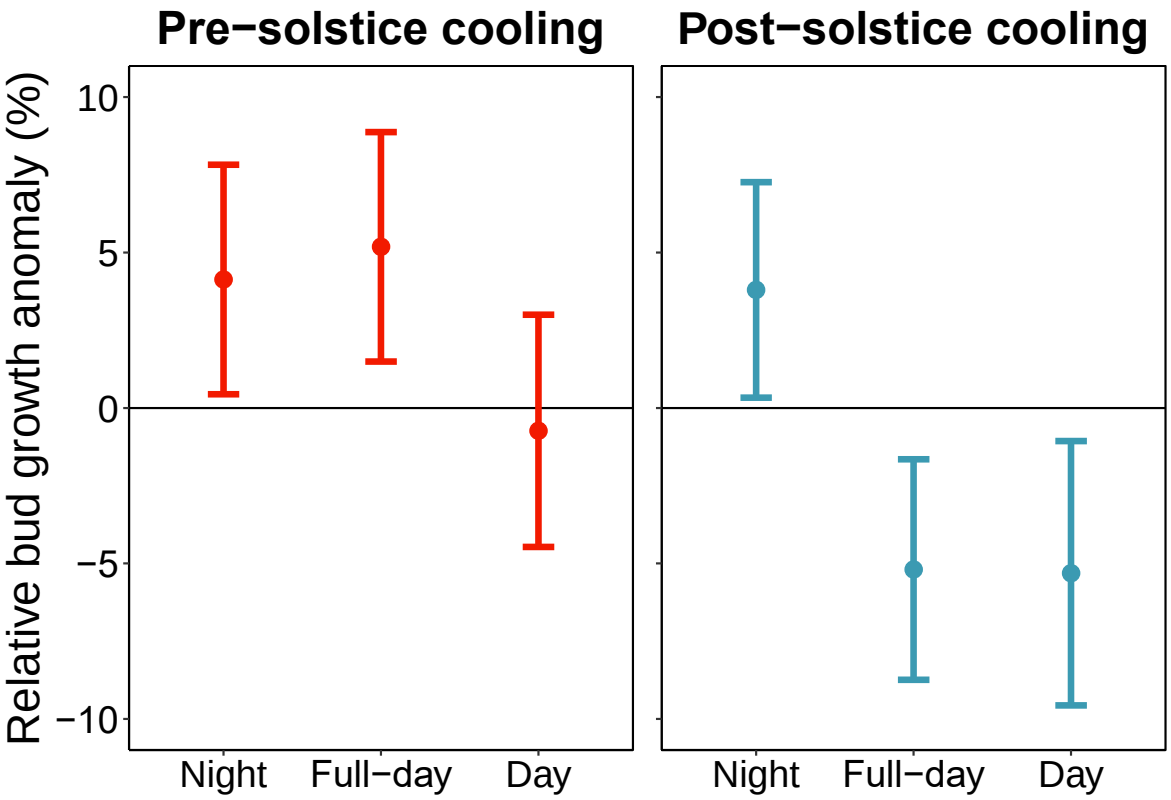

Figure S8. Effects of pre- and post-solstice daytime, nighttime and full-day cooling on relative bud growth in *Fagus sylvatica* (experiment 2). Pre-solstice (22 May to 21 June) and post-solstice (22 June to 21 July) cooling treatments were applied. Full-day cooling trees were continuously cooled to 8°C, day cooling trees were cooled to 8°C in the day and kept at 20°C at night, night cooling trees were cooled to 8°C at night and kept at 20°C in the day. Analyses show effect size means ± 95% confidence intervals from linear mixed models, including treatment as fixed effect and bud-type (apical vs lateral) as random effect.

