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Asymmetric effects of cooler and warmer winters on beech phenology last beyond spring

Running head: Temperature legacy effect on tree phenology

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Abstract

In temperate trees, the timings of plant growth onset and cessation affect biogeochemical cycles, water and energy balance. Currently, phenological studies largely focus on specific phenophases and on their responses to warming. How differently spring phenology responds to the warming and cooling, and affects the subsequent phases, has not been yet investigated in trees. Here, we exposed saplings of *Fagus sylvatica* L. to warmer and cooler climate during the winter 2013–2014 by conducting a reciprocal transplant experiment between two elevations (1340 vs. 371 m a.s.l., ca. 6 °C difference) in the Swiss Jura mountains. To test the legacy effects of earlier or later budburst on the budset timing, saplings were moved back to their original elevation shortly after the occurrence of budburst in spring 2014. One degree decrease of air temperature in winter/spring resulted in a delay of 10.9 days in budburst dates, whereas one degree of warming advanced the date by 8.8 days. Interestingly, we also found an asymmetric effect of the warmer winter vs. cooler winter on the budset timing in late summer. Budset of saplings that experienced a cooler winter was delayed by 31 days compared to the control, whereas it was delayed by only 10 days in saplings that experienced a warmer winter. Budburst timing in 2015 was not significantly impacted by the artificial advance or delay of the budburst timing in 2014, indicating that the legacy effects of the different phenophases might be reset during each winter. Adapting phenological models to the whole annual phenological cycle, and considering the different response to cooling and warming, would improve predictions of tree phenology under future climate warming conditions.

Introduction

Phenology of temperate and boreal trees plays a crucial role in ecosystems structure and functioning (Fu *et al.*, 2016). An advance or a delay in the beginning or/and end of tree growth may considerably impact biogeochemical cycles and therefore feedback on the global earth's climate (Cleland *et al.*, 2007; Peñuelas *et al.*, 2009; Richardson *et al.*, 2013; IPCC, 2014; Ma *et al.*, 2016; Wolf *et al.*, 2016). For instance, increasing temperatures have extended the growing season of temperate and boreal trees and resulted in a larger carbon uptake (Keenan *et al.*, 2014). Earlier leaf-out as a result of warmer spring may even compensate for the carbon loss occurring in more frequent dry summers (Wolf *et al.*, 2016). In addition, since phenological response to temperature and photoperiod was shown to highly

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differ among coexisting species, climate change will likely affect the competition among species (Vitasse *et al.*, 2011) and ultimately lead to shifts in species distributions (Chuine, 2010). Long-term phenological observations and experiments showed that spring tree phenology does not only depends on warm temperatures in spring because chilling temperatures during winter control the dormancy release, so that spring phenophases respond in a non-linear manner to temperature increase (Morin *et al.*, 2010; Fu *et al.*, 2015b). While much attention has been paid to the effect of warming on trees, tree phenological responses to cooling have got little attention in the scientific literature (but see a recent study conducted on alpine meadows Li *et al.*, 2016). In particular, the advance of spring phenophases in response to global warming may put leaves in colder conditions that in turn may affect the subsequent phenophases. Understanding how plant phenology will respond to both warmer and cooler conditions is thus essential to predict the overall effect of ongoing climate change on plants.

Numerous studies have reported significant phenological shifts worldwide in response to ongoing climate warming (Menzel & Fabian, 1999; Menzel *et al.*, 2006; Fu *et al.*, 2014a). Over the period 1980–2012, the dates of spring leaf phenology have advanced by 6 to 19 days in Europe and North America, respectively (Fu *et al.*, 2015a). But warmer climate may not necessarily induce earlier leaf-out in spring because temperature has a dual role that affects both dormancy release in winter and the initiation of bud development in spring (Chuine, 2010; Harrington & Gould, 2015). It has been recently reported that warmer temperature recorded during the last two decades has resulted in an increase in forcing requirement for budburst of temperate trees in Europe, which is likely the result of insufficient chilling requirement for a full dormancy release or an increasing photoperiodic limitation (Fu *et al.*, 2015b). For example, for *Fagus sylvatica L.* (European beech), photoperiod plays an important role in regulating the timing of budburst, which then limits the extent of the phenological response to global warming (Vitasse & Basler, 2013; Basler & Körner, 2014). The complex interaction between chilling, forcing temperatures and photoperiod involved in bud dormancy is probably the reason why numerous questions remain open to predict future spring phenology. For instance, there is no agreement on how the expected decrease of chilling in winter will affect the budburst timing in the coming decades or which range of chilling temperatures are actually efficient to break the bud dormancy. In contrast to spring phenology, bud set and leaf senescence in temperate trees have received fewer attention and the environmental cues that influence autumn senescence

are still poorly understood (Gallinat *et al.*, 2015; Panchen *et al.*, 2015). Photoperiod and temperature are considered as the main drivers of growth cessation and leaf senescence (Delpierre *et al.*, 2016), but precipitation and nutrient availability may also play a non-negligible role (Panchen *et al.*, 2015; Liu *et al.*, 2016).

