Winter temperatures dominate spring phenological responses to warming

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Abstract

1

2   Decades of fundamental research on woody plant species highlight three major cues that shape spring

phenological events: chilling, forcing, and photoperiod *(e.g., 1; 2; 3).* Increasing research on the phenolog-
ical impacts of climate change has led to debate over how common these cues are across species, and—if
prevalent—whether chilling and/or photoperiod cues may be slowing phenological responses to warming
in recent years *(4; 5; 6; 7).* Here we use a global meta-analysis of all published controlled environment
studies to test for the relative effects of these cues across 203 species. We find almost all species show
strong responses to all three cues, with chilling being the strongest (2 times greater than forcing), and
photoperiod the weakest (0.7 relative to forcing). Simple forecasts from our findings for a well-studied
region (Central Europe) suggest that spring phenology will continue to advance. Stalling effects of chilling
generally appear above 4°C warming for most locations, and thus are unlikely to underlie apparently slow-
ing phenological responses. Our results unify both sides of the debate over phenological cues: while all
species may respond to all cues strongly in experimental conditions, in current environmental conditions
the dominant signal of climate change is from increased forcing.

Main text

For decades, plant phenology has been one of the most reported and consistent biological imprints of climate
change *(8):* many temperate plants are leafing and flowering days to weeks earlier with rising temperatures
(9; 10). Understanding such shifts is important as phenology shapes community assembly and a suite of
ecosystem services, including pollination and carbon sequestration, and scales up to impact projections of
climate change itself *(11).*

As research interest in phenology has progressed, critical discrepancies and uncertainties in our understanding
have emerged. Though responses to warming are consistent on average, they show substantial variation
among species and sites *(12).* Furthermore, long-term observational studies provide increasing evidence that
sensitivities of phenology to temperature are weakening in recent decades *(13; 14),* especially in Europe, where
researchers suggest that responses to multiple environmental cues underlie declining temperature sensitivities
(6).

Fundamental research in phenology outlines how three major environmental cues, chilling (cool temperatures,
generally occurring in the fall and late winter), forcing (warm temperatures, generally occurring in the late
winter and early spring), and photoperiod (daylength), provide multiple routes to budburst each spring,
depending on the environment *(15).* For example, in some species a cool winter will lower the amount of
forcing required to trigger budburst, compared to a warmer winter *(16).* Additionally, photoperiod may

[32] trigger budburst, given low chilling and/or forcing *(7; 17; 18)*. Research suggests that all three cues may

[33] affect spring phenology for many temperate woody species *(3; 17; 19)*, which could have critical forecasting

[34] implications—predicting delays in spring phenology as increased warming reduces chilling in some areas *(20)*

[35] or where earlier budburst shortens the experienced photoperiod. However, there is strong debate, with some

[36] research suggesting some cues—such as photoperiod—may be effectively absent in some species, but dominate

[37] in others *(7; 5)*.

[38] Resolving this debate requires overcoming major hurdles to estimate responses to each cue. Studies at-

[39] tempting to estimate cues using long-term observational data *(e.g., 7; 21)* generally fail to overcome the

[40] fundamental challenge that cues are strongly correlated in nature (*e.g.*, during the transition from winter

[41] to spring at temperate latitudes, forcing, and photoperiod usually increase in step). In contrast to observa-

[42] tional studies, controlled environment experiments can break down correlations between the cues. Note that

[43] controlled environment studies differ from field warming experiments, which are designed to test higher tem-

[44] peratures in natural conditions (12), because they are designed to create conditions less often seen in nature,

[45] but which can critically help researchers identify cues. These experiments—most often conducted in growth

[46] chambers or similar systems to control temperature and light (Fig. 1)—have been conducted for decades and

[47] their accurate respresentation of phenology in the natural world has been tested (22). They have produced

[48] contrasting results, however, potentially due to differences in focal species or study sites *(7; 18; 23; 24; 25)*.

