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ORIGINAL PAPER

Chilling temperature and photoperiod influence the timing of bud burst in juvenile *Betula pubescens* Ehrh. and *Populus tremula* L. trees

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Abstract

• *Key message* This research demonstrates that inadequate chilling has the potential to slow both the rate and speed of bud burst of two important European forest species; *Betula pubescens* and *Populus tremula*.

• *Context* The timing of bud burst in deciduous trees has been widely used as an indicator of spring warming. However, winter chilling conditions can have a significant impact on the timing of bud burst. If trees receive insufficient chilling, there may be a delay in bud burst even if spring temperatures rise. Therefore, it is important to understand the effect of chilling on spring phenology of trees.

• *Methods* Here, we exposed juvenile (3–6-year old) birch (*B. pubescens* Ehrh.) and aspen (*P. tremula* L.) trees to a range of photoperiods, chilling durations and forcing temperatures in controlled environment chambers to assess the impact on the timing of bud burst.

• *Results* Analysis of variance demonstrated that longer chilling duration resulted in earlier bud burst in both species, and less thermal time was needed to reach maximum bud burst. Therefore, in warmer winters bud burst in spring may be expected to be delayed if insufficient chilling is received. However, longer photoperiod may, at least in part, compensate for shorter chilling duration. These results suggest that models predicting climate warming impacts on phenology should take winter chilling into account when considering the timing of bud burst in deciduous species.

Keywords Bud burst · Temperature · Chilling · Photoperiod · Birch · Aspen

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1 Introduction

Bud dormancy is an important stage in the development of trees in temperate regions, where survival depends on the ability to cease and resume growth according to seasonal environmental variations. But rather than a uniform state of inhibited growth, pioneering work carried out by Champagnat (1974, 1983) and later by Lang et al. (1987) and Arora et al. (2003) revealed dormancy to be a gradual transition of different physiological conditions, namely para-dormancy, endo-dormancy and eco-dormancy. These conditions are respectively (i) an inhibition of growth in the buds induced by plant tissues outside the buds, (ii) an inhibition from within the bud and (iii) a restriction of growth controlled by the environment (Arora et al. 2003). For most deciduous tree species, dormancy is induced mainly by short photoperiods during late summer and autumn (Håbjørg 1972; Heide 1974), while the breaking of (endo-)dormancy is mainly triggered by winter chilling temperatures (Sarvas 1972; Champagnat 1974,

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1983, 1993). The importance of the role of winter chilling in dormancy release has been documented since the 1920s (Coville 1920). Insufficient chilling during warm winters causes the endo-dormancy phase to extend and decrease more slowly (Sarvas 1972; Cannell and Smith 1983).

The state of chilling has yet to be clearly defined, as each species and even ecotype within a species can differ in their chilling requirements (e.g. Myking and Heide 1995; Li et al. 2003; Laube et al. 2014). It is usually expressed as the sum of hours or days below a certain threshold temperature, typically 10 or 12 °C, and above 0 °C (Körner 2006; Battey 2000). After the breaking of endo-dormancy, plants enter the eco-dormancy phase as they become receptive to warm spring temperatures to resume metabolism and growth activity. Also, the heat sum requirements (or thermal time, cumulative temperatures ≥10 °C) to release eco-dormancy varies between species, and there is a negative interaction between the level of chilling and the heat sum needed for bud burst to occur. For example, with more chilling, less heat is needed for flushing (Heide 1993a; Viherä-Aarnio et al. 2014). Besides temperature, photoperiod also plays a role in dormancy release, especially in higher latitudes where the need for a long photoperiod for bud burst to occur prevents a temperature-induced break of dormancy too early in the season (Körner 2006).

Overall, bud development in temperate trees is controlled by a combination of (i) chilling (temperature and duration), (ii) photoperiod and (iii) spring or forcing temperatures. Although extensive literature exists on the effects of temperature and photoperiod on plants (e.g. Heide 1993a; Cleland et al. 2007; Caffarra et al. 2011a), the interactions between these factors and the differences between (and within) species requires further clarification to improve predictions of phenology responses to a warmer climate. Different sensitivities to photoperiod may constrain some species' ability to respond to warmer temperatures (Basler and Körner 2012). Furthermore, the interactions between photoperiod and temperature are not fixed, meaning that particularly warm temperatures can partially override photoperiod controls, and particularly long days can partially override chilling requirements (Heide 1993a; Caffarra et al. 2011a). Since the timing of phenological events has an effect on survival and reproduction of trees, selective pressures and the responses are ultimately the result of adaptation to particular environments. Different species are therefore expected to have different phenological responses, which are important to understand for estimating the impact of climate change at an ecosystem level. Many earlier experimental studies were carried out on a single species (e.g. Håbjørg 1972; Heide 1993a; Li et al. 2003), but more recent ones have incorporated a wide range of deciduous and conifer trees (such as, Morin et al. 2010; Basler and Körner 2012, 2014; Fu et al. 2012; Laube et al. 2014).

Bud burst is often used as a measure of dormancy release, even though it is only one of the results of the dormancy release process. Gene function and cell metabolism are reactivated before bud burst becomes apparent (Arora et al. 2003). Physiological signals such as hormone and sugar levels induce cellular level processes within the plant such as cambium growth in the stem and meristem development within the buds even before bud elongation becomes visible (Horvath et al. 2003). Bud burst is however the most clear and observable phenological stage in tree species such as birch and aspen. Therefore, because of its indicator value for climate change (Menzel et al. 2006), bud burst is a key phenophase that is monitored in many phenology networks around the world. It is however not only important to monitor bud burst over the long term, but also to understand the mechanisms that underlie this phenological event.

Since both chilling and forcing temperatures have a strong influence on the timing of bud burst of a wide range of tree species it is important to understand how future climate warming may impact bud burst. Recent studies have demonstrated how the interaction between chilling accumulation and forcing requirements result in a nonlinear response in the timing of bud burst to rising spring temperature (Morin et al. 2010; Fu et al. 2012, 2015; Vitasse and Basler 2013b). Furthermore, the interaction between chilling and photoperiod on bud burst may also be expected to change as temperatures continue to rise. Indeed, many interactions that cannot be predicted at present are expected to arise in the future in response to changing climatic conditions (Laube et al. 2014).

