This document is the accepted manuscript version of the following article:
Baumgarten, F., Zohner, C., Gessler, A., & Vitasse, Y. (2021). Chilled to be forced:
the best dose to wake up buds from winter dormancy. *New Phytologist*.
<https://doi.org/10.1111/nph.17270>

MR FREDERIK BAUMGARTEN (Orcid ID : 0000-0002-8284-8384)

DR CONSTANTIN MARIO ZOHNER (Orcid ID : 0000-0002-8302-4854)

PROF. ARTHUR GESSLER (Orcid ID : 0000-0002-1910-9589)

Article type : MS - Regular Manuscript

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

Frederik Baumgarten¹, Constantin Zohner², Arthur Gessler^{1,3} & Yann Vitasse¹

¹ Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Zürcherstr. 111, 8903
Birmensdorf, Switzerland

² Institute of Integrative Biology, ETH Zurich (Swiss Federal Institute of Technology),
Universitätsstrasse 16, 8092 Zurich, Switzerland

³ Institute of Terrestrial Ecosystems, ETH Zurich (Swiss Federal Institute of Technology),
Universitätsstrasse 16, 8092 Zurich, Switzerland

Orcid:

Arthur Gessler: 0000-0002-1910-9589

Yann Vitasse: 0000-0002-7454-505X

Constantin Zohner: 0000-0002-8302-4854

Frederik Baumgarten: 0000-0002-8284-8384

Author for correspondence:

Frederik Baumgarten

Tel: +41-32-739-2057

Email: frederik.baumgarten@wsl.ch

Received: 12 November 2020

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/NPH.17270](https://doi.org/10.1111/NPH.17270)

This article is protected by copyright. All rights reserved

Accepted: 2 February 2021

Total word count:	6306	No of figures:	5
Summary:	200	No of tables:	1
Introduction:	1062	No of Supporting Information	5
Material and Methods:	1919		
Results:	953		
Discussion:	2368		

Keywords: budburst, chilling, climate chamber experiments, climate warming, dormancy, forcing, temperature response, tree phenology.

Paper type: Original Research

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 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 1'170 twig cuttings to a range of temperatures from -2° to 10°C for 1, 3, 6, and 12 weeks.
- We found that freezing temperatures were most effective for half of the species or as effective as chilling temperatures up to 10°C, i.e. 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 around 4-6°C, instead revealing strong sensitivity to a large range of temperatures. These findings could be valuable for improving phenological models and predicting future spring phenology in a warming world.

Introduction

The time when the 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 the tree 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 maximize competitive ability. In essence, the timing of spring leaf-out is the result of a trade-off between minimizing the risk of frost damage and maximizing resource uptake (nutrients, water, CO₂, 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 (Korner & Basler, 2010; Delpierre *et al.*, 2016). Although longer photoperiods have been shown to advance budburst and accelerate cell development in a few species (Basler & Korner, 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’) in temperate, boreal and even subtropical trees for dormancy release and budburst (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 ‘endo-dormancy’, while ‘eco-dormancy’ 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 use hereafter the term ‘dormancy’ whenever we refer to a general state of bud rest. Once dormancy has been induced, a negative relationship has been commonly found between the amount of heat (forcing) required for budburst and the exposure time to chilling temperatures either experimentally (Cannell & Smith, 1983; Murray *et al.*, 1989; reviewed in Ettinger *et al.*, 2020) or 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 around 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.* (1987a, b) assumes that effective chilling ranges between 0 and 7.2 °C with an optimum at 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 holds for the majority of temperate trees. In addition, some experimental evidence from Scandinavia suggests that the upper range of effective chilling range lays around 12 °C for Loblolly Pines (Garber, 1983) and for Birch (Myking & Heide, 1995). 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 to bud 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 likely 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 bud burst 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. A total of 1’170 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 that allows 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 expect the range of efficient chilling for dormancy release to be wider than commonly assumed including sub-zero temperatures. We further expect 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 expect that 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 show 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, hereafter, 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 and Basler (2013)). The chilling requirement of the species has been only rarely investigated but was observed by (Laube *et al.*, 2014) to be sensitive to chilling until end of winter. Forcing requirement after long chilling (until 14 March) was found by the same authors to be 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 a.s.l.). The mean annual air temperature recorded at the nearest climate station was 9.8°C and the mean annual precipitation was 1080 mm (mean 1990 – 2020 recorded at Zurich Fluntern, 555 m

