1   **Daylength helps temperate deciduous trees to leaf-out at the optimal time**

2

3   **Short title:** Daylength guarantees leaf-out at right time

4

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27 **Abstract:**

28 Global warming has led to substantially earlier spring leaf-out in temperate-zone

29 deciduous trees. The interactive effects of temperature and daylength underlying this

30 warming response remain unclear, yet need to be accurately represented by Earth

31 System models to improve projections of the carbon and energy balances of temperate

32 forests and the associated feedbacks to the Earth's climate system. We studied the

33 control of leaf-out by daylength and temperature using data from six tree species

34 across 2377 European phenology observation sites (www.pep725.eu), each with at

35 least 30 years of observations. We found that, in addition to- and independent of the

36 known effect of chilling, daylength correlates negatively with the heat requirement for

37 leaf-out in all studied species. In warm springs when leaf out is early, days are short

38 and the heat requirement is higher than in an average spring, which mitigates the

39 warming-induced advancement of leaf-out and protects the tree against precocious

40 leaf-out and the associated risks of late frosts. In contrast, longer-than-average

41 daylength (in cold springs when leaf-out is late) reduce the heat requirement for leaf-

42 out, ensuring that trees do not leaf-out too late and miss out on large amounts of solar

43 energy. These results provide the first large-scale empirical evidence of a widespread

44 daylength effect on the temperature sensitivity of leaf-out phenology in temperate

45 deciduous trees.

Introduction

The timing of leaf-out co-determines the growth, reproductive success and

competitiveness of temperate deciduous trees and thus strongly affects their fitness

and distribution (Chuine, 2010). Global warming has led to substantially earlier spring

leaf-out (Menzel *et al.*, 2006, Parmesan & Yohe, 2003, Peñuelas & Filella, 2001),

although this advance is declining (Fu *et al.*, 2015). These changes in spring

phenology may influence terrestrial ecosystem fluxes of carbon, water, nutrient and

energy in a short term (Keenan *et al.*, 2014, Myneni *et al.*, 1997, Piao *et al.*, 2017).

Mechanistic understanding of the leaf-out process is, however, far from complete

(Chuine *et al.*, 2010, Flynn & Wolkovich, 2018, Körner & Basler, 2010, Laube *et al.*,

2014, Zohner *et al.*, 2016), which challenges the projected impacts of climate change

on ecosystems by dynamic global vegetation models (Richardson *et al.*, 2012). A

better understanding of the ecophysiological processes controlling leaf-out phenology

is thus essential for improving our understanding of the responses of ecosystems to

the ongoing climate change and the subsequent feedbacks to the climate system, as

well as explaining the slow-down of the warming-induced advance in leaf out.

A species' optimal leaf-out date results from natural selection that optimizes the

species' fitness under given environmental conditions, such as avoiding freezing

damage (Lenz et al, 2016), ensuring flowering synchrony among species (Elzinga,

Atlan and Biere, 2007; Zohner, Mo & Renner, 2018) and maximizing the length of

the remaining season for light and nutrient resources (competition with other trees) as

68   well as for tissues maturation (Körner *et al*, 2016). In temperate and boreal regions,

69   temperature, including both cold winter temperatures (chilling requirement) and warm

70   spring temperatures (heat requirement), and daylength interact to realize leaf out

71   around the optimal date (Flynn & Wolkovich, 2018, Körner & Basler, 2010).

72   Chilling accumulates over autumn and winter, and when the accumulated chilling

73   exceeds the chilling requirement, endodormancy (the first stage of dormancy (Lang,

74   1987)) is broken and buds enter the second dormancy stage: ecodormancy (Chuine &

75   Régnière, 2017, Hänninen, 2016). During ecodormancy, meristem cells begin to

76   grow, a process that is accelerated by warm temperatures and a gradually increasing

77   daylength (Hänninen, 2016). Inter-annual variation of these three environmental

78   drivers is strongly correlated (e.g. a warm winter reduces chilling and increases heat

79   supply, and the earlier leaf-out associates with short daylength). As a result, the direct

80   effect of daylength on spring phenology and its eventual interactions with chilling and

81   the heat requirement remain unclear and highly debated (Chuine *et al.*, 2010, Flynn &

82   Wolkovich, 2018, Körner & Basler, 2010, Laube *et al.*, 2014, Zohner *et al.*, 2016). In

83   this study, based on a large set of in situ phenology observations across Europe

84   (www.pep725.eu), we propose a framework to unravel the effect of daylength on leaf-

85   out phenology of temperate-zone deciduous trees and test the hypothesis that

86   daylength affects the leaf-out process by altering the heat requirement at any given

87   chilling accumulation.

88

89   We start by assuming that trees are characterized by an optimal, climate-dependent,

90   daylength (Figure. 1). Occasional late frost events give a competitive disadvantage to

91   individuals that leaf-out earlier than the species' optimal daylength, while reduced

92   light harvesting gives a competitive disadvantage to individuals that leaf-out later than

93   the optimum period. As such, an optimal date of leaf-out exists for a given species,

94   determined by a trade-off between maximizing annual carbon and nutrient uptake to

95   ensure competitive advantage by lengthening the duration of the ground cover period

96   (earlier leaf out is preferred) and reducing the risk of late frost damage after leaf out

97   (later leaf out gives more security and is thus preferred (Allstadt *et al.*, 2015, Liu *et*

98   *al.*, 2018) (Figure. 1a). In the long term, carbon assimilation and competitiveness are

99   determined by the lowest of these two cost functions, and the optimal leaf out date

100   occurs where the minimum cost yields the highest carbon assimilation and

101   competitiveness. Experimental studies have revealed a nonlinear relation between

102   accumulated chilling and the heat required for leaves to flush (typically quantified as

103   growing degree day units, GDD) (Figure. 1b). Daylength is hypothesized to act as a

104   cue controlling the sensitivity of meristem cell growth to warm temperatures, thereby

105   altering the apparent relationship between chilling and GDD requirement. Their non-

106   linear relation becomes steeper when days are shorter than optimal (short daylength,

107   Figure. 1c) and less steep when days are longer than optimal (long daylength, Figure.

108   1d).

109

110   Two substantially different impacts of sub-optimal daylength on leaf-out date are thus

111   theoretically possible: (i) Shorter than optimal daylength reduces the temperature

112   sensitivity (increases the GDD requirement), thereby avoiding precocious leaf-out that

113   would increase the risk of frost damage, (*i.e.* the short daylength effect, Figure. 1c).

114   (ii) Longer than optimal daylength increases the temperature sensitivity (reduces the

115   GDD requirement), effectively avoiding belated leaf-out at a time when solar

116   radiation is high and thus ideal for photosynthesis (*i.e.* the long daylength effect,

117   Figure. 1d).
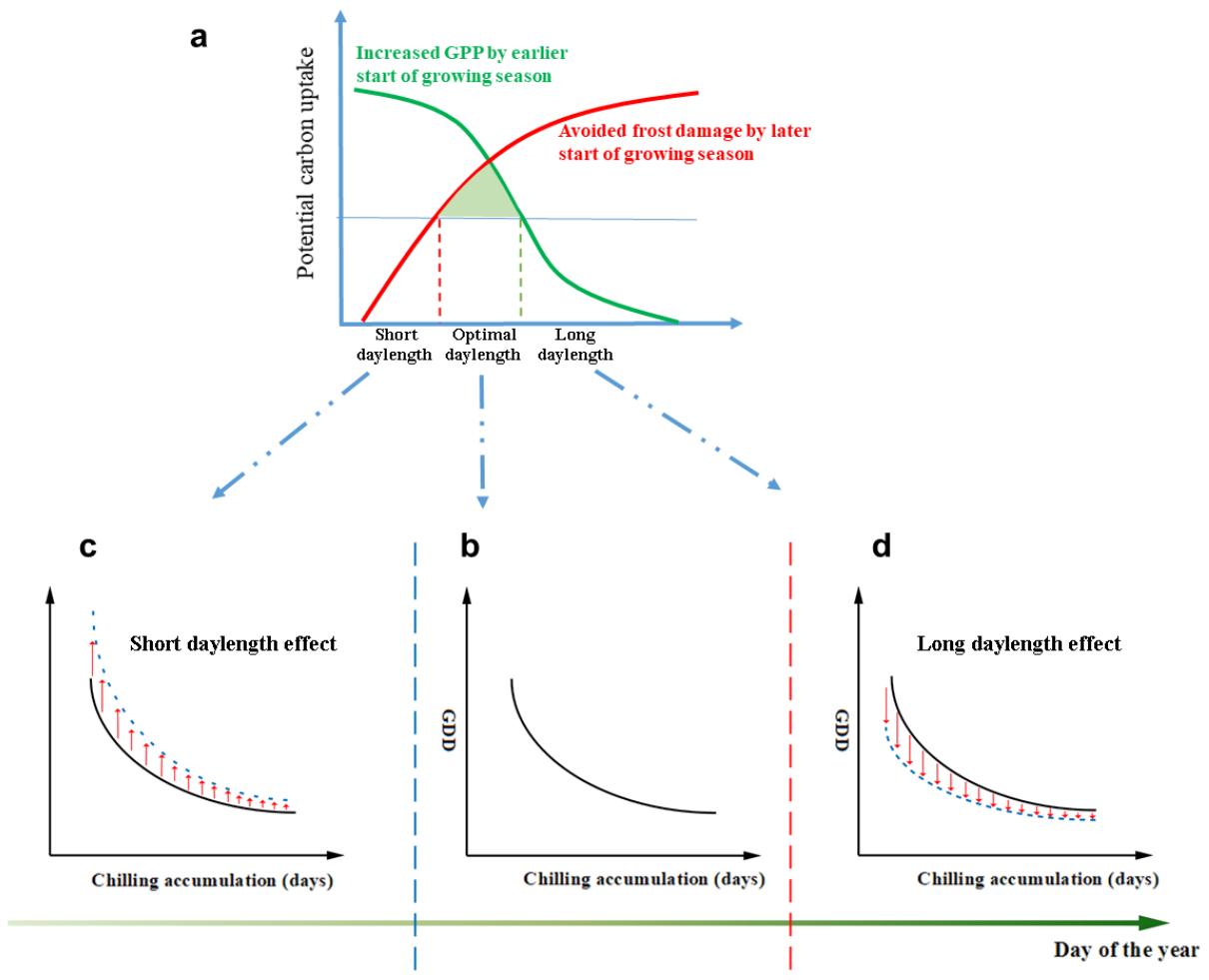
118   *Figure 1. (**a**) Conceptual scheme depicting why an optimal daylength for tree leaf-out*