The work presented here builds on previously published research carried out by Caffarra and Donnelly (2010) and Caffarra et al. (2011a, b) who investigated the effects of forcing temperature and photoperiod on the rates of dormancy release and induction in *Betula pubescens*, suggesting that further research was needed on chilling effects. In light of this, the aim of this study was to quantify the primary effects and interactions of temperature (in the form of chilling) and photoperiod on the timing of bud burst in both *B. pubescens* and *Populus tremula*, using controlled environment experiments. The results will form part of a framework for use in process-based phenological models. Furthermore, the results will have implications for forestry and conservation, because as winter temperatures become milder due to climate change, critical chilling thresholds might not be reached in the future. This is particularly important for forest populations growing at the warm margins of their distribution range.

2 Materials and methods

2.1 Plant material

2.1.1 B. pubescens Ehrh. (Birch)

The birch clone used here, originated from Germany and was grown at the John F. Kennedy Arboretum (New Ross, Co.

Wexford, Ireland), which is part of the International Phenological Gardens (IPG) network. Samples were vegetatively propagated (further details in Caffarra and Donnelly 2010) in 2003 and 2004 as described by Hartmann et al. (1997). The juvenile birch trees used in these experiments ranged in height from 30 to 45 cm and were kept outdoors in 1 L plastic pots containing John Innes No. 3 compost prior to use.

2.1.2 P. tremula L. (Aspen)

The aspen trees were commercially cloned juvenile trees purchased from Cappagh nurseries (Aughrim, Co. Wicklow, Ireland; EU Plant Passport 7238). The clones were propagated from root cuttings and were grown outdoors at the site of the nursery. For the experiments, the 3- to 4-year-old trees with heights between 80 and 120 cm were transplanted into plastic pots with a diameter of 15–20 cm containing John Innes No. 3 compost and were kept outdoors.

There was no overlap in plants being used in the experiments; all trees used were exposed to natural temperature and photoperiod in the year prior to commencement of the experiments. For the second year's experiments, aspen trees of the same age and size as the previous year were purchased from the nursery, and the birch trees used were from the same propagation batches as before. However, the birch trees were therefore 1 year older. The average viable number of buds per aspen tree was 59, ranging between 24 and 152 buds per tree, and the average number of buds for birch trees was 16 (between 4 and 41 buds per tree).

2.1.3 Experimental design

3 Experiment 1

In the autumn and winter of 2009–2010, experiments investigating dormancy release were conducted on thirty 3–4-year-old aspen trees and thirty 5–6-year-old birch trees to determine the effects of photoperiod and chilling requirement on the timing and percentage of bud burst. The experiments consisted of two phases.

The first phase began on 25th November 2009, with 15 trees of each species which had been kept outdoors since 1st August 2009, to receive natural chilling in ambient conditions. On the day of transfer into the growth chambers, the trees had only partially fulfilled their chilling requirements: they had undergone 35 chilling days (number of days with average temperature ≤ 10 °C since 1st September, which is a rough estimation of the amount of chilling; Myking and Heide 1995). Three different photoperiod conditions (8, 12 and 16 h of daylight; Table 1) were created in a growth chamber (Conviron PGR15).

The lights in the growth chamber were switched off automatically at night between 1 and 9 a.m. Five trees from each species were subjected to the different photoperiod lengths by covering them with non-transparent black plastic at different times of day: ten trees (five aspen and five birch trees) were covered at 5 p.m. resulting in an 8-h photoperiod, another ten trees (five aspen and five birch trees) were covered at 9 p.m. resulting in a 12-h photoperiod and the remaining trees remained uncovered resulting in a 16-h photoperiod treatment. The temperature in the growth chamber was kept constant at 10 °C. Relative humidity was kept constant at 50 %, and the plants were watered every other day. The temperature underneath the plastic was checked regularly and was not found to be considerably higher (<1 °C) than 10 °C. The dates when first bud burst occurred and when 50 % and 100 % of all buds had burst on a particular tree were recorded and observations were made daily, for a period of 123 days. Bud burst was defined as the first appearance of green leaf tips from between the opening bud scales (defined as stage 3 in Murray et al. 1989, Fig. 1).

The second phase of the experiment began on 18th January 2010. The 30 plants (15 aspen and 15 birch) used in the second experimental phase had received their full chilling requirement in natural conditions before being placed in the growth chamber. The same number of replicates (i.e. five trees per condition), and the same photoperiod, forcing temperature, humidity

Table 1 Details of the treatments received in each of the experiments during two seasons

Experiment	Plants		Chilling treatment		Forcing treatment		Recording	
	Species	Number per treatment	Temperature and photoperiod	Duration (days)	Temperature (°C)	Photoperiod (h)	Frequency	Percentage bud burst
1	Populus tremula Betula pubescens	5	Natural	35, 82	10	8, 12, 16	Daily	1st, 50 %, 100 %
2	P. tremula B. pubescens	8	Natural	25, 53, 76	20	10, 16	Every 2nd day	Exact count

"Chilling duration" is expressed in days with average daily temperature ≤ 10 °C, starting from 1st September

Natural non-controlled temperatures and photoperiods, received outdoors
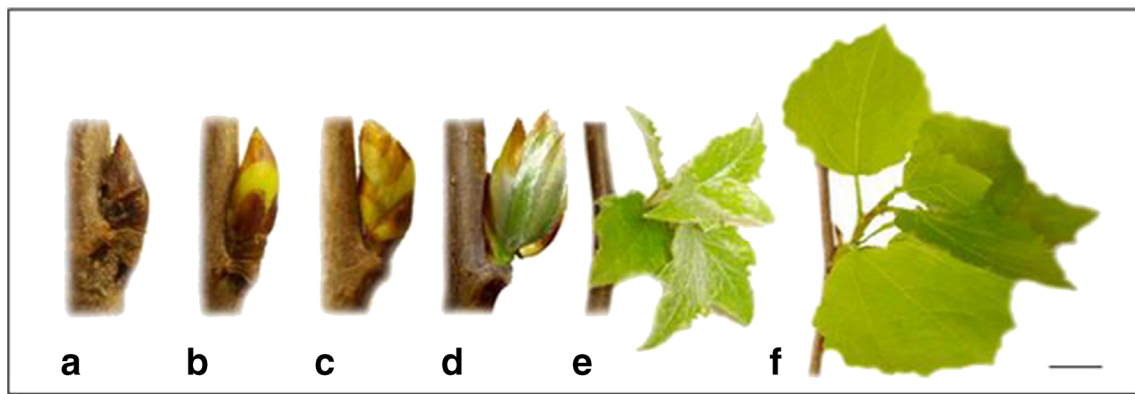
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Fig. 1 Stages of bud burst as observed in *Populus tremula* in experiment 1. **a** Dormant bud, **b**–**c** swelling and greening of the bud scales, **d** green leaf tissue visible from between the scales (bud burst), **e** leaf unfolding and **f** juvenile leaves. *Scale bars* **a**–**d**, 2 mm, **e**, 5 mm and **f**, 8 mm

and watering conditions were used as in the first phase of the experiment. Observations were made for 68 days.