Most of the studies focus on a single phenophase and therefore do not consider any potential effect of one phenophase to the other phenophases occurring within the same growing season (Delpierre *et al.*, 2016) or even from one year to the next one, i.e. the so-called carryover effect. Yet, growing evidences show that the different phenophases are not independent from each other (Fu *et al.*, 2014b; Keenan & Richardson, 2015; Delpierre *et al.*, 2016). Based on a manipulative warming experiment, Fu *et al.* (2014c) found a carryover effect of spring over autumn phenology or even to the next year spring phenology. Considering that most experiments have been performed in climate chambers by manipulating temperature and photoperiod (Sherry *et al.*, 2007; Basler & Korner, 2012; Chung *et al.*, 2013; Fu *et al.*, 2013; 2014b), experiments conducted in natural conditions could provide precious insights to further explore these mechanisms and improve our understanding of the potential carryover effect of one phenophase to the next one.

In this study, we conducted a reciprocal transplant experiment between two elevations (1340 m a.s.l. vs. 371 m a.s.l.) in the Swiss Jura mountains using saplings of *Fagus sylvatica* L. The upward and downward translocation allowed us to simulate cooler or warmer temperatures during winter and early spring. Then, the saplings were moved back to their original elevations shortly after budburst in order to test the legacy effects of the budburst timing on the timing of budset in autumn and next year budburst. More specifically, we expect (i) an impact of warmer- and cooler-induced winter on autumn phenology through shifts in the beginning of growth onset, (ii) a stronger effect of a cooler winter than a warmer winter both on spring phenology and on the legacy effects for autumn phenology, (iii) a carryover effect of autumn phenology on next year spring phenology.

Materials and methods

Study sites and experimental design

The experiment was conducted at two sites, at low and high elevation in the Swiss Jura mountains, in order to simulate warmer (transplant from the higher site to the lower site) and cooler (transplant from lower to higher site) conditions. The high elevation site (Alpage des Amburnex: N46°54', E6°23') was located at 1340 m a.s.l. and has a mean annual rainfall of ca. 1450 mm and a mean annual temperature of 5.5°C (2009 – 2015). The low elevation site (Bois Chamblard: N46°27', E6°24') was situated at 371m a.s.l. and has a mean annual rainfall of 1180 mm with a mean annual temperature of 11.4 °C (2009–2015). Hereafter, these two sites are referred to as “high site” and “low site” according to their elevation.

The figure 1 represents the configuration of the experiment at different time of the experiment that took place from autumn 2013 to spring 2015. In early September 2013, 60 six years old saplings of *Fagus sylvatica* L. (European beech) were collected in the vicinity of the two study sites, referred hereafter as the donor forests (Fig. 1a), with 30 saplings from each donor forest. The saplings were selected for having similar height and morphology (diameter of 7 to 9 mm and height of 70 to 80 cm). When the plants were collected, we paid particular attention not to disturb the roots of the saplings by taking as much as possible untouched soil around the roots, in order to minimize transplantation stress. These saplings with their clods of about 30 cm diameter and 30 cm depth were then planted in pots (22 liters, 35 cm of diameter and 40 cm depth) filled with their local soil. The transplant experiment (Fig. 1b) was conducted on the week from the 23rd to 27th of September 2013, as follow: at the low site, 15 out of the 30 potted saplings served as a control for the cooling treatment and were kept at the low site in a protected common garden (low site common garden) during the whole experiment. They are referred hereafter as *control cooling* (CC). The other 15 saplings were moved to the high site in another protected common garden (high site common garden) to experience a cooler climate and are referred hereafter as *cooling* (C). Similarly, at the high site, 15 out of the 30 potted saplings served as a control for the warming treatment and were kept at the high site common garden during the whole experiment. They are referred hereafter as *control warming* (CW). The other 15 individuals were moved to the low site common garden to experience a warmer climate and are referred hereafter as *warming* (W). All pots were placed in an open area near the donor forest and buried into the ground, so that the top

of the pots was about the soil surface. We monitored bud development in spring 2014, and approximately two weeks after budburst for each individual tree separately, they were transplanted back to their original site. The timing of two weeks allowed the leaves of each sapling to reach a minimum of maturation to increase their freezing resistance once back at its original site, especially important for the saplings going back to the high elevation site. By that time, the tree reached the phenological stage 4 (fully unfolded leaf) but the shoot elongation was only at its beginning. At the end, all the potted trees were moved back to their original site from April 20th to May 9th and from May 29th to June 7th at the low and high elevation sites, respectively (Fig. 1c). With this procedure, treatments and controls were under the same environmental conditions during the growing season 2014 and only differed in their budburst timing and previous winter conditions. It is also noteworthy that the elevational gradient allowed to induce contrasting temperature regime (mean difference of 4.2°C for the period from October 2013 to April 2014 between the two sites), while keeping similar day length between the two sites. Saplings only received water from natural rainfall. Except three individuals at the low site, all individuals survived during the whole experimental period up to spring 2015.