119   *exists within which the competitiveness of a species is maximized and how this is*

120   *realized. Tree competitiveness is increased by earlier start of the growing season,*

121   *which maximizes annual carbon and nutrient uptake and reduces that of the*

122   *neighbors, but is subject to a trade-off with avoiding the risk of late frost-induced*

123   *damage for which a later start of growing season is preferred. The green filled area*

124   *represents the leaf-out period that ensures the most secure, high competitiveness and*

125   *carbon uptake. This study provides evidence that the spring leaf-out process requires*

126   *less warm temperatures (lower heat requirement; GDD), and thus becomes more*

127   *temperature responsive, as daylength increases. As such, daylength aids in*

128   *constraining leaf-out within the optimal period in both cold and warm springs. (**b**) In*

129   *very warm springs, when leaf-out is early, trees minimize the advance of leaf-out*

130   *because their temperature sensitivity is low under short daylength. This is reflected in*

131   *an increased GDD requirement and results in trees being protected against late frost*

132   *events, i.e. the "short daylength effect": (**c**) Under optimal daylength the GDD*

133   *required for leaf-out is mainly determined by the chilling accumulated during*

134   *endodormancy; (**d**) In very cold springs, when leaf-out is late, trees minimize the*

135   *delay of leaf-out because their temperature sensitivity becomes greater under*

136   *increasing daylength. This high temperature sensitivity is reflected in the reduced*

137   *GDD requirement, and protects trees against leafing-out too late, i.e. the "long*

138   *daylength effect".*

139

140 These two postulated consequences of daylength impacts have not been strongly

141 supported by empirical evidence. The short daylength effect in early spring, to our

142 knowledge, has not yet been empirically documented, whereas the long daylength

143 effect in late spring has been experimentally evidenced in earlier studies, albeit only

144 on cut twigs or saplings (Flynn & Wolkovich, 2018, Körner & Basler, 2010, Laube *et*

145 *al.*, 2014, Malyshev *et al.*, 2018) and not yet on mature trees. We therefore set out to

146 show the consequences of both a short and a long daylength on spring leaf-out of

147 mature trees of temperate deciduous tree species, to assess how widespread these two

148 effects are across these species, to quantify the sensitivities of the GDD requirement

149 to sub- and supra-optimal daylength and, last, to determine the relative importance of

150 chilling and daylength as controls of the leaf-out process.

151

152 **Materials and methods**

153 We tested the daylength effect on mature trees using data from 2377 sites of the

154 European phenological network (http://www.pep725.eu/) (Templ *et al.*, 2018). The date

155 of leaf-out had been recorded at each site for at least 30 years between 1950 to 2016,

156 but in most cases observations were available for many more years. Six deciduous tree

157 species were selected (for which sufficient observational data were available): *Fagus*

158 *sylvatica* (beech), *Aesculus hippocastanum* (horse chestnut), *Betula pendula* (birch),

159 *Fraxinus excelsior* (ash), *Quercus robur* (oak) and *Tilia cordata* (lime). In total 509,284

160 individual observations from 12348 site-species combinations at 2377 sites were used.

161 The sites mainly occurred in moderate climates in Central Europe (Supplementary

162 Figure 1 and 2). The leaf-out dates were defined based on the BBCH code (Biologische

163 Bundesanstalt, Bundessortenamt und Chemische Industrie, BBCH = 11, first visible

164 leaf stalk) (Templ *et al.*, 2018). We first determined the preseason length for each

165 species at each site as the period before leaf-out for which the partial correlation

166 coefficient between leaf-out and air temperature was highest (Fu *et al*, 2015). Using

167 this optimal preseason, we then calculated the GDD requirement for each species at

168 each site and in each year. We defined the GDD requirement as an integration of daily

169 mean temperature (*Tt*) above a temperature threshold (*Tth*) throughout the preseason

170 with the mean leaf-out dates as the end:

171 $$GDD = Tt - Tth, \qquad if \ Tt > Tth \qquad (1)$$

172 where *Tth* is the threshold temperature for GDD accumulation and *Tt* is the mean daily

173 temperature. We used a threshold *Tth* of 5 ℃. We also tested a temperature threshold

174 of 0 ℃, which produced very similar results. To best the robustness of the results, we

175 further calculated the GDD from the 1st December to the date of leaf-out for each

176 species at each site, and found very similar results (Supplementary Figure 3). We

177 therefore only report results using the threshold of 5 ℃ and the preseason

178 Chilling occurs at low, yet non-freezing temperatures and the number of days with

179 mean temperature between 0 and 5 ℃ was suggested as a good proxy for chilling

180 accumulation, although inter-species variation in the chilling efficiency of different

181 temperatures is probably high. Chilling requirement is a physiological parameter that

182 corresponds to the amount of chilling needed to break endodormancy and enter the

183 ecodormancy. In the present study, chilling was calculated as the number of days (CD)

184   when daily temperature was between 0 and 5 ℃ from 1 September in the previous year

185   until the day of leaf-out. We also tested another approach, using 0 ℃ and 10 ℃ as

186   temperature thresholds counting all days with mean temperatures between these

187   thresholds, which produced very similar results. Similar results were also obtained

188   when below-freezing temperatures were included, calculating as the number of days

189   when daily temperature below 5 ℃ (Supplementary Figure 4) or 7 ℃ (Supplementary

190   Figure 5), and similar results were obtained. We therefore only report the results based

191   on the chilling accumulation using the 0 ℃ - 5 ℃ temperature range.

192   Daylength at the day of leaf-out (DL) was calculated as a function of latitude and DOY:

193
$$DL = 24 - \frac{24}{\pi} \cos^{-1}\left[\frac{\sin\frac{0.8333\pi}{180} + \sin\frac{L\pi}{180}\sin\varphi}{\cos\frac{L\pi}{180} * \cos\varphi} \right] \tag{2}$$

194
$$\varphi = \sin^{-1}(0.29795 * \cos\theta) \tag{3}$$

195
$$\theta = 0.2163108 + 2 * \tan^{-1}\left(0.9671396 * \tan\left(0.0086 * (DOY - 186)\right)\right) \tag{4}$$

196   where *L* is the latitude of the phenological site.

197   The daily mean air temperature at each site was derived from a gridded climatic data

198   set of daily mean temperature at 0.25 º spatial resolution (approximately 25 km, ERA-

199   WATCH) (Fu *et al.*, 2014).

200

201   **The sensitivity of GDD requirement to chilling and daylength**

202   We calculated cumulative chilling, the GDD requirement and daylength at the day of

203   leaf-out for each year at each site. For each individual tree, we divided the data into

204   four subsets according to chilling accumulation, *i.e.* case 1: lowest chilling

205   accumulation: $CD < CDmean - 1$ standard deviation of CD (CDsd); case 2: low

206   chilling accumulation: $CDmean-CDsd < CD < CDmean$; case 3: high chilling

207   accumulation: $CDmean < CD < CDmean + CDsd$, and case 4: highest chilling

208   accumulation: $CD > CDmean + CDsd$. Within each CD subset, we subsequently

209   calculated the GDD requirement for three daylength conditions, i.e. leaf-out under

210   short-daylength conditions ($DL < DLmean - 0.75 DLsd$), under long-daylength

211   conditions ($DL > DLmean + 0.75 DLsd$) and under average-daylength conditions

212   ($DLmean - 0.5 DLsd < DL < DLmean + 0.5 DLsd$). The differences in mean GDD

213   requirement for leaf-out among the DL groups were tested using independent t-tests

214   for each chilling case and each species. Furthermore, we calculated the daylength

215   sensitivity of GDD as the slope of the linear regression between GDD and DL, and

216   then the average of the four daylength sensitivities was determined for each species at

217   each site. Using similar methodology, we divided the data into four subsets according

218   to DL for each individual tree, *i.e.* case 1: shortest daylength: $DL < DLmean - DLsd$;

219   case 2: short daylength: $DLmean - DLsd < DL < DLmean$; case 3: long daylength:

220   $DLmean < DL < DLmean + DLsd$, and case 4: longest daylength: $DL > DLmean +$

221   DLsd, and then calculated the chilling sensitivity of GDD for each species at each

222   site. To compare the relative importance of chilling versus that of daylength as

11

223   determinants of the GDD requirement for leaf-out, we first normalized the daylength

224   and chilling sensitivity, respectively, using a min-max normalization for each species:

225
$$Si = \frac{(Si - Smin)}{(Smax - Smin)} \tag{5}$$

226   Where Si is the daylength or chilling sensitivity at site i, Smin and Smax are the

227   minimum and maximum observed values of the daylength - or chilling sensitivity

228   across all trees of that species. Then, the mean and standard deviation of all

229   normalized sensitivities was calculated for each species. Histograms were used to

230   show the distribution of sensitivities across all trees for each species.

