

Three times greater weight of daytime than of night-time temperature on leaf unfolding phenology in temperate trees

Yongshuo H. Fu^{1,2}, Yongjie Liu¹, Hans J. De Boeck¹, Annette Menzel^{3,4}, Ivan Nijs¹, Marc Peaucelle⁵, Josep Peñuelas^{6,7}, Shilong Piao^{2,8} and Ivan A. Janssens¹

¹Centre of Excellence GCE (Global Change Ecology), Department of Biology, University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Belgium; ²Sino-French Institute for Earth System Science, College of Urban and Environmental Sciences, Peking University, Beijing 100871, China; ³Ecoclimatology, Technische Universität München, 85354 Freising, Germany; ⁴Institute for Advanced Study, Technische Universität München, Lichtenbergstraße 2a, 85748 Garching, Germany; ⁵Laboratoire des Sciences du Climat et de l'Environnement, CEA CNRS UVSQ, Gif-sur-Yvette, France; ⁶CREAF, Cerdanyola del Vallès, Barcelona, 08193 Catalonia, Spain; ⁷CSIC, Global Ecology Unit CREAM-CSIC-UAB, Cerdanyola del Vallès, Barcelona, 11 08193 Catalonia, Spain; ⁸Key Laboratory of Alpine Ecology and Biodiversity, Institute of Tibetan Plateau Research, Chinese Academy of Sciences, Beijing 100085, China

Summary

Author for correspondence:

Yongshuo H. Fu

Tel: +32 3 265 2254

Email: Yongshuo.fu@uantwerpen.be

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- The phenology of spring leaf unfolding plays a key role in the structure and functioning of ecosystems. The classical concept of heat requirement (growing degree days) for leaf unfolding was developed hundreds of years ago, but this model does not include the recently reported greater importance of daytime than night-time temperature.
- A manipulative experiment on daytime vs night-time warming with saplings of three species of temperate deciduous trees was conducted and a Bayesian method was applied to explore the different effects of daytime and night-time temperatures on spring phenology.
- We found that both daytime and night-time warming significantly advanced leaf unfolding, but the sensitivities to increased daytime and night-time temperatures differed significantly. Trees were most sensitive to daytime warming (7.4 ± 0.9 , 4.8 ± 0.3 and 4.8 ± 0.2 d advancement per degree Celsius warming ($d\ ^\circ C^{-1}$) for birch, oak and beech, respectively) and least sensitive to night-time warming (5.5 ± 0.9 , 3.3 ± 0.3 and 2.1 ± 0.9 d $^\circ C^{-1}$). Interestingly, a Bayesian analysis found that the impact of daytime temperature on leaf unfolding was approximately three times higher than that of night-time temperatures.
- Night-time global temperature is increasing faster than daytime temperature, so model projections of future spring phenology should incorporate the effects of these different temperatures.

Introduction

Plant phenology is highly sensitive to climate change (Menzel *et al.*, 2006; Schwartz *et al.*, 2006; Jeong *et al.*, 2011; Fu *et al.*, 2014b). Warming-induced phenological changes can affect ecosystemic structure and functioning by extending the length of the growing season (Cleland *et al.*, 2007; Piao *et al.*, 2008), changing species composition (Chuine, 2010) or altering the interaction between plants and animals (Hunter, 1992; Memmott *et al.*, 2007), and even between plants and climate (Peñuelas *et al.*, 2009). The underlying physiological mechanisms nevertheless remain unclear (Hänninen & Kramer, 2007; Chuine *et al.*, 2013; Fu *et al.*, 2013). A better understanding of plant phenological processes is required to predict the response of plants to future climate change (Richardson *et al.*, 2013; Fu *et al.*, 2015b).

Several centuries ago, Réaumur proposed that the date of leaf unfolding could be linked to the accumulated temperature in a preceding period, generally called heat requirement or growing degree days (GDDs) (Réaumur, 1735). Other environmental

factors have also recently been associated with leaf unfolding, such as winter chilling (Murray *et al.*, 1989; Fu *et al.*, 2013; Laube *et al.*, 2014a), photoperiod (Körner & Basler, 2010; Way & Montgomery, 2014), humidity (Laube *et al.*, 2014b), precipitation (Peñuelas *et al.*, 2004; Fu *et al.*, 2014c) and conditions in the previous year (Fu *et al.*, 2014a). Taking advantage of these findings, models that include not only GDD but also chilling and photoperiod (Hänninen & Kramer, 2007; Chuine *et al.*, 2013) have been widely used to predict leaf unfolding and its response to climate change. Such predictions for leaf unfolding, however, still vary considerably and differ largely from *in situ* observations, resulting in substantial uncertainty when predicting ecosystemic responses of carbon and water balances to climate change (Levis & Bonan, 2004; Kucharik *et al.*, 2006; Piao *et al.*, 2007; Richardson *et al.*, 2012). Current phenological models may thus not capture all the main mechanisms involved (Chuine *et al.*, 2013; Fu *et al.*, 2015a). A key weakness could be the accuracy of estimation of the heat requirement, which has received less attention in previous studies. The unit of heat requirement,

