

Late spring freezes coupled with warming winters alter temperate tree phenology and growth

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Summary

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Received: 17 December 2020

Accepted: 13 April 2021

New Phytologist (2021) **231**: 987–995
doi: 10.1111/nph.17416

Key words: climate change, budburst, false spring, forest recruitment, phenology, spring freeze, temperate, temporal reassembly.

- Spring phenology is advancing with warming but late spring freezes may not advance at the same rate, potentially leading to an increase in freezes after trees initiate budburst. Research suggests warming winters may delay budburst through reduced chilling, which may cause plants to leafout more slowly, thus decreasing spring freeze tolerance.
- Here, we assessed the effects of late spring freezes and reduced over-winter chilling on sapling phenology, growth and tissue traits, across eight temperate tree and shrub species in a laboratory experiment.
- We found that spring freezes delayed leafout – extending the period of greatest risk for freeze damage – increased damage to the shoot apical meristem, and decreased leaf toughness and leaf thickness. Longer chilling accelerated budburst and leafout, even under spring freeze conditions. Thus, chilling compensated for the adverse effects of late spring freezes on phenology. Despite the effects of spring freezes and chilling on phenology, we did not see any major reordering in the sequence of species leafout.
- Our results suggest climate change may impact forest communities not through temporal reassembly, but rather through impacts on phenology and growth from the coupled effects of late spring freezes and decreased over-winter chilling under climate change.

Introduction

The timing of spring in temperate deciduous forests shapes plant and animal communities and influences ecosystem services and forest management. With increasing temperatures, spring phenology (i.e. budburst and leafout, which are strongly cued by temperature) is advancing, reshaping ecosystem dynamics. In one major example, advancing spring phenology has led to increased carbon uptake across temperate forests, which are essential carbon sinks that combat the negative effects of climate change (Keenan *et al.*, 2014). However, climate change could diminish or reverse these positive effects on carbon storage, specifically through cold snaps during the spring and increasing temperatures in the winter.

While climate change has warmed the Northern Hemisphere, cold snaps and freezing events are still occurring. These weather events can have major impacts on plant development each spring. One such event is a late spring freeze (Marino *et al.*, 2011), often defined as when temperatures drop below freezing (i.e. below -2.2°C , Schwartz *et al.*, 2002) after budburst has initiated. Damage from late spring freeze events can have cascading effects within a forest, from changes to nutrient cycling and carbon uptake to shifts in seedling recruitment (Hufkens *et al.*, 2012; Richardson *et al.*, 2013; Klosterman *et al.*, 2018). Furthermore, late spring freezes can increase the probability of additional freezes within a growing season by extending the period in which

plants are most at risk – the time between budburst and leafout, what we refer to as the ‘duration of vegetative risk’. This may result in a positive feedback loop: observational studies suggest plants take longer to reflush leaves after a late spring freeze – up to 38 d (Gu *et al.*, 2008; Augspurger, 2009, 2013; Menzel *et al.*, 2015) – which could lead to additional late spring freezes in a season (Augspurger, 2009). As late spring freezes are predicted to increase in certain regions with increased climate change (Ault *et al.*, 2015; Liu *et al.*, 2018; Zohner *et al.*, 2020), understanding their impacts is essential for robust forest management strategies and climate forecasting (Kral-O’Brien *et al.*, 2019).

Warmer winters may also play a critical role in the future of forests as they directly impact one of the major cues plants use to time budburst: over-winter low temperatures (chilling), in addition to increasing spring temperatures (forcing) and longer daylengths (Chuine *et al.*, 2016). Many temperate plant species have evolved chilling requirements to avoid leafout during warm snaps in the middle of the winter, but with climate change, chilling requirements may not be met. If chilling is not met, plants may leafout much slower or incompletely, which can in turn affect freeze tolerance. Thus, predicting climate change impacts on late spring freezes may also require understanding the interplay of warming winters and late spring freeze risk in forest tree species.

Understanding how winter chilling and late spring freezes may affect future forests requires knowing whether this interaction varies across species within a community. This is especially true if species have evolved along a trade-off of risking spring freezes for early access to resources. While a single-species perspective may predict all individuals to require high levels of chilling to delay budburst and ultimately diminish late spring freeze risk, competition between species for nutrients, water and light resources in the early spring probably pushes some species – and some individuals within species – to leafout earlier (Augspurger, 2013). Young trees and understory species generally initiate budburst before canopy trees to benefit from higher light levels (Augspurger, 2008; Vitasse *et al.*, 2013), which potentially puts these species and individuals at higher risk of freeze damage (Vitasse *et al.*, 2014). Thus, successful forest recruitment requires seedlings and saplings to minimize late spring freeze risk while maximizing growth.

The combination of species- and lifestage-level differences in response to late spring freezes, chilling and climate change could reshape the temporal assembly of forest communities. Species typically leafout in a similar sequence, with understory species leafing out earlier and higher canopy trees leafing out last, but many studies predict substantial shifts in chronological order and reassembly of species' leafout with climate change (Laube *et al.*, 2013; Roberts *et al.*, 2015). The effects of warming on phenological cues, especially on chilling and forcing (through warming winters and springs, respectively), combined with its effects on late spring freezes, could alter competition and recruitment among forest species for early-season resources. Such shifts could in turn impact species diversity and carbon uptake in temperate forests.

Here, we assessed the effects of over-winter chilling length and late spring freezes on sapling phenology and growth across eight temperate tree and shrub species. We exposed individuals to different levels of over-winter chilling crossed with a late spring freeze event in growth chambers, then followed individuals for a growing season in a glasshouse to understand: how does over-winter chilling impact phenology, growth and physical leaf traits; how do late spring freeze events impact phenology, growth and physical leaf traits; and how does the interaction between chilling and late spring freezes impact community structure and phenological order?

Materials and Methods

Plant selection and material

We selected eight temperate woody plant tree and shrub species (Supporting Information Table S1) that span varying spring phenologies (i.e. early to later leafout), are not generally used as crops or ornamental species, and are common in middle and eastern North American forests: *Acer saccharinum*, *Alnus incana rugosa*, *Betula papyrifera*, *Betula populifolia*, *Cornus racemosa*, *Salix purpurea*, *Sorbus americana* and *Viburnum dentatum* (we originally included two additional species – *Fagus grandifolia* and *Nyssa sylvatica*, but the plants were not delivered in a usable condition

and thus we excluded them from the experiment). We used 48 dormant, 1-yr-old, bare root saplings – each measuring 6–12 inches – for each species from Cold Stream Farm LLC (Freesoil, MI, USA; 44°6'N, 86°12'W) for a total of 384 individuals. Upon receipt, on 21 November 2018, plants were potted in 656 ml deepots with Fafard #3B Metro Mix soil and placed in growth chambers at the Weld Hill Research Building of the Arnold Arboretum (Boston, MA, USA; 42°17'N, 71°8' W) at 4°C for different durations depending on chilling treatment (explained below).

