Temperature and photoperiod drive spring phenology across all species in a temperate forest community

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Summary:

(1) Accurate predictions of spring plant phenology with climate change are critical for projections of growing seasons, plant communities and a number of ecosystem services, including carbon storage. Progress towards prediction, however, has been slow because the major cues known to drive phenology—temperature (including winter chilling and spring forcing) and photoperiod—generally covary in nature and may interact, making accurate predictions of plant responses to climate change complex and non-linear. Alternatively, recent work suggests many species may be dominated by one cue, which would make predictions much simpler.

(2) We manipulated all three cues across 28 woody species from two North American forests.

(3) All species responded to all cues examined. Chilling exerted a strong effect, especially on budburst (15.8 days), with responses to forcing and photoperiod greatest for leafout (19.1 and 11.2 days, respectively). Interactions between chilling and forcing suggest each cue may compensate somewhat for the other. Cues varied across species, leading to staggered leafout within each community and supporting the idea that phenology is a critical aspect of species' temporal niches.

(4) Our results suggest that predicting the spring phenology of communities will be difficult, as all species we studied could have complex, non-linear responses to future warming.

Plant phenology—the timing of recurring life history events, such as leafout and flowering—is critical to the structure and function of ecosystems (Cleland *et al.*, 2007). Spring plant phenology in particular drives local ecosystem properties, from the length of the growing season to energy balance between land and atmosphere, and scales up to impact global carbon cycles (Richardson *et al.*, 2009).

Phenology is also one of the major biological indicators of climate change, with plant phenology shifting earlier across the globe 4-6 days/°C with warming (IPCC, 2014). While this average response is strikingly consistent when considered across diverse datasets (Wolkovich *et al.*, 2012), it masks considerable variation. Variation is extreme when examined across species (Wolkovich *et al.*, 2014), but additional variation can be seen within species over space (Kramer *et al.*, 2017; Vitasse *et al.*, 2013) and time (Fu *et al.*, 2015; Yu *et al.*, 2010). Understanding this variation has been the goal of much recent work (Donnelly *et al.*, 2017; Laube *et al.*, 2015; Rutishauser *et al.*, 2008; Zohner *et al.*, 2017), with research focusing on two major linked aims: (1) identifying and quantifying the environmental cues that drive spring phenology (i.e., vegetative budburst and subsequent leaf development—leafout), and (2) identifying what drives variation in cues between different species.

Decades of study on wild species spring phenology—mainly focused on temperate woody species—show that three major cues underlie budburst and leafout: warm spring temperatures (forcing), increasing daylength (photoperiod), and length and intensity of winter temperature (chilling). Across studies, increasing temperatures in the spring appear to be a dominant factor that controls spring phenology; yet many of these studies have been observational—making it nearly impossible to tease out the co-varying effects of longer days and reduced cold temperatures, which generally reduce chilling (Chuine, 2000; Cook *et al.*, 2012). By contrast, experiments from controlled environments (e.g., growth chambers) have highlighted the additional importance of photoperiod and chilling (Caffarra *et al.*, 2011; Falusi & Calamassi, 1996; Foley *et al.*, 2009; Ghelardini *et al.*, 2010; Heide, 1993a), with longer days and increased chilling leading to more rapid budburst (Caffarra & Donnelly, 2011). Many of these cues are known to interact: photoperiod and chilling can together determine spring phenology through their complex impacts on dormancy release (Chuine, 2000), insufficient chilling may be offset by additional forcing, and photoperiod and chilling often interact, as a long photoperiod enhances cell growth, compensating for a lack of chilling during plants' winter dormancy (Caffarra *et al.*, 2011; Heide, 1993a; Myking & Heide, 1995).

Yet, while such complexities have been identified in some species, a growing body of hypotheses and experimental studies has suggested many species are dominated by one cue and may lack any response to other cues (Körner & Basler, 2010). If true, this would have critical implications for predicting responses to climate change. Species dominated by a forcing cue would be predicted to continue to advance their leafout timing with warming, while species with strong photoperiod cues would instead stop advancing at some threshold point (Körner & Basler, 2010). This could lead to major separation in the phenology of communities, as some species shift earlier while others change little, with cascading consequences for species coexistence and invasion. Alternatively, if all three cues—forcing, photoperiod, and chilling—are present and interact then predictions would be far more complex (Chuine & Cour, 1999). A species experiencing a mild winter with insufficient chilling (as predicted with climate change) could still break bud, but it would require longer photoperiods and/or warmer temperatures (Heide, 1993a) than it has in the historical record—a trend increasingly seen in long-term observational records (e.g, Carter *et al.*, 2017; Fu *et al.*, 2015). If such complex cues are seen in all species within a community it could mean community phenology may shift more in step, with no dramatic separation between species.