Phenological observations

Bud development was monitored during springs 2014 and 2015 using 5 distinct stages: dormant bud (0), swollen bud (1), budburst (2), leaves folded in a single plan (3) and at least one leaf fully unfolded (4), as commonly used for deciduous trees (e.g. Vitasse *et al.*, 2014b). The observations were conducted on the apical bud of each sapling in each site by the same observer three times a week from March 24th 2014 until all saplings reached the stage 4. In summer/autumn 2014, while all transplanted saplings were back in their site of origin, we monitored budset on the shoot apical meristem three times a week using 4 different stages: ongoing leaf development (0), new formed green and soft bud (1), small and brown bud (2), elongated (> 1cm) and brown bud (3). Budset timing was defined when a given sapling reached the stage 3, which was taken as a proxy for the cessation of the primary growth. Leaf coloration and leaf fall were also monitored but we chose to use only bud set data in this study as a proxy for growth cessation. The growing season length for each sapling was defined as the time in days between budburst and bud set.

Climatic data

Relative air humidity, soil and air temperature were recorded hourly in each site using EM50 data-loggers (Decagon Devices, Inc., Pullman, WA, USA). Air temperature sensors (VP3, Decagon Devices, Inc., Pullman, WA, USA) were placed at the average height of the saplings (i.e. 60 cm from the ground). Note that the high elevation site was characterized by a snow cover up to ca. 1 meter from end of December to mid-March. Thus, saplings at the high site were covered by snow during some period in winter and may have experienced cooler temperatures than a few meters aloft. However, our temperature logger positioned at the tree canopy sapling capture the temperature as experienced by buds. Although numerous studies consider chilling temperatures to be comprised between 0 and +5 °C, we considered temperatures between -1 and +5 °C as efficient for chilling, because when the saplings of the high site were covered by snow air temperature was slightly below 0 °C and we assumed the temperature induced by snow to contribute to the chilling requirement. In order to assess the requirement of chilling and forcing temperatures for budburst, we calculated chilling hours as follows: we cumulated the chilling hours received from 1 November until budburst both in 2014 and in 2015. To evaluate the forcing temperature requirement to budburst, we calculated the number of hours above 5°C from 1st of February to the budburst date both 2014 and 2015.

Growth and bud morphology

For each sapling, we measured the stem apical diameter (mm), the stem basal diameter at 3 cm from the ground (mm), and the stem length (cm). The four longest branches were labelled by colored strings, and their diameter and length were also measured. All growth parameters were assessed at the beginning and at the end of the growing season 2014. Additional measurements of the length and diameter of 5 buds were performed for each sapling including the apical and four surrounded buds at the end of the growing season 2014, as well as the total number of buds per saplings. The diameter of the buds was measured at one-third of the bud length from the base. All diameter, as well as bud length measurements were done with a digital caliper (MarCal 16 EWR, accuracy 0.01 mm) while the stem and main branches length were measured with a tape (accuracy 0.1 cm).

We calculated the volume of the stem and branches assuming a truncated cone shape:

$$V_i = \pi * \frac{D^2 + D * d + d^2}{12} (mm^3)$$

where V is the volume of the stem or branch *i*, “D” is the basal diameter of the stem and “d” is the apical diameter of the stem.

The total volume of one individual sapling was then calculated as the sum of the volumes of the stem and the four main branches.

$$V_{tot} = V_{Stem} + V_{Branch1} + V_{Branch2} + V_{Branch3} + V_{Branch4} (mm^3)$$

The increase in the stem length and total volume was calculated as the difference between measurements conducted at the end (EGS) and at the beginning (BGS) of the growing season.

$$\Delta V = V_{EGS-2014} - V_{BGS-2014} (mm^3)$$

$$\Delta SL = SL_{EGS-2014} - SL_{BGS-2014} (mm)$$

where “ ΔV ” is the total volume increase and “ ΔSL ” is the stem length increment.

Non-Structural Carbohydrates (NSC) analysis

The NSC are defined as low molecular weight sugars (glucose, fructose and sucrose) plus starch. In November 2014, when leaves of all saplings were senescent (i.e. either colored or fallen), we collected one woody twig of 6 cm from each sapling (i.e. 58 samples) corresponding to the tissue produced in 2014. Within 2 hours after collection, the samples were heated at the laboratory in a microwave oven at 800 W for three times 25 seconds, allowing to denature enzymes and stop microbial activity. Samples were then immediately dried to constant weight at 60 °C. NSC was analyzed as described in Hoch *et al.* (2002) using an enzymatic digest technique with subsequent spectrophometric glucose tests (Li *et al.*, 2002). Samples were boiled in distilled water and after centrifugation, treated with invertase and isomerase to convert fructose and sucrose into glucose. Glucose was converted to gluconate-6-phosphate using a Hexosekinase reaction kit (Sigma Diagnostics, St. Louis, Mo., USA). The insoluble material was kept at 40°C with the dialyzed crude enzyme clarase from

Aspergillus oryzae to break down starch to glucose. Starch and sugar standards as well as laboratory standard of plant powder were used as controls for analyses. Finally, sugar, starch and NSC concentrations (%) were calculated on a dry matter basis.

