Woody plant phenological responses are strongly associated with key functional traits

Deirdre Loughnan[1], Faith A M Jones[1], Geoffrey Legault[1], Mira Garner[2], Darwin Sodhi[3], Daniel Buonaiuto[4], Catherine Chamberlain[5], Ignacio Morales Castilla [6], Ailene Ettinger[7], and E M Wolkovich[1]

March 25, 2022

[1] Department of Forest and Conservation, Faculty of Forestry, University of British Columbia, 2424 Main Mall Vancouver, BC Canada V6T 1Z4.

[2] Arnold Arboretum of Harvard University, 1300 Centre Street, Boston, Massachusetts, USA;

[3] Organismic & Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, Massachusetts, USA;

[4] XXXX

[5] XXXX

[6] Edificio Ciencias, Campus Universitario 28805 Alcalá de Henares, Madrid, Spain

[7] XXXX

Corresponding Author: Deirdre Loughnan deirdre.loughnan@ubc.ca

1   Summary

Key Words: Budburst phenology, functional traits, Trees, climate chagne

2   Introduction

Climate change is altering the timing of species phenologies, with changes in temporal niches reshaping ecological communities and interactions between species. In temperate systems, the observed advances in plant phenological events, such as budburst, leafout, and flowering times, are associated with changes in seasonal temperatures, particularly warming winter and spring conditions (Menzel et al., 2006; Fitter and Fitter, 2002). But despite this strong general trend, phenological responses vary across species and geographically, and we have yet to fully understand the underlying mechanisms driving observed differences (Chuine et al., 2010; Morin et al., 2009). As the effects of climate change become more pronounced, understanding these relationships is of increasing importance if we are to predict and preserve the diversity and services found in temperate forest ecosystems.

While we have yet to identify all drivers of selection on phenologies, considerable work has shown the importance of three abiotic cues – chilling, forcing, and photoperiod – as the primary drivers of budburst and leafout in temperate deciduous species (Basler and Körner, 2014; Chuine et al., 2016; **?**; Flynn and Wolkovich, 2018). For budburst to occur, species must experience extended period of cold temperatures to break dormancy (Cooke et al., 2012), where species with higher chill requirements budburst later in the season. Spring forcing temperatures, or the temperatures needed to cue species to initiate growth after dormancy release, are also changing as temperatures warm and the timing at which suitable temperature thresholds are met occur earlier within the season (citation). Photoperiod cues can also determine a species ability to initiate growth (Basler and Körner, 2014; Zohner et al., 2020). Species with strong photoperiod requirements are, however, expected to be more constrained in their ability to track changes in temperature and may face fitness costs and novel species interactions as a result (**?**). Previous studies support the general trend of advancing budburst in response to each cue, but with considerable variation in the relative importance of different cues across species (Chuine et al., 2016; Flynn and Wolkovich, 2018). Some woody plant species, for example, require less forcing to budburst after experiencing a cool winter with more chilling, while also having the ability to compensate for low chilling with high forcing conditions or longer photoperiods (Laube et al., 2014; Harrington and Gould, 2015; Flynn and Wolkovich, 2018; Caffarra and Donnelly, 2011; Basler and Körner, 2014; Zohner et al., 2016). Evidence for the role of photoperiod is largely species specific (Heide, 1993; Basler and Körner, 2014; Singh et al., 2017; Zohner et al., 2016), with few studies testing for its importance across species in a community (but see Flynn and Wolkovich (2018)). Species that are less dependent on photoperiod cues and able to track trends in temperatures may benefit from greater intra-annual phenotypic plasticity resulting in greater fitness outcomes under increasingly variable climates (citation?). Despite the insights that identifying these proximate drivers have provided, we still lack a generalizable and mechanistic understanding of why species and populations differ in their cue use that. Further insight on this topic is needed to predict future changes in species sensitivities and community structure.

In our efforts to understand variation in spring phenological timing, researchers have tested several potential mechanisms to identify the drivers of species cue responses. Work exploring drivers of intraspecific cue use, for example, has found age or the development stage of woody plants to be important. Younger life stages, including both seedlings and younger understory trees, budburst earlier than mature individuals in the canopy (Vitasse, 2013; Seiwa and Kikuzawa, 1991). These trends reflect both differences in the temperature sensitivities across life stages and effects of ontogenic changes as trees mature (Vitasse, 2013; Seiwa and Kikuzawa, 1991). Interspecific differences in cues, however, have been studied in relation to species' phylogenetic relatedness. Work on this topic has found strong

evidence for events like flowering-time and budburst to be consistent within taxonomic families, sug-
gesting conservatism in the genetic and physiological mechanisms that determine species phenologies
(Kochmer and Handel, 1986; Davies et al., 2013; Gougherty and Gougherty, 2018). Studies of woody
plant phenologies across species ranges have also highlighted the importance of local adaptations, with
the presence of gradients in phenological responses and presumably cue use at northern range limits
(Lechowicz, 1984; Chuine and Beaubin, 2001; Chuine et al., 2010). In temperate systems for exam-
ple, greater temperature variation in North America was associated with higher chilling requirements
and more conservative phenological responses (Zohner et al., 2017). Studies testing for trends in cues
responses across species latitudinal ranges have also observed stronger responses to photoperiod cues
at lower latitudes (Zohner et al., 2016). Exploring these potential drivers of plant phenologies have
illustrated the nuanced nature of phenology in shaping diverse communities, but they are still limited
in the degree to which they explain the variation we observe across species and ecosystems.

Taking a functional trait approach to phenological research could help explain the variation in cue use
across species and geographically (Flynn and Wolkovich, 2018; Osada, 2017). Early work on functional
traits used trait data from diverse global assemblages of deciduous plants to identify associations be-
tween traits, common growth strategies, and different niche space (Westoby, 1998; Wright et al., 2004;
Chave et al., 2009). The resulting leaf-height-seed scheme and the more extensive leaf economic spec-
trum found direct associations between several trait values and gradients in species growth rates and
competitive abilities (Westoby, 1998; Wright et al., 2004; Díaz et al., 2016; Chave et al., 2009; Funk
et al., 2016). While reproductive phenological traits have been identified as ecologically important for
many years (??), few studies have explored their role in the larger trait framework. Spring pheno-
logical traits, such as budburst and leafout, define the beginning of the growing season and period of
photosynthesis, and therefore also have the potential to correlate with established growth strategies.
Support for the existence of trade-offs in budburst dates and traits related to growth and resource
use have been observed across plant functional groups and habitat types in a handful of studies. For
example, several studies have found deciduous woody species with smaller vessel diameters and dif-
fuse or semi-ring-porous xylem structures to leaf out earlier than species with larger vessels, as this
anatomy reduces the risk of embolism during freezing events (?Lechowicz, 1984). In testing relation-
ships between budburst and leaf traits of deciduous tree species in Japan, (Osada, 2017) found positive
correlations between budburst date and leaf area, leaf mass, and nitrogen content by both mass and
area, while (Sun et al., 2006) found deciduous species with high leaf mass per area (a trait that is the
inverse of specific leaf area) to budburst earlier in deciduous oak forests in eastern China. Variation
in leafout can also relate to species heights, both intraspecifically and across functional groups, with
shorter individuals or understory species leafing out earlier than taller individuals or canopy species
(??). To date, however, research in this area has focused on individuals at local scales, or few traits
for a small number of species, limiting our ability to draw more general and causal inferences. There
is also a lack of studies linking traits directly to cue sensitivity rather than phenological date. The
likely associations between cue sensitivity, phenological events, and growth strategies may allow for
more generalizable trends across species and sites, and better account for species variability in key
environmental cue use.