Research to date shows cues clearly vary across species, and recent efforts have focused on understanding and predicting this variation. Studies have focused on attributes of species: native/exotic (Willis *et al.*, 2010), the successional stage (i.e., pioneer or climax communities) to which species traditionally belong (Basler & Körner, 2012; Laube *et al.*, 2014), and a variety of possibly related traits (Lechowicz, 1984; Polgar *et al.*, 2014). Most of these studies hinge on an often implicit assumption that phenology—by helping define the temporal niche of a species—is a critical axis along which plant species assemble within communities (Gotelli & Graves, 1996; Loreau & de Mazancourt, 2008). Support for this hypothesis comes from work showing that phenology is often staggered within communities, and from the special case of plant invasions, where research suggests that climate change has provided open temporal niche space for new species to occupy (Willis *et al.*, 2010; Wolkovich *et al.*, 2013). As the abiotic environment is not the sole contributor to plant performance, considering a suite of co-occurring species together is key for making progress in understanding the role phenology plays in shifts in community composition and ecosystem functioning (Cleland *et al.*, 2007).

Improved understanding and predictions of phenology with climate change would benefit from a fuller understanding of the interacting environmental cues that drive phenology within (and eventually across) communities. To this aim we studied how forcing, photoperiod and chilling cues vary in their impact on spring phenology across a community of 28 woody plant species from two temperate forest locations (Tables S1), separated by 4° latitude. We used clipped dormant branches, which have been shown to approximate whole plant responses (Vitasse & Basler, 2014), and forced them in controlled environments that varied forcing temperatures, photoperiod and chilling. We predicted that: (1) Cues would vary across species, driving staggered leafout across the spring, and (2) within-species cues would trade off, such that some species would be dominated by one or another cue, while others would show a mix of cues. To our knowledge this is the first multi-species study to assess all three cues in one experiment through a controlled environment approach; while several studies have done this for one species (Skuterud *et al.*, 1994; Søgaard *et al.*, 2008; Sønsteby & Heide, 2014; Worrall & Mergen, 1967), other studies of all three cues have used separate experiments (e.g., Caffarra & Donnelly, 2011) or relied on field sampling to assess one or more cues (e.g, Basler & Körner, 2012; Laube *et al.*, 2014; Zohner *et al.*, 2016).

Materials & Methods

Field sampling

Woody plant cuttings were made in January 2015 for 28 species at Harvard Forest (HF, 42.5°N, 72.2°W) and the Station de Biologie des Laurentides in St-Hippolyte, Québec (SH, 45.9°N, 74.0°W). The typical late January temperatures are -3.4 and -22°C, respectively, with daylengths (across the year) ranging from 9 to 15.25 hours and 8.5 to 15.75 hours. Weather station data from each field site were obtained for calculations of chilling units (see Table S7).

Species were chosen based on the dominant forest vegetation at each site, aiming to maximize the number of shared species between the two sites. Of the 28 species, at least 19 occurred at both sites. For each species, up to 15 representative healthy, mature individuals with branches accessible by pole pruners from the ground were tagged in late summer and fall 2014. In winter 2015, six individuals were located and 4-16 cuttings taken from each individual, depending on size of the individual and number of treatments to be applied. Cuttings were kept cold and transported back to the Arnold Arboretum in Boston, MA.

Growth Chamber Study

Cuttings were placed in growth chambers at the Arnold Arboretum in Erlenmeyer flasks with distilled water; water was changed every 7-10 days. The bases of cuttings were re-cut at each water change under water to prevent callusing. For 11 of the 28 species, sufficient cuttings were obtained from each individual tree to apply the full set of 12 experimental treatments: 2 temperature (20°C / 10°C warm vs. 15°C / 5°C cool) x 2 photoperiod (12 vs. 8 h) x 3 chilling (no additional chilling, additional 30 d at 4°C, or 30 d at 1.5°C) treatments. For the remaining 17 species, sufficient cuttings were obtained only to apply the temperature and photoperiod treatments, without the additional chilling levels. The total number of cuttings for a given species thus ranged from 24 to 144, depending on presence at each site and application of the chilling treatment (Fig. S1). Lighting was a combination of halogen incandescent bulbs and T5HO fluorescent lamps with the lamploft adjusted to provide roughly 400 $\mu mol/m^2/s$ as measured by Apogee QSO-A5E quantum PAR light sensors in each chamber (sensor set to the height of the cuttings). Treatments were rotated across chambers every two weeks, as was flask position within chamber, to remove any possible effects of chamber or flask position.

Phenology of the cuttings was assessed using a BBCH scale, modified for use in trees (Finn *et al.*, 2007), with observations on each of the 2,136 cuttings made every 2-3 days for the course of the 82-day experiment, a total of 48 observation days. The phenological stages assessed in the present study are budburst, defined as beginning of sprouting or bud breaking or shoot emergence (Code 07 in Finn *et al.*, 2007) and leafout, defined as first leaves unfolded (Code 11 in Finn *et al.*, 2007). Additional stages up to flowering and stem elongation were also recorded and we provide a photographic guide to help visualize stages across species (Savas *et al.*, 2017). In total, we made over 19,320 phenological observations at the cutting level.

Statistical analysis

We analyzed our data using Bayesian hierarchical models because they allowed us to best-estimate responses to our full experimental design at both the species and across-species levels. In particular this approach takes into account the effect, variance and sample size for each species. For the two phenological responses measured, days to budburst and leafout, we fit mixed-effects hierarchical models using site, warming, photoperiod, and chilling treatments, and all two-way interactions as predictors (fixed effects) and species as modeled groups (random effects). This approach allowed us to calculate the posterior probabilities of the effects for each of the abiotic drivers individually and interactively across all species sampled. The models were fit using the programming languages `Stan` (Carpenter *et al.*, 2016)(`www.mc-stan.org`), accessed via the *rstan* package (version 2.15.1) in R (R Development Core Team, 2017), version 3.3.3. Stan provides efficient MCMC sampling via a No-U-Turn Hamiltonian Monte Carlo approach (more details can be found in Gelman *et al.* (2014) and in Carpenter *et al.* (2016)).