Growth chamber and glasshouse conditions

Individuals were randomly selected for one of six experimental treatments from a full factorial design of late spring freeze (two levels: presence or absence of late spring freeze) × chilling (three levels: 8, 6 or 4 wk of chilling at 4°C with an 8 h photoperiod, lighting was a combination of T5HO fluorescent lamps with halogen incandescent bulbs at c. 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$; see Table S2 for conversion of chilling duration to chilling hours, Utah model and chill portions). Individuals were rotated within and among growth chambers every 2 wk to minimize bias from possible growth chamber effects.

Once each chilling treatment was completed, we moved individuals to a glasshouse with mean daytime temperature of 15°C and a mean nighttime temperature of 10°C, and a daily photoperiod of 12 h (lighting was from LEDs VYPRx Plus glasshouse fixtures at c. 159 $\mu\text{mol m}^{-2} \text{s}^{-1}$) throughout the spring until all individuals reached full leaf expansion. After all individuals of all species reached full leaf expansion, glasshouse temperatures and photoperiods were kept ambient, and all individuals were up-potted to 983 ml deepots and fertilized with Scotts 15-9-12 Osmocote Plus 5-6. Due to experimental and facility constraints, all individuals received the same late summer glasshouse conditions; this included changes in temperature and photoperiod generally associated with dormancy onset. Therefore, individuals that received longer chilling treatments (6 and 8 wk of chilling) effectively experienced shorter growing season conditions.

Phenology and late spring freeze treatment

We recorded phenology (using the BBCH scale, which defines and describes the phenological development stages (Meier, 2001)) every 2–3 d through full leaf expansion. The duration of vegetative risk (Chamberlain *et al.*, 2019) was measured as the duration in days between budburst date (BBCH stage 07, which is defined as the 'beginning of sprouting or bud breaking; shoot emergence'; Finn *et al.*, 2007) and the date of full leaf expansion (BBCH stage 19, defined as 'leaf expansion complete' or when all petioles have emerged and the leaves have completely unfolded) for each individual. Individuals in the 'late spring freeze' treatment were placed in a growth chamber set to mimic a late spring freeze event during budburst, defined as once at least 50% of the buds were at BBCH stage 07 but the individual had not yet reached BBCH stage 19 (i.e. each sapling was exposed to a late spring freeze based on its individual phenological timing). Late

spring freeze treatments lasted *c.* 14 h, beginning at 18:00 h; temperatures were ramped down to -3°C for 3 h (Fig. 1). At *c.* 08:00 h the following day, we placed late spring freeze individuals back in the glasshouse. Once the leaves from the first flush on all individuals reached full leaf expansion (BBCH stage 19), we recorded phenology weekly until 1 August 2019, then every 2–3 d again to monitor fall phenology. Our intention was to observe the delay and damage to the original leaves. We monitored all individuals until complete budset.

Growth measurements

We measured shoot growth as a metric of plant height three times throughout the growing season: the day an individual reached full leaf expansion (BBCH stage 19), 60 d after full leafout and when an individual reached complete budset. The final shoot growth was measured as the difference in height on the date of complete budset from the height on the date of full leaf expansion.

We measured the Chl content of four leaves on each individual 60 d after full leafout using an atLEAF CHL PLUS Chlorophyll meter, converting Chl content to mg cm^{-2} using the atLEAF CHL PLUS conversion tool. We measured leaf thickness using a Shars Digital Micrometer (accurate to 0.001 mm) and leaf toughness in newtons using a Shimpo Digital Force Gauge on two leaves for each individual 60 d after full leafout. Additionally, we qualitatively measured damage to the shoot apical meristem (score of 0/1), where a score of 1 consisted of complete damage or disruption of growth in the main stem and resulted in early dormancy induction or reliance on lateral shoot growth (see Methods S1: ‘Measuring shoot apical meristem damage’ and Fig. S1 for more details). Finally, we harvested each plant after it reached complete budset to dry, separate and weigh below- and aboveground biomass (including leaves).

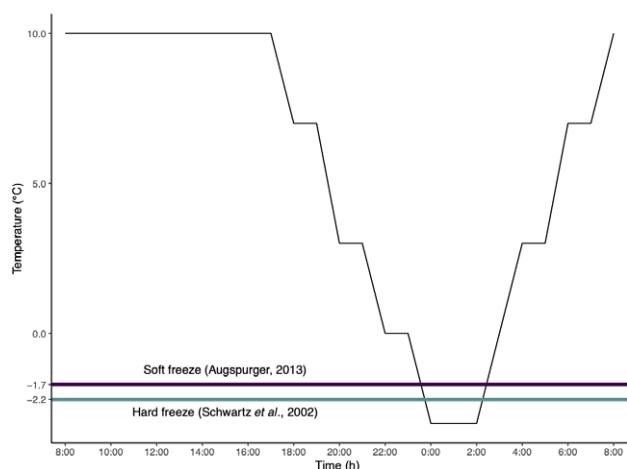


Fig. 1 Late spring freeze treatment temperature regime in the growth chamber. Lines show estimated freeze levels for a soft freeze (defined as temperatures falling below -1.7°C after budburst, when damage is known to occur in certain portions of a forest; Augspurger, 2013) or a hard freeze (defined as temperatures falling below -2.2°C , when damage is known to occur across more species and larger portions of forest habitats; Schwartz *et al.*, 2002).

Data analysis

We used Bayesian hierarchical models (with the BRMS package, v.2.3.1, Bürkner, 2017) in R (v.3.3.1, R Development Core Team, 2017) to estimate the effects of chilling duration, late spring freeze treatment and all two-way interactions as predictors on: duration of vegetative risk, growing season length, shoot apical meristem damage, total shoot growth, total biomass, Chl content, leaf toughness and leaf thickness. We modeled species hierarchically as grouping factors, which generated an estimate and posterior distribution for each species as well as an overall response across the eight species used in our experiment (see Methods S2: ‘Data analysis and model equations’ for more details). We ran four chains, each with 4000 iterations, of which 2500 were warm-up iterations, for a total of 6000 posterior samples for each predictor for each model using weakly informative priors (increasing priors three-fold did not impact our results). We evaluated our model performance based on \widehat{R} values that were close to 1.0, checked chain convergence and posterior predictive checks visually (Gelman *et al.*, 2014), and made sure all models had zero divergent transitions. Our models generally had high n_{eff} (6000 for most parameters, but as low as 1400 for a couple of parameters in the shoot apical meristem model).

