

¹ Late spring freezes coupled with warming winters alter temperate
² tree phenology and growth

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²²

²³ **Summary**

- ²⁴ • Spring phenology is advancing with warming but late spring freezes may not advance at the same rate,
²⁵ potentially leading to an increase in freezes after trees initiate budburst. Research suggests warming
²⁶ winters may delay budburst through reduced chilling, which may cause plants to leaf out more slowly,
²⁷ thus decreasing spring freeze tolerance.
- ²⁸ • Here, we assessed the effects of late spring freezes and reduced over-winter chilling on sapling phenology,
²⁹ growth and tissue traits, across eight temperate tree and shrub species in a laboratory experiment.
- ³⁰ • We found that spring freezes delayed leafout—extending the period of greatest risk for freeze damage—
³¹ and increased damage to the shoot apical meristem, decreased leaf toughness and leaf thickness. Longer
³² chilling accelerated budburst and leafout, even under spring freeze conditions. Thus chilling compen-
³³ sated for the adverse effects of late spring freezes on phenology. Despite the effects of spring freezes
³⁴ and chilling on phenology, we did not see any major re-ordering in the sequence of species leafout.
- ³⁵ • Our results suggest climate change may impact forest communities not through temporal reassembly,
³⁶ but rather through impacts on phenology and growth from the coupled effects of late spring freezes and
³⁷ decreased over-winter chilling under climate change.

³⁸ *Keywords:* false spring, climate change, phenology, spring freeze, forest recruitment, temporal reassembly,
³⁹ budburst, temperate

⁴⁰ **Introduction**

⁴¹ The timing of spring in temperate deciduous forests shapes plant and animal communities and influences
⁴² ecosystem services and forest management. With warming temperatures, spring phenology (i.e., budburst
⁴³ and leafout, which are strongly cued by temperature) is advancing, reshaping ecosystem dynamics. In one
⁴⁴ major example, advancing spring phenology has led to increased carbon uptake across temperate forests,

45 which are essential carbon sinks that combat the negative effects of climate change (Keenan *et al.*, 2014).
46 But climate change could diminish or reverse these positive effects on carbon storage: specifically through
47 cold snaps during the spring and warming temperatures in the winter.

48 While climate change has warmed the Northern Hemisphere, cold snaps and freezing events are still occurring.

49 These weather events can have major impacts on plant development each spring. One such event is a late
50 spring freeze (Marino *et al.*, 2011), often defined as when temperatures drop below freezing (i.e., below -
51 2.2°C, Schwartz *et al.*, 2002) after budburst has initiated. Damage from late spring freeze events can have
52 cascading effects within a forest, from changes to nutrient cycling and carbon uptake to shifts in seedling
53 recruitment (Hufkens *et al.*, 2012; Richardson *et al.*, 2013; Klosterman *et al.*, 2018). Furthermore, late spring
54 freezes can increase the probability of additional freezes within a growing season by extending the period in
55 which plants are most at risk—the time between budburst and leafout, what we refer to as the ‘duration of
56 vegetative risk’. This may result in a positive feedback loop: observational studies suggest plants take longer
57 to re-flush leaves after a late spring freeze—up to 38 days (Gu *et al.*, 2008; Augspurger, 2009, 2013; Menzel
58 *et al.*, 2015)—which could lead to additional late spring freezes in a season (Augspurger, 2009). As late spring
59 freezes are predicted to increase in certain regions with increased climate change (Ault *et al.*, 2015; Liu *et al.*,
60 2018; Zohner *et al.*, 2020) understanding their impacts is essential for robust forest management strategies
61 and climate forecasting (Kral-O’Brien *et al.*, 2019).

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

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

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

88 Here, we assessed the effects of over-winter chilling length and late spring freezes on sapling phenology and
89 growth across eight temperate tree and shrub species. We exposed individuals to different levels of over-
90 winter chilling crossed with a late spring freeze event in growth chambers, then followed individuals for a
91 growing season in a greenhouse to understand: (1) How does over-winter chilling impact phenology, growth
92 and physical leaf traits, (2) how do late spring freeze events impact phenology, growth and physical leaf traits,
93 and (3) how does the interaction between chilling and late spring freezes impact community structure and
94 phenological order?