4 Experiment 2

As in the previous year, an experiment investigating dormancy release was conducted in the autumn and winter of 2010–2011. For this experiment, forty-eight 3–4-year-old aspen trees and forty-eight 6–7-year-old birch trees were used to determine the effects of photoperiod and chilling requirement on the timing and percentage of bud burst. Experiment 2 involved three phases, consistent with three chilling duration conditions (25, 53 and 76 days in ambient conditions) (Table 1).

On 4th November 2010, the first batch of 16 aspen and 16 birch trees were transferred from outdoor conditions to two controlled environment chambers (Conviron PGR15 and Conviron A1000), wherein two different photoperiod conditions were automatically regulated (10 and 16 h of day light, with eight trees of each species in each treatment). The temperature was kept constant at 20 °C and relative humidity at 50 %. Observations of the exact number of buds that had burst on a particular tree were recorded every other day, for 101 days. During the months of September and October 2010, the plants had received 25 chilling days, before being transferred into experimental conditions (Table 1). One of the growth chambers had a thermostat failure in the beginning of December 2010, which resulted in the plants in this chamber receiving a heat shock treatment of up to 40 °C for several hours. Bud burst was recorded for these plants, and the results of this treatment (10 h photoperiod treatment in birch) will be discussed and compared but cannot be analysed with the results from the 'regular' treatments.

For the second phase, 32 trees were transferred from ambient into controlled conditions (two Conviron A1000 growth chambers) on 12th December 2010. At this date, the trees had

undergone 53 chilling days (Table 1). Sixteen trees from each species were used, of which eight received a photoperiod of 10 h and the other eight received 16 h. Photoperiod, forcing temperature, humidity, watering treatment and observation frequency were the same as in the first phase. The plants were monitored for 63 days.

The last 32 trees had fulfilled their chilling requirements with 76 days of chilling (Table 1) and were transferred into experimental conditions in the Conviron A1000 and PGR15 growth chambers on 14th of January. Observations were recorded for 30 days, and photoperiod, forcing temperature, humidity and watering regime were the same as previously described.

4.1 Chilling unit calculations

Daily chilling units were calculated using the equation below, described in the DORMPHOT model (Caffarra et al. 2011b) for *B. pubescens*. The chilling state CS(t) at day t, is given by the daily accumulation of chilling units:

$$CS(t) = \sum_{t_d}^{t} \frac{1}{1 + e^{aC(T_t - cC)^2 + (T_t - cC)}} \tag{1}$$

where t_d is the starting day of chilling accumulation (set as 1st September of the year preceding bud burst), T_t is the average daily temperature on day t and aC and cC are function parameters, estimated as respectively 0.03 and 13.89 for *B. pubescens*. The critical threshold (C_{crit}; or fulfilment of chilling requirements) for *B. pubescens* was determined at CS(t)=55.35 (Caffarra et al. 2011b).

For *P. tremula*, no species-specific models for chilling requirement are available; therefore, a general method for calculating chilling units for temperate trees was used. Chilling units were estimated as 1 if the average daily temperature was between 0 and 12 °C, and estimated as 0 if temperatures were negative or above 12 °C, starting from 1st September (e.g. Battey 2000; Myking and Heide 1995).

4.2 Meteorological data

Daily maximum and minimum temperatures for the outdoor chilling conditions were recorded at the Met Éireann meteorological station at Dublin Airport (53° 25′ N, 06° 16′ W, 74 m a.s.l.), 4.2 km from the location where the plants were stored.

4.3 Thermal time calculations

An important difference between experiments 1 and 2 was the forcing temperature used (Table 1): 10 and 20 °C, respectively. Both experiments could therefore only be compared if the heat units received by the plants were standardised. This was done by calculating the thermal time (TT) for each data point recorded for bud burst and by using this measure in analysis instead of the number of days to bud burst.

The thermal time to bud burst was calculated as the accumulated degree days above 0 °C since transfer into experimental conditions or since 1st January for plants that were transferred after this date. The thermal time to bud burst was calculated for each bud burst recorded. Buds that did not flush during the observation period were recorded as flushing after this period for the purpose of statistical analysis (123 and 68 days for experiment 1 and 101, 63 and 30 days for experiment 2). The TT to first, 50 % and 100 % bud bursts was subjected to a univariate two-way analysis of variance (ANOVA), with chilling, photoperiod and interaction as fixed effects.

4.4 Statistical analyses

The timing of bud burst was also recorded as the number of days to three different stages of bud burst within a single tree: first, 50 % and 100 % bud bursts. Smaller differences between the stages were measured when bud burst was faster (fast succession in days), compared with higher differences when bud burst was slower (more days needed to reach the next stage). Observations were made several times on the same trees and were therefore not independent. Repeated-measure ANOVA was used to determine which of the explanatory variables (*chilling* or *photoperiod*) had the greatest influence on differences in days between the stages within a tree.

The two response variables, days to bud burst (*BBD*) and percentage of bud burst (*percentage*), were analysed in relation to two explanatory variables: the environmental factors (1) duration of chilling in natural conditions (*chilling*) and (2) photoperiod length during forcing (*photoperiod*). Other response variables used were TT, the thermal time to bud burst and *slope*, the slope of a regression line between observation points (number of days to stage of bud burst vs. stage of bud burst: first, 50 % and 100 % bud bursts).

The test statistics used were (i) repeated-measure ANOVA (Tabachnick and Fidell 2007) and (ii) mixed between within-subject ANOVA. The mixed ANOVA design is a combination of between-group (i.e. standard) ANOVA and repeated-measure ANOVA. This design is used when the repeated measure is carried out on different levels (Pallant 2007), for instance as in the levels of bud burst in experiment 1 (first, 50 % and 100 % bud bursts). For tests where the repeated measures were irrelevant, for instance when *slope* was used as the response variable, a standard ANOVA was performed. All statistical analyses were carried out using SPSS 18.0 and R 2.12.2. Graphs were constructed using R.2.12.2 and SigmaPlot 12.0.