231

232   **Results and discussion**

233   In agreement with a multitude of previous studies (Cannell & Smith, 1983, Fu *et al.*,

234   2016, Laube *et al.*, 2014), we found that all studied tree species showed lower GDD

235   requirement under higher chilling conditions (Figure 2 and Supplementary Table 1).

236   Interestingly, we also observed that in all six species and within each CD group, the

237   GDD requirement for leaf-out was statistically significantly higher under short- than

238   average-daylength conditions, and significantly lower under long- than average-

239   daylength conditions (using paired *t* test, $P<0.001$, Figure 2, and Supplementary

240   Figure 6 and Supplementary Table 2 as an example at high chilling accumulation).

241   Contrasting results of the photoperiod effect were reported in experimental studies

242   (Heide 1993; Laube *et al*, 2014). However, almost all of these studies are based on

243   cuttings or saplings in manipulative experiments, and using constant day length rather

244   natural continuous changes in day length (e.g. Zohner *et al*, 2016; Laube *et al*.,

245   2014)., and young trees often behave opportunistically and exhibit earlier leaf-out

246   than mature trees of the same species. Using trees of different ontogenetic stages

247   might thus explain part of the differences among previous studies. In the present

248   study, we selected six species that belong to five families (Fagaceae, Betulaceae,

249   Malaceae, Sapindaceae, Oleaceae), some of which phylogenetically quite distinct

250   (Supplementary Figure 4). Because every tested species (all six species for which

251   sufficient observations were available) exhibited very similar daylength responses, we

252   postulate the widespread existence of a daylength effect among temperate zone

253   deciduous tree species, at least among European temperate-zone tree species. In line

254   with our findings, a recent study found consistent daylength effects on leaf-out

255   phenology exists across 28 woody species in a North American temperate forest

256   (Flynn & Wolkovich, 2018).

257   For each individual tree, we calculated the sensitivity of the GDD requirement for

258   leaf-out to changes in daylength. On average across all species and averaged over four

259   different chilling intensities, compared to the GDD requirement under average

260   daylength conditions, each one-hour decrease in daylength (comparable to the

261   observed inter-annual range) increased GDD by 37°C-days, i.*e.* by 26%

262   (Supplementary Figure 8a and b), while a 1-h increase in daylength decreased the

263   GDD requirement by 31°C-days, *i.e.* by 22% (Supplementary Figures 8a and b). We

264   further compared the relative sensitivity of the GDD requirement for leaf-out to

265   chilling and daylength (comparing the GDD response to one standard deviation of the

266   observed variation in either chilling or daylength), and observed species-specific

267   sensitivity differences among the six study species (Figure. 3).
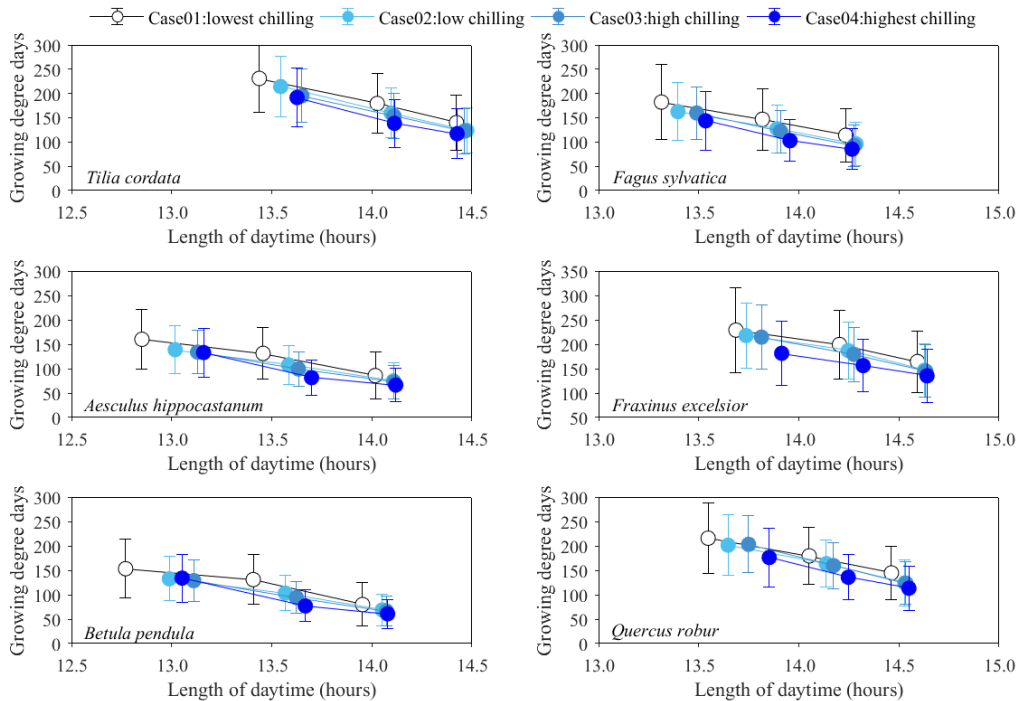

Figure 2. *Dependence of GDD requirement of leaf-out on daylength under constant*

269   *chilling conditions for six tree species. Dependencies are shown for four different*

270   *chilling intensities (see Methods).*

271

272   In detail, sensitivity to daylength was larger than the sensitivity to chilling in four out

273   of six species: *Betula pendula*, *Aesculus hippocastanum*, *Tilia cordata* and *Fraxinus*

274   *excelsior*, while no difference was detected in *Quercus robur*. *Fagus sylvatica* also

275   exhibited a pronounced sensitivity to daylength, but its sensitivity to chilling was even

276   greater (Figure. 3). *Fagus sylvatica* is indeed known as a highly chilling-sensitive

277   species (Kramer, 1994, Malyshev *et al.*, 2018). Our findings thus confirm that

278   daylength is an important co-regulator of leaf-out in mature temperate deciduous trees

14

279   (Flynn & Wolkovich, 2018, Körner & Basler, 2010), and further suggest that

280   daylength likely affects the leaf-out process indirectly by altering the non-linear

281   relationship between chilling and GDD requirement. These results also support the

282   hypothesis that the shorter daylength due to earlier leaf-out in spring contributes to the

283   declining apparent temperature sensitivity of leaf-out in European temperate

284   deciduous trees (Fu *et al*. 2015). These mechanisms are conceptualized in Figure. 4.

285   With climate warming, the GDD requirement increases due to reduced chilling, but

286   GDD supply increases more (Figure. 4, panel a). As a result, GDD supply equals

287   GDD requirement earlier in the year (visualized as an excess GDD supply in Figure 4,

288   panel a), which drives earlier leaf-out. The associated shorter daylength, however,

289   further increases the GDD requirement and thereby restricts the advance of leaf-out

290   (Figure. 4, panel b) and reduces the temperature sensitivity of leaf-out with climate