⁹⁵ Materials and Methods

⁹⁶ Plant selection and material

⁹⁷ We selected eight temperate woody plant tree and shrub species (Table S1) that span varying spring phe-
⁹⁸ nologies (i.e., early to later leafout), are not generally used as crops or ornamental species and are common in
⁹⁹ middle and eastern North American forests: *Acer saccharinum*, *Alnus incana rugosa*, *Betula papyrifera*, *Be-*
¹⁰⁰ *tula populifolia*, *Cornus racemosa*, *Salix purpurea*, *Sorbus americana*, and *Viburnum dentatum* (we originally
¹⁰¹ included two additional species—*Fagus grandifolia* and *Nyssa sylvatica*, but the plants were not delivered in
¹⁰² a usable condition and thus we excluded them from the experiment). We used 48 dormant, one year old,
¹⁰³ bare root saplings—each measuring 6-12 inches—for each species from Cold Stream Farm LLC (Freesoil, MI;
¹⁰⁴ 44°6' N -86°12' W) for a total of 384 individuals. Upon receipt, on 21 November 2018, plants were potted in
¹⁰⁵ 656 ml deepots with Fafard #3B Metro Mix soil and placed in growth chambers at the Weld Hill Research
¹⁰⁶ Building of the Arnold Arboretum (Boston, MA; 42°17' N -71°8' W) at 4°C for different durations depending
¹⁰⁷ on chilling treatment (explained below).

¹⁰⁸ Growth chamber and greenhouse conditions

¹⁰⁹ Individuals were randomly selected for one of six experimental treatments from a full factorial design of
¹¹⁰ late spring freeze (two levels: presence or absence of late spring freeze) x chilling (three levels: eight, six or
¹¹¹ four weeks of chilling at 4°C with eight hour photoperiod, lighting was a combination of T5HO fluorescent
¹¹² lamps with halogen incandescent bulbs at roughly $250 \mu\text{mol m}^{-2}\text{s}^{-1}$; see Table S2 for conversion of chilling
¹¹³ duration to chilling hours, Utah model and chill portions). Individuals were rotated within and among growth
¹¹⁴ chambers every two weeks to minimize bias from possible growth chamber effects.

¹¹⁵ Once each chilling treatment was completed, we moved individuals to a greenhouse with mean daytime
¹¹⁶ temperature of 15°C and a mean nighttime temperature of 10°C, and a photoperiod of 12 hour days (lighting
¹¹⁷ was from LEDs VYPRx Plus greenhouse fixtures at approximately $159 \mu\text{mol m}^{-2}\text{s}^{-1}$) throughout the spring

118 until all individuals reached full leaf expansion. After all individuals of all species reached full leaf expansion,
119 greenhouse temperatures and photoperiods were kept ambient, and all individuals were up-potted to 983 ml
120 deepots and fertilized with SCOTTS 15-9-12 Osmocote Plus 5-6. Due to experimental and facility constraints,
121 all individuals received the same late summer greenhouse conditions; this included changes in temperature and
122 photoperiod generally associated with dormancy onset. Therefore, individuals that received longer chilling
123 treatments (six and eight weeks of chilling), effectively experienced shorter growing season conditions.

124 **Phenology and late spring freeze treatment**

125 We recorded phenology (using the BBCH scale, which defines and describes the phenological development
126 stages, Meier, 2001) every 2-3 days through full leaf expansion. The duration of vegetative risk (Chamberlain
127 *et al.*, 2019) was measured as the duration in days between budburst date (BBCH stage 07, which is defined
128 as the “beginning of sprouting or bud breaking; shoot emergence”; Finn *et al.*, 2007) and the date of full leaf
129 expansion (BBCH stage 19, defined as “leaf expansion complete” or when all petioles have emerged and the
130 leaves have completely unfolded) for each individual. Individuals in the ‘late spring freeze’ treatment were
131 placed in a growth chamber set to mimic a late spring freeze event during budburst, defined as once at least
132 50% of the buds were at BBCH stage 07 but the individual had not yet reached BBCH stage 19 (that is, each
133 sapling was exposed to a late spring freeze based on its individual phenological timing). Late spring freeze
134 treatments lasted approximately 14 hours, beginning at 18:00; temperatures were ramped down to -3°C for
135 three hours (Figure 1). At approximately 08:00 the following day, we placed late spring freeze individuals
136 back in the greenhouse. Once the leaves from the first flush on all individuals reached full leaf expansion
137 (BBCH stage 19), we recorded phenology weekly until 1 August 2019, then every 2-3 days again to monitor
138 fall phenology. Our intention was to observe the delay and damage to the original leaves. We monitored all
139 individuals until complete budset.