The model was fit as follows:

$$
\begin{aligned}
y_i \sim N(\alpha_{sp[i]} &+ \beta_{site_{sp[i]}} + \beta_{forcing_{sp[i]}} + \beta_{photoperiod_{sp[i]}} + \beta_{chilling1_{sp[i]}} + \beta_{chilling2_{sp[i]}} \\
&+ \beta_{forcing \times photoperiod_{sp[i]}} + \beta_{forcing \times site_{sp[i]}} + \beta_{photoperiod \times site_{sp[i]}} \\
&+ \beta_{forcing \times chilling1_{sp[i]}} + \beta_{forcing \times chilling2_{sp[i]}} \\
&+ \beta_{photoperiod \times chilling1_{sp[i]}} + \beta_{photoperiod \times chilling2_{sp[i]}} \\
&+ \beta_{site \times chilling1_{sp[i]}} + \beta_{site \times chilling2_{sp[i]}})
\end{aligned}
$$

The α and each of the 14 β coefficients were modeled at the species level, as follows:

1. $\beta_{site_{sp}} \sim N(\mu_{site}, {\sigma^2}_{site})$

...

14. $\beta_{site \times chilling2_{sp}} \sim N(\mu_{site \times chilling2}, {\sigma^2}_{site \times chilling2})$

For the μ and σ parameters, weakly informative priors were chosen (increasing the priors three-fold did not change the model results). We validated that our model code could return valid parameter values using test data.

We ran four chains simultaneously, with 4 000 warm-up iterations followed by 3 997 sampling iterations, resulting in 15 998 posterior samples for each parameter. We used a non-centered parameterization on all interaction terms and assessed good model performance through $\hat{R}$ close to 1 and high n_{eff} (15 998 for most parameters, but as low as 2440 for several parameters) as well as visual consideration of chain convergence and posteriors (Gelman *et al.*, 2014).

In our figures we show means ± 50% credible intervals from this model. We used 50% intervals because of our focus here is on the most likely value for each parameter (e.g., estimated response to forcing) and because they are computationally stable (Carpenter *et al.*, 2016; Gelman *et al.*, 2014). For those interested in a more traditional significance-testing approach (which is not our focus here), we provide 95% credible intervals in Table S3-S6. To assess relationships between responses to forcing, chilling and photoperiod across species we fit a simple linear relationship to each of the 1000 last iterations and report the mean and 50% credible intervals from the resulting distribution.

Results

Budburst success

Across all treatments, 9.8% of the cuttings did not break bud, while an additional 10.4% did not reach the leafout stage following budburst (see *Budburst and leafout success* in the Supporting Information). Variation was highest due to species identity, but removal of the five species with the lowest budburst or leafout success did not qualitatively affect the results and quantitatively most estimates changed by less than 10% (see *Budburst and leafout success* in the Supporting Information, Tables S2-S4 and Fig. S2-S3) .

Days to budburst and leafout

Higher forcing temperatures, longer photoperiod (12 vs. 8 h), and additional chilling all caused large advances in budburst and leafout (Fig. 1, Tables S5-S6). Forcing temperatures (20°C / 10°C warm vs. 15°C / 5°C cool) and chilling (no additional chilling, additional 30 d at 4°C, or 30 d at 1.5°C) caused the largest advances in budburst and leafout, and these two effects offset one another, as shown by their interactive delayed response (Fig. 1). Effects of chilling at 4°C were greater than forcing for all species (for which chilling was assessed) for budburst and for most species for leafout (effects of forcing were greater than chilling for *Acer saccharum, Populus grandidentata, Quercus rubra, Viburnum lantanoides*). Effects of forcing were greater than effects of photoperiod for all species for budburst, and for all but one species for leafout (*Prunus pensylvanica*). We found similar effects of chilling across two different base temperatures, with only minor differences: responses to the colder (1.5°C) chilling treatment were similar or more muted compared to responses to the warmer (4°C) chilling treatment (Fig. 1, S5-S6, Tables S5-S6, see also Fig. S9).

Responses to cues were qualitatively similar across both budburst and leafout, but quantitatively varied greatly between the two phenophases (Fig. 1-2), with responses generally greater for leafout (Fig. 1). This change was dramatic for the response to forcing and photoperiod where the advance in days more than doubled from budburst to leafout (Fig. 1, Table S5-S6).

These three factors (forcing, chilling and photoperiod) did show some degree of substitutability, meaning, for example, that a lack of chilling could be made up for by an increase in forcing. These are indicated by the positive two-way interactions: for example, while forcing or chilling at 4°C alone would advance leafout (-19 or -17 days, respectively), their combined effect would lead to an advance of only 26 days because of their interaction (10 days, see Table S5). Chilling and forcing temperature are more substitutable than chilling and photoperiod, for both budburst and leafout, while forcing and photoperiod showed virtually no substitutability for budburst and only a small amount for photoperiod (Fig. 1).