that is, daily GDD, is generally calculated by using the average daily temperature in excess of a base temperature (Miller *et al.*, 2001). The weight of daytime and night-time temperatures in calculating GDD is thus the same. Recent studies, however, have suggested that spring leaf phenology may be more dependent on daytime than on night-time temperature (Hanes, 2014; Piao *et al.*, 2015). Specifically, both studies found that the spring phenology, that is, both *in situ* leaf unfolding (only maple trees in Hanes (2014)) and remote-sensing-based green-up onset over the northern hemisphere (Piao *et al.*, 2015), are more related to daily maximum temperature than to minimum temperature. This implies an asymmetric influence of daytime vs night-time temperatures on the heat requirement of leaf unfolding.

Spring phenology, especially of forest tree species, affects the global carbon and water balance and climatic feedbacks (Pan *et al.*, 2011; Richardson *et al.*, 2013). To explore the different effects of daytime and night-time temperatures on spring phenology, well designed experiments deliver increased process understanding (De Boeck *et al.*, 2015). This, in turn, could be used to improve the phenology models, leading to more accurate projections of ecosystem responses, especially against a background of ongoing climate change. Indeed, the global minimum temperature has increased about twice as fast as the maximum temperature since 1950 (IPCC, 2014), rendering it even more pertinent to differentiate between daytime and night-time temperature changes. Only a few night-time warming experiments have been conducted, focusing on crops and grassland (Dhakhwa & Campbell, 1998; Peng *et al.*, 2004; Xia & Wan, 2012), but (to our knowledge) the effects of daytime and night-time warming on the tree phenology of leaf unfolding have not been experimentally investigated.

We therefore designed a novel manipulative experiment to unveil potential differential effects of daytime vs night-time warming on leaf phenology. We used saplings of three species of temperate deciduous trees: birch (*Betula pendula*), oak (*Quercus robur*) and beech (*Fagus sylvatica*), which are common species in Europe and characterized by different heat requirements. The objectives of this study were to determine and quantify the different effects of daytime and night-time warming on leaf unfolding in these three temperate tree species.

Materials and Methods

Experimental design and phenological measurements

The experiment was conducted at the Drie Eiken campus of the University of Antwerp (Belgium, 51°19'N, 4°21'E). We transplanted local birch (*Betula pendula* L.), oak (*Quercus robur* L.) and beech (*Fagus sylvatica* L.) saplings 1 m in height into plastic pots (diameter 25 cm, depth 40 cm) on 4 December 2014 and moved them into 12 outdoor climate-controlled chambers on 1 January 2015. These saplings were bought from a nursery close to our experimental site. Grown from seeds collected in a local forest, saplings had been cultivated in the same field for 1 yr before being transplanted into pots and used in our experiment. The pots were filled with an organic substrate with a pH of 6.0%

and 20% organic matter (Universal Potting Soil, Viano, Aalst, Belgium). Seven saplings each of beech, oak and birch were placed in each chamber, totaling 84 saplings per species. The chambers could be artificially warmed in a controlled manner up to 9°C above fluctuating ambient temperatures using a centralized heating system (Fu *et al.*, 2013). They were sunlit, facing south, with a transparent polycarbonate plate at the top 4 mm thick (light absorption = 15%; Fu *et al.*, 2013). The interior surface area was 200 × 150 cm, the height was 180 cm on the south side and 200 cm on the north side (Supporting Information Fig. S1). Temperature sensors (Siemens, type QFA66, Berlin, Germany) were used to continuously monitor the air temperature inside each chamber, logging every 30 min and stored as hourly data. Over the experimental period (from 1 January 2015 until leaf unfolding in spring 2015), the saplings were watered once or twice per wk as soon as the topsoil appeared dry.