a.s.l. ~8 km away from the study site). Using the same temperature dataset, the mean long-term air temperature over the winter season (December–February) was 1.5°C, with a mean temperature of the coldest month (January) of ~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 years). 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). A total of 34 twigs (29 on 19 Oct, 1 on 29 Nov and 4 on 17 Jan) of ~60 cm length with intact buds were harvested for each of the five selected individuals per species (total of 1'020 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 ~50 cm and placed into plastic boxes with deionized water to minimize bacterial development (Fig. S1). We then labelled and grouped the twigs so that each of the 5 individuals (replicates) per species was represented in every temperature treatment. The different temperature treatments started one week after the sampling on 19 October and one day 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 ~0.5 cm) and placed into fresh deionized 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 5 individual trees of the same six species on five additional occasions (05. Oct., 25. Oct., 09. Nov., 23. Nov. and 04. Jan., 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 = ~100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, measured by LI-COR Li189 quantum PAR light sensor at bud height) were set to 8 h photoperiod (shortest day at this latitude) and were exchanged between the chambers every week to remove a potential effect of light intensity (<20 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of difference). 20 cuttings per species corresponding to four times five replicates were placed in each of the seven climate chamber. After 1, 3, 6 and 12 week(s) 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, i.e., placed in a large climate chamber at 20 °C and 16h photoperiod (halogen lamps, photosynthetic photon flux density PPFD = 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ 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 ~70% throughout the experiment. Twigs collected at the last sampling date on 17 January 2019, were only exposed to 0, 4, and 8 °C for 6 weeks (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 $\pm$ sd). Because of this quite stable temperature we quantified the amount of forcing as the number of days to budburst at 20 °C.

Throughout the experiment, temperatures in all treatments were recorded using temperature loggers (MX2203, Onset Computer Corporation, 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 Tab. 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 (bud burst), bud scales were open and leaves were partially visible; at stage 3 (leaf-out), leaves had fully emerged from the buds but were still folded, crinkled or pendant, depending on species; and at stage 4 (leaf unfolded), at least one leaf was fully unfolded. For 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. Three weeks 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 5 buds for *Acer*, 13 for *Fagus*, *Quercus* and *Tilia*, 18 for *Betula* and 48 for *Larix*. Because *Quercus* breaks only about 40% of all buds during the first flush (personal observations on attached branches from adult trees) budburst percentage of this species was standardized 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 (Table S1 and S2). Eventually the 0 °C chilling treatment led to slight increased mortality in twigs of *Betula*, *Tilia* and *Larix* most probably due to frequent thawing-freezing occurrences. No or little chilling exposure caused most twig cuttings of *Acer*, *Tilia* and *Betula* to desiccate after months of forcing, although some cuttings managed to flush after ~180 days of forcing at 20 °C, demonstrating that cuttings can survive that long. For cuttings that desiccated after several month in theses 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 as much as one month did not affect the observed patterns.

Data analysis and statistics

For the analysis, we focused on the phenological stage 2 (i.e. bud burst) because this stage represents 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 analyses of days to budburst and budburst percentage (see next paragraph), we used data from the main sampling on 19 Oct. (1, 3, 6 and 12 weeks of artificial chilling) and from the maximum chilling treatment sampled on 17 January (12 weeks of natural chilling plus 6 weeks of artificial chilling at 0° and 4°C; see Fig. 1), which corresponds to 18 weeks 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 bud burst and chilling exposure as follows:

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

where a is the asymptote (corresponding to the days to budburst after saturating amount 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 weeks total chilling) to avoid impossible (negative) values, whereas the other parameters were fitted by the model.