Data analysis

The experiment followed a one factor design with repeated measurements. The studied factor consisted in 4 treatments: C = cooling, CC= control cooling, W = warming, CW = control warming. The experimental unit was a pot with a single individual, which is also the observational unit. For the analysis of budburst, chilling and forcing hours, each plant was observed twice, on year 1 (2014) and on year 2 (2015), while bud set was observed only during year 1. Year is therefore a within factor and is considered in the analysis as repeated measurement. The design was slightly unbalanced due to some mortality in the warming treatment: treatments C and CC with n = 15 individuals, treatment CW with n = 12 and treatment W with n = 14 individuals. An analysis of variance was performed for testing the treatment effect (either cooling or warming) on budburst and bud set timing, on chilling and forcing hours requirement, as well as on NSC content. Tukey tests adapted for repeated measurement designs were used to compare the statistical differences among the treatments. The relation between chilling and forcing was analyzed with linear regressions, both through the means of the different treatments (n = 4) over the two years 2014 and 2015 and through the individual sapling data. Growth and bud morphology parameters were analyzed using an analysis of variance at one factor for both treatments separately.

All analyses were performed using R 2.5.3 (R Core Team, 2013) using the R-package 'easyanova' (Arnhold, 2013) to perform the Anova and calculate the Tukey tests.

Results

Budburst in spring 2014 after a cooling or a warming winter

The mean budburst date in the cooling treatment (C; saplings transplanted to the high site) was significantly later than in the control treatment (CC; saplings kept at the low site), with a mean delay of 46 ± 2 days (Fig. 2a). Likewise, the mean budburst date for saplings in the

warming treatment (W; saplings transplanted to low site) in 2014 was significantly advanced (mean 37 ± 2 days) compared to the control warming (CW; saplings kept at the high site) (Fig. 2a). No difference was detected in the budburst timing of saplings at the high elevation site between the control warming and the cooling treatment, whereas at the low site budburst occurred 8 days earlier in the control cooling than in the warming treatment ($p < 0.001$).

The magnitude of the impact of warming and cooling on the timing of budburst was significantly different. Given the temperature difference of 4.2 °C (see first section of Material and Methods) between the two sites during the reciprocal transplantation, i.e. from October 2013 to April 2014, the cooling treatment showed a delay of 10.9 days per degree decrease of air temperature measured at sapling height, whereas the warming treatment showed an advance of 8.8 days per degree increase of air temperature (Fig. 2a).

Although saplings under cooling control (CC) and warming treatments (W) at the low site experienced similar duration of chilling, i.e. 2051 and 2055 hours respectively, from 1st of November to budburst (Fig. 2b), saplings under warming treatment required 189 additional forcing hours to budburst as compared to saplings under the cooling control ($p < 0.001$; Fig. 2b). By contrast, saplings of the cooling treatment (C) and warming control (WC) at the high site required almost the same duration of forcing hours to budburst, i.e. 560 and 566 hours, respectively (Fig. 2b).

Carryover effects of the timing of budburst on budset, and next year budburst

The cooling treatment in winter 2013-2014 induced a significant delay of 31 days in bud set in autumn 2014 ($p < 0.001$; Fig. 3a) while the warming treatment induced an advance of 10 days in bud set compared to the control, but not significant (Fig. 3b). Interestingly, we observed the same trend on the leaf senescence date as no effect of treatment, warming or cooling, was found on the time lag between bud set to leaf senescence at each site (Table 1). Nevertheless, the two populations showed differences in their time lag, with 27 to 33 days for the control cooling (CC) and the cooling treatments (C), and 2 to 6 days for the control warming (CW) and the warming treatment (W), respectively. In details, we found that 10

days delay in spring budburst induced by the cooling treatment was associated with 6.7 days delay in the timing of budset in autumn compared to the control, whereas no significant change of the budset timing was observed in the warming treatment with 10 days advance in budburst associated to -2.7 days in the budset date (Fig. 3b).

In spring 2015, i.e. one year after the saplings were moved back to their original sites, we did not find significant differences in the budburst date between the cooling treatment and its control (Fig. 3a) or in their forcing requirement (1041 and 1053 hours, respectively; insert Fig. 3a). Moreover, despite 4 days advance in the warming treatment (Fig. 3b) that required 68 forcing hours less than the control (insert Fig. 3b), the shifts, i.e. both budburst timing and forcing requirement, were not significant ($p = 0.16$) between the warming and control treatments. Nonetheless, during that second spring, the first individuals that tended to budburst first were all from the warming treatment, as shown in the figure 4. Actually, frost damages were observed on the developing new leaves at the high elevation site on May 11th 2015 (red arrow, Fig. 4), and at that time, among the damaged individuals, 7 saplings out of 14 from the warming treatment reached budburst, while only 3 over 12 reached the same stage for the control warming saplings (Fig. 4). Before this specific date, we found that air temperature (blue line, Fig.4) dropped 3 times to freezing temperatures, up to -2.5 °C. After this date when temperatures got warmer, all saplings from the warming treatment reached budburst by the 17th of May, while the rest of the saplings from the control warming treatment reached this stage by the 26th of May.

