Full Paper

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Chilled to be forced: the best dose to wake up buds from winter dormancy

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Summary

- Over the last decades, spring leaf-out of temperate and boreal trees has substantially advanced in response to global warming, affecting terrestrial biogeochemical fluxes and the Earth's climate system. However, it remains unclear whether leaf-out will continue to advance with further warming because species' effective chilling temperatures, as well as the amount of chilling time required to break dormancy, are still largely unknown for most forest tree species.
- Here, we assessed the progress of winter dormancy and quantified the efficiency of different chilling temperatures in six dominant temperate European tree species by exposing 1170 twig cuttings to a range of temperatures from −2°C to 10°C for 1, 3, 6 or 12 wk.
- We found that freezing temperatures were most effective for half of the species or as effective as chilling temperatures up to 10°C, that is, leading to minimum thermal time to and maximum success of budburst. Interestingly, chilling duration had a much larger effect on dormancy release than absolute chilling temperature.
- Our experimental results challenge the common assumption that optimal chilling temperatures range *c.* 4–6°C, instead revealing strong sensitivity to a large range of temperatures. These findings are valuable for improving phenological models and predicting future spring phenology in a warming world.

Introduction

The time when leaves emerge from the bud in spring is a critical period in the seasonal cycle of temperate and boreal plants. The development and reproductive success of trees depends on the timing of this phenological event in relation to climatic conditions (Zohner *et al.*, 2018). Temperate and boreal trees have therefore developed complex mechanisms to forecast spring onset and maximise competitive ability. In essence, the timing of spring leaf-out is the result of a trade-off between minimising the risk of frost damage and maximising resource uptake (nutrients, water, CO_2 and light) (Vitasse *et al.*, 2014b). Temperature and photoperiod are thought to be the main environmental cues used by trees to regulate the physiological progression of winter dormancy (Körner & Basler, 2010; Delpierre *et al.*, 2016). Although longer photoperiods have been shown to advance budburst and accelerate cell development in a few species (Basler & Körner, 2014; Fu *et al.*, 2019), its effect on dormancy release seems negligible in most temperate species (Laube *et al.*, 2014; Zohner *et al.*, 2016; Fadón *et al.*, 2020). By contrast, numerous studies have highlighted the importance of winter temperatures slightly above freezing (referred to as 'chilling temperatures') for dormancy release and budburst in temperate, boreal and even subtropical trees (Way & Montgomery, 2015; Flynn & Wolkovich, 2018; Du *et al.*, 2019). Already a century ago, Coville (1920) proposed a physiological explanation, whereby chilling temperatures stimulate the degradation of starch into soluble sugars and induce an osmotic gradient allowing for water influx to the bud cells.

The period in late autumn and winter during which growth is not possible regardless of temperature conditions has been commonly referred to as 'endodormancy', while 'ecodormancy' is the subsequent state in which buds are able to flush when sufficiently exposed to warm temperatures (Lang *et al.* (1987); for an overview of the biochemical perspective see Anderson *et al.* (2001)). For clarity's sake we hereafter use the term 'dormancy' whenever we refer to a general state of bud rest. Once dormancy has been induced, a negative relationship has commonly been found between the amount of heat (forcing) required for budburst and the exposure time to chilling temperatures both experimentally (Cannell & Smith, 1983; Murray *et al.*, 1989; reviewed in Ettinger *et al.*, 2020) and empirically *in situ* over large spatial or temporal gradients (Wenden *et al.*, 2020). An accurate representation of winter chilling in phenological models is crucial for future projections of spring phenology (Gauzere *et al.*, 2019; Asse *et al.*, 2020). However, empirical knowledge on the effective

chilling temperature ranges is still missing for most temperate tree species (Chuine *et al.*, 2016). Because of these uncertainties, phenological models substantially differ in their implementation of effective chilling temperature ranges (Fadón *et al.*, 2020). For instance, the 'Utah model', widely used in agricultural sciences, assumes that chilling temperatures between 2.5 and 12.5°C are efficient to break dormancy with a maximum efficiency at *c.* 7°C (Richardson *et al.*, 1974). When temperatures fall outside of this range, the model assumes no, or even a negative, effect on dormancy release. Similarly, the 'dynamic model' based on the experimental results from Fishman *et al.* (1987) assumes that effective chilling ranges between 0 and 7.2°C with an optimum temperature of 6°C. Interestingly, most models assume that freezing temperatures have no effect on dormancy release or even reverse the chilling effect (reviewed in Hänninen, 2016), but no experimental study to date has proven that this is true for the majority of temperate trees. In addition, some experimental evidence from Scandinavia suggests that the upper range of effective chilling range lies *c.* 12°C for Loblolly Pines (Garber, 1983) and for Birch (Myking & Heide, 1995). More experimental knowledge about effective chilling temperature ranges across a variety of species is therefore urgently needed to improve the performance of phenology models and to forecast spring phenology under future climate change.

In addition, studies that address the effect of winter chilling on spring leaf-out phenology commonly focus on the amount of forcing required for buds to burst in response to varying chilling conditions. However, a lack of chilling does not only affect the time of budburst but could potentially impact tree vitality by decreasing bud survival (the proportion of buds that open in spring), which is a good indicator for yield in fruit trees (Petri & Leite, 2003; Campoy *et al.*, 2019), yet only rarely monitored and/or accounted for in forest trees (but see Man *et al.* (2017)). As the climate continues to change and winters get warmer, the importance of chilling for spring phenology is likely to increase, because a lack of chilling might lower the sensitivity of trees to spring warming due to insufficient release of dormancy (Fu *et al.*, 2015; Asse *et al.*, 2018). This reduction of chilling may be most pronounced at the southern distribution ranges of temperate tree species, where winters are already mild and are likely to be above the optimal range of chilling efficiency (Luedeling *et al.*, 2011).

In this study, we assessed the efficiency and optimal duration of a range of chilling temperatures to both reduce the time to budburst and increase the percentage of budburst (as a proxy for dormancy depth and bud vitality) in six major European tree species, namely *Betula pendula* Roth, *Larix decidua* Mill., *Tilia cordata* Mill., *Acer pseudoplatanus* L., *Quercus robur* L. and *Fagus sylvatica* L. In total, 1170 twig cuttings were collected during autumn and winter and were exposed to natural and artificial chilling conditions. This allowed us to calculate species-specific parameters of dormancy progression, such as maximum dormancy depth, velocity of dormancy release and minimal forcing requirement for budburst to address the following questions:
(**1**) What is the (optimal) range of chilling temperatures to reduce dormancy depth and increase bud vitality?
(**2**) Are there species-specific differences in the parameters of dormancy progression related to the species' phenological strategy (i.e. early, intermediate or late flushing species)?

Based on recent evidence from phenological modelling (Wang *et al.*, 2020; Zohner *et al.*, 2020), we expected the temperature range of efficient chilling for dormancy release to be wider than commonly assumed, including subzero temperatures. We further expected that key parameters of dormancy progression show substantial interspecific variation depending on species' phenological strategy. Assuming that chilling requirements are sufficiently met at the study site, we expected that the minimum forcing requirement would reflect the order of flushing among species.