140 **Growth measurements**

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

145 We measured the chlorophyll content of four leaves on each individual 60 days after full leaf out using an
146 atLEAF CHL PLUS Chlorophyll meter, converting chlorophyll content to mg cm⁻² using the atLEAF CHL
147 PLUS conversion tool. We measured leaf thickness using a Shars Digital Micrometer (accurate to 0.001mm)
148 and leaf toughness in Newtons using a Shimpo Digital Force Gauge on two leaves for each individual 60 days
149 after full leaf out. Additionally, we qualitatively measured damage to the shoot apical meristem (score of
150 0/1), where a score of 1 consisted of complete damage or disruption of growth in the main stem and resulted
151 in early dormancy induction or reliance on lateral shoot growth (see supplemental materials ‘Methods S1:
152 Measuring shoot apical meristem damage’ and Figure S1 for more details). Finally, we harvested each plant
153 after it reached complete budset to dry, separate and weigh belowground and aboveground biomass (including
154 leaves).

155 **Data analysis**

156 We used Bayesian hierarchical models (with the brms package, version 2.3.1, Bürkner, 2017) in R (version
157 3.3.1, R Development Core Team, 2017) to estimate the effects of chilling duration, late spring freeze treatment
158 and all two-way interactions as predictors on: (1) duration of vegetative risk, (2) growing season length, (3)
159 shoot apical meristem damage, (4) total growth, (5) total biomass, (6) chlorophyll content, (7) leaf toughness
160 and (8) leaf thickness. We modeled species hierarchically as grouping factors, which generated an estimate
161 and posterior distribution for each species as well as an overall response across the eight species used in our
162 experiment (see supplemental materials ‘Methods S2: Data analysis and model equations’ for more details).
163 We ran four chains, each with 4 000 iterations, of which 2 500 were warm-up iterations, for a total of 6

164 000 posterior samples for each predictor for each model using weakly informative priors (increasing priors
165 three-fold did not impact our results). We evaluated our model performance based on \hat{R} values that were
166 close to 1.0, checked chain convergence and posterior predictive checks visually (Gelman *et al.*, 2014), and
167 made sure all models had zero divergent transitions. Our models generally had high n_{eff} (6 000 for most
168 parameters, but as low as 1 400 for a couple of parameters in the shoot apical meristem model).

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

182 Results

183 Chilling durations impacted individual phenology. As seen in many other studies (Chuine *et al.*, 2001; Laube
184 *et al.*, 2013; Ettinger *et al.*, 2020), we found decreases in chilling delayed day of budburst by 4.8 ± 1.8 days for
185 six weeks of chilling and by 7.6 ± 1.8 days for four weeks of chilling (as mentioned above, all values are given
186 relative to the no late spring freeze under eight weeks of chilling treatment; Table S11). Shorter chilling also
187 slowed the rate of leafout, increasing the duration of vegetative risk under both shorter chilling treatments

188 (6 weeks: 15.7%, 2.1 ± 1.4 days; 4 weeks: using 90% uncertainty intervals, 20.1%, 2.7 ± 2.5 days, 96%
189 uncertainty intervals, 2.7 ± 3.3 days; Figure 2a, Table S12 and Table S3). Decreased chilling shortened the
190 growing season for individuals exposed to four weeks of chilling but not for individuals exposed to six weeks
191 of chilling (6 weeks: 0.9%, 2.5 ± 8.0 days; 4 weeks: using 90% uncertainty intervals -3.4%, -9.7 ± 8.2 days,
192 96% uncertainty intervals -9.7 ± 10.4 days; Figure 2b and Table S4), due to constraints on our experimental
193 design: individuals in longer chilling treatments were put in the greenhouse two to four weeks later than the
194 four weeks of chilling treatment, but all individuals experienced the same ambient late summer conditions
195 when in the greenhouse, including shifts in temperature and light associated in the late summer/autumn that
196 generally trigger dormancy onset; thus shortening the growing season for individuals that experienced longer
197 chilling.

198 Late spring freezes also impacted individual phenology. Individuals exposed to the late spring freeze treatment
199 had longer durations of vegetative risk (8 weeks: 26.9%, 3.6 ± 1.3 days). Effects on the duration of vegetative
200 risk from late spring freeze treatments and from decreased chilling were generally additive, resulting in slightly
201 longer durations given late spring freeze with six weeks of chilling (31.3%, 4.2 ± 1.8 days) and even longer
202 durations of vegetative risk for individuals exposed to a late spring freeze with only four weeks of chilling
203 (42.5%, 5.7 ± 1.8 days, Figure 2a and Table S3).

