Variation in leaf flushing date influences autumnal senescence and next year's flushing date in two temperate tree species

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Recent temperature increases have elicited strong phenological shifts in temperate tree species, with subsequent effects on photosynthesis. Here, we assess the impact of advanced leaf flushing in a winter warming experiment on the current year's senescence and next year's leaf flushing dates in two common tree species: *Quercus robur* L. and *Fagus sylvatica* L. Results suggest that earlier leaf flushing translated into earlier senescence, thereby partially offsetting the lengthening of the growing season. Moreover, saplings that were warmed in winter–spring 2009–2010 still exhibited earlier leaf flushing in 2011, even though the saplings had been exposed to similar ambient conditions for almost 1 y. Interestingly, for both species similar trends were found in mature trees using a long-term series of phenological records gathered from various locations in Europe. We hypothesize that this long-term legacy effect is related to an advancement of the endormancy phase (chilling phase) in response to the earlier autumnal senescence. Given the importance of phenology in plant and ecosystem functioning, and the prediction of more frequent extremely warm winters, our observations and postulated underlying mechanisms should be tested in other species.

climate change | tree phenology | spring flushing | leaf senescence

Leaf phenology of temperate trees has recently received particular attention because of its sensitivity to the ongoing climate change (1–3), and because of its crucial role in the forest ecosystem, water and carbon balances, and species distribution (4–6).

A wide variety of methods, such as long-term phenological records (7), indirect measurements of ecosystem greening by remote sensing using satellites or webcam digital images (8–10), and modeling approaches (11–13), have been applied to monitor and study phenological changes. These different approaches, conducted at different spatial scales (from individual plants to biomes), have documented a clear advancement of leaf flushing in temperate climate zones and, to a lesser extent, a delay in leaf senescence (14, 15). Furthermore, various temperature manipulation experiments have simulated the impact of future winter warming on leaf phenology and confirmed an advancement in the timing of leaf flushing in response to warming (16–18). However, the response of leaf flushing to climate warming is highly nonlinear (16, 19, 20), because trees also depend on cold temperatures to break bud dormancy (21–23). This chilling requirement may not (fully) be met in a warming climate, especially at the southern edges of species distribution ranges (5, 24, 25).

Most previous phenological studies have focused on specific phenophases, but how a phenological change (e.g., advanced leaf flushing) affects subsequent phenological events is rarely investigated. Nonetheless, the annual growth cycle of boreal and temperate trees forms an integrated system, where one phenophase in the cycle can affect the subsequent phases (26, 27). Such carryover effects have already been detected in fruit and nut

trees, where winter warming resulted in insufficient chilling (28, 29), which subsequently postponed the onset of flowering, with an associated negative impact on crop yields and crop quality (30, 31). Heide (32) also found that delayed senescence in warm autumns delayed spring leaf flushing in the following year in boreal trees. To our knowledge, however, no study has explored the lagged effect of winter warming-induced earlier leaf flushing on the current year's senescence and on leaf flushing dates after one growing season.

In this study, we exposed young trees to manipulated winter temperature to assess the legacy effect of warming-induced variation in leaf flushing (spring 2010) on the timing of leaf senescence (autumn 2010) and flushing in the following year (spring 2011) in two common deciduous and late-successional temperate tree species: pedunculate oak (*Quercus robur* L.) and European beech (*Fagus sylvatica* L.). Specifically, we tested the hypothesis that the physiological impact of winter warming lasts longer than the current growing season. To confirm our experimental results on young trees, we further explored the legacy effects on mature trees of these two study species using the long-term phenological observations of the European phenology network (www.pep725.eu).

Results

Impact of Winter and Spring Warming on Current Year's Growth. Leaf flushing and leaf senescence in 2010 were both advanced by the

> ### Significance
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> Leaf phenology of temperate ecosystems is shifting in response to global warming. This affects surface albedo, ecosystem carbon balance, and evapotranspiration, and the response of leaf phenology to climatic drivers has therefore received particular interest. However, despite considerable effort, models have failed to accurately reproduce phenology patterns, likely because mechanistic understanding is incomplete. Here, we show that earlier leaf flushing in response to a warm winter translated into earlier leaf senescence and even earlier leaf flushing in the following year. This legacy effect of winter warming on leaf phenology has important implications for understanding and modelling leaf phenology and its impact on ecosystem functioning, especially in relation to global warming, and is likely to open new research lines.

