Ontogenic changes rather than difference in temperature cause understory trees to leaf out earlier

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Summary

- In a temperate climate, understory trees leaf out earlier than canopy trees, but the cause of this discrepancy remains unclear. This study aims to investigate whether this discrepancy results from ontogenic changes or from microclimatic differences.
- Seedlings of five deciduous tree species were grown in spring 2012 in the understory and at canopy height using a 45-m-high construction crane built into a mature mixed forest in the foothills of the Swiss Jura Mountains. The leaf development of these seedlings, as well as conspecific adults, was compared, taking into account the corresponding microclimate.
- The date of leaf unfolding occurred 10–40 d earlier in seedlings grown at canopy level than in conspecific adults. Seedlings grown in the understory flushed *c.* 6 d later than those grown at canopy height, which can be attributed to the warmer temperatures recorded at canopy height (*c.* 1°C warmer).
- This study demonstrates that later leaf emergence of canopy trees compared with understory trees results from ontogenic changes and not from the vertical thermal profile that exists within forests. This study warns against the assumption that phenological data obtained in warming and photoperiod experiments on juvenile trees can be used for the prediction of forest response to climate warming.

Introduction

As sessile organisms, plants inevitably experience a wide range of environmental conditions during their lifetime as a result of climatic variations from year to year and of microenvironmental changes. This is particularly true for long-lived woody species, such as trees, which usually grow through the forest understory with limited light before reaching the canopy. Trees have therefore developed ontogenic changes in their physiology and morphology to cope with the changing microenvironmental conditions during their lifetime. Thus, in order to optimize light utilization, mature leaves produced at different stages of tree ontogeny (i.e. seedlings, saplings and adult trees) generally differ in their photosynthetic capacity and light response, morphology and anatomy (e.g. Cavender-Bares & Bazzaz, 2000; Ishida *et al.*, 2005; Mediavilla & Escudero, 2009; Thomas, 2010). For example, a general pattern found in temperate deciduous tree species is an increase in leaf mass per unit area (LMA) from seedling or sapling to adult life stage (Thomas & Winner, 2002). However, despite some evidence that a genetic component is involved in the control of age- and size-related changes in foliar morphology and physiology (reviewed in Day *et al.*, 2002), it remains unclear whether such morphological and physiological changes depend directly on tree life stage (tree age-related changes) or simply reflect microenvironmental differences (environmentally induced developmental changes).

Although differences in leaf physiology and morphology between juvenile tree stages and mature trees have been explored intensively, few studies have focused on leaf phenology patterns within tree species among different life stages. Among these studies, results generally highlight that understory trees in temperate forests exhibit earlier bud burst in spring than do conspecific adults (Gill *et al.*, 1998; Seiwa, 1999a,b; Augspurger & Bartlett, 2003; Augspurger, 2004), with some exceptions found, such as in *Fagus grandifolia*, *Fagus crenata* and *Betula lenta* (Tomita & Seiwa, 2004; Richardson & O'Keefe, 2009). Earlier flushing of understory trees allows them to take advantage of the optimal light conditions before canopy closure, which has been considered as a mechanism enabling seedlings to thrive in the understory despite low light availability in summer (Gill *et al.*, 1998; Seiwa, 1999a; Augspurger *et al.*, 2005; Kwit *et al.*, 2010). For instance, by experimentally shading saplings of *Aesculus glabra* and *Acer saccharum* for *c.* 1 month before canopy closure during three consecutive years in Illinois (USA), Augspurger (2008) found a significant decrease in survival and growth rate relative to unshaded saplings. In the Great Smoky Mountains National Park (USA), Lopez *et al.* (2008) found that the early-leafing species intercepted 45–80% of their growing season photon flux before canopy closure. However, it remains unclear whether the phenological difference between seedlings and adults is related directly to tree age, as suggested by Seiwa (1999a,b), or is induced microclimatically by the vertical