204 Late spring freezes impacted growth habit and shoot growth but not total biomass. Across all chilling
205 treatments, late spring freezes led to more damage to the shoot apical meristem (8 weeks: 50.0% increase
206 in probability of damage under late spring freeze treatment or 2.0 ± 1.4 ; 6 weeks: 37.5% or 1.5 ± 2.1 ; and
207 4 weeks: 50.0% or 2.0 ± 2.2 ; Figure 2c and Table S5). There was little change in shoot growth over the
208 growing season across all chilling treatments under late spring freeze conditions (8 weeks: 9.4%, 5.1 ± 7.0
209 cm; 6 weeks: -0.7%, -0.4 ± 10.3 cm; 4 weeks: 8.7%, 4.7 ± 9.0 cm) but shoot growth increased with four
210 weeks of chilling durations (4 weeks: 20.4%, 11.0 ± 6.6 cm; Figure 2d and Table S6). There was very little
211 change in total biomass under late spring freeze conditions (compared to the no late spring freeze) across all
212 treatments (8 weeks: -7.0%, -3.4 ± 4.6 g; 6 weeks: -7.4%, -3.6 ± 4.7 g; 4 weeks: (5.9%, 2.9 ± 6.5 g); Figure

²¹³ **2e** and Table S7).

²¹⁴ Late spring freezes also affected physical leaf traits. Late spring freeze treatments decreased leaf toughness
²¹⁵ across the eight and six weeks of chilling treatments (8 weeks: -7.5%, -0.03 ± 0.03 N; 6 weeks: -7.5%, -0.03 ±
²¹⁶ 0.04 N) and decreases in chilling led to increased toughness for the four weeks of chilling (4 weeks: 12.5%, 0.05
²¹⁷ ± 0.04 N; Figure 2f and Table S8). There was little change in chlorophyll content in leaves under late spring
²¹⁸ freeze conditions with eight and six weeks of chilling (8 weeks: -1.3%, -0.4 ± 1.3 mg cm⁻²; and 6 weeks: -4.8%,
²¹⁹ -1.5 ± 1.9 mg cm⁻²) and there was little change with decreased chilling durations on chlorophyll content (6
²²⁰ weeks: -0.2%, -0.06 ± 1.4 mg cm⁻²; and 4 weeks: 1.3%, 0.4 ± 1.4 mg cm⁻²; Figure S2 and Table S9). Late
²²¹ spring freezes led to decreased leaf thickness in the eight weeks of chilling treatment, but there was little
²²² change for the six and four weeks of chilling treatments (8 weeks: using 90% uncertainty intervals -5.5%, -7.4
²²³ ± 6.3 μm 96% uncertainty intervals, -7.4 ± 8.2 μm); 6 weeks: 3.7%, 4.9 ± 8.9 μm; and 4 weeks: -0.5%, -0.7
²²⁴ ± 9.0 μm) and decreased chilling durations led to increased leaf thickness for six weeks of chilling but not
²²⁵ for four weeks of chilling (6 weeks: using 90% uncertainty intervals, 11.5%, 15.4 ± 12.4 μm 96% uncertainty
²²⁶ intervals, 15.4 ± 16.2 μm; and 4 weeks: 6.1%, 8.2 ± 12.0 μm; Figure 2g and Table S10).

²²⁷ Late spring freezes and chilling treatments were generally consistent across species, though not always. Du-
²²⁸ ration of vegetative risk decreased for most species with longer chilling (i.e., the eight weeks), except for
²²⁹ *Salix purpurea*, which experienced shorter durations of vegetative risk with shorter chilling (Figure 2a). Late
²³⁰ spring freezes led to meristem damage across all species except for *Betula populifolia* and *Sorbus americana*.
²³¹ Additionally, *Viburnum dentatum* experienced consistent meristem damage under all treatments (Figure 2c).
²³² Effects on leaf thickness were especially variable across species under the longer chilling treatments, specif-
²³³ ically with *Sorbus americana* and *Viburnum dentatum* having thicker leaves with increased chilling (Figure
²³⁴ 2g).

²³⁵ Despite large treatment effects on phenology, we found no major effects on phenological rank within the
²³⁶ community. Order of leafout timing was consistent across all treatments, with *Salix purpurea* always being
²³⁷ first to leafout, followed by *Betula papyrifera*, *B. populifolia* and *Cornus racemosa*, followed by *Alnus rugosa*,

Sorbus americana, *Viburnum dentatum* and *Acer saccharinum* (Figure 3). *Viburnum dentatum* was the only species to change rank across treatments, though it was consistently grouped with the later-leafout species. Order of budset timing was also consistent across all treatments, with *Cornus racemosa* and *Sorbus americana* being first to set bud, followed by *Betula papyrifera*, *Acer saccharinum* and *Viburnum dentatum*, followed by *B. populifolia*, *Salix purpurea* and *Alnus rugosa* (Figure S3). *Acer saccharinum* was the only species to change budset rank across treatments, though it was grouped consistently with *Betula papyrifera* and *Viburnum 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 chilling. Our results confirmed the major features of late spring freezes (plant damage) and chilling (advancing spring phenology) 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., *Salix purpurea*, a species which shows complex responses to longer chilling that may be associated with re-acclimation of cold hardiness; Kalberer *et al.*, 2006; Arora & Rowland, 2011). With late