Materials and Methods

Study species and site

We selected six deciduous tree species that are native to Europe and showed contrasting spring phenology, namely *Betula pendula* Roth, *Larix decidua* Mill., *Tilia cordata* Mill., *Acer pseudoplatanus* L. *Quercus robur* L. and *Fagus sylvatica* L. For clarity and brevity, from this point forwards, we refer to each species by its genus name. *Betula* and *Larix* are early successional species with a typical early flushing strategy (Asse *et al.*, 2018). *Acer*, *Quercus* and *Fagus* are late flushing species in the area (Lenz *et al.*, 2013) whereas *Tilia* shows intermediate leaf-out dates (Lenz *et al.*, 2016). *Fagus* is known from the literature to be sensitive to photoperiod and to require a high amount of chilling to fully break its winter dormancy (reviewed in Vitasse & Basler, 2013). The chilling requirement of the species has rarely been investigated, but was observed by Laube *et al.* (2014) to be sensitive to chilling until end of winter. The same authors showed that forcing requirement after long chilling (until 14 March) is rather low for the pioneer species *Betula* and *Larix*, intermediate for *Fagus* and *Quercus* and highest for *Acer*.

Twig cuttings of the six species were harvested during autumn and winter 2018/2019 in a mature mixed forest near Zurich at the foothill of the Uetliberg mountain (47°21′22″N, 8°27′35″E; 550 m above sea level (asl)). The mean annual air temperature recorded at the nearest climate station was 9.8°C and the mean annual precipitation was 1080 mm (1990–2020 mean recorded at Zurich Fluntern, 555 m asl. *c.* 8 km away from the study site). Using the same temperature dataset, the mean long-term air temperature over the winter season (December to February) was 1.5°C, with a mean temperature of the coldest month (January) of *c.* 1°C. The autumn/winter 2018–2019, was particularly warm with a mean winter temperature recorded on site (Hobo Logger MX2203, TidbiT) of 2.5°C (Fig. 1).

Sampling

For each species, we selected five healthy adult trees (>10 m height, >50 yr). The main experiment consisted of three sampling dates that took place on 19 October 2018 (main harvest), 29 November 2018 and 17 January 2019, which may correspond to unchilled, partially and fully chilled buds depending on species

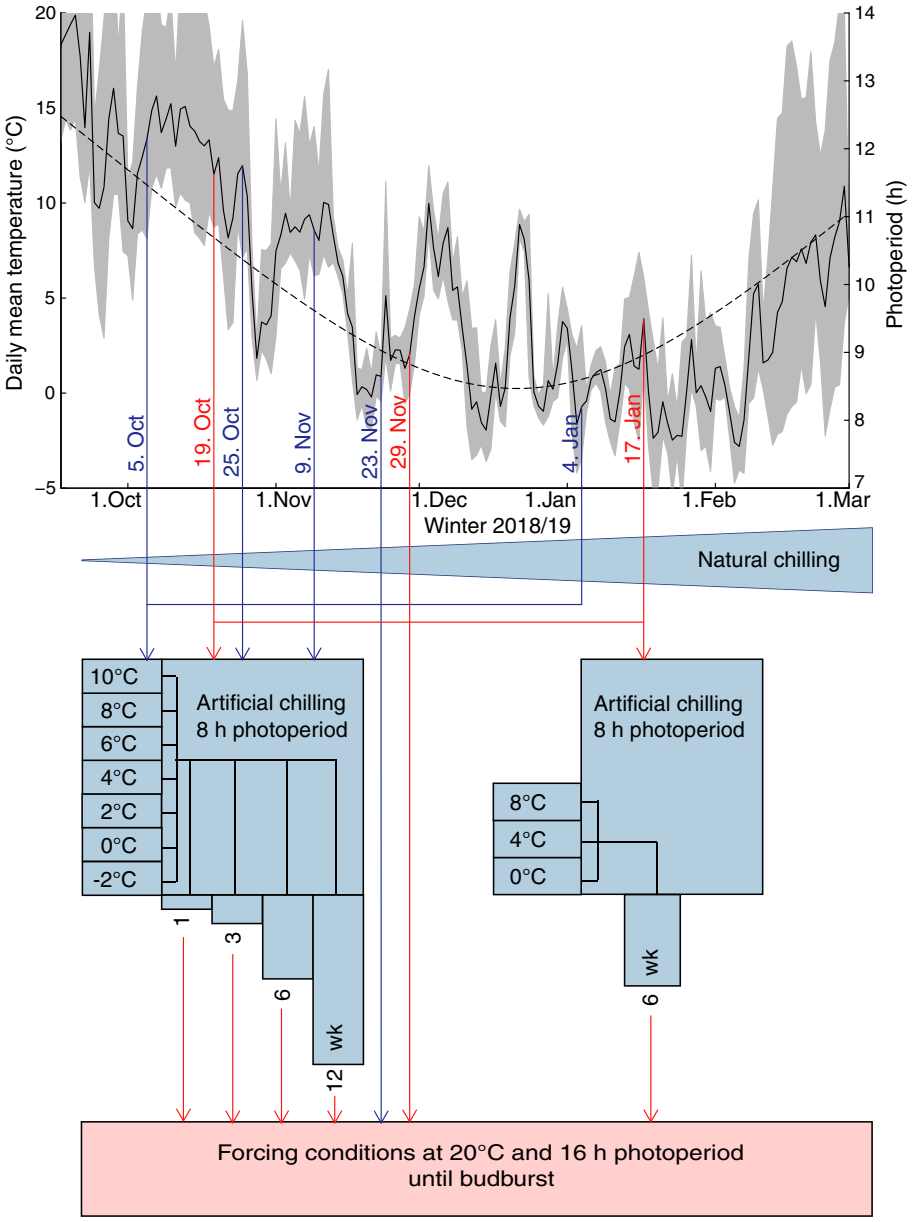

Fig. 1 Daily mean (black line), minimum and maximum (shaded area) air temperature and photoperiod (black dashed line) at the study site near Zurich, Switzerland over the winter season 2018/2019 along with the experimental design. Red vertical lines correspond to the three sampling dates of the main experiment. At each sampling date a set of cuttings was directly transferred into a forcing chamber at 20°C and monitored subsequently until budburst. Twigs sampled on 19 October 2018 were additionally exposed to seven chilling temperatures (from −2°C to +10°C) during 1, 3, 6 or 12 wk before being moved into the forcing chamber. Cuttings collected on 17 January 2019 were exposed to three chilling temperatures (0, 4 and 8°C) for 6 wk. Blue vertical lines correspond to an additional set of trees that were sampled on additional five dates and twigs were then directly exposed to forcing conditions to study the progress of dormancy depth under natural conditions.