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+6 °C winter–spring warming for both species ($n = 10$ and $n = 5$, respectively, $P < 0.01$, Fig. 1). Leaf senescence occurred 15 and 18 d earlier in the +6 °C winter–spring-warming treatment for oak and beech, respectively. Per day of advance in leaf flushing, leaf senescence was advanced by 0.36 and 0.47 d for oak and beech, respectively. In agreement with these experimental results on young trees, we also observed an indication for a relationship between leaf flushing and leaf senescence dates in the long-term phenological observations on mature trees of these two study species. After removing the effect of preseason temperature in the partial correlation analysis, the timing of leaf senescence was positively correlated with spring leaf flushing dates in around 70% of study sites, and more than 27% were significant, with mean partial correlation coefficient $r = 0.1$ (PEP network results, Fig. 2, red histograms). In contrast, only 30% of all sites showed negative correlations, and only 9% (oak) and 7% (beech) were significant. The reader should be aware that in our manipulation experiment all trees were exposed to the same growth conditions and only differed in winter temperature and leaf flushing date, whereas the trees in the phenology database not only differed in leaf flushing date but also in growth conditions during the growing season. Taking this variability into account, observing such a clear pattern in the dataset toward positive partial correlations between leaf flushing and senescence dates was remarkable.

Despite the earlier leaf senescence in our manipulation experiment, the length of the growing season was significantly extended ($n = 5$, $P_{unadj} < 0.01$) in the +6 °C winter–spring-warming treatment for both oak (+27 d) and beech (+20 d), which was due to the larger shift in leaf flushing dates (Table 1). The advanced leaf flushing in 2010 in the +6 °C winter–spring-warming treatment was also associated with some physiological and morphological changes, which were substantial for oak (increased leaf number, higher leaf area per tree, and higher starch accumulation, $n = 5$, all $P_{unadj} < 0.05$), but less for beech (significant increase in leaf number, $n = 5$, $P_{unadj} < 0.05$ and a tendency to increase leaf area per tree and total sugar content, $n = 5$, $P_{unadj} < 0.10$) (Table 1). No significant difference was found between the +6 °C winter–spring-warming treatment and ambient treatment for the other growth and physiological traits

Fig. 2. Frequency distribution of partial correlation coefficients between leaf senescence and leaf flushing (red), after controlling for preseason temperature, and between leaf senescence and preseason temperature (blue), after controlling for leaf flushing, for two study species: *F. sylvatica* L. (*Upper*) and *Q. robur* L. (*Lower*). Mean values of the partial correlation coefficients across all phenology stations (n), and percentages of the total number of positive correlations, as well as the percentages of statistically significant correlations (in parentheses), are also provided.

measured [i.e., stem diameter and height increment, specific leaf area (SLA), light-saturated net photosynthetic capacity (A_{max}), bud diameter and length, bud numbers, and stem N and C concentration].

Impact of Winter–Spring Warming on Flushing Dates After One Growing Season. A significant positive linear relation was found between leaf flushing dates observed in 2010 (directly after the 2009–2010 warming treatments) and in 2011 for both oak and beech (Fig. 3). This suggests that warming-induced advanced leaf flushing leads to earlier leaf flushing in the following year as well, even though saplings from all treatments had been exposed to the same ambient conditions for almost one full year (post-leaf flushing 2010–April 2011). For example, in 2011, leaf flushing of saplings exposed to the +6 °C winter–spring-warming treatment in 2009–2010 occurred 6 d earlier than leaf flushing of saplings in the ambient treatment for oak ($n = 5$, $P_{unadj} = 0.002$) and tended to occur also earlier for beech (5 d; $n = 5$, $P_{unadj} = 0.06$). The relatively flat slope of the linear regression was due to the different range in leaf flushing dates between the two years. In 2010, the warming treatments of winter and spring 2009–2010 enforced a wide range of leaf flushing dates, whereas the ambient conditions of winter 2010–2011, similar for all trees, resulted in a narrow range of leaf flushing dates (Fig. 3).