5 Results

5.1 Meteorological parameters

Monthly mean temperatures (Fig. 2a) and the accumulation of chilling units (Fig. 2b, c) during the experiments differed between the 2 years of the study (experiments 1 and 2). In September and October, average monthly temperatures were lower during the chilling period of experiment 1 (Fig. 2a). Because lower temperatures accelerate dormancy induction and release, dormancy release was more advanced during the first year of the experiments for *B. pubescens* (Fig. 2b). For *P. tremula*, a more crude method was used for the calculation of chilling units and the difference in chilling accumulation
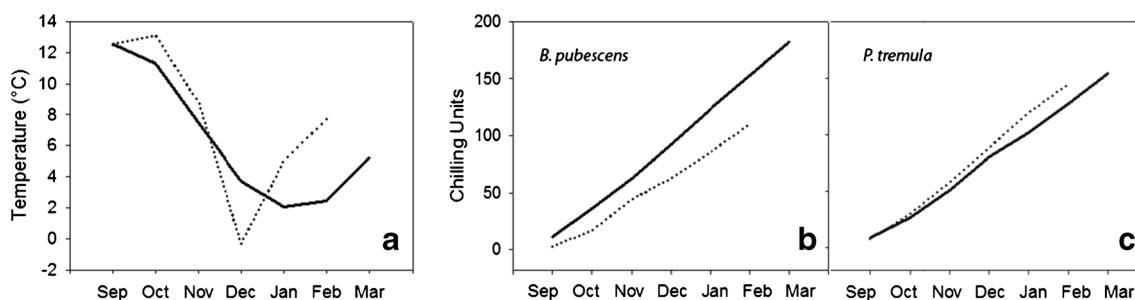

Fig. 2 Mean monthly temperature at Dublin Airport (**a**) and accumulated chilling units (averaged per month) for *Betula pubescens* (**b**) and *Populus tremula* (**c**) during autumn and winter of 2009–2010 (experiment 1, *black line*) and 2010–2011 (experiment 2, *dotted line*)

between both experiments was smaller than for *B. pubescens* (Fig. 2b, c). This is important for comparisons between both experiments: not only forcing temperatures were different but also chilling temperatures.

5.2 The impact of chilling and photoperiod on the timing of bud burst

In order to compare the timing of bud burst between experiments 1 and 2, only the points of first, 50 % and 100 % bud bursts were analysed. Heat shock birch samples (experiment 2) were omitted from the analyses, as was the condition with the shortest chilling duration in experiment 1 for *P. tremula*. ANOVA revealed significant main effects for chilling duration on the timing of bud burst for *B. pubescens* in experiment 1 and for *P. tremula* in experiment 2 ($p=0.000$) (Table 2). For *B. pubescens*, the significance level for *chilling* was close to 0.05 ($p=0.067$). Long photoperiods tended to reduce the number of days to bud burst although this trend was only statistically significant ($p=0.073$, 0.096 and 0.064) at the 0.10 level. An interaction between *chilling* and *photoperiod* had a significant effect on the number of days to bud burst ($p=0.011$) in *B. pubescens*, showing that the effect of chilling duration varied with different photoperiods.

Figure 3 shows the timing of bud burst in experiment 2. It is clear how chilling duration had an effect on the number of days to bud burst in *P. tremula* (Fig. 3c, d). In particular, the shortest chilling condition had a much shallower slope compared with the other two chilling conditions, indicating a slower progression of bud burst. In *B. pubescens*, no significant effects were observed for chilling on the timing of bud burst (Tables 2 and 3; Fig. 3a, b): the sigmoid curves have a similar shape and steepness, except for the minimal chilling condition in Fig. 3b, indicating an initial surge in bud burst, after which a slower progression of bud burst occurred. This pattern is more than likely due to the heat shock that the trees received early on in the experiment.

Significant ($p<0.001$) effects of chilling, photoperiod and the interaction of chilling and photoperiod on the timing of first bud burst were found for *B. pubescens* in experiment 2 (Table 3). Also for *P. tremula*, a significant ($p<0.001$) influence of chilling was observed on first bud burst date in experiment 2. For days to 50 % bud burst, a stage often recorded in phenological monitoring programmes, chilling duration had a significant influence on the timing of bud burst for both species in experiment 2. There was also a significant effect of photoperiod on *B. pubescens* in experiment 1 and for *P. tremula* in experiment 2. In all analyses, missing values (non-flushing buds) were considered as flushing at the latest

Table 2 Average values (±standard deviation) for the timing (number of days) of bud burst (the slope of a linear regression line between first, 50 % and 100 % bud bursts) for conditions with different chilling durations (CD; chilling days) and photoperiod lengths (Photo) and analysis of variance results for the effects of photoperiod (P), chilling duration (C) and interaction between both effects (C×P) on the timing of bud burst

Photo (h)	Average values			ANOVA results				
	CD (days)	*Betula pubescens*	*Populus tremula*	Effect	*B. pubescens*		*P. tremula*	
					F (*df*)	p	F (*df*)	p
Experiment 1								
8	35	15.0±6.4	–	C	55.21 (1)	**<0.001**	–	–
8	82	3.9±2.5	8.6±2.6					
12	35	18.3±2.7	–	P	2.94 (2)	0.073	0.67 (2)	0.537
12	82	2.8±0.8	7.3±2.9					
16	35	8.6±4.3	–	C×P	5.48 (2)	**0.011**	–	–
16	82	4.4±3.2	9.6±2.3					
Experiment 2								
10	25	–	42.9±19.2	C	2.96 (2)	0.067	22.10 (2)	**<0.001**
10	53	4.6±1.4	22.4±19.6					
10	76	6.5±1.2	6.3±2.2	P	2.95 (1)	0.096	3.64 (1)	0.064
16	25	6.5±3.1	32.0±16.0					
16	53	4.6±2.7	11.1±6.7	C×P	2.95 (1)	0.096	0.94 (2)	0.401
16	76	3.8±1.1	6.3±2.2					