(Fig. 1). In total, 34 twigs (29 on 19 October 2018, 1 on 29 November 2018 and 4 on 17 January 2019) of *c.* 60 cm length with intact buds were harvested for each of the five selected individuals per species (total of 1020 twigs) with a 7-m long pole pruner. Twig cuttings were put into wetted plastic bags in the field (to prevent desiccation), recut and placed in tap water on the same day. All remaining leaves were removed manually to prevent drying from transpiration. All twigs were pruned to a length of *c.* 50 cm and placed into plastic boxes with deionised water to minimise bacterial development (Supporting Information Fig. S1). We then labelled and grouped the twigs so that each of the five individuals (replicates) per species was represented in every temperature treatment. The different temperature treatments started 1 wk after the sampling on 19 October 2018 and 1 d after all other sampling dates. During the twig preparation, twigs were kept outside at ambient temperature. To prevent vessel occlusion during the experiment, the base of each cutting was recut (by *c.* 0.5 cm) and placed into fresh deionised water every week for the 20°C forcing treatment or every second week for the chilling treatments.

To obtain complementary information about the progress of dormancy under natural conditions, we sampled another five individual trees of the same six species on five additional dates (5 October 2018, 25 October 2018, 9 November 2018, 23 November 2018 and 4 January 2019; Fig. 1). At each sampling date, these twigs were directly placed into forcing conditions at 20°C (see below for details about climatic conditions in the forcing chamber) to quantify the forcing requirement to budburst as a proxy for dormancy depth under natural conditions.

Chilling and forcing conditions

The whole study design is depicted in Fig. 1. To test the chilling efficiency at different temperatures, the twigs sampled on 19 October 2018 (main harvest) were exposed to constant temperatures from −2°C to 10°C with 2°C steps using seven climate chambers. Light sources (halogen lamps, photosynthetic photon flux density PPFD = *c.* 100 μmol m^2 s^{-1}, measured by Li-Cor Li189 quantum photosynthetically active radiation (PAR) light sensor at bud height) were set to an 8 h photoperiod (shortest day at this latitude) and were exchanged between the chambers every week to remove the potential effect of light intensity (<20 μmol m^2 s^{-1} of difference). In total, 20 cuttings per species, corresponding to 4 × 5 replicates, were placed in each of the 7 climate chambers. After 1, 3, 6 or 12 wk of exposure to each chilling temperature treatment, five twigs per species, corresponding to the five studied individuals, were transferred from each climate chamber to forcing conditions, that is, placed in a large climate chamber at 20°C under a 16 h photoperiod (halogen lamps, photosynthetic photon flux density (PPFD) = 50 μmol m^2 s^{-1} at bud height) to remove potential photoperiod limitations (longest day at this latitude). To prevent bud desiccation, the relative air humidity in the chambers was kept at *c.* 70% throughout the experiment. Twigs collected at the last sampling date on 17 January 2019, were only exposed to 0, 4 or 8°C for 6 wk (maximum chilling; Fig. 1).

Mean air temperature in the forcing chamber ranged between 19.3°C and 20.4°C at bud height (19.7 ± 0.2°C; mean ± SD). Because of this stable temperature, we quantified the amount of forcing as the number of days to budburst.

Throughout the experiment, temperatures in all treatments were recorded using temperature loggers (MX2203; Onset Computer Corp., Bourne, MA, USA). All climate chambers provided stable temperature treatments, and actual temperatures were within ± 0.5°C of the target value, except for the warmest chilling treatment, which exceeded the target temperature on average by 1.2°C; see Table 1).

Phenology monitoring

Bud development of every twig was monitored every third or fourth day (twice a week) using a four-stage categorical scale (Vitasse *et al.*, 2013): at stage 0 (dormant bud), no bud development was visible; at stage 1 (bud swelling), buds were swollen and/or elongating; at stage 2 (budburst), bud scales were open and leaves were partially visible; at stage 3 (leaf-out), leaves had fully emerged from the buds but were still folded, crinkled or pendant, depending on species; and at stage 4 (leaf unfolded), at least one leaf was fully unfolded. For the deciduous conifer *Larix decidua*, we stopped monitoring at stage 3, when the emerging needles start to spread from each other. The day of year for each stage was recorded for the earliest bud per twig. At 3 wk after the first bud of a twig reached the budburst stage, we assessed the percentage of buds that reached at least stage 2 (budburst), as a proxy for twig vitality, taking all buds per twig into account. The number of buds per twig varied across species with on average five buds for *Acer*, 13 for *Fagus*, *Quercus* and *Tilia*, 18 for *Betula* and 48 for *Larix*. Because *Quercus* breaks only *c.* 40% of all buds during the first flush (personal observations on attached branches from adult trees) budburst percentage of this species was standardised relative to this maximum value under natural conditions.

Each twig was recut and examined for intact buds and vessels at weekly intervals. The water in the −2°C chamber was completely frozen, but did not cause freezing damage to the buds, nor did it cause increased mortality rates (Tables S1, S2). Eventually the 0°C chilling treatment led to a slightly increased mortality in twigs of *Betula*, *Tilia* and *Larix* most probably due to frequent freeze-thaw events. Little or no chilling exposure caused most twig cuttings of *Acer*, *Tilia* and *Betula* to desiccate after months of forcing, although some cuttings managed to flush after *c.* 180 d of forcing at 20°C, demonstrating that cuttings can survive for this long. For cuttings that desiccated after several months in these critically low chilling conditions, the date of desiccation (visual inspection) was used as a proxy for budburst (earliest possible date of budburst) and a budburst percentage of 0% was assigned. This allowed us to improve the fit of the forcing–chilling as well as the vitality–chilling curve in the range of low chilling. Because of the exceptionally high forcing requirements under minimal chilling, delaying this date by as much as 1 month did not affect the observed patterns.

Data analysis and statistics

For the analysis, we focused on phenological stage 2 (i.e. budburst) because this stage represented the earliest clearly visible indicator of leaf emergence for all species. In addition, later stages may depend on the availability of resources (established water and nutrient fluxes), which may be limited when using twig cuttings. For the analysis of days to budburst and budburst percentage (see next paragraph), we used data from the main sampling on 19 October 2018 (1, 3, 6 or 12 wk of artificial chilling) and from the maximum chilling treatment sampled on 17 January 2019 (12 wk of natural chilling plus 6 wk of artificial chilling at 0° and 4°C; see Fig. 1), which corresponded to 18 wk total chilling. Additionally, the same analyses were performed using the control cuttings (only natural chilling) from all sampling dates.

Days to budburst

Based on the existing literature (e.g. Murray *et al.*, 1989), we fitted a negative exponential function to examine the relationship between forcing required to budburst and chilling exposure as follows:

Table 1 Means and standard deviations of the temperature measured inside the climate chambers.

Temperature treatment (°C)	−2	0	2	4	6	8	10
Mean ± SD of actual temperature (°C)	−1.7 ± 1.1	0.1 ± 1.1	2.1 ± 0.7	3.6 ± 0.8	5.7 ± 0.6	7.6 ± 0.7	11.2 ± 3.5

$$f(t) = a + ie^{rt}$$

where a is the asymptote (corresponding to the days to budburst after saturating amounts of chilling), i is the intercept (corresponding to the days to budburst without any chilling), r is the rate of decay and t is the chilling time in weeks. The asymptote was approximated by the mean days to budburst after the longest chilling exposure (18 wk total chilling) to avoid impossible (negative) values, whereas the other parameters were fitted by the model.