thermal gradient occurring from the ground to the canopy, as suggested by Augspurger (2004). This is a crucial knowledge gap, because many phenological studies have been conducted with seedlings or saplings, especially experimental studies manipulating temperature and photoperiod, and, to date, no reasonable proof has been found of whether results from these studies can be scaled to adult trees, in particular, when such data are used to predict forest phenology under continued climate change. In other words, does the phenology of juvenile and adult trees respond similarly to environmental conditions? The lack of experimental comparison between seedlings growing in the understory or in the canopy is obviously a result of the practical difficulties in growing seedlings in tall tree crowns. In this study, I took advantage of a 45-m-high tower crane built in a mixed forest in the foothills of the Jura Mountains to compare the timing of leaf emergence of seedlings grown at both ground level and canopy height, for five deciduous tree species exhibiting contrasting phenology. I aim to answer two main questions: (1) to what extent do juvenile trees differ in their leaf unfolding date compared with conspecific adult trees? (2) Are these differences caused by a distinct contrast in sensitivity to environmental drivers between the two life stages (ontogenic changes) or are they a result of microclimatic variation (environmentally induced developmental shifts)?

Materials and Methods

Study site and study species

The experiment was conducted in a mature mixed forest stand (c. 110 yr old) near the village of Hofstetten (47°28′N, 7°30′E, 570–580 m asl), located 12 km south-west of Basle, Switzerland. Soils are of the rendzina type on calcareous bedrock. The dominant tree species are *Fagus sylvatica* L. and *Picea abies* L. *Acer campestre* L., *Acer pseudoplatanus* L., *Carpinus betulus* L., *Fraxinus excelsior* L., *Prunus avium* L. and *Tilia platyphyllos* Scop. occur as companion species. The site is situated on a north-facing slope with no access to the ground water table and has essentially rocky subsoil at 40–90 cm below the surface. The mean annual air temperature recorded on a long-term series in the nearest climate station was 10.3°C and the mean annual precipitation was 810 mm (1970–2011 recorded at Binningen, 316 m asl, c. 10 km distant from the study site). Using the same temperature dataset, the

mean air temperature over the c. 6-month growing season from April to October was 15.6°C, with a mean temperature for the warmest month (July) of 19.3°C. The winters are mild with the mean temperature of the coldest month (January) being c. 1.5°C. At the study site, there are usually only a few weeks of slight snow cover during mid-winter.

I selected *Prunus avium*, *Tilia platyphyllos*, *Fagus sylvatica*, *Acer pseudoplatanus* and *Fraxinus excelsior* for the experiment, first because they are typical tree species of a central European forest and, second, because they exhibit large differences in their timing of leaf unfolding, with *P. avium* flushing earliest and *F. excelsior* latest. For clarity and brevity, hereafter I refer to each species by its genus.
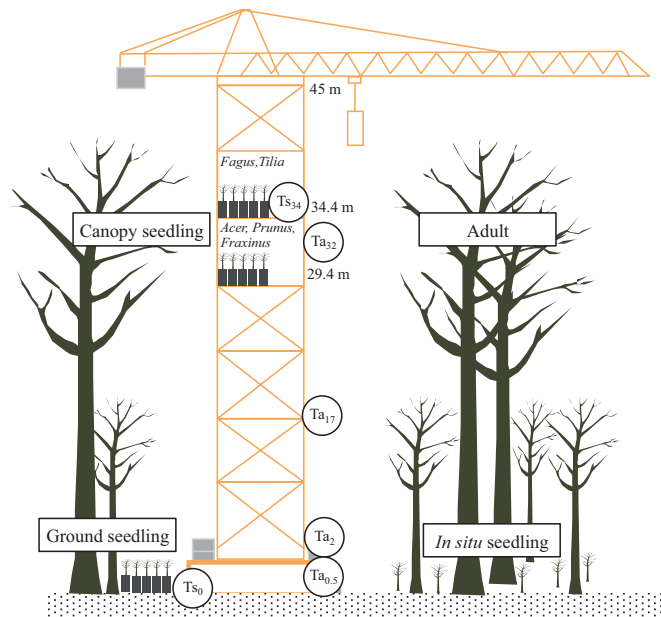
Experimental design