Significant *p* values are in bold

Fig. 3 Percentage of bud burst in *Betula pubescens* (**a**, **b**) and *Populus tremula* (**c**, **d**) clones against number of days after transfer into experimental conditions in experiment 2; **a**, **c** 16 h daylength; **b**, **d** 10 h daylength. *Heavy eight-pointed rectilinear black star*, minimal chilling; *empty circle*, intermediate chilling; *black down-pointing small triangle*, maximal chilling
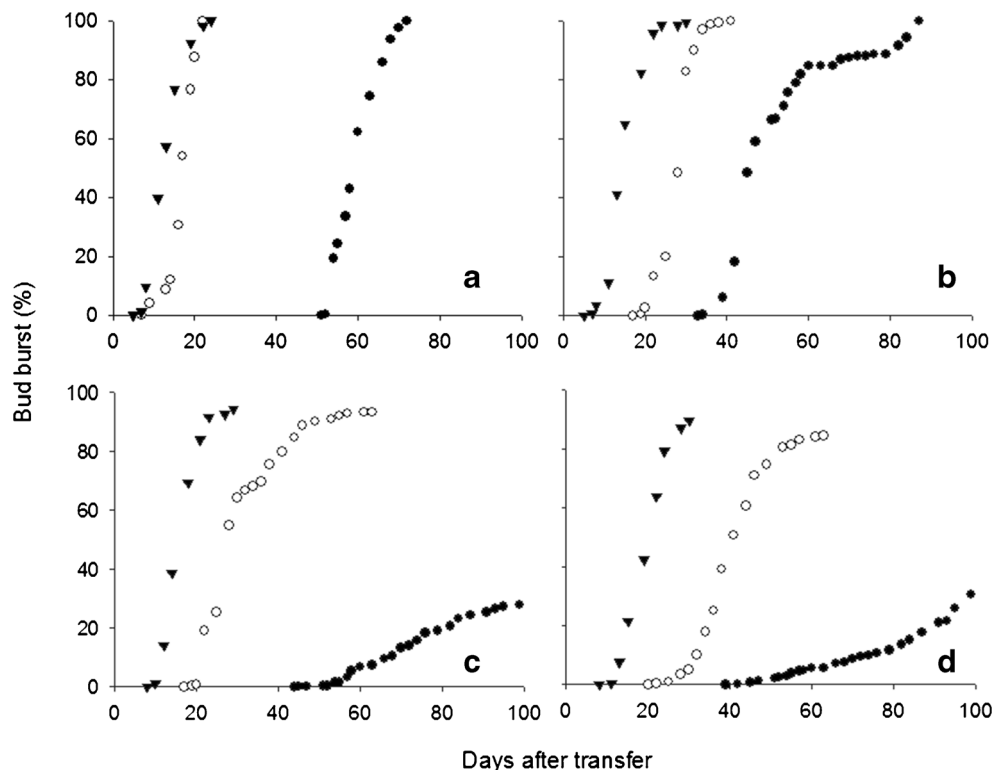
date of the experimental recording time, except for *P. tremula* in experiment 1, where bud flush was extremely rare after the shortest chilling duration. Therefore, the effect of chilling duration could not be measured for 50 and 100 % bud bursts in experiment 1. In experiment 2 however, the higher forcing temperature resulted in a higher percentage of bud burst, even after shorter chilling durations, and both chilling and photoperiod had significant effects on the timing of 50 and 100 % bud bursts for *P. tremula*. However, the condition with the shortest chilling duration in experiment 2 had to be omitted

from the 100 % bud burst analysis for *P. tremula* due to the low number of plants reaching this stage. *B. pubescens* reached 100 % bud burst at a timing that was influenced by chilling and photoperiod in both experiments and by interaction of both effects in experiment 1 (Table 3).

5.3 Effect of chilling on thermal time to bud burst

The effect of chilling duration on the timing of bud burst is illustrated in Fig. 4. Comparing the mean values in Fig. 4, it is

Table 3 Univariate two-way ANOVA results for the timing of three stages of bud burst (first, 50 % and 100 % bud bursts) in species *Betula pubescens* and *Populus tremula*, in two different experiments with varying chilling durations (C; two conditions in experiment 1 and three conditions in experiment 2) and under different photoperiods (P; three conditions in experiment 1 and two conditions in experiment 2)

Effect	1st bud burst				50 % bud burst				100 % bud burst			
	B. pubescens		*P. tremula*		*B. pubescens*		*P. tremula*		*B. pubescens*		*P. tremula*	
	F (df)	p	F (df)	p	F (df)	p	F (df)	p	F (df)	p	F (df)	p
Experiment 1												
C	0.37 (1)	0.547	1.97 (1)	0.174	1.33 (1)	0.261	–	–	10.12 (1)	**0.004**	–	–
P	0.21 (2)	0.808	0.76 (2)	0.481	3.86 (2)	**0.036**	0.03 (2)	0.973	5.30 (2)	**0.013**	0.71 (2)	0.52
C×P	0.05 (2)	0.955	0.34 (2)	0.716	1.74 (2)	0.199	–	–	4.62 (2)	**0.021**	–	–
Experiment 2												
C	50.88 (1)	**<0.001**	86.6 (2)	**<0.001**	13.17 (1)	**<0.001**	211 (2)	**<0.001**	268 (2)	**<0.001**	7.83 (1)	**0.009**
P	13.83 (1)	**0.001**	0.03 (1)	0.855	0.02 (1)	0.901	6.05 (1)	**0.018**	18.45 (1)	**<0.001**	5.03 (1)	**0.033**
C×P	14.76 (1)	**0.001**	2.02 (1)	0.146	10.05 (1)	0.818	2.15 (1)	0.129	1.18 (1)	0.287	0.79 (1)	0.383

Significant p values are in bold (<0.05)