The two forest sites showed similar responses, with only a very minor possible delay in overall timing for the northern site, and a more pronounced effect of site through its interaction with chilling (Fig. 1, Tables S5-6). The full effect of chilling depended on site, with a larger impact of site on the lower chilling temperature (1.5°C). Effects of forcing temperatures and photoperiod were not clearly impacted by site.

At the community level we found that all species were responsive to all cues (forcing temperatures, photoperiod and chilling, Fig. 3, S4-5), with each species having slightly different cues such that each species would budburst and leafout at a distinct time compared to other species (Fig. 3). Across species responses to forcing and photoperiod were related for budburst (mean slope of 0.31, CI of 0.15-0.48) and leafout (mean slope of 0.45, CI of 0.26-0.66), whereas responses between forcing and chilling were very weak (budburst: mean slope of 0.12, CI of 0.04-0.20; leafout: mean slope of 0.11, CI of 0.04-0.20). Early species tended to show the smallest responses to all cues, suggesting they bud burst and leaf out early because they require lower amounts of spring forcing, shorter days and chilling to start growth each season. In contrast, mid and late species relied on a varying mix of cues to drive their spring phenology: for example, *Populus grandidentata* showed a relatively strong response to forcing and chilling, but a milder response to photoperiod, while *Fagus grandifolia* had a strong response to forcing and photoperiod and a much smaller response to chilling.

Discussion

We found that all species responded to all three cues—spring forcing, winter chilling and photoperiod—suggesting that future spring phenology with continued warming will most likely be complex. While some observational responses to date have suggested a potentially linear phenological response to warming (Ellwood *et al.*, 2013) our results provide support for decades of research that find spring phenology depends on a complex suite of multiple interactive cues (e.g., Caffarra *et al.*, 2011; Heide, 1993b). All three cues individually advanced budburst and leafout, with the effects of chilling and forcing showing the largest interactive effect. The interactive effects of forcing and chilling have been noted repeatedly before (e.g., Caffarra *et al.*, 2011; Heide, 1993a) and highlight that insufficient chilling can be overcome by additional forcing—a hypothesis suggested by recent studies that have found shifting temperature sensitivities in observational data over time (Fu *et al.*, 2015; Yu *et al.*, 2010). Photoperiod and forcing also showed an interactive effect, but mainly for leafout and much smaller than either the singular effects of forcing or photoperiod (Figure 1).

Phenological cues: Multiple cues and interactive effects

In contrast to our expectations that within a species cues would trade off (i.e., a species could be dominated by one cue), we found that species tended to show similar cues, especially between forcing and photoperiod (Fig. 2a,c). Thus, a species with a strong response to forcing temperatures generally also had a strong response to photoperiod and, similarly, a species with a comparatively weak response to forcing also had a weaker response to photoperiod. This was also seen somewhat with chilling (Fig. 2b,d), though we have fewer species with which to assess the relationship (see Methods). There was substantial variation, however, such that if only a small subset of species had been included in the study, it might have been concluded that a trade-off between photoperiod sensitivity and warming sensitivity would exist. For example, *Fagus grandifolia* exhibited a relatively limited response to warming but substantial photoperiod sensitivity, while *Rhamnus frangula* showed a relatively limited response to photoperiod but substantial warming sensitivity.

These results also suggest that simple classification of some species as 'sensitive' or 'insensitive' or similar bins (e.g., 'high', 'low', 'no' sensitivity) to any cue would be artificial, as species did not form distinct clusters (Fig. 2). Previous studies have classified some of our studied species as non- or low-responsive to photoperiod (e.g., *Alnus incana, Aronia melanocarpa* in Zohner *et al.*, 2016), but we found these species were responsive to photoperiod and that any one species' cues were only slightly different from the cues of several other species, yielding no clear way to define such binary classifications. This is despite the fact that our species spanned a diversity of genera, including canopy and understory species. We did find that shrubs tended to show smaller budburst responses to photoperiod (Fig. 2a) than many trees, but this was not seen for leafout (Fig. 2c).

Our finding that all species responded strongly to all three cues is at odds with some recently published work (Basler & Körner, 2012; Laube *et al.*, 2014; Zohner *et al.*, 2016), but is coherent with many other studies (e.g., Heide, 1993a; Worrall & Mergen, 1967), with related process-based models of woody plant phenology (Chuine *et al.*, 2016, 2000) and with recent trends in long-term data (e.g, Carter *et al.*, 2017; Fu *et al.*, 2015). The contrasting results may be due to varying methodologies and study aims. Our study used samples collected from the field in January—when species in these locations had likely not fully met requirements for any cue—then used controlled environments (growth chambers) to manipulate all three cues at once. Thus, one possible reason for our contrasting findings could be that none of cuttings experienced full chilling in our experimental design, though the estimated chilling units in our treatments with additional chilling were generally much higher than estimated chilling accumulated in the field at each site in the same year (Table S7) and we found only small effects of additional chilling in a follow-up experiment the following year (Fig. S9). Experimental chilling, however, is highly artificial and fails to replicate the daily, hourly and finer temporal variation in temperature that plants experience in the field as they accumulate chilling (Erez *et al.*, 1988; Luedeling *et al.*, 2009).