In November 2011, 18 seedlings, 2–5-yr old, were harvested for each study species, within 150 m around the crane, and immediately replanted in square containers (14 cm wide × 23 cm deep) containing 3 l of the local forest soil. Depending on the species, the height of the seedlings ranged between 10 and 26 cm, with a stem diameter from 0.20 to 0.27 cm (Table 1). All 90 containers (18 × 5 species) containing seedlings were then buried slightly in the soil and left in the forest understory over winter. Immediately after snow melt and a substantial cold period (Fig. 2a), on 24 February 2012, half of the containers were moved to two elevated positions in the forest canopy layer using a 45-m-high construction crane built in the forest in 1999 (Pepin & Körner, 2002). Seedlings of the two tallest adult species, *Fagus* and *Tilia*, were placed on a platform at 34.4 m above the ground, whereas *Acer*, *Fraxinus* and *Prunus* were placed on a lower platform, at 29.4 m above the ground (Fig. 1). These seedlings are referred to hereafter as 'canopy seedlings'. The remaining containers were placed in the understory at c. 20 m distant from the base of the crane at ground level. These seedlings are referred to hereafter as 'ground seedlings' (Fig. 1). In both canopy and ground seedling groups, all containers were installed in plastic boxes with drainage holes, and the nine replicates of each species were divided into three subplots.

In winter 2011, I selected and tagged nine mature trees of each of the study species within 150 m around the crane. These adult trees are referred to hereafter as 'adults' (Fig. 1). The average height of the selected trees ranged from 28 to 36 m for *Prunus*

Table 1 Height and diameter of the monitored adult and seedling tree species

Species	Adult		Ground seedling		Canopy seedling		*In situ* seedling	
	Height (m)	dbh (10^{-2} m)	Height (10^{-2} m)	Diameter (10^{-3} m)	Height (10^{-2} m)	Diameter (10^{-3} m)	Height (10^{-2} m)	Diameter (10^{-3} m)
Prunus avium	28.2 ± 2.0	33 ± 4	20.2 ± 2.3	2.7 ± 0.3	26.2 ± 2.1	2.1 ± 0.2	19.9 ± 7.0	2.5 ± 0.5
Tilia platyphyllos	31.9 ± 0.9	55 ± 5	13.4 ± 1.9	2.5 ± 0.3	12.3 ± 2.0	2.3 ± 0.3	40.8 ± 6.0	4.8 ± 0.5
Fagus sylvatica	35.9 ± 1.1	65 ± 3	20.1 ± 1.3	2.2 ± 0.2	18.2 ± 1.3	2.0 ± 0.1	28.8 ± 1.9	2.1 ± 0.1
Acer pseudoplatanus	29.6 ± 0.5	44 ± 3	10.5 ± 0.6	2.4 ± 0.1	11.5 ± 1.3	2.4 ± 0.2	29.0 ± 3.3	3.3 ± 0.2
Fraxinus excelsior	29.2 ± 1.9	43 ± 5	9.9 ± 1.6	2.0 ± 0.3	12.7 ± 2.0	2.4 ± 0.2	30.0 ± 4.1	3.5 ± 0.4

dbh, diameter at breast height.

Fig. 1 The experimental design used to compare seedling and adult tree phenology. Ta_{32}, Ta_{17}, Ta_2 and $Ta_{0.5}$ correspond to the air temperature recorded at 32, 17, 2 and 0.5 m height during the experiment. Ts_{34} and Ts_0 correspond to the soil temperature recorded in the container at 10 cm depth for canopy seedlings and ground seedlings, respectively.

and *Fagus*, respectively, matching closely the height of the platforms used inside the crane tower to position the seedlings (Table 1). To avoid any water limitation during bud burst, all the containers were watered when necessary (four times in March and twice in April). In early spring 2012, nine *in situ* seedlings for each species were selected and tagged within 150 m around the crane. These latter seedlings, called hereafter '*in situ* seedlings', were used for comparison with the ground seedling group as a control of the potential effect of transplantation, as well as to provide the natural pattern of leaf emergence timing between understory and canopy trees.