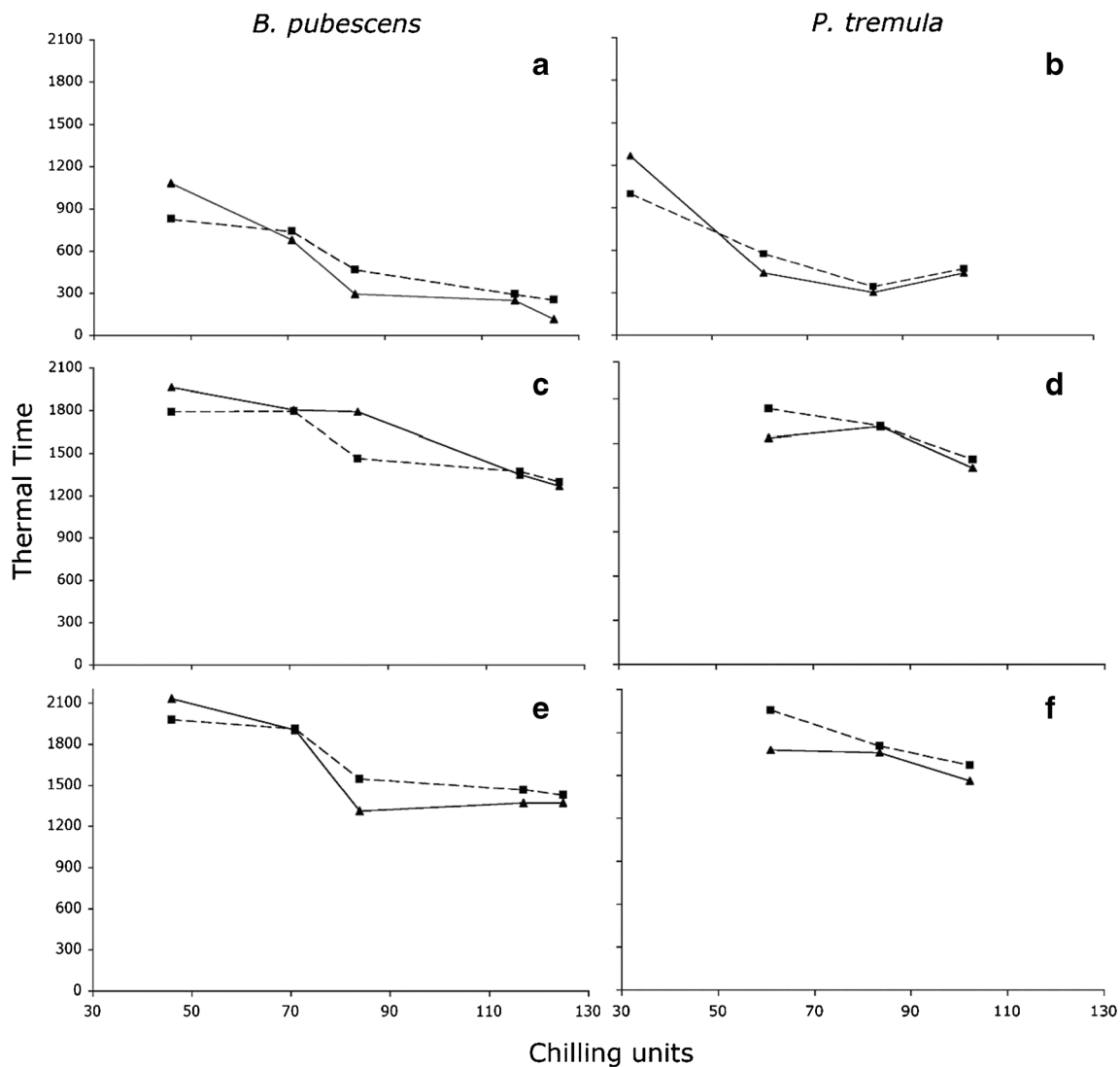**Fig. 4** Average thermal time (expressed in degree days >0 °C) to **a**, **b** first, **c**, **d** 50 % and **e**, **f** 100 % bud bursts for *Betula pubescens* (**a**, **c** and **e**) and *Populus tremula* (**b**, **d** and **f**) clones subjected to a total of five chilling conditions for *B. pubescens* (46, 71, 84, 117 and 125 chilling units) and a total of four chilling conditions for *P. tremula* (33, 60–61, 84 and 103 chilling units). *Squares*, thermal time to bud burst averaged over conditions with 8–10 h photoperiod, *Triangles*, thermal time to bud burst averaged over 12–16 h photoperiod. Data are from both experiments 1 and 2

clear that overall chilling duration had an effect on the timing of bud burst to the three stages. Thermal time to bud burst decreased after longer chilling periods for all stages, even though chilling was more frequently a significant factor on the timing of the different stages of bud burst in experiment 2 than in experiment 1 (Table 3).

5.4 Overall effects of chilling and photoperiod on the timing of bud burst

Experiment 1 A between-subject multivariate test with Wilk's lambda was used to test whether the individual trees showed differences in the timing of bud burst over the three stages and revealed a significant interaction between bud burst and chilling ($p<0.05$, Tables 4 and 5). The interaction between

chilling and photoperiod was not significant. There was a substantial main effect for stages of bud burst and for chilling ($p<0.001$), and also a significant influence of photoperiod ($p<0.05$), indicating that the birch trees needed a significantly different number of days to reach each stage of bud burst, and this difference was due to the different chilling and photoperiod treatments. A significant interaction between timing of bud burst and chilling was found ($p<0.001$) and also an interaction between all three variables tested ($p<0.05$), although the effect of photoperiod on days to each level of bud burst was minor ($p=0.177$). This implies that the trees responded differently to the chilling conditions for different stages of bud burst, for instance chilling had a more significant effect on the 100 % bud burst level than on the other two levels of bud burst in *B. pubescens* (Table 3) (Table 6).

Table 4 Average values of number of days to stage of bud burst (BB) with standard deviation, for different chilling and photoperiod conditions of *Betula pubescens* in experiment 1

	Partial chilling			Full chilling		
	Photoperiod					
	8 h	12 h	16 h	8 h	12 h	16 h
BB						
1st	77.4±9.3	73.6±10.9	63.0±13.6	80.8±2.8	78.2±1.5	76.0±3.5
50 %	90.4±16.5	92.6±10.7	74.5±9.1	84.2±4.6	82.0±1.2	79.4±4.3
100 %	97.0±17.3	107±11.6	80.3±10.9	88.6±7.8	83.8±1.3	84.8±9.7

Experiment 2 All bud burst data, excluding the heat shock treatment of *B. pubescens*, were used in a mixed between within-subject ANOVA analysis. All effects tested, with the exception of the main effect of photoperiod for *B. pubescens*, but including all interaction effects were highly significant ($p<0.001$) (Table 5). These results imply that chilling duration had a significant effect on the date of bud burst, across all buds and in both species tested. The effect of chilling on the day of bud burst was strongly dependent ($p<0.001$) on the photoperiod during forcing for both species. For *P. tremula*, the main effect of photoperiod length during forcing was also significant on flushing dates for all buds, regardless of which tree the buds belonged to. As mentioned, for *B. pubescens*, photoperiod had no significant effect, which might have been due to the exclusion of the trees that received the heat shock treatment.