We report mean estimated effect sizes in two ways. First, we give the percentage change (decrease/increase), which provides an estimate of relative change comparable across different response variables: for continuous response values this is the estimated mean effect (from the posterior) divided by the intercept (no late spring freeze under 8 wk of chilling treatment), while for binomial response values we converted to probability percentages and then back converted to the original scale by multiplying by two standard deviations following Gelman & Hill (2006). Second, we present the mean effect in natural units alongside a \pm uncertainty estimate calculated from the 90% uncertainty interval of the posterior (taking the average of the 5% and 95% posterior estimates; the posteriors were generally symmetrical), except for values from the simple linear models which are reported as means \pm SE. We use 90% uncertainty intervals because they are computationally stable (Gelman & Hill, 2006; Carpenter *et al.*, 2017), and we provide 96% uncertainty intervals in Tables S3–S10. We report treatments as having an effect when the 90% uncertainty intervals do not overlap zero. Most reported treatment effects do not overlap zero at the 96% uncertainty intervals either; when they do we report both the 90% and the 96% uncertainty intervals.

Results

Chilling durations impacted individual phenology. As seen in many other studies (Chuine *et al.*, 2001; Laube *et al.*, 2013; Ettinger *et al.*, 2020), we found that decreases in chilling delayed day of budburst by 4.8 ± 1.8 d for 6 wk of chilling and by 7.6 ± 1.8 d for 4 wk of chilling (as mentioned above, all values are given relative to the no late spring freeze under 8 wk of chilling treatment; Table S11). Shorter chilling also slowed the rate of leafout, increasing the duration of vegetative risk under both

shorter chilling treatments (6 wk: 15.7%, 2.1 ± 1.4 d; 4 wk: using 90% uncertainty intervals, 20.1%, 2.7 ± 2.5 d, 96% uncertainty intervals, 2.7 ± 3.3 d; Fig. 2a; Tables S3 and S12). Decreased chilling shortened the growing season for individuals exposed to 4 wk of chilling but not for individuals exposed to 6 wk of chilling (6 wk: 0.9%, 2.5 ± 8.0 d; 4 wk: using 90% uncertainty intervals -3.4% , -9.7 ± 8.2 d, 96% uncertainty intervals -9.7 ± 10.4 d; Fig. 2b; Table S4), due to constraints on our experimental design: individuals in longer chilling treatments were put in the glasshouse 2–4 wk later than the 4 wk of chilling treatment, but all individuals experienced the same ambient late summer conditions when in the glasshouse, including shifts in temperature and light associated with late summer/autumn that generally trigger dormancy onset. This shortened the growing season for individuals that experienced longer chilling.

Late spring freezes also impacted individual phenology. Individuals exposed to the late spring freeze treatment had longer durations of vegetative risk (8 wk: 26.9%, 3.6 ± 1.3 d). Effects on the duration of vegetative risk from late spring freeze treatments and from decreased chilling were generally additive, resulting in slightly longer durations given late spring freeze with 6 wk of chilling (31.3%, 4.2 ± 1.8 d) and even longer durations of vegetative risk for individuals exposed to a late spring freeze with only 4 wk of chilling (42.5%, 5.7 ± 1.8 d, Fig. 2a; Table S3).

Late spring freezes impacted growth habit and shoot growth but not total biomass. Across all chilling treatments, late spring

freezes led to more damage to the shoot apical meristem (8 wk: 50.0% increase in probability of damage under late spring freeze treatment or 2.0 ± 1.4 ; 6 wk: 37.5% or 1.5 ± 2.1 ; and 4 wk: 50.0% or 2.0 ± 2.2 ; Fig. 2c; Table S5). There was little change in shoot growth over the growing season across all chilling treatments under late spring freeze conditions (8 wk: 9.4%, 5.1 ± 7.0 cm; 6 wk: -0.7% , -0.4 ± 10.3 cm; 4 wk: 8.7%, 4.7 ± 9.0 cm) but shoot growth increased with 4 wk of chilling durations (4 wk: 20.4%, 11.0 ± 6.6 cm; Fig. 2d; Table S6). There was very little change in total biomass under late spring freeze conditions (compared to the no late spring freeze) across all treatments (8 wk: -7.0% , -3.4 ± 4.6 g; 6 wk: -7.4% , -3.6 ± 4.7 g; 4 wk: 5.9%, 2.9 ± 6.5 g; Fig. 2e; Table S7).

Late spring freezes also affected physical leaf traits. Late spring freeze treatments decreased leaf toughness across the 8 and 6 wk of chilling treatments (8 wk: -7.5% , -0.03 ± 0.03 N; 6 wk: -7.5% , -0.03 ± 0.04 N) and decreases in chilling led to increased toughness for the 4 wk of chilling (4 wk: 12.5%, 0.05 ± 0.04 N; Fig. 2f; Table S8). There was little change in Chl content in leaves under late spring freeze conditions with 8 and 6 wk of chilling (8 wk: -1.3% , -0.4 ± 1.3 mg cm $^{-2}$; and 6 wk: -4.8% , -1.5 ± 1.9 mg cm $^{-2}$) and there was little change with decreased chilling durations on Chl content (6 wk: -0.2% , -0.06 ± 1.4 mg cm $^{-2}$; and 4 wk: 1.3%, 0.4 ± 1.4 mg cm $^{-2}$; Fig. S2; Table S9). Late spring freezes led to decreased leaf thickness in the 8 wk of chilling treatment, but there was little change