Vertical microclimate

Air temperature was recorded hourly using data loggers (TidBit v2 UTBI-001, Onset Computer Corporation, Bourne, MA, USA) at four different heights above ground level (Fig. 1). Two loggers were placed in the understory: at 0.5 m (approximately seedling height, referred to hereafter as $Ta_{0.5}$) and 2 m (Ta_2) above the ground. The two other loggers were mounted to the north side of the crane tower at *c.* 17 m (Ta_{17}) and *c.* 32 m (approximately canopy height, Ta_{32}; Fig. 1) height. All loggers were positioned under a white double-layered, aerated plastic shelter to prevent any exposure to rain or to direct sunlight. Soil temperature was also recorded hourly in dummy containers at −10 cm depth, both at ground level (Ts_0) and at canopy height (Ts_{34}, Fig. 1). To compare cumulative degree hours among the different loggers recording air temperature, I accumulated the temperature difference for every hour above a 5°C threshold, starting on 24 February 2012 (when containers were placed at the canopy height) until the end of the flushing period (mid-May

2012). Before and after the study period, all loggers were immersed for 24 h in an ice–water bath for 0°C calibration and cross-checking of the sensors for identical readings. During the calibration, deviations did not exceed 0.11°C among the different loggers, meeting the manufacturer's specifications.

Phenological observations

For each group of seedlings (i.e. *in situ*, ground and canopy seedlings) and adult trees, I monitored bud development twice a week from the end of February to the end of May 2012. I used a categorical scale from '0' (no bud activity) to '4' (leaves out and flat). At stage 1, buds were swollen and/or elongating; at stage 2, buds were open and leaves were partially visible; at stage 3, leaves had fully emerged from the buds, but were still folded, crinkled or pendant, depending on the species; at stage 4, at least one leaf was fully unfolded. For seedlings, I considered the apical bud only and the leaf unfolding date was reached for each seedling when the apical bud reached stage 3, which was estimated by linear interpolation when necessary (i.e. when this stage occurred in between two monitoring dates). Some seedlings were damaged by mice during the winter of 2010 and were not included in the analyses (10 individuals in total, reducing the number of replicates to seven for *Tilia* and eight for the other species for both canopy and ground seedlings). For the selected adult trees, I considered the bulk of the foliage and assessed the proportion of the buds having reached the most advanced phenological stage. Observations were made using binoculars (magnifying power: 10×30 with Image Stabilization technology) at a distance of *c.* 15 m from each selected tree. Then, in order to minimize bias in comparing the phenology of seedlings and adults, I converted the continuous phenological scores recorded for adults into a categorical scale as follows: each phenological score from '1' to '4' was considered to be reached by a given adult tree when at least 10% of the buds were at the corresponding stage. For each adult tree, the date of leaf unfolding was then considered to be reached when the individual was noted to be at stage 3 (10% of the buds had fulfilled stage 3). This date was estimated by linear interpolation when necessary.

Data analysis

A two-way analysis of variance was performed to compare the date of leaf unfolding among species and experimental groups (i.e. ground seedling, canopy seedling, *in situ* seedling and adult). All factors were treated as fixed factors. Species was considered as a fixed effect because the species were specifically chosen for their differences in the timing of flushing (early-, intermediate- and late-flushing species). Before analysis, the data were log-transformed to homogenize variances and meet the assumption of normality, as recommended for continuous positive data measured on an interval scale (Keene, 1995). Within species, the mean leaf unfolding date (corresponding to stage 3) was compared among the four experimental groups using Tukey's honestly significant difference (HSD) test. All statistical analyses were performed using the software JMP 5.0.1.2 (SAS Institute, Cary, NC, USA).

Results

Vertical temperature profile

The cumulative degree hours above 5°C during early spring indicated that the air temperature became gradually warmer from the ground level to the canopy layer (Fig. 2b). For instance, on 1 April 2012, that is, *c.* 1 month after the beginning of the experiment, the cumulative degree hours reached 3341°C h at 32 m height, 3097°C h at 17 m height, 2696°C h at 2 m height and 2539°C h at 0.5 m in the understory (Fig. 2b). From the beginning of the experiment to the end of the flushing period (24 February to 20 May 2012), the air temperature at the canopy layer was 0.5, 1 and 0.8°C warmer than the air temperature at 0.5 m height for daily minimum, mean and maximum, respectively, and the soil temperature was, on average, 1.4°C warmer in containers of canopy seedlings than in containers of ground seedlings (Fig. 2b).
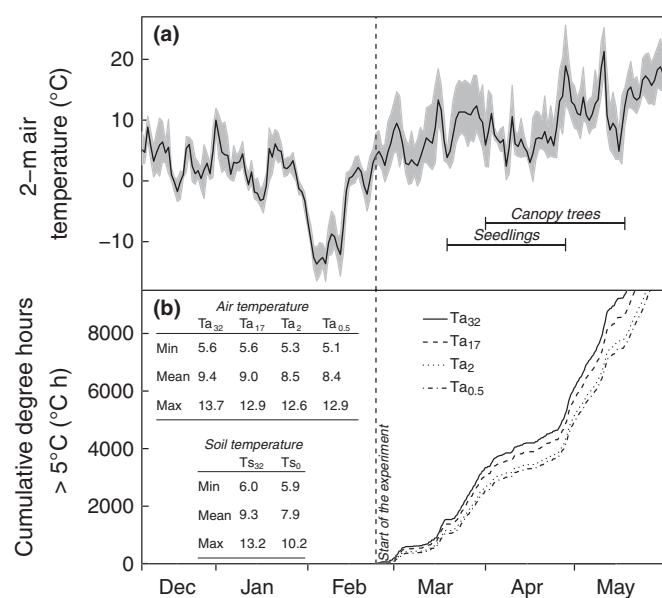
Phenological differences among species