To date, there have been numerous studies investigating the relationships between climate and func-
tional traits and a wealth of literature on the separate effects of climate cues as drivers of phenology.
However, the selective pressures shaping species traits under variable temperatures are also likely to
act on species responses to phenological cues and define a species temporal niche. Species with a
more acquisitive life-strategy have shorter rates of return on resource investments and the ability to
take advantage of the greater abundance of soil nutrients and light early in the growing season. Such
species face a lesser cost in initiating phenological events too early, as they can recover from early
season damage (cite Cat's paper?). For example, some acquisitive species produce leaves with high
leaf nitrogen content and Specific Leaf Area (SLA) and can take advantage of greater light availability
by having higher rates of photosynthesis (Wright et al., 2004; Pereira and Des Marais, 2020), while

124 also limiting the costs of tissue production (**???**). Acquisitive-strategy species also invest less in their
125 wood structure, having shorter heights and lower stem densities (Laughlin et al., 2010). Species that
126 budburst earlier in the growing season require less spring forcing and winter chilling, and shorter pho-
127 toperiods (Flynn and Wolkovich, 2018), allowing them to grow under less competition for light and
128 soil resources. The suite of traits of acquisitive species contrasts with more conservative life-strategy
129 species that exhibit slower, more competitive growth strategies that benefit from slower rates of return
130 on resource investment and the longer retention of leaf tissue. A greater requirement for cue unit
131 accumulation to trigger phenological events should align with a more conservative life-strategy as such
132 species seek to avoid damage due to premature development.
133
134 In this study, we test for associations between plant phenological responses to environmental cues
135 and common functional traits. Budburst data for tree species in controlled environmental studies was
136 selected from the Observed Spring Phenology Response in Experimental Environments (OSPREE)
137 database and paired with functional trait data from the TRY and BIEN databases. This data was
138 used to explicitly test for the relative differences in functional traits and the timing of budburst in
139 response to experimental forcing, chilling, and photoperiod cues. Drawing on previous work and the
140 broader trait literature, we predict that species that respond less strongly to chilling, forcing, and
141 photoperiod conditions are more likely to have traits associated with acquisitive growth but low com-
142 petitiveness, as reflected by high SLA, high leaf nitrogen content per mass (LNC), shorter heights, and
143 lower seed mass. In contrast, species that are more responsive to chilling, forcing and photoperiods
144 will have traits more associated with conservative growth and higher competitive abilities, such as low
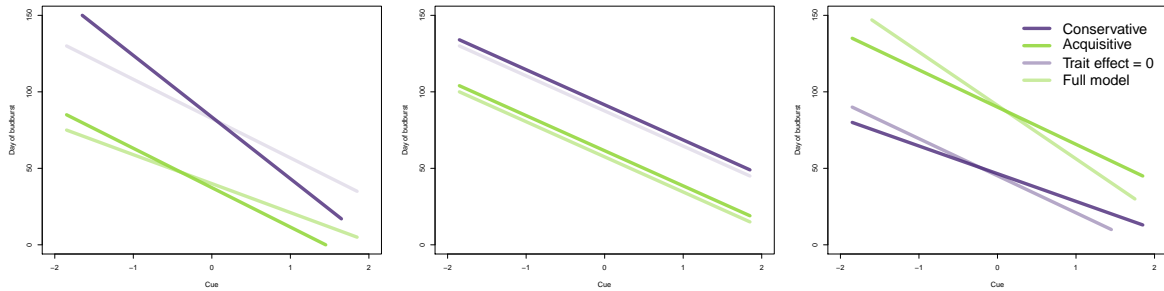145 SLA, low LNC, greater heights and heavier seeds.
146

Figure 1: Conceptual figures

147 Using tree height as an illustrative example, we predict taller trees to be more conservative in their
148 growth strategies and shorter species to budburst earlier and exhibit a more acquisitive growth strat-
149 egy. Previous work on cue responses in woody species have consistently observed negative responses
150 to stronger cues, resulting in advanced budburst, and therefore we expect that the estimated cue re-
151 sponses from our models would all be negative. Under this assumption, there could be three possible
152 trends in the relationships between cue and trait effects on budburst date. If phenological responses
153 align with trait variation associated axes of acquisitive to conservative growth, we predict there to be
154 a negative trait effect, resulting in a steeper negative slope in the cue response, and a stronger cue
155 response and advance in budburst dates with higher cues (Fig. 1). This is illustrated by the steeper
156 slope of the solid lines for both the conservative and acquisitive species in (Fig. 1). If the more con-
157 servative species have later budburst dates than the more acquisitive species, we should also observe
158 a negative correlation between the trait effect and the cue slopes. It is important to note that the
159 smaller differences in slope estimates for when the trait effect is zero and the full model observed for
160 species with low traits is due to the magnitude of the trait value and not a difference in strength of the
161 response. If functional traits have no relation to budburst phenology, the trait effect will be estimated

162 as zero and we could expect to see no difference in the slopes of full model and cue only model (Fig. 1).
163 Finally, if our model estimates a positive trait effect, potentially as a result of a trade-off in selection
164 for budburst phenology and resource use or competitiveness, we predict the slopes of our full model to
165 be less steep than the cue only model (Fig. 1).
166

3   Methods

168 For our analysis, we combined phenological data from the OSPREE database (**?**) with functional trait
169 data from the TRY(cite) and BIEN (cite) trait databases.
170

171 The OSPREE database contains woody, deciduous species phenological data for which experimental
172 data on phenological cues is available, and the phylogenetic relationship is well estimated. First
173 published in 2019, this database has since been updated, and now includes the review of an additional
174 623 and 270 new publications from each of the following search terms:

175 - (budburst OR leaf-out) AND (photoperiod OR daylength) AND temperature*

176 - (budburst OR leaf-out) AND dorman*.

177 From this subsequent review, we an additional 12 papers met our selection criteria. For additional
178 information on the construction of the OSPREE database and methods of cue estimates, see (**?**). Our
179 analysis used all available budburst data for our 37 focal species, with the data originating from 28
180 unique studies.
181

182 Both TRY and BIEN are large databases compiling plant trait data across many individuals, species,
183 and studies. Initially, we began by searching both databases for all available trait data for all 234
184 species represented in the OSPREE database.
185

186 Data was also obtained from the BIEN database using the BIEN R package (**?**). Data were requested
187 or downloaded in December 2018. Our full trait datasets included data on 96 species and ten traits
188 from the TRY database and 34 species and seven traits from the BIEN database. For our analysis,
189 however, we only included trait data from adult individuals with a minimum height of 1.42 m and
190 we removed all data from experiments or growing in non-natural habitats. Traits were also grouped
191 where appropriate, for example, separate entries for SLA values with petioles, without petioles, and for
192 which no petiole presence was specified were all categorized as a single trait in our analysis (see Table
193 S1). Duplicated data across the datasets were removed (n= 434905). Finally, we subsetted the data to
194 include only species for which we had a complete dataset for each species and trait. This resulted in a
195 dataset of only 26 species and six functional traits. To test for correlations in our six traits and further
196 refine our trait selection, we applied a PCA. The principle component explained 32.2% of variation
197 while the second explained 23.4% of the variation (Fig. S1). Given the strong association between the
198 SLA and LDMC leaf traits, and similarly between stem specific density (SSD) and height, we further
199 reduced the number of traits in our analysis to include only height, seed mass, LNC, and SLA. By
200 including only these four traits, we were able to increase the number of species we could include in our
201 analysis as we had had at least one trait measurement for 37 species (height n = 47781, seed mass n
202 = 281, LNC n = 3853, SLA n = 7656). Given the abundance of height data and overrepresentation
203 of height measurements for six of our focal species, we randomly sampled 3000 height measurements
204 for each of these species to include in our analysis (n = 27318). This reduces the effect of trait values
205 from these frequently measured species from overwhelming the partial pooling effect in our model. In
206 addition we excluded seed mass data from the HE Marx dataset from BIEN, as it consisted of only
207 one value, making it challenging to include the study level effect in our model.
208