Interestingly, the positive correlation between two years' leaf flushing dates observed in our warming experiment was also found in mature trees in the long-term field-based phenology observations. The results of partial correlation analysis suggested that, consistent with previous studies, the timing of leaf flushing is associated negatively with preseason temperature in almost all sites (99.6%; significant relationship in 90% of the sites; Fig. 4, blue histograms). This confirms that warmer spring temperatures almost always advance the timing of leaf flushing. However, after removing this dominant impact of preseason temperature, for both oak and beech we also found that the current year's timing of leaf flushing is associated positively with the previous year's leaf flushing dates. This positive correlation was found in ~80% of the study stations, of which 40% exhibited a statistically significant positive partial correlation. In contrast, only 20% of all sites show negative correlations, and only 3% (oak) and 5% (beech) were significant (Fig. 4, red histograms).

Fig. 1. Leaf flushing and leaf senescence dates for both oak and beech in 2010 in ambient and +6 °C warming treatments during the winter–spring season of 2009–2010. Values are means ±1 SE for each treatment ($n = 10$ for leaf flushing and $n = 5$ for leaf senescence). The degrees of freedom are 1, 19 (for leaf flushing) and 1, 9 (for leaf senescence). The F values are 128.6 and 228.7 (12.0 and 20.2) for leaf flushing (leaf senescence) between ambient and +6 °C winter–spring-warming treatment for oak and beech, respectively. Different letters denote a significant difference (at $P < 0.05$); standard font is used for oak and italic is used for beech.

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Table 1. Growth parameters of saplings of oak and beech in +6 °C winter–spring-warming and ambient treatments

	Oak				Beech			
Growth traits	+6 °C	Ambient	P_{unadj}	P_{adj}	+6 °C	Ambient	P_{unadj}	P_{adj}
Leaf phenology								
Growing season lengthin 2010, d	193 (3)	166 (2)	**<0.001**	**<0.001**	176 (3)	156 (2)	**<0.001**	**<0.001**
Date leaf flushing 2011, DOY	103 (3)	109 (2)	**0.002**	**0.03**	111 (3)	116 (1)	**0.06**	0.49
Growth traits								
Diameter increment 2010, cm	0.26 (0.09)	0.18 (0.07)	0.52	0.95	0.17 (0.10)	0.13 (0.04)	0.40	0.87
Height increment 2010, cm	15.4 (1.5)	14.4 (4.1)	0.83	1.00	16.0 (2.4)	11.3 (2.9)	0.45	0.90
Leaf/bud and morphological traits								
SLA, $cm^2 \cdot g^{-1}$	168.3 (6.2)	193.4 (9.1)	0.14	0.82	245.1 (12.1)	204.3 (15.3)	0.12	0.81
Total leaf area, cm^2	1404.4 (145.1)	579.3 (22)	**0.002**	**0.03**	1,685.5 (180.8)	1334.9 (14.2)	**0.10**	0.75
Total leaf number	218 (32)	84 (4)	**0.01**	0.12	248 (34)	179 (18)	**0.05**	0.46
Bud diameter, mm	2.2 (0.1)	2.0 (0.1)	0.28	0.98	1.9 (0.2)	1.6 (0.2)	0.20	0.94
Bud length, mm	13.7 (1.0)	10.7 (0.5)	0.49	1.00	3.2 (0.5)	2.7 (0.4)	0.12	0.80
Total bud number	160 (16)	199 (17)	0.13	0.86	112 (14)	162 (19)	0.13	0.80
Physiological traits								
Stem N concentration, %	1.9 (0.6)	1.4 (0.6)	0.74	1.00	0.63 (0.1)	0.53 (0.1)	0.11	0.78
Stem C concentration, %	47.2 (0.3)	47.8 (0.3)	0.92	1.00	46.5 (0.3)	46.8 (0.3)	0.56	1.00
A_{max}, $\mu mol \cdot m^{-2} \cdot s^{-1}$	6.4 (2.5)	7.0 (1.9)	0.37	1.00	11.4 (0.4)	14.2 (1.5)	0.69	1.00
Wood starch content, $mg \cdot tree^{-1}$	104.1 (1.1)	84.6 (1.9)	**0.02**	0.25	108.9 (1.6)	97.9 (1.2)	0.14	0.86
Wood total sugar content, $mg \cdot tree^{-1}$	308.6 (5.1)	262.4 (6.9)	0.18	0.93	314.5 (3.7)	279.1 (3.8)	**0.08**	0.60