Interestingly, for all saplings in each treatment over 2014 and 2015, the duration of forcing hours required to budburst was negatively correlated to the duration of chilling hours experienced by the saplings ($R^2 = 0.89$, $p < 0.001$). The more chilling experienced by the saplings, the less forcing was required to budburst (Fig. 5).

Carryover effect on growth, bud morphology and non-structural carbohydrates (NSC)

Stem length increment and total growth in volume during the growing season 2014 were found to be associated with the previous year's warming-cooling treatments (Table 2). Cooling treatment (C) had a negative impact on both parameters. Although saplings in the cooling treatment experienced the growing season at the low site (as they were moved back

to low elevation shortly after budburst), the cooler winter and early spring conditions and delayed-induced budburst had significantly decreased their growth (i.e. volume growth and stem length increment; Table 2) comparing to saplings of the cooling control (CC) ($p < 0.001$). At high elevation, the difference in growth between warming (W) and control warming (CW) were less marked (with 5.0 and 7.8 cm³ in volume growth for W and CW, respectively) but the stem length increased consistently ($0.01 < p < 0.001$) between CW and W (with 3.8 and 7.1 mm, respectively). The number of buds was not statistically affected by both treatment warming and cooling compared to their respective controls. Interestingly, saplings from the cooling treatment (C) had smaller buds ($p < 0.001$), in length and diameter, after the end of the growing season, than those from the control treatment (CC). Conversely, saplings from the warming treatment (W) had longer and larger buds ($0.05 < p < 0.01$), compared to their control (CW).

The NSC concentration did not show significant differences between warming/cooling treatments and their controls (Fig. 6). Indeed, in spite of the substantial shift of budburst timing due to the cooling or warming treatments, the proportion of NSC stored in the twigs produced during the current growing season reached similar levels. The weak differences seem to root more in the origin of saplings (donor site) rather than the result of the treatments. Saplings coming from the lower site tended to have higher concentrations of total NSC and higher starch proportion compared to sugar. Only saplings from the warming treatment produced as much sugars as starch, showing a significant change in the proportion of sugar ($p < 0.001$) (Fig. 6; Table S1), compared to the other treatments. Furthermore, no apparent relationships were observed between the total NSC content and the growing season length 2014 (GSL), as the population from the low site (CC and C), showing the higher NSC content, had a GSL comprised between 123 and 137 days ($p < 0.06$), while the population from the high site (CW and W) showed a GSL between 131 and 159 days ($p < 0.001$; Fig. 6). Interestingly, the cooling and the warming treatment did not affect the GSL in the same way, i.e. cooling reduced the GSL by 14 days whereas warming increased the GSL by 28 days.

Discussion

Using an original transplant experiment from a ~1000 m-elevation gradient allowed us to induce natural warming and cooling to European beech saplings. We showed that spring budburst phenology has a significant but different response to warming and cooling. In particular, we found that beech trees had a greater budburst response to cooling than to warming, i.e. ~11 days delay vs. ~9 days advance per degree cooling and warming, respectively. Interestingly the induced advance or delay in the budburst due to the downward or upward transplantation significantly affects the budset timing in the following autumn, while the saplings were growing at their site of origin during the duration of the growing season. Earlier budburst correlated with earlier budset timing and *vice versa*. However, the magnitude of this legacy effect of spring phenology over autumn phenology differed between the warming and the cooling treatment with higher legacy effect found in the cooling treatment. Additionally, this asymmetric effect of warming and cooling is reflected on the growing season length (GSL), where cooling reduced the GSL by 14 days whereas warming increased GSL by 28 days. Our study provides evidences in natural conditions of the carryover effect of spring phenophases over following phenophases in a temperate tree.

Asymmetric budburst response to cooling and warming

Our study showed that one degree of temperature change induced a larger phenological shift in the cooling treatment than in the warming treatment. This result is consistent with the theory that warmer temperatures may reduce the duration of chilling to fully release winter endodormancy and therefore increase the duration of forcing required to budburst. Similarly, Fu *et al.* (2013) found no further advance in the leaf-out timing of oak and beech when temperature was artificially warmed by 6°C compared to the 5°C warming, in which earlier leaf-out was still observed. European beech is known to have a high chilling requirement for the dormancy release and to interact with photoperiod (Vitasse & Basler, 2013). The population from high elevation was exposed to a warming of about 6°C. We therefore suggest that spring phenology in the warming treatment has responded less than in the cooling treatment due to a lack of chilling exposure or too short photoperiod that has reduced the sensitivity of buds to respond to forcing temperatures (Basler & Körner, 2014). To our knowledge, only one study has shown an asymmetric phenological response to warming and cooling by moving plants in different climate conditions, but on alpine meadows (Li *et al.*,

2016). The authors showed that prolonged phenological stage, such as flowering, induced by transplantation to warmer locations, lead to longer reproductive phases and activity period, whereas cooler conditions led to shorter vegetative and reproductive phases. Studies using natural climatic gradient ‘as experiments by nature’ are particularly relevant as plant phenological responses to artificial warming in experiments were found to significantly differ from long-term series of observations (Wolkovich *et al.*, 2012).