Three replicate chambers were maintained at ambient temperature for the entire duration of the experiment (control treatment), and the other nine chambers were used for three replicates of three warming treatments: whole-day warming, i.e. continuously warmed by 2°C above ambient over the entire experimental period; daytime warming only; and night-time warming only. Daytime and night-time were defined as the periods from sunrise to sunset and sunset to sunrise, respectively (see Fig. 1a). A sample of the four treatments is shown in Fig. 1(b). The daily temperature settings of the daytime and night-time warming treatments were changed based on daily GDDs to ensure that these were identical among the three warming treatments. Indeed, the mean temperatures of the three warming treatments were very similar: daytime warming, 10.4°C; night-time warming, 10.2°C; and whole-day warming, 10.3°C. The daily GDD was calculated as:

$$\text{GDD} = T_{\text{mean}} - T_{\text{base}} \quad \text{if } T_{\text{mean}} > T_{\text{base}} \quad \text{Eqn 1}$$

$$T_{\text{mean}} = \left(\sum_{\text{hour}=1}^{24} T_{\text{hour}} \right) / 24 \quad \text{Eqn 2}$$

where T_{mean} is average daily temperature and T_{hour} is hourly temperature. T_{base} is the base temperature, set at 0°C in the present study as used by Sarvas (1972). Similar results were observed using 5°C as the base temperature (not shown). The chambers provided continuous warming, with no significant differences in GDD accumulation among the three warming treatments from 1 January to the end of May 2015, as programmed (Fig. 1c).

Phenological traits were observed on the terminal bud of each sapling. Leaf unfolding was defined as the date when the entire leaf blade and stalk were visible (Fig. S1), following the guidelines in Fu *et al.* (2013). Monitoring began on 1 February and was repeated every 2 d.

Model description and calibration

Three models of growing degree hours (GDHs) were parameterized to predict the date of leaf unfolding, that is: the classical GDH model (GDH_{classical}), which assumes that a certain number

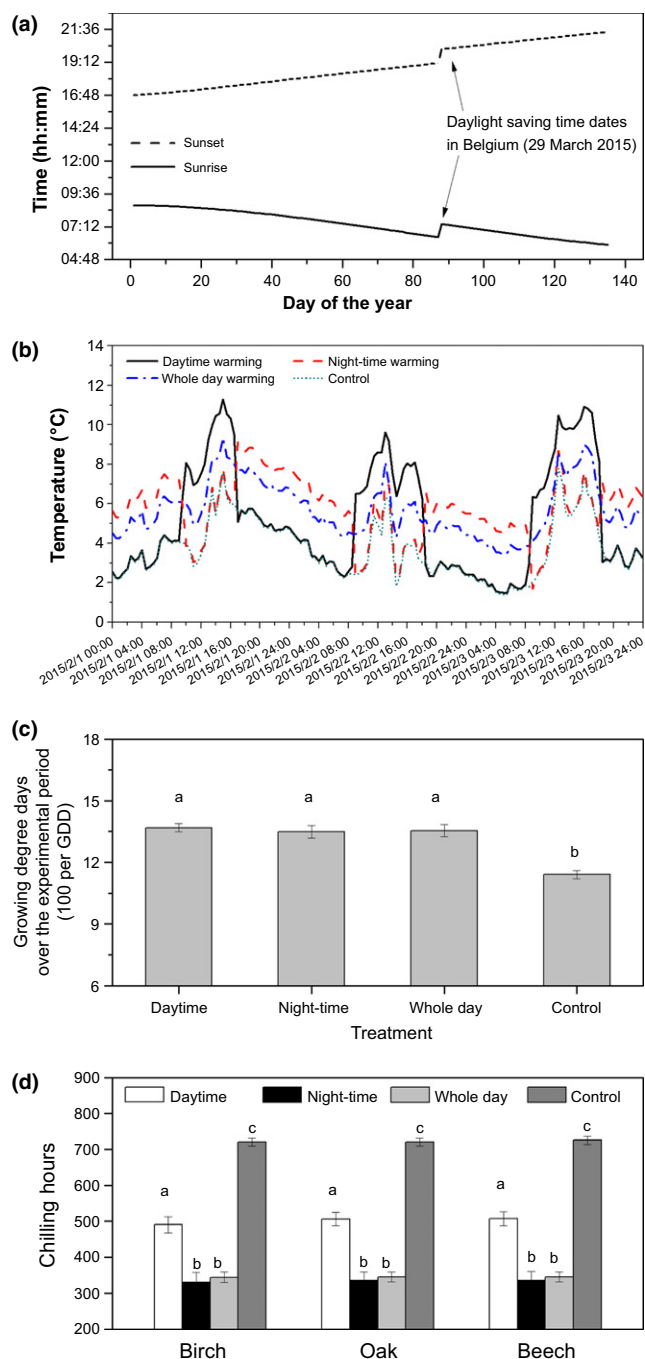


Fig. 1 (a) Timing of sunrise and sunset during the experimental period from 1 January to the end date of leaf unfolding. (b) A sample of the variation in air temperature during 3 d (year/month/day hh:mm) in all treatments, that is, whole-day warming, daytime warming only, night-time warming only and control. (c) The mean growing degree days (GDDs) \pm 1 SD in each treatment, accumulated from 1 January to the end date of May 2015. (d) Changes of chilling \pm 1 SD among the four treatments during the experimental period from 1 January to the end date of leaf unfolding. The chilling accumulation was calculated as chilling hours for hourly temperatures between 0 and 5°C, as in a previous study (Fu *et al.*, 2015b). Different letters denote significant differences ($P < 0.05$).