[49] Resolving these discrepancies is critical to accurate predictions of spring phenology, especially as continued

[50] climate change will yield warmer temperatures than has been experienced in at least 150 years and warming

[51] will occur at a rapid pace (26; 27; 28; 29; 30).

[52] Here, we use a meta-analytic to leverage nearly 40 years of controlled environment studies to understand how

[53] chilling, forcing, and photoperiod determine budburst timing in woody species. We reviewed 201 papers from

[54] controlled environment studies, then extracted data from all experiments that reported budburst responses,

[55] yielding data from 72 studies across 39 years and 203 species (Fig. S1, Tables S1, S2). The resulting Observed

[56] Spring Phenology Responses in Experimental Environments (OSPREE) database includes only studies for

[57] which we could identify forcing, photoperiod, and chilling treatments quantitatively and includes a mix of

[58] studies where plant tissue was grown in greenhouses or brought in from the field and exposed to experimental

[59] conditions (Fig. 1 31). As chilling was rarely reported, we calculated chilling when possible, using a common

[60] but approximate method *(32)*, in which chilling does not accumulate below 1.4°C or at high temperatures

[61] (throughout the main text we use the term 'chill unit,' see Supplemental Materials, especially Table **??**, for

62 details).

63 We estimated the effects of chilling, forcing, and photoperiod using a Bayesian hierarchical model. Our model

64 averages over interactive effects of predictors, as it includes only main effects; it estimates both species-level

65 responses (generally yielding more accurate estimates for well-studied species, such as *Fagus sylvatica* and

66 *Betula pendula*), and the distribution from which they are drawn, yielding an estimate of the overall response

67 across species (see Methods). Some species are only represented in one dataset in the OSPREE database,

68 making it impossible to statistically differentiate between species, study, and treatment effects for these taxa.

69 To address this, we combined species found in only one study into "complexes" at the level of genera—such

70 that each taxonomic unit we use in our model occurs across multiple studies (and treatments, see the *The*

71 *Observed Spring Phenology Responses in Experimental Environments (OSPREE) database* section in the

72 Supplemental Materials for details. Our main budburst model can be summarized by the following equation,

73 in which i represents each unique observation, sp is the species or species complex grouping, α represents

74 the intercept, β terms represent slope estimates, and y is the days to budburst since forcing conditions were

75 applied.

$$y_i = \alpha_{sp[i]} + \beta_{forcing_{sp[i]}} + \beta_{photoperiod_{sp[i]}} + \beta_{chilling_{sp[i]}} + \epsilon_i$$

,

$$\epsilon_i \sim N(0, \sigma_y^2)$$

The α and each of the three β coefficients were modeled at the species level, as follows:

$$\alpha_{sp} \sim N(\mu_\alpha, \sigma_\alpha)$$

$$\beta_{forcing_{sp}} \sim N(\mu_{forcing}, \sigma_{forcing})$$

$$\beta_{photoperiod_{sp}} \sim N(\mu_{photoperiod}, \sigma_{photoperiod})$$

$$\beta_{chilling_{sp}} \sim N(\mu_{chilling}, \sigma_{chilling})$$

[76] We applied this 'main' model to both a dataset with 203 species ('all species'), as well as with 67 species

[77] grouped into 37 species or species complex groupings (Tables S3, S4). We fit several additional models,

[78] including a model testing latitude effectsand and one testing effects of chilling study design (see *Models* in

[79] the Supplemental Materials for model equations and other details).