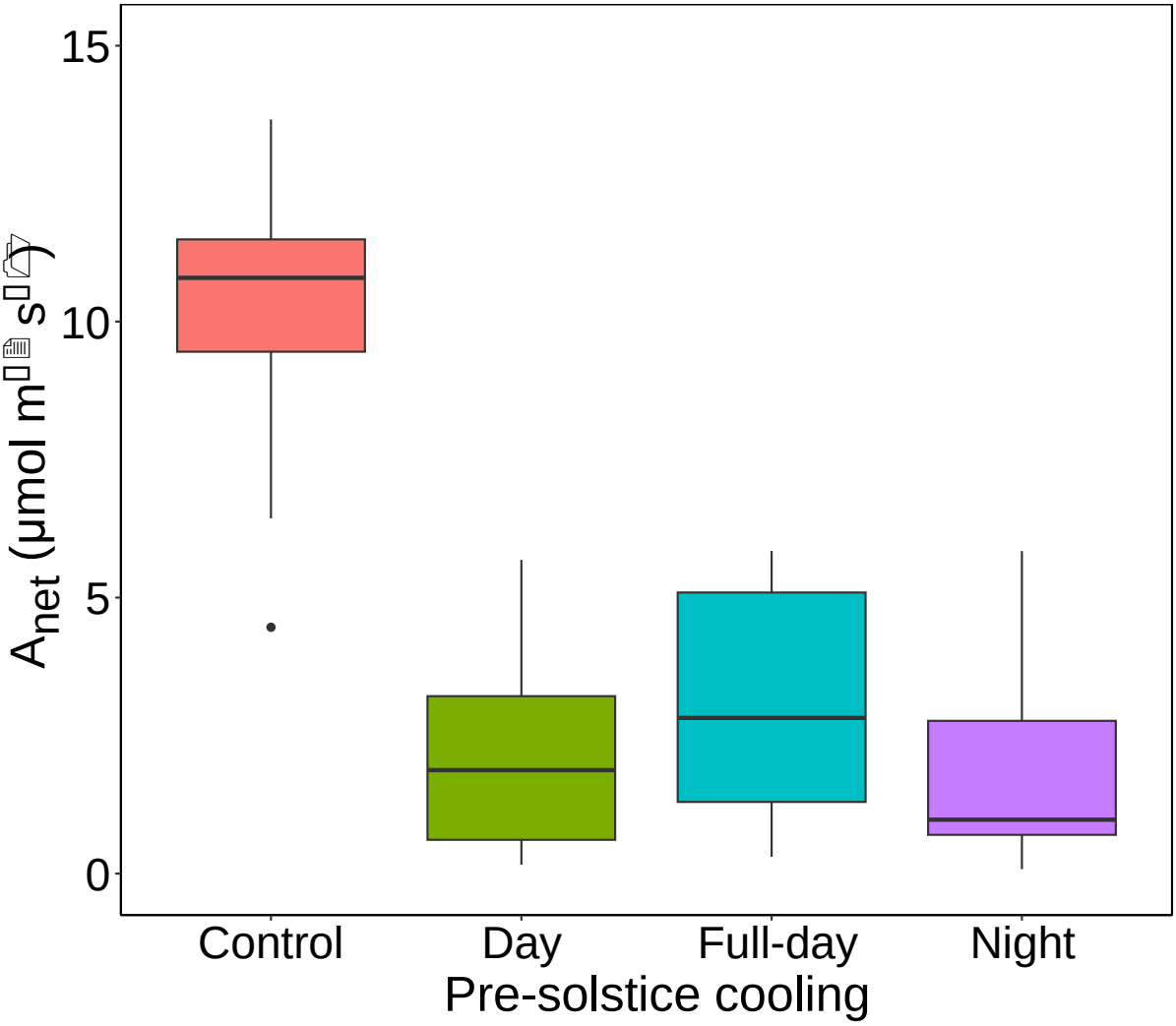

Figure S9. Photosynthesis rates of *Fagus sylvatica* trees under pre-solstice ambient conditions, daytime, nighttime and full-day cooling in July (experiment 2). Absolute leaf-level photosynthesis (net CO_2 assimilation).