Interestingly, we found that during the first spring 2014, saplings in warming treatment required 189 forcing hours more than the control cooling, in spite they were under the same conditions during winter, and hence received the same duration of chilling hours. This difference in forcing requirement to budburst at the lowest site may be caused by a genetic differentiation between low and high elevation populations as it is often found for the timing of budburst (Vitasse *et al.*, 2009a; 2009b). Nevertheless, the difference between the two populations vanished at the high site and might be due to genes vs environment interactions (Vitasse *et al.*, 2013; 2014c). Indeed, Vitasse *et al.* (2013) found a higher differentiation in the timing of budburst between different populations of beech trees in their lower elevation common garden, and like in our study, this differentiation vanished or was reduced at the high elevation common garden. It could be that under warmer conditions (low elevation), warm-adapted individuals for which dormancy is released can start their growth, while cold-adapted individuals get less chilling and therefore need more forcing requirement (Fig. 5). Conversely, under colder climate (high elevation), even if some individuals are released from dormancy – i.e. warm-adapted population - and have fulfilled their chilling requirement, temperatures are still too cold, so that when temperature gets finally warmer, all populations start at the same time. The fact that phenological processes are not only affected by climatic differences across space, but also by underlying geographic variations in plant genetics, due to long-term climatic adaptation, has been already described on a broader scale and larger number of temperate species (Liang, 2016). This author demonstrates that spring phenology of colder climate-adapted populations can be either advanced compared to warmer climate-adapted populations through lower thermal requirements, or delayed because of higher chilling demands for dormancy release. Our results may also be explained by a carryover effect from the previous growing season since warming saplings originate from a forest located at higher elevation.

One phenophase can affect subsequent phenophases

The induced delay or advance of budburst had a strong impact on the timing of budset, used here as a proxy for primary growth cessation. The carry-over effect of spring phenology over budset timing and leaf senescence was different in the two treatments: a delay in budburst induced by a cooler winter had more effect on budset timing than an advance of budburst induced by a warmer winter. This, again, highlights that effects of cooler conditions on phenological events will not simply mirror effects of warmer conditions in the opposite direction (Li *et al.*, 2016). It is noteworthy that the time lag between bud set and leaf senescence timing was not affected by either treatment but differed in duration between the two populations. This could be explained by genetic differentiation between low and high populations as high populations may have evolved to start senescence and subsequent cold acclimation earlier than low elevational populations (Arora *et al.*, 2003; Vitasse *et al.*, 2014a). Our findings confirm that spring phenophases could affect autumn phenophases, as recently found in experimental conditions (Fu *et al.*, 2014c; Keenan & Richardson, 2015; Delpierre *et al.*, 2016). The relationship between spring and bud set or autumn phenology might be explained by the effect of NSC storage (Fu *et al.*, 2014c). In agreement with Fu *et al.* (2014c), no significant difference in the NSC concentration was found between the treatments and their respective controls. Saplings in control and treatment were under the same environmental conditions during the whole growing season but differ in their spring phenology (due to warmer or cooler induced winter conditions). Thus, irrespective of the growing season beech trees maintain a minimum of NSC content. The dynamic of NSC storage on branchlet may be seen as a balance between promoting primary growth or accumulating reserves in case of stress. Noteworthy, plants from the cooling treatment have grown 84% less (total volume growth) than the control even though they have spent the majority of the growing season in the same conditions at low elevation but have finally accumulated slightly more reserves than saplings in the control. This result suggests that an artificial delay of budburst promotes the accumulation of sugars until reaching a minimum threshold before to allocate carbon to growth. Similarly, in the warming treatment, saplings did not have higher NSC content even though they leaf-out earlier and had extended their growing season and growth was therefore promoted (stem elongation increased by 186% in comparison to the control). In these favorable conditions, the minimum threshold of NSC accumulation must have been reached way earlier, leaving more time for growth. Our study therefore supports the hypothesis of a minimum threshold of NSC reserves to ensure tree

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survival (Nardini *et al.*, 2016), tree resilience against stress conditions (Hartmann & Trumbore, 2016) and enough reserves in the twigs for next spring phenology, which strongly rely on the breakdown of branchlet starch as the main carbon source for budburst and leaf development (Klein *et al.*, 2016). The relation between NSC content and bud set or senescence is unclear, but trees may have a specific requirement of NSC storage that would inhibit growth and promote bud set, which might be slightly different among the two provenances (Herold, 1980). A possible explanation is that once the trees fulfill their NSC requirement (storage capacity) they start the senescence process, because an excess of NSC may inhibit photosynthesis (Gent & Seginer, 2012; Seginer & Gent, 2014). Further investigations about the dynamics of NSC accumulation during this period would be relevant to better understand to what extent autumn phenology plays a role in the carbon pools of trees (but see Klein & Hoch, 2015; Klein *et al.*, 2016).