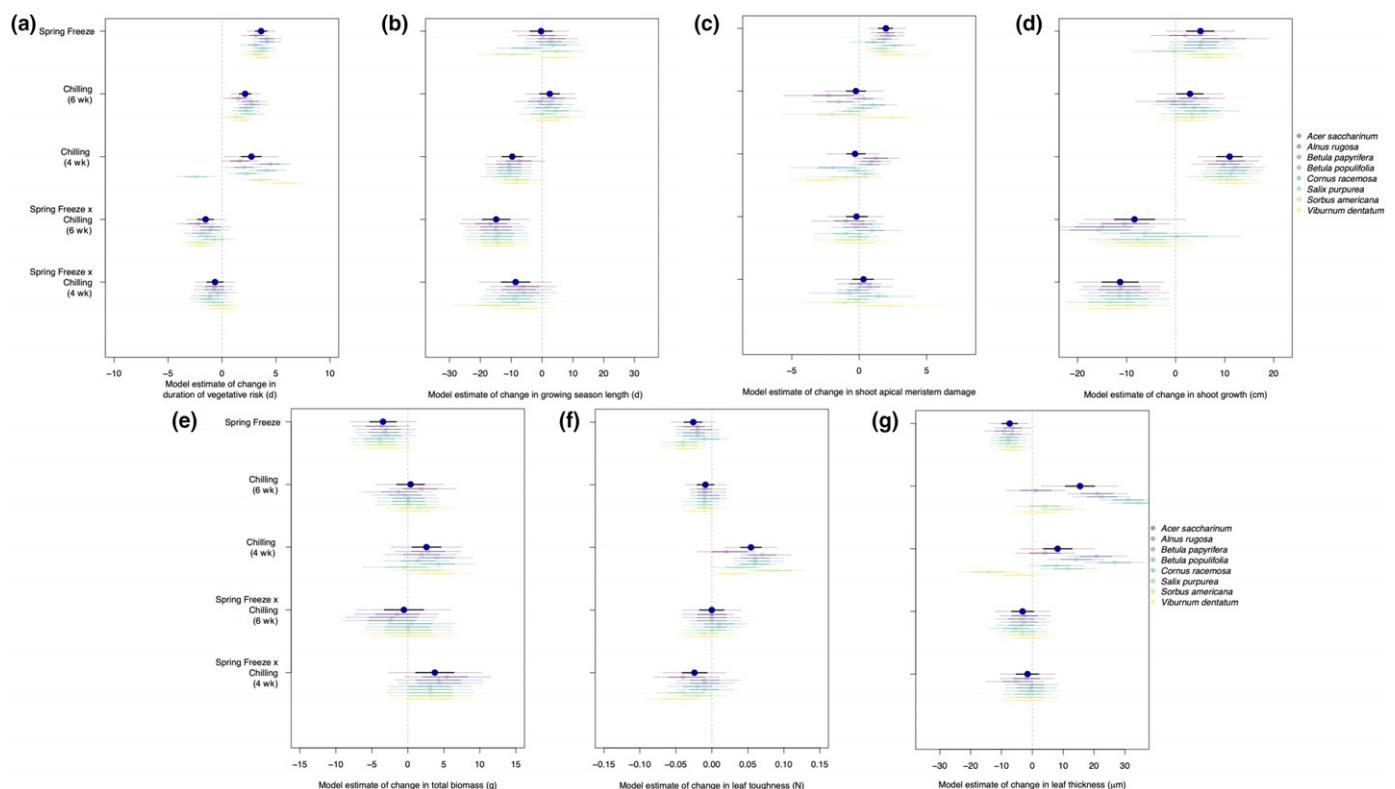


Fig. 2 Effects of late spring freeze treatment, 6 wk of chilling and 4 wk of chilling relative to 8 wk of chilling with no late spring freeze on (a) the duration of vegetative risk (d), (b) growing season length (d), (c) shoot apical meristem damage, (d) total shoot growth as a metric of height (cm), (e) total above- and belowground biomass (g), (f) leaf toughness (N) and (g) leaf thickness (μ m). Dots and thin lines show means and 90% uncertainty intervals and thicker lines show 50% uncertainty intervals. Larger blue dots represent overall estimates across all species, while smaller dots are estimates for each species.

for the 6 and 4 wk of chilling treatments (8 wk: using 90% uncertainty intervals – 5.5%, $-7.4 \pm 6.3 \mu\text{m}$, 96% uncertainty intervals, $-7.4 \pm 8.2 \mu\text{m}$; 6 wk: 3.7%, $4.9 \pm 8.9 \mu\text{m}$; and 4 wk: -0.5%, $-0.7 \pm 9.0 \mu\text{m}$) and decreased chilling durations led to increased leaf thickness for 6 wk of chilling but not for 4 wk of chilling (6 wk: using 90% uncertainty intervals, 11.5%, $15.4 \pm 12.4 \mu\text{m}$, 96% uncertainty intervals, $15.4 \pm 16.2 \mu\text{m}$; and 4 wk: 6.1%, $8.2 \pm 12.0 \mu\text{m}$; Fig. 2g; Table S10).

Late spring freezes and chilling treatments were generally consistent across species, though not always. The duration of vegetative risk decreased for most species with longer chilling (i.e. 8 wk), except for *S. purpurea*, which experienced shorter durations of vegetative risk with shorter chilling (Fig. 2a). Late spring freezes led to meristem damage across all species except for *B. populifolia* and *S. americana*. Additionally, *V. dentatum* experienced consistent meristem damage under all treatments (Fig. 2c). The effects on leaf thickness were especially variable across species under the longer chilling treatments, specifically with *S. americana* and *V. dentatum* having thicker leaves with increased chilling (Fig. 2g).

Despite large treatment effects on phenology, we found no major effects on phenological rank within the community. The order of leafout timing was consistent across all treatments, with *S. purpurea* always being first to leafout, followed by *B. papyrifera*, *B. populifolia* and *C. racemosa*, followed by *A. rugosa*, *S. americana*, *V. dentatum* and *A. saccharinum* (Fig. 3). *Viburnum dentatum* was the only species to change rank across treatments, though it was consistently grouped with the later-leafout species. The order of budset timing was also consistent across all treatments, with *C. racemosa* and *S. americana* being first to set bud, followed by *B. papyrifera*, *A. saccharinum* and *V. dentatum*, followed by *B. populifolia*, *S. purpurea* and *A. rugosa* (Fig. S3). *Acer saccharinum* was the only species to change budset rank across treatments, though it was grouped consistently with *B. papyrifera* and *V. dentatum*.

Discussion

Our experiment examined the consequences of two major interactive effects of climate change across eight deciduous forest tree species – late spring freezes and over-winter chilling. Our results confirmed the major features of late spring freezes (plant damage) and chilling (advancing spring phenology), and then highlighted how these two effects altered multiple aspects of plant phenology, plant growth and leaf traits. Importantly, we found late spring freezes and decreased chilling have additive effects on the duration of vegetative risk. This suggests that the combination of increased late spring freezes and warmer winters could be especially detrimental to forest communities by extending the period of lowest freeze resistance and making multiple late spring freeze events more common with warming.

Late spring freezes and chilling influence risk, damage and growth

Chilling length greatly influences spring phenology during the critical budburst to leafout phases (Chuine *et al.*, 2001; Laube *et al.*, 2013), and thus may compensate for the detrimental effects of late spring freezes on phenology. Increased chilling consistently shortened the duration of vegetative risk across species, except for one outlier species (i.e. *S. purpurea*, a species which shows complex responses to longer chilling that may be associated with reacclimation of cold hardiness; Kalberer *et al.*, 2006; Arora & Rowland, 2011). With late spring freezes lengthening the duration of vegetative risk, the risk of multiple late spring freezes occurring during this sensitive period in one season increases. Under our experimental conditions – where we applied a late spring freeze event at the same developmental phase for all individuals (effectively decoupling the covariation between risk and budburst timing in natural conditions, where frost risk generally decreases over time each spring and thus is lower for