Budburst percentage

The success of budburst (budburst percentage) in relation to chilling exposure was modelled using a Gompertz growth function as follows:

$$f(t) = ae^{-be^{rt}}$$

where a is the asymptote (corresponding to the budburst percentage after saturating amounts of chilling), b is the shift along the x-axis, r is the growth rate and t is the chilling time in weeks. The asymptote was fixed at 100%, assuming that all species reach this target value when species-specific chilling requirements were fully satisfied.

The effect of the different chilling temperatures on days to budburst and budburst percentage was tested using nonlinear least square (nls) models. Chilling temperature was only further considered if it significantly improved the model.

To compare chilling requirements among species, we extracted three key parameters from these models in terms of days to budburst at 20°C (forcing) and budburst percentage (vitality):

(**1**) 'Maximum dormancy depth' was defined as the forcing requirement until budburst and budburst percentage under low chilling (1 wk) to identify both the sensitivity to forcing temperatures and the (in)ability to flush during early winter (true endodormancy).

(**2**) 'Minimum forcing' was defined as the forcing requirement until budburst and budburst percentage under high chilling (*c.* 18 wk) to approximate the flushing performance when dormancy is completely released.

(**3**) 'Velocity of dormancy release' was defined as the rate of decay extracted from the exponential model of the forcing–-chilling curve and the growth rate extracted from the Gompertz model of the vitality–chilling relationship.

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

Results

Days to budburst at 20°C under varying chilling duration and temperature

After short (1 wk) exposure to chilling conditions, *Acer* and *Tilia* required on average *c.* 180 d to budburst, whereas *Betula* and *Fagus* required *c.* 60 d to budburst (Fig. 2a) and *Quercus* and *Larix* required only 35 and 20 d to budburst, respectively (Fig. 2a). For *Acer*, *Tilia* and *Betula*, days to budburst decreased rapidly towards the asymptote of minimum forcing. By contrast, *Fagus*, *Quercus* and *Larix* showed a weaker response to chilling duration, with forcing to budburst just or still not approaching the asymptote, even after 18 wk of chilling (Fig. 2a). The rate of decay was significantly lower (faster decrease in forcing) at −2°C and 0°C in *Acer*, *Tilia*, *Betula* and *Fagus*, whereas similar chilling efficiencies for all tested temperatures between −2°C and +10°C were found for *Quercus* and *Larix* (Fig. 2a, see also Fig. 4; Table S3 to compare model parameters).

Under natural chilling, days to budburst significantly decreased in all species to a minimum that differed only marginally between 4 January and 17 January 2019, indicating that chilling requirement is likely to be fully satisfied (Fig. 2b).

Percentage of budburst under varying chilling duration and temperature

Budburst percentage significantly increased with longer exposure to artificial chilling conditions in all species irrespective of the chilling temperature applied (Fig. 3a). For instance, budburst percentages of *Acer*, *Tilia* and *Betula* were mostly below 20% after 1 wk of artificial chilling and increased in a sigmoid-like relationship with increasing chilling duration up to nearly 100% after 18 wk (Fig. 3a). This increase was especially pronounced under lowest chilling temperatures (−2°C and 0°C) in *Acer* and *Betula* (Fig. 3a; see also section below 'Efficiency of chilling temperatures to release dormancy'). Budburst percentages of *Quercus*, *Fagus* and *Larix* increased slower and linearly with increasing chilling exposure to reach maximum values *c.* 75% after the longest chilling exposure. *Quercus* surprisingly performed worse under the longest chilling treatment.

Interestingly, under natural conditions, budburst percentages of *Acer*, *Tilia* and *Betula* remained close to 0% until 9 November 2018 (i.e. DOY 313; Fig. 3b). However, after this date, budburst percentages increased rapidly and reached almost 100% for *Tilia* and *Betula* and *c.* 65% for *Acer*. By contrast *Fagus*, *Quercus* and *Larix* were already able to budburst with budburst percentages *c.* 20% under lowest chilling conditions, which slowly increased with the sampling date. By the last sampling date on 17 January, *Larix* and *Quercus* reached values close to 100% while *Fagus* only reached *c.* 60%.

Efficiency of chilling temperatures to release dormancy

Chilling temperature treatments had a substantial and significant effect on the rate of decay of forcing required to budburst in *Acer*, *Betula* and *Tilia* (Fig. 4; Tables S3, S4) with −2°C and 0°C triggering a faster decline of days to budburst (lower rate of decay). The chilling duration required to reduce the forcing requirement to budburst by 50% was shortened by 15 d (*Acer*), 10 d (*Betula*) and 4 d (*Tilia*) for the −2°C treatment compared with all temperature treatments pooled. Surprisingly, also chilling temperatures up to 10°C reduced days to budburst in all species.