As expected, the five study species exhibited contrasted timing of bud development (Table 2, Fig. 3). Interestingly, they exhibited the same ranking among the three seedling categories, but

Fig. 2 (a) Two-meter air temperature recorded from mid-December to the end of the flushing period. The thick line corresponds to the daily mean temperatures surrounded by a gray area denoting the daily minimum and daily maximum temperatures. The flushing period of canopy trees and *in situ* seedlings is indicated within the diagram and corresponds to the period from the first individual to flush until the last individual flushed. (b) Cumulative degree hours above 5°C from the beginning of the experiment (24 February 2012) to the date of the last individual flushing (mid-May 2012) in the understory at 0.5 and 0.2 m, at subcanopy level (*c.* 17 m height in the crane tower) and at canopy height (*c.* 32 m height in the crane tower). The inset table shows the mean of the daily minimum, mean and maximum air temperatures during the same period for each of the four height levels: 0.5 m ($Ta_{0.5}$), 2 m (Ta_2), 17 m (Ta_{17}) and 32 m (Ta_{32}), together with the soil temperature recording during the same period in the containers of seedlings grown in the understory (Ts_0) and at canopy height (Ts_{34}).
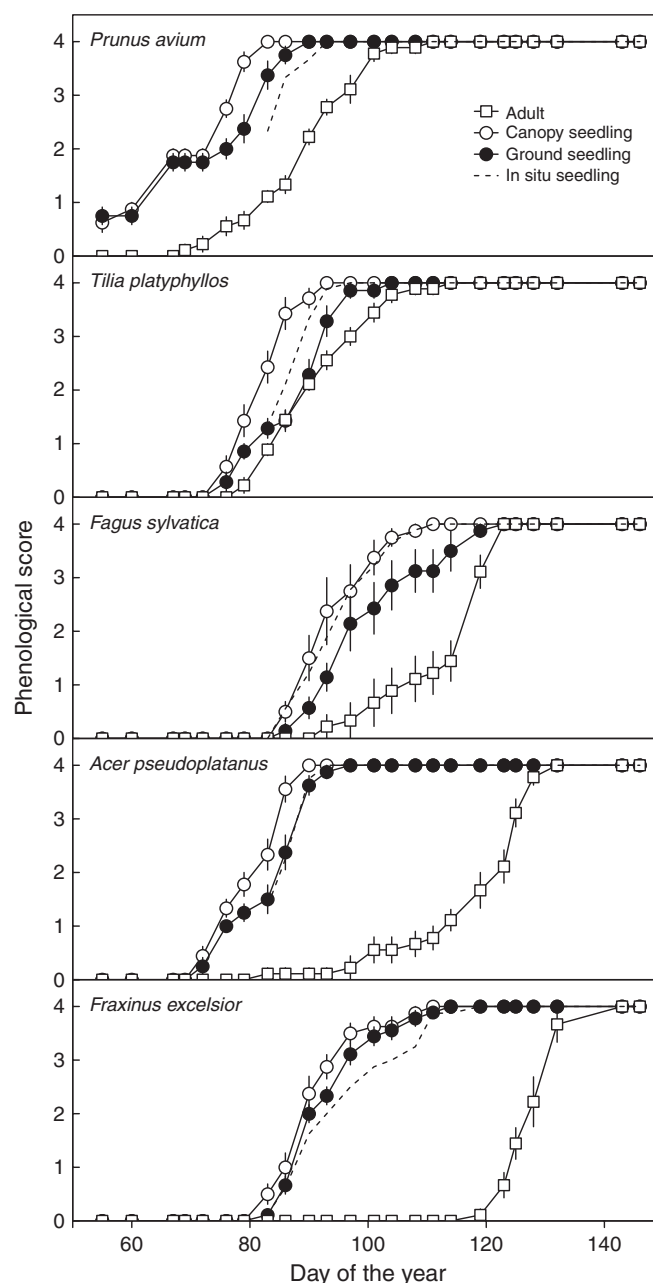
Table 2 Summary of the analysis of variance performed on the date of leaf unfolding across species and experimental groups (i.e. adult, ground seedling, canopy seedling and *in situ* seedling)