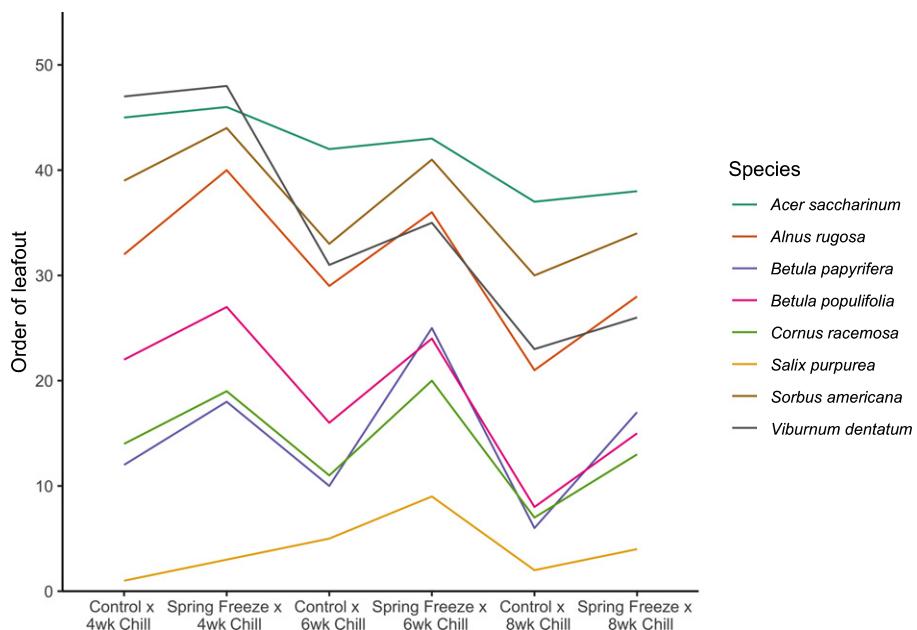


Fig. 3 Rank order of leafout across all species using mean trends.

later budbursting individuals) – we found that chilling can compensate for this increase in duration of vegetative risk (with increased chilling, the duration of vegetative risk did not increase under late spring freeze conditions). This suggests chilling is equally or more important for saplings in terms of limiting exposure to multiple late spring freezes. With climate change and increasing temperatures, over-winter chilling is anticipated to decrease (Laube *et al.*, 2013) and late spring freezes are predicted to increase in certain regions (Ault *et al.*, 2015; Liu *et al.*, 2018). This combination could greatly impact plant performance and survival, and shape species distributions due to the lengthening period of lowest freeze resistance and increased risk of multiple freeze events in one season, which could ultimately affect ecosystem processes, such as carbon uptake and nutrient cycling.

Our results suggest the combination of late spring freezes and shifts in chilling impact sapling growth. We found that late spring freezes impacted sapling growth through damage to the shoot apical meristem consistently across most species, regardless of a species phenological order (i.e. early vs late budburst). This is in contrast with past studies that have found early-budburst species can withstand lower temperature thresholds (Lenz *et al.*, 2013; Muffler *et al.*, 2016), and may be due to the different species we studied or differences in methodology (e.g. many studies freeze leaves while we froze entire saplings). Damage to the shoot apical meristem can lead to reliance on lateral shoot growth, rendering inefficient growth patterns and – if damage is significant within a forest stand – can lead to declines in recruitment (Rhodes & Clair, 2018). Our results supported that late spring freezes may reshape growth patterns, but showed it was highly dependent on chilling. We found growth was reduced when late spring freezes were combined with longer chilling, but this did not translate directly into lower biomass; instead biomass was fairly constant with slight reductions under treatments that showed the most growth (lower chilling \times late spring freeze). Given these results it is likely that lateral shoot growth accounted for reduced apical shoot growth, though we did not measure lateral shoot growth to confirm this. Thus, the combination of warming through reduced chilling and increased late spring freezes could impact both plant growth and tissue investment patterns and – subsequently – affect overall sapling performance and competition among saplings.

Layered onto shifts in performance via changes in the growth patterns of saplings, we found both late spring freezes and chilling impacted important leaf traits. Leaf traits can help capture the challenge plants face in balancing leaf construction costs – where thicker, tougher leaves may have higher costs to build but pay off via greater resistance to herbivory and other biotic and abiotic damage (Wright *et al.*, 2004; Onoda *et al.*, 2011) – against growth potential – where thinner, less tough leaves may allow rapid growth, or in the case of late spring freezes, rapid regrowth, to take advantage of available resources including light (Edwards *et al.*, 2014; Diáz *et al.*, 2016). Our experiment considered only a small slice of these complex traits (which vary across clades, habitats and a growing season, McKown *et al.*, 2013), but suggest consistent shifts in leaf quality due to late spring freezes,

which decreased leaf toughness and leaf thickness. However, we also found that increased chilling levels consistently decreased leaf toughness, with increased chilling combined with late spring freezes weakly increasing toughness; together, this suggests a complex role of both late spring freezes and chilling in determining leaf traits. These results – though focused on saplings – support increasing work that suggests impacts of climate change on phenology may not simply mean longer growing seasons and thus increased carbon storage, but may be complicated by shifts in leaf traits, photosynthetic capacity and other plant metrics (Bauerle *et al.*, 2012; Stinziiano & Way, 2017; Huang *et al.*, 2020) that could alter predictions. Further studies that test whether these results hold for adult trees (to date studies suggest there is no difference between saplings and adults, Cuervo-Alarcon *et al.*, 2018), and consider additional traits, such as the secondary compounds and total phenolic content (Ayres, 1993; Webber & Mason, 2016), as well as photosynthetic rate of the leaves, will be critical to determine the singular and interactive effects of chilling and late spring freezes on leaf traits to predict changes with warming at a larger scale.

Late spring freezes and chilling did not reshape temporal assembly

Climate model projections and experimental studies with very low chilling predict substantial shifts in species leafout order under future climate change conditions (Laube *et al.*, 2013; Roberts *et al.*, 2015); other studies using long-term phenology observations, however, suggest leafout phenology order is consistent across years (Wesolowski & Rowinski, 2006). We did not find major shifts in species leafout order – consistent with observational studies (Wesolowski & Rowinski, 2006) – except for *V. dentatum*, though it still leafed out within the late-leafout group of species across all treatments. Additionally, effects of late spring freeze and chilling treatments did not obviously impact growth or leaf traits based on rank, except for leaf thickness where species generally grouped by leafout order. Our results are also in line with some experimental studies: for example, in one full factorial growth chamber experiment, most treatments did not lead to substantial phenological reordering, except when individuals experienced little to no field chilling (Laube *et al.*, 2013). Therefore, our study tentatively supports multiple lines of evidence that suggest climate change may not cause major reassembly of forest communities due to winter warming or increases in late spring freezes. These conclusions, however, extend only as far our experimental design applies. Our late spring freeze treatment caused noncatastrophic damage to most individuals and may not scale up to some of the dramatic events observed in natural systems (Gu *et al.*, 2008; Augspurger, 2009). Additionally, our chilling treatments may not capture the full range of future shifts in chilling (current forecasts for chilling vary highly across regions, see Fraga *et al.*, 2019), especially as chilling is not well understood (Nanninga *et al.*, 2017) and may depend on fall temperatures, diurnal temperature ranges and other factors our design did not consider (Dennis, 2003). Our findings that chilling has cascading effects on phenology, growth and leaf traits suggest we

need to better understand over-winter chilling to best translate laboratory studies to the field and to make accurate predictions.