Bud burst percentage

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

$$f(t) = ae^{-br^t}$$

where a is the asymptote (corresponding to the budburst percentage after saturating amount 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 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 week) to identify both the sensitivity to forcing temperatures and the (in)ability to flush during early winter (true endo-dormancy).
- 2) 'Minimum forcing' was defined as the forcing requirement until budburst and budburst percentage under high chilling (~18 weeks) 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-week) exposure to chilling conditions, *Acer* and *Tilia* required on average ~180 days to budburst, whereas *Betula* and *Fagus* required ~60 days to budburst (Fig. 2a) and *Quercus* and *Larix* required only 35 and 20 days to budburst, respectively (Fig. 2a). For *Acer*, *Tilia*, and *Betula*, days to budburst decreased rapidly towards the asymptote of minimum forcing. In 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 weeks of chilling (Fig. 2a). The rate of decay was significantly lower (faster decrease in forcing) at -2 and 0 °C in *Acer*, *Tilia*, *Betula* and *Fagus*, whereas similar chilling-efficiencies for all tested temperatures between -2 and +10 °C were found for *Quercus* and *Larix* (Fig. 2a, see also Fig. 4 and 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 4th and 17th 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 week of artificial chilling and increased in a sigmoid-like relationship with increasing chilling duration up to nearly 100% after 18 weeks (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 around 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 ~65% for *Acer*. In contrast *Fagus*, *Quercus* and *Larix* were already able to budburst with budburst percentages around 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 ~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, Table S3 and 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 days (*Acer*), 10 days (*Betula*) and 4 days (*Tilia*) for the -2 °C treatment compared to all temperature treatments pooled. Surprisingly also chilling temperatures up to 10° C reduced days to budburst in all species. *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 freezing/thawing 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 similar 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 summarized in Fig. 5. For this comparison all chilling temperature treatments were pooled.

Maximum dormancy depth: *Acer* and *Tilia* showed by far the deepest dormancy (estimated after one week of chilling) with a forcing requirement of ~6 months at 20 °C until bud burst and a budburst percentage close to 0% (see Fig. 5). *Larix* was able to burst a few buds (budburst percentage=13%) after only ~3 weeks of forcing. In *Betula* and *Fagus*, ~25% of buds bursted after

~10 weeks of forcing. *Quercus* revealed a surprisingly shallow dormancy, flushing after ~5 weeks of forcing with a budburst percentage of ~50%.

Minimum forcing: The variation among species in the amount of forcing required for budburst after 18 weeks 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 days at 20°C, whereas *Larix* required 11 days, *Tilia* 18 days, *Quercus* 23 days, *Acer* 26 days and *Fagus* 27 days. Budburst percentages were close to 100%, except for *Larix* and *Fagus* in which budburst percentages ranged only around 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 days 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 to satisfy 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 when accounting for the range of efficient chilling temperature 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) than by the actual temperature 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, which do not necessarily reflect their ranking in the time of budburst in the study area. In fact, we found minimum forcing requirements to best reflect 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 endo- and eco-dormancy phases, does not seem to hold for the studied species. First, for *Fagus*, *Quercus* and *Larix* we did not detect a true endo-dormancy phase since 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 after the 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 hours, budburst percentages increased immediately at any temperature tested, even at 10°C. This suggests that the artificial chamber conditions could have 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 (8h) 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 ~9.3h for Zurich), followed by a steep increase of 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; Hanninen & Tanino, 2011; Bauerle *et al.*, 2012), but to our knowledge has never been shown to release dormancy.

Species-specific strategies to synchronize 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 optimize 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, e.g. *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 for seedlings by Murray *et al.* (1989) 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, having 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, i.e., 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 flush at a more consistent date of similar frost probability despite of varying interannual winter temperatures (Lenz *et al.*, 2016).

The third dormancy characteristic we evaluate here is the minimum forcing requirement, i.e., the amount of forcing required for budburst when dormancy is fully released. This ‘minimum forcing’ greatly varies among species and can cause a difference in the timing of flushing of more than three weeks (~6 days of minimum forcing for *Larix* and ~25 days 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), e.g., *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 in order to further improve phenological models. The species order of minimum forcing requirement reflects well the natural sequence of species’ flushing dates, hence 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). A wider range of effective chilling temperature, as found in this study, suggests a further advancement in leaf-out

timing as climate warming progresses, except perhaps in the southernmost part of species distributions (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 confirms 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 weeks represents a winter with above-average chilling days at the study site, it is noticeable that *Fagus* and *Larix* seem to still not have reached 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 *et al.* in 1989 in south England. Also, Vitasse and Basler (2013) suggested that beech populations require very high chilling exposure to reach their minimum heat requirement for budburst, which seems to be achieved only at elevations >1600 m a.s.l. in the Pyrenees mountains. A recent study by Asse *et al.* (2020) also confirms 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 to artificial chilling in climate chambers. This suggests that chilling temperatures outside of the tested range, especially below -2 °C, may also contribute to release dormancy in *Larix decidua*, a species which 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 in order 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 thus 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

including freezing temperatures predicted observed leaf-out dates more accurate 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, e.g. 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 parameterized 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 having warmer climate (Luedeling *et al.*, 2011; Melke, 2015). Other experimental studies have focused on a few model species such as *Populus* under a unique experimental setup, which makes it extremely difficult to compare and transfer it to other species (Ettinger *et al.*, 2019). In fact, our results show that co-existing species have different sensitivity to chilling temperature exposure and temperature values which calls for developing models with species-specific assumptions.