In contrast to our use of experimental manipulation of all three major cues, many other studies have used multiple field sampling dates (i.e., sampling once every several weeks across the winter) to assess the effect of one cue, most often chilling (Laube *et al.*, 2014; Weinberger, 1950; Zohner *et al.*, 2017), combined with controlled environments that manipulate the other cues (most often forcing and photoperiod). This is done based on the assumption that chilling accumulates across a winter season, yet forcing temperatures and photoperiod generally increase as well—meaning it may be hard to assess fully any one cue using this method. Studies using this method may thus underestimate the full suite of cues used to control spring phenology.

Study design plays an important role in all controlled environment phenology studies and can easily affect the findings and predictive utility of such studies in many ways. Studies that repetitively sample throughout the winter may less accurately measure each cue, but have the advantage of providing more realistic environmental conditions by capturing realistic shifts in all three cues across the winter-spring season (Basler & Körner, 2012), and thus play an important role in predicting near-term impacts of climate change. In contrast, many studies (e.g., Caffarra *et al.*, 2011; Laube *et al.*, 2014) create more extreme changes in cues to assess better whether a cue is present. For example, our study used a more extreme photoperiod difference between the two treatments, which may better detect photoperiod responses. Our four-hour photoperiod difference corresponds to a temporal change of 10-12 weeks in the spring at our two sites (see Supplemental Materials), which, while extreme, is not an impossible change given projected warming and variation seen to date (Stocker *et al.*, 2013; Wolkovich *et al.*, 2012). The drawback of this approach, however, is that the design is much more artificial in its climate and, given the extreme treatments, may be less relevant for near-term projections and difficult to robustly extrapolate to future conditions. Such designs may be more useful for rough estimates of longer-term predictions of phenological responses with climate change and/or for use in parameterizing process-based models, which often use a mix of results from observations and experiments.

Further, most phenological studies face limitations on how fully they can assess cues because of limited understanding of dormancy and its release preceding budburst (Chuine *et al.*, 2016; Cooke *et al.*, 2012). Because dormancy release cannot be easily assessed (Chuine *et al.*, 2016) most studies to date using individuals sampled from the field do not fully know at what stage in endo- or eco-dormancy an individual is before the experiment or exactly how much chilling or forcing has been received. At our sampling date in January all individuals would have received some degree already of all three cues, but still responded significantly to all treatments. In particular our finding that all species responded to chilling suggests our sampled individuals must have all still been in endodormancy, since major responses to chilling are not expected after plants have moved from endo to ecodormancy (Chuine *et al.*, 2016). Our sampling date may also have affected our findings with regard to site effects. Chilling was the only factor to show noticeable differences due to site effects, which could indicate that chilling requirements vary across populations due to local adaptation, or it could be due to the field chilling experienced before we took cuttings for our experiments (see Table S4). Additionally, our finding of no major difference in the two different chilling temperatures could indicate that plants cannot assess chilling temperatures below some threshold (Coville, 1920; Guy, 2014; Harrington *et al.*, 2010), or accumulate chilling at a similar rate for the two temperatures we selected (e.g., Harrington & Gould, 2015), or that most met their chilling requirements at the higher chilling treatment. If the latter is true, then studies that sample much earlier in the season should find an elevated response to lower chilling temperatures. We performed a similar chilling experiment the following year and again found similar responses to chilling temperatures of 1°and 4°C (Figure S9). Finally, our limited knowledge of what controls dormancy release also makes determining which temperatures are 'forcing' and which are 'chilling' difficult. Chilling is often assumed to happen below 5°C, but may occur also between 5-10°C (or higher) depending on the species and study (Harrington & Gould, 2015; Luedeling *et al.*, 2013). This may make our low forcing treatment (15/5°C) a possible nighttime chilling treatment, depending on the species and exact conditions. This design, however, had the benefit of holding the diurnal temperature range—which has been suggested to alter budburst timing (Rossi & Isabel, 2017)—constant across treatments.

Phenological cues at the community scale

At the community level we found that each species had a unique suite of cues, leading to a generally staggered leafout (Fig. 3). This provides support for the idea that spring phenology is an important component of the temporal niche (Gotelli & Graves, 1996; Loreau & de Mazancourt, 2008). Species cues varied depending on the phenophase considered, meaning species' responses also shuffled between the two stages (Fig. 2). This fundamentally means that the species that bursts bud first will not necessarily leaf out first. Such differences may be because budburst and leafout represent fundamentally different responses (Basler & Körner, 2014): budburst is cued by forcing and photoperiod, whereas leafout generally requires biosynthesis, thus forcing and photoperiod may act more as limiting factors than cues for leafout.

These quantitatively diverging findings for each phenophase suggest complex dynamics in the early season within a community of woody plant species. Increasing evidence suggests the period between budburst and leafout is when plants are at greatest risk of tissue loss from frost (Lenz *et al.*, 2013), and these new insights have come at the same time that research suggests risk of frost damage may increase with climate change (Augspurger, 2009; Dai *et al.*, 2013). For early-season species in particular, this period may be critical to their current and future performance. Our results suggest that the cues for each stage are not identical and support other work suggesting cues on bud swelling and budburst may be distinct from the cues governing the development afterwards (Basler & Körner, 2014). Understanding budburst is particularly difficult as it is the first observable event after a series of unobservable (but see Rinne *et al.*, 2011), yet important physiological events required for budburst (Caffarra *et al.*, 2011; Vitasse *et al.*, 2014) and our results echo calls for increased research in this topic (Chuine *et al.*, 2016), which spans molecular, cellular and whole plant areas of study (Morin *et al.*, 2009; Rinne *et al.*, 2011; Singh *et al.*, 2017).