Data are the average values obtained from five saplings per treatment (SE in parentheses). P_{unadj} is significance level (as obtained by one-way ANOVA test), and P_{adj} is adjusted values after correction for Bonferroni-based multiple comparisons, which take into account possible correlations between the growth traits. Bold text indicates significance at the 10% level. The SLA, A_{max}, and leaf area were measured on July 22, 2010; the other physiological traits were measured in December 2010 when all leaves were senesced. DOY, day of the year.

Discussion

Legacy Effect of Winter and Spring Warming. In this study, we found that an advancement of flushing date in response to a warmer winter is influencing flushing dates even 1 y later. This suggests that the physiological impact of winter warming lasts longer than the current growing season. Two mutually nonexclusive hypotheses can explain this carryover effect of altered leaf flushing dates on next year's leaf flushing. The first hypothesis, supported by both our experimental data and the long-term phenological records of the European phenological network, is that the legacy effect of earlier leaf flushing operates through earlier senescence, allowing an extended period for chilling accumulation. The second hypothesis is that shifts in sugar metabolism play a role in this carryover effect. Both hypotheses are discussed below in detail.

Spring flushing is highly dependent on both cold (chilling) and warm (forcing) temperatures, corresponding to two dormancy phases: endodormancy (the period during which the plant remains dormant owing to internal factors) and ecodormancy (the period during which the plant remains dormant owing to external, environmental conditions) (33). Once the chilling requirement is fulfilled, trees enter the ecodormancy phase and flush when a certain amount of warmth has accumulated. Chilling has been found to be important for oak and beech trees (20, 23, 24), our two study species, with beech having a particularly high chilling requirement (34). In this study, the earlier leaf senescence observed in the warm treatments applied during previous winter/ early spring (Table 1 and Fig. 1) might have allowed buds to enter endodormancy earlier in the fall, leading to an earlier start of chilling accumulation. This implied that the chilling requirement was also met earlier, advancing the break of ecodormancy and thereby the onset date of forcing accumulation in the following spring. In other words, the endodormancy phase might have occurred earlier, which was beneficial for earlier leaf flushing in the next year (35).

Leaf senescence of the saplings exposed to the +6 °C treatment occurred around 20 d earlier than leaf senescence of the ambient saplings for both oak and beech. During these 20 d, we noticed that the temperature dropped below 5 °C at night, which

is generally assumed as the optimum temperature for chilling accumulation. This suggests that the earlier leaf senescence led to earlier occurrence of the endodormancy phase, and hence an earlier accumulation of (or exposure to) chilling temperatures required to break dormancy.

The second possible reason for the earlier leaf flushing in 2011 in the trees that were warmed in winter 2009–2010 might be the higher contents of nonstructural carbohydrates, that is, total sugars in beech and starch in oak, in response to the longer growing season. A recent study found that the total plant carbohydrate content has a close relationship with the leaf flushing process (36), maybe through hormonal control. We observed only weak evidence of larger starch/total sugar content associated with earlier leaf flushing in our experiment (Table 1). However, our sample size was small (only five saplings per treatment) and larger sample sizes are needed to draw more firm conclusions. However, how the nonstructural carbohydrates regulate leaf phenology remains poorly understood (37, 38). In this perspective, the use of a process-based forest ecosystem model, such as CASTANEA (39) or EMERGent (40), could be useful to test the relation between simulated carbohydrate reserves and leaf dynamics.