of GDHs ($\text{GDH}_{\text{requirement}}$) are needed to trigger leaf unfolding; an improved GDH model ($\text{GDH}_{\text{day-night}}$), which weights daytime and night-time temperatures differently for GDH

accumulation; and a $\text{GDD}_{\text{nonlinear}}$ model that assumes an exponential function to calculate GDH accumulation. All models assume that leaves unfold when $\text{GDH}_{\text{requirement}}$ is reached.

The accumulation of GDH in the models starts on a fixed day t (1 January, as used by Murray *et al.* (1989)) until the date of leaf unfolding (LU). During this period, GDHs accumulate when the hourly temperature (T_{hour}) exceeds a base temperature (T_{base}) by using a linear relationship:

$$\text{GDH}_{\text{classical}} = \sum_t \left(\sum_{\text{hour}=1}^{24} (T_{\text{hour}} - T_{\text{base}}) \right) \quad \text{if } T_{\text{hour}} > T_{\text{base}} \quad \text{Eqn 3}$$

In the $\text{GDH}_{\text{day-night}}$ model, we defined the effect of daytime temperature on leaf unfolding has a larger effect than night-time temperature, and two new parameters are thus added to weight the impact of daytime (K_a , prior parameter distribution: 0.5–1) and night-time (K_b , prior parameter distribution: 0–0.5) temperatures on GDH accumulation:

$$\text{GDH}_{\text{day-night}} = \sum_t \left(\sum_{\text{hour}=1}^{24} \left(K_a * (T_{\text{hour_daytime}} - T_{\text{base}}) + K_b * (T_{\text{hour_night-time}} - T_{\text{base}}) \right) \right) \quad \text{if } T_{\text{hour}} > T_{\text{base}} \quad \text{Eqn 4}$$

where $T_{\text{hour_daytime}}$ and $T_{\text{hour_night-time}}$ are the hourly temperatures (T_{hour}) during the daytime and night-time, respectively.

The accumulation of GDHs in the $\text{GDH}_{\text{nonlinear}}$ model starts on 1 January, as in the $\text{GDH}_{\text{classical}}$ and $\text{GDH}_{\text{day-night}}$ models, until the date of leaf unfolding. During this period, GDHs accumulate when the hourly temperature (T_{hour}) exceeds a base temperature (T_{base}) by using an exponential function of hourly temperature:

$$\text{GDH}_{\text{nonlinear}} = \sum_t \left(\sum_{\text{hour}=1}^{24} \left(K * T_{\text{hour}} * (T_{\text{hour}} - T_{\text{base}}) \right) \right) \quad \text{if } T_{\text{hour}} > T_{\text{base}} \quad \text{Eqn 5}$$

where K is the parameter range from 0 to 1.

The models were parameterized by a Bayesian Markov chain Monte Carlo method. This Bayesian calibration walks through the coefficient space of each parameter (prior distribution) in such a way that the collection of visited points forms a set of parameter coefficients (posterior distribution). The model was run 10^4 times with different parameter coefficients sampled from the prior distribution of the coefficients. This method has previously been used to parameterize phenological models; Fu *et al.* (2012) provides a detailed description. Information for the parameters K_a and K_b was lacking, so flat distributions were defined as the prior parameter distribution (Van Oijen *et al.*, 2005). We quantified the weights of daytime and night-time

temperatures on daily GDH accumulation by calculating the $K_a : K_b$ ratio for each run; the distributions of the ratios are presented as histograms.

Data analysis

The mean date of leaf unfolding and the GDH requirement were analyzed using all saplings per treatment, that is, the mean values were averaged across all saplings of each treatment (in three chambers). The differences between the mean date of leaf unfolding under warming treatments and under controls were defined as the advancement of leaf unfolding under warming treatments. As chamber is a nested factor, we specified the nested effects of the chambers in the runs of the linear mixed models and found that the chambers did not significantly affect the results. Tukey's *post hoc* honest significant difference tests were applied to test pairwise comparisons of the advancement, GDH requirement and temperature sensitivity of leaf unfolding among treatments. The temperature sensitivity of leaf unfolding (S_T) was calculated using linear regression analyses (ordinary least-squares regressions) of the dates of leaf unfolding against mean air temperature from 1 January to the day of leaf unfolding for each species and each warming treatment. All statistical analyses were conducted using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). The effectiveness of the phenological models was evaluated with the root-mean-square error (RMSE) of the difference between the predicted and observed dates of leaf unfolding:

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^n (\text{Sim}_i - \text{Obs}_i)^2}{n - 1}} \quad \text{Eqn 6}$$

where Obs_i and Sim_i are the mean observed and simulated dates of leaf unfolding in chamber i , respectively, and n is the number of dates.