[80] Across studies, all cues—chilling, forcing, and photoperiod—advance budburst phenology (Fig. 2, Tables S3,

[81] S4). Chilling was the strongest cue (-8.89 days/standard unit [95% CI: -12.03 - -5.8] or -2.84 days per chill unit

[82] [95% CI: -3.73 - -1.97]), followed by forcing (-4.36 days/standard unit [95% CI: -6.6 - -2.1] or -0.79 days per °C

[83] of warming, [95% CI: -1.16 - -0.41]), and photoperiod (-3.15 days/standard unit [95% CI: -5.53 - -0.74] or -0.54

[84] days per hour of daylight [95% CI:-0.93- -0.17]; Figs. S4, 3, S8; Tables S3, S4; see Supplemental Materials for

[85] more details). While photoperiod had the smallest effect among the three cues, our results contrast with the

[86] extensive literature suggesting photoperiod is an unimportant cue for many species *(7; 5)*—instead we found

[87] it was surprisingly large, even when accounting for its interaction with latitude (i.e., the latitude from which

[88] plant material was collected prior to being placed in experimental conditions; see Supplemental Materials

[89] for details, especially Figs. S3, S11, Table S5). It was also generally consistent across species (variance

[90] = 5.24 days per hour of daylength in the standardized model), only deviating in *Fagus sylvatica*, a species

[91] well-known for having a large response to photoperiod (which we also found, Figs. 2, S3). Species responses

[92] to chilling were slightly more variable (variance = 7.36 days per chill unit in the standardized model, Fig. 2)

[93] than responses to forcing (variance = 5.67 days per forcing unit in the standardized model Fig. 2).

[94] As temperature is fundamentally altered by climate change, our finding that different ends of the temperature

[95] spectrum—chilling and forcing—have the strongest effects on budburst suggests that understanding these cues

[96] will be critical for forecasting phenology with climate change. Many previous studies attribute advances in

[97] budburst to increased forcing *(10; 16; 17; 33)*. Our results, however, suggest that, across 203 species and

[98] 72 controlled environment studies around the world, chilling has a greater effect on budburst than forcing

[99] (Figs. 2, S3, S7; Tables S3-S5). This has not been widely suggested previously, perhaps because little work

[100] has directly manipulated chilling, and the few studies that have were designed to compare chilling versus

[101] photoperiod effects *(e.g., 7; 17; 19; 23)*, not chilling versus forcing effects. A caveat to our estimates there

[102] are likely complex interactions between chilling, photoperiod, and forcing that we were not able to quantify

[103] in this meta-analysis; indeed, experimental studies do not generally test for interactions between all three

[104] cues. Further, many additional factors can affect phenological responses, including ontogeny (Table S7, 21), ,

[105] and separating species-specific endo- and eco-dormancy requirements (Fig. 1) needs additional physiological

research.

A simple interpretation of our results supports the hypotheses that chilling and photoperiod cues may underlie declining sensitivities to warming in long-term Central European data *(6; 13; 14)*. Under these hypotheses, warming increases forcing and thus advances budburst, but such advances become muted if warming also causes declines in chilling and shorter photoperiods experienced near the timing of budburst *(5)*. This basic agreement between our results and long-term observational trends integrates across experimental conditions that encompass more extreme scenarios than may be seen in nature (Fig. S4, 3). A more robust comparison requires examining predictions under conditions closer to those found in nature.

Reinterpreting our model using climate and phenology data that have led to observations of declining temperature sensitivities in Central Europe suggests that chilling and photoperiod are unlikely to cause the observed declines in sensitivity. Our model predicts such declines only at extreme warming for most sites (see Supplemental Materials). In contrast to the common hypothesis that plants experience less chilling with global warming, we found that—for many sites—total estimated chilling increased with warming (Fig. S9A, C), though this varied with local climate prior to warming (Figs. S6 - S10). Portions of Central Europe have experienced more dramatic warming in winter versus summer *(34)*; yet even if warming uniquely occurs in the winter, our results suggest that delays due to decreased chilling only occur at warming above at least 4°C for most sites, though responses vary by species (Fig. 4, S9, S6). At high warming, predicted declines in sensitivity were due to declines in chilling—photoperiod had comparatively little effect on budburst day of year, even for the photosensitive species *F. sylvatica* (Fig. S11).