Implications of the Carryover Effect of Early Leaf Flushing. The results of this study have important implications for understanding and opening new research avenues on the self-regulated control of leaf flushing and senescence and the future leaf flushing changes under intense winter–spring warming. The timing of leaf flushing in temperate regions is well known to depend on preceding winter and spring temperature across many tree species (7, 11). Nonlinear responses of leaf flushing to climate warming have been reported (16, 19, 20), and the underlying mechanism is likely that the winter chilling requirements are not completely fulfilled in the warmer conditions (21, 22, 25). This study contributes to our understanding of leaf phenology by showing that the previous year's winter temperature also influences the current year's leaf flushing process. Models taking into account this phenomenon are likely to improve their interannual simulation of leaf flushing and growth

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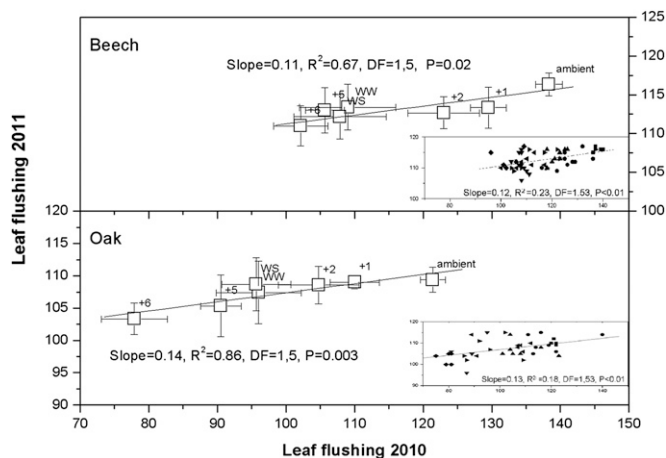
Fig. 3. Linear regression between leaf flushing dates in 2010 and in 2011 of oak and beech. Slope indicates the slope of the linear regression. R^2 indicates the coefficient of determination. DF is the degrees of freedom. The larger leaf flushing variation in spring 2010 was caused by the prior different warming manipulation. Between leaf flushing in 2010 and that in 2011 all saplings were exposed to the same climate (in the field). Different symbols or numbers correspond to different warming treatments from December 1, 2009 until leaf flushing in spring 2010. Winter–spring-warming treatments (+1 °C, +2 °C, +5 °C, and +6 °C) are shown. The main graphs show the regression through the means per treatment for both oak and beech; insets show the linear regression using the individual saplings data. WS, spring-only warming treatment (+6 °C during the spring period only, mid-February to leaf flushing); WW, winter-only warming treatment (+6 °C during the winter period only, December 1 to mid-February).

dynamics at least for late successional deciduous species, such as oak and beech, in future warmer climate conditions. The carryover effect of the previous winter temperature may be particularly beneficial for ecosystem C sequestration because it can be associated with an advanced leaf flushing and longer growing season. It is known that the C balance of terrestrial ecosystems is particularly sensitive to the changes at the edges of the growing season, such as during leaf flushing (6, 41, 42). The advanced spring flushing would likely improve the C uptake (43) if late frost does not occur (44). However, these feedbacks might not take place if the legacy effect of winter warming on leaf flushing is counterbalanced by a negative effect of winter warming during endo-dormancy [insufficient chilling leading to an increase of forcing requirement (22)]. Thus, the impact of the legacy effect of warming-induced earlier leaf flushing might be tightly linked to the magnitude and impact of the chilling deficit.