	df	F	P value
Species	4	149.1	<0.0001
Experimental groups	3	282.0	<0.0001
Species × experimental groups	12	20.0	<0.0001

F values and significance are provided. df, degree of freedom; *F*, *F* values from Fisher's test.

Fig. 3 The progress of bud and leaf development in spring for the two experimental groups of seedlings in containers (i.e. seedlings grown in the understory, 'Ground seedling', and at canopy height, 'Canopy seedling'), seedlings monitored *in situ* ('*In situ* seedling') and adult canopy trees ('Adult'). Bars represent standard errors.

different ranking between the seedling and adult categories (Figs 3, 4). For instance, although *Acer* was ranked as the second latest species to flush in the adult category, it was ranked as the second earliest species to flush in the seedling category (Fig. 4). The mean discrepancy between the leaf unfolding date of the earliest and the latest species was also greater within adult trees (*c.* 36 d between *Fraxinus* and *Prunus*) than within seedling categories (15–24 d between *Fagus* and *Prunus*).

Phenological differences among the four experimental groups

No significant difference was detected in the date of leaf unfolding between *in situ* and ground seedlings in any of the five tree species, indicating a negligible effect of transplantation (Fig. 4). For all species, the date of leaf unfolding in adult trees occurred remarkably later than in all the three categories of seedlings (Figs 3, 4). The comparison between the phenology of canopy seedling and adult permits a comparison of life stage effects under otherwise similar environmental conditions. For all species, adult trees flushed significantly later than seedlings grown at canopy height, on average 25 d later, with substantial differences among species: 10 d in *Tilia*, between 17 and 20 d in *Prunus* and *Fagus*, and >37 d in *Fraxinus* and *Acer* (Fig. 4). *In situ* seedlings also flushed 6–36 d earlier than adult trees (Fig. 4). The comparison between canopy and ground seedlings provides the opportunity to test the potential effect of microclimate induced by the vertical temperature gradient. In all studied species, the date of leaf unfolding was systematically earlier in canopy than in ground seedlings (mean of 5.5 d, Figs 3, 4). Within species, this discrepancy was significant for *Prunus*, *Tilia* and *Acer*, but not for *Fagus* and *Fraxinus*, which are also the two latest species in seedling categories (Fig. 4).