Our predictions leave open the question of what underlies declining sensitivities across Europe, but one possibility is that it may be a statistical artifact of how temperature sensitivities are calculated. Physiologically, budburst is triggered by the accumulation of forcing temperatures during the spring *(15; 35)*. However, researchers today often estimate temperature sensitivities from long-term observational data using a linear regression of annual budburst date versus mean spring temperature, or other aggregated temperature metrics *(e.g., 12)*. This approach has the benefit of yielding an easily interpretable metric—days change per °C—but will estimate systematically lower sensitivities given warmer daily temperatures, even with no change in the underlying cues (Fig. S12). We found the declining sensitivities observed in European data are of the same magnitude as those predicted from a statistical artifact (sensitivity declines of 0.8 ± 0.3 days/°C in European data versus 0.9 ± 0.5 days/°C in simulations), and the data also show a related decline in leafout variance that would not be immediately predicted from shifting cues *(see Potential statistical artifacts in declines of*

temperature sensitivity in observational long-term data in the Supplemental Materials and *36,* for further details). This statistical artifact is likely not confined to phenological studies; it should apply to any research using a similar days/°C metric to estimate an underlying thermal accumulation model where the thermal sum per day is non-stationary, as is the case with climate change.

A consistent result of our findings—across both the experimental and future in situ environmental conditions— is the importance of chilling. Yet chilling and its related physiological stage, endodormancy, are not well understood *(15)*. Models of how species accumulate chilling are poorly developed for forest trees, with few relevant tests evaluating the particular temperatures at which species do or do not accumulate chilling. Instead, researchers generally rely on models developed for perennial fruit trees (*i.e.*, Utah *(32)* and chill portions *(37)*, both of which were developed for peach species). These models are themselves *hypotheses* for how chilling may accumulate and produce dormancy release, but are likely to be inaccurate for many species *(38)*.

Progress on developing chilling models for wild species is especially slow, as only a small portion of studies (13 of the total 72 studies) manipulate chilling directly. Instead many studies estimate chilling effects through sequential removal of tissue from the field followed by exposure to 'forcing' conditions (Fig.1a,1b, 25 out of 72; the remaining studies did not appear to manipulate chilling), with the assumption that tissues collected later experience more chilling *(39)*. This method benefits from more natural chilling conditions but introduces other challenges: first, chilling duration may not always co-vary with the magnitude of total accumulated chilling *(38)*, and, second, photoperiod and other factors also change over time. Indeed, we found that sequential-removal studies tended to result in later budburst, weaker effects of chilling and stronger effects of forcing compared to estimates from studies that directly manipulated chilling (Fig. S7, Table S6, *(39; 40)*, suggesting a study's design of chilling manipulation impacts both forcing and chilling estimates. An improved understanding of chilling could in turn alter our understanding of forcing. Although researchers often define 'chilling' and 'forcing' treatments based on temperatures, physiologically plants appear to accumulate forcing mainly after chilling requirements have been met, thus identifying processes that plants undergo when accumulating chilling versus forcing will be critical for the most accurate forecasts *(15)*.

Our results unify decades of experimental studies, which have shown the importance of chilling, forcing, and daylength to determining budburst timing, with long-term observational data, where forcing appears to dominate responses to recent warming. We do not find strong evidence for delaying budburst in the near future, and suggest that recent observed declines in temperature sensitivity may be related to statistical

artifacts from estimating complex cues from observational data. Instead, our predictions suggest budburst will continue to advance in many well-studied European regions in the future. The most dramatic changes will come from regions where winter warming causes dramatic decreases in chilling, with implications for ecosystem services related to phenology.