We did not find a significant carryover effect on next year spring phenology in both cooling and warming treatment, on the contrary to what was observed in previous warming experiment (Fu *et al.*, 2014c), though a none-negligible advance in the number of trees that reached budburst was observed for the saplings that experienced a warmer winter the year before, compared to their control. It is likely that this advance disappeared because of a freezing event that occurred shortly after the first signs of budburst, buffering the potential differences between the treatments, and also indicating that despite a potential memory effect from a previous warm winter/spring the year before (2013/2014), the climatic variables of the previous months (2015) were the dominant drivers of spring phenology.

Relationship between chilling and forcing requirement

Our study also supports the negative relationship between the duration of chilling and the forcing requirement for budburst: the more chilling beech saplings experienced, the less forcing they required to budburst. Even if the number of chilling hours was quite high at the high site (around 2700 h), trees required a significant duration of forcing (around 550 hours) to budburst, which is supposed to be a minimum heat requirement when chilling exceeds requirement for a full dormancy break. At the low site, beech saplings required significantly more forcing hours to budburst (around 1000 hours). The low number of chilling hours received compared to the plants placed at the high elevation site (around 2000 hours compared to 2700 hours) together with the short photoperiod during early spring may have enhanced the forcing requirement to budburst, especially as this species has been shown to

require long duration of chilling to fully break dormancy and its sensitivity to forcing temperatures is higher under longer photoperiod (Vitasse & Basler, 2013; Basler & Körner, 2014). This control of chilling and photoperiod has been seen as a mechanism to avoid a too early flushing when late winter temperatures get warmer and therefore limit the risk of young leaves to be exposed to late spring frost (Vitasse *et al.*, 2014b). This is particularly important because emerging leaves are the most sensitive stage to freezing temperatures (Lenz *et al.*, 2013).

Limitations of the study

Although the phenology of saplings may not represent the phenology of adult trees (Vitasse, 2013), assessing their response to climatic variability is relevant since young trees represent the next generation in mature forests. The carryover effect of one phenophase to the next ones might be less pronounced for saplings compared to mature trees for which other factors such as flowering and seed formation might play a regulating role. Furthermore, other limitations such as precipitation or soil nutrient availability that were not controlled in our experiment may have also affected the timing of budset.

The legacy effects of earlier or late budburst on next year spring phenology is not fully discarded as late spring frosts observed in May in our study may have buffered the possible differences as previously mentioned. Because our study was focused on the legacy effect of the timing of budburst on the next phenophases, our experimental design was not appropriate to test the legacy effect of autumn phenology on next year spring phenology. The timing of leaf senescence and/or budset may postpone the dormancy period and therefore influenced spring phenology on the following year, possibly counterbalancing the effect of global warming (Heide, 2003). A similar experiment focusing on the legacy effect of the timing of growth cessation would therefore be relevant to complement our understanding of relationships between phenophases.

Our study focused on a single species which is dominant in Europe but very particular in terms of phenology compared to co-existing species (Vitasse & Basler, 2013). Investigating the legacy effect of spring phenology over autumn phenophases in a array of species would be crucial to know whether we can generalize our results to other species and to better understand the future phenological responses of trees to ongoing climate warming.

In conclusion, we found that budburst timing of beech trees responds more to cooling than to warming. Furthermore, the induced delay or advance of budburst had a strong impact on the timing of budset, used here as a proxy for primary growth cessation, with again a stronger legacy effect of a cooler winter than of a warmer winter. As shown using in situ long-term series of observations (Keenan *et al.*, 2014), we suggest that the potential delay in senescence processes due to global warming might be smaller than expected because of this positive relationship between spring budburst timing and autumn phenophases. Our study also supports that a carryover effect could either modify the temperature sensitivity of the buds, i.e. changing the chilling and forcing requirements for dormancy break and initiation of bud development, respectively or postpone the different phenophases. Overall, our experimental study provides direct evidence that, besides the abiotic factors, the internal biotic effects should be considered in phenological models to improve predictions of trees response to climate change and models performance.

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Tables

Table 1. Time-lag expressed in days between the date of bud set and the date of leaf senescence for control cooling (CC) and cooling (C) treatments at the low site and of control warming (CW) and warming (W) treatments at the high site in autumn 2014. Additionally to the bud set, leaf coloration and leaf fall were also monitored and we considered individual sapling as senescent when 50% of its leaves were colored or fallen according to the methodology employed in Vitasse *et al.* (2009b). Data are means \pm 1 SE with $n = 10$.