Limitations of the study

Chilling temperature treatments at -2 and 0 °C showed largest variation especially in terms of bud burst percentage. Cuttings either performed above average (reflected by lower forcing requirements and partly higher budburst percentages) or desiccated presumably because of freezing/thawing events that may have disrupted the water column in the vessels for which the applied method appears challenging. Twigs that were exposed to no or little 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 proofing that cuttings can survive for that long. Another method consists in removing the apical bud to release lateral buds from the apical dominance (Cline, 1997; Malyshev, 2020). While this

may be appropriate to estimate the peak of dormancy, this study aimed to quantify how the sensitivity to chilling and forcing changes during the dormancy for adult trees as realistically as possible (without altering apical buds influence). By focussing on stage 2 (budburst) the consequences of erratic bud burst might not be fully visible. However, we continued the monitoring until stage 4 and assessed (after another 3 weeks) the percentage of all buds per twig that reached at the stage of budburst. These two indicators provide both a sensitive (first bud bursts) and a robust (budburst percentage) indicator, which both reflect the same patterns of dormancy progression. In addition, the method used here has been proven to be reliable in reflecting the phenology of the donor trees (Vitasse & Basler, 2014; Menzel *et al.*, 2020) and was recently 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 stem 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 and Heide (1995) for latitudinal differences in the baseline of forcing for *Betula pubescens* and *B. pendula*).

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

Conclusion

Our study demonstrates 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 should therefore account for all chilling temperatures below 10°C, especially temperatures slightly below or above zero in temperate and boreal regions. We further show 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 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 correlate 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 toward identifying the lower and upper chilling temperature thresholds to (efficiently) release dormancy (below -2 and above 10 °C) as well as the efficiency of forcing temperatures.

Acknowledgments

The research leading to these results was funded by the Velux Foundation (Project grant No. 1119)

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 CZ substantially contributed to the manuscript.

References

- Anderson JV, Chao WS, Horvath DP. 2001.** A current review on the regulation of dormancy in vegetative buds. *Weed Science* **49**(5): 581-589.
- Asse D, Chuine I, Vitasse Y, Yoccoz NG, Delpierre N, Badeau V, Delestrade A, Randin CF. 2018.** Warmer winters reduce the advance of tree spring phenology induced by warmer springs in the Alps. *Agricultural and Forest Meteorology* **252**: 220-230.
- Asse D, Randin CF, Bonhomme M, Delestrade A, Chuine I. 2020.** Process-based models outcompete correlative models in projecting spring phenology of trees in a future warmer climate. *Agricultural and Forest Meteorology* **285**: 107931.
- Basler D, Korner C. 2014.** Photoperiod and temperature responses of bud swelling and bud burst in four temperate forest tree species. *Tree Physiol* **34**(4): 377-388.
- Bauerle WL, Oren R, Way DA, Qian SS, Stoy PC, Thornton PE, Bowden JD, Hoffman FM, Reynolds RF. 2012.** Photoperiodic regulation of the seasonal pattern of photosynthetic capacity and the implications for carbon cycling. *Proc Natl Acad Sci U S A* **109**(22): 8612-8617.