Phenological rank remained consistent across all of our late spring freeze and chilling treatments – where all species were affected equally. In nature, however, not all of our study species are at equal risk of late spring freezes, with early-budburst species (e.g. *S. americana* or *B. papyrifera*) generally more at risk than later-budburst species (e.g. *A. saccharinum* or *V. dentatum*). This suggests that climate change could reshape forest communities, though not directly through temporal assembly. Instead, our results suggest that change may come from the effect of late spring freeze and reduced chilling on physical damage, with potential impacts on physical leaf traits.

Late spring freezes and predicting forest recruitment

Our results from a laboratory experiment combined with previous work across diverse scales (Vitasse *et al.*, 2013; Yeaman *et al.*, 2016) highlight the complexity of predicting future forest recruitment with climate change. Changes in the growth patterns and leaf traits that we observed combined with advances in our molecular understanding of phenology (Satake *et al.*, 2013; Wilczek *et al.*, 2014; Fournier-Level *et al.*, 2016) suggest continued efforts to understand multivariate trait syndromes, and how these syndromes shift across climate and development (Diáz *et al.*, 2016; McKown *et al.*, 2013), may prove critical. While we studied a small set of traits, additional work could consider traits that may lower the risk of late spring freeze damage to temperate trees, such as increased ‘packability’ of leaf primordia in winter buds, which allows for more rapid leafout (Edwards *et al.*, 2017), increased trichome density on young leaves to protect leaf tissue against freezes (Prozherina *et al.*, 2003; Agrawal *et al.*, 2004) and buds with decreased water content to increase freeze tolerance (Beck *et al.*, 2007; Morin *et al.*, 2007; Norgaard Nielsen & Rasmussen, 2009; Poirier *et al.*, 2010; Kathke & Bruelheide, 2011; Hofmann & Bruelheide, 2015; Muffler *et al.*, 2016). Future cross-species studies could in turn leverage advances in our understanding of how phenological and freeze tolerance traits may relate. While we selected species for early vs late leafout, future studies would ideally select species across trait syndromes.

Scaling from laboratory and field experiments, such as ours, to robust forecasts of future forests will require integrating across lifestages, species and up to macroecological scales (McGill *et al.*, 2019). Over-winter chilling decreases with climate change will probably put saplings – which generally leafout earlier (Augspurger, 2009) – at greater risk of damage from late spring freeze events. This could lead to dieback of saplings, especially of early-budbursting species and/or individuals, which our results show could see increases in the durations of vegetative risk from the dual effects of lower chilling and heightened late spring freeze risk. Loss or reduction of early-budburst species could in turn have widespread consequences for competition among species and lifestages and reshape forest demographics in complex ways. Such complexity will likely be hard to predict without more accurate models of forest competition and development (Moorecroft

et al., 2001; Fisher *et al.*, 2018). Understanding how late spring freezes are changing and how equally – or not – these effects impact different species, especially their seedlings and saplings, is a crucial first step for future projections.

Acknowledgements

We would like to thank all of the growth facilities staff at the Weld Hill Research Building with special thanks to K. Woodruff, F. Rosin and W. Daly for their continued dedication to the project and glasshouse and laboratory assistance. We also thank all reviewers, D. Buonaiuto, A. Ettinger, J. Gersony, D. Loughnan, A. Manandhar and D. Sodhi for their continued feedback and insights that helped improve the experimental design, questions and manuscript.

Author contributions

CJC and EMW conceived the study, identified species to use in the study and determined which phenological and growth measurements to observe. CJC performed the analyses and produced all figures and tables. CJC wrote the paper, and both authors edited it.

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Data availability

Data and code from the analyses are available via KNB (<https://knb.ecoinformatics.org/view/doi:10.5063/F47MJ4>). Raw data, Stan model code and output are available on GitHub and will be provided upon request.

References

- Agrawal AA, Conner JK, Stinchcombe JR. 2004. Evolution of plant resistance and tolerance to frost damage. *Ecology Letters* 7: 1199–1208.
- Arora R, Rowland LJ. 2011. Physiological research on winter-hardiness: deacclimation resistance, reacclimation ability, photoprotection strategies, and a cold acclimation protocol design. *HortScience* 46: 1070–1078.
- Augspurger CK. 2008. Early spring leafout enhances growth and survival of saplings in a temperate deciduous forest. *Oecologia* 156: 281–286.
- Augspurger CK. 2009. Spring 2007 warmth and frost: phenology, damage and refoliation in a temperate deciduous forest. *Functional Ecology* 23: 1031–1039.
- Augspurger CK. 2013. Reconstructing patterns of temperature, phenology, and frost damage over 124 years: spring damage risk is increasing. *Ecology* 94: 41–50.
- Ault TR, Schwartz MD, Zurita-Milla R, Weltzin JF, Betancourt JL. 2015. Trends and natural variability of spring onset in the coterminous United States as evaluated by a new gridded dataset of spring indices. *Journal of Climate* 28: 8363–8378.
- Ayres MP. 1993. Plant defense, herbivory, and climate change. In: Kareiva PM, Kingsolver JG, Huey RB, eds. *Biotic interactions and global change*, vol. 75. Sunderland, MA, USA: Sinauer Associates 75–94.
- Bauerle WL, Oren R, Way DA, Qian SS, Stoy PC, Thornton PE, Bowden JD, Hoffman FM, Reynolds RF. 2012. Photoperiodic regulation of the seasonal