Discussion

This study revealed that seedlings of temperate tree species have an earlier spring phenology than conspecific canopy trees, and this discrepancy was enhanced when they were moved up to the canopy height. The phenological discrepancy was remarkable, as it reached up to more than a month depending on species. Thus, I invalidated the hypothesis that seedlings exhibit earlier phenology as a result of a vertical temperature gradient, but rather demonstrated that the difference in phenology can only be caused by ontogenic differences between seedlings and adults. Here, I observed earlier flushing in seedlings grown at canopy height relative to those grown at ground level (on average 5.5 d earlier). Augspurger (2004) found the opposite pattern in *Aesculus glabra* when moving seedlings to the top of a barn roof (7 m height), and suggested that the earlier flushing of the understory relative to conspecific canopy trees may result from the vertical temperature gradient occurring from the ground to the canopy. To my knowledge, this is the only study in which seedlings have been translocated to an elevated position, although this upward shift was not sufficient to reach the canopy height (20–25 m). However, the results found here are not inconsistent with the results
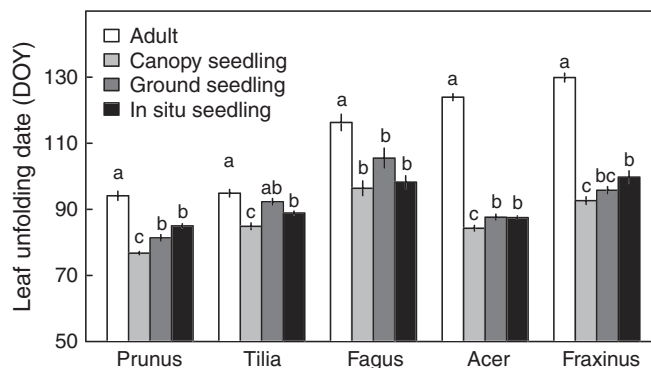
Fig. 4 Leaf unfolding date (based on the phenological score '3') of the four experimental groups (i.e. Ground seedling, Canopy seedling, *In situ* seedling and Adult) for the five examined tree species (*Fagus sylvatica*, *Acer pseudoplatanus*, *Fraxinus excelsior*, *Prunus avium* and *Tilia platyphyllos*). Different letters within species among the experimental groups indicate significant differences in the leaf unfolding dates (Tukey's honestly significant difference (HSD) test). Bars represent standard errors. DOY, day of the year.

obtained from the previous study as, in both cases, the vertical temperature profile may explain the phenological shift found between seedlings grown at ground level and those grown in an upper vertical position: in the previous study, temperature was recorded as 0.4–0.7°C colder above the roof than in the understory, delaying the phenology of the seedlings growing above the roof, whereas, in the present study, the temperature recorded at canopy layer was, on average, 1°C warmer than in the understory, resulting in a slightly earlier flushing of seedlings grown at canopy height relative to seedlings grown in the understory. The vertical temperature gradient within forests is expected to increase from understory to crown area during periods of strong positive net radiation (in spring and summer) and to decrease in winter when the radiation balance is negative, although this pattern is strongly related to different factors, such as slope exposure, stand density and species composition of the canopy (Geiger *et al.*, 2003). Here, I found that the mean, minimum and maximum temperatures increased gradually from ground to canopy height irrespective of the season, but this effect was more pronounced in spring and summer (data not shown). A similar vertical temperature profile has also been reported in mixed conifer forest in Sierra Nevada (Rambo & North, 2009). The vertical temperature pattern found here in the mixed study forest might partly result from the topography of the site (north-facing slope), which makes the incoming radiation heat the top of the trees earlier in the morning than it does for the understory environment. The phenological shift of −3 to −9 d found here for a *c.* 1°C difference in mean spring temperature is similar to that found in previous studies, which observed a phenological shift in seedlings of *Fagus sylvatica*, *Acer pseudoplatanus*, *Prunus avium*, *Fraxinus excelsior* and *Quercus petraea* ranging from −2.4 to −6.3 d per degree of warming in early spring (Vitasse *et al.*, 2010, 2013).

Earlier flushing in seedlings and saplings has been interpreted as an opportunistic strategy to capture sufficient light for growth before canopy closure. Overall, my results support the hypothesis of a shift in timing of leaf emergence with increasing tree age in