- Campoy JA, Darbyshire R, Dirlewanger E, Quero-Garcia J, Wenden B. 2019.** Yield potential definition of the chilling requirement reveals likely underestimation of the risk of climate change on winter chill accumulation. *Int J Biometeorol* **63**(2): 183-192.
- Cannell MGR. 1997.** Spring phenology of trees and frost avoidance*. *Weather* **52**(2): 46-52.
- Cannell MGR, Smith RI. 1983.** Thermal time, chill days and prediction of budburst in *Picea sitchensis*. *Journal of Applied Ecology* **20**(3): 951-963.
- Chuine I. 2010.** Why does phenology drive species distribution? *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**(1555): 3149-3160.
- Chuine I, Bonhomme M, Legave JM, Garcia de Cortazar-Atauri I, Charrier G, Lacointe A, Ameglio T. 2016.** Can phenological models predict tree phenology accurately in the future? The unrevealed hurdle of endodormancy break. *Glob Chang Biol* **22**(10): 3444-3460.
- Chuine I, Garcia de Cortazar Atauri I, Hänninen H, Kramer K 2013.** Plant development models. *Phenology: an integrated Environmental Science*: Springer, Dordrecht, 2013. S. 275-293.
- Cline M. 1997.** Concepts and terminology of apical dominance. *Am J Bot* **84**(8): 1064.
- Cooke JE, Eriksson ME, Junttila O. 2012.** The dynamic nature of bud dormancy in trees: environmental control and molecular mechanisms. *Plant Cell Environ* **35**(10): 1707-1728.
- Coville FV. 1920.** The Influence of Cold in Stimulating the Growth of Plants. *Proc Natl Acad Sci USA* **6**(7): 434-435.
- Delpierre N, Dufrêne E, Soudani K, Ulrich E, Cecchini S, Boé J, François C. 2009.** Modelling interannual and spatial variability of leaf senescence for three deciduous tree species in France. *Agricultural and Forest Meteorology* **149**(6-7): 938-948.
- Delpierre N, Vitasse Y, Chuine I, Guillemot J, Bazot S, Rutishauser T, Rathgeber CBK. 2016.** Temperate and boreal forest tree phenology: from organ-scale processes to terrestrial ecosystem models. *Annals of Forest Science* **73**(1): 5-25.
- Du Y, Pan Y, Ma K. 2019.** Moderate chilling requirement controls budburst for subtropical species in China. *Agricultural and Forest Meteorology* **278**: 107693.
- Ettinger A, Chamberlain C, Morales-Castilla I, Buonaiuto D, Flynn D, Savas T, Samaha J, Wolkovich E. 2020.** Winter temperatures predominate in spring phenological responses to warming. *Nature Climate Change*: 10(12), 1137-1142.

Ettinger A, Chuine I, Cook B, Dukes J, Ellison A, Johnston M, Panetta A, Rollinson C, Vitasse Y, Wolkovich E. 2019. How do climate change experiments alter plot-scale climate? *Ecology Letters* **22**(4): 748-763.

Fadón E, Fernandez E, Behn H, Luedeling E. 2020. A Conceptual Framework for Winter Dormancy in Deciduous Trees. *Agronomy* **10**(2): 241.

Fishman S, Erez A, Couvillon G. 1987. The temperature dependence of dormancy breaking in plants: computer simulation of processes studied under controlled temperatures. *Journal of Theoretical Biology* **126**(3): 309-321.

Flynn DFB, Wolkovich EM. 2018. Temperature and photoperiod drive spring phenology across all species in a temperate forest community. *New Phytol* **219**(4): 1353-1362.

Fu YH, Campioli M, Deckmyn G, Janssens IA. 2013. Sensitivity of leaf unfolding to experimental warming in three temperate tree species. *Agricultural and Forest Meteorology* **181**(0): 125-132.

Fu YH, Liu Y, De Boeck HJ, Menzel A, Nijs I, Peaucelle M, Peñuelas J, Piao S, Janssens IA. 2016. Three times greater weight of daytime than of night-time temperature on leaf unfolding phenology in temperate trees. *New Phytologist* **212**(3): 590-597.

Fu YH, Zhang X, Piao S, Hao F, Geng X, Vitasse Y, Zohner C, Peñuelas J, Janssens IA. 2019. Daylength helps temperate deciduous trees to leaf-out at the optimal time. *Global Change Biology* **25**(7): 2410-2418.

Fu YH, Zhao H, Piao S, Peaucelle M, Peng S, Zhou G, Ciais P, Huang M, Menzel A, Penuelas J, et al. 2015. Declining global warming effects on the phenology of spring leaf unfolding. *Nature* **526**(7571): 104-107.

Garber MP. 1983. Effects of chilling and photoperiod on dormancy release of container-grown loblolly pine seedlings. *Canadian Journal of Forest Research* **13**(6): 1265-1270.

Gauzere J, Lucas C, Ronce O, Davi H, Chuine I. 2019. Sensitivity analysis of tree phenology models reveals increasing sensitivity of their predictions to winter chilling temperature and photoperiod with warming climate. *Ecological Modelling* **411**: 108805.