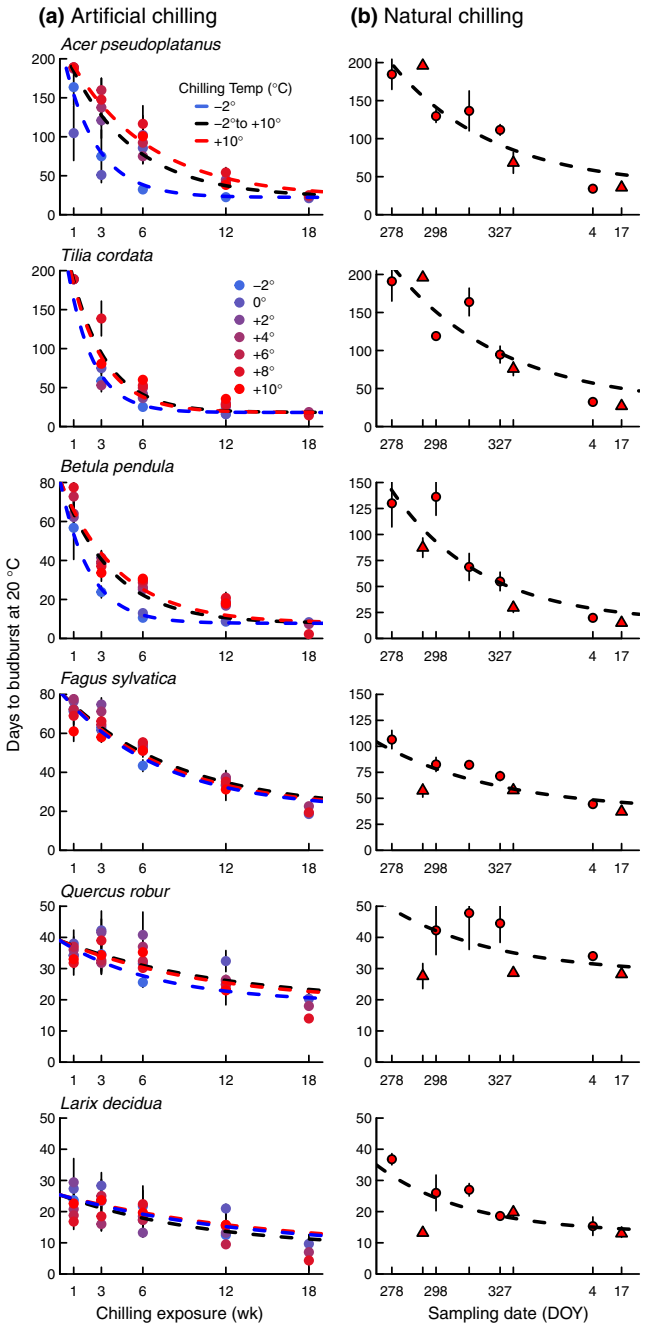

Fig. 2 Number of days at 20°C required to budburst in response to different duration of either artificial (a) or natural (b) chilling exposure. Each dot represents the mean of the five replicates with the corresponding standard error per temperature treatment. Curves were estimated using exponential models for pooled temperatures (black dashed line) as well as for the highest (red dashed line) and lowest (blue dashed line) temperature treatment for the artificial chilling treatments. Twigs from the main sampling on 19 October 2018 (before substantial natural chilling occurred) were used except for the 18 wk treatment that corresponds to the last sampling conducted on 17 January 2019 with additional 6 wk of artificial chilling. Twigs that were exposed to natural chilling originated either from the same trees used in the main experiment (triangles) or from an additional set of trees within the same population (circles). Note the difference in the y-axis scale among the different species.

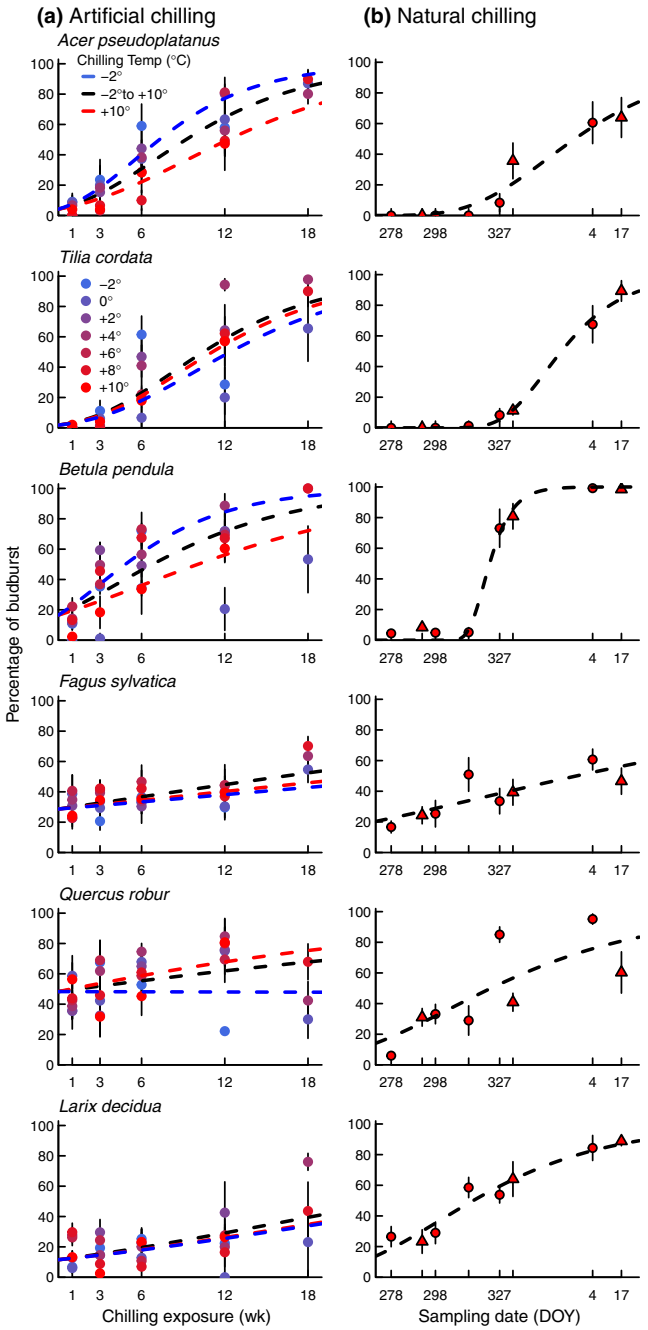

Fig. 3 Percentage of budburst (as a proxy for vitality) in response to artificial chilling (a) or natural chilling (b). Each dot represents the mean of the five replicates with the corresponding standard error per temperature treatment. Curves were estimated using Gompertz models for pooled temperatures (black dashed line) as well as for the highest (red dashed line) and lowest (blue dashed line) temperature treatment for the artificial treatments. Twigs from the main sampling on 19 October 2018 (before substantial natural chilling occurred) were used except for the 18 wk treatment that corresponds to the last sampling on 17 January 2019 with additional 6 wk of artificial chilling. This maximum chilling treatment was used to estimate minimum forcing requirements to budburst. Twigs that were exposed to natural chilling originated either from the same trees used in the main experiment (triangles) or from an additional set of trees within the same population (circles). DOY, day of year.