Joint model of trait and phenology

To understand the implications of linking traits directly to cue responses, we developed a joint hierarchical Bayesian model. Our model is composed of two sub-models, a trait model and a phenology model, that are co-estimated and linked by a shared parameter. Since each trait varied in the number of studies in which it is included as well as the number of individuals for which it is measured, we chose to model each trait separately. The first part of the model is a hierarchical intercept only model where the response variable $Y_{i,j}$ is the observed trait value of species i from study j, and is assumed to be normally distributed. We further assume that the observed trait value is composed of a "grand" species trait value $\alpha_{\text{trait},i}$ that is shared across all individuals of a species and that is independent of environment, a hierarchical grouping term on the intercept for study, $\alpha_{\text{study},j}$, to account for study-level differences in environment or observation methods, and random error. This results in the following sub-model for each trait:

$$Y_{i,j} \sim \mathcal{N}(\mu_{i,j}, \sigma_{\text{trait}}) \tag{1}$$

where σ_{trait} represents random error in the trait value (i.e., independent of study or species) and:

$$\mu_{i,j} = \alpha_{\text{trait},i} + \alpha_{\text{study},j} \tag{2}$$

with:

$$\boldsymbol{\alpha}_{\text{trait}} = \{\alpha_{\text{trait},1}, \ldots, \alpha_{\text{trait},n}\}^T \text{ such that } \boldsymbol{\alpha}_{\text{trait}} \sim \mathcal{N}(\mu_{\alpha_{\text{trait}}}, \sigma_{\alpha_{\text{trait}}}) \tag{3}$$
$$\boldsymbol{\alpha}_{\text{study}} = \{\alpha_{\text{study},1}, \ldots, \alpha_{\text{study},n}\}^T \text{ such that } \boldsymbol{\alpha}_{\text{study}} \sim \mathcal{N}(0, \sigma_{\alpha_{\text{study}}})$$

Parameters $\mu_{\alpha_{\text{trait}}}$ and $\sigma_{\alpha_{\text{trait}}}$ represent the mean trait value across all species and the standard deviation in trait values between species, respectively. The mean effect of study is assumed to be centered at 0 with standard deviation $\sigma_{\alpha_{\text{study}}}$.

The second part of the joint model is a hierarchical linear model where the normally distributed response variable $Z_{i,k}$ is the day of budburst for species i experiencing forcing (F_k), chilling (C_k), and photoperiod (P_k). This sub-model is linked to the trait sub-model via the shared parameters $\alpha_{\text{trait},i}$, representing the "grand" trait values of species that are independent of study. The overall structure of the phenology sub-model is similar to that of Ettinger et al. (https://doi.org/10.1038/s41558-020-00917-3), except species' responses to forcing ($\beta_{\text{force},i}$), chilling ($\beta_{\text{chill},i}$), and photoperiod ($\beta_{\text{photo},i}$) are treated not as single parameters but as a combination of parameters, a species-specific response that is independent of its trait value (e.g., $\alpha_{\text{force},i}$) and an effect of its trait value (e.g., $\beta_{\text{trait.force}}$) that is multiplied by $\alpha_{\text{trait},i}$ and does not differ between species. In other words, species responses to cues interact with their "grand" trait values, and we assume this interaction is independent of species identity. The phenology sub-model can thus be written as:

$$Z_{i,k} \sim \mathcal{N}(\mu_{i,k}, \sigma_{\text{pheno}}) \tag{4}$$

where σ_{pheno} represents random error in budburst day and:

$$\mu_{i,k} = \alpha_{\text{pheno},i} + \beta_{\text{force},i} \times F_k + \beta_{\text{chill},i} \times C_k + \beta_{\text{photo},i} \times P_k \tag{5}$$

with:

$$\beta_{\text{force},i} = \alpha_{\text{force},i} + \beta_{\text{trait.force}} \times \alpha_{\text{trait},i} \tag{6}$$
$$\beta_{\text{chill},i} = \alpha_{\text{chill},i} + \beta_{\text{trait.chill}} \times \alpha_{\text{trait},i}$$
$$\beta_{\text{photo},i} = \alpha_{\text{photo},i} + \beta_{\text{trait.photo}} \times \alpha_{\text{trait},i}$$

and all species-specific parameters are, as in the trait sub-model, given hierarchical structure whereby:

$$\boldsymbol{\alpha}_{\text{pheno}} = \{\alpha_{\text{pheno},1}, \ldots, \alpha_{\text{pheno},n}\}^T \text{ such that } \boldsymbol{\alpha}_{\text{pheno}} \sim \mathcal{N}(\mu_{\alpha_{\text{pheno}}}, \sigma_{\alpha_{\text{pheno}}}) \qquad (7)$$

$$\boldsymbol{\alpha}_{\text{force}} = \{\alpha_{\text{force},1}, \ldots, \alpha_{\text{force},n}\}^T \text{ such that } \boldsymbol{\alpha}_{\text{force}} \sim \mathcal{N}(\mu_{\alpha_{\text{force}}}, \sigma_{\alpha_{\text{force}}})$$

$$\boldsymbol{\alpha}_{\text{chill}} = \{\alpha_{\text{chill},1}, \ldots, \alpha_{\text{chill},n}\}^T \text{ such that } \boldsymbol{\alpha}_{\text{chill}} \sim \mathcal{N}(\mu_{\alpha_{\text{chill}}}, \sigma_{\alpha_{\text{chill}}})$$

$$\boldsymbol{\alpha}_{\text{photo}} = \{\alpha_{\text{photo},1}, \ldots, \alpha_{\text{photo},n}\}^T \text{ such that } \boldsymbol{\alpha}_{\text{photo}} \sim \mathcal{N}(\mu_{\alpha_{\text{photo}}}, \sigma_{\alpha_{\text{photo}}})$$

Parameters $\mu_{\alpha_{\text{pheno}}}$, $\mu_{\alpha_{\text{force}}}$, $\mu_{\alpha_{\text{chill}}}$, $\mu_{\alpha_{\text{photo}}}$ represent the mean budburst day, response to forcing, response to chilling, and response to photo period across all species, respectively. Parameters $\sigma_{\alpha_{\text{pheno}}}$, $\sigma_{\alpha_{\text{force}}}$, $\sigma_{\alpha_{\text{chill}}}$, $\sigma_{\alpha_{\text{photo}}}$ are the standard deviations between species.

Forcing, chilling, and photoperiod (F_k, C_k, P_k) were z-scored to account for differences in the scale of predictors across studies (**?**), as well as differences in the natural units for the cues. We assumed parameters had weakly informative prior distributions (generally normal or half-normal distributions) that we obtained from a series of prior predictive checks where the objective was to produce a wide but also plausible range of trait and phenology values (e.g., budburst dates between days $0 - 365$). The joint model was coded in the Stan programming language (Stan citation) and fit to the trait and phenology data (see above) using using the rstan package (version, citation). For all traits, model fits were deemed valid based on *Stan's* diagnostic metrics, including no divergences across 1000 iterations, high effective sample size (n_eff), and scale reduction factor $\hat{R}$ close to 1 across 4 chains. We quantify 90% credible interval of posterior distributions using the highest probability density index.

Finally, we used a phylogenetic generalized least-squares regression model (PGLS) to test the relationship between day of budburst and individual traits. This analysis allowed us to test for phylogenetic non-independence in the phenology-trait relationship (**?**). We obtained a rooted phylogenetic tree by pruning the tree developed by (**?**) and performed the PGLS analysis using the mean trait values and mean posterior estimates of the cue responses from our joint model. The PGLS was run using the "Caper" package in R (**?**).

4   Results

In modelling each individual traits' relationship with phenological cues, our models estiamted the simple geometric mean for each species-trait combination to fall within the posterior estimations from our models (Fig. 2).There were a limited number of species in the height model where the simple geometric mean fell outside of predicted species means after accounting for the effect of study, for example *Quercus ilex*, *Quercus petraea*, *Quercus coccifera*, *Aesculus hippocastanum*, and *Rhamnus cathartica*.These model estimates may also reflect our use of a normal distribution for our traits. While the normal distribution can result in the estimation of ecologically unrealistic zero or negative values, we used this distribution as it strikes a good balance between being biologically realistic and computationally viable. In general our model produced realistic trait estimates that usually did not cross a zero threshold.