291   warming.
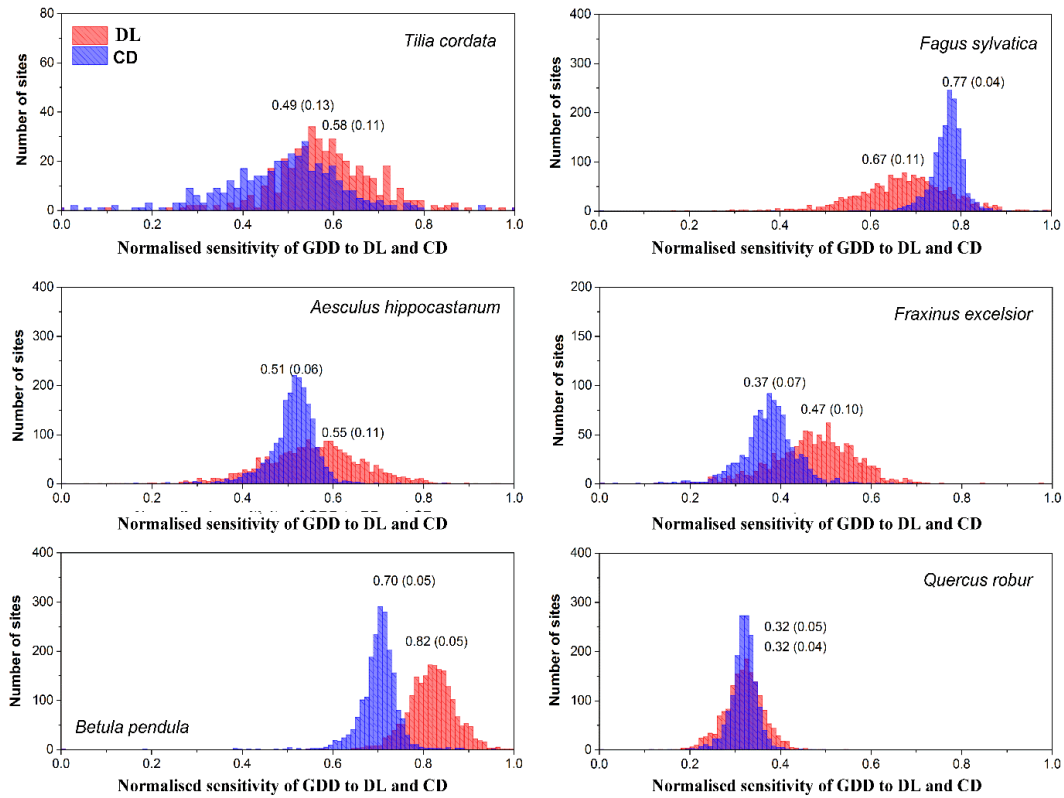
Figure 3. Histograms of the Growing Degree Day (GDD) sensitivity to changes in daylength (DL, in red) and in chilling (CD, in blue) across all individual trees of six deciduous tree species. Sensitivity was calculated as the change in GDD per one standard deviation in the observed DL and CD, respectively, and is therefore coined 'normalized sensitivity'. mean sensitivities and standard deviations (in brackets) are provided.

16
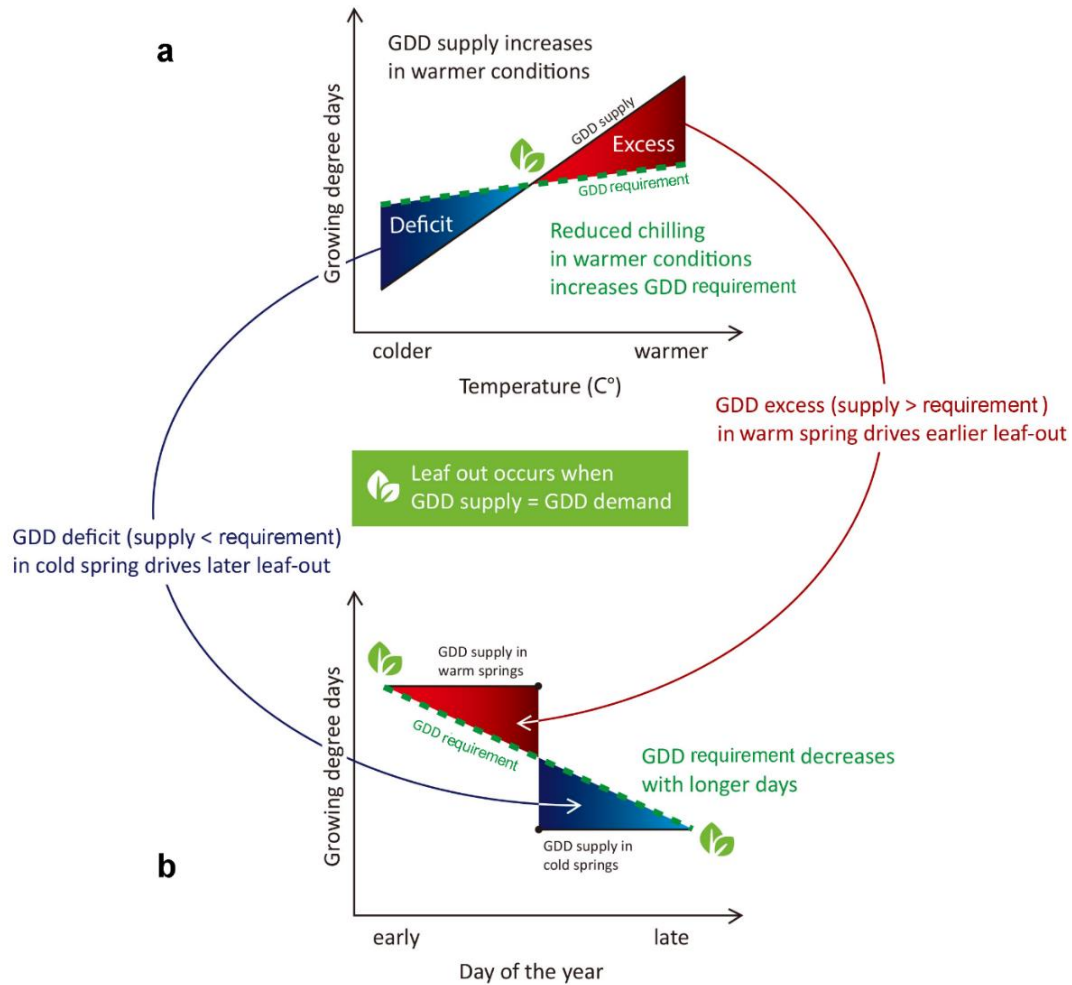
Figure. 4. *Conceptual scheme summarizing how daylength helps deciduous trees to*

*leaf-out within or close to the optimal period. (**a**) Leaf-out occurs when the supply of*

warm temperatures (GDD supply: accumulated daily growing degree days, GDD;

black line) equals the GDD requirement (physiological parameter to trigger leaf-out).

Note that the X axes indicate the climate from cold to warm spring. The more chilling

is accumulated, the lower the GDD requirement (green dashed line; for simplicity

reasons we here assume a linear relation). In cold springs, the deficit in GDD supply

*drives a delay in leaf-out date (blue areas in panels **a** and **b**). (**b**) The associated*

increasing daylength, however, increases the temperature sensitivity and thereby

causes a decline in the GDD requirement, with leaf-out occurring when GDD supply

equals the declining GDD requirement. In contrast, in warm springs (red areas in

*panels **a** and **b**), GDD supply typically exceeds the chilling-induced GDD requirement*

earlier in the year, but the short daylength earlier in spring induces a low

temperature sensitivity and thereby an increased GDD requirement, which minimizes

the warming-induced advance of leaf-out. Note that the X axes indicate the leaf-out

timing from early to late.

Daylength thus acts as an environmental cue, counteracting the advancing impact of

global warming, and helping trees to leaf-out close to their optimal date. As daylength

increases from early to late spring, we observed that the GDD requirement also

decreases under similar chilling conditions. Similar responses, supporting our

findings, were previously reported in experimental studies using cuttings, although the

daylength difference among treatments was very large in these studies (Zohner *et al.*,

2016). There are also other studies that reported a decreased photoperiod effect with

increasing chilling accumulation (Laube *et al*, 2014; Hänninen 2016), which may be

because the increasing chilling ensures leaf out at the optimal daylength, when the

photoperiod effect is minimal. We observed that the GDD requirement decreases

consistently from very short to very long daylength, suggesting that one single

physiological mechanism may suffice to explain the protective effect of daylength

against both early as well as late leaf-out. We speculate that daylength directly

impacts on the temperature sensitivity of cell development to warming. By keeping

the calculation of GDD constant with daylength, the increase in temperature

330   sensitivity with increasing daylength is mathematically translated into a reduced GDD

331   requirement. However, we acknowledge that the heat signal required by the

332   meristems to initiate leaf-out may not be directly altered by daylength, but that the

333   heat signal reception may become more efficient with increasing daylength. Our data,

334   unfortunately, do not allow unraveling the underlying physiological mechanism.

335

336   We further speculate that the daylength control over the GDD requirement depends on

337   the start date of the ecodormancy phase relative to the date when optimal daylength

338   thresholds are reached or passed. When ecodormancy begins late relative to the

339   optimum daylength thresholds, the temperature sensitivity of cell development is

340   elevated, resulting in reduced GDD requirement to force leaf-out. In contrast, when

341   ecodormancy starts earlier than the target daylength threshold, the temperature

342   sensitivity of cell development may remain low, but not zero, until the date when the

343   optimal daylength threshold is passed. The starting date of ecodormancy, however,

344   cannot easily be determined empirically and is therefore typically ignored in

345   phenology studies, explaining why the relation between daylength and the start of

346   ecodormancy remains poorly understood (Chuine *et al.*, 2016), despite their

347   importance for pushing the field beyond the state of the art (Hänninen, 2016). To our

348   knowledge, neither the start date of ecodormancy, *i.e.* the start date of GDD

349   accumulation, nor the optimal daylength thresholds have been well studied (but see

350   Chuine *et al*, 2016). As long as dormancy remains poorly understood, also the

351   estimations of chilling and forcing units remain uncertain. For example, the duration

352   of the chilling accumulation period and the start date of the heat accumulation period,

353   as well as their interactions are still unclear. Similarly, the optimal temperature ranges

354   for chilling accumulation and the temperature threshold above which GDD's start to

355   accumulate, as well as the length of GDD accumulation are poorly understood.