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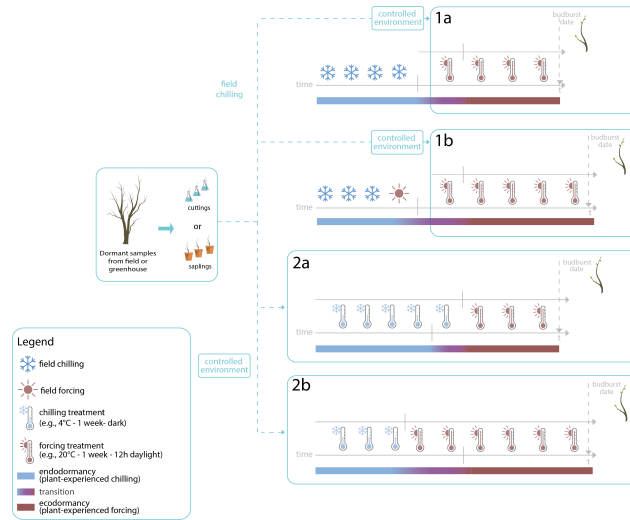
Acknowledgements

Figures

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Figure 1: **Controlled environment experiments that test temperature effects on woody plant phenology** often manipulate temperature cues, which include chilling and forcing. Chilling is typically manipulated via either field methods (1a,1b), in which plant material is collected after different amounts of chilling have been experienced, or experimental methods (2a,2b) in which plant material is place in controlled environment chambers set to different chilling temperatures and/or durations. Chilling treatments are designed to break plant enodormancy, after which forcing treatments are imposed by moving plant material to warmer temperatures ('eco-dormancy') that allow budburst to occur. A challenge with controlled environment studies is that species-specific chilling requirements are rarely known, so the experimental manipulations may not always align with what is experienced by the plant. Panel 1a shows an example in which an experimental manipulation aligns with plant-experienced endo- and eco-dormancy. Panels 1b shows an example in which endo-dormancy is broken while the plant is still in field 'chilling' conditions but is experiencing forcing (eco-dormancy). Add something about Panel 2. Photoperiod is often simulataneously manipulated during forcing treatments (not shown).
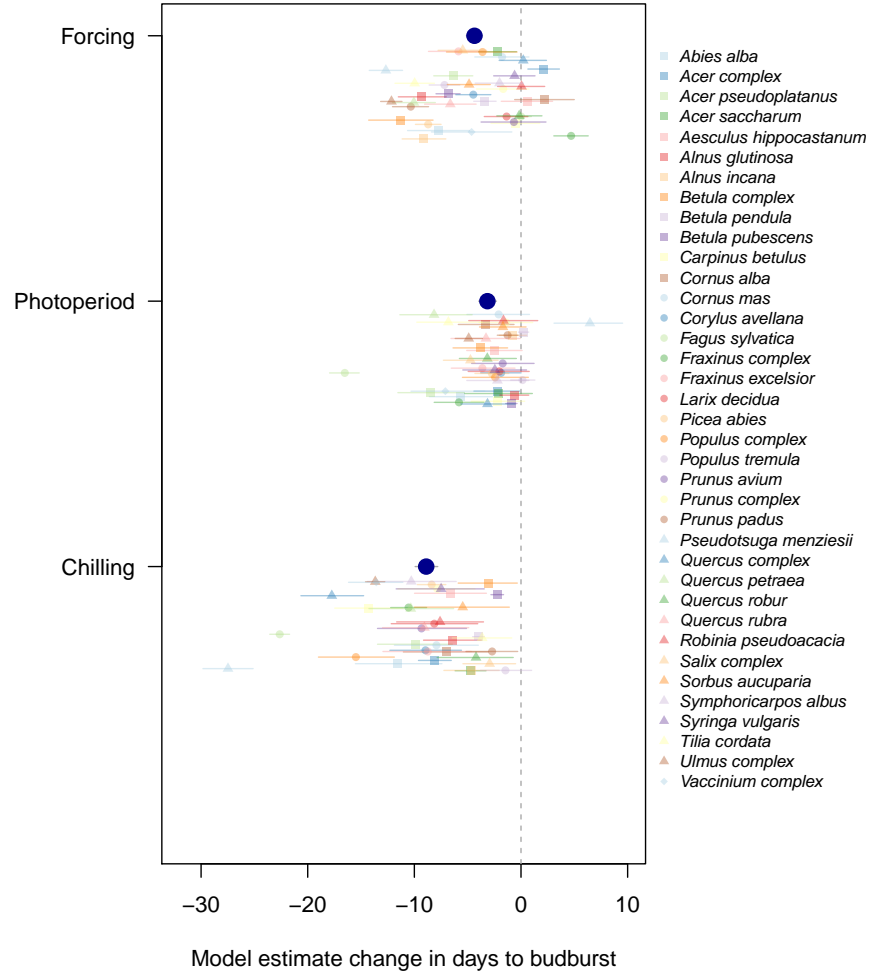