Results

The three warming treatments significantly advanced the date of leaf unfolding for all three species, but the amount of advancement differed significantly ($P < 0.05$) among the treatments (Fig. 2a), except for oak where the influence of night-time and whole-day warming was the same. Despite the similar GDDs among the treatments, daytime warming advanced the dates of leaf unfolding most, with average advancements of 13.4 ± 4.1 , 8.5 ± 2.5 and 8.3 ± 3.5 d for birch, oak and beech, respectively. The advancement was shortest under night-time warming but was still significant (8.8 ± 3.0 , 5.1 ± 3.8 and 2.9 ± 3.4 d for birch, oak and beech, respectively), with whole-day warming eliciting an intermediate response (11.2 ± 5.2 , 6.4 ± 4.4 and 5.6 ± 4.5 d, respectively).

The temperature sensitivity of leaf unfolding (change in $\text{d } ^\circ\text{C}^{-1}$ warming) logically also differed significantly among the three warming treatments (Fig. 2b). The trees of all three species were most sensitive to daytime warming (average rates of

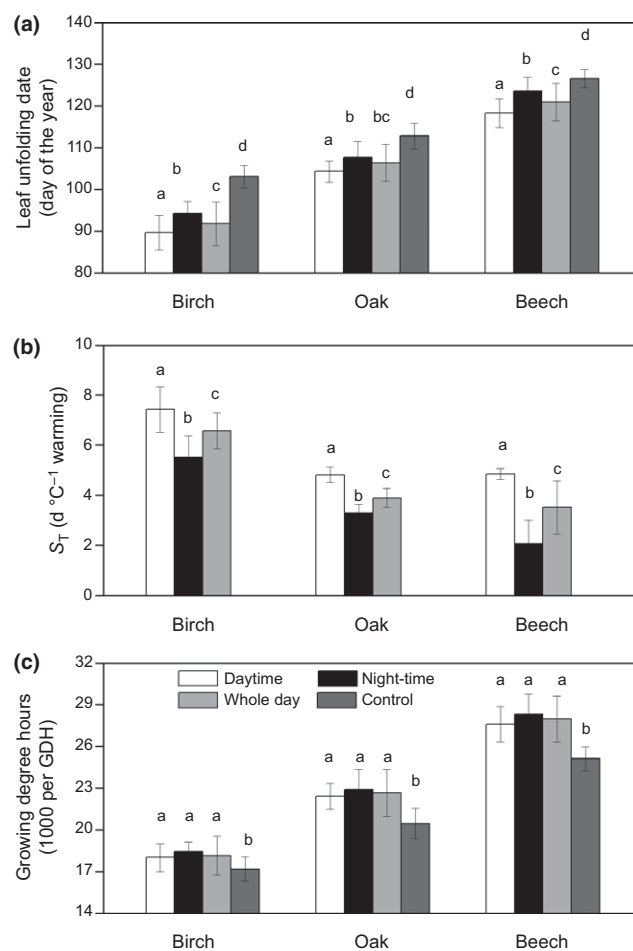


Fig. 2 (a–c) Mean date of leaf unfolding ± 1 SD (a), temperature sensitivity (S_T) ± 1 SD (b) and mean accumulation of growing degree hours (GDHs) ± 1 SD (c) calculated from 1 January to the date of leaf unfolding for each tree species. Different letters denote significant differences ($P < 0.05$) in the dates of leaf unfolding and GDHs among the four treatments.

advancement of 7.4 ± 0.9 , 4.8 ± 0.3 and 4.8 ± 0.2 d $^\circ\text{C}^{-1}$ for birch, oak and beech, respectively) and least sensitive to night-time warming (5.5 ± 0.9 , 3.3 ± 0.3 and 2.1 ± 0.9 d $^\circ\text{C}^{-1}$, respectively), with whole-day warming intermediate (6.6 ± 0.7 , 3.9 ± 0.4 and 3.5 ± 1.1 d $^\circ\text{C}^{-1}$, respectively).

Interestingly, the GDH calculated from 1 January 2014 to the day of leaf unfolding were significantly larger for trees in the three warming treatments than for those in the control treatments for all three species (Fig. 2c), even though the dates of leaf unfolding were significantly advanced for the trees in the warming treatments (Fig. 2a). Among the three warming treatments (i.e. daytime, night-time and whole-day warming), we found that earlier leaf unfolding was associated with less GDH accumulation, although the GDH accumulation did not differ significantly among the three warming treatments. This suggests that the weight of daytime and night-time temperatures in calculating GDH should be different. Specifically, daytime temperatures may play a more important role than night-time temperatures in leaf unfolding.