Site	Treatment	Time lag	
		(bud set to senescence)	
Low	C	33 \pm 5.4	ns
	CC	27 \pm 3.9	
High	W	6 \pm 2.4	ns
	CW	2 \pm 3.5	

Table 2. Growth and bud morphology parameters measured at the beginning and at the end of the growing season 2014 for a given site and treatments. C refers to the cooling treatment and represents the trees moved to high elevation during winter/spring 2013-2014 and then moved back to their original site at low elevation, and CC refers to the control cooling treatment in which remained during the whole experiment at the low site. W refers to the warming treatment and represents the trees moved to low elevation during winter/spring 2013-2014 and then moved back to their original site at high elevation, and CW refers to the control warming in which remained during the whole experiment at the high site. Data represents mean \pm 1 SE with $n = 10$. The stars denote a significant difference (at $p < 0.001$) between treatment at the different timings while *ns* is used for non-significant difference.

Site	Treatment	Growth parameter						Bud morphology			
		Total volume growth (cm ³)	<i>p</i>	Stem length increment (mm)	<i>p</i>	Bud number	<i>p</i>	Length (mm)	<i>p</i>	Diameter (mm)	<i>p</i>
Low	C	3.7 \pm 0.8	***	2.5 \pm 0.8	***	46 \pm 8	ns	11.2 \pm 0.6	***	1.9 \pm 0.1	***
	CC	23.2 \pm 3.4		14.1 \pm 2.4		68 \pm 8		17.9 \pm 0.8		2.7 \pm 0.1	
High	W	5.0 \pm 0.9	ns	7.1 \pm 0.9	**	78 \pm 10	ns	9.2 \pm 0.4	*	1.8 \pm 0.1	*
	CW	7.8 \pm 1.4		3.8 \pm 0.5		76 \pm 8		7.9 \pm 0.3		1.6 \pm 0.1	

Figure captions

Fig. 1 Experimental design of the transplant experiment before (a), during (b) and after the transplantation (c). The scheme represents the situation of the saplings of the different treatments at their different locations during the experiment. C refers to the cooling treatment and represents the trees moved to high elevation during winter/spring 2013-2014 (b) and then moved back to their original (donor) low elevation site (c). CC refers to the control cooling treatment in which trees remained during the whole experiment at the low site. W refers to the warming treatment and represent the trees moved to low elevation during winter/spring 2013-2014 (b) and then moved back to their original site at high elevation (c). CW refers to the control warming treatment in which trees remained during the whole experiment at the high site.

Fig. 2 Budburst timing in 2014 (day of year) of European beech (a) and number of forcing hours and chilling hours required to budburst for each site (low and high elevation) and treatment (b). C refers to the cooling treatment and represents the trees moved to high elevation during winter/spring 2013-2014 and then moved back to their original site at low elevation. CC refers to the control cooling treatment in which trees remained during the whole experiment at the low site. W refers to the warming treatment and represent the trees moved to low elevation during winter/spring 2013-2014 and then moved back to their original site at high elevation. CW refers to the control warming treatment in which trees remained during the whole experiment at the high site. Bars represent mean values \pm 1 SE for each treatment ($n = 15$). Different letters in the histograms (a) denote significant differences (at $p < 0.05$). The square brackets above the histograms (b) are used to compare chilling hours and forcing hours per treatment at the same site, respectively, with *ns* for non-significant difference and *** for $p < 0.001$.

Fig. 3 Bud set timing in 2014 and budburst timing in 2015 (day of year) of control cooling (CC) and cooling (C) treatments at low elevation (a) and of control warming (CW) and warming (W) treatments at high elevation (b). Number of forcing and chilling hours required to budburst, respectively to their treatments, are indicated in the inserts. Bars represent the mean values \pm 1 SE for each treatment ($n = 12$ for CW, $n = 14$ for W and $n = 15$ for CC and C). The stars denote a significant difference (at $p < 0.001$) between treatment at the different timings while *ns* is used for non-significant difference.

Fig. 4 Air temperature (blue line) and cumulative number of trees (histograms) that have reached the budburst stage for the warming treatment and control warming in spring 2015 at the high site. The red arrow indicates when frost-damaged leaves (brownish young leaves) were observed in the field.

Fig. 5 Relationship between the accumulated number of chilling hours received (for air temperature between -1 and +5 °C) from the 1st November to budburst, and the number of forcing hours required to budburst, calculated as the sum of hours above 5 °C from the 1st of February to budburst date for each treatment in both 2014 and 2015 (linear regression). Air temperature was recorded at the canopy level, i.e. around 60 cm above the ground.

Fig. 6 Percentage of non-structural carbohydrates (sugar and starch) and growing season length 2014 (numbers above histograms) of control cooling (CC) and cooling (C) treatments at the low site and of control warming (CW) and warming (W) treatments at the high site. Bars represent the mean values \pm 1 SE (n = 10), for which significance level between treatments and their controls are indicated for the total non-structural carbohydrates (sugar + starch content).