356   Different assumptions can, however, lead to contrasting and sometimes illogical

357   results. For example, an increased GDD requirement is obtained when leaf out is very

358   late and the GDD is calculated over a fixed number of days prior to leaf out (see

359   Supplementary Figure 9). Studies focusing on ecophysiological experiments are thus

360   urgently needed to fully understand spring phenology and enable the development of

361   reliable phenology synthesis studies and –models (Chuine & Régnière, 2017,

362   Hänninen *et al.*, 2019).

363

364   Climate warming-induced spring phenology advances substantially alter regional and

365   global biogeochemical cycles and climate systems (Forzieri *et al.*, 2017, Myneni *et*

366   *al.*, 1997, Peñuelas & Filella, 2009). However, as the daylength effect reduces the

367   temperature sensitivity of leaf-out in warmer years, slowing down the advancing rate

368   of leaf-out, it thereby also reduces the warming-induced extension of ground cover,

369   and the carbon uptake, evapotranspiration and albedo. This study found that all

370   investigated temperate-zone deciduous tree species (the six species for which

371   sufficient observational data were available) use daylength as a signal to help ensuring

372   that leaf-out occurs close to a species-dependent optimal time of the year, by

373   increasing the GDD requirement for leaf-out when daylength is too short, and

374   reducing the GDD requirement as daylength becomes too long.

375

376   **References**

377   Allstadt AJ, Vavrus SJ, Heglund PJ, Pidgeon AM, Thogmartin WE, Radeloff VC (2015) Spring
378   plant phenology and false springs in the conterminous US during the 21st century.
379   Environmental Research Letters, **10**, 104008.

380   Cannell M, Smith R (1983) Thermal time, chill days and prediction of budburst in Picea
381   sitchensis. Journal of Applied Ecology, 951-963.

382   Chuine I (2010) Why does phenology drive species distribution? Philosophical Transactions
383   of the Royal Society B: Biological Sciences, **365**, 3149-3160.

384   Chuine I, Bonhomme M, Legave JM, García De Cortázar‐Atauri I, Charrier G, Lacointe A, Amé
385   glio T (2016) Can phenological models predict tree phenology accurately in the
386   future? The unrevealed hurdle of endodormancy break. Global Change Biology, **22**,
387   3444-3460.

388   Chuine I, Morin X, Bugmann H (2010) Warming, Photoperiods, and Tree Phenology. Science,
389   **329**, 277-278.

390   Chuine I, Régnière J (2017) Process-Based Models of Phenology for Plants and Animals.
391   Annual Review of Ecology, Evolution, and Systematics, **48**, 159-182.

392   Elzinga JA, Atlan A, Biere A, Gigord L, Weis AE, Bernasconi G. 2007. Time after time:
393   flowering phenology and biotic interactions. Trends in Ecology and Evolution, **22**,
394   432-439.

395   Flynn DFB, Wolkovich EM (2018) Temperature and photoperiod drive spring phenology
396   across all species in a temperate forest community. New Phytologist, **219**, 1353-
397   1362.

398   Forzieri G, Alkama R, Miralles DG, Cescatti A (2017) Satellites reveal contrasting responses of
399   regional climate to the widespread greening of Earth. Science, **356**, 1180-1184.

400   Fu YH, Campioli M, Vitasse Y *et al.* (2014) Variation in leaf flushing date influences autumnal
401   senescence and next year's flushing date in two temperate tree species. Proceedings
402   of the National Academy of Sciences of the United States of America, **111**, 7355-
403   7360.

404   Fu YH, Liu Y, De Boeck HJ *et al.* (2016) Three times greater weight of daytime than of night‐
405   time temperature on leaf unfolding phenology in temperate trees. New Phytologist,
406   **212**, 590-597.

407   Fu YH, Zhao HF, Piao SL *et al.* (2015) Declining global warming effects on the phenology of
408   spring leaf unfolding. Nature*, ***526**, 104-107.

409   Hänninen H (2016) Boreal and temperate trees in a changing climate. Netherlands, Springer
410   Business Media, Dordrecht.

411   Hänninen H, Kramer K, Tanino K, Zhang R, Wu J, Fu YH (2019) Experiments are necessary in
412   process-based tree phenology modelling. Trends in Plant Science, **24**, 199-209.

413   Heide O.M. (1993) Day length and thermal time responses of budburst during dormancy
414   release in some northern deciduous trees, Physiologia plantarum, **88**, 531–540.

415   Keenan TF, Gray J, Friedl MA *et al.* (2014) Net carbon uptake has increased through
416   warming-induced changes in temperate forest phenology. Nature Climate Change*, ***4**,
417   598.

418   Körner C, Basler D (2010) Phenology Under Global Warming. Science*, ***327**, 1461-1462.

419   Körner C, Basler D, Hoch G, Kollas C, Lenz A, Randin CF, Vitasse Y, Zimmermann NE (2016)
420   Where, why and how? Explaining the low-temperature range limits of temperate
421   tree species. Journal of Ecology, **104**, 1076-1088

422   Kramer K (1994) Selecting a model to predict the onset of growth of Fagus sylvatica. Journal
423   of Applied Ecology, **31**, 172-181.

424   Lang GA (1987) Dormancy: a new universal terminology. HortScience, **22**, 817-820.

425   Laube J, Sparks TH, Estrella N, Höfler J, Ankerst DP, Menzel A (2014) Chilling outweighs
426   photoperiod in preventing precocious spring development. Global Change Biology*,*
427   **20**, 170-182.

428   Lenz A, Hoch G, Körner C, Vitasse Y (2016) Convergence of leaf-out towards minimum risk of
429   freezing damage in temperate trees, Functional Ecology, **9**, 1480-1490.

430   Liu Q, Piao S, Janssens IA *et al.* (2018) Extension of the growing season increases vegetation
431   exposure to frost. Nature Communications*, ***9**, 426.

432   Malyshev AV, Henry HA, Bolte A, Khan MaA, Kreyling J (2018) Temporal photoperiod
433   sensitivity and forcing requirements for budburst in temperate tree seedlings.
434   Agricultural and Forest Meteorology*, ***248**, 82-90.

435   Menzel A, Sparks TH, Estrella N *et al.* (2006) European phenological response to climate
436   change matches the warming pattern. Global Change Biology*, ***12**, 1969-1976.

437   Myneni RB, Keeling C, Tucker CJ, Asrar G, Nemani RR (1997) Increased plant growth in the
438   northern high latitudes from 1981 to 1991. Nature*, ***386**, 698.

439   Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across
440   natural systems. Nature*, ***421**, 37-42.

441   Peñuelas J, Filella I (2001) Responses to a warming world. Science*, ***294**, 793-795.

442   Peñuelas J, Filella I (2009) Phenology feedbacks on climate change. Science*, **324**, 887-888.

443   Piao S, Liu Z, Wang T *et al.* (2017) Weakening temperature control on the interannual
444   variations of spring carbon uptake across northern lands. Nature Climate Change*, **7**,
445   359.

446   Richardson AD, Anderson RS, Arain MA *et al.* (2012) Terrestrial biosphere models need
447   better representation of vegetation phenology: results from the North American
448   Carbon Program Site Synthesis. Global Change Biology*, **18**, 566-584.

449   Templ B, Koch E, Bolmgren K, Ungersbock M, Paul A, Scheifinger H (2018) Pan European
450   Phenological database (PEP725): a single point of access for European data.
451   International journal of biometeorology*, **62**, 1-5.

452   Vitasse Y, Basler D (2013) What role for photoperiod in the bud burst phenology of European
453   beech. European Journal of Forest Research*, **132**, 1-8.

454   Vitasse Y (2013) Ontogenic changes rather than difference in temperature cause understory
455   trees to leaf out earlier. New phytologist, **198**, 149-155.

456   Zohner CM, Benito BM, Svenning J-C, Renner SS (2016) Day length unlikely to constrain
457   climate-driven shifts in leaf-out times of northern woody plants. Nature Climate
458   Change*, **6**, 1120-1123.

459   Zohner, C. M., Mo, L., & Renner, S. S. (2018). Global warming reduces leaf-out and
460   flowering synchrony among individuals. eLife, **7**, e40214.

Supplementary Materials

479 **Supplementary Legends:**

480 **Supplementary Table1.** The mean GDD requirement of leaf-out in different

481 daylength conditions, *i.e.* DLearly, short daylength effect, DLmid, average length in

482 daylength and DLlate, long daylength effect, under same chilling accumulation

483 conditions (case) for six tree species. Four chilling condition were studies, *e.g.*

484 case01, chilling lowest; case02, chilling low, case03, chilling high and case04,

485 chilling highest (see Methods for details). n indicates the number of trees.