The parameterization of our model allowed for the separation of species and study level effects for each trait. Species height values were distributed with a standard deviation of 5.9 m (90% credible interval: 4.7, 7.1), and study height values were distributed with a standard deviation of 7.5 m (90% credible interval: 5.6, 9.4). The strong effects of study level variation are most apparent for in the height estimates of...

Species SLA estimates were distributed with a standard deviation of 7.8 (90% credible interval: 6.1, 9.3), and study SLA estimates were distributed with a standard deviation of 3.3 (90% credible interval: 1.8, 4.8). The highest estimated mean species SLA estimate in our dataset was for *Acer pensylvanicum*, and the lowest estimated mean LNC estimate was for *Quercus coccifera*.

Species $\log_{10}$ seed mass values were distributed with a standard deviation of 1.6 (90% credible interval: 1.3, 1.9), and study $\log_{10}$ seed mass values were distributed with a standard deviation of 1 (90% credible interval: 0.8, 1.1). The highest estimated mean species $\log_{10}$ seed mass estimate in our dataset was for *Juglans cinerea*, which translates as a seed mass of approximately 10000mg. The lowest estimated mean $\log_{10}$ seed mass estimate was for *Betula populifolia*, which translates as a seed mass of approximately 0.1 mg.

Finally, mean LNC was estimated as 22.6 (90% credible interval: 20.4, 25). Species LNC values were distributed with a standard deviation of 5.1 (90% credible interval: 4.1, 6), and study LNC values were distributed with a standard deviation of 3.5 (90% credible interval: 2.1, 5.1). The highest estimated mean species LNC estimate in our dataset was 39 (90% credible interval: 41.5, 36.8) for *Prunus persica*, and the lowest estimated mean LNC estimate was 13 (90% credible interval: 16.5, 10.4) for *Hamamelis virginiana*.

The relationship between species height and cue responses was varied across the three cues, with increasing tall trees having more positive responses to warming, but negative responses to both chilling and photoperiod (Fig. 5). Of the three cues, height had the strongest influence on chilling, with an estimated interaction parameter of -0.5 (90% credible interval: -1, -0.1). The response to photoperiod was also negative, but the photoperiod slope was smaller at -0.3 (90% credible interval: -0.6, 0). Interestingly, forcing had a positive slope, at 0.2 (90% credible interval: -0.1, 0.5). The positive response to forcing indicates that tall trees, like *Acer pseudoplantanus*, have more positive slopes in the full model compared to models with a zero trait effect, and a much greater difference in their slopes than shorter trees like *Corylus avettana* (Fig.6). In contrast, the negative responses to chilling and photoperiod mean that the full model for taller trees will have a more negative slope than that of the model with zero trait effects.

We also found variation in the relationship between SLA and each of the three cue responses (Fig. 5), with increaing SLA leading responses to forcing and chilling to be more positive, but negative responses with increasing photoperiod. Chilling was most strongly influenced by SLA (interaction parameter: 0.3: 90% credible interval: -0.1, 0.7). Species' responses to forcing were also positively correlated with SLA values (interaction parameter: 0.2, 90% credible interval: -0.1, 0.4), however, responses to photoperiod were negative (interaction parameter: -0.2, 90% credible interval: -0.4, 0). For species with high SLA values, such as *Fagus grandifolia*, this positive trait effect in forcing and chilling responses produced a more positive slope in the full model relative to the slope when the trait effect is set to zero. The difference in the slopes of species with leaves with low SLA, such as *Quercus ilex*, is much smaller (Fig.6). The relatively small trait effect of photoperiod is reflected in the smaller difference in the slopes between the full model and the model without the effect of trait (Fig.6).

As we predicted, species log seed mass had a negative relationship with all three cue responses 5. As we observed for other trait models, seed mass had the largest influence on species responses to chilling (interaction parameter: -1.1: 90% credible interval: -2.8, 0.7). The influence of seed mass on forcing and photoperiod were also negative but relativley negligible, with slopes of -0.3 (90% credible interval: -1.4, 0.8) and -0.6 (90% credible interval: -1.6, 0.4) respectivley. The negative trait effect estimated from our model produced more negative slopes, however, given the nature of the log transformation of the seed mass data, the resulting estimated day of budburst can be positive. This is observed in the response of the large seeded species *Aesculus hippocastanum* to forcing cues 6. The negative effect of the seed mass trait still produced a more negative slope in the full model compared to the slope of the model with a zero trait effect for each of the three cues (Fig.6). The slope differences of small seeded species, like *Alnus glutinosa*, was considerably smaller. Our model also estimated the trait effect of photoperiod to produce similar slopes for both the full model and the model with a trait effect set to zero, indicating a relatively small effect of this cue (Fig.6).

Finally, the relationship between LNC and cue responses was also in line with our predictions, producing a positive association between LNC and cue responses (Fig.5). Chilling was most influenced by LNC (interaction parameter: 0.7 :90% credible interval: 0.2, 1.2), followed by the response to forcing (interaction parameter: 0.5: 90% credible interval: 0.1, 0.9), while photoperiod again had the smallest
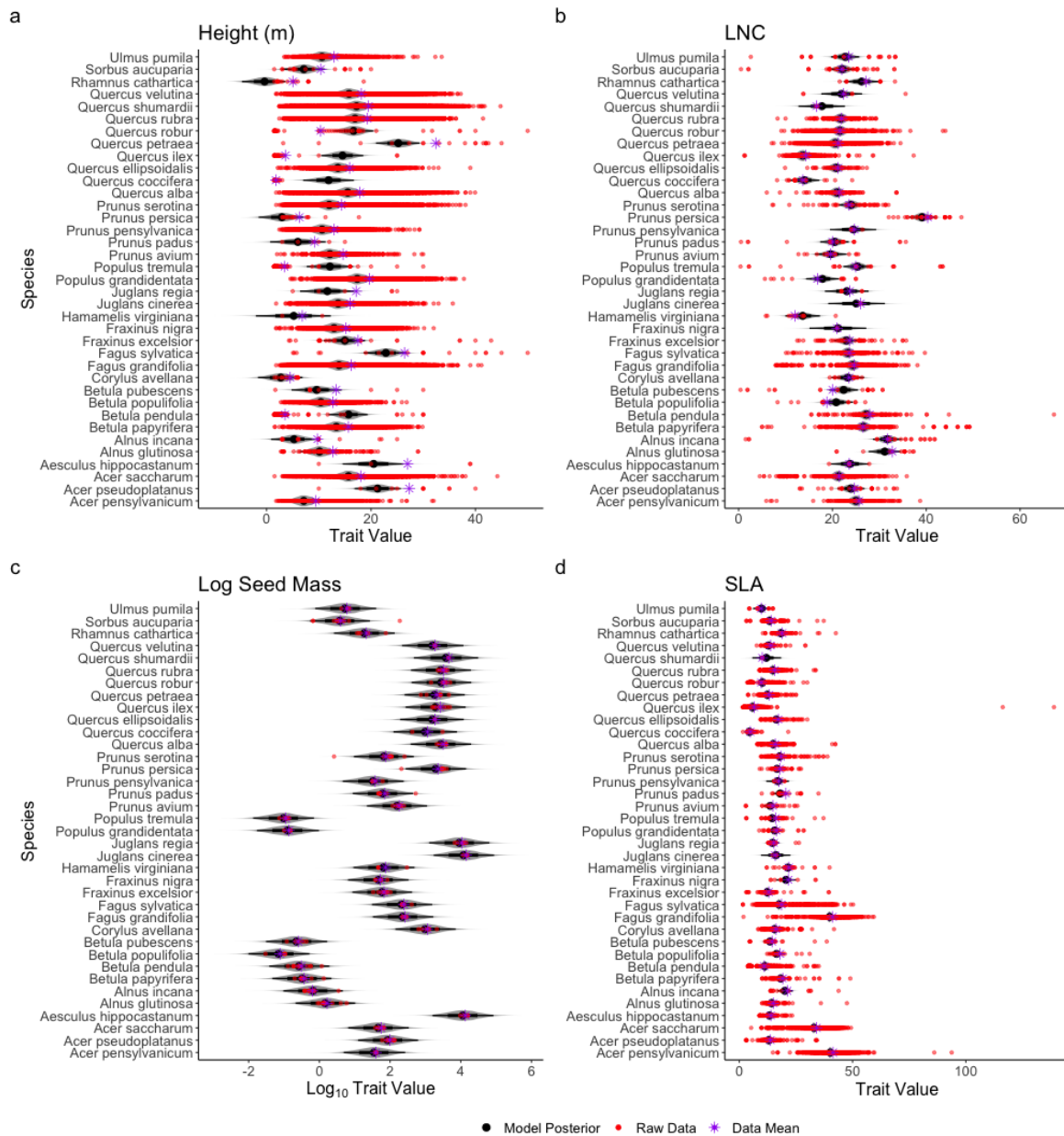
Figure 2: Raw data and posterior esti.