- pattern of photosynthetic capacity and the implications for carbon cycling. *Proceedings of the National Academy of Sciences, USA* 109: 8612–8617.
- Beck EH, Fettig S, Knake C, Hartig K, Bhattacharai T. 2007. Specific and unspecific responses of plants to cold and drought stress. *Journal of Biosciences* 32: 501–510.
- Bürkner PC. 2017. brms: an R package for Bayesian multilevel models. *Journal of Statistical Software* 80: 1–28.
- Carpenter B, Gelman A, Hoffman M, Lee D, Goodrich B, Betancourt M, Brubaker MA, Guo J, Li P, Allen R. 2017. Stan: a probabilistic programming language. *Journal of Statistical Software* 76. doi: 10.18637/jss.v076.i01
- Chamberlain CJ, Cook BI, de Cortazar Atauri IG, Wolkovich EM. 2019. Rethinking false spring risk. *Global Change Biology* 25: 2209–2220.
- Chuine I, Aitken SN, Ying CC. 2001. Temperature thresholds of shoot elongation in provenances of *Pinus contorta*. *Canadian Journal of Forest Research* 31: 1444–1455.
- Chuine I, Bonhomme M, Legave JM, García de Cortázar-Atauri I, Charrier G, Lacointe A, Améglio T. 2016. Can phenological models predict tree phenology accurately in the future? The unrevealed hurdle of endodormancy break. *Global Change Biology* 22: 3444–3460.
- Cuervo-Alarcon L, Arend M, Müller M, Sperisen C, Finkeldey R, Krutovsky KV. 2018. Genetic variation and signatures of natural selection in populations of European beech (*Fagus sylvatica* L.) along precipitation gradients. *Tree Genetics & Genomes* 14: 84.
- Dennis F. 2003. Problems in standardizing methods for evaluating the chilling requirements for the breaking of dormancy in buds of woody plants. *HortScience* 38: 347–350.
- Díaz S, Kattge J, Cornelissen JHC, Wright IJ, Lavorel S, Dray S, Reu B, Kleyer M, Wirth C, Colin Prentice I et al. 2016. The global spectrum of plant form and function. *Nature* 529: 167–171.
- Edwards EJ, Chatelet DS, Sack L, Donoghue MJ. 2014. Leaf life span and the leaf economic spectrum in the context of whole plant architecture. *Journal of Ecology* 102: 328–336.
- Edwards EJ, Chatelet DS, Spriggs EL, Johnson ES, Schlutius C, Donoghue MJ. 2017. Correlation, causation, and the evolution of leaf teeth: a reply to Givnish and Kriebel. *American Journal of Botany* 104: 509–515.
- Ettinger AK, Chamberlain CJ, Morales-Castilla I, Buonaiuto DM, Flynn DFB, Savas T, Samaha JA, Wolkovich EM. 2020. Winter temperatures predominate in spring phenological responses to warming. *Nature Climate Change* 10: 1137–1142.
- Finn G, Straszewski A, Peterson V. 2007. A general growth stage key for describing trees and woody plants. *Annals of Applied Biology* 151: 127–131.
- Fisher RA, Koven CD, Anderegg WRL, Christoffersen BO, Dietze MC, Farrior CE, Holm JA, Hurt GC, Knox RG, Lawrence PJ et al. 2018. Vegetation demographics in earth system models: a review of progress and priorities. *Global Change Biology* 24: 35–54.
- Fournier-Level A, Perry EO, Wang JA, Braun PT, Migneault A, Cooper MD, Metcalf CJE, Schmitt J. 2016. Predicting the evolutionary dynamics of seasonal adaptation to novel climates in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* 113: E2812–E2821.
- Fraga H, Pinto JG, JaA S. 2019. Climate change projections for chilling and heat forcing conditions in European vineyards and olive orchards: a multi-model assessment. *Climatic Change* 152: 179–193.
- Gelman A, Carlin JB, Stern HS, Dunson DB, Vehtari A, Rubin DB. 2014. *Bayesian data analysis, 3rd edn*. New York, NY, USA: CRC Press.
- Gelman A, Hill J. 2006. *Data analysis using regression and multilevel/hierarchical models*. Cambridge, UK: Cambridge University Press.
- Gu L, Hanson PJ, Post WM, Kaiser DP, Yang B, Nemani R, Pallardy SG, Meyers T. 2008. The 2007 Eastern US spring freeze: increased cold damage in a warming world. *BioScience* 58: 253.
- Hofmann M, Bruelheide H. 2015. Frost hardiness of tree species is independent of phenology and macroclimatic niche. *Journal of Biosciences* 40: 147–157.
- Huang JG, Ma Q, Rossi S, Biondi F, Deslauriers A, Fonti P, Liang E, Mäkinen H, Oberhuber W, Rathgeber CBK et al. 2020. Photoperiod and temperature as dominant environmental drivers triggering secondary growth resumption in northern hemisphere conifers. *Proceedings of the National Academy of Sciences, USA* 117: 20645–20652.
- Hufkens K, Friedl MA, Keenan TF, Sonnentag O, Bailey A, O'Keefe J, Richardson AD. 2012. Ecological impacts of a widespread frost event following early spring leaf-out. *Global Change Biology* 18: 2365–2377.
- Kalberer SR, Wisniewski M, Arora R. 2006. Deacclimation and reacclimation of cold-hardy plants: current understanding and emerging concepts. *Plant Science* 171: 3–16.
- Kathke S, Bruelheide H. 2011. Differences in frost hardiness of two Norway spruce morphotypes growing at Mt. Brocken, Germany. *Flora – Morphology, Distribution, Functional Ecology of Plants* 206: 120–126.
- Keenan TF, Gray J, Friedl MA, Toomey M, Bohrer G, Hollinger DY, Munger JW, O'Keefe J, Schmid HP, Wing IS et al. 2014. Net carbon uptake has increased through warming-induced changes in temperate forest phenology. *Nature Climate Change* 4: 598–604.
- Klosterman S, Hufkens K, Richardson AD. 2018. Later springs green-up faster: the relation between onset and completion of green-up in deciduous forests of North America. *International Journal of Biometeorology* 62: 1645–1655.
- Kral-O'Brien KC, O'Brien PL, Harmon JP. 2019. Need for false spring research in the Northern Great Plains, USA. *Agricultural & Environmental Letters* 4: 1–5.
- Laube J, Sparks TH, Estrella N, Höfler J, Ankerst DP, Menzel A. 2013. Chilling outweighs photoperiod in preventing precocious spring development. *Global Change Biology* 20: 170–182.
- Lenz A, Hoch G, Vitasse Y, Körner C. 2013. European deciduous trees exhibit similar safety margins against damage by spring freeze events along elevational gradients. *New Phytologist* 200: 1166–1175.
- Liu Q, Piao S, Janssens IA, Fu Y, Peng S, Lian X, Ciais P, Myneni RB, Peñuelas J, Wang T. 2018. Extension of the growing season increases vegetation exposure to frost. *Nature Communications* 9: 426.
- Marino GP, Kaiser DP, Gu L, Ricciuto DM. 2011. Reconstruction of false spring occurrences over the southeastern United States, 1901–2007: an increasing risk of spring freeze damage? *Environmental Research Letters* 6: 24015.
- McGill BJ, Chase JM, Hortal J, Overcast I, Rominger AJ, Rosindell J, Borges PAV, Emerson BC, Etienne RS, Hickerson MJ et al. 2019. Unifying macroecology and macroevolution to answer fundamental questions about biodiversity. *Global Ecology and Biogeography* 28: 1925–1936.
- McKown AD, Guy RD, Kláspéte J, Geraldes A, Friedmann M, Cronk QCB, El-Kassaby YA, Mansfield SD, Douglas CJ. 2013. Geographical and environmental gradients shape phenotypic trait variation and genetic structure in *Populus trichocarpa*. *New Phytologist* 201: 1263–1276.
- Meier Ü. 2001. Growth stages of mono- and dicotyledonous plants. BBCH Monograph Edited by Uwe Meier Federal Biological Research Centre for Agriculture and Forestry. *Agriculture* 12: 141–147.
- Menzel A, Helm R, Zang C. 2015. Patterns of late spring frost leaf damage and recovery in a European beech (*Fagus sylvatica* L.) stand in south-eastern Germany based on repeated digital photographs. *Frontiers in Plant Science* 6: 110.
- Moorecroft PR, Hurtt GC, Pacala SW. 2001. A method for scaling vegetation dynamics: The Ecosystem Demography Model (ED). *Ecological Monographs* 71: 557–585.
- Morin X, Ameglio T, Ahas R, Kurz-Besson C, Lanta V, Lebourgeois F, Miglietta F, Chuine I. 2007. Variation in cold hardiness and carbohydrate concentration from dormancy induction to bud burst among provenances of three European oak species. *Tree Physiology* 27: 817–825.
- Muffler L, Beierkuhnlein C, Aas G, Jentsch A, Schweiger AH, Zohner C, Kreyling J. 2016. Distribution ranges and spring phenology explain late frost sensitivity in 170 woody plants from the Northern Hemisphere. *Global Ecology and Biogeography* 25: 1061–1071.
- Nanninga C, Buyarski CR, Pretorius AM, Montgomery RA. 2017. Increased exposure to chilling advances the time to budburst in North American tree species. *Tree Physiology* 37: 1727–1738.
- Norgaard Nielsen CC, Rasmussen HN. 2009. Frost hardening and dehardening in *Abies procera* and other conifers under differing temperature regimes and warm-spell treatments. *Forestry* 82: 43–59.
- Onoda Y, Westoby M, Adler PB, Choong AMF, Clissold FJ, Cornelissen JHC, Díaz S, Dominy NJ, Elgart A, Enrico L et al. 2011. Global patterns of leaf mechanical properties. *Ecology Letters* 14: 301–312.