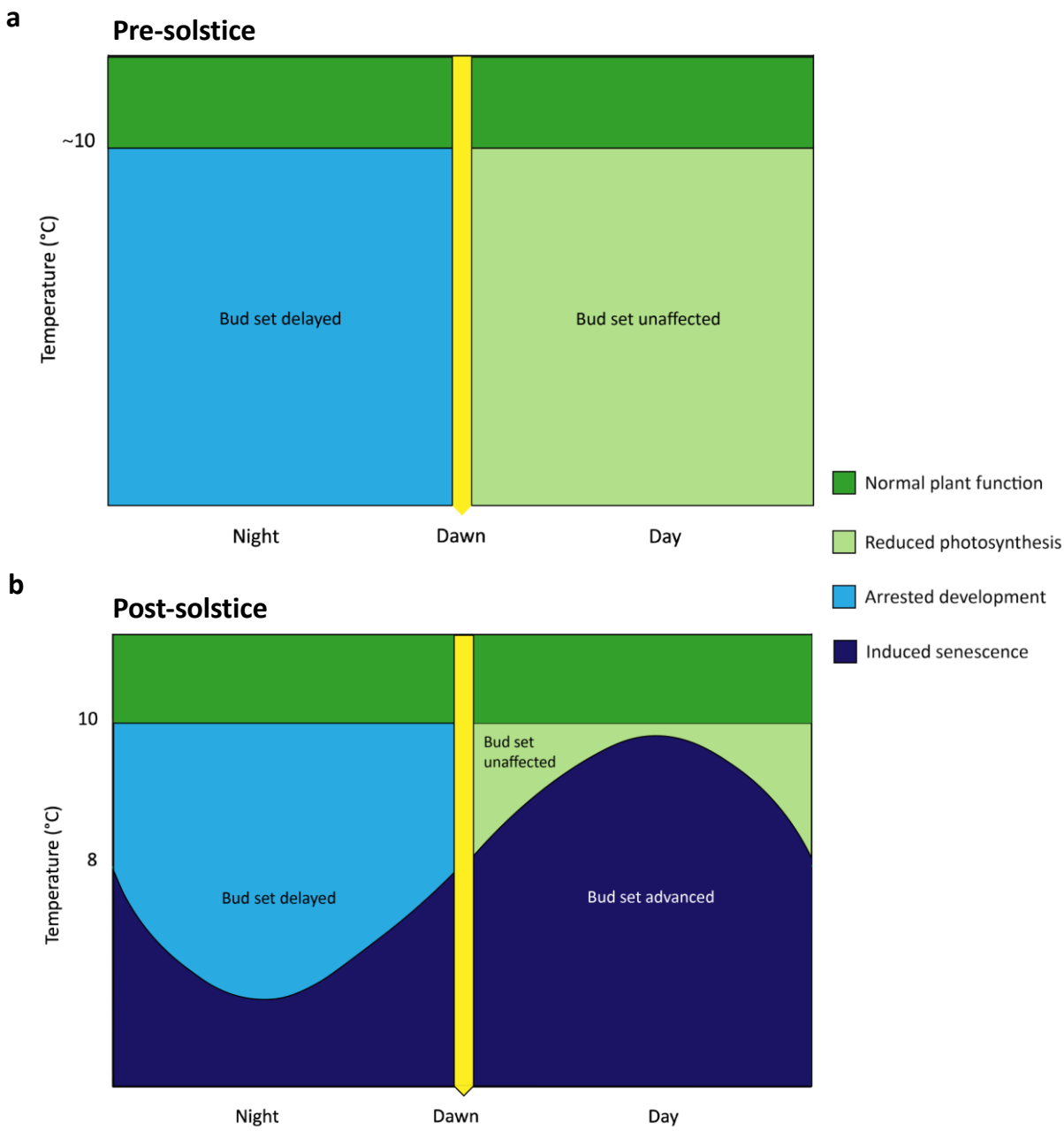

Figure S10. Conceptual model of pre- and post-solstice effects of day and nighttime temperatures on the autumn phenology of *Fagus sylvatica*. The colours of the shaded areas represent the effect of cooling to different temperatures at specific times of the day. Above a certain threshold, plants function normally, and cooling has little effect on the timing of primary growth cessation (green; here depicted as 10°C, n.b. this is an intended simplification and does not necessarily accurately represent the reality). Light blue areas show combinations that lead to arrested development, which delays primary growth cessation. Pale green areas depict combinations that reduce photosynthesis and potentially growth but do not affect phenology. Dark purple areas depict combinations that lead to the induction of senescence and overwintering processes, therefore advancing primary growth cessation. **a)** Before the solstice, lengthening days give temperate trees a reliable cue of the favourable conditions to come, so there is no temperature that can induce senescence at this time of year (Kramer, 1936). Nighttime cooling (< ~10°C) slows down beech's developmental processes such as meristematic activity, tissue expansion and maturation, which leads to a growth and development debt (Zweifel *et al.*, 2021). As the days are sufficiently warm, photosynthesis is unaffected. This means that the trees accumulate excess carbon that can only be used later in the growing season to make up for this developmental debt, thus delaying

primary growth cessation. Daytime cooling (< ~10°C) slows development to a lesser extent, however, it also reduces carbon gain by inhibiting photosynthesis but not respiration. Due to carbon limitation, affected trees may prioritise development over growth, so buds may be shorter but there is no resulting delay to primary growth cessation. **b)** After the solstice and due to shortening days, trees become increasingly sensitive to cooling, especially during the day. Therefore, daytime temperatures < ~10°C are likely to induce overwintering responses and therefore advance autumn phenology. However, due to typical daily cycles of temperature always reaching their minimum during the night, trees are less susceptible to cooling in the night than in the day. At night, temperatures between ~4°C and ~10°C slow the trees' developmental processes, leading to delayed primary growth cessation.