Our community-level findings may help build on our understanding of what factors ultimately shape each species' mix of cues for budburst and leafout. Recent work has addressed this issue by examining how attributes such native/invasive status, climatic range, or climatic history predict cues (e.g., Laube *et al.*, 2015; Zohner *et al.*, 2017). Building on these insights will require improved understanding of phenology's role in defining a species niche and controlling its inclusion in a community. For example, assembly theory suggests early-active species could out-compete later-active species through priority effects, which would produce communities where all species leafout early. When this is not the case (as in our data and many other systems) trade-offs may explain variation in phenology at the community-level (Chesson & Huntly, 1997). In temperate forests one dominant hypothesis for this trade-off is that early-active species should also have traits that allow them to survive or avoid tissue loss to frost (Sakai & Larcher, 1987) while later-active species would need traits that allow them to be competitive for resources after other species have already had access to resource pools (e.g., soil nutrients or light). Testing these hypotheses requires matched trait and phenology data with a focus on careful measures of frost sensitivity (e.g., the minimum temperatures tissues can experience without damage) and traits related to competition (e.g., resource uptake metrics or growth rates under varied nutrient and competitive environment regimes).

Conclusions

Across the two communities we studied, our results suggest species within a community have staggered budburst and leafout due to a mix of all three major environmental cues: forcing temperatures, photoperiod and chilling. In contrast to our hypothesis (and others', e.g., Körner & Basler, 2010), we found no evidence of any species being dominated by one or another cue; instead, species tended to show similar cues, especially between forcing and photoperiod cues. Thus, accurately predicting the phenology of any one of our studied species under diverse environmental conditions would require considering how all three cues will change in concert. This is an especially difficult task given that climate change projections that could be used to robustly estimate future forcing and chilling are difficult to obtain (e.g., many projections do not provide daily estimates). Shifting climate has already clearly altered forcing and potentially chilling across the globe (IPCC, 2014; Stocker *et al.*, 2013) with trends expected to continue and possibly accelerate. In contrast, photoperiod has not, and will not, shift; however plants that advance their leafout will experience large shifts in photoperiod. These trends combined with our results mean that all 28 species we studied could potentially show complex, non-linear responses in the future, with cascading community and ecosystem consequences.

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Author Contributions:

DFBF and EMW conceived of the study design, performed analyses and wrote the paper. DFBF also carried out the experiment.

Data, Code & Model Output:

Raw data will be available via the Harvard Forest Data Archive upon publication and are available to all reviewers upon request. Stan model code and output provided upon request.

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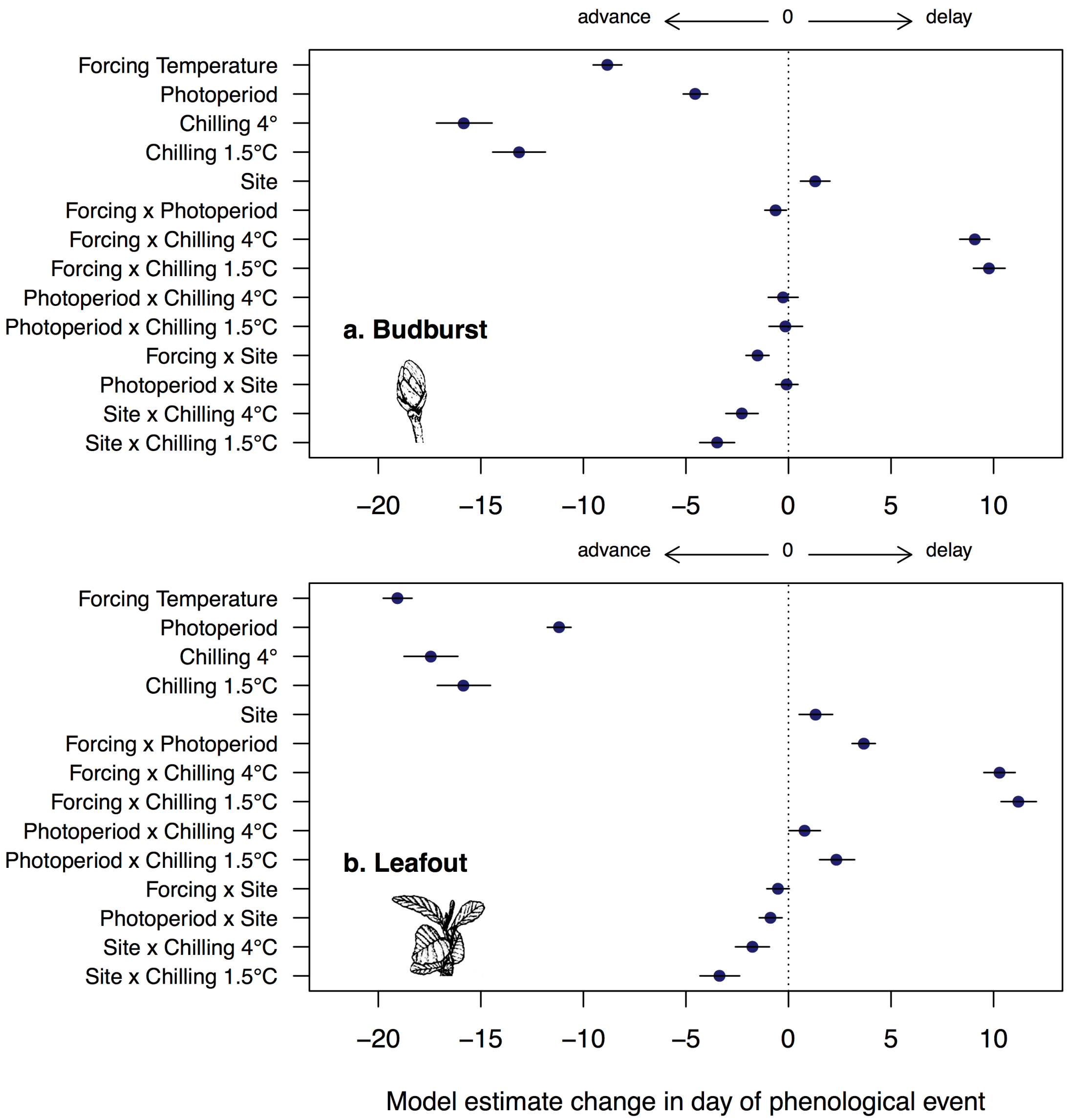