order to enhance performance throughout the life cycle of the tree (Seiwa, 1999a). Although risky in relation to late spring frosts, the advanced ontogenesis allows seedlings and saplings to start their development in optimal light conditions before canopy closure, enhancing their growth and, subsequently, their competitive ability (Gill *et al.*, 1998; Augspurger, 2008; Lopez *et al.*, 2008). However, later bud burst in adults reduces the likelihood of frost damage, particularly in reproductive organs, thus exerting a fitness benefit. Accordingly, my results highlight a remarkably large phenological difference between seedlings and adults within species, probably enhanced by a warm period in early spring (end of March) allowing seedlings to flush, followed by a cooler period in the first half of April, slowing the bud development of adults (the last 2 wk of March were, on average, 3°C warmer than the first 2 wk of April, Fig. 2a). Further investigations are necessary to examine whether there is only an absolute difference between phenology of understory and conspecific canopy trees or, more likely, whether they also differ in their phenological response to the main environmental factors regulating bud burst of temperate tree species, that is, air temperature, and, for some late successional species, a combination of temperature and photoperiod (Körner & Basler, 2010; Polgar & Primack, 2011). Yet, the second hypothesis seems to be more plausible as, here, I found that the ranking of the date of leaf emergence among species was similar within the three categories of seedling, but different in the adult group. This suggests that the dormancy cycle of the buds may be affected by the age of the plant (Cooke *et al.*, 2012). In temperate tree species, the thermal time to bud burst is known to decrease with increased duration of previous chilling, down to a minimal thermal time when buds are considered to be 'fully chilled' (Murray *et al.*, 1989; Falusi & Calamassi, 1996; Cannell, 1997; Hannerz *et al.*, 2003). This relationship has been documented for three of the five tree species selected in this study, namely *P. avium*, *T. platyphyllos* and *F. sylvatica* (Lyr *et al.*, 1970; Murray *et al.*, 1989), with *P. avium* probably having the lowest requirement of chilling for a full rest completion, whereas *F. sylvatica* is known to have a high requirement of chilling for dormancy release as well as a photoperiodic control (reviewed in Vitasse & Basler, 2013). In our study, the vertical gradient of temperature from the ground to the canopy might have led to different fulfilment of chilling requirement between understory and canopy trees. Yet, this is unlikely, because the vertical profile of temperature found here was less pronounced in winter during endodormancy than in early spring during the experiment (0.37°C warmer at the canopy during December 2011–January 2012, data not shown). Furthermore, in the study site (north-facing slope at *c.* 580 m asl), the amount of chilling was likely to exceed the chilling requirement of the study species to fully release bud dormancy (see air temperature during winter in Fig. 2). More likely, seedlings may have a shallower bud dormancy than mature trees, resulting in a lower requirement of chilling for dormancy release and/or a lesser interaction with the increase in photoperiod in early spring for photoperiod-sensitive tree species, and so could flush earlier in response to a temperature increase in spring. In other words, bud rest completion is likely to occur later for adults than for juvenile trees, as suggested by Hanninen *et al.* (2007).

As, in natural conditions, mature trees may currently not experience sufficient unusual climatic events (such as a lack of chilling in winter) to accurately calibrate phenological models for future predictions, there is a renewed interest in the performance of experimental studies to improve the calibration of the parameters of such models by manipulating chilling and forcing temperatures in controlled growth chambers (e.g. Harrington *et al.*, 2010; Morin *et al.*, 2010; Fu *et al.*, 2012), or even photoperiod, as future climate is expected to be warmer at a shorter photoperiod (Caffarra *et al.*, 2011a,b; Basler & Körner, 2012). For obvious practical reasons, these studies are conducted on juvenile trees or cuttings (but see Hanninen *et al.*, 2007). The results obtained from these studies may therefore not be valuable for the inference of model parameters for the prediction of forest phenology in the future, because of the strong ontogenic effects operating on tree phenology, as demonstrated by the present study. Further investigations need to be performed on cuttings and rooted cuttings obtained from adult trees to examine whether their phenology responds similarly to environmental conditions as adult trees, and therefore whether they can be used as a substitute for mature trees in warming and photoperiod experiments to infer the phenological responses of forests to climate change (Basler & Körner, 2012).

Conclusion

The present study aimed to remind ecologists that all basic knowledge on the role of chilling, forcing temperatures and photoperiod on the timing of bud burst of temperate tree species, to a large extent, has been gained from juvenile trees. Because of the strong ontogenic effects acting on phenology, as demonstrated here, this knowledge may not be valuable for the inference of the phenological responses of forests to climate change. However, investigations of the phenological responses to climate change of both seedling and adult life stages are relevant to assess the impact of climate change on the fitness and growth of tree populations. On the one hand, seedling phenology may be crucial in the survival of a given population, as seedlings appear to be more opportunistic to track spring temperatures and therefore may be more exposed to late frost events in the future. On the other hand, shifts in adult phenology affect considerably tree growth and competitive abilities, which may change among tree species growing in mixed forest stands as the climate becomes warmer. I recommend that particular caution should be taken when phenological models are calibrated on juvenile trees rather than on adults to predict forest phenology under continued climate warming.

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