Relationship Between Spring Flushing and Autumn Senescence. Our ancillary measurements on leaf senescence were available for only a limited number of trees and therefore decisive conclusions cannot be drawn. Nevertheless, using the European phenology network dataset, after removing the dominant climate effect in the partial correlation analysis a similar pattern was found in mature trees over long-term series of phenological records. Our results therefore open the door for new insights in our understanding of the drivers of autumnal leaf senescence in temperate trees. The most widely accepted mechanism underlying the onset of leaf senescence in deciduous trees is represented by the environmental control hypothesis, which proposes that leaf senescence is triggered when the unfavorable autumn season comes, that is, a decrease in day length (45), in temperature (25, 46–49), or in both day length and temperature (50). However, our study shows that in both oak and beech saplings leaf senescence started significantly later for trees maintained at ambient conditions during the previous winter than trees exposed to

winter warming, despite the fact that all saplings experienced identical conditions during the growing season. This suggests that the environmental cues alone cannot fully explain the onset of leaf senescence, although they may dominate the variability in senescence date. Further research should confirm these first findings and analyze the physiological drivers of this carryover phenomenon that influences or overrules the environmental control of leaf senescence. In particular, it needs to be verified whether sink limitation partially controls leaf senescence. We observed a larger starch/total sugar content in saplings exhibiting earliest leaf senescence, which might indicate that leaf senescence occurs once the sugar content has reached the maximum carbohydrate storage capacity (51, 52).

Limits and Conclusion

In our experiment we used saplings from a single genotype of two species and grew them in soil fertilized and irrigated in the same way, and in the same ambient light regime (photoperiod). Although the elimination of these potentially confounding determinants of leaf flushing (49, 53) allowed us to detect and elucidate the legacy effects in leaf phenological processes, our study has some shortcomings that future studies need to address. For example, further investigations are necessary to check whether these legacy effects are present in more tree species, especially in indeterminate growth species, because both *F. sylvatica* and *Q. robur* exhibit determinate flushing behavior. The European phenology database included one species exhibiting this growth pattern with sufficient replication to allow a partial correlation analysis, birch (*Betula pendula*), and for this species we found similar, but weaker, patterns compared with the patterns for the two species included in this study (Fig. S1). More experimental results are therefore needed to identify how general our observations are across tree species.

In conclusion, our study suggests that the physiological impact of winter warming lasts longer than the subsequent growing season. The legacy effect of earlier leaf flushing on autumnal leaf senescence and even on leaf flushing after one growing season calls for a renewed attention on the variables responsible for the
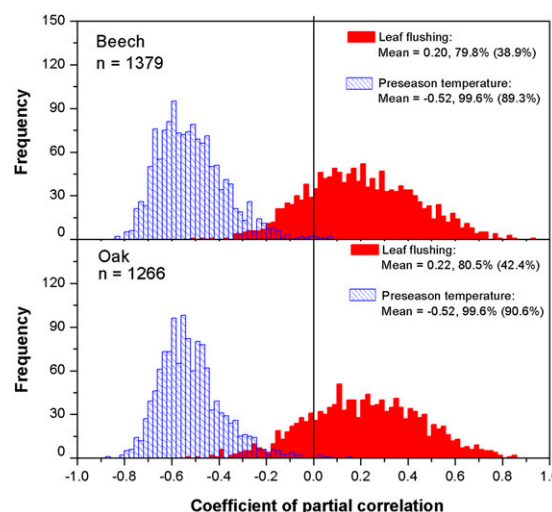
Fig. 4. Frequency distribution of partial correlation coefficients between consecutive years' leaf flushing dates (red), after controlling for preseason temperature, and between leaf flushing and preseason temperature (blue), after controlling for leaf flushing in the previous year, for two study species: *F. sylvatica* L. (*Upper*) and *Q. robur* L. (*Lower*). Mean values of the partial correlation coefficients across all phenology stations (n) and percentages of the total number of positive correlations, as well as the percentages of statistically significant correlations (in parentheses), are also provided.

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interseasonal and interannual "tree memory", such as, for instance, C reserves. Furthermore, developers of leaf flushing models should be aware that meteorological winter/spring conditions (temperature, daylength, humidity, etc.) are not the only drivers of the leaf-flushing timing, but that the previous year's meteorological conditions can play also a significant role by shifting the different dormancy phases.