Figure 1: Effects of forcing temperature, photoperiod, chilling and site on budburst (a) and leafout (b) days across 28 species. Dots and bars show mean and 50% credible intervals from a Bayesian hierarchical model that also incorporated species-level variations (see Tables S2-S3; Figs. 1, S2-S3). Advances in phenology are shown by negative numbers; delays are shown as positive. Forcing temperatures and photoperiods were two levels each (see Methods), and chilling treatments were applied for 30 days. Budburst and leafout images from Finn *et al.* (2007).

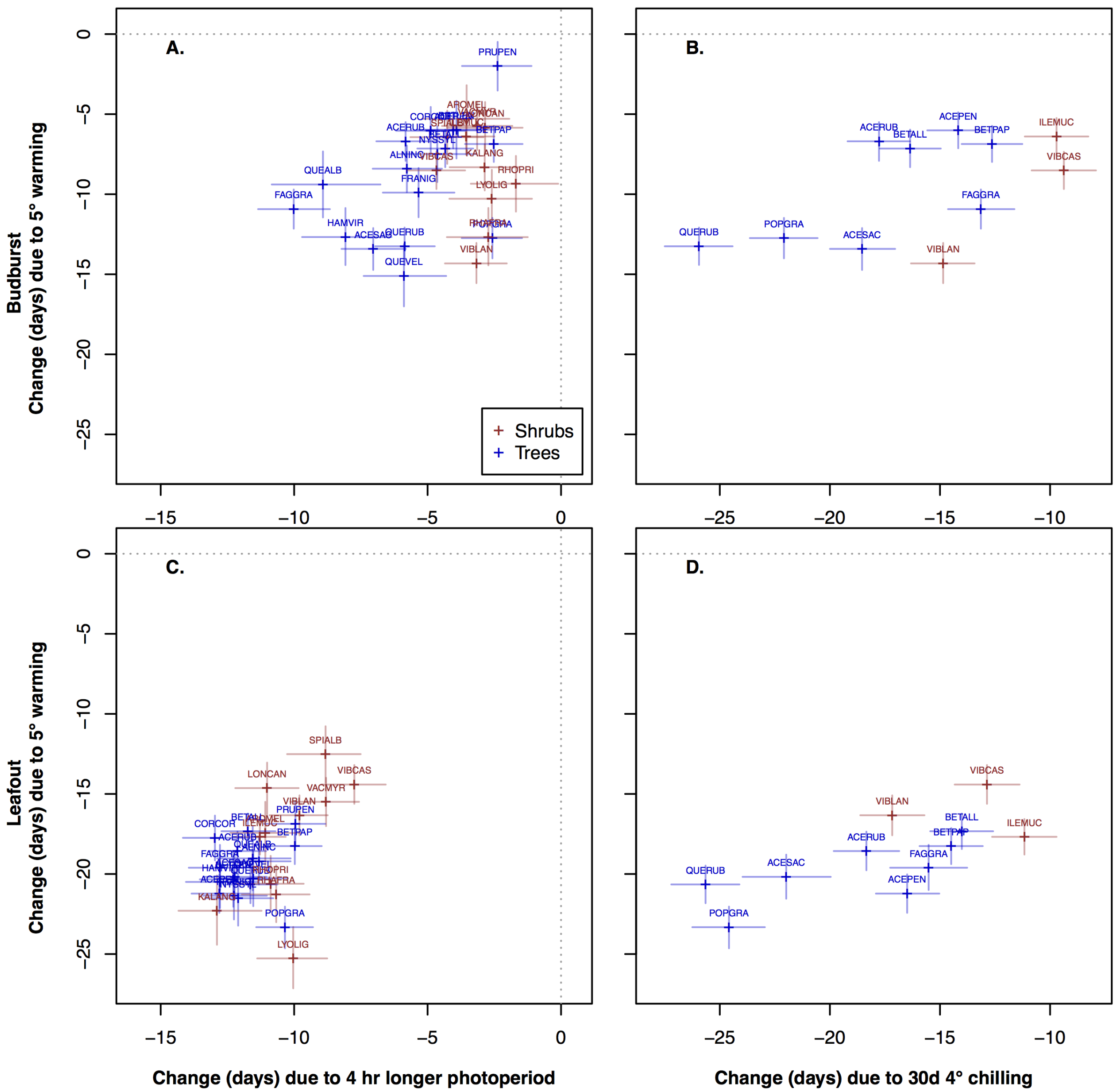

Figure 2: Effects of photoperiod, temperature and chilling across species (shrub species shown in red, tree species in blue): Crosses and bars show mean and 50% credible intervals from a Bayesian