influence (interaction parameter: 0.3 :90% credible interval: 0, 0.7). For species like *Alnus glutinosa* that produce leaves with high LNC, the positive trait effect resulted in a more positive slope in the full model relative to the slope of the zero trait effect model. In contrast, species with low LNC like *Quercus ilex* have a much smaller difference in their slopes (Fig.6). The model estimated trait effect of LNC for photoperiod is relatively small, producing less of a difference between the full model and the zero trait effect model (Fig.6).

The results of the PGLS analysis suggest there are mostly non-significant phylogenetic relationships between each of our four traits and chilling responses, as well as between height and forcing, and seed mass and forcing cues (SM tableX). There were no phylogenetic relationships between SLA or LNC
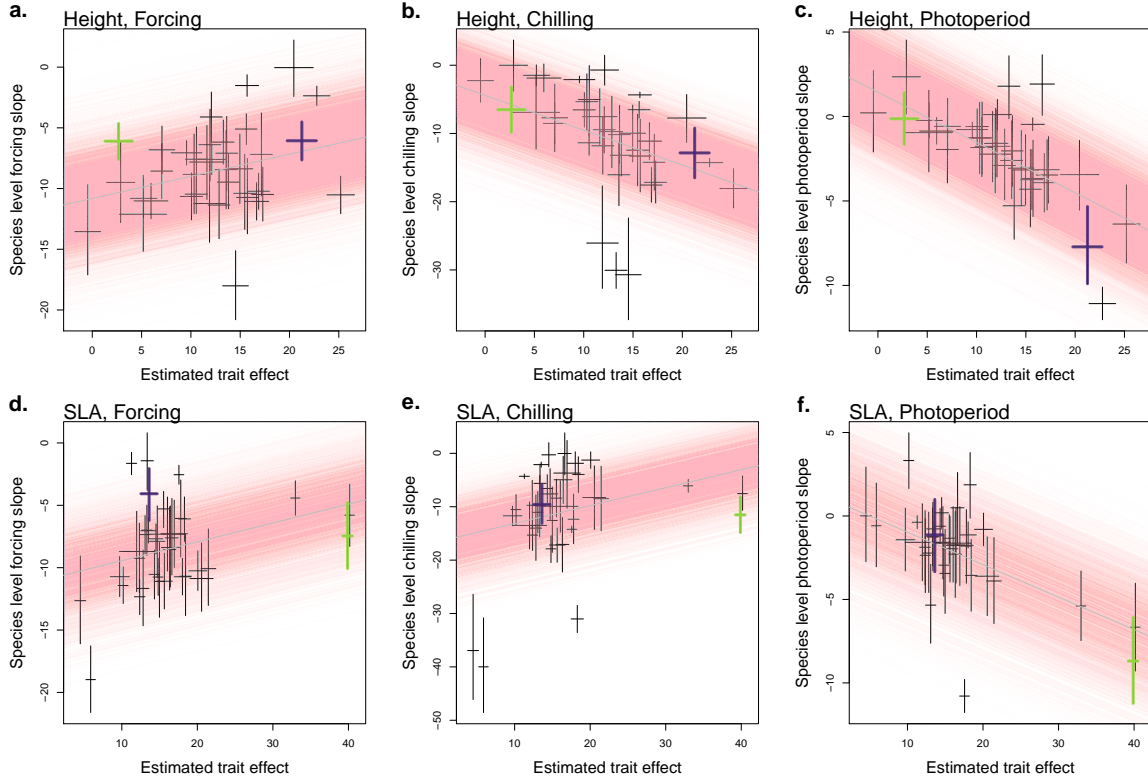
Figure 3: Trait relationships with cue slopes

and forcing, or for any trait and photoperiod cues (SM tableX). While these results suggest there is some phylogenetic effect influencing the cue response of species for these traits, we were unable to further incorporate these effects into our current analysis given the complexity of our model.

5   Discussion

We found that the functional traits of our assemblage of woody plant species did influence plant responses to phenological cues. In general, our findings suggest species with high SLA and LNC leaf traits, low heights, and small average seed masses advance their budburst dates in response to increasing chilling cues, compared to low trait values. Interestingly, the relationships of traits to forcing and photoperiod cues were only partially in line with our predictions. Trees with high SLA and LNC and small seeds did correlate with advances in budburst as forcing increased. However, the response of forcing cues to height contrasted our predictions, with taller trees requiring less forcing temperatures to advane their budburst. Our model estimates also contrasted our predictions for SLA and photoperiod, with species with leaves with high SLA values having a stronger cue response and requiring longer photoperiods to advance budburst. The remaining traits did follow our predictions, with tall species with large seeds and leaves with low LNC having greater responses to longer photoperiods.

Traits consistently had the largest influence on chilling cues, suggesting they play a role in species transition from winter dormancy (endodormancy) and their avoidance of false spring events. As we predicted, there were strong negative relationships between chilling cues and height and seed mass (Fig.6), indicating that species that are tall and produce large seeds do have greater chilling requirements to budburst and will budburst later in the growing season. This is supported by the identity of species with the largest heights and seed masses, including species in the genus *Quercus* and *Fagus*
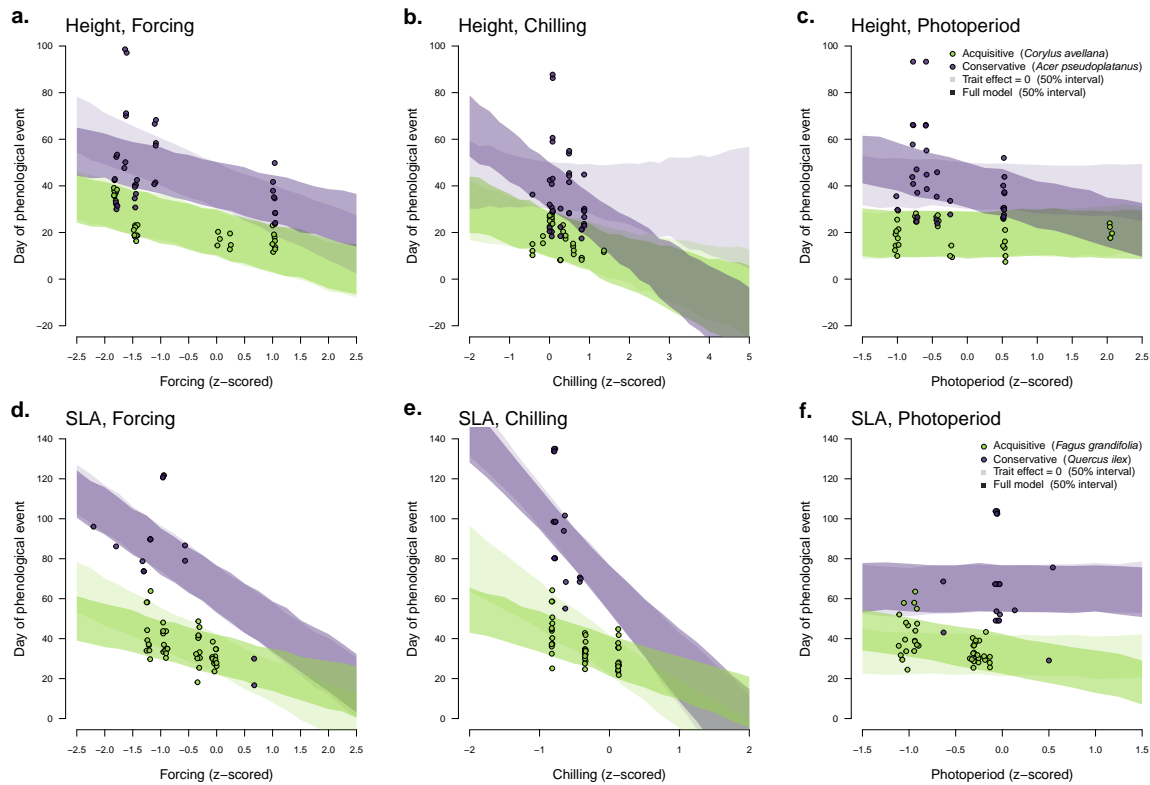
Figure 4: Estimated cue responses for acquisitive and conservative spp.