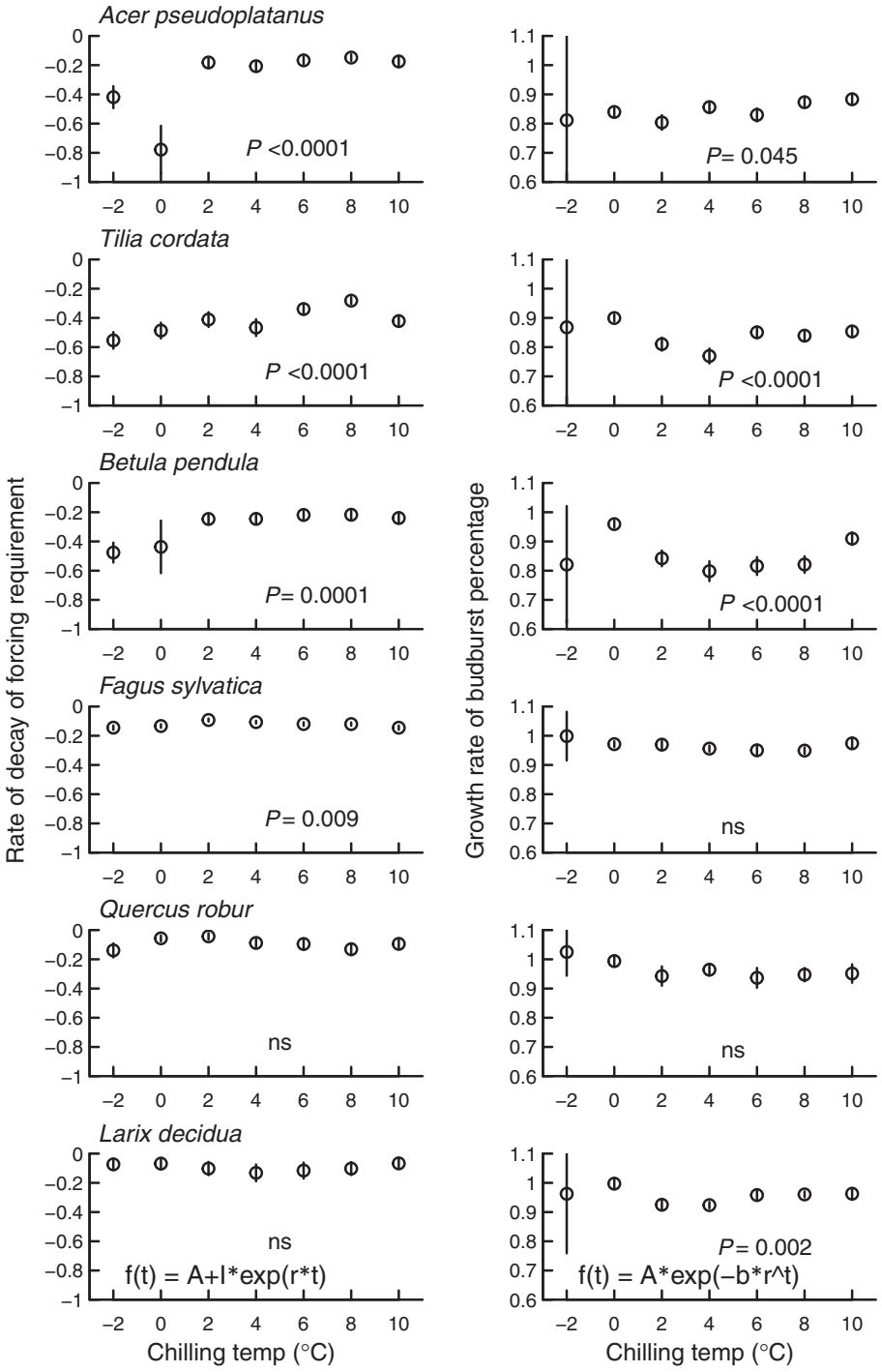

Fig. 4 Rate of decay of forcing requirements to budburst (left column) and growth rate of budburst percentage (right column) estimated for different chilling temperatures. Faster dormancy release is indicated by a low value of decay rates and a high value of growth rates (model formula indicated in the lowest panels). *P*-values indicate a significant difference among the seven chilling temperature treatments (ANOVA between nonlinear least square models with and without chilling temperature as additional parameter; ns, not significant). Error bars indicate ± 1SE.

Fagus, *Quercus* and *Larix* showed only marginal differences among all applied chilling temperatures with moderate rates of decay found for all temperatures (Fig. 4).

The increase of budburst percentages among the chilling temperature treatments showed a similar, but weaker, pattern with a high variation for the lowest chilling temperature treatments as a result of higher mortality (desiccation), presumably because of freeze–thaw events that may have disrupted the water column in the vessels. Lowest values (indicating steepest growth curves) were found at −2°C for *Acer* and *Betula* and 4°C for *Tilia* and *Larix* (Fig. 4). *Quercus* and *Fagus* performed similarly across the full range of tested chilling temperatures.

Differences in chilling requirements among the study species

To tease apart the differences in chilling requirements among species, the three indicators introduced in the method section are summarised in Fig. 5. For this comparison all chilling temperature treatments were pooled.

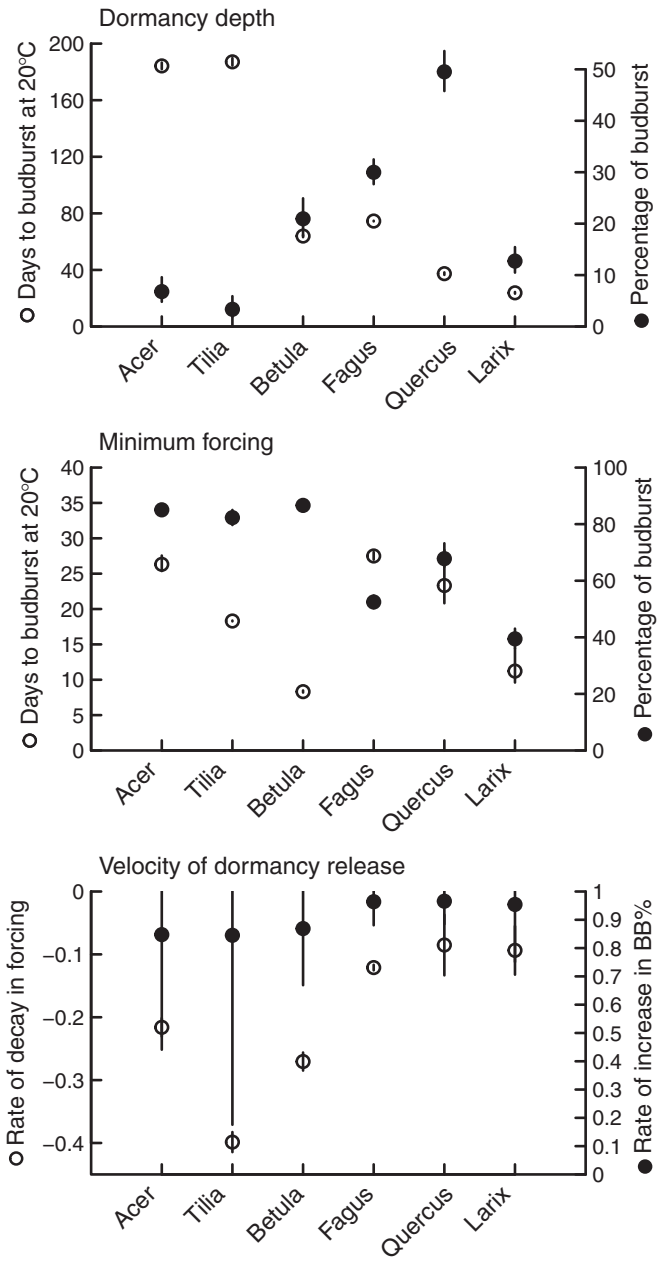

Fig. 5 Comparison of three dormancy related indicators among the six study species. Dormancy depth, days to budburst at 20°C and percentage of budburst after 1 wk of artificial chilling; minimum forcing, days to budburst at 20°C and percentage of budburst after 18 wk of artificial chilling; velocity of dormancy release, decay and growth rates extracted from the exponential and Gompertz models for the forcing–chilling and vitality–chilling relationships, respectively (pooled chilling temperatures). Note the different scales of the y-axis. Error bars indicate ± 1SE. BB, budburst.

Maximum dormancy depth: *Acer* and *Tilia* showed by far the deepest dormancy (estimated after 1 wk of chilling) with a forcing requirement of *c.* 6 months at 20°C until budburst and a budburst percentage close to 0% (see Fig. 5). *Larix* was able to burst a few buds (budburst percentage = 13%) after only *c.* 3 wk of forcing. In *Betula* and *Fagus*, *c.* 25% of buds burst after *c.* 10 wk of forcing. *Quercus* revealed a surprisingly shallow dormancy, flushing after *c.* 5 wk of forcing with a budburst percentage of *c.* 50%.

Minimum forcing: The variation among species in the amount of forcing required for budburst after 18 wk of total chilling was similar to the among-species variation in leaf-out dates observed under natural conditions at the study site. *Betula* was able to flush after only 8 d at 20°C, whereas *Larix* required 11 d, *Tilia* 18 d, *Quercus* 23 d, *Acer* 26 d and *Fagus* 27 d. Budburst percentages were close to 100%, except for *Larix* and *Fagus* in which budburst percentages ranged only *c.* 50% (Fig. 5).