486 **Supplementary Table 2.** The difference in mean GDD requirement of leaf-out

487 between the daylength treatments, e.g. under short daylength: GDDshort DL; under

488 optimal daylength: GDDavgDL; under long daylength: GDDlongDL) under the same

489 chilling conditions in the low chilling accumulation group.

490 **Supplementary figure 1.** Distribution of the selected phenological sites.

491 **Supplementary figure 2**. (**a**) The climate of selected phenological sites. One circle

492 indicates one site. (**b**) and (**c**) the annual temperature and precipitation across all

493 selected sites.

494 **Supplementary figure 3**. Dependence of GDD requirement of leaf-out on daylength

495 under constant chilling conditions for six tree species. Dependencies are shown for four

496 different chilling intensities (see Methods). The GDD was calculated as daily sum of

497 the daily temperature above 50C over the period from 1st September to the date of leaf-

498 out

499 **Supplementary figure 4**. Dependence of GDD requirement of leaf-out on daylength

500 under constant chilling conditions for six tree species. Dependencies are shown for

501 four different chilling intensities (see Methods). The chilling was calculated as day

502   when the daily temperature below 50C over the period from 1st September to the date

503   of leaf-out.

504   **Supplementary figure 5**. Dependence of GDD requirement of leaf-out on daylength

505   under constant chilling conditions for six tree species. Dependencies are shown for

506   four different chilling intensities (see Methods). The chilling was calculated as day

507   when the daily temperature below 70C over the period from 1st September to the date

508   of leaf-out.

509   **Supplementary figure 6.** Mean GDD requirement of leaf-out in the daylength

510   treatments (left panels) and under the same chilling (days) conditions (right panels) in

511   the low chilling accumulation group. The numbers in the right panels are the mean

512   chilling days in each of the three daylength regimes in the left panels (using the same

513   colors). The GDD requirement was calculated as explained in Methods. Letters, *i.e.* a,

514   b and c, indicate statistically significant differences (at $P < 0.001$).

515   **Supplementary figure 7.** Phylogenetic tree of European forest tree species and the

516   family names were provided with different color. The figure was modified from study

517   of Sardans *et al.,* 2015. The species that selected in the present study were marked with

518   pink boxes.

519   **Supplementary figure 8.** Changes in absolute and relative values of GDD requirement

520   for spring leaf-out (across all chilling conditions) under one hour shorter (black) and

521   longer (white) daylengths for each and all of the six studied species.

522   **Supplementary figure 9.** Dependence of GDD requirement of leaf-out on daylength

523   under constant chilling conditions for six tree species. The GDD was calculated using

524   a fixed length prior to leaf-out for each species at each site. Dependencies are shown

525   for four different chilling intensities (see Methods).

532

Species (n)	Chilling conditions	DLearly (hours)	DLmid (hours)	DLlate (hours)	DLearly		DLmid		DLlate	
					mean	std	mean	std	mean	std
Aesculus hippocastanum (2069)	case01	12.9	13.5	14.0	160.1	60.6	131.3	52.5	85.9	48.1
	case02	13.1	13.6	14.1	138.9	48.9	107.5	39.3	74.9	36.3
	case03	13.0	13.6	14.1	133.9	44.9	99.4	35.6	72.3	34.0
	case04	13.2	13.7	14.1	133.0	49.8	81.6	35.7	66.4	34.1
Betula pendula (2052)	case01	12.8	13.4	14.0	153.3	60.4	131	50.9	79.8	44.4
	case02	13.1	13.7	14.1	134.0	48.9	77.0	32.4	60.8	29.4
	case03	13.1	13.6	14.1	128.9	42.2	94.2	32.6	66.2	30.3
	case04	13	13.6	14.1	132.6	45.3	103.4	35.7	68.9	32.2
Fagus sylvatica (1588)	case01	13.3	13.8	14.2	182.4	77.9	146.8	63.2	113.9	55.2
	case02	13.4	13.9	14.3	162.6	59.5	127.4	49.4	96.2	44.7
	case03	13.5	13.9	14.3	159.8	54.5	122.0	43.8	92.5	42.3
	case04	13.5	14	14.3	143.7	60.2	102.8	42.8	85.3	41.8
Fraxinus excelsior (1044)	case01	13.7	14.2	14.6	229.0	87.1	199.4	70.4	164.5	62.9
	case02	13.7	14.2	14.6	217.7	67.1	187.1	58.7	146.7	55.0
	case03	13.8	14.3	14.6	214.6	65.9	179.6	55.5	144.7	53.7
	case04	13.9	14.3	14.6	181.4	65.6	156.2	54.4	135.3	54.7
Quercus robur (1686)	case01	13.5	14.1	14.5	215.9	72.9	179.5	58	144.5	55.3
	case02	13.6	14.1	14.5	203.3	58.0	159.6	46.9	123.5	43.6
	case03	13.7	14.2	14.5	201.5	62.1	163.7	48.7	124.3	46.8
	case04	13.9	14.2	14.6	176.3	60.2	135.8	46.8	113.2	46
Tilia cordata (438)	case01	13.2	13.9	14.3	231.0	69.4	179.4	61.4	140.0	57.4
	case02	13.4	14.0	14.4	214.3	62.3	159.7	51.4	123.9	46.9
	case03	13.5	14.0	14.4	195.4	55.3	153.2	46.2	122.8	46.5
	case04	13.5	14.0	14.3	191.7	61.2	138.6	49.2	116.9	51.2

Supplementary Table 2. The difference in mean GDD requirement of leaf-out between the

534 daylength treatments, e.g. under short daylength: GDDshort DL; under optimal daylength:

535 GDDavgDL; under long daylength: GDDlongDL) under the same chilling conditions in the

536 low chilling accumulation group.

537

Species	GDD shortDL vs. GDDavgDL			GDD longDL vs. GDDavgDL		
	t	P	df	t	P	df
Aesculus hippocastanum	32.986	<0.001	1928	-45.943	<0.001	1928
Betula pendula	33.118	<0.001	970	-32.853	<0.001	970
Fagus sylvatica	31.793	<0.001	1491	-36.568	<0.001	1491
Fraxinus excelsior	19.682	<0.001	970	-32.853	<0.001	970
Quercus robur	33.999	<0.001	1569	-40.378	<0.001	1569
Tilia cordata	22.646	<0.001	396	-18.238	<0.001	396