260 spring freezes lengthening the duration of vegetative risk, the risk of multiple late spring freezes occurring
261 during this sensitive period in one season increases. Under our experimental conditions—where we applied
262 a late spring freeze event at the same developmental phase for all individuals (effectively decoupling the
263 covariation between risk and budburst timing in natural conditions, where frost risk generally decreases over
264 time each spring and thus is lower for later budbursting individuals)—we found that chilling can compensate
265 for this increase in duration of vegetative risk (with increased chilling, the duration of vegetative risk did not
266 increase under late spring freeze conditions). This suggests chilling is equally or more important for saplings
267 in terms of limiting exposure to multiple late spring freezes. With climate change and warming temperatures,
268 over-winter chilling is anticipated to decrease (Laube *et al.*, 2013) and late spring freezes are predicted to
269 increase in certain regions (Ault *et al.*, 2015; Liu *et al.*, 2018). This combination could greatly impact plant
270 performance, survival and shape species distributions due to the lengthening period of lowest freeze resistance
271 and increased risk of multiple freeze events in one season, which could ultimately affect ecosystem processes,
272 such as carbon uptake and nutrient cycling.

273 Our results suggest the combination of late spring freezes and shifts in chilling impact sapling growth. We
274 found that late spring freezes impacted sapling growth through damage to the shoot apical meristem consis-
275 tently across most species, regardless of a species phenological order (i.e., early versus late budburst). This
276 is in contrast with past studies that have found early-budburst species can withstand lower temperature
277 thresholds (Lenz *et al.*, 2013; Muffler *et al.*, 2016), and may be due to the different species we studied or dif-
278 ferences in methodology (e.g., many studies freeze leaves while we froze entire saplings). Damage to the shoot
279 apical meristem can lead to reliance on lateral shoot growth, rendering inefficient growth patterns and—if
280 damage is significant within a forest stand—can lead to declines in recruitment (Rhodes & Clair, 2018). Our
281 results supported that late spring freezes may reshape growth patterns, but showed it was highly dependent
282 on chilling. We found growth was reduced when late spring freezes were combined with longer chilling, but
283 this did not translate directly into lower biomass; instead biomass was fairly constant with slight reductions
284 under treatments that showed the most growth (lower chilling x late spring freeze). Given these results it is
285 likely that lateral shoot growth accounted for reduced apical shoot growth, though we did not measure lat-

²⁸⁶ eral shoot growth to confirm this. Thus the combination of warming through reduced chilling and increased
²⁸⁷ late spring freezes could impact both plant growth and tissue investment patterns and—subsequently—affect
²⁸⁸ overall sapling performance and competition among saplings.

²⁸⁹ Layered onto shifts in performance via changes in the growth patterns of saplings, we found both late spring
²⁹⁰ freezes and chilling impacted important leaf traits. Leaf traits can help capture the challenge plants face in
²⁹¹ balancing leaf construction costs—where thicker, tougher leaves may have higher costs to build but pay off
²⁹² via greater resistance to herbivory and other biotic and abiotic damage (Wright *et al.*, 2004; Onoda *et al.*,
²⁹³ 2011)—against growth potential—where thinner, less tough leaves may allow rapid growth, or in the case of
²⁹⁴ late spring freezes, rapid regrowth, to take advantage of available resources including light (Edwards *et al.*,
²⁹⁵ 2014; Díaz *et al.*, 2016). Our experiment considered only a small slice of these complex traits (which vary
²⁹⁶ across clades, habitats and a growing season, McKown *et al.*, 2013), but suggest consistent shifts in leaf
²⁹⁷ quality due to late spring freezes, which decreased leaf toughness and leaf thickness. However, we also found
²⁹⁸ that increased chilling levels consistently decreased leaf toughness, with increased chilling combined with late
²⁹⁹ spring freezes weakly increasing toughness; altogether suggesting a complex role of both late spring freezes
³⁰⁰ and chilling in determining leaf traits. These results—though focused on saplings—support increasing work
³⁰¹ that suggests climate change's impacts on phenology may not simply mean longer growing seasons and thus
³⁰² increased carbon storage, but may be complicated by shifts in leaf traits, photosynthetic capacity and other
³⁰³ plant metrics (Bauerle *et al.*, 2012; Stinziano & Way, 2017; Huang *et al.*, 2020; Zani *et al.*, 2020) that could
³⁰⁴ alter predictions. Further studies, that test whether these results hold for adult trees (to date studies suggest
³⁰⁵ there is no difference between saplings and adults, Cuervo-Alarcon *et al.*, 2018), and consider additional
³⁰⁶ traits, such as the secondary compounds and total phenolic content (Ayres, 1993; Webber & Mason, 2016),
³⁰⁷ as well as photosynthetic rate of the leaves, will be critical to determine the singular and interactive effects
³⁰⁸ of chilling and late spring freezes on leaf traits to predict changes with warming at a larger scale.