Velocity of dormancy release: Dormancy was released fastest (indicated by the rate of decay in forcing required to budburst) in *Tilia*, followed by *Betula* and *Acer* reducing their forcing amount by 50% in only 15, 24 and 31 d of chilling, respectively (Fig. 5). For *Fagus*, *Quercus* and *Larix*, dormancy was released slower, not or just reaching their lowest forcing requirement after the longest chilling exposure. This pattern was also reflected in the growth rate of the percentage of budburst, although with greater variance especially in *Acer*, *Tilia* and *Betula* due to the different growth rates among the chilling temperature treatments (Fig. 5).

Discussion

By demonstrating that freezing temperatures are most efficient for satisfying chilling requirements for half of the studied species, and as efficient as any other temperature up to 10°C for the other three species, this study suggests that most of the current phenological models are based on spurious assumptions about the range of effective chilling temperatures to reduce dormancy depth. The large range of effective chilling temperatures detected here suggests that dormancy release and subsequent budburst is, to a much greater extent, determined by chilling quantity (i.e. length of exposure) rather than by the actual temperature that trees experience. These findings could serve to improve phenological modelling as many previous models have misrepresented the relevant chilling temperature range (Wang *et al.*, 2020).

Our results also underline the diversity of sensitivities to chilling and the dormancy depth among co-existing species that do not necessarily reflect their ranking in 'time of budburst' in the study area. In fact, we found that minimum forcing requirements best reflected the natural sequence of flushing at the study site, starting with *Larix* and *Betula* (late March), followed by *Tilia* (mid-April) and the late successional species *Acer* (late April), *Quercus* and *Fagus* (both early May).

Dormancy as a dynamic process

Although widely used in phenology research, the classical definition of dormancy from Lang *et al.* (1987), with a clear distinction between endodormancy and ecodormancy phases, does not seem to hold for the studied species. First, for *Fagus*, *Quercus* and *Larix*, we did not detect a true endodormancy phase as budburst was possible even without any chilling exposure, unlike the three other species. Second, both days to budburst and budburst percentage gradually approached an optimum value, reflecting a

continuous, dynamic dormancy progression as proposed by Cooke *et al.* (2012), rather than the two distinct phases.

Under natural chilling conditions, budburst percentages of cuttings from *Acer*, *Tilia* and *Betula* remained at 0% until temperature dropped below zero on 9 November 2018 for the first time in autumn. However, when twigs of the same species (and tree individuals) were exposed to artificial chilling conditions and a photoperiod of 8 h, budburst percentages increased immediately at any temperature tested, even at 10°C. This suggests that the artificial chamber conditions stimulated dormancy release, possibly by accelerating the effect of chilling accumulation in these artificial treatments. Among possible other causes (lower light intensity, no temperature fluctuations), we suggest that the short daylength (8 h) applied may be the most plausible explanation. We therefore conclude that buds of *Acer*, *Tilia* and *Betula* start to release dormancy and become sensitive to chilling only once a critically short photoperiod has been reached in autumn. Under natural conditions this photoperiod seemed to be reached around mid-November (i.e. a photoperiod of *c.* 9.3h for Zurich), followed by a steep increase in budburst percentage. A short photoperiod has been widely reported to induce dormancy, indicated by cessation of growth and photosynthetic activity (Noodén & Weber, 1978; Delpierre *et al.*, 2009; Hänninen & Tanino, 2011; Bauerle *et al.*, 2012) but, to our knowledge, short days have never been shown to release dormancy.

Species-specific strategies to synchronise budburst timing with climatic fluctuations

Our results highlight that co-existing tree species have developed distinct strategies to prevent leaf emergence during winter and to optimise the timing of leaf-out in spring. It appears that multiple measures are needed to adequately quantify the dormancy period and its variation among species.

First, (maximum) dormancy depth can substantially vary among species, for example *Quercus* and *Larix* were quite sensitive to forcing at any time during winter, whereas buds of *Acer* and *Tilia* remained completely 'locked' until they were exposed to a considerable amount of chilling. Similar differences in the dormancy depth among species have been reported by Murray *et al.* (1989) for seedlings, with *Larix decidua* showing a shallow and *Fagus sylvatica* and *Betula pendula* a deep dormancy depth under natural conditions in UK. A more recent study from northern Germany showed a peak in the dormancy in seedlings of *Acer pseudoplatanus*, *Tilia cordata* and *Quercus robur* in early December, whereas no peak was detected for *Fagus sylvatica* and *Larix decidua* (Malyshev *et al.*, 2018). However, dormancy levels were generally much lower than the ones found in this study. Ontogenetic effects could explain this discrepancy, as seedlings have been shown to be more opportunistic than adult trees, with lower chilling and forcing requirements (Vitasse, 2013; Vitasse *et al.*, 2014a; Osada & Hiura, 2019).

The second key characteristic of dormancy progression is the relationship between forcing and chilling, that is how fast the amount of forcing required to budburst decreases with increasing exposure to chilling (velocity of dormancy release). The rate of decay of the forcing–chilling curve shapes the velocity of dormancy release and allows species such as *Betula* and *Tilia* to respond more quickly to an early warm spell in spring, despite a deep dormancy during early winter. The nonlinear response to chilling has been proposed to reflect the nonlinear increase in spring temperature (Cannell, 1997). This allows trees to leaf-out at a more consistent date when long-term probabilities of freezing events match the species-specific freezing resistances, despite varying interannual winter temperatures (Lenz *et al.*, 2016).

The third dormancy characteristic we evaluated here was the minimum forcing requirement, that is the amount of forcing required for budburst when dormancy is fully released. This 'minimum forcing' greatly varied among species and caused a difference in the timing of flushing of more than 3 wk (*c.* 6 d of minimum forcing for *Larix* and *c.* 25 d for *Acer* at constant 20°C). These different forcing minima might partly reflect differences in the lower thresholds of temperature sensitivity (Fu *et al.*, 2013), for example *Betula* might already accumulate forcing temperatures slightly above 0°C, whereas *Quercus* might only be sensitive to higher temperatures due to its more southern distribution range. Further experiments are necessary to identify the efficiency of forcing temperatures to release ecodormancy to further improve phenological models. The species order of minimum forcing requirement reflects well the natural sequence of species' flushing dates, therefore the longest chilling exposure applied in this study was clearly enough to meet natural chilling conditions (Laube *et al.*, 2014). This confirms the overwhelming effect of forcing temperatures in explaining leaf-out timing of major trees in the temperate zone where chilling requirements are fulfilled (Ettinger *et al.*, 2020). The wide range of effective chilling temperatures we found in this study suggests a further advancement in leaf-out timing as climate warming progresses, except perhaps in species' southernmost distribution ranges (Luedeling *et al.*, 2012). It is remarkable that *Quercus robur* is one of the latest flushing species in central Europe despite its low chilling and forcing requirements. In more oceanic climates, such as south western France, *Quercus petraea* appears to be one of the earliest flushing species (end of March, several weeks before beech), which confirmed that deciduous oaks can indeed substantially advance budburst under higher forcing and lower chilling conditions (Vitasse *et al.*, 2009).