538 **Supplementary figure 1.** Distribution of the selected phenological sites.
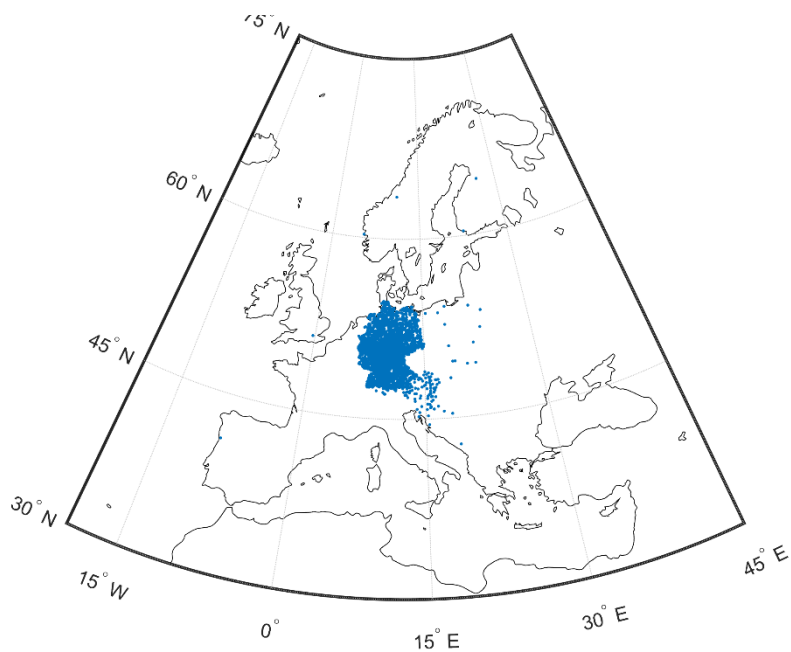
Supplementary figure 2. (**a**) The climate of selected phenological sites. One circle
540 indicates one site. (**b**) and (**c**) the annual temperature and precipitation across all
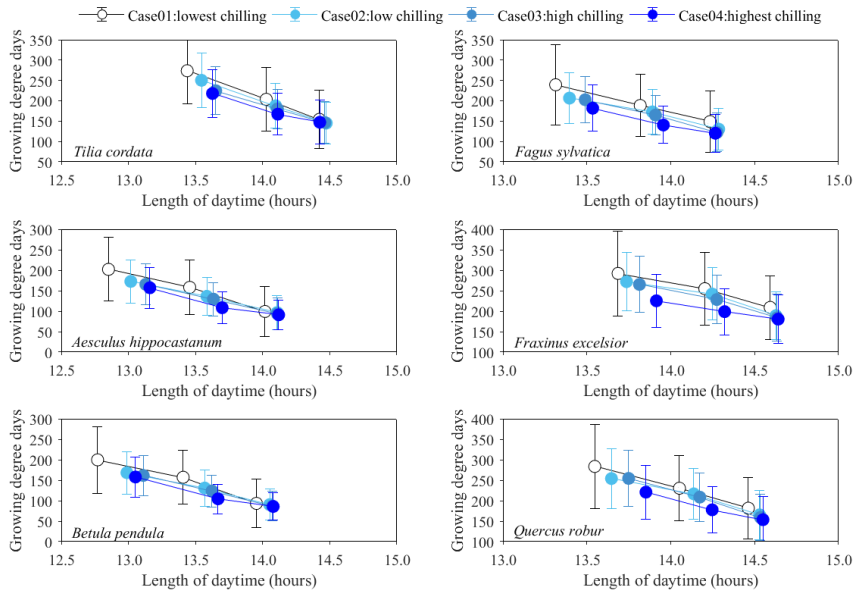541 selected sites.
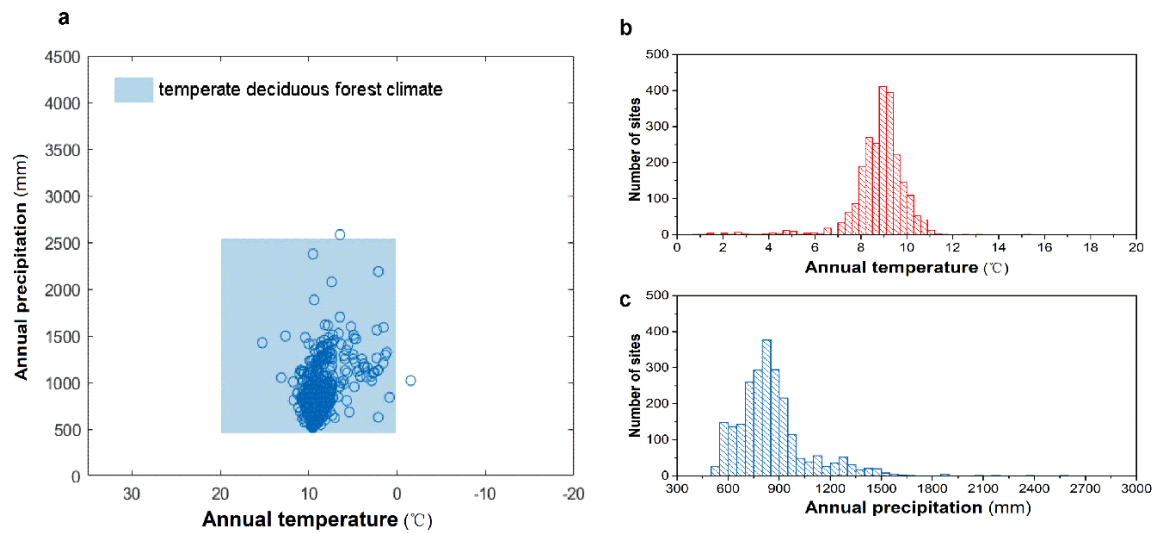
542   **Supplementary figure 3.** Dependence of GDD requirement of leaf-out on daylength

543   under constant chilling conditions for six tree species. Dependencies are shown for

544   four different chilling intensities (see Methods). The chilling was calculated as day

545   when the daily temperature below 5^0C over the period from 1st September to the date

546   of leaf-out.
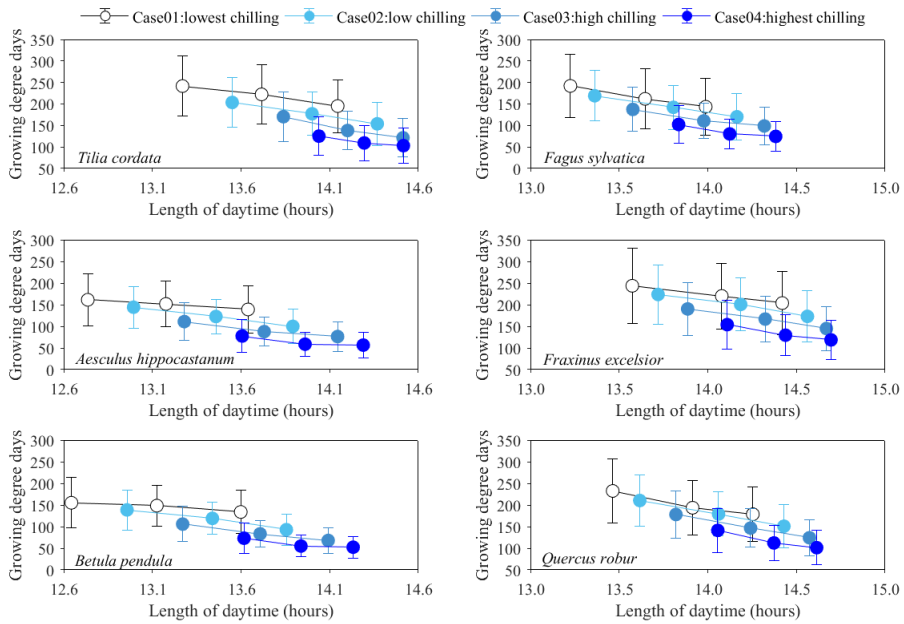
547

548   **Supplementary figure 4.** Dependence of GDD requirement of leaf-out on daylength

549   under constant chilling conditions for six tree species. Dependencies are shown for

550   four different chilling intensities (see Methods). The chilling was calculated as day

551   when the daily temperature below 7^0C over the period from 1st September to the date

552   of leaf-out.

553

554   **Supplementary figure 4.** Dependence of GDD requirement of leaf-out on daylength
555   under constant chilling conditions for six tree species. Dependencies are shown for
556   four different chilling intensities (see Methods). The GDD was calculated as daily
557   sum of the daily temperature above 5^0C over the period from 1st December to the date
558   of leaf-out

559
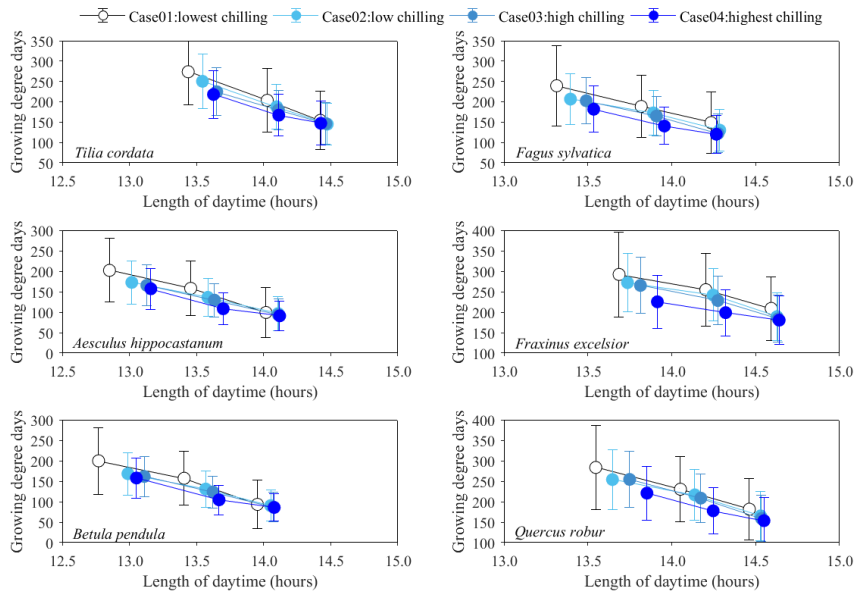
Supplementary figure 6. Mean GDD requirement of leaf-out in the daylength treatments (left panels) and under the same chilling (days) conditions (right panels) in the low chilling accumulation group. The numbers in the right panels are the mean chilling days in each of the three daylength regimes in the left panels (using the same colors). The GDD requirement was calculated as explained in Methods. Letters, *i.e.* a, b and c, indicate statistically significant differences (at *P* < 0.001).
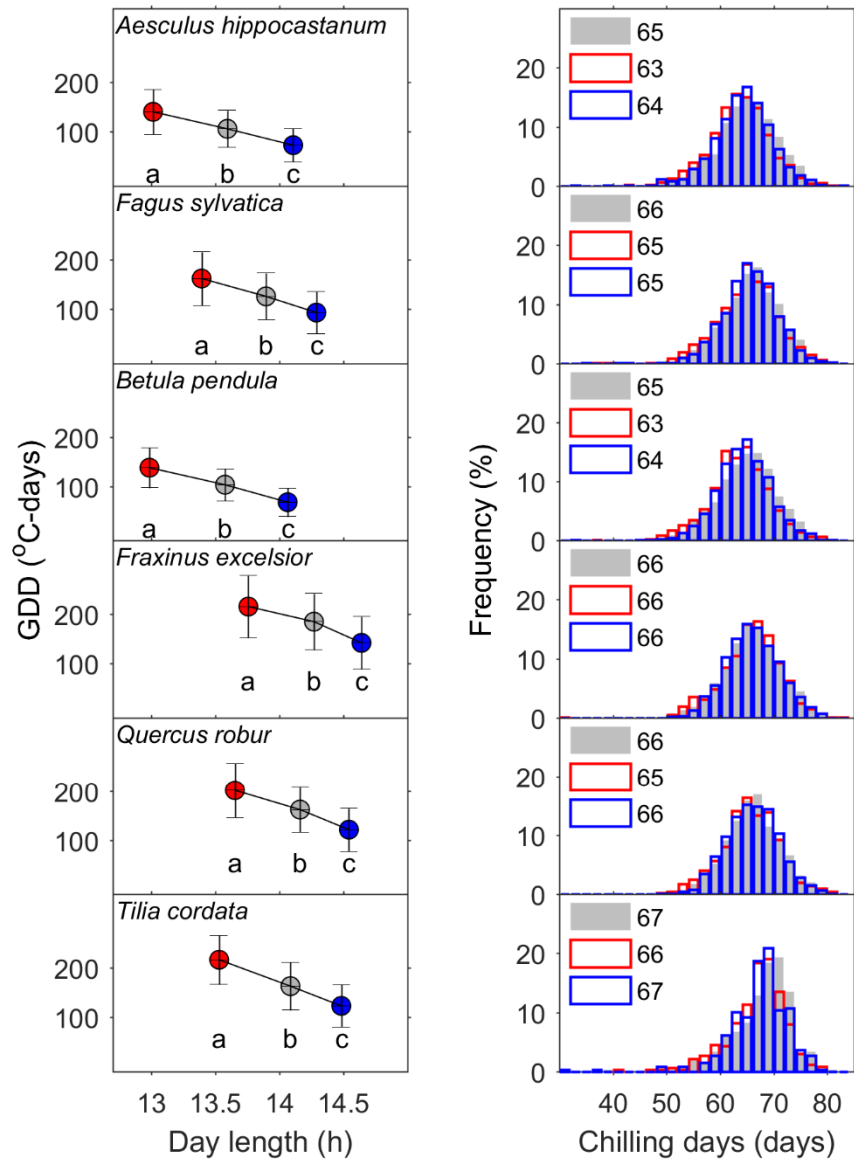
Supplementary figure 7. Phylogenetic tree of European forest tree species and the family