The modeling supported this finding. The performance of the GDH_{day-night} model (which incorporated the differential effects of daytime and night-time temperatures) was a substantial improvement over the GDH_{classical} model. The RMSEs of the GDH_{day-night} model were only 1 day for all three species, compared with 3 d for the GDH model (Fig. 3a–c). The posterior distribution of the $K_a:K_b$ ratio was similar for the three species, with medians near 3 (Fig. 3d–f), indicating that the weight of daytime temperature on the accumulation of GDHs was three times larger than the weight of night-time temperature. The GDH_{nonlinear} model performed better than the classical GDD model, but still less well than the GDH_{day-night} model (Fig. 4).

Discussion

A trend towards earlier leaf unfolding with currently increasing temperatures has been widely documented for plants in cold and temperate regions (Peñuelas *et al.*, 2002; Menzel *et al.*, 2006; Schwartz *et al.*, 2006; Fu *et al.*, 2014b). The phenological response to climatic warming, however, is nonlinear (Chuine *et al.*, 2010; Fu *et al.*, 2015b; Marchin *et al.*, 2015), and a significant reduction in temperature sensitivity of leaf unfolding since the 1980s, attributed to winter warming and potentially to photoperiod limitation, has recently been reported (Fu *et al.*, 2015b). Here, we provide experimental evidence of an additional mechanism contributing to the