309 **Late spring freezes and chilling did not reshape temporal assembly**

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

331 Phenological rank remained consistent across all of our late spring freeze and chilling treatments—where
332 all species were affected equally. In nature, however, not all of our study species are at equal risk of late
333 spring freezes, with early-budburst species (e.g., *Salix americana* or *Betula papyrifera*) generally more at

334 risk than later-budburst species (e.g., *Acer saccharinum* or *Viburnum dentatum*). This suggests that climate
335 change could reshape forest communities, though not directly through temporal assembly. Instead, our results
336 suggest that change may come from the effect of late spring freeze and reduced chilling on physical damage,
337 with potential impacts on physical leaf traits.

338 Late spring freezes and predicting forest recruitment

339 Our results from a laboratory experiment combined with previous work across diverse scales (Vitasse *et al.*,
340 2013; Yeaman *et al.*, 2016) highlight the complexity of predicting future forest recruitment with climate
341 change. Changes in the growth patterns and leaf traits that we observed combined with advances in our
342 molecular understanding of phenology (Satake *et al.*, 2013; Wilczek *et al.*, 2014; Fournier-Level *et al.*, 2016)
343 suggest continued efforts to understand multivariate trait syndromes, and how these syndromes shift across
344 climate and development (Díaz *et al.*, 2016; McKown *et al.*, 2013), may prove critical. While we studied a
345 small set of traits, additional work could consider traits that may lower temperate trees' risk of late spring
346 freeze damage, such as increased 'packability' of leaf primordia in winter buds, which allows for more rapid
347 leafout (Edwards *et al.*, 2017), increased trichome density on young leaves to protect leaf tissue against
348 freezes (Prozherina *et al.*, 2003; Agrawal *et al.*, 2004) and buds with decreased water content to increase
349 freeze tolerance (Beck *et al.*, 2007; Morin *et al.*, 2007; Norgaard Nielsen & Rasmussen, 2009; Poirier *et al.*,
350 2010; Kathke & Bruelheide, 2011; Hofmann & Bruelheide, 2015; Muffler *et al.*, 2016). Future cross-species
351 studies could in turn leverage advances in our understanding of how phenological and freeze tolerance traits
352 may relate. While we selected species for early- versus late-leafout, future studies would ideally select species
353 across trait syndromes.

354 Scaling from laboratory and field experiments, such as ours, to robust forecasts of future forests will require
355 integrating across lifestages, species, and up to macroecological scales (McGill *et al.*, 2019). Over-winter
356 chilling decreases with climate change will likely put saplings—which generally leafout earlier than later
357 lifestages to gain access to light (Augspurger, 2009)—at greater risk of damage from late spring freeze events.

358 This could lead to dieback of saplings, most especially of early-budbursting species and/or individuals, which
359 our results show could see increases in durations of vegetative risk from the dual effects of lower chilling
360 and heightened late spring freeze risk. Loss or reduction of early-budburst species would in turn likely
361 have widespread consequences for competition among species and lifestages and reshape forest demographics
362 in complex ways. Such complexity will likely be hard to predict without more accurate models of forest
363 competition and development (Moorcroft *et al.*, 2001; Fisher *et al.*, 2018). Understanding how late spring
364 freezes are changing and how equally—or not—these effects impact different species, especially their seedlings
365 and saplings, is a crucial first-step for future projections.

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371 design, questions and manuscript.

372 Author Contribution

373 C.J.C. and E.M.W. conceived of the study, identified species to use in the study and determined which
374 phenological and growth measurements to observe. C.J.C. performed the analyses and produced all figures
375 and tables. C.J.C. wrote the paper, and both authors edited it.

³⁷⁶ **Data Availability**

³⁷⁷ Data and code from the analyses is available via KNB (<https://knb.ecoinformatics.org/view/doi:10.5063/F47MJ4.>). Raw data, Stan model code and output are available on GitHub and provided upon request.