sylvatica, which are well known late budbursting species (cite studies showing these species are late).
Our model estimates for photoperiod across the four traits also support the findings of previous studies
of phenology that observed responses to photoperiod cues to be weakest (citation). To our surprise, the
influence of LNC on photoperiod cues was weaker than it's cue response to forcing and photoperiod.
This is a trait most commonly associated with photosynthetic potential and a proxy for the amount
of nitrogen rich rubisco enzymes within a leaf (citation). The lack of a relationship therefore suggests
that factors other than daylength, such as herbivore pressure, may be selecting for this functional trait.
Our study relating functional traits to phenology is unique from previous research in that we are
drawing associations between the traits and the cue responses that define woody species phenologies.
In general our model estimates are...

-traits are contributing to cue responses bc the lines are all different

Discuss the DF paper again

- What do our results suggest for the relationship between cue use and traits? - Species responses
  to forcing, chilling, and photoperiod cues are influenced by species functional traits. - generally in
  line with previous studies of phenological cues, cue responses were all negative leading to strong
  advances in bb date

    – Do we find relationships between cues and traits?

    – Do these trends agree with an acquisitive/conservative tradeoff?

- How do our results relate to previous studies? Huang et al. 2018 - found several growth strategies
  - all combinations of early- fast, early-slow, late-fast etc - but looked at flowering Osada 2017
  - bb later for sp with greater LMA, thickness, Narea – driven by differences across deciduous

11

and evergreen spp; Deciduous alone: bb positively correlated with leaf mass, area, vessel diam in cross spp comparisons

- What do our results suggest for the bigger picture? Sun, S., D. Jin, and R. Li. 2006. LMA neg correl with leafout; larger LMA = earlier

  - How might traits constrain/facilitate future shifts in phenology? - our findings do support the idea that phenology is an important functional trait
  - How might ecosystem functioning shift if species track temperature? How to our results relate to seasonality and frost risk?
  - What does it mean if more competitive/invasive species respond to warming and start bb earlier - outcompete species and lead to compressed temporal niche?
  - Relate our results to invasion success

- Limitations/strengths?

  - we assume stronger cues mean earlier bb but really it's more complicated than this
  - broad approach means lose detail and compromise - traits come from different populations to the phenology data
  - disconnect between trait data - observational - and phenology data that is in a controlled environment
  - limited data may have reduced diversity of traits/strategies - may not be enough to detect predicted trends – reframe this as less of a limitation and more of a future direction
  - Why we think mean height values were different from geometric mean values for some species. Talk about the influence of accounting for the study effect.

The trait data used in this experiment was collected independently of the phenological cue experiments, from individuals in diverse ecosystems and community contexts. While this limits our ability to understand the relationships between cue use and traits at the population and individual level, our approach allowed us to test for general trends that scale across populations and can inform future more fine scale research.

References

Basler, D., and C. Körner. 2014. Photoperiod and temperature responses of bud swelling and bud burst in four temperate forest tree species. Tree Physiology 34:377–388.

Caffarra, A., and A. Donnelly. 2011. The ecological significance of phenology in four different tree species: Effects of light and temperature on bud burst. International Journal of Biometeorology 55:711–721.

Chave, J., D. Coomes, S. Jansen, S. L. Lewis, N. G. Swenson, and A. E. Zanne. 2009. Towards a worldwide wood economics spectrum. Ecology Letters 12:351–366.

Chuine, I., and E. G. Beaubin. 2001. Phenology is a major determinant of tree species range. Ecology Letters 4:500–510.

Chuine, I., M. Bonhomme, J. M. Legave, I. García de Cortázar-Atauri, G. Charrier, A. Lacointe, and T. Améglio. 2016. Can phenological models predict tree phenology accurately in the future? The unrevealed hurdle of endodormancy break. Global change biology 22:3444–3460.

Chuine, I., X. Morin, and H. Bugmann. 2010. Warming, photoperiotls, and tree phenology. Science 329:277–278.

Cooke, J. E., M. E. Eriksson, and O. Junttila. 2012. The dynamic nature of bud dormancy in trees: Environmental control and molecular mechanisms. Plant, Cell and Environment 35:1707–1728.

Davies, T. J., E. M. Wolkovich, N. J. Kraft, N. Salamin, J. M. Allen, T. R. Ault, J. L. Betancourt, K. Bolmgren, E. E. Cleland, B. I. Cook, T. M. Crimmins, S. J. Mazer, G. J. McCabe, S. Pau, J. Regetz, M. D. Schwartz, and S. E. Travers. 2013. Phylogenetic conservatism in plant phenology.

Díaz, S., J. Kattge, J. H. Cornelissen, I. J. Wright, S. Lavorel, S. Dray, B. Reu, M. Kleyer, C. Wirth, I. Colin Prentice, E. Garnier, G. Bönisch, M. Westoby, H. Poorter, P. B. Reich, A. T. Moles, J. Dickie, A. N. Gillison, A. E. Zanne, J. Chave, S. Joseph Wright, S. N. Sheremet Ev, H. Jactel, C. Baraloto, B. Cerabolini, S. Pierce, B. Shipley, D. Kirkup, F. Casanoves, J. S. Joswig, A. Günther, V. Falczuk, N. Rüger, M. D. Mahecha, and L. D. Gorné. 2016. The global spectrum of plant form and function. Nature 529:167–171.

Fitter, A. H., and R. S. Fitter. 2002. Rapid changes in flowering time in British plants. Science 296:1689–1691.

Flynn, D. F. B., and E. M. Wolkovich. 2018. Temperature and photoperiod drive spring phenology across all species in a temperate forest community. New Phytologist 219:1353–1362.

Funk, J. L., J. E. Larson, G. M. Ames, B. J. Butterfield, J. Cavender-Bares, J. Firn, D. C. Laughlin, A. E. Sutton-Grier, L. Williams, and J. Wright. 2016. Revisiting the Holy Grail: Using plant functional traits to understand ecological processes. Biological Reviews 92:1156–1173.

Gougherty, A. V., and S. W. Gougherty. 2018. Sequence of flower and leaf emergence in deciduous trees is linked to ecological traits , phylogenetics , and climate. New Phytologist 220:121–131.

Harrington, C. A., and P. J. Gould. 2015. Tradeoffs between chilling and forcing in satisfying dormancy requirements for Pacific Northwest tree species. Frontiers in Plant Science 6:1–12.

Heide, O. M. 1993. Daylength and thermal time responses of budburst during dormancy release in some northern deciduous trees. Physiologia Plantarum 88:531–540.

Kochmer, J. P., and S. N. Handel. 1986. Constraints and Competition in the Evolution of Flowering Phenology. Ecological Monographs 56:303–325.