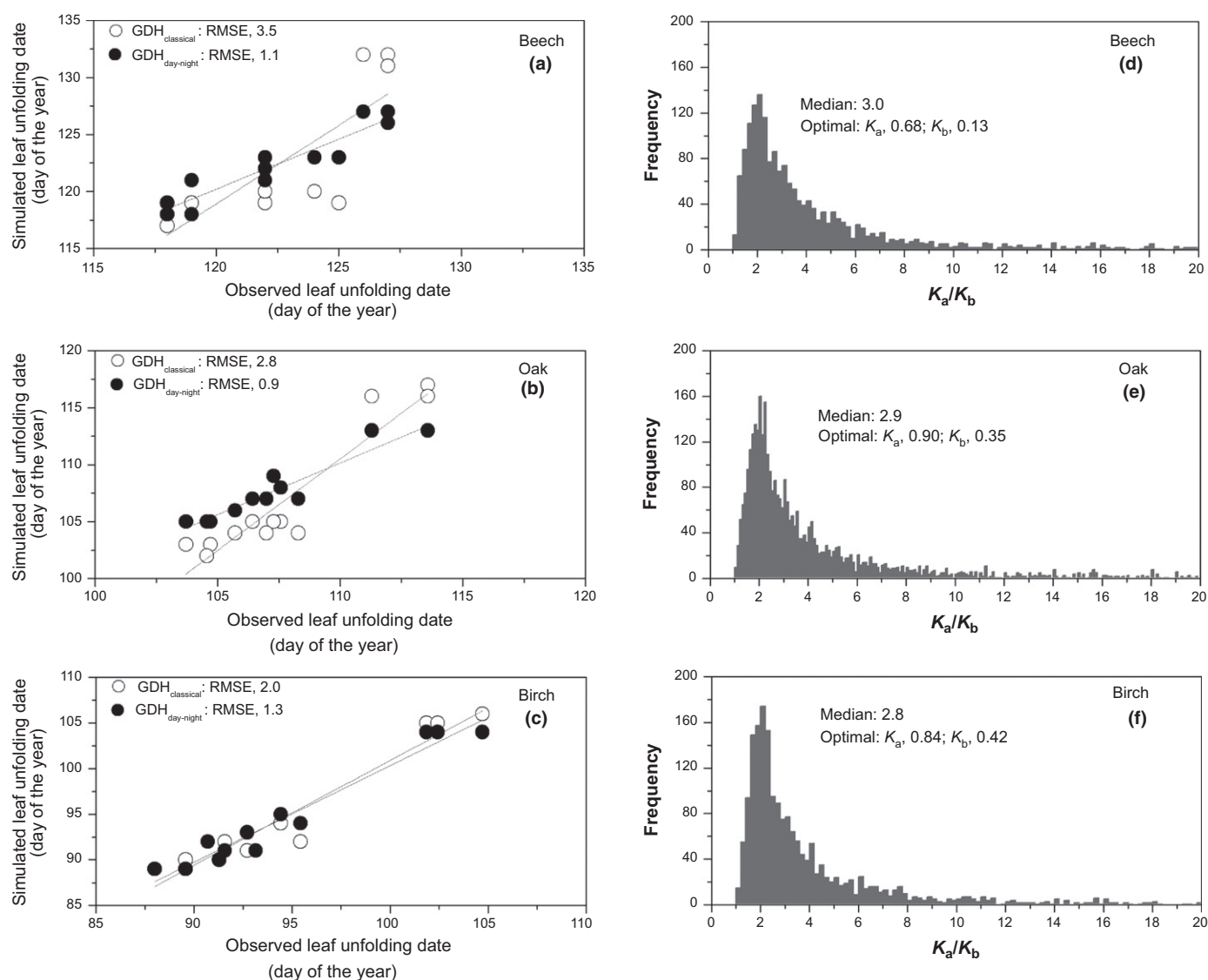


Fig. 3 Comparison of observed and predicted dates of leaf unfolding for the models for growing degree hours (GDH_{classical} and GDH_{day-night}) for: (a) beech; (b) oak; (c) birch. Compared with the GDH_{classical} model, two new parameters were added to GDH_{day-night} to weight the impacts of daytime (K_a) and night-time (K_b) temperatures on GDH accumulation. The root-mean-square errors (RMSEs) are provided for each model and species. The histograms show the distributions of the $K_a:K_b$ ratios of the GDH_{day-night} model across all Markov chain Monte Carlo runs for: (d) beech; (e) oak; (f) birch. The medians are provided for each species.

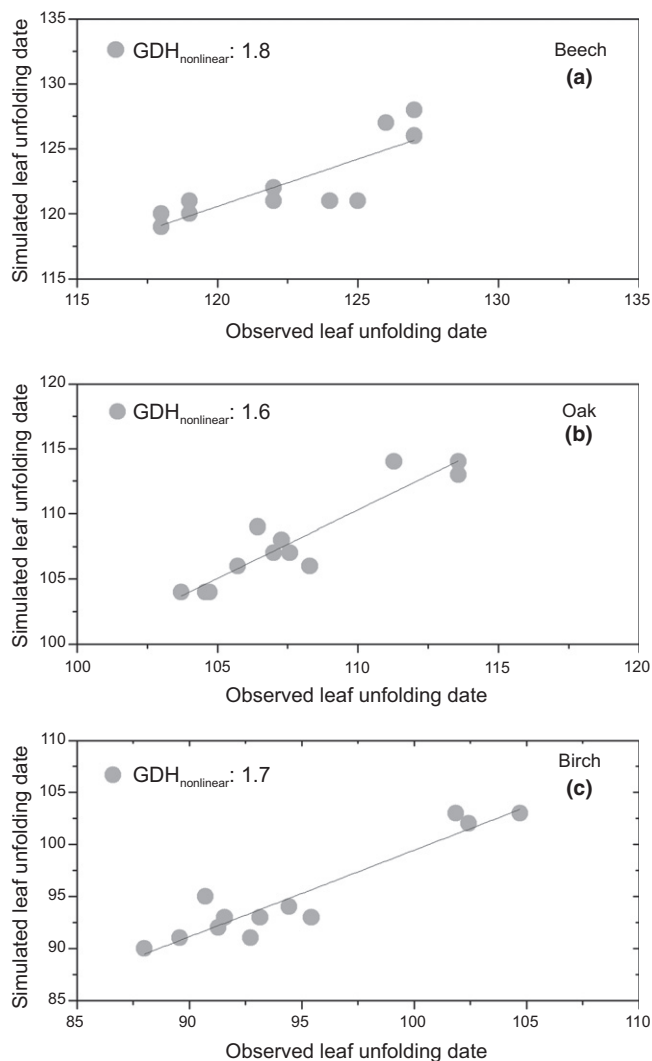


Fig. 4 Comparison of observed and predicted dates of leaf unfolding with the nonlinear growing degree hours model ($GDH_{nonlinear}$) for beech (a), oak (b) and birch (c). The root-mean-square errors (RMSE) are provided for each species.

nonlinearity of the temperature response to warming: a three-fold difference in the sensitivity of leaf unfolding to increases in daytime vs night-time temperatures. Our result is consistent with the main conclusion of a recent study by Piao *et al.* (2015), which demonstrated that spring leaf phenological dates were mostly correlated with gridded daytime temperatures. Another finding, however, differed between this large-scale analysis based on remote sensing and our analysis based on saplings of three tree species: we found that night-time warming also significantly advanced the dates of leaf unfolding, albeit at a much lower rate compared with daytime warming. The experimentally warmed night-time temperatures in our experiment, though, were often higher than the threshold temperature for heat accumulation, which was generally not the case in the study by Piao *et al.* (2015), which analyzed differences in ambient temperature.