Hänninen H 2016. The Annual Phenological Cycle. *Boreal and Temperate Trees in a Changing Climate*. Dordrecht: Springer Netherlands, 35-138.

Hanninen H, Tanino K. 2011. Tree seasonality in a warming climate. *Trends Plant Sci* **16**(8): 412-416.

- Korner C, Basler D. 2010.** Plant science. Phenology under global warming. *Science* **327**(5972): 1461-1462.
- Lang GA, Early JD, Martin GC, Darnell RL. 1987.** Endo-, para-, and ecodormancy: physiological terminology and classification for dormancy research. *Hortscience* **22**(3): 371-377.
- Laube J, Sparks TH, Estrella N, Hofler J, Ankerst DP, Menzel A. 2014.** Chilling outweighs photoperiod in preventing precocious spring development. *Glob Chang Biol* **20**(1): 170-182.
- Lenz A, Hoch G, Körner C, Vitasse Y. 2016.** Convergence of leaf-out timing towards minimum risk of freezing damage in temperate trees. *Functional Ecology* **30**(9): 1480-1490.
- Lenz A, Hoch G, Vitasse Y, Korner C. 2013.** European deciduous trees exhibit similar safety margins against damage by spring freeze events along elevational gradients. *New Phytol* **200**(4): 1166-1175.
- Luedeling E, Blanke M, Gebauer J 2012.** Chilling challenges in a warming world. *II International Symposium on Horticulture in Europe 1099*. 901-907.
- Luedeling E, Girvetz EH, Semenov MA, Brown PH. 2011.** Climate change affects winter chill for temperate fruit and nut trees. *PLoS ONE* **6**(5): e20155.
- Malyshev AV. 2020.** Warming Events Advance or Delay Spring Phenology by Affecting Bud Dormancy Depth in Trees. *Front Plant Sci* **11**: 856.
- Malyshev AV, Henry HAL, Bolte A, Khan MASA, Kreyling J. 2018.** Temporal photoperiod sensitivity and forcing requirements for budburst in temperate tree seedlings. *Agricultural and Forest Meteorology* **248**: 82-90.
- Man RZ, Lu PX, Dang QL. 2017.** Insufficient Chilling Effects Vary among Boreal Tree Species and Chilling Duration. *Frontiers in Plant Science* **8**: 1354.
- Melke A. 2015.** The physiology of chilling temperature requirements for dormancy release and bud-break in temperate fruit trees grown at mild winter tropical climate. *Journal of Plant Studies* **4**(2), doi:10.5539/jps.v4n2p110.
- Menzel A, Yuan Y, Hamann A, Ohl U, Matiu M. 2020.** Chilling and Forcing From Cut Twigs- How to Simplify Phenological Experiments for Citizen Science. *Front Plant Sci* **11**: 561413.
- Morin X, Viner D, Chuine I. 2008.** Tree species range shifts at a continental scale: new predictive insights from a process-based model. *Journal of Ecology* **96**(4): 784-794.

- Murray MB, Cannell MGR, Smith RI. 1989.** Date of budburst of fifteen tree species in Britain following climatic warming. *Journal of Applied Ecology* **26**(2): 693-700.
- Myking T, Heide OM. 1995.** Dormancy release and chilling requirement of buds of latitudinal ecotypes of *Betula pendula* and *B. pubescens*. *Tree Physiol* **15**(11): 697-704.
- Noodén L, Weber J 1978.** Environmental and hormonal control of dormancy in terminal buds of plants In: Dormancy and developmental arrest. Clutter ME ed: Academic Press, New York, S. 221-268.
- Osada N, Hiura T. 2019.** Intraspecific differences in spring leaf phenology in relation to tree size in temperate deciduous trees. *Tree Physiol* **39**(5): 782-791.
- Petri JL, Leite GB 2003.** Consequences of insufficient winter chilling on apple tree bud-break. *VII International Symposium on Temperate Zone Fruits in the Tropics and Subtropics* 662. 53-60.
- Richardson EA, Seeley SD, Walker DR. 1974.** A model for estimating the completion of rest for "Redhaven" and "Elberta" peach trees. *Hortscience U.S.A.*; DA.; VOL. 9; NO 4; PP. 331-332
- Vitasse Y. 2013.** Ontogenic changes rather than difference in temperature cause understory trees to leaf out earlier. *New Phytol* **198**(1): 149-155.
- Vitasse Y, Basler D. 2013.** What role for photoperiod in the bud burst phenology of European beech. *European Journal of Forest Research* **132**(1): 1-8.
- Vitasse Y, Basler D. 2014.** Is the use of cuttings a good proxy to explore phenological responses of temperate forests in warming and photoperiod experiments? *Tree Physiology* **34**(2): 174-183.
- Vitasse Y, Hoch G, Randin CF, Lenz A, Kollas C, Scheepens JF, Korner C. 2013.** Elevational adaptation and plasticity in seedling phenology of temperate deciduous tree species. *Oecologia* **171**(3): 663-678.
- Vitasse Y, Lenz A, Hoch G, Korner C. 2014a.** Earlier leaf-out rather than difference in freezing resistance puts juvenile trees at greater risk of damage than adult trees. *Journal of Ecology* **102**(4): 981-988.
- Vitasse Y, Lenz A, Korner C. 2014b.** The interaction between freezing tolerance and phenology in temperate deciduous trees. *Front Plant Sci* **5**(541): 541.