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- 545 Table S1: Description of species used in this study.
- 546 Table S2: Chill units under growth chamber conditions.
- 547 Table S3: Summary of model with effects of treatments across species on duration of vegetative risk.
- 548 Table S4: Summary of model with effects of treatments across species on growing season length.
- 549 Table S5: Summary of model with effects of treatments across species on meristem damage.
- 550 Table S6: Summary of model with effects of treatments across species on total season growth in height (cm).
- 551 Table S7: Summary of model with effects of treatments across species on total biomass (g).
- 552 Table S8: Summary of model with effects of treatments across species on leaf toughness (N).
- 553 Table S9: Summary of model with effects of treatments across species on leaf chlorophyll content (mg/cm²).
- 554 Table S10: Summary of model with effects of treatments across species on leaf thickness (μm).
- 555 Table S11: Summary of simple linear regression model of day of budburst across chilling treatments.
- 556 Table S12: Summary of simple linear regression model of duration of vegetative across chilling treatments.
- 557
- 558 Figure S1: Evidence of shoot apical meristem damage, quantified as 1.
- 559 Figure S2: Model estimates of effects on chlorophyll content (mg/cm²).
- 560 Figure S3: Rank order of budset across all species using mean trends.
- 561
- 562 Methods S1: Methods for measuring shoot apical meristem damage.
- 563 Methods S2: Methods for data analysis and model equations.
- 564

565 **Tables and Figures**

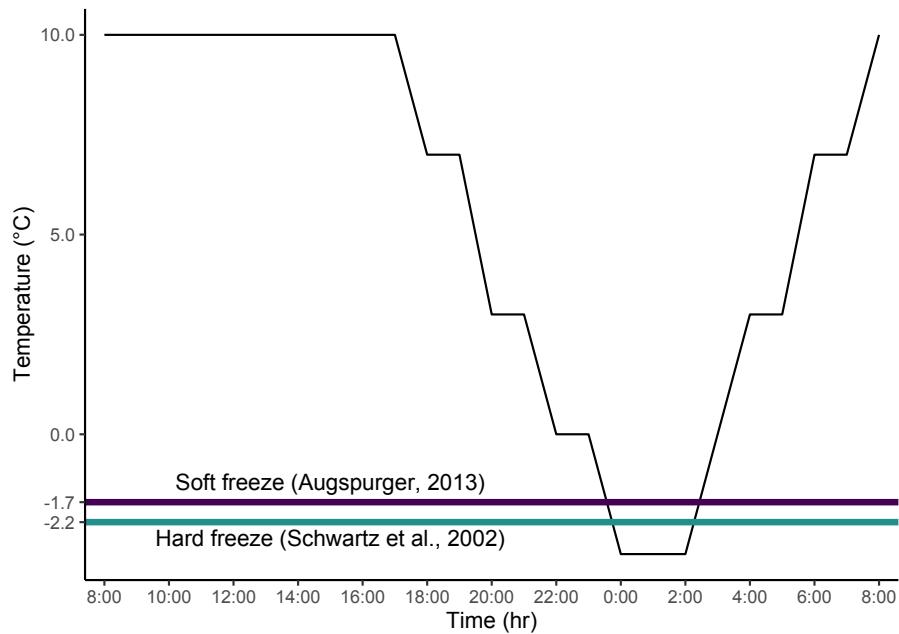


Figure 1: Late spring freeze treatment temperature regime in the growth chamber. Lines show estimated freeze levels for a soft freeze (defined as temperatures falling below -1.7°C after budburst, when damage is known to occur in certain portions of a forest; Augspurger, 2013) or a hard freeze (defined 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).