The threefold greater impact of daytime vs night-time temperatures on leaf unfolding suggests that weighted hourly

temperatures are more appropriate for calculating heat accumulation than daily mean temperatures. This implies that the GDD-based phenological models currently used would not accurately predict leaf unfolding in future climates, as night-time temperatures are projected to increase faster than daytime temperatures (IPCC, 2014). Better predictions of spring leaf-out timing could be achieved by acknowledging the nonlinear relationship between diurnal temperature and heat accumulation for leaf unfolding we uncovered.

The asymmetric effect of daytime and night-time temperature on the phenology of leaf unfolding is clear. Why leaf unfolding is more dependent on daytime than night-time temperature nonetheless remains unclear. We propose two nonmutually exclusive hypotheses to explain these results.

First, the response of heat accumulation to temperature may be nonlinear instead of linear, as assumed in the original GDD concept. Daytime temperatures are much higher and would thus logically contribute more to heat accumulation if the temperature response was nonlinear. Indeed, we found that the $GDH_{nonlinear}$ model performed better than the classical GDD model, but still less well than the $GDH_{day-night}$ model (Fig. 4), suggesting that the nonlinear response of heat accumulation to temperature may explain part of the asymmetric effect of daytime and night-time temperature on leaf unfolding, but this proposal is admittedly incomplete.

Second, daytime solar radiation warms meristems directly and more than air temperature, especially under clear skies (Savvides *et al.*, 2014). Leaf unfolding is probably more directly related to meristem temperature than to air temperature (Grace *et al.*, 1989). The difference between meristem and air temperature is typically much smaller at night than during the daytime, and the sign of this temperature difference may even change. Calculating GDD using only the air temperature may therefore introduce an error that underestimates the true temperature-driving phenology during daytime. The underlying mechanisms of how radiation and temperature affect leaf unfolding by the accumulation of heat remain incompletely understood and require additional manipulation experiments.

In addition to the heat requirement, a chilling requirement and photoperiod can contribute to the timing of leaf unfolding in temperate tree species (Cannell & Smith, 1983; Murray *et al.*, 1989; Körner & Basler, 2010; Fu *et al.*, 2014c; Laube *et al.*, 2014a; Way & Montgomery, 2014). The saplings in our study were grown under the same photoperiodic conditions, eliminating any effect of photoperiod. The concept of chilling assumes that a certain amount of low temperature (the chilling requirement) is needed to end endodormancy, and sufficient warm temperatures (heat requirement) are subsequently necessary to trigger leaf unfolding (Cannell & Smith, 1983; Hänninen, 1990; Hänninen & Kramer, 2007; Chuine *et al.*, 2013). The chilling and heat requirements are negatively correlated, with more chilling decreasing the heat requirements for leaf unfolding (Murray *et al.*, 1989; Fu *et al.*, 2014c). The specifics of this correlation remain unclear, which leads to differences among phenological models (Chuine, 2000). In the present study, all warming treatments significantly reduced the amount of chilling hours

compared with the controls (Fig. 1d). The chilling hours under night-time warming were significantly reduced compared with those in the daytime warming treatment, but the heat requirement did not differ significantly between these two treatments (Fig. 2c), suggesting that the chilling requirement may have already been fulfilled in all saplings before the onset of all warming treatments. In other words, it is unlikely that further chilling was needed after the release from endodormancy, even if temperatures were suited to contributing to further chilling accumulation. Trees may therefore sequentially accumulate chilling and heat during endodormancy and ecodormancy, respectively, providing support for the sequential model of chilling and heating for predicting leaf unfolding.

In conclusion, this study has demonstrated that leaf unfolding in temperate tree species advanced with both daytime and night-time warming, but the sensitivity to increased daytime or night-time temperatures differed significantly. The impact of increases in daytime temperature on leaf unfolding via heat accumulation was approximately three times higher than that of increases in night-time temperatures. To our knowledge, this is the first time that direct experimental evidence has been provided for asymmetric daytime–night-time warming effects. The nonlinear response of leaf unfolding to climatic warming might be more pronounced as a result of a nonuniform diurnal temperature warming with a faster increase in night-time than in daytime temperature (IPCC, 2014). The asymmetric effect of daytime and night-time warming on leaf unfolding should be incorporated into models of leaf unfolding to improve phenological predictions and our understanding of the responses of global carbon and water cycles to the ongoing climate change. This requires more well-designed experiments to identify the generality of our results by focusing on the underlying mechanisms.

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Author contributions

Y.H.F., S.P. and I.A.J. designed the research. Y.H.F. and Y.L. performed the experiments. Y.H.F. and I.A.J. wrote the manuscript. Y.H.F., Y.L., H.J.D.B., A.M., I.N., M.P., J.P., S.P. and I.A.J. contributed to the interpretation of the results and to the text.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Fig. S1 The climate-controlled chambers used in the study at the University of Antwerp, Belgium.

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