Laube, J., T. H. Sparks, N. Estrella, J. Höfler, D. P. Ankerst, and A. Menzel. 2014. Chilling outweighs photoperiod in preventing precocious spring development. Global Change Biology 20:170–182.

Laughlin, D. C., J. J. Leppert, M. M. Moore, and C. H. Sieg. 2010. A multi-trait test of the leaf-height-seed plant strategy scheme with 133 species from a pine forest flora. Functional Ecology 24:493–501.

Lechowicz, M. J. 1984. Why Do Temperate Deciduous Trees Leaf Out at Different Times? Adaptation and Ecology of Forest Communities. The American Naturalist 124:821–842.

Menzel, A., T. H. Sparks, N. Estrella, E. Koch, A. Aaasa, R. Ahas, K. Alm-Kübler, P. Bissolli, O. Braslavská, A. Briede, F. M. Chmielewski, Z. Crepinsek, Y. Curnel, Å. Dahl, C. Defila, A. Donnelly, Y. Filella, K. Jatczak, F. Måge, A. Mestre, Ø. Nordli, J. Peñuelas, P. Pirinen, V. Remišová, H. Scheifinger, M. Striz, A. Susnik, A. J. Van Vliet, F. E. Wielgolaski, S. Zach, and A. Zust. 2006. European phenological response to climate change matches the warming pattern. Global Change Biology 12:1969–1976.

Morin, X., M. J. Lechowicz, C. Augspurger, J. O'keefe, D. Viner, and I. Chuine. 2009. Leaf phenology in 22 North American tree species during the 21st century. Global Change Biology 15:961–975.

Osada, N. 2017. Relationships between the timing of budburst, plant traits, and distribution of 24 coexisting woody species in a warm-temperate forest in Japan. American Journal of Botany 104:550–558.

Pereira, C. G., and D. L. Des Marais. 2020. The genetic basis of plant functional traits and the evolution of plant-environment interactions. International Journal of Plant Sciences 181:56–74.

Seiwa, K., and K. Kikuzawa. 1991. Phenology of tree seedlings in relation to seed size. Canadian Journal of Botany 69:532–538.

Singh, R. K., T. Svystun, B. AlDahmash, A. M. Jonsson, and R. P. Bhalerao. 2017. Photoperiod- and temperature-mediated control of phenology in trees – a molecular perspective.

Sun, S., D. Jin, and R. Li. 2006. Leaf emergence in relation to leaf traits in temperate woody species in East-Chinese Quercus fabri forests. Acta Oecologica 30:212–222.

Vitasse, Y. 2013. Ontogenic changes rather than difference in temperature cause understory trees to leaf out earlier. New Phytologist 198:149–155.

Westoby, M. 1998. A leaf-height-seed (LHS) plant ecology strategy scheme. Plant and Soil 199:213–227.

Wright, I. J., M. Westoby, P. B. Reich, J. Oleksyn, D. D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J. H. C. Cornellissen, M. Diemer, J. Flexas, J. Gulias, E. Garnier, M. L. Navas, C. Roumet, P. K. Groom, B. B. Lamont, K. Hikosaka, T. Lee, W. Lee, C. Lusk, J. J. Midgley, Ü. Niinemets, H. Osada, H. Poorter, P. Pool, E. J. Veneklaas, L. Prior, V. I. Pyankov, S. C. Thomas, M. G. Tjoelker, and R. Villar. 2004. The worldwide leaf economics spectrum. Nature 428:821–827.

Zohner, C. M., B. M. Benito, J. D. Fridley, J. C. Svenning, and S. S. Renner. 2017. Spring predictability explains different leaf-out strategies in the woody floras of North America, Europe and East Asia. Ecology Letters 20:452–460.

Zohner, C. M., B. M. Benito, J. C. Svenning, and S. S. Renner. 2016. Day length unlikely to constrain climate-driven shifts in leaf-out times of northern woody plants. Nature Climate Change 6:1120–1123.

Zohner, C. M., L. Mo, T. A. M. Pugh, J.-F. Bastin, and T. W. Crowther. 2020. Interactive climate factors restrict future increases in spring productivity of temperate and boreal trees. Global Change Biology pages 1–14.
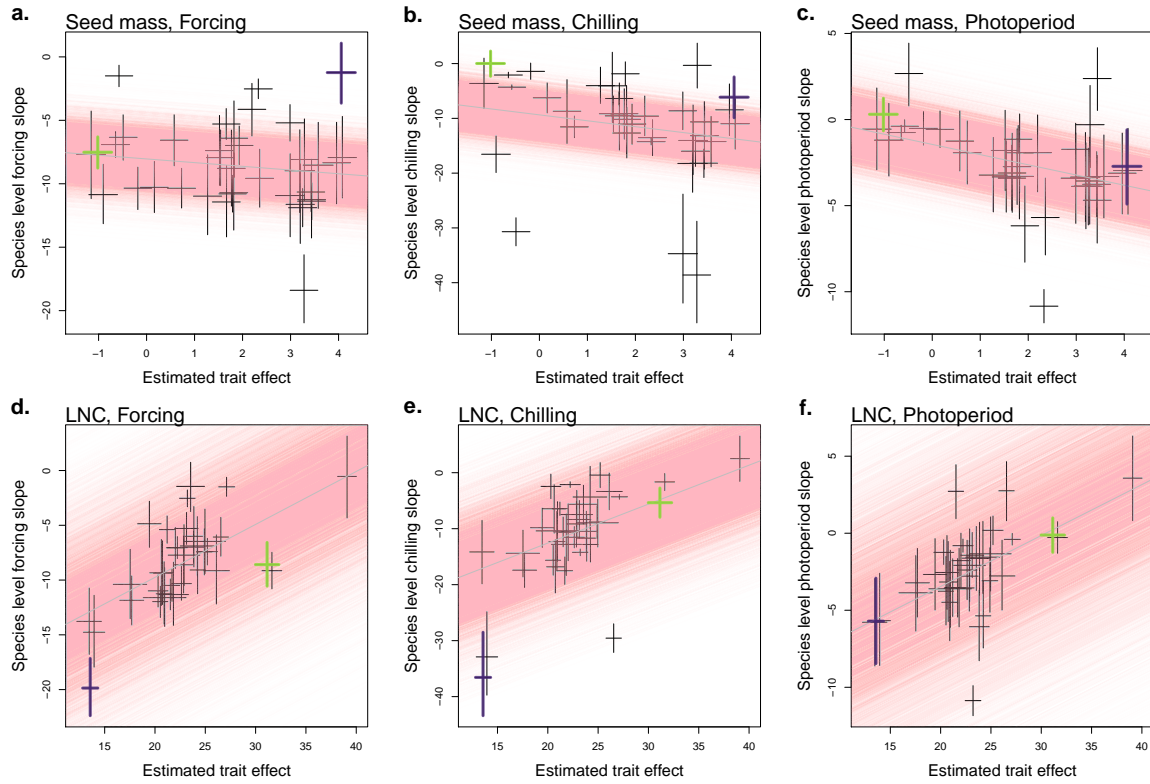
6   Supplementary Material
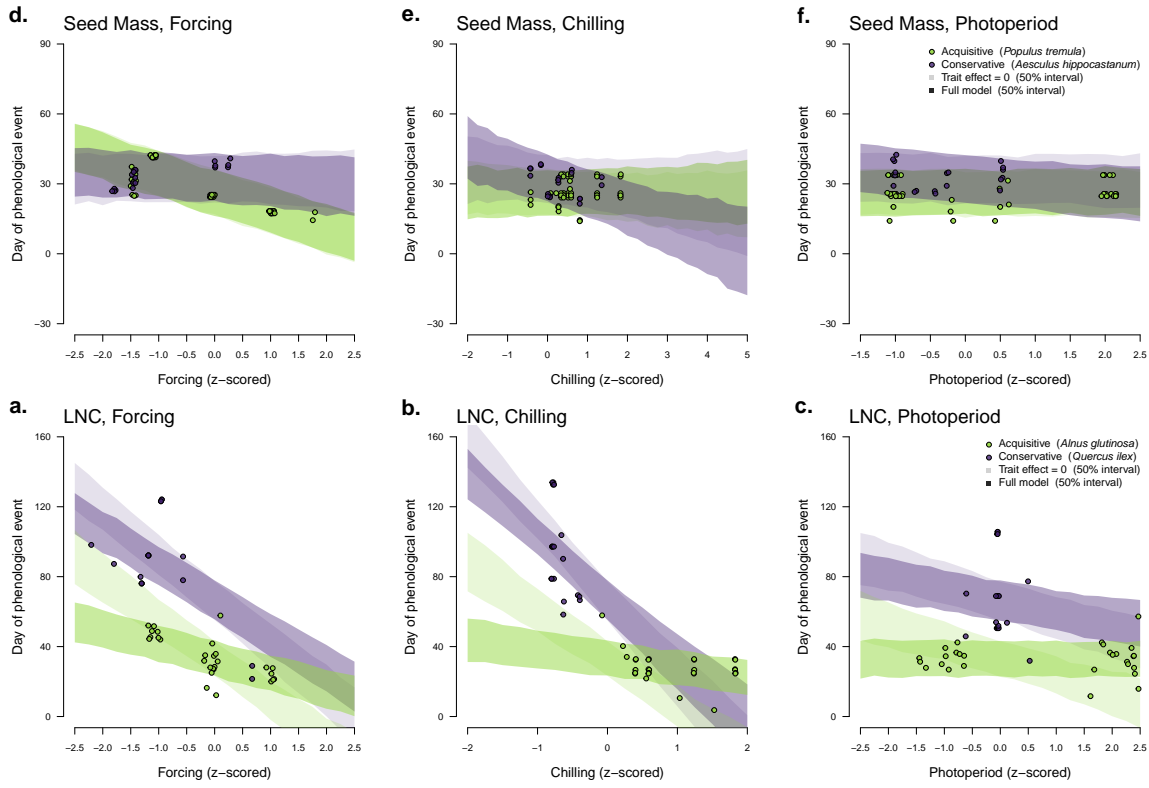
Figure 5: Trait relationships with cue slopes