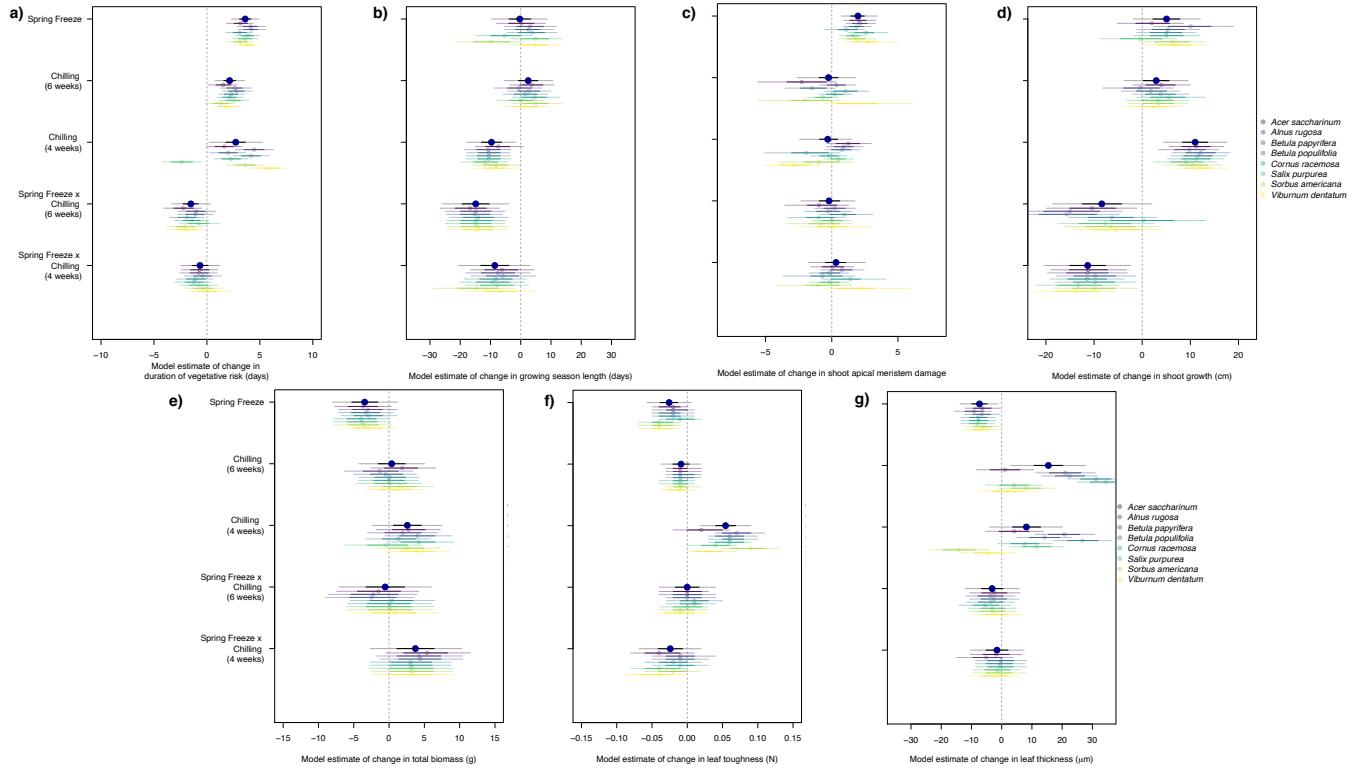


Figure 2: Effects of late spring freeze treatment, six weeks of chilling and four weeks of chilling relative to eight weeks of chilling with no late spring freeze on a) duration of vegetative risk (days), b) growing season length (days), c) shoot apical meristem damage, d) total shoot growth as a metric of height (cm), e) total aboveground and belowground biomass (g), f) leaf toughness (N) and g) leaf thickness (μm). 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.

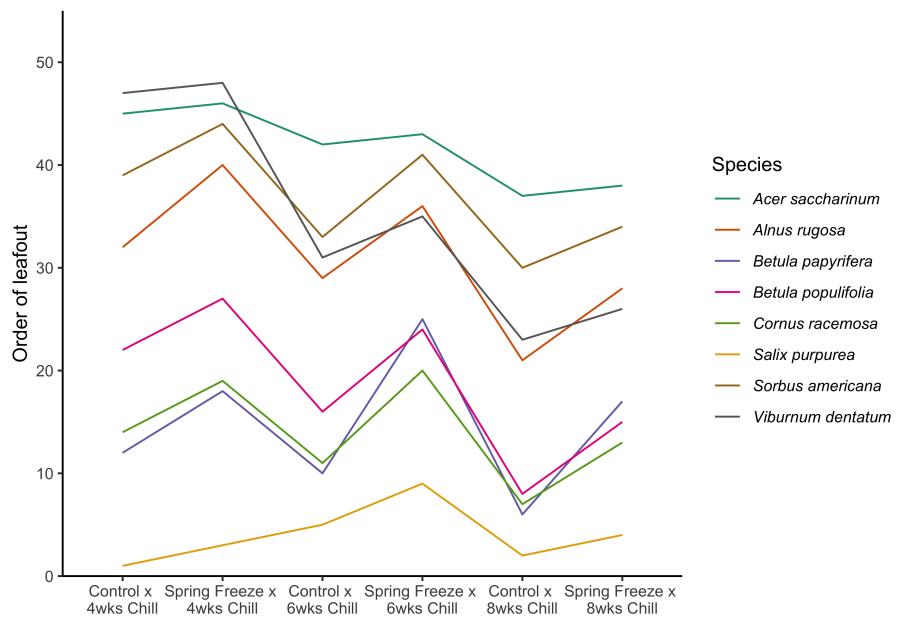


Figure 3: Rank order of leafout across all species using mean trends.