Figure 2: **Estimated effects of chilling, forcing, and photoperiod on budburst timing across 67 species in 42 controlled environment studies.** Using standardized units, which allow comparisons across cues, we show that most species (smaller symbols) are responsive to most cues, with chilling being the strongest cue when considering overall estimates across species (larger, dark blue circles). Overall estimates shown here were generally similar to other model formulations, including using data from 203 species, and using different methods for calculating chilling (Figs. S3, S7; Tables S3-S5). Lines represent 50% uncertainty intervals (other intervals provided in Tables S3-S5)
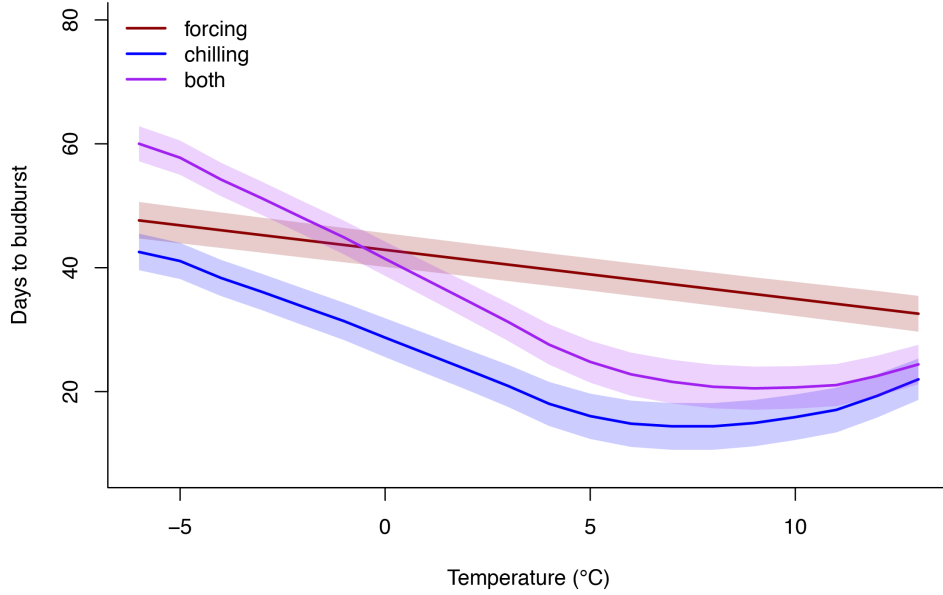
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Figure 3: **Estimates of budburst across a range of forcing temperatures and estimated chilling** (converted to a representative mean temperature, see *Estimating chilling* in the Supplemental Materials) based on overall estimates of chilling and forcing effects (Fig. 1). Figure 4S is a three-dimensional version that shows all possible combinations of chilling, forcing, and budburst across the experimental conditions. We predict budburst timing based on forcing temperature and estimated chilling (converted to a mean temperature, see *Estimating chilling*). Note that days to budburst is relative to experimental methods and thus not comparable to day of year in the field, shading represents 50% credible intervals. We show the effect of chilling temperature on budburst, with