- Vitasse Y, Porte AJ, Kremer A, Michalet R, Delzon S. 2009.** Responses of canopy duration to temperature changes in four temperate tree species: relative contributions of spring and autumn leaf phenology. *Oecologia* **161**(1): 187-198.
- Wang HJ, Wu CY, Ciais P, Penuelas J, Dai JH, Fu YS, Ge QS. 2020.** Overestimation of the effect of climatic warming on spring phenology due to misrepresentation of chilling. *Nature communications* **11**(1): 1-9.
- Way DA, Montgomery RA. 2015.** Photoperiod constraints on tree phenology, performance and migration in a warming world. *Plant Cell Environ* **38**(9): 1725-1736.
- Wenden B, Mariadassou M, Chmielewski FM, Vitasse Y. 2020.** Shifts in the temperature-sensitive periods for spring phenology in European beech and pedunculate oak clones across latitudes and over recent decades. *Glob Chang Biol* **26**(3): 1808-1819.
- Zohner CM, Benito BM, Svenning J-C, Renner SS. 2016.** Day length unlikely to constrain climate-driven shifts in leaf-out times of northern woody plants. *Nature Climate Change* **6**(12): 1120-1123.
- Zohner CM, Mo L, Pugh TAM, Bastin JF, Crowther TW. 2020.** Interactive climate factors restrict future increases in spring productivity of temperate and boreal trees. *Glob Chang Biol* **26**(7): 4042-4055.
- Zohner CM, Mo LD, Renner SS. 2018.** Global warming reduces leaf-out and flowering synchrony among individuals. *Elife* **7**: e40214.

Supporting information

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

Table S2 Mortality rates for twigs of the main experiment sampled on 16. Oct 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 bud burst percentage in response to artificial and natural chilling.

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

Tables

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

Temp treatment (°C)	-2	0	2	4	6	8	10
Mean±sd of actual temp (°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

Figure legends

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 weeks before to be moved into the forcing chamber. Cuttings collected on 17 January 2019 were exposed to 3 chilling temperatures (0, 4 and 8 °C) for 6 weeks. Blue vertical lines correspond to an additional set of trees that were sampled on additional 5 dates and twigs were then directly exposed to forcing conditions to study the progress of dormancy depth under natural conditions.

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 weeks which correspond to the last sampling conducted on 17 January 2019 with additional 6 weeks of artificial chilling. Twigs that were exposed to natural chilling originate from either 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 weeks which

correspond to the last sampling on 17 January 2019 with additional 6 weeks of artificial chilling. This maximum chilling treatment was used to estimate minimum forcing requirements to budburst. Twigs that were exposed to natural chilling originate from either the same trees used in the main experiment (triangles) or from an additional set of trees within the same population (circles).

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 non-linear least square models with and without chilling temperature as additional parameter; n.s. for not significant). Errorbars indicate $\pm 1\text{SE}$.

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 week of artificial chilling; Minimum forcing, days to budburst at 20 °C and percentage of budburst after 18 weeks 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 relationship, respectively (pooled chilling temperatures). Note the different scales of the y-axis. Errorbars indicate $\pm 1\text{SE}$. BB, budburst.