hierarchical model (see Tables S2-S3; Figs. 1, S2-S3). For visualization purposes, species names are represented by the first three letters of the genus and first three letters of the species epithet (see Table S1 for full species names and Fig. S5-S6 for additional versions of figure).

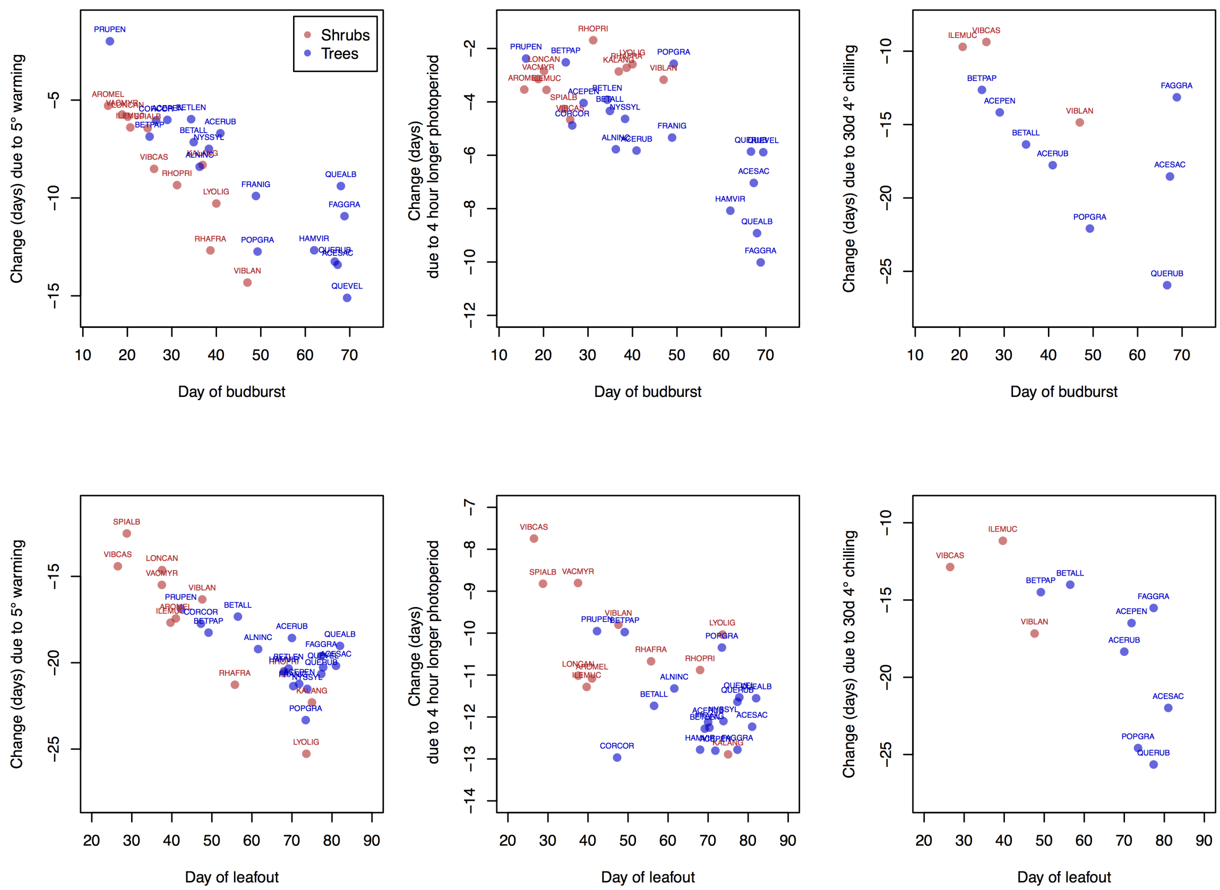

Figure 3: Effects of photoperiod, temperature and chilling across species compared to day of budburst (upper panels) or leafout (lower panels): we show mean estimates of sensitivity to warming, photoperiod, and chilling from a Bayesian hierarchical model (see Tables S2-S3; Figs. 1, S2-S3). For visualization purposes, species names are represented by the first three letters of the genus and first three letters of the species epithet (see Table S1 for full species names).