- Poirier M, Lacointe A, Ameglio T. 2010. A semi-physiological model of cold hardening and dehardening in walnut stem. *Tree Physiology* 30: 1555–1569.
- Prozherina N, Freiwald V, Rousi M, Oksanen E. 2003. Interactive effect of springtime frost and elevated ozone on early growth, foliar injuries and leaf structure of birch (*Betula pendula*). *New Phytologist* 159: 623–636.
- R Development Core Team. 2017. *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Rhodes AC, Clair SBS. 2018. Measures of browse damage and indexes of ungulate abundance to quantify their impacts on aspen forest regeneration. *Ecological Indicators* 89: 648–655.
- Richardson AD, Keenan TF, Migliavacca M, Ryu Y, Sonnentag O, Toomey M. 2013. Climate change, phenology, and phenological control of vegetation feedbacks to the climate system. *Agricultural and Forest Meteorology* 169: 156–173.
- Roberts AM, Tansey C, Smithers RJ, Phillimore AB. 2015. Predicting a change in the order of spring phenology in temperate forests. *Global Change Biology* 21: 2603–2611.
- Satake A, Kawagoe T, Saburi Y, Chiba Y, Sakurai G, Kudoh H. 2013. Forecasting flowering phenology under climate warming by modelling the regulatory dynamics of flowering-time genes. *Nature Communications* 4: 2303.
- Schwartz MD, Reed BC, White MA. 2002. Assessing satellite-derived start-of-season measures in the conterminous USA. *International Journal of Climatology* 22: 1793–1805.
- Stinziano JR, Way DA. 2017. Autumn photosynthetic decline and growth cessation in seedlings of white spruce are decoupled under warming and photoperiod manipulations. *Plant, Cell & Environment* 40: 1296–1316.
- Vitasse Y, Hoch G, Randin CF, Lenz A, Kollas C, Scheepens JF, Körner C. 2013. Elevational adaptation and plasticity in seedling phenology of temperate deciduous tree species. *Oecologia* 171: 663–678.
- Vitasse Y, Lenz A, Hoch G, Körner C. 2014. Earlier leaf-out rather than difference in freezing resistance puts juvenile trees at greater risk of damage than adult trees. *Journal of Ecology* 102: 981–988.
- Webber JF, Mason CM. 2016. Utility of the colorimetric Folin-Ciocalteu and aluminum complexation assays for quantifying secondary metabolite variation among wild sunflowers. *Helia* 39: 157–167.
- Wesolowski T, Rowinski P. 2006. Timing of bud burst and tree-leaf development in a multispecies temperate forest. *Forest Ecology and Management* 237: 387–393.
- Wilczek AM, Cooper MD, Korves TM, Schmitt J. 2014. Lagging adaptation to warming climate in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* 111: 7906–7913.
- Wright IJ, Reich PB, Westoby M ADD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M et al. 2004. The worldwide leaf economics spectrum. *Nature* 428: 821–827.
- Yeaman S, Hodgins KA, Lotterhos KE, Suren H, Nadeau S, Degner JC, Nurkowski KA, Smets P, Wang T, Gray LK et al. 2016. Convergent local adaptation to climate in distantly related conifers. *Science* 353: 1431–1433.
- Zohner CM, Mo L, Renner SS, Svenning JC, Vitasse Y, Benito BM, Ordóñez A, Baumgarten F, Bastin JF, Sebald V et al. 2020. Late-spring frost risk between 1959 and 2017 decreased in North America but increased in Europe and Asia. *Proceedings of the National Academy of Sciences, USA* 117: 12192–12200.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Evidence of shoot apical meristem damage, quantified as 1.

Fig. S2 Model estimates of the effects on Chl content (mg cm^{-2}).

Fig. S3 Rank order of budset across all species using mean trends.

Methods S1 Methods for measuring shoot apical meristem damage.

Methods S2 Methods for data analysis and model equations.

Table S1 Description of species used in this study.

Table S2 Chill units under growth chamber conditions.

Table S3 Summary of the model with effects of treatments across species on the duration of vegetative risk.

Table S4 Summary of the model with effects of treatments across species on growing season length.

Table S5 Summary of the model with effects of treatments across species on meristem damage.

Table S6 Summary of the model with effects of treatments across species on total season growth in height (cm).

Table S7 Summary of the model with effects of treatments across species on total biomass (g).

Table S8 Summary of the model with effects of treatments across species on leaf toughness (N).

Table S9 Summary of the model with effects of treatments across species on leaf Chl content (mg cm^{-2}).

Table S10 Summary of the model with effects of treatments across species on leaf thickness (μm).

Table S11 Summary of the simple linear regression model of day of budburst across chilling treatments.

Table S12 Summary of the simple linear regression model of duration of vegetative across chilling treatments.

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