6 Discussion

This study reports on the experimental effects of two environmental factors on the timing of dormancy release, in the form of bud burst, in two important forest species native to Ireland and Europe: *B. pubescens* Ehrh. and *P. tremula* L. However, a number of notable limitations to the experimental setup must be considered before discussing the results in a wider context. Firstly, the limited number of temperature and photoperiod treatments used resulted from the fact that only two controlled environment chambers, with few regulatory controls, were available. Therefore, we used a number of contrasting (high and low) conditions for each environmental parameter in order to maximise the available resources. We were unable to simulate the gradual increase in temperature and photoperiod plants receive under natural conditions and recognise the less than ideal nature of the experimental setup. In addition, the fact that we used a limited number of juvenile trees also reduces the realism somewhat. A recent study suggests that experiments using cuttings from adult trees provide more realistic data on the timing of bud burst than juvenile trees (Vitasse 2013). Nevertheless, we are confident that the results demonstrate the impact of chilling on the timing of bud burst of juvenile trees and the importance of the interaction with photoperiod for these two species. These quantifiable effects, which have often been underestimated, should lead to an improved performance of phenological models. Furthermore, the results have potential implications for forest conservation and management practices, and for the commercial growth of these two species used as renewable energy resources, particularly as temperatures are projected to rise in future.

6.1 The effect of chilling duration on bud burst

An overall decrease in the time taken to reach bud burst in response to increasing duration of chilling was found at different forcing temperatures and for both species *B. pubescens* and *P. tremula*. This confirms previous results for a range of deciduous species (e.g. Heide 1993a; Murray et al. 1989; Fu et al. 2013; Dantec et al. 2014; Laube et al. 2014; Viherä-Aarnio et al. 2014) and is an indication that dormancy release, as dormancy itself, is not a fixed state, but involves continuous physiological changes within the plant. The largest differences in bud burst timing occurred in the early stages of chilling, which supports the use of an inverse exponential function to explain the relationship between accumulated chilling units and heat unit requirements for bud burst (Murray et al. 1989; Chuine et al. 1999; Ghelardini et al. 2009).

Table 5 Multivariate mixed between within-subject ANOVA results for the fixed factors effects on timing of bud burst

Fixed effects	Wilk's lambda		
	df	F	p
BB	2	85.58	**0.000**
C	1	329.13	**0.000**
P	2	3.71	**0.043**
BB×C	2	25.84	**0.000**
BB×P	4	1.67	0.177
C×P	2	2.35	0.121
BB×C×P	4	3.04	**0.029**

A Wilk's lambda statistic was used for the multivariate test. Significant *p* levels are in bold

BB bud burst stage, *C* chilling condition, *P* photoperiod

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Table 6 Experimental results (mean number of days to bud burst and standard deviations) and mixed between within-subject ANOVA results of type III test for the fixed effects on the timing of bud burst for *Betula pubescens* and *Populus tremula* clones in experiment 2

| | Number of days to bud burst | | | | | | Mixed ANOVA results | | | |
| | P (10 h) | | | P (16 h) | | | Fixed effects | Type III sum of squares | | |
	MC	IC	FC	MC	IC	FC		df	F	p
B. pubescens										
Mean	–	28	13	56	28	18	C	2	5051.5	**<0.001**
St. Dev.	–	3	3	4	6	4	P	1	0.81	0.37
							C×P	2	219.14	**<0.001**
P. tremula										
Mean	52	41	20	57	17	14	C	2	9009.3	**<0.001**
St. Dev.	5	6	4	2	2	4	P	1	305.09	**<0.001**
							C×P	2	134.64	**<0.001**

P photoperiod, *MC* minimal chilling, *IC* intermediate chilling, *FC* full chilling, *C* chilling duration. Significant *p* values (<0.001) are in bold

Chilling duration not only had a significant effect on the initial timing of bud burst but also on the time it took to reach 50 and 100 %. This effect was more pronounced for *P. tremula* than for *B. pubescens*: the trees receiving the shortest chilling durations only advanced very slowly to a higher percentage of bud burst and even failed to reach 40 % bud burst within the monitoring time of 103 days. Such an extended period of bud burst for incompletely chilled plants may negatively impact the fitness of the plant but may also affect the availability of resources for herbivores feeding on new leaves.

Whether chilling requirements for temperate trees, in Europe, are currently sufficient for full dormancy release has been considered in recent literature. For example, Vitasse et al. (2011), using a range of phenological models, suggested that current chilling conditions are adequate to fulfil the chilling requirements of a number of deciduous species (including *Fagus sylvatica*) but that this may change during the current century as winter temperatures increase. A recent study examining the impact of chilling and forcing temperatures on leaf unfolding of oak (*Quercus petraea*) and beech (*F. sylvatica*) revealed that beech required a greater amount of chilling than oak (Dantec et al. 2014). The authors concluded that winter chilling was currently insufficient for full dormancy release, particularly for beech, and that populations growing in the warmest margins of their distribution will likely be faced with similar conditions as global temperature continues to rise. Indeed, Zohner and Renner (2014) came to a similar conclusion suggesting that as the number of chill days decreased towards 2050, forcing requirements will increase concurrently thus leading to potential changes in phenology especially for species with a high chilling requirement. Other studies (Fu et al. 2013, 2015) reported a reduction in winter chilling resulted in a greater thermal requirement for bud burst to occur in beech (*F. sylvatica*), oak (*Quercus robur*) and birch (*Betula pendula*), a similar conclusion was drawn by Laube

et al. (2014). In the current study, birch showed a high percentage of bud burst, even after short chilling periods, while many of the aspen trees failed to show signs of dormancy release under the same conditions. This suggests that *P. tremula* has a higher chilling requirement than *B. pubescens*. But also in fully chilled plants, a longer time was needed for bud burst to occur in *P. tremula*, compared with *B. pubescens*. This implies that *P. tremula* also has a greater thermal requirement, or alternatively, it is possible that full chilling requirements were not met for *P. tremula* at the latest date of transfer into experimental conditions, resulting in an incomplete release of dormancy. Further testing is needed to assess the real chilling requirement of *P. tremula*. These studies suggest that chilling requirements are currently being fulfilled for some species but not for others but that it is highly lightly that in future more species will experience inadequate chilling for the full release of dormancy which will impact the plants ability to respond to rising spring temperatures.

This research has shown that for *B. pubescens* the minimal chilling requirement was met in early-December but that chilling may not have been completed even by late-January for *P. tremula*, since not all aspen trees that were chilled until the 18th January reached 100 % bud burst. Heide (1993a) observed a similar phenomenon whereby bud burst for *B. pubescens* was earlier compared with *P. tremula* in natural outdoor conditions.