Materials and Methods

Experiment. Seventy saplings (3–4 y old) of both single-genotype oak and beech, originating from a local nursery, were subjected to different warming treatments in climate-controlled, sunlit growth chambers (20, 54) from December 1, 2009 until leaf flushing in spring 2010 at the University of Antwerp (51°19′ N, 4°21′E). Up to the start, as well as after the temperature manipulations, all nursery-grown saplings were placed outside and subjected to uniform conditions (i.e., equal fertilization, irrigation, and light conditions). Saplings were transplanted into plastic pots (diameter 25 cm, depth 30 cm) with sandy soil in late November 2009 and sufficient slow-release fertilizer was added with 100 g·m^{-2}. The composition of the slow-release fertilizer was 13–10–20 for N, P, and K, respectively (all in percentage). The saplings were watered as soon as the topsoil seemed dry, normally once or twice a week.

Treatments comprised different intensities of winter–spring warming (+1, +2, +5, or +6 °C above ambient temperatures), winter-only warming (December 1–mid-February, +6 °C), and spring-only warming (mid-February–leaf flushing, +6 °C), and an ambient treatment (+0 °C) in which saplings were kept out of the chambers (for details on warming and chambers see Table S1 and Fig. S2). The chambers provided a stable warming treatment and actual warming was within ±5% of the prescribed value (20, 54). As expected, these different warming treatments elicited different leaf flushing dates in spring 2010 (up to 40-d variation in leaf flushing date for both oak and beech; for more details see refs. 20 and 23). Each sapling was moved out of the chambers to a nearby field in spring 2010, as soon as flushing of the first leaves was complete (i.e., at different time depending on the phenology of each sapling). Within the field, saplings from all treatments were arranged randomly in rows (with 50 cm between rows), fertilized again, and irrigated as soon as the topsoil seemed dry. All saplings were kept together in the field until spring 2011, except for 15 saplings of both species that were harvested destructively in late November 2010 to measure a range of physiological and growth traits in the ambient, +2 °C, and +6 °C winter–spring-warming treatments (details are discussed below). Unfortunately, data were lost for the +2 °C treatment. Leaf flushing dates were thus recorded on 70 saplings per species in spring 2010 and on 55 saplings per species in 2011 (Table S1).

Measurements of Phenology, Growth, and Traits. Buds of oak and beech were formed in late summer, in line with previous studies (55, 56). Leaf flushing observations were conducted on the terminal bud of each individual sapling, according to the following phenology scale: (1) undeveloped bud: bud still in winter dormancy; (2) swollen bud: green or elongated bud with broken scales; (3) leaf flushing: leaf bases still hidden in bud scales but leaf tips detached from the bud axis; and (4) leaf unfolded: the entire leaf blade and the leaf stalk were visible. Monitoring started on February 1 in 2010 and March 1 in 2011 and was repeated every 3 d (between stages 1 and 2) and every 2 d (between stages 2 and 3), always at the same time (2:00–3:00 PM). In this study, we used the starting date of stage 3 to determine leaf flushing date. We obtained exactly the same results when using stage 2. This is likely due to the fact that stage 3 followed stage 2 within 2–5 d across all treatments and species. To simplify, only results using stage 3 were presented. Stem diameter (at 20 cm above the soil) and height were measured for all saplings on December 1, 2009 and November 20, 2010.

In autumn 2010, leaf senescence was recorded for five saplings in the ambient (+0 °C) and +6 °C winter–spring-warming treatments. The selected saplings from these contrasted treatments covered the extremes of observed leaf flushing dates in 2010 for both oak and beech. Leaf senescence was defined as the date at which half of the leaves were colored or dropped, following the method described in Vitasse et al. (57). Growing season length was quantified for individual trees as the difference between days of the year of senescence and leaf flushing.