Figure 6: Estimated cue responses for acquisitive and conservative spp.

Table 1: Height model estimates

Variable	mean	sd	X2.5.	X50.	X97.5.	Rhat
mu_grand	12.62	1.83	8.95	12.63	16.21	1.00
muPhenoSp	32.13	2.69	26.94	32.12	37.43	1.00
muForceSp	-10.81	2.81	-16.34	-10.77	-5.33	1.00
muChillSp	-4.42	4.05	-12.71	-4.35	3.34	1.00
muPhotoSp	1.44	2.23	-2.98	1.44	5.77	1.00
betaTraitxForce	0.18	0.19	-0.21	0.18	0.56	1.00
betaTraitxChill	-0.51	0.28	-1.04	-0.52	0.06	1.00
betaTraitxPhoto	-0.30	0.16	-0.62	-0.30	0.02	1.00
sigma_sp	5.91	0.76	4.61	5.84	7.58	1.00
sigma_study	7.51	1.20	5.49	7.38	10.24	1.00
sigma_traity	5.39	0.02	5.36	5.39	5.43	1.00
sigmaPhenoSp	15.17	2.07	11.23	15.11	19.42	1.00
sigmaForceSp	4.95	1.18	2.99	4.84	7.56	1.00
sigmaChillSp	8.63	2.19	5.25	8.33	13.72	1.00
sigmaPhotoSp	3.45	0.93	1.87	3.36	5.51	1.00
sigmapheno_y	14.22	0.25	13.74	14.22	14.72	1.00

Table 2: SLA model estimates

Variable	mean	sd	X2.5.	X50.	X97.5.	Rhat
mu_grand	16.54	1.57	13.51	16.53	19.54	1.01
muPhenoSp	31.39	2.51	26.51	31.35	36.45	1.00
muForceSp	-10.95	2.67	-16.44	-10.89	-5.87	1.01
muChillSp	-16.49	4.62	-26.03	-16.33	-7.86	1.01
muPhotoSp	0.97	2.56	-4.29	1.02	5.74	1.02
betaTraitxForce	0.15	0.15	-0.13	0.15	0.45	1.01
betaTraitxChill	0.34	0.25	-0.12	0.33	0.84	1.01
betaTraitxPhoto	-0.19	0.14	-0.47	-0.19	0.10	1.02
sigma_sp	7.78	0.97	6.12	7.70	9.89	1.00
sigma_study	3.27	0.96	1.82	3.12	5.49	1.00
sigma_traity	6.17	0.05	6.07	6.16	6.26	1.00
sigmaPhenoSp	13.96	2.10	10.03	13.91	18.20	1.00
sigmaForceSp	4.91	1.13	3.07	4.79	7.43	1.00
sigmaChillSp	10.48	2.29	6.60	10.28	15.35	1.00
sigmaPhotoSp	3.72	0.89	2.24	3.64	5.75	1.00
sigmapheno_y	14.21	0.26	13.71	14.21	14.72	1.00

Table 3: Seed mass model estimates

Variable	mean	sd	X2.5.	X50.	X97.5.	Rhat
mu_grand	1.84	0.48	0.90	1.84	2.77	1.00
muPhenoSp	31.43	2.70	26.33	31.40	36.84	1.00
muForceSp	-8.04	1.57	-11.19	-8.03	-4.98	1.00
muChillSp	-9.36	2.79	-15.05	-9.28	-4.02	1.00
muPhotoSp	-1.44	1.27	-3.90	-1.47	1.06	1.00
betaTraitxForce	-0.29	0.67	-1.58	-0.29	1.03	1.00
betaTraitxChill	-1.08	1.09	-3.20	-1.09	1.07	1.00
betaTraitxPhoto	-0.59	0.58	-1.74	-0.59	0.54	1.00
sigma_sp	1.62	0.19	1.30	1.60	2.03	1.00
sigma_study	0.97	0.10	0.77	0.97	1.16	1.00
sigma_traity	0.25	0.01	0.23	0.25	0.27	1.00
sigmaPhenoSp	14.93	2.29	10.62	14.89	19.61	1.00
sigmaForceSp	4.92	0.99	3.18	4.85	7.06	1.00
sigmaChillSp	10.65	2.53	6.44	10.37	16.20	1.00
sigmaPhotoSp	3.76	0.91	2.23	3.67	5.80	1.00
sigmapheno_y	14.16	0.25	13.69	14.15	14.64	1.00

Table 4: LNC model estimates

Variable	mean	sd	X2.5.	X50.	X97.5.	Rhat
mu_grand	22.65	1.41	19.90	22.65	25.44	1.00
muPhenoSp	31.21	2.51	26.35	31.15	36.32	1.00
muForceSp	-19.42	5.45	-30.39	-19.50	-8.61	1.01
muChillSp	-26.48	7.09	-40.56	-26.52	-12.15	1.00
muPhotoSp	-10.07	4.89	-19.99	-10.02	-0.60	1.01
betaTraitxForce	0.48	0.23	0.02	0.48	0.95	1.01
betaTraitxChill	0.70	0.30	0.09	0.70	1.30	1.00
betaTraitxPhoto	0.33	0.20	-0.06	0.33	0.73	1.01
sigma_sp	5.12	0.61	4.05	5.07	6.44	1.00
sigma_study	3.54	0.97	2.07	3.40	5.78	1.00
sigma_traity	5.13	0.06	5.02	5.13	5.25	1.00
sigmaPhenoSp	14.07	1.96	10.46	13.96	18.13	1.00
sigmaForceSp	4.51	1.03	2.70	4.42	6.76	1.00
sigmaChillSp	8.92	2.02	5.73	8.63	13.60	1.00
sigmaPhotoSp	3.85	0.88	2.37	3.77	5.80	1.00
sigmapheno_y	14.22	0.26	13.73	14.21	14.73	1.00