forcing kept at the mean level across all experiments (16°C); the effect of forcing temperature with chilling kept at the mean level across all experiments (1324 chilling units), and the effect of varying both chilling and forcing temperatures simultaneously.
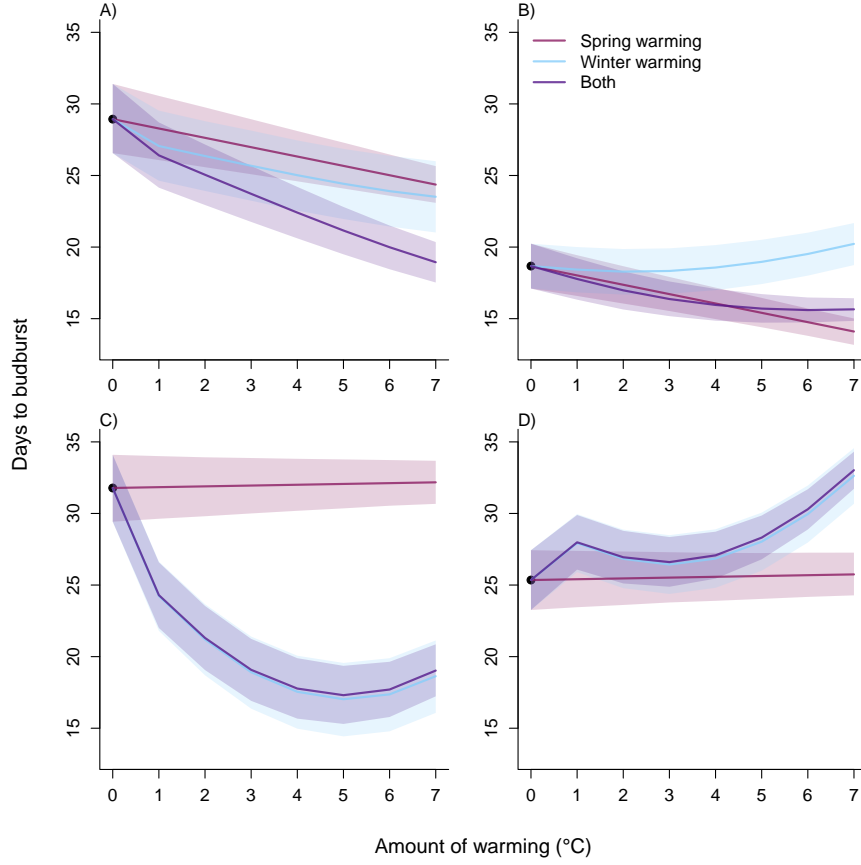
Figure 4: **Implications of warming on budburst timing varies across species and sites**, depending strongly on pre-warming climate conditions related to chilling for each site. Here we show species-level estimates from our model (see Fig. 1 in the main text) for the two most common species in the OSPREE database: *Betula pendula* (A,B) and *Fagus sylvatica* (C,D). We compare estimates of budburst assuming varying levels of winter warming (*i.e.*, which affectins chilling, Fig. S10), with forcing kept at the mean pre-warming level, to estimates assuming varying levels of spring warming (*i.e.*, forcing) with chilling kept at mean pre-warming levels, to estimates with winter and spring warming occurring simultaneously. For sites in A (46.8°N, 12.8°E) and C (48.8°N, 15.4°E) chilling decreases with warming, leading to greater advances in budburst (compared to forcing alone). For the sites in B (48.3°N, 15.8°E) and D (46.7, 15.8°E), on the other hand, warming increases chilling, leading to smaller advances, and eventually, delays with substantial warming. See Supplemental Materials, especially Figs. S6 - S10, for details. Fig. S9 shows all possible combinations of winter and spring warming in a three-dimensional plot.