names were provided with different color. The figure was modified from study of Sardans *et*

al., 2015. The species that selected in the present study were marked with boxes.
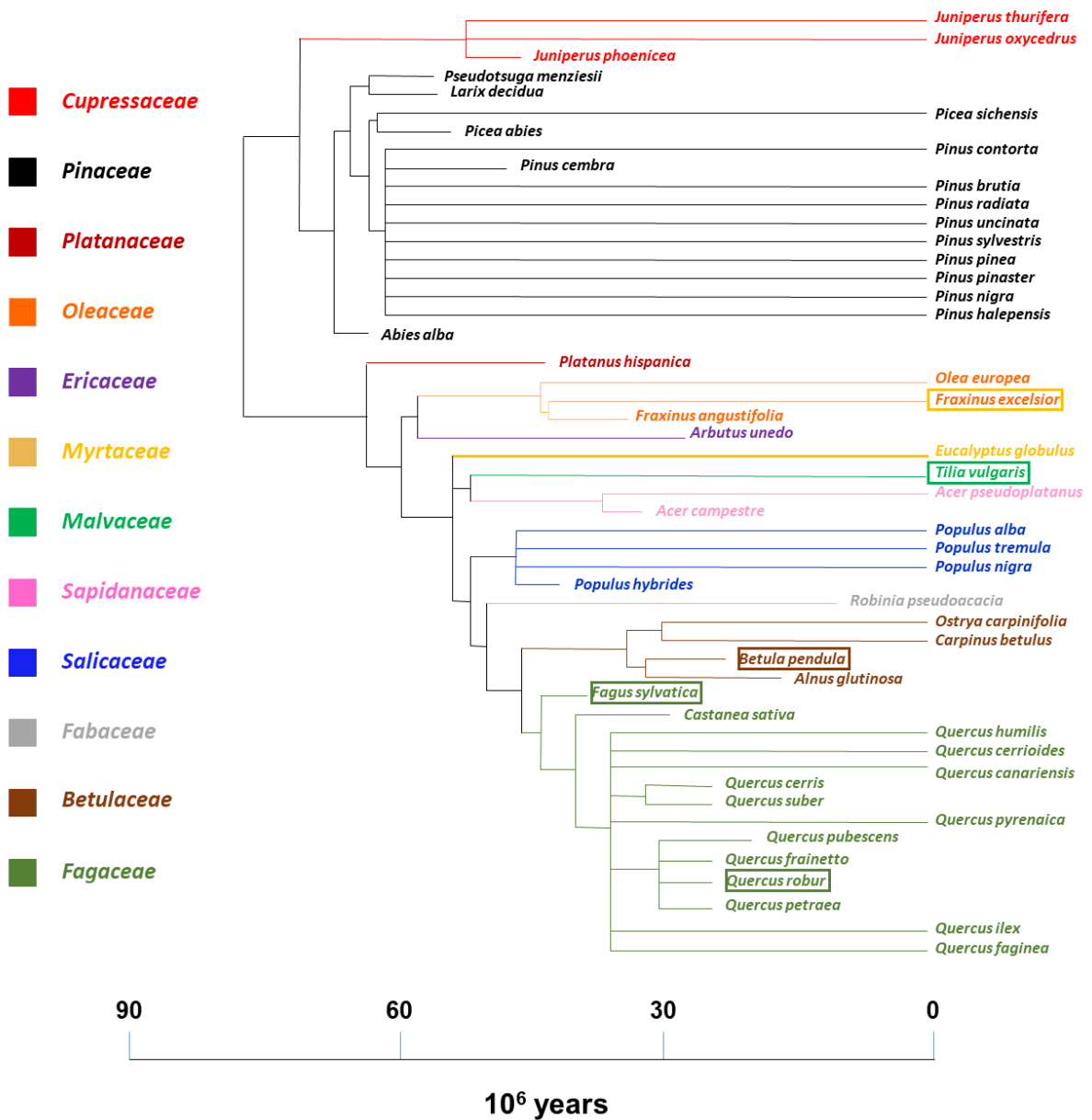
Supplementary figure 8. Changes in absolute (a) and relative (b) values of GDD
requirement for spring leaf-out (across all chilling conditions) under one hour shorter (black)
and longer (white) daylength for each and all of the six studied species.
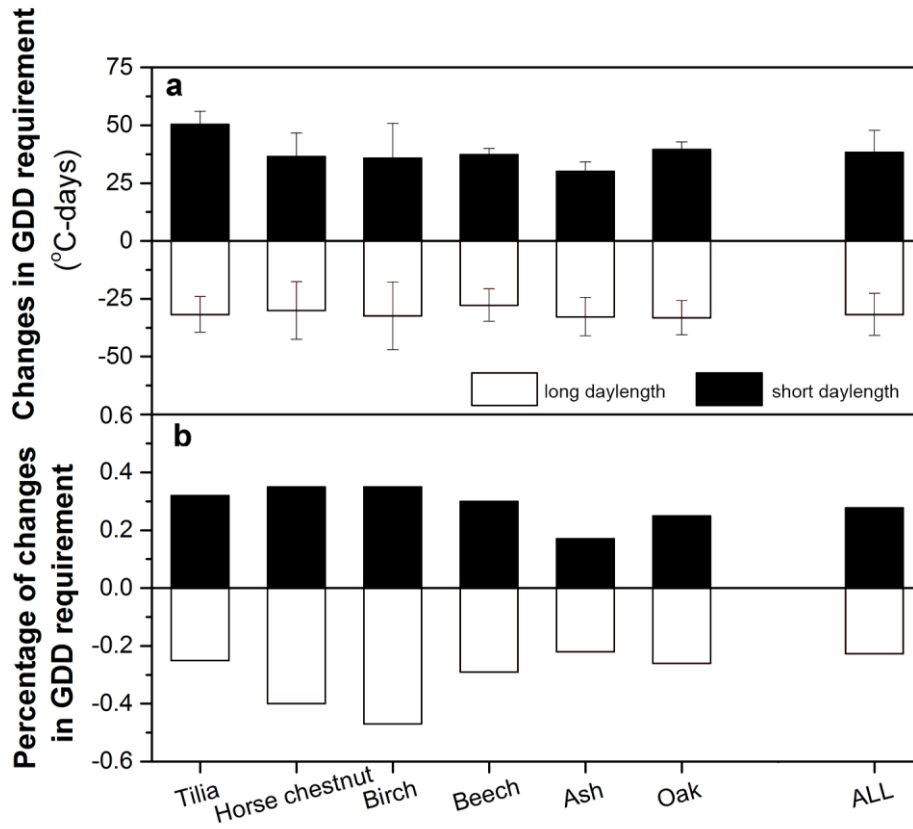
Supplementary figure 9. The distribution of normalized daylength (DL, in red) and chilling
(CD, in blue) sensitivity of GDD requirement for leaf-out for all study species. The histograms
show the distribution across all sites and the mean sensitivities and standard deviations (in
brackets) are provided. The GDD was calculated from the 1st Jan to the date of leaf-out for each
year of each species at each site.

37