The fact that in experiment 1 we used a forcing temperature of 10 °C, which also falls within the active chilling range might have confounded the calculation of the actual chilling units received by plants (as plants were given extra chilling units during forcing). Thus, we prefer to make a general qualitative comment on the fact that long chilling results in a qualitatively lower forcing requirement for bud burst rather than quantify the amount of decrease in thermal time per extra chilling unit. Even though the plants might be partially subjected to an 'internal

clock' for their development which depends more on chronological time than accumulated temperature units, we know from a vast literature on the subject (see Battey 2000 for a general review) that a certain amount of chilling temperatures is needed in order for bud burst to occur satisfactorily (in high percentages and synchronously). The disentangling of this 'internal clock' component from the chilling component of dormancy warrants further research.

6.2 Interaction effects between chilling duration and photoperiod length

Overall, fully chilled plants were found to be less sensitive to photoperiod variations, as indicated by the many significant values found for interaction effects between chilling duration and photoperiod length. In experiment 2 (20 °C forcing temperature), some significant interaction effects were found for both species examined. In contrast, Heide (1993b) did not report any interaction effects between chilling duration and photoperiod in a range of forest species, including *B. pubescens* and *P. tremula*. However, Heide (1993b) used cuttings in his experiments, as opposed to juvenile trees in these experiments. Interestingly, the use of saplings (*Carpinus betulus*, *F. sylvatica*, *Acer pseudoplatanus*) in controlled experimental conditions have been shown to bud burst slightly earlier than adult trees or cuttings (Vitasse and Basler 2013a). This suggests that care must be taken when comparing results from different data collection methods and extrapolation of responses from one life stage to another may not be appropriate (Morin et al. 2010). Furthermore, Vitasse (2013) advises caution when using results from juvenile trees, as opposed to adults, when calibrating phenological models due to the strong ontogenic differences in phenology between different life stages.

6.3 The effect of photoperiod on bud burst

Because temperature is a variable measurement, it can sometimes be an unreliable indicator for favourable growing conditions for plants, particularly in temperate regions where a late frost can have a detrimental effect on the vulnerable tissues of newly opened leaf and flower buds. Also in subsequent seasons after frost damage, a delay in bud break, reduction in shoot and canopy growth, and a reduction in flowering and fruit production have been reported (Bokhorst et al. 2009). Therefore, many plants in these regions use an extra security factor against the breaking of dormancy happening too early in the season: photoperiodism (Körner 2006; Way and Montgomery 2014). However, to what extent a particular species' phenology is controlled by photoperiod can vary: for instance, some species with little or no day length control are *Sorbus aucuparia* L., *Rubus* spp. and *C. betulus* L. (Heide 1993a, b). Also

for elm (*Ulmus* spp.), photoperiod had no effect on dormancy release (Ghelardini et al. 2009). A recent study by Laube et al. (2014) reported that long photoperiod only slightly advanced bud burst in 30 % of species tested. However, for *F. sylvatica* photoperiod seemed to act as a limiting factor during the winter months, reducing early dormancy release and frost risk (Caffarra and Donnelly 2010), and also *Prunus* species exhibited a photoperiod response in leaf formation (Heide 2008). Because deciduous trees vary greatly in their sensitivity to photoperiod, predicting how this will influence their phenological response to future warmer temperature requires further investigation to determine the species that might be more successful in migrating and those that may be more suitable for forestry purposes (Way and Montgomery 2014).

Overall, few significant effects of photoperiod length on the timing of bud burst were found between the two species studied here and no effect of photoperiod was found on the timing to reach first, 50 % and 100 % bud bursts: the advancement of bud burst within a single tree was not different for longer photoperiods compared with shorter photoperiods. In experiment 1 (with low forcing temperature), the birch trees responded significantly differently to different photoperiods in days to bud burst, while in experiment 2 (high forcing temperature), only the aspen trees showed an effect of photoperiod. A photoperiod sensitivity of birch trees, particularly in the early stages of the chilling period, was reported in earlier studies (Håbjørg 1972; Li et al. 2003; Caffarra and Donnelly 2010), and therefore, it is surprising that experiment 2 did not provide evidence for a significant effect of photoperiod on bud burst in *B. pubescens*. A possible explanation why *B. pubescens* failed to show sensitivity to photoperiod, may be due to the 10-h photoperiod condition that received a heat shock after short chilling duration and whilst in forcing conditions, but before any buds had flushed. Because of this treatment, bud burst occurred earlier than expected and although this condition was omitted from the analyses, any significant effect that would have been present might have been levelled out by the removal of this condition.

Where interactions between photoperiod and chilling were significant, long photoperiods had an effect on time to bud burst after short chilling durations, reducing the number of days to bud burst. However, this effect could not fully substitute for an incomplete fulfilling of the chilling requirements and disappears altogether when chilling requirements are completely fulfilled. This conclusion follows previous findings such as in *B. pubescens* and *B. pendula* clones (Myking and Heide 1995), and in Norway spruce (Søgaard et al. 2008), and is an interesting finding that provides further evidence for the importance of chilling temperature in the current debate on environmental drivers of tree phenology (Chuine et al. 2010; Körner and Basler 2010).

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7 Conclusions

In the current study, very different responses in the timing of dormancy release were observed between two species subjected to the same environmental conditions. Both *B. pubescens* and *P. tremula* are considered to be opportunistic pioneer species and the variation in responses between these two species suggest a slight nuance in the hypothesis that climate change affects the phenology of early-successional tree species more than that of late successional species (Caffarra and Donnelly 2010; Körner and Basler 2010) and that a thorough understanding of the effects of chilling conditions is needed at a species level and for more species than have been studied thus far.

Since the photoperiod effect was weaker for longer chilling durations, the possible effects of a climatic warming include earlier bud burst and therefore a higher risk of frost damage on newly emerged buds. This would be an unfavourable trend for birch, with an earlier bud burst compared with aspen and with an ability to reach bud burst even after incomplete chilling, short photoperiods and a forcing temperature of 10 °C. Indeed, *B. pubescens* has already shown a highly variable start of growing season over the decade 1981–1990, compared with other species in the IPG network (Caffarra and Donnelly 2010). The risks for *P. tremula* may lie in an extended period of dormancy release or even a failure to reach dormancy release due to milder winters. Many other forest species are likely to have a similar response to chilling, and models predicting the impacts of climate change on a large scale should not only consider earlier bud burst, but also take a possibility of delayed bud burst into account for some species. Overall, our results highlight the complexities involved in quantifying the environmental drivers of bud burst and species-specific nature of the progression of bud burst. However, these results are expected to help improve the predictive performance of the DORMPHOT model and other similar processed-based phenology model.

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