We compared a wide range of physiological and morphological traits between the saplings maintained at ambient conditions and those exposed to the +6 °C winter–spring-warming treatment, which had exhibited much earlier leaf flushing. Total leaf number per tree, A_{max}, SLA, and total leaf area per tree were measured on July 22, 2010. A_{max} was measured at

photosynthetically active radiation = 2,000 µmol·m^{-2}·s^{-1} with a portable open photosynthetic system (LI-6400; Li-Cor). To calculate the SLA (expressed as the ratio of leaf area to leaf dry mass, square centimeters per gram), five leaves were collected from each individual in each treatment. The area of each leaf was measured with a planimeter and then all of the leaves were dried at 70 °C for 3 d to determine the dry weight. Furthermore, we measured the length of each leaf from each sapling and calculated the total leaf area per tree using an allometric function relating leaf length to leaf area, using the data derived from the leaves used to determine SLA. In early December 2010 the total number of buds per individual tree was counted and the size (length and diameter) of the five uppermost apical buds was measured. Branches, stems, and roots were weighed separately to obtain the total fresh biomass (for beech, most senesced leaves were still attached). Subsamples thereof were then dried at 70 °C for 3 d and weighed again to obtain the dry biomass, from which the dry/fresh weight ratio was determined that was used to determine total dry weight per tree.

A 5-cm segment of stem, root, and branch was taken from each sapling and analyzed for carbohydrate content and C and N concentration. The starch and sugar contents were measured by the anthrone method (58). Sugars and carbohydrates were extracted from dried and ground plant material. First, soluble sugars were extracted with aqueous ethanol, and then starch was extracted with 80% ethanol. The concentrations of total sugars (soluble sugars + insoluble sugars) and starch were expressed as milligrams of glucose equivalents per gram of dry weight. The C and N concentrations were measured with a dynamic flush combustion method in a NC 2100 Soil Analyzer (Carlo Erba Strumentazione).

Data Analysis. The relation between the 2010 and 2011 leaf flushing dates was analyzed with a linear regression, both through the means of the different treatments ($n = 7$) and through the individual sapling data ($n = 55$). One-way ANOVA was used to evaluate the difference between +6 °C winter–spring warming and ambient treatments of leaf flushing ($n = 10$), leaf senescence ($n = 5$), and growing season length ($n = 5$), as well as the physiological and morphological traits ($n = 5$), all in 2010. Additionally, a Bonferroni-based correction for multiple comparisons was applied (59), taking into account possible correlations between the growth traits. The adjusted P value is

$$P_{adj} = 1 - \left(1 - P_{unadj}(k)^{g(k)}\right) \quad \text{where} \quad g(k) = M^{1-r(k)},$$

where $P_{unadj}(k)$ is the unadjusted P value for the kth growth trait, $r(k)$ is the mean correlation among the outcomes other than outcome k, and M is the number of growth traits being tested. P values <0.05 were considered significant and values <0.10 as indicating a tendency. All statistical analyses were conducted using SPSS 16.0 (SPSS Inc).

Testing the Legacy Effect on Mature Trees. We further explored the legacy effects of phenological events on mature trees of the two same species, *Q. robur* L. (oak) and *F. sylvatica* L. (beech), using data from the European phenology network (www.pep725.eu) at more than 1,000 locations for each species (Fig. S3). The leaf flushing and leaf senescence dates were defined according to the BBCH (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) code (60).

Preseason temperature is known to determine the timing of spring leaf flushing (7), as well as the timing of leaf senescence (61). To test the correlation between the leaf flushing dates in two consecutive years, as well as between the dates of the current year's leaf flushing and the current year's leaf senescence, we applied a partial correlation analysis to remove the covariate effects of preseason temperature. This method has been successfully applied to remove the covariate effects between study factors in other ecological studies (62, 63). The preseason periods were defined as 90 d preceding the day of leaf senescence or leaf flushing. The selected sites from the European phenology database each had more than 30 y of leaf flushing observations for which also the previous year's leaf flushing date was recorded (needed for testing the correlation between flushing dates in consecutive years) or included at least 30 y of observations on both leaf flushing and leaf senescence dates within the same year (needed for testing the correlation between the current year's leaf flushing vs. leaf senescence) during the period 1950–2011. The daily mean air temperature of each site was derived from a gridded climate dataset of daily mean temperature at 0.25° spatial resolution (~25 km, ERAWATCH).

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