Given that the longest chilling exposure of 18 wk represents a winter with above-average chilling days at the study site, it is noticeable that, even under 18 wk chilling, *Fagus* and *Larix* did not reach their maximum flushing performance (both days to budburst and budburst percentage). Our result therefore suggest that chilling requirements at this site are not fully completed in these species, a finding that was also reported for beech by Murray and colleagues in 1989 in southern England. Also, Vitasse & Basler (2013) suggested that beech populations require very high chilling exposure to reach their minimum heat requirement for budburst, which seemed to be achieved only at elevations > 1600 m asl in the Pyrenees mountains. A recent study by Asse *et al.* (2020) also confirmed this high chilling requirement for *Larix*, predicting trends towards later dates of budburst at low elevations in the French Alps during unusual warm winters due to insufficient chilling. Our study shows that, despite the same

twig-cutting methodology, chilling under natural conditions at the study site led to a higher budburst percentage in *Larix* compared with artificial chilling in climate chambers. This suggests that chilling temperatures outside the tested range, especially below −2°C, may also contribute to release dormancy in *Larix decidua*, a species that has its core distribution in the subalpine belt in the Alps.

Implications for modelling

Our results suggest that a revision of the existing assumptions about the range and efficiency of chilling temperatures of temperate tree species is urgently needed to improve the predictions of phenological models. State-of-the-art phenology models use different ranges of chilling for temperate forest trees with either constant or weighted efficiencies to release dormancy based on previous studies (Coville, 1920; Richardson *et al.*, 1974; Cannell & Smith, 1983; Murray *et al.*, 1989). Leaf-out advancement (and therefore carbon uptake) of 30 perennial species was recently found to be largely overestimated in all models that do not account for freezing temperatures (e.g. by assuming a chilling range > 0°C) (Wang *et al.*, 2020). Models that included freezing temperatures predicted observed leaf-out dates more accurately, especially in mid and higher latitudes where winter mean temperatures are below 0°C.

The amount of chilling required to induce sensitivity of buds to forcing temperatures and the amount of forcing temperatures required for budburst are two parameters that are generally inferred statistically for forest trees (i.e. by selecting a combination of parameters showing the best model performance, for example Chuine *et al.* (2013)). However a better understanding of the fundamental processes during dormancy (especially the understanding of dormancy break) has been shown recently to substantially improve phenological predictions, especially because global warming is causing temperature conditions outside the 'normal' range, under which most models are calibrated (Asse *et al.*, 2020). Only adequately parameterised models will therefore be able to predict future phenology and distribution ranges of temperate trees in a warmer climate in which chilling may increasingly become limiting (Morin *et al.*, 2008; Chuine, 2010; Chuine *et al.*, 2016; Gauzere *et al.*, 2019). In fruit trees, model parameters representing chilling and forcing requirements have been more accurately evaluated (experimentally and under natural conditions) due to the economic dimension of producing fruit in countries that have warmer climate (Luedeling *et al.*, 2011; Melke, 2015). Other experimental studies have focused on a few model species such as *Populus* under unique experimental setups, which makes it difficult to compare the results to other species (Ettinger *et al.*, 2019). In fact, our results show that co-existing species differ in their sensitivities to chilling exposure and thus call for developing models with species-specific assumptions.

Limitations of the study

Chilling temperature treatments at −2°C and 0°C showed the largest variation, especially in terms of budburst percentage. Cuttings either performed above average (reflected by lower forcing requirements and partly higher budburst percentages) or were desiccated, presumably because freeze-thaw events may have disrupted the water column in the vessels, and for which the applied method appears challenging. Twigs that were exposed to little or no chilling showed high mortality rates, meaning that they desiccated before sufficient forcing could be accumulated to permit budburst. However, some cuttings indeed revealed their forcing requirements at this critically low chilling exposure by flushing after half a year of exposure at constant 20°C, thereby proving that cuttings can survive such a long period. Another method consists in removing the apical bud to release lateral buds from apical dominance (Cline, 1997; Malyshev, 2020). While this may be appropriate when estimating the peak of dormancy, this study aimed to quantify how the sensitivity to chilling and forcing changes during dormancy for adult trees (without altering apical bud influence).

By focussing on stage 2 (budburst) the consequences of erratic budburst might not be fully visible. However, we continued to monitor until stage 4 and assessed (after another 3 wk) the percentage of all buds per twig that reached this budburst stage. These two indicators provide both a sensitive (time to earliest budburst) and a robust (budburst percentage) indicator, which both reflected the same patterns of dormancy progression. In addition, the method used here has been proved to be reliable in reflecting the phenology of the donor trees (Vitasse & Basler, 2014; Menzel *et al.*, 2020) and has been applied to better understand chilling and photoperiod effects on temperate (Laube *et al.*, 2014; Flynn & Wolkovich, 2018), and even subtropical, trees (Du *et al.*, 2019). As the trees examined in this study were taken from only one population near Zurich, it is possible that the results of both chilling and forcing requirements found here may vary within the species distribution range (e.g. see Myking & Heide (1995) for latitudinal differences in the lower temperature threshold of effective forcing for *Betula pubescens* and *B. pendula*).

The constant photoperiod in both chilling (8 h) and forcing (16 h) treatments may have influenced dormancy progression and forcing accumulation, because forcing accumulation is more efficient during daytime (Fu *et al.*, 2016). However, this setting allowed us to remove any potential interacting effects of photoperiod, as found experimentally for many American tree species (Flynn & Wolkovich, 2018).

Conclusion

Our study demonstrated that all chilling temperatures in the range of −2°C to +10°C effectively released dormancy for all studied species, with half of the species exhibiting the highest chilling sensitivity at −2°C. Chilling duration outweighed the importance of absolute chilling temperatures, and phenological models of temperate and boreal trees should therefore account for all chilling temperatures below 10°C, especially temperatures slightly below or above zero. We further showed that multiple key parameters are relevant to improve our understanding of a tree's dormancy cycle. Among these parameters, maximum depth

of dormancy, velocity of dormancy release and minimum forcing are critical and may serve as the basis for process-based phenology modelling (Gauzere *et al.*, 2019). For all indicators of dormancy progression, it was found that only the minimum forcing requirement correlated with the time of flushing in natural conditions, confirming that forcing in spring is currently the main factor explaining the timing of leaf-out among species. To further improve phenological forecasts, future experimental efforts should be directed towards identifying the lower and upper chilling temperature thresholds to (efficiently) release dormancy (below −2°C and above 10°C) as well as the efficiency of forcing temperatures.

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Author contributions

FB and YV planned and designed the experiment. FB conducted the experiment, analysed the data and drafted the manuscript with the advice and assistance of YV. AG and CMZ substantially contributed to the manuscript.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Experimental setup of the experiment (chilling and forcing chambers).

Table S1 Mortality rates for twigs of the main experiment sampled on 16 October for different artificial chilling exposures.

Table S2 Mortality rates for twigs of the main experiment sampled on 16 October for different artificial chilling temperatures.

Table S3 Model parameters for days to budburst in response to artificial and natural chilling.

Table S4 Model parameters for budburst percentage in